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The Role of the Catecholaminergic Projections to the Forebrain in the Modulation of Autonomic Responses to Stress

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ABSTRACT

The Role of the Catecholaminergic Projections to the Forebrain in the Modulation of Autonomic Responses to Stress

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Exposure of animals to noxious or stressful stimuli increases heart rate (HR) and blood pressure through activation of the autonomic nervous system (ANS). Stressors also elicit the release of the neuromodulatory catecholamines dopamine (DA) and noradrenaline (NA) in several regions of the forebrain. Although many of these regions project to brainstem nuclei involved in the control of autonomic output, the functions of the catecholamines in the modulation of responses to stressful stimuli mediated by the ANS are not known.

To resolve this issue, a series of experiments was carried out to examine the effects of injections of agonists and antagonists of DAergic and NAergic receptors into four regions of the forebrain innervated by the catecholaminergic projections, on an autonomically-mediated response to stress, the increase in HR induced by tail pinch, in rats anesthetized with urethane. The regions tested included the medial frontal cortex (MFC), agranular insular cortex (AIC), nucleus accumbens (NAC) and the central nucleus of the amygdala (CeA).

Injections of an antagonist of β-adrenoceptors into each of these regions reduced the magnitude of the increase in HR induced by tail pinch. Injections of an agonist of β-adrenoceptors in the MFC, AIC and NAC

increased basal HR but did not affect the pinch response. Injections of drugs acting at α-adrenoceptors altered neither of these parameters, but injections of a combination of agonists of α- and β-adrenoceptors made into the NAC markedly increased the magnitude of the response to pinch.

When injected alone, agonists or antagonists of DAergic receptors were largely without effect on basal HR and the response to pinch. However, injections of a combination of a D_2 antagonist and an agonist of the β-adrenoceptor into the AIC significantly increased the magnitude of the pinch response.

These results provide the first clear evidence that catecholamines released in the forebrain during basal conditions and in response to stress are important modulators of autonomic output. NA, primarily through actions on β-adrenoceptors, exerts the most salient influence, serving to facilitate the output of the ANS during both of these conditions. In the case of the NAC, this NAergic influence may be facilitated, in turn, by the stimulation of α-adrenoceptors. Although DA in these regions may not have a salient influence on autonomic output, it may, via the stimulation of D_2 receptors, modulate the actions of NA on β-adrenoceptors in at least the AIC.

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In the rat, noxious stimuli elicit characteristic behavioral and physiological responses. Behaviorally, the rat may become more vigilant and alert and certain classes of behavior may be suppressed. Exposure to stressors also results in characteristic changes in critical functions such as heart rate (HR), blood pressure, respiration, digestive activity and neuroendocrine activity. The constellation of behavioral and physiological changes seen in response to noxious stimuli is known as the stress response (Chrousos & Gold, 1992; Glavin, 1985; Swanson, 1991).

Many of the physiological responses elicited by stress are mediated by the autonomic nervous system (ANS). The ANS is foremost among the pathways through which the central nervous system (CNS) can influence the activity of organ systems in the periphery, such as the heart and the vasculature. The projections of the ANS to target organs originate within the CNS from preganglionic nuclei located in the brainstem and spinal cord. Structures higher in the CNS can influence autonomic output via projections to the preganglionic nuclei. The neural circuitry mediating these descending influences on autonomic output has been a subject of intense study (Chrousos & Gold, 1992; Loewy, 1991; Oppenheimer & Cechetto, 1990).

Changes in the activity of many pathways in the CNS also occur in response to stress. Of major interest are the forward-projecting groups of neurons located in the midbrain and medulla that contain the neuromodulatory catecholamines dopamine (DA) and noradrenaline (NA). The electrical activity of the neurons of origin of these projections increase in response to stressors, resulting in the increased release of catecholamines from their terminals in the forebrain. The responses of the catecholamine-containing systems to stress have been the subject of study for many years (Cenci, Kalen, Mandel & Bjorklund, 1992). Despite this fact, their function in

behavioral and physiological responses to stress remains unclear. It is known, however, that many areas of the forebrain that are innervated by the catecholaminergic systems project, both directly and indirectly to nuclei in the hypothalamus, medulla and spinal cord involved in the control of autonomic output (Loewy, 1991). It may be suggested, therefore, that the release of catecholamines in the forebrain modulates the basal and stress-induced activity of the ANS via an action on these descending projections.

The findings from several types of studies provide support for this notion. Stimulation of the medial frontal cortex (MFC) or agranular insular cortex (AIC), two such forebrain regions, reduce basal HR and blood pressure. Stimulation of the central nucleus of the amygdala (CeA), on the other hand, increases basal HR and blood pressure and induces ulcers of the stomach, an organ also controlled by the ANS (al Maskati & Zbrozyna, 1989; Hardy & Holmes, 1988; Henke, 1985; Iwata, Chida & LeDoux, 1987).

Output from these areas of the forebrain also appear to modulate evoked increases in autonomic outflow. Stimulation of the MFC blocks the increases in HR and blood pressure induced by the electrical stimulation of the lateral hypothalamus or amygdala. Electrolytic lesions of the MFC or CeA decrease the severity of gastric ulcers induced by exposure to an intense, prolonged stressor, while similar lesions of the CeA have been observed to reduce the magnitude of the HR and blood pressure responses to acute stressors (al Maskati & Zbrozyna, 1989; Coover, Murison & Jellestad, 1992; Roozendaal, Koolhaas & Bohus, 1991; Sullivan & Henke, 1986). The modulation of the influence of these nuclei on autonomic output is poorly understood.

There is evidence to suggest that the catecholaminergic projections to these forebrain regions modulate the basal activity of the ANS. Injections of

NA into the CeA increase blood pressure, while injections of NA into the AIC result in changes in metabolic activity thought to be mediated by the ANS (Leonzio, Card, Zaspal, Price, Timmermans & Schwaber, 1987; McGregor, Menendez, Atrens & Lin, 1991; Ohta, Watanabe & Ueki, 1991).

Evidence that the catecholaminergic projections to these regions modulate autonomic responses to stress is sparse. The most clear evidence for such a possibility suggest a role for catecholamines in the CeA in gastric ulceration induced by stress. Injections of NA or DA into the CeA were found to reduce, while injections of blockers of these receptors increased the severity of these ulcers (Glavin et al., 1991; Ray, Henke & Sullivan, 1987, 1988).

Taken together, these findings suggest that the projections from forebrain nuclei innervated by the catecholaminergic systems can influence the output of the ANS, in both the basal condition and when it is activated. Catecholamines in these nuclei may, furthermore, modulate the influence of the projections descending from these regions on autonomic output.

The release of catecholamines in the forebrain may also modulate autonomic responses to stress indirectly by altering the perceptual qualities of noxious stimuli. The projections to the nucleus accumbens (NAC) have been the most studied in this regard. For example, injections of the catecholamine-releasing agent, amphetamine, directly into the NAC, exerts analgesic effects in behavioral tests of sensitivity to painful stimuli (Altier & Stewart, 1993).

Taken together, the results of these studies implicate the catecholaminergic innervation of the forebrain in the modulation of the output of the ANS. These are the ideas that will be explored in the present thesis, and which provide the basis for the experiments that were carried out. It is hypothesized that the release of catecholamines in the forebrain can serve

inhibitory or facilitatory functions in the modulation of autonomic output during basal conditions and when it is increased in response to noxious or stressful stimuli.

The Catecholaminergic Projections to the Forebrain

The DAergic and NAergic projections to the forebrain have been the subject of intensive study for many years. As a result, a reasonably complete picture of their functional anatomy has been developed. This is especially true in the case of the MFC and NAC. Although the anatomy of the catecholaminergic projections to the AIC and CeA has been clearly delineated, the electrophysiological effects of catecholamines on neurons in these regions are poorly understood.

The Dopamine Systems

Anatomy of the Dopamine Systems

The cell bodies of the midbrain DA systems originate in the ventral tegmental area (VTA) and the adjacent substantia nigra pars compacta of the ventral midbrain. The DAergic neurons in these nuclei project rostrally through the medial forebrain bundle to innervate the forebrain. Although there is some overlap in the fields of projection of these two structures, the VTA projects primarily to limbic regions such as the frontal cortex, the NAC and the CeA, while the substantia nigra projects primarily to structures of the basal ganglia, such as the caudate-putamen (Dahlstrom & Fuxe, 1964; Fallon & Loughlin, 1987; Graybiel & Ragsdale, 1983; Moore & Bloom, 1978; Oades & Halliday, 1987).

The Mesocortical Dopamine Projection

DAergic afferents to the frontal cortex arise primarily from medial regions of the VTA. In the rat, these projections terminate mainly in cortical layers V and VI, although a sparse projection to layers I, II and III also exists. Immunocytochemical evidence suggests the DAergic fibers found in the frontal cortex terminate mainly on the dendrites and spines of pyramidal cells, and form mainly symmetric or inhibitory synapses. A smaller proportion of DA terminals are found on interneurons in the frontal cortex. This pattern of DAergic innervation is similar for both the medial and lateral regions of the frontal cortex including the MFC and AIC (Berger, Thierry, Tassin & Moyne, 1976; Descarries, Lemay, Doucet & Berger, 1986; Fallon & Loughlin, 1987; Febvret, Berger, Gaspar & Verney, 1991; Swanson, 1982; Thierry, Blanc, Sobel, Stinus & Glowinski, 1973; Vincent, Khan & Benes, 1993).

In the frontal cortex, the distribution of receptors for DA matches the distribution of DAergic fibers and terminals. D_1 and D_2 receptors are especially enriched in layers V and VI of both the medial and lateral regions of the frontal cortex. These receptors appear to be localized, at least in the MFC, on pyramidal cells as well as on interneurons, and there is evidence for the colocalization of D_1 and D_2 receptors on both types of cells (Bouthenet, Martres, Sales & Schwartz, 1987; Dawson, Barone, Sidhu, Wamsley & Chase, 1988; Fremeau, Duncan, Fornaretto, Dearry, Gingrich, Breese & Caron, 1991; Girault, Horiuchi, Gustafson, Rosen & Greengard, 1990; Mansour, Meador-Woodruff, Bunzow, Civelli, Akil & Watson, 1990; Martres, Sales, Bouthenet & Schwartz, 1985; Reader, Briere, Gottberg, Diop & Grondin, 1988; Vincent et al., 1993).

The cell bodies and dendrites of the pyramidal neurons that project

cortically and subcortically are located primarily in layers V and VI of the frontal cortex. That their distribution overlaps with the terminal zones of the DA projection and DA receptor fields suggests that DA released in these regions modulates both the laterally-directed and descending projections of the frontal cortices (Berger, 1992; Cassel & Wright, 1986; Ferino, Thierry, Saffroy & Glowinski, 1987; Krettek & Price, 1977; Vogt, Rosene & Peters, 1981).

The Mesoaccumbens Dopamine Projection

The NAC receives a dense DAergic projection from the VTA. In the NAC, DAergic fibers have been observed to form synapses on medium spiny neurons, which are the output cells of the NAC, as well as on locally-projecting interneurons. Levels of D_1 receptors and proteins related to the D_1 receptor are very high throughout the NAC. D_2 receptors and their associated mRNA, in contrast, are more sparsely distributed. The results of studies using electron microscopy and in situ hybridization suggest that D_1 and D_2 receptors are found on medium spiny neurons and on interneurons containing gamma aminobutyric acid (GABA) or acetylcholine. D_1 and D_2 receptors may also be colocalized on the same neurons in this region. Subpopulations of D_1 and D_2 receptors may also be located on DA terminals as well as on the terminals of other afferent projections to the NAC, such as glutamatergic projections arising from the cortex; these receptors may modulate the release of these transmitters. It is likely that, as has been found in the caudate-putamen, the majority of DA receptors in the NAC are located on the dendrites and cell bodies of neurons, rather than on the terminals of its afferents (Allin, Russel, Lamm & Taljaard, 1988; Altar & Hauser, 1987; Bouthenet et al., 1987; Dawson et al., 1988; Fremeau et al., 1991; Graham, Crossman & Woodruff, 1990; Gustafson, Ouimet & Greengaard, 1989; Mansour et al., 1990; Marshall, Navarrete & Joyce, 1989; Sesack, Aoki & Pickel,

1994; Walaas & Oiumet, 1989; Zahm, 1989, 1991).

The Mesoamygdaloid Dopamine Projection

The CeA receives a moderately dense DAergic projection from the VTA-substantia nigra region and from the more posterior A8 cell group in the retrorubral field. The topography of the DAergic projection to, and the location of DAergic receptors in the CeA is poorly understood. Both DA and tyrosine hydroxylase, an enzyme involved in the synthesis of DA are enriched in the lateral division of the CeA. It has been generally found that the lateral CeA possesses relatively higher densities of D_1 receptor binding and D_1 receptor-associated proteins compared to the medial region. This is in opposition to the observation that the mRNA for the D_1 receptor is enriched in the medial CeA and is largely absent laterally. Whether this mismatch reflects the actual distribution of these cellular elements or is due to technical limitations inherent in such studies is not clear. D_2 receptors are also present in the CeA and appear to be most prominent laterally. Little is known about the synaptic connections of the DAergic fibers with the cells of the CeA. It is known, however, that although the lateral CeA contains few neurons that project to sites outside the amygdala, it does project heavily to the medial CeA, the division that contains the projection cells of this nucleus. The localization of DAergic fibers and receptors to the lateral, but not the medial CeA suggests, therefore, that the influence of DA on the output neurons of the CeA may be indirect (Bouthenet et al., 1987; Cassel & Gray, 1989b; Dawson et al., 1988; Freedman & Cassel, 1994; Fremeau et al., 1991; Gustafson & Greengaard, 1990; Leviel, Charriere, Fayada & Guibert, 1986; Loughlin & Fallon, 1983; Roder & Ciriello, 1993).

The Effects of Stress on the Dopamine Systems

Electrophysiological and neurochemical studies have shown that

exposure to stressors activates the DA systems. Stressors such as tail pinch increase the firing of DAergic cells in the VTA (Maeda & Mogenson, 1982; Mantz, Thierry & Glowinski, 1989). These findings are corroborated by both *in vivo* and *ex vivo* studies showing that stress increases the release and turnover of DA in the projection fields of the VTA neurons in the MFC, AIC, NAC and CeA (Abercrombie, Keefe, DiFrischia & Zigmond, 1989; Cenci et al., 1992; Claustre, Rivy, Dennis & Scatton, 1986; Coco, Kuhn, Ely & Kilts, 1992; D'Angio, Serrano, Driscoll & Scatton, 1988; Herman, Guillonneau, Dantzer, Scatton, Semerdjian-Rouquier & Le Moal, 1982; Imperato, Angelucci, Casolini, Zocchi & Puglisi-Allegra, 1992; Lavielle, Tassin, Thierry, Blanc, Herve, Barthelmy & Glowinski, 1978; MacLennan, Pelleymounter, Atmadja, Jakubovic, Maier & Fibiger, 1989).

The stress-evoked release of DA in at least the MFC appears to be dependent on both the increased firing of DAergic neurons. Perfusion of dialysis probes implanted in the MFC or NAC with either the sodium channel blocker tetrodotoxin or calcium-free dialysate reduces the stress-induced release of DA in these sites (Moghaddam, Roth & Bunney, 1990; Moghaddam, 1993).

Part of the stress-induced release of DA may be mediated at the level of the DA terminals by glutamatergic mechanisms. Glutamate is released in the MFC in response to stress (Moghaddam, 1993), possibly from corticopetal projections that synapse on the terminals of the mesocortical DA projection. Perfusion of dialysis probes implanted in the MFC with antagonists of the glutamate receptor reduces the stress-induced release of DA in this brain region (Jedema & Moghaddam, 1994), suggesting that at least part of the stress-induced release of DA in the MFC is dependent on glutamatergic afferents. Although glutamatergic terminals are present in other regions of the brain

such as the NAC, AIC and CeA, and locally applied glutamate is known to evoke the release of DA in at least the NAC, the role of glutamate in the stress-evoked release of DA in these other regions is not known (Carter, 1980; Cassel & Wright, 1986; Imperato, Honore & Jensen, 1990; Takagishi & Chiba, 1991).

The mesocortical DA projection may be the most sensitive to the effects of stressors in terms of the level of noxious stimulation required to elicit DA release, at least when compared to the mesoaccumbens projection. The application of more intense or longer-lasting stressors appears to be necessary to evoke the release of DA in the NAC (Abercrombie et al., 1989; Cenci et al., 1992; Imperato, Puglisi-Allegra, Casolini & Angelucci, 1991; Keefe, Stricker, Zigmond & Abercrombie, 1990; Moghaddam et al., 1990).

Electrophysiology of Cells in the Terminal Fields of the Dopamine Systems

The role of DA as a modulator of the spontaneous and evoked activity of neurons in the MFC and NAC has been the subject of numerous studies. The effects of DA on neurons in the AIC and CeA, on the other hand are not known.

Effects of Dopamine on Neurons of the Frontal Cortex

The primary effect of the stimulation of DA receptors located on pyramidal cells in the frontal cortex is hyperpolarization and a resulting inhibition of firing. Stimulation of the VTA, a treatment that releases DA in the MFC, markedly reduces the spontaneous firing of neurons in the MFC. This inhibitory effect of VTA stimulation is reduced by lesions of the ascending DA projections or by the iontophoretic administration of antagonists of D_2, but not D_1 receptors onto the MFC cells prior to VTA stimulation (Ferron, Thierry, Le Douarin & Glowinski, 1984; Godbout, Mantz,

Pirot, Glowinski & Thierry, 1991; Mantz, Milla, Glowinski & Thierry, 1988; Peterson, Olsta & Matthews, 1990; Peterson, St. Mary & Harding, 1987; Pirot, Godbout, Mantz, Tassin, Glowinski & Thierry, 1992).

The local iontophoresis of DA also inhibits the spontaneous activity of cells in the MFC (Godbout et al., 1991; Pirot et al., 1992; Sesack & Bunney, 1989; Yang & Mogenson, 1990). Sesack & Bunney (1989) found that the inhibitory effect of DA was greatly reduced by the co-iontophoresis of antagonists of the D_2 receptor, while D_1 antagonists had a much weaker effect. These results are supported in part by those of Godbout et al. (1991), who found that the iontophoresis of D_2, but not D_1 receptor antagonists blocked the inhibitory effects of iontophoretically-applied DA in the MFC. In contrast to the observations made by Sesack & Bunney (1989), Godbout et al. (1991) did find that the effects of an iontophoretically-applied D_2 agonist mimicked those of DA. In agreement with Sesack & Bunney (1989), and Godbout et al. (1991), Parfitt, Gratton & Bickford-Wimer (1990) found that the local microinjection of a D_2 agonist was much more effective in reducing the firing rate of MFC cells than was a D_1 agonist. They found, in addition, that D_1 and D_2 agonists did not act synergistically when co-injected.

The results of several studies suggest that DA is an important modulator of the evoked firing of pyramidal cells in the MFC. Stimulation of the VTA has been shown to inhibit the firing of MFC cells induced by stimulation of the mediodorsal nucleus of the thalamus (MD), a site that sends excitatory glutamatergic projections to the MFC. The firing of cells in the MFC induced by noxious stimuli such as tail pinch was similarly inhibited by stimulation of the VTA. The subtype of the DA receptor mediating the inhibitory effects of DA on the evoked firing of cells were not examined (Mantz et al., 1988; Thierry, Godbout, Mantz & Glowinski, 1990).

Taken together, these studies suggest that the most important effect of DA on the activity of pyramidal cells in the MFC is inhibition. Stimulation of the VTA or the local application of DA or DAergic agonists has been consistently shown to reduce both the spontaneous and evoked firing of pyramidal cells in the MFC. The D_2 receptor appears to be the primary mediator of these inhibitory effects of DA, at least in the case of spontaneous activity. The D_1 receptor may also mediate part of the inhibitory effects of DA, but its role in this appears to be smaller than that of the D_2 receptor.

Effects of Dopamine on Neurons of the Nucleus Accumbens

As with MFC neurons, the stimulation of the VTA or the local iontophoresis of DA inhibits the spontaneous activity of neurons in the NAC. D_2 receptors appear to be the primary mediator of these inhibitory effects of DA (O'Donnell & grace, 1994; Parfitt et al., 1990), although a few studies have shown that D_1 receptors may also be involved (Sasa, Hara & Takaori, 1991; Parfitt et al., 1990).

A series of studies have provided evidence for an interaction of D_1 with D_2 receptors in the production of the inhibitory effects of DA in the NAC. The depletion of DA by α-methyl paratyrosine was shown to greatly reduce the inhibitory effect of a D_2 agonist applied by iontophoresis, an effect that was reinstated by the co-iontophoresis of an electrophysiologically inactive dose of a D_1 agonist. The depletion of DA did not affect the inhibitory response to the iontophoresis of a higher dose of a D_1 agonist. These results suggest that the stimulation of D_1 receptors alone can inhibit the spontaneous firing of cells in the NAC, and that the co-stimulation of D_1 receptors is necessary for the expression of the inhibition of firing seen when D_2 receptors are stimulated (Hu, Wachtel, Galloway & White, 1990; Johansen, Hu & White, 1991; Wachtel, Hu, Galloway & White, 1989; White, 1987; White

& Wang, 1986).

DA has also been shown to modulate the evoked activity of neurons in the NAC. The activation of NAC neurons induced by stimulation of the parafascicular thalamus, hippocampus, amygdala or frontal cortex is reduced when the VTA is concurrently stimulated (Hara, Sasa & Takaori, 1989; Liang, Wu, Yim & Mogenson, 1991; Sasa, Hara & Takaori, 1991; West & Michael, 1990; Yang & Mogenson, 1984; Yim & Mogenson, 1982). Hara et al. (1989) showed that the inhibitory effect of VTA stimulation on the evoked activity of NAC neurons could be mimicked by the iontophoretic application of either D_1 or D_2 agonists onto the NAC cells. The effect of VTA stimulation was blocked by the iontophoresis of D_1, but not D_2 receptor antagonists onto the NAC cells. These results are in agreement with those of studies showing that D_1 receptor stimulation is inhibitory on the activity of NAC neurons, and may be necessary for the expression of the inhibitory effects D_2 receptor stimulation. Other work, carried out both *in vivo* and *in vitro* , has demonstrated that, in general, D_2 agonists exert a more potent inhibitory effect on the evoked activity of cells in the NAC than do D_1 agonists. These studies, however, did not specifically address the possibility that the effects of stimulation of D_2 receptors is dependent on co-stimulation of D_1 receptors (Liang et al., 1991; O'Donnell & Grace, 1994; Pennartz, Dolleman-Van der Weel, Kitai & Lopes de Silva, 1992; Qiao, Dougherty, Wiggins & Dafny, 1990).

The Noradrenaline Systems

Anatomy of the Noradrenaline Systems

The cell bodies of the NAergic projections to the forebrain are found in

the LC and several nuclei located in the tegmental field of the brainstem. The NAergic cells of the tegmental nuclei are intermixed with cells that contain adrenaline. The LC, on the other hand, is composed of only NAergic cells.

The NAC and CeA receive NAergic projections from both the LC and the lateral tegmental groups, while the NAergic innervation of the cerebral cortex is derived solely from the LC. In addition to their projections to the forebrain, the NAergic nuclei project to sites in the pons, medulla and spinal cord (Cunningham & Sawchenko, 1988; Grzanna & Fritschy, 1991; Guyenet, 1991; Jones, 1991; Roder & Ciriello, 1994).

The Coeruleocortical Noradrenaline Projection

As with the rest of the cerebral cortex, the frontal cortex receives NA-containing projections from the LC. These projections are widespread and relatively diffuse in their sites of termination. NAergic fibers are found throughout all cortical layers but appear to be most prominent in superficial layers. β_1 and both α_1 and α_2 receptors are found in the frontal cortex. β_1 receptors occur mainly in superficial layers, while α adrenoceptors of both subtypes are sparsely and diffusely distributed throughout all layers of the frontal cortex. NAergic receptors have been found on both pyramidal cells and on interneurons in the frontal cortex. Synapses made by NAergic fibers have been difficult to verify in any area of the cortex due, possibly, to the extremely fine nature of the fibers or labeling methods of poor sensitivity. The hypothesis that NA exerts its postsynaptic effects in a paracrine manner, by diffusing to receptors located some distance from the terminals may also account for the difficulty in demonstrating NAergic synapses. A substantial proportion of β-adrenoceptors may also be localized on glial cells in the frontal cortex (Dahlstrom & Fuxe, 1964; Descarries, LeMay, Doucet & Berger, 1988; Jones, 1991; Jones & Palacios, 1991; Sargent Jones, Gauger & Davis, 1985;

Stone & Ariano, 1989; Wanaka,Kiyama, Murakami, Matsumoto, Malbon & Tohyama, 1989).

The Coeruleo-Accumbal and Tegmento-Accumbal Noradrenaline Projections

Compared with the DAergic projection, relatively little is known about the NAergic innervation of the NAC. The NAergic innervation of the NAC originates from both the LC and the tegmental cell groups, and these two projections do not appear to be compartmentalized regarding their areas of termination in the NAC. NAergic fibers appear to be diffusely distributed throughout the extent of the NAC, but, as in the case of the cortex, synapses made by the NAergic fibers are difficult to verify in this nucleus. Both β_1 and α adrenoceptors of both subtypes are found in the NAC. The distribution of these receptors in the NAC is described as sparse and diffuse (Allin et al., 1988; Grzanna & Fritschy, 1991; Jones & Palacios, 1991; Sargent Jones et al., 1985; Wanaka et al., 1989).

The Coeruleo-Amygdalar and Tegmento-Amygdalar Noradrenaline Projections

The CeA receives NAergic projections from the LC and the tegmental cell groups. NAergic fibers and dopamine beta hydroxylase, the NA-synthesizing enzyme, appear to be most prominent in medial areas of the CeA. The synaptic connectivity of the NAergic fibers in the CeA is not known. β_1, α_1 and α_2-adrenoceptors have been demonstrated in the CeA, but their localization has not been clearly delineated. The fact that NAergic fibers and the synthesizing enzyme for NA are found primarily in the medial division of the CeA suggests that this transmitter plays an important role in the output of this nucleus, since this region contains the projection cells of the CeA (Grzanna & Fritschy, 1991; Jones & Palacios, 1991; Petrov, Krukoff & Jhamandas, 1993; Roder & Ciriello, 1993; Sargent Jones, Gauger & Davis, 1985;

Wanaka et al., 1989).

The Effects of Stress on the Noradrenaline Systems

Stress activates the NAergic projections to the forebrain. Exposure to acute stress increases the firing of NAergic cells in the LC, an effect that is probably mediated through a glutamatergic mechanism in the region of the cell body (Adams & Foote, 1988; Hajos & Engberg, 1990). Consistent with these findings is the observation that acute stressors increase both the turnover of NA and the rate of tyrosine hydroxylation in the LC. Similar increases in the turnover of NA have been observed in the tegmental cell groups known to project to the NAC and CeA, suggesting that these projections are also activated in response to stress (Lachuer, Gaillet, Barbagli, Buda & Tappaz, 1991).

The enhanced firing and metabolic activity of NAergic cells induced by stress is reflected in the increased release and turnover of NA in regions of the brain that they innervate. The stress-induced release of NA has been demonstrated using the technique of microdialysis in the MFC, NAC and CeA (Cenci et al., 1992; Pacak, Palkovits, Kvetnansky, Fukuhara, Armando, Kopin & Goldstein, 1993). The effects of stress on the release of NA in the AIC have not been tested using microdialysis, but a study measuring levels of NA and its metabolites in tissue samples from this region after stress revealed changes in NA turnover consistent with increased release (Herman et al., 1982).

Effects of Noradrenaline on Neurons of the Frontal Cortex

Both the electrical stimulation of the LC and the local iontophoresis of NA have been shown to inhibit the spontaneous activity of pyramidal cells in the MFC. This inhibitory effect of the iontophoresis of NA onto MFC cells was blocked by co-iontophoresis of antagonist of the β-adrenoceptor and mimicked by co-iontophoresis of β-adrenergic agonists. In contrast, the co-iontophoresis of α antagonists had no effect on the inhibitory responses shown by cortical cells to the iontophoretic application of NA (Godbout et al., 1991).

The effects of LC stimulation on the evoked activity of neurons in the MFC appear to be more subtle. Although stimulation of the LC has been shown to reduce the rate of firing of MFC neurons evoked by either MD stimulation or noxious stimuli such as tail pinch, the LC stimulation has a greater effect on the background firing of the cells. The fact that the spontaneous firing of cells is reduced while the evoked firing of cells is relatively unaffected by stimulation of the LC has led to the interpretation that the stimulation of NA receptors in the MFC may actually facilitate neurotransmission by increasing the signal to noise ratio (Mantz et al., 1988; Thierry et al., 1990).

In general, these results agree with those of electrophysiological studies of the role of NA in other regions of the cortex suggesting that the stimulation of adrenoceptors modulates the postsynaptic effects of other transmitters. For example, the iontophoresis of NA onto cells of the somatosensory cortex can unmask the excitatory effects of subthreshold doses of iontophoretically-applied glutamate or acetylcholine, transmitters that

commonly excite cortical cells at higher doses. In support of this finding, the firing of cells in the somatosensory cortex induced by tactile stimulation is also enhanced by the local iontophoresis of NA (Foehring, Schwindt & Crill, 1989; Law-Tho, Crepel & Hirsch, 1993; Mouradian, Sessler & Waterhouse, 1991; Radisavljevik, Cepeda, Peacock, Buchwald & Levine, 1994).

There is controversy surrounding the identity of the adrenoceptor subtype underlying these facilitatory effects of NA in the cerebral cortex. Mouradian et al. (1991) found that the local iontophoresis of an agonist of the α_1-adrenoceptor unmasked the excitatory effects of subthreshold pulses of glutamate in a manner similar to NA. The effects of NA were, furthermore, blocked by antagonists of the α_1-adrenoceptor, and shown to be mediated by the second messenger system coupled to this receptor. In this study, the local iontophoresis of β-adrenergic drugs and second messengers linked to the β-adrenoceptor had no effect on the NA-induced potentiation of the response to glutamate. In contrast, Radisavljevik et al. (1994) showed that the potentiating effect of iontophoretically-applied NA on the glutamate-induced firing of cortical cells was mimicked by an agonist of the β-adrenoceptor and blocked by the corresponding antagonist. Drugs acting specifically at α -adrenoceptors were not tested in this study. The reasons for the discrepant findings described in these two studies are not clear, as they utilized similar experimental preparations.

The results from a recent series of studies using the technique of c-Fos immunocytochemistry also shed light on the role of NA in the activation of MFC neurons induced by stress. C-Fos is an immediate early gene whose expression in neurons is widely used as an index of their activation. It was found that either 6-OHDA lesions of the LC or the systemic injection of an antagonist of the β-adrenoceptor blocked the increased expression of c-Fos in

neurons in the MFC induced by either stress or the administration of yohimbine, a drug that stimulates the release of NA (Bing, Stone, Zhang & Filer, 1991; Stone, Zhang, John, Filer & Bing, 1993). These results suggest that NA released during stress mediates, at least in part, the activation of neurons in the MFC via the stimulation of β-adrenoceptors. Although this finding suggests that NA facilitates the activation of MFC neurons, it must be pointed out that the expression of c-Fos is only detectable with stressors of relatively long duration. It is possible that NA plays a different role in the earlier-appearing activational responses of MFC neurons to stressors.

Work by this same group suggests that glia may be a primary target of the NAergic innervation of the MFC (Stone & Ariano, 1989; Stone, John, Bing & Zhang, 1992). They found that the inhibition of glial metabolism blocked the increases in cyclic adenosine monophosphate (cAMP), the cellular second messenger associated with the β-adrenoceptor, induced by an agonist of the β-adrenoceptor in both *in vivo* and *in vitro* preparations . The destruction of neurons with an excitotoxin, on the other hand, did not affect the cAMP response to the agonist. These results are in agreement with anatomical work demonstrating that many β-adrenoceptors are located on glia in the MFC and other cortical regions. It is nevertheless clear that drugs acting at the β-adrenoceptor exert potent effects on the activity of neurons in the cortex. Stone suggests two explanations for this apparent discrepancy. First, separate populations of β-adrenoceptors may exist in the MFC, one on glia, coupled positively to adenylate cyclase, and one on neurons, not coupled to adenylate cyclase but still capable of altering the firing of the neurons. Another possibility is that the stimulation of β-adrenoceptors on glia induces them to release a neuroactive substance that, in turn, affects neuronal activity. Recent results suggest that cAMP itself may be this substance as it is readily detectable

via microdialysis in the extracellular space in response to the stimulation of β-adrenoceptors or to stress (Egawa, Hoebel & Stone, 1988; Stone & John, 1992). Stone further suggests that the cAMP may then enter the neurons and thus influence their activity. These results, taken together with those from electrophysiological and neuroanatomical studies, suggests that the stimulation of β-adrenoceptors is clearly able to exert potent effects on the activity of neurons in the MFC, whether they are localized on neurons or glia.

Effects of Noradrenaline on Neurons of the Nucleus Accumbens

In comparison to DA, much less is known about the role of NA in the electrical activity of NAC neurons. Unemoto, Sasa & Takaori (1985a, 1985b) found that stimulation of the LC or the local iontophoresis of NA reduced the firing of neurons in the NAC induced by stimulation of the hippocampus. This effect of LC stimulation was blocked by the local iontophoresis of antagonists of the β-adrenoceptor onto the NAC cells, but not by the similar application of antagonists of the α-adrenoceptor. The excitation of NAC cells induced by stimulation of the parafascicular thalamus was blocked by neither stimulation off the LC nor the iontophoretic application of NA. These results suggest that NA acting at the β-adrenoceptor inhibits the evoked activity of neurons in the NAC and that this NAergic influence is specific to separate populations of NAC cells. The role of NA in the spontaneous firing of neurons in the NAC was not assessed in this study.

Effects of Noradrenaline on Neurons of the Central Nucleus of the Amygdala

Only one study appears to have been carried out on the influence of NA on the electrophysiological activity of neurons in the CeA. Rainnie, Fernhout & Shinnick-Gallagher (1992) found, *in vitro* , that the hyperpolarization of CeA neurons that occurs after they fire was blocked by bath application of NA. This observation suggests that NA has the net effect

of facilitating the activity of CeA neurons. This is in keeping with the actions of NA demonstrated on the evoked activity of cells in the cerebral cortex.

Taken together, the results of these studies suggest that the catecholamines DA and NA influence the activity of neurons in many forebrain nuclei. Both of these transmitters primarily act to inhibit the spontaneous activity of neurons. In the case of evoked activity, most research shows that DA is inhibitory. NA has been found to inhibit the evoked activity of neurons in some studies, while others provide evidence that the activation of NAergic receptors potentiates the postsynaptic actions of excitatory neurotransmitters. These facilitatory effects of NA have been most often demonstrated in the cerebral cortex.

The inhibitory effects of DA appear to be mediated by receptors of the D_2 subtype, although the stimulation of D_1 receptors has also been observed to result in inhibition. There is also evidence for the interaction of D_1 with D_2 receptors in the production of these inhibitory effects of DA. The stimulation of D_1 receptors may be required for the expression of the inhibitory effects of D_2 receptors.

The inhibitory effects of NA on neuronal activity in at least the MFC and NAC appears to be mediated by stimulation of β-adrenoceptors. β-adrenoceptors appear to be the primary mediators of the facilitatory effects of NA on the evoked activity of cortical neurons, although one recent study also demonstrated that the α-adrenoceptor participates in this.

The Autonomic Nervous System

The ANS is a primary regulator of vital functions critical to homeostasis. It controls the activity of cardiac and smooth muscle, the

exocrine glands, thermogenetic mechanisms and has an indirect, but powerful influence on metabolism through its control of the release of hormones from the pancreas and adrenal medulla. Its cell bodies of origin, from which the projections to target organs originate, are known as preganglionic neurons and are found in the medulla oblongata of the brainstem and in the spinal cord. These preganglionic neurons, in turn, receive projections from nuclei located throughout the CNS (Dampney, 1994).

The ANS mediates many reflexes involved in homeostasis. Sensory neurons innervating autonomically-controlled organs route information from receptors on the organs regarding the state of vital parameters, such as the pressure and pH of the blood, to nuclei in the spinal cord and brainstem. Afferents from neurons subserving pain and touch in muscle and skin also project to these nuclei. These nuclei, in turn, integrate this afferent information and relay it outward through the effector pathways of the ANS. As will be discussed in the following sections, projections from many regions higher in the neuraxis influence the output of these autonomic nuclei (Dampney, 1994; Guyenet, 1991; Loewy, 1991).

The ANS comprises the sympathetic and parasympathetic divisions. Organs controlled by the ANS are most often innervated by both the sympathetic and parasympathetic divisions. In most cases, such as the cardiovascular system, the sympathetic division exerts an excitatory influence on the activity of target organs, while the parasympathetic division is inhibitory. The outflow of the two systems maintains vital functions in a homeostatic balance in both the resting state and under conditions when the system is challenged, such as during exposure to a stressor (Loewy, 1991).

The Sympathetic Nervous System

Sympathetic effector or motor pathways to target organs originate from preganglionic nuclei located in the spinal cord. Preganglionic neurons of the sympathetic system project to target organs via a pathway containing two synapses. The preganglionic neurons contain acetylcholine, and project to "postganglionic" neurons grouped in clusters, or ganglia, adjacent to the spine. The postganglionic neurons contain NA and project directly to the target organ (Loewy, 1991).

The projections of the preganglionic neurons to the sympathetic ganglia are organized in a very loose somatotopic manner, with preganglionic fibers projecting to ganglia controlling the head originating from rostral areas of the spinal cord, and those innervating more caudally-located structures from caudal regions. This apparently loose organization belies the specificity with which the sympathetic motor systems can, in certain circumstances, selectively influence individual target organs (Dampney, 1994).

The preganglionic nuclei in the spinal cord are the final relay centers in the CNS through which the motor outflow to sympathetically-controlled organs is routed. They receive direct projections from the paraventricular nucleus of the hypothalamus and from nuclei in the brainstem including the A5 adrenergic cell group, the caudal raphe nuclei and the rostral ventrolateral medulla (RVLM). Of these nuclei, the RVLM plays the most important role in the sympathetic control of cardiovascular output, as the neurons in this region furnish an excitatory drive on the preganglionic neurons (Guyenet, 1991; Stornetta, Morrison, Ruggiero & Reis, 1989).

The Parasympathetic Nervous System

Parasympathetic motor pathways are organized in a manner similar to

those of the sympathetic division. The postganglionic neurons of the parasympathetic system use acetylcholine as a transmitter, however, and their cell bodies are usually located on the wall of the target organ, rather than alongside the spinal cord (Dampney, 1994).

The cell bodies of the preganglionic neurons of the parasympathetic nervous system are located in the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus in the medulla. Projections from both of these nuclei descend through the vagus nerve to their ganglion cell targets. Projections from the dorsal motor nucleus of the vagus terminate in ganglia that control the mucous glands and musculature of the upper gastrointestinal tract, while those from the nucleus ambiguus terminate in the cardiac ganglion and provide parasympathetic, inhibitory control of the heart, especially in terms of HR. The dorsal motor nucleus of the vagus also projects to the cardiac ganglia, but a salient role for this projection in the control of the heart has not been demonstrated (Loewy; 1991).

Projections to the nucleus ambiguus are diverse. The most important sources of its afferents include the CeA, the paraventricular, dorsomedial, lateral and posterior hypothalamic nuclei, the bed nucleus of the stria terminalis, the central gray region, the substantia innominata, the parabrachial nucleus, the nucleus of the solitary tract (NTS) and the mesencephalic reticular formation(Dampney, 1994; Loewy, 1991).

Autonomic Control of Cardiovascular Responses to Stress

Exposure to noxious or stressful stimuli results in increases in HR and blood pressure. The increased outflow through the sympathetic division of the ANS appears to be the most important mediator of these cardiovascular effects of stress (Chrousos & Gold, 1992; Dampney, 1994; Loewy, 1991).

The anatomy and physiology of the sympathetic pathways mediating cardiovascular responses to noxious stimuli have been partially characterized. Stornetta et al. (1989) provided evidence that the RVLM is a key part of the circuit underlying the activation of cardiovascular output induced by the noxious stimulation of peripheral nerves. This response, also known as the somatic pressor reflex, can also be elicited by intense electrical or mechanical stimulation of the skin, limbs or tail in awake or anesthetized animals. The afferent nerves thus stimulated terminate on neurons in the spinal cord that project, in turn, directly to the RVLM, a region that provides the most important excitatory drive on the sympathetic preganglionic neurons involved in cardiovascular output. Stornetta et al. (1989) found that lesions of the RVLM blocked this reflex. These results suggest the possibility that the descending projections from the forebrain may exert their modulatory influence on cardiovascular activation induced by stressors through effects on this circuit. This circuit is consistent with the finding that noxious stimulation of this type increases the firing of RVLM neurons.

The neurons in the RVLM that project to the preganglionic nuclei are also controlled by projections from many areas higher in the neuraxis. One such region is the NTS. The NTS, like the RVLM receives projections from both afferent nerves innervating somatic and visceral sites and from more rostral regions of the CNS including the frontal cortex and CeA. The NTS, in turn, projects to the RVLM. These projections from the NTS are known to be of prime importance in the control of the activity of RVLM neurons. As will be discussed in the following sections, there is evidence that the NTS functions as a critical center through which projections from forebrain nuclei are relayed to the RVLM.

The parasympathetic division of the ANS is also activated by stressors.

Its role in cardiovascular activation induced by noxious stimuli appears to be less important than the sympathetic nervous system. Part of the parasympathetic activation induced by stress may occur secondary to the increased sympathetic outflow. A clear example of this is the transient slowing of HR that occurs in response to sympathetically-mediated increases in blood pressure. Increases in blood pressure are detected by receptors in the periphery; this afferent information feeds back to, and activates the parasympathetic motor nuclei whose descending projections are responsible, in part, for the reduction in HR that occurs to counter the increased blood pressure. This example underscores the mutual relationship the sympathetic and parasympathetic divisions of the ANS share; peripheral events brought about by activation of one division can feed back and activate the other.

Influence of Forebrain Nuclei on Autonomic Output

The catecholaminergic systems innervate forebrain regions known to project to nuclei in the hypothalamus and brainstem involved in the control of the ANS. It is possible that these catecholaminergic projections modulate autonomic responses to stress via an influence of the activity of these descending projections.

In order to examine the influence of the catecholaminergic systems on responses to stress mediated by the ANS, it is necessary to understand the descending projections from their terminal fields in terms of their anatomy and the nature of the signals they conduct to centers involved in the control of the ANS. Does neurotransmission in these pathways serve to facilitate or inhibit the output of the ANS?

Of the forebrain regions examined in the present investigation, the projections from the MFC, AIC and CeA have been the most thoroughly

investigated in terms of their role in the control of autonomic output. The NAC, on the other hand, has been comparatively little-studied in this regard. The following sections will deal with the known neuroanatomy and possible functions of these forebrain nuclei in the modulation of both the basal and evoked output of the ANS.

The Frontal Cortex

The frontal cortex of the rat is the homologue of the dorsolateral prefrontal cortex in the primate, a cortical region known to be involved in the highest orders of information processing and behavior (Berger, Gaspar & Verney, 1991; Kolb, 1984). It is classically defined as the cortical projection zone of the MD, although not all regions of the frontal cortex receive afferents from this area of the thalamus.

The frontal cortex is subdivided into a number of regions based on neuronal morphology and connectivity with other cortical and subcortical sites. In the rat, the frontal cortex comprises the MFC (the cingulate, prelimbic and infralimbic cortices) on the medial bank of the frontal poles, the precentral cortex located centrally and rostrally, and the orbital and agranular insular cortex (AIC) located on the lateral aspect of the frontal poles (Conde, Audinat, Maire-Lepoivre & Crepel, 1990; Freedman & Cassel, 1991; Krettek & Price, 1977; Vog et al., 1981).

The afferent sources and efferent targets of the frontal cortex are diverse. In addition to its DAergic and NAergic input from the brainstem and its input from the midline thalamus, the frontal cortex receives cholinergic projections from the basal forebrain and serotonergic projections from the raphe nuclei. The frontal cortex is also reciprocally connected with the amygdalar nuclei, including the CeA, and with the hypothalamus,

including the lateral and posterolateral nuclei. The two regions of the frontal cortex examined in the present thesis, the MFC, especially its infralimbic subfield and the AIC project directly to autonomic centers in the brainstem. The component fields of the frontal cortex are also richly interconnected among themselves (Hurley, Herbert, Moga & Saper, 1991; Sesack, Deutch, Roth & Bunney, 1989; Takagishi & Chiba, 1991).

The Medial Frontal Cortex

Autonomic Projections of the Medial Frontal Cortex. The infralimbic region of the MFC has recently been shown to project directly to several nuclei in the midbrain, pons and medulla involved in the regulation of autonomic output. Efferents from this region descend to the central gray region, the parabrachial nucleus, the NTS, the dorsal motor nucleus of the vagus, the RVLM, the nucleus ambiguus and the sympathetic preganglionic neurons in the spinal cord. Other important targets of infralimbic efferents include most other regions of the frontal cortex, the amygdalar nuclei, the midline thalamus, and the lateral, suprachiasmatic and medial preoptic nuclei of the hypothalamus. Although there is evidence that other regions of the MFC, such as the prelimbic cortex, project to pontine and medullary nuclei involved in autonomic control, the most recent evidence suggests that the projections from the infralimbic region are the most numerous (Hurley et al., 1991; Sesack et al., 1989; Takagishi & Chiba, 1991).

The Medial Frontal Cortex and Autonomic Function. Stimulation of medial regions of the frontal cortex, especially the infralimbic zone result in changes in basal HR and blood pressure. Al Maskati & Zbrozyna (1989) found that stimulation of the infralimbic cortex, but not more dorsal areas such as the prelimbic cortex led to decreases in basal HR and blood pressure in

anesthetized rats. Hardy & Holmes (1988), on the other hand, observed similar decreases in blood pressure, but no change in HR following stimulation of the infralimbic region. This may have been due to the fact that Al Maskati & Zbrozyna (1989) stimulated the cortex at a higher frequency. In agreement with these findings, Burns & Wyss (1985) found that stimulation of any site within the MFC resulted in decreases in blood pressure, with the most pronounced responses occurring when stimulation was applied to ventral regions, such as the infralimbic cortex. These hypotensive responses to infralimbic stimulation were blocked when sympathetic, but not parasympathetic output was disrupted (Hardy & Holmes, 1988). These latter results suggest, importantly, that the inhibitory effects of stimulation of the MFC are mediated by a reduction in sympathetic output.

The functions of the MFC in the cardiovascular activation induced by either stressors or the stimulation of other brain regions have also been studied. Al Maskati & Zbrozyna (1989) and Zbrozyna & Westwood (1991) found that electrical or chemical stimulation of either the prelimbic or infralimbic zones of the MFC blocked the increases in HR and blood pressure induced by stimulation of "sympathoexcitatory" brain regions such as the basolateral amygdala. In the case of prelimbic stimulation, inhibition of the response was seen in the absence of effects on basal HR and blood pressure. As described previously, stimulation of the infralimbic region also decreased basal HR and blood pressure.

In keeping with the results from these stimulation studies, Ruit & Neafsey (1988) found that ablation of both the prelimbic and infralimbic cortex blocked the decreases in HR and blood pressure seen with stimulation of the hippocampus, a structure known to send excitatory projections to the frontal cortex.

These results contrast with the findings of a more recent lesion study carried out by the same group indicating that the MFC does not play a role in the modulation of cardiovascular responses to stressors. Frysztak & Neafsey (1994) found that aspiration of the prelimbic and infralimbic cortex did not affect cardiovascular responses to footshock. A possible explanation of this discrepancy is that in the period between lesion and testing of animals in the Frysztak & Neafsey study (1994), compensatory changes in the circuitry controlling the cardiovascular system may have occurred. This notion is partially supported by the fact that Ruit & Neafsey (1988) uncovered an inhibitory function of the MFC in cardiovascular output when lesions were made on the day of testing.

Interestingly, Frysztak & Neafsey (1994) found that although the lesions did not alter the response to footshock, they did affect the cardiovascular responses elicited by a cue previously associated with this stressor. They observed, furthermore, that the administration of atropine reversed the effects of the MFC lesions on the conditioned response. These findings suggest that the MFC modulates cardiovascular responses to conditioned, but not unconditioned stressors through effects on parasympathetic output.

The results of a study that examined a non-cardiovascular index of ANS function further suggests a role for the MFC in the modulation of autonomic output. Sullivan & Henke (1986) showed that aspiration of the MFC reduced the incidence of ulcers of the stomach induced by an intense, prolonged stressor. The division of the ANS affected by these lesions is, unfortunately, difficult to infer from their effects on gastric ulceration. Although the parasympathetic nervous system is known to be the primary pathway through which stressors produce gastric ulceration, the lesions may also have produced their effects through actions on sympathetic output.

Exposure to the stressor used in these experiments results in an initial inhibition of parasympathetic activity, resulting in decreases in gastric parameters such as peristalsis and acid secretion. After the cessation of stress, however, a rebound increase in parasympathetic activity is observed, that is manifest in a hyperactivation of gastric processes. This hyperactivation has been shown to underlie the development of the ulcers.

A number of hypothetical mechanisms can be proposed to explain the effects of MFC lesions on the ulcer response. The lesions of the MFC may have decreased the incidence of ulcers by reducing the magnitude of the rebound in parasympathetic activity. This may have occurred through a direct effect on parasympathetic output after the exposure to stress, or secondary to a reduction of the early-appearing parasympathetic inhibition. The lesions may also have conferred protection via effects on sympathetic output, as gastric activity is also controlled by this division of the ANS. These hypotheses remain to be verified.

Only one study has specifically examined the role of the catecholaminergic projections to the MFC in the control of autonomic output. McGregor, Menendez & Atrens (1990) found no significant alterations in metabolism or thermogenesis after making injections of either NA or α-adrenergic drugs into the MFC. These results suggest that the NAergic projections to the MFC do not play a strong role in the output of the ANS involved in the control of metabolism or thermogenesis.

Taken together, the results of these studies suggest that projections originating from the MFC participate in the modulation of autonomic output. The strongest evidence, from stimulation studies, suggests that the projections of the MFC, especially the infralimbic region, are inhibitory on the basal output of the cardiovascular system and are mediated by an inhibition

of sympathetic output. In the case of cardiovascular responses induced by stress, the function of the MFC is less clear. In the one study that specifically examined this, lesions of the MFC did not affect cardiovascular responses to footshock. Other studies have demonstrated, however, that the electrical stimulation of the MFC blocks the cardiovascular activation induced by the stimulation of other brain regions.

The Agranular Insular Cortex

Autonomic Projections of the Agranular Insular Cortex. The lateral bank of the frontal pole, which includes the AIC has long been described as visceral cortex due to its direct projections to a number of nuclei known to be involved in the control of the ANS. The projections, to a large extent, parallel those of the infralimbic cortex. Projections descend to the amygdalar nuclei, thalamus, hypothalamus, and central gray region. Projections to pontine and medullary regions known to be involved in the control of autonomic output include the NTS, parabrachial nucleus, dorsal motor nucleus of the vagus, RVLM and the preganglionic nuclei in the spinal cord (Loewy, 1991; Sesack et al., 1989).

The Agranular Insular Cortex and Autonomic Function. Oppenheimer & Cechetto (1990) have shown that insular regions of cortex are organized along a rostrocaudal gradient in terms of cardiovascular responses to electrical stimulation. Stimulation of rostral regions increases HR and blood pressure, while stimulation of more posterior regions decreases these parameters. Unfortunately, these detailed mapping studies evaluated only the more posterior regions of the insular cortex that are sparsely innervated by the DAergic projections. The electrical stimulation of more rostral regions of the insular cortex, including the AIC, have, on the other hand, been shown to

decrease HR and blood pressure (Hardy & Holmes, 1988; Hardy & Mack, 1990; Sun, 1992). The results of pharmacological experiments have shown that these effects of AIC stimulation are mediated by effects on the output of the sympathetic nervous system (Hardy & Holmes, 1988).

These inhibitory responses to stimulation of the AIC may be mediated by an indirect projection to the RVLM via the lateral hypothalamus and NTS. Hardy & Mack (1990) found that injections of lidocaine into the lateral hypothalamus or NTS blocked the hypotension and bradycardia induced by stimulation of the AIC. Fiber-sparing lesions of the lateral hypothalamus also blocked the cardiovascular effects of such stimulation, offering support for the presence of a hypothalamic synapse in the pathway. These results are supported by those of Sun (1988), who found that the cells in the RVLM that exert a facilitatory drive on autonomic output were inhibited by stimulation of the AIC.

In agreement with these findings, a study that measured other indexes autonomic activity presented evidence that the output of the AIC functions to inhibit sympathetic output. McGregor et al. (1990) showed that injections of a glutamate agonist into the AIC resulted in changes in metabolism and heat production consistent with reduced sympathetic output.

Only one study has examined the effects of NAergic manipulations in the AIC on autonomically-mediated processes. McGregor et al. (1991) found that injections of NA into this region caused changes in metabolism and thermogenesis suggestive of sympathetic inhibition.

These anatomical and functional studies suggest that the projections from the AIC modulate cardiovascular output in an inhibitory manner in the basal condition. The influence of at least the AIC on cardiovascular output may be mediated by a projection to the RVLM, or to the NTS, a structure that

projects in turn to the RVLM. No studies have examined the role of AIC in stress-induced increases in cardiovascular output. As in the case of the MFC, it appears that these effects of AIC stimulation on the basal activity of the cardiovascular system are mediated by reductions in sympathetic output.

Taken together, the results of these studies suggest that activity in the projections descending from the MFC and AIC function to inhibit cardiovascular output in the basal state via reductions in sympathetic output. In the case of the AIC, these effects may be mediated by a projection to the RVLM, with intervening synapses in the hypothalamus and NTS. The analogous experiments on the projection mediating the inhibitory effect of stimulation of the MFC on cardiovascular activity have not been carried out. It is clear, however, that the decreases in HR and blood pressure seen with stimulation of the MFC is mediated by inhibition of sympathetic output. The fact that neuroanatomical studies demonstrate that the projections of the MFC to brainstem nuclei are, to a large extent, analogous to those of the AIC suggests that the cardiovascular effects of MFC and AIC stimulation may be mediated by inhibitory projections to the RVLM.

Less is known of the function of the frontal cortex in cardiovascular responses to noxious or stressful stimuli. The one study which addressed this issue demonstrated that lesions of the MFC did not affect cardiovascular responses to a footshock stressor. In contrast, stimulation of the MFC has been shown to reduce the cardiovascular activation induced by the stimulation of other brain regions. The functions of the AIC in evoked cardiovascular output have not been examined.

The Nucleus Accumbens

The NAC is classically thought of as part of a circuit mediating locomotor activity and motivational processes. Its functions in the control of autonomic output have not been studied directly. There is, however, anatomical evidence suggesting that the NAC is connected with nuclei involved in the control of the ANS.

The NAC receives, in addition to its DAergic and NAergic afferents, projections from the medial thalamus, hypothalamus, hippocampus, amygdala and cortex, including the frontal cortex. The major efferent targets of the NAC are the pallidum and the VTA-substantia nigra region.

Autonomic Projections of the Nucleus Accumbens. The parabrachial nucleus appears to be the only autonomic center that receives direct projections from the NAC. The NAC may, however, influence autonomic function via indirect projections to other autonomic nuclei. For example, the ventral pallidum, the major target of projections from the NAC also projects to the perifornical hypothalamus, a region that, in turn, projects directly to the preganglionic neurons of the sympathetic nervous system in the spinal cord (Alheid & Heimer, 1988; Fuller, Russchen & Price, 1987; Grove, 1988; Merideth & Wouterlood, 1990; Merideth, Wouterlood & Pattiselanno, 1990).

The Nucleus Accumbens and Autonomic Function. There is little evidence that the projections from the NAC exert an important influence on autonomic output. Although a few studies have shown that the electrical stimulation of this region can influence HR and blood pressure, more recent research suggests that these effects were mediated by stimulation of corticofugal fibers that course through the NAC, and not by pathways originating in the NAC (Al Maskati & Zbrozyna, 1989).

A series of studies suggest that the NAC may be involved in the

control of gastric activity. Xing, Balaban, Seaton, Washington & Kauffman (1991) showed that injections of neurotensin directly into the NAC reduced the incidence of gastric ulcers induced by a strong stressor. DA was shown to modulate this action, as either 6-OHDA lesions of the VTA or the coinjection of haloperidol into the NAC blocked the protective effects of neurotensin.

The results of a recent study provide less direct evidence that the DAergic projection to the NAC may influence the output of the ANS. With the sudden cessation of opiate administration in animals that have been treated chronically with such drugs, a profound rebound activation of the autonomic nervous system occurs, which is manifest in increased HR, blood pressure and lacrimation. Several behavioral symptoms also occur, which may be at least partially dependent on autonomic activation. To date, the effects of catecholaminergic manipulations on the autonomic symptoms of the opiate withdrawal syndrome have not been examined. Harris & Aston-Jones (1994) have, however, shown that many of the behavioral components of the syndrome can be blocked by the administration of agonists D_2 receptor either systemically, or directly into the NAC, prior to the precipitation of the withdrawal response with an antagonist of opiate receptors. It was further shown that the administration of a D_2 antagonist directly into the NAC of opiate-treated rats elicited many withdrawal behaviors. Taken together, the results from this experiment suggest that the activation of the DAergic projection to the NAC can inhibit at least the behavioral manifestations of a syndrome characterized by a high degree of autonomic activity.

Catecholaminergic projections to the NAC may also influence autonomic responses to noxious or stressful stimuli indirectly, by modulating the perceived salience of these stimuli. Altier & Stewart (1994) found that injections of amphetamine into the NAC reduced the number of pain-related

behaviors shown by rats in response to injections of formalin into the hind paw.

Although the NAC projects directly and indirectly to nuclei involved in autonomic control, there is little evidence suggesting that it participates in the modulation of autonomic output. The results of a few studies suggest that the DAergic projections to the NAC are indirectly involved in the modulation of gastric activity, an autonomically-controlled process. The functions of the NAergic projections to the NAC in the output of the ANS have not been studied.

The Central Nucleus of the Amygdala

The CeA is connected with nuclei distributed throughout the neuraxis. A number of studies have shown that it is involved in the activation of physiological and behavioral responses to emotionally significant stimuli, including noxious or stressful events.

In addition to its DAergic and NAergic afferents, the CeA receives extensive and largely reciprocal projections from the frontal cortex, including the infralimbic, prelimbic, cingulate and insular zones. The CeA differs from the other amygdalar nuclei in that its input from the cortex is considerably more extensive. Other major sources of projections to the CeA include the hippocampus, hypothalamus, thalamus, parabrachial nucleus, NTS, central gray region and the serotonergic raphe nuclei.

Autonomic Projections of the Central Nucleus of the Amygdala. The CeA may influence the activity of the ANS via direct projections to hypothalamic, pontine and medullary nuclei involved in autonomic control. These targets of CeA projection neurons include the paraventricular nucleus of the hypothalamus, central gray region, parabrachial nucleus, the RVLM, the NTS

and the vagal nuclei (Alheid & Heimer, 1988; Cassel, Chittick, Siegel & Wright, 1989; Cassel & Wright, 1986; Danielsen, Magnuson & Gray, 1989; Gray, Carney & Magnuson, 1989; LeDoux, Farb & Ruggierio, 1990; Roder & Ciriello, 1993; Uryu, Okumura, Shibasaki & Sakanaka, 1992; Wallace, Magnuson & Gray, 1989, 1992; Zardetto-Smith, Moga, Magnuson & Gray).

The Central Nucleus of the Amygdala and Autonomic Function. Electrical or chemical stimulation of the CeA in awake rats has been shown to alter the output of the ANS in the basal state. This activation has been characterized as resembling the defence reaction seen when animals are exposed to noxious or threatening stimuli. These increases in HR and blood pressure appear to be mediated primarily by increases in the output of the sympathetic nervous system. Stimulation of the CeA of anesthetized animals, in contrast, decreases HR and blood pressure. The reasons for these differential effects of stimulation are not clear, although it has been suggested that anesthetics block the vasoconstriction of vascular beds in the periphery necessary for the production of at least the pressor responses (Gelsema, Agarwal & Calaresu, 1989; Iwata, Chida & LeDoux, 1987).

Several studies have provided evidence that the output of the CeA influences the activity of autonomic projections controlling the stomach during both basal conditions and in response to stressors. Electrical stimulation of the CeA induces gastric ulceration, while electrolytic lesions of this nucleus reduce the incidence of gastric ulcers induced by stressors. As described previously, the autonomic pathways contributing to this gastric ulceration are uncertain.

The catecholaminergic innervation of the CeA has been shown to modulate the influence of the CeA on the changes in gastric activity induced by stress. Intra-CeA injections of agonists of either DA or NA reduce the

incidence of gastric ulcers induced by exposure to a strong stressor. The protective effects of DA in the CeA were further shown to be mediated primarily by the stimulation of receptors of the D_1 type, and those of NA by the stimulation of β-adrenoceptors (Ray, Henke & Sullivan, 1990). These results suggest that the release of DA and NA in the CeA in response to stress protects against gastric ulceration. The autonomic mechanism underlying these effects are not known, however. As described earlier, the effects of a particular manipulation on the activity of the sympathetic and parasympathetic divisions of the ANS is difficult to infer from its on the development of ulcers. These manipulations may affect the ulcer response through actions on the sympathetic or parasympathetic divisions of the ANS.

Two studies have examined the role of catecholamines in the CeA on cardiovascular activity during basal conditions. Injections of NA into the CeA were found to increase blood pressure in awake, unrestrained animals, suggesting that the stimulation of NAergic receptors in this nucleus increases sympathetic output (Leonzio et al., 1987; Ohta et al., 1989). Ohta et al. (1989) observed, on the other hand, a marked increase in blood pressure and a decrease in HR following injections of NA into the CeA. Since the dose of NA used in the Ohta experiment was about twice that of the Leonzio et al. (1987) study, and exerted a strong pressor effect, it may be suggested that the decrease in HR observed by Ohta et al. (1989) was a reflexive response to the large increase in blood pressure. Although the autonomic pathways underlying these responses were not assessed, these results are consistent with the notion that the stimulation of NAergic receptors in the CeA increases the output of the sympathetic nervous system.

In summary, the electrical or chemical stimulation of the CeA evokes increases in HR and blood pressure in awake animals that are mediated

primarily by increases in sympathetic output. The NAergic projections to the CeA appear to facilitate this excitatory drive on cardiovascular output. Catecholamines in the CeA also appear to modulate the output of autonomic pathways underlying gastric activity. Injections of either DA or NA into this nucleus reduces the incidence of gastric ulcers induced by stress. The autonomic pathways contributing to these latter effects are not known.

The Present Experiments

The results of these studies implicate the MFC, AIC, NAC and CeA in the regulation of autonomic output. These regions project directly and indirectly to nuclei in the medulla and spinal cord involved in the control of the ANS. The projections from these nuclei appear to have a functional role in the modulation of autonomic output, as their stimulation or lesion can alter such autonomic parameters as HR, blood pressure and gastric activity, both during basal conditions and in response to stress.

A number of studies have provided evidence that the influence of these nuclei on autonomic responses is under the modulatory control of their DAergic and NAergic afferents. For example, injections of NA into the CeA increase blood pressure, while injections of DA or NA into this same nucleus can reduce the incidence of ulcers of the stomach. Aside from these studies, the role of the catecholaminergic projections to individual nuclei in the forebrain in the modulation of autonomic responses, during basal conditions or in response to stress has not been systematically studied.

The experiments carried out in the present thesis sought to overcome the limitations of these previous studies and systematically address the role of the DAergic and NAergic projections to the MFC, AIC, NAC and CeA in the basal and stress-induced output of the sympathetic nervous system.

If the release of DA and NA in these forebrain regions is involved in the modulation of sympathetic output, a number of hypotheses about its action can be entertained. Catecholaminergic neurotransmission in these sites may influence basal sympathetic tone. Another possibility is that the catecholaminergic projections exert a modulatory influence on the increases in sympathetic output induced by noxious or stressful stimuli.

The experiments described in this thesis were done to evaluate these hypotheses. In these experiments, carried out on anesthetized rats, the effects of injections of DAergic and NAergic drugs into the MFC, AIC, NAC and CeA on basal HR and the increase in HR induced by tail pinch, a sympathetically-mediated response, were assessed.

MATERIALS AND METHODS

Subjects

A total of 131 male Wistar rats (Charles River, St. Constant Quebec) weighing 300-325g were housed in an animal room maintained at 22 degrees Celsius (light phase 0800h to 2000h). Rat chow and tap water were available ad libitum. Experiments were carried out between 1000h and 1800h.

Surgery and HR Recording

Rats were anesthetized with urethane (1.5 g/kg, Sigma) administered intraperitoneally (i.p.). Anesthesia was maintained throughout the experiment with supplemental doses of urethane (250 mg/kg, i.p.) as required. Core temperature was continuously monitored and maintained between 37.0 and 37.5 degrees C with a homeothermic heating blanket (Harvard Apparatus).

Electrocardiographic (ECG) electrodes made from safety pins were implanted transcutaneously, one on each flank overlying the ribs and one on the back overlying the scapulae. The signal from the three ECG electrodes was amplified and filtered (BMA 100, World Precision Instruments) and then routed to a MacLab amplifier connected to a Macintosh computer used to record HR from the ECG signal with MacLab software. The average HR was recorded every 1.5 s.

Rats were then placed in a stereotaxic frame (David Kopf, Tujunga, CA), the scalp was cut and retracted and stereotaxically-placed holes were drilled over the right MFC, AIC, NAC and CeA with a manual drill affixed to the stereotaxic carrier. The dura overlying each of the four sites was then punctured with a 22 gauge needle. Cannulae were aimed at the following

coordinates, relative to bregma, with the skull flat. MFC: A/P=3.0 mm, LAT=.8 mm, D/V=-5.5 mm; AIC: A/P=2.2 mm, LAT=4.2 mm, D/V=-6.3 mm. NAC: A/P=2.2 mm, LAT=1.2 mm, D/V=-7.4 mm. CeA: A/P=-2.2 mm, LAT=4.4 mm, D/V=-8.2 mm.

Procedure

Prior to testing, 5 to 7 tail pinches, each 10 s in duration, separated by at least 5 min were administered 2 cm from the base of the tail with an adjustable tubing clamp (VWR). The tension of the clamp was adjusted until reproducible tachycardiac responses of 10 to 30 beats per min were obtained in response to a 10 s tail pinch.

Prior to the recording of HR, a 28 gauge injection cannula connected to a 1 µl syringe (Hamilton, Reno, NV) with plastic tubing was stereotaxically lowered to the site being tested. HR was recorded continuously following the placement of the cannula. Five minutes after placement of the injection cannula, testing was carried out as follows:

Predrug Phase. During the predrug phase, baseline HR was collected for 1 min. Two 10 s tail pinches separated by 5 min were then administered.

Intracranial Injections: Five min after the second pinch in the predrug phase, drug (10 to 40 nmol) was injected into the test site in a volume of 0.5 µl over 45 s.

Postdrug Phase. Five min after the intracranial injections, two 10 s tail pinches separated by 5 min were administered. The recording of HR continued until 5 min after the second tail pinch.

At this point, the injection cannula was withdrawn and the stability of the tachycardiac response to tail pinch was confirmed, with adjustments being made to the tubing clamp if necessary. Twenty minutes after the injection

cannula was withdrawn, and at least 10 min after the last test pinch, the injection cannula was placed in the next of the 4 sites and testing was initiated as described above. Using this procedure, two rats were tested simultaneously.

The effects of injection of drugs into the MFC, AIC, NAC and CeA on the HR responses to tail pinch were assessed at each site in each rat in an order counterbalanced across subjects.

Drugs

Drugs were prepared freshly every day and were dissolved in physiological saline with the exception of phentolamine, which was dissolved in distilled water.

The following drugs were used.

D_1 agonist: SKF82958 ((±)-6-Chloro-N-allyl-1-phenyl-2,3,4,5-tetrahydro-1h-3-benzazepine hydrobromide (RBI).

D_1 antagonist: SKF83566 (±)-7-Bromo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1h-3-benzazepine hydrochloride (RBI).

D_2 agonist: quinpirole (_trans_-(-)-4aR-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride (Lilly).

D_2 antagonist: Raclopride tartrate (Astra).

β-adrenoceptor agonist: isoproterenol hydrochloride (Sigma).

β-adrenoceptor antagonist: propranolol (±)-1-(Isopropylamino)-3-(1-napthyloxy)-2-propanol hydrochloride) (ICN).

α-adrenoceptor agonist: phenylephrine hydrochloride (RBI).

α-adrenoceptor antagonist: phentolamine hydrochloride (Sigma).

Choice of Urethane as Anesthetic. These experiments were carried out on rats anesthetized with urethane. Urethane was chosen because it is known to spare autonomically-mediated responses to painful or noxious stimuli. Urethane also has minimal depressant actions on the firing of CNS neurons, in contrast to other commonly used anesthetics such as the barbiturates (Maggi & Meli, 1986a, 1986b). This latter characteristic was thought to be important due to the fact that DA and NA are known to be primarily inhibitory on neuronal firing. Anesthetics known to reduce the basal activity of neurons might be expected to mask the effects of drugs mediated by neuronal inhibition.

Histology

At the end of each experiment, animals were administered an overdose of sodium pentobarbital (Somnotol) and perfused transcardially with physiological saline followed by 10% formaldehyde. The brains were removed and stored in 10% sucrose-formaldehyde prior to subsequent slicing into 30 μm sections in the coronal plane on a freezing microtome. Mounted sections were then stained using formal thionin, and the placement of cannulae in the four sites in each rat were drawn onto representative plates from the atlas of Swanson (1992).

Presentation of Data

Average HR was recorded continuously over 1.5 s intervals throughout testing. To simplify the analysis of results, these data were reduced in that samples of HR were taken at representative points from each of the two pinches administered in the predrug and postdrug phases. HR for was sampled at three points, the baseline prior to each pinch (BASE), the peak

HR induced by each pinch (PEAK) and the HR 2.5 min after each pinch, as a measure of recovery (RECOV). Figure 1a, shows an example of an unreduced HR record. The same data sampled according to this procedure is shown in Figure 1b.

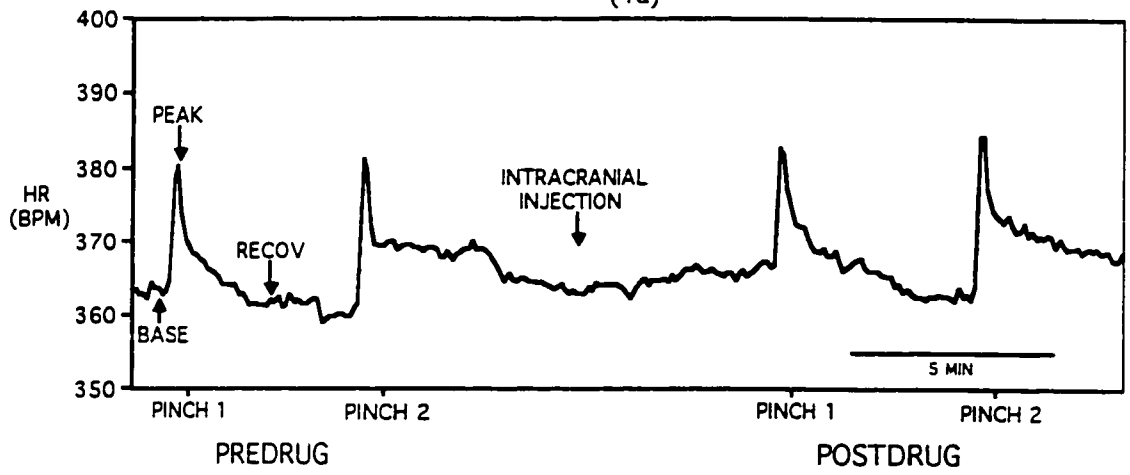
For presentation, the data from individual animals were converted to change scores from their initial baseline HR recorded prior to the first pinch in the predrug phase (ΔHR). The group means and standard errors of the mean (S.E.M.) were calculated from these data. Data from the postdrug phase were then superimposed on those from the predrug phase to demonstrate the effects of the drug injections on basal HR and on the responses to tail pinch. This is illustrated in Figure 1c.

Statistics

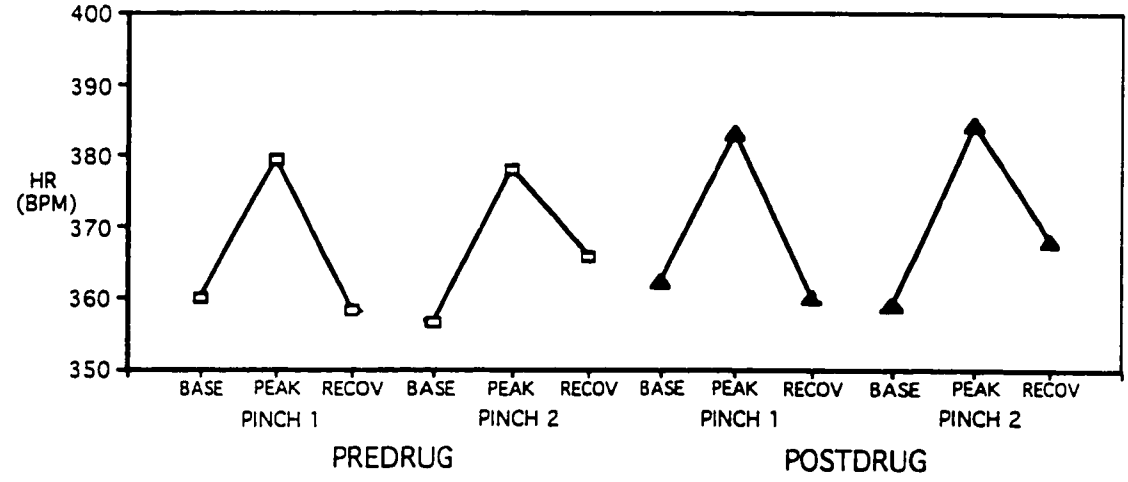
Analyses of variance (ANOVA) using repeated measures were carried out on the absolute HR values for each drug or drug combination at each site. The factors and levels of this 2x2x3 design were drug phase (predrug and postdrug), pinch number (first pinch and second pinch), and sample (BASE, POST and RECOV). The technique of simple effects was used to assess the effect of drug on baseline HR and on the response to tail pinch. Statistics were carried out using BMDP software on a VAX mainframe computer.

Figure 1 An example of a plot of the raw HR data sampled every 1.5 s, prior to data reduction (1a). Figure 1b shows the data in 1a after the data reduction procedure. For presentation, the reduced data from individual animals were converted to change scores from their initial baseline HR (ΔHR), from which means and standard errors were calculated. The means and standard errors from the predrug phase were then superimposed on those from the postdrug phase (1c).

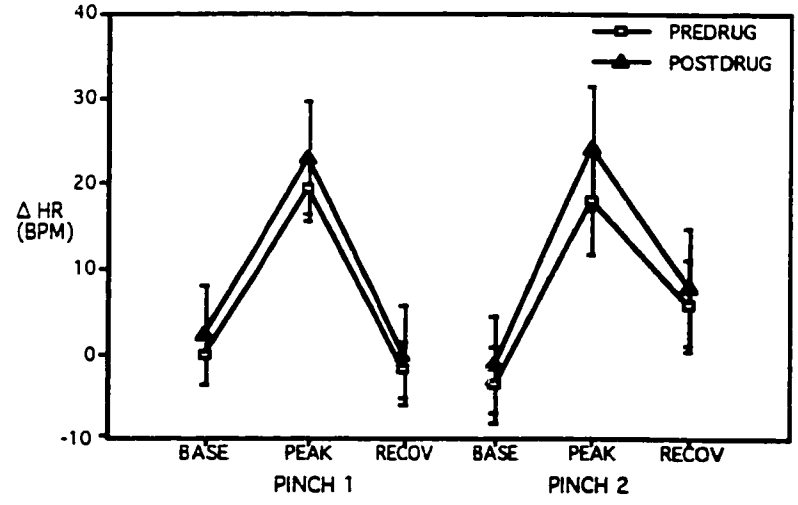
(1a)


400
390
380
370
360
350
HR
(BPM)
PEAK
RECOV
BASE
INTRACRANIAL
INJECTION
5 MIN
PINCH 1
PINCH 2
PINCH 1
PINCH 2
PREDRUG
POSTDRUG

(1b)


400
390
380
370
360
350
HR
(BPM)
BASE
PEAK
RECOV
BASE
PEAK
RECOV
BASE
PEAK
RECOV
BASE
PEAK
RECOV
PINCH 1
PINCH 2
PINCH 1
PINCH 2
PREDRUG
POSTDRUG

(1c)


40
30
20
10
0
-10
Δ HR
(BPM)
PREDRUG
POSTDRUG
BASE
PEAK
RECOV
BASE
PEAK
RECOV
PINCH 1
PINCH 2

RESULTS

Histology

The placements of cannulae in the MFC, AIC, NAC and CeA of animals that contributed data to the analyses are depicted in Appendix 1.

Statistical Procedure Used in Analyses

Analyses of simple effects and of simple interactions were carried out in conjunction with the ANOVA to assess the effects of drug injections on baseline HR and on the response to pinch. Due to the nature of the experiment, a number of analytical strategies were possible. The procedure that was used to analyze these data will therefore be briefly described.

It was anticipated that intracranial injections of drugs could affect HR and the response to tail pinch in a number of ways. The first could be an effect on baseline HR (BASE), without affecting the response to tail pinch. The second could be an effect on the magnitude of the pinch response (PEAK). Finally, the drug might facilitate or inhibit the recovery of HR after the pinch (RECOV).

Changes in baseline HR induced by the drug injections were assessed with the test of the main effect of drug from the omnibus ANOVA. This test was supplemented with tests of the simple effect of drug at the baseline prior to each pinch.

Drug-induced changes in the response to pinch were first assessed with the test of the drug x sample interaction from the ANOVA. A significant drug x sample interaction could result from either a drug-induced change in the magnitude of the increase in HR caused by tail pinch or in the recovery of HR after the pinch. Where appropriate, drug x sample interaction was

further analyzed with tests of the simple effects of this interaction at each pinch.

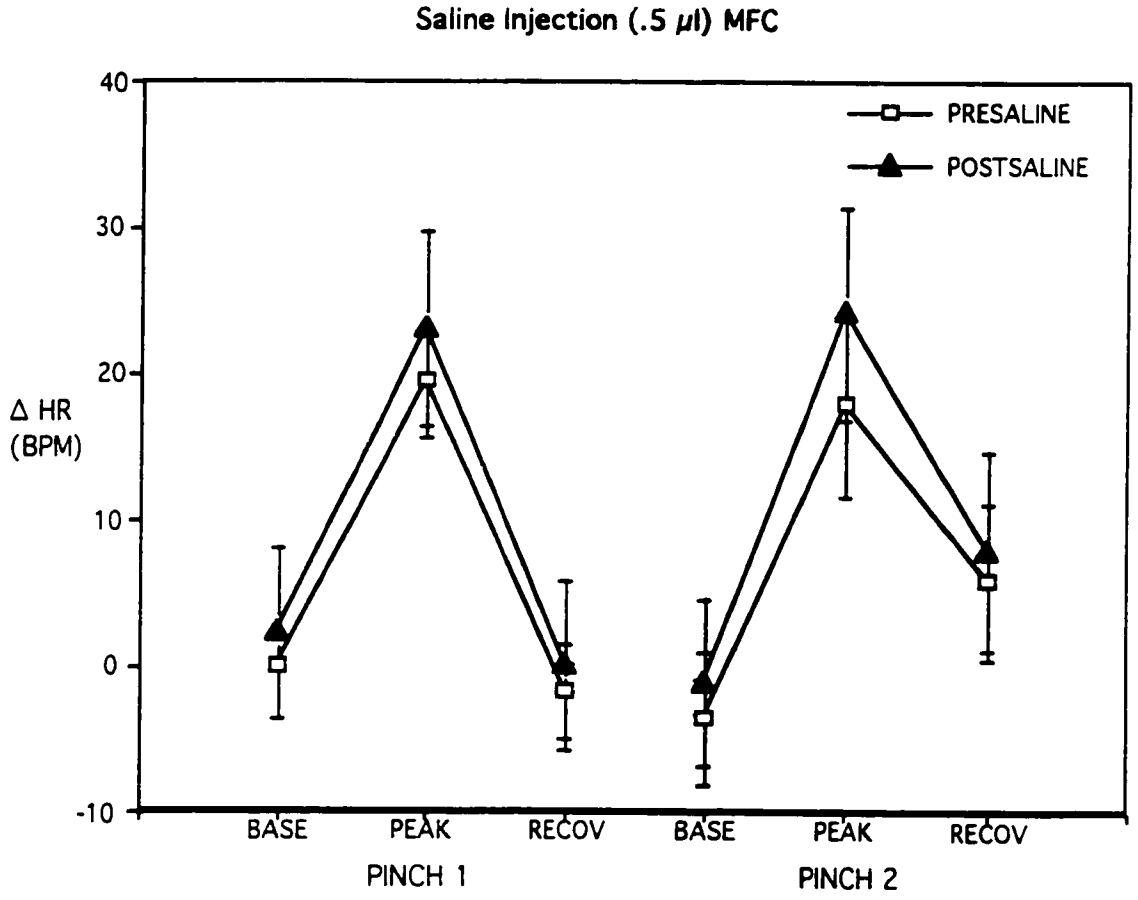
Two additional effects of drug that could be observed were dependent on the effect of pinch. The effect of pinch was sometimes manifest in an increase in baseline HR prior to the second pinch and a potentiated response to the second pinch, including both an increase in the magnitude of the peak response and a slower recovery. Such phenomenon could be reflected in a significant main effect of pinch, and in a significant pinch x sample interaction, respectively. Although these effects of pinch were observed in some experiments, they were absent in many others. As a result of this inconsistency, the drug x pinch and the drug x pinch x sample interactions were found to be unreliable measures of drug effects. It was decided, therefore, to assign less importance to these interactions involving the factor of pinch when carrying out the analyses.

Effects of Injections of Saline

To control for the non-specific effects of intracranial injections on baseline HR and on the response to tail pinch, a group of animals was run that received injections of physiological saline (0.5 µl) into the MFC (n=9), AIC (n=7), NAC (n=6) and CeA (n=8). Experiments on these animals were carried out using the same procedure with which animals receiving injections of drugs were tested. The results from the experiment assessing the effects of saline injections into the MFC will be described in detail. Plots of the results from the experiments examining the effects of saline injections into the AIC, NAC and CeA are presented separately in Appendix 2.

The effects of injections of saline into the MFC are shown in Figure 2. As expected, tail pinch for 10 s increased HR. This is reflected in a significant

Figure 2 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min administered prior to (PRESALINE), and following (POSTSALINE) injections of .5 μl of saline into the MFC.


Saline Injection (.5 μl) MFC
PRESALINE
POSTSALINE
Δ HR
(BPM)
40
30
20
10
0
-10
BASE
PEAK
RECOV
BASE
PEAK
RECOV
PINCH 1
PINCH 2

effect of sample [$F(2, 16)=25.32$, $p < .0001$]. Injections of saline into the MFC did not significantly alter baseline HR, as reflected in the result of the test of the main effect of drug from the ANOVA [$F(1,8)=.76$, $p > .40$]. This is further supported by the fact that the result of the test of the simple effect of drug at the baseline prior to the first pinch was not significant [$F(1,48)=.27$, $p > .60$].

Saline injections into the MFC did not affect the magnitude of the response to tail pinch. This is demonstrated by the result of the test of the drug x sample interaction from the ANOVA [$F(1,16)=1.78$, $p > .20$], and is further substantiated by the results of tests of simple effects, when the drug x sample interaction was assessed at each pinch [First pinch: $F(2,32)=.27$, $p > .76$, Second pinch: $F(2,32)=1.61$, $p > .21$]. The lack of effect of saline injected into the MFC on baseline HR or on the magnitude of the response to pinch is further demonstrated by the fact that the results of tests of the simple effects of drug at each sample in each pinch were not significant [For the 6 tests: $F(1,48)=2.07$, $p > .16$].

As described previously, the main effect of pinch as well as the interactions including this factor were not used in the analysis of the effects of drug injections. The results of these tests, will however be described here in the analysis of the data from the saline control experiment for the sake of completeness. Although the main effect of pinch was not statistically significant [$F(1,8)=.20$, $p > .66$], the pinch x sample interaction was [$F(2,16)=8.04$, $p < .005$], reflecting the fact that the magnitude of the response to pinch tended to be greater in the second pinch compared to the first. The peak increase in HR tended to be larger, and the recovery of HR tended to be slower after the second pinch. Such potentiation was observed in both the predrug and postdrug phases. This is reflected in the results of separate tests of the simple effect of the pinch x sample interaction in the predrug and postdrug

phases [Predrug phase: $F(2,32)=5.80$, $p < .01$, Postdrug phase: $F(2,32)=5.38$, $p < .05$].

The results of this experiment that used saline as a control for the effects of injection, demonstrate that the design of the experiment provided meaningful data in that the injection did not significantly affect either baseline HR or the response to tail pinch. The results of the experiment demonstrate, furthermore, that the two pinches administered in the predrug phase affected neither baseline HR nor the magnitude of the response to the two pinches administered in the postdrug phase.

Effects of Injections of Dopaminergic Drugs

Effects of Injections of the D_1 Receptor Agonist SKF82958

Highlights

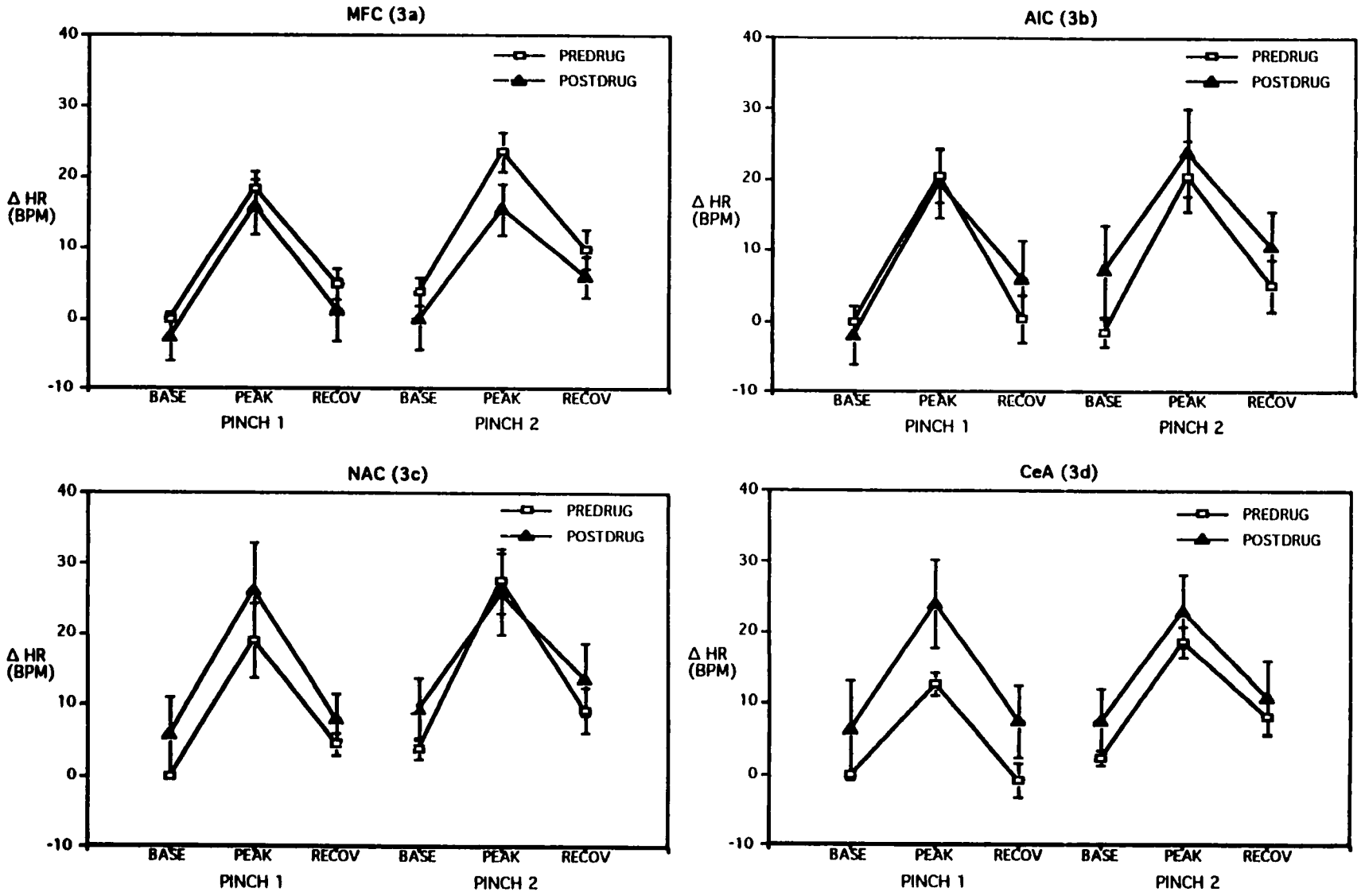
The effects of injections of 20 nmol of the D_1 receptor agonist SKF82958 into the MFC (n=9), AIC (n=7), NAC (n=9) or CeA (n=5) on baseline HR and the response to pinch are shown in Figure 3. In summary, injections of SKF82958 did not significantly alter baseline HR or the response to tail pinch when injected into any of the four sites tested. There was, however, a general tendency for HR to be higher when the D_1 agonist was injected into the AIC, NAC and CeA, and a slight tendency to be lower when it was injected into the MFC.

Medial Frontal Cortex

As shown in Figure 3a, injections of SKF82958 into the MFC did not significantly alter baseline HR. This is demonstrated by the result of the test

Figure 3 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 20 nmol of the D_1 receptor agonist SKF82958 into the MFC (3a), AIC (3b), NAC (3c) or CeA (3d).

SKF82958 (20 nmol)



of the main effect of drug [$F(1,8)=1.54$, $p > .24$]. In support of this, the result of the test of the simple effect of drug at the baseline prior to the first pinch was not significant [$F(1,48)=.39$, $p > .54$]. After the first pinch in the postdrug phase, however, HR tended to be lower. The test of the drug x sample interaction showed that SKF82958 did not affect the response to tail pinch [$F(2,16)=.37$, $p > .69$].

Agranular Insular Cortex

Figure 3b shows that injections of SKF82958 into the AIC did not affect baseline HR significantly as revealed by the test of the main effect of drug [$F(1,6)=.40$, $p > .55$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,36)=.12$, $p > .73$]. There was, however, a tendency for HR to remain elevated after the first pinch in the postdrug phase. These injections did not affect the response to tail pinch as revealed by the test of the drug x sample interaction [$F(2,12)=.95$, $p > .41$].

Nucleus Accumbens

Injections of SKF82958 into the NAC did not significantly affect baseline HR as shown by the results of tests of the main effect of drug [$F(1,8)=2.36$, $p > .16$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,48)=2.31$, $p > .14$], despite the tendency for HR to be elevated after the drug injection (Figure 3c). These injections did not significantly affect the response to tail pinch as shown by the result of the test of the drug x sample interaction [$F(2,16)=.53$, $p > .59$].

Central Nucleus of the Amygdala

Although injections of SKF82958 into the CeA tended to increase baseline HR, especially during the first pinch, this trend was not statistically significant as shown by the result of the test of the main effect of drug [$F(1,4)=2.24$, $p > .20$], and by the test of the simple effect of drug at the baseline

prior to the first pinch [F(1,24)=1.59, p > .24] (Figure 3d). Injections of this drug did not significantly affect the response to tail pinch as assessed by the test of the drug x sample interaction [F(2,8)=.39, p > .69], despite a tendency for the magnitude of the first pinch to be larger after drug injection.

Effects of Injections of the D_1 Receptor Antagonist SKF83566

Highlights

The effects of injections of 20 nmol of the D_1 receptor antagonist SKF83566 into the MFC (n=7), AIC (n=7), NAC (n=8) or CeA (n=6) on baseline HR and the response to pinch are shown in Figure 4. SKF83566 increased baseline HR significantly when injected into the NAC. Injections of this drug into the MFC, AIC and CeA also increased baseline HR, but these effects were not statistically significant. SKF83566 did not affect the magnitude of the response to tail pinch when it was injected into any of the four sites.

Medial Frontal Cortex

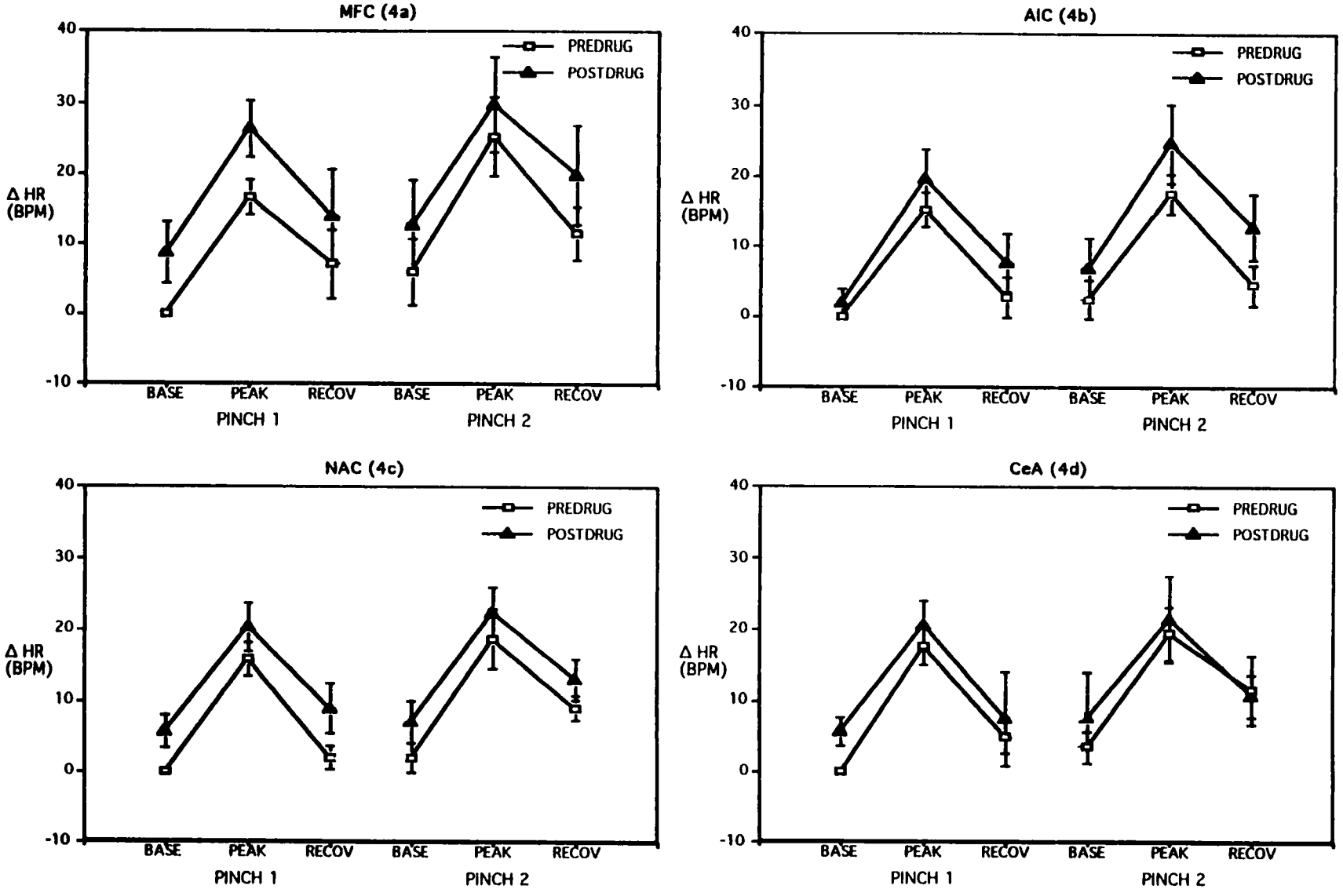
In Figure 4a, it is shown that the injection of SKF83566 into the MFC tended to increase baseline HR, but not to a statistically significant degree, as reflected in both the main effect of drug [F(1,6)=4.69, p < .10] and the simple effect of drug at the baseline prior to the first pinch [F(1,36)=4.55, p < .10]. Injections of SKF83566 into the MFC did not affect the response to tail pinch as revealed by the test of the drug x sample interaction [F(2,12)=.07, p > .93].

Agranular Insular Cortex

SKF83566 injections into the AIC (Figure 4b) did not significantly affect baseline HR as revealed by the test of the main effect of drug [F(1,6)=3.41, p > .11] and of the simple effect of drug at the baseline prior to the first pinch

Figure 4 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 20 nmol of the D_1 receptor antagonist SKF83566 into the MFC (4a), AIC (4b), NAC (4c) or CeA (4d).

SKF83566 (20 nmol)



[F(1,36)=.31, p > .58]. HR did, however, tend to be higher after the injection of SKF83566 into the AIC, especially after the first pinch. The response to tail pinch was not affected by these injections, as shown by the result of the test of the drug x sample interaction [F(2,12)=.02, p > .22].

Nucleus Accumbens

Injections of SKF83566 into the NAC (Figure 4c) increased baseline HR. This fact was reflected in a significant main effect of drug [F(1,7)=8.69, p < .05], and in the result of the test of the simple effect of drug at the baseline prior to the first pinch [F(1,42)=5.36, p < .05]. Injections of SKF83566 did not affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [F(2,14)=.44, p > .65].

Central Nucleus of the Amygdala

As seen in Figure 4d, injections of SKF83566 into the CeA did not significantly affect baseline HR as shown by the results of the tests of the main effect of drug [F(1,5)=2.04, p > .21] and of the simple effect of drug at the baseline prior to the first pinch [F(1,30)=2.60, p > .12]. There was, however, a slight tendency towards increased baseline HR after these injections. Injections of this drug into the CeA did not alter the response to pinch, as shown by the result of the test of the drug x sample interaction [F(2,10)=1.59, p > .25].

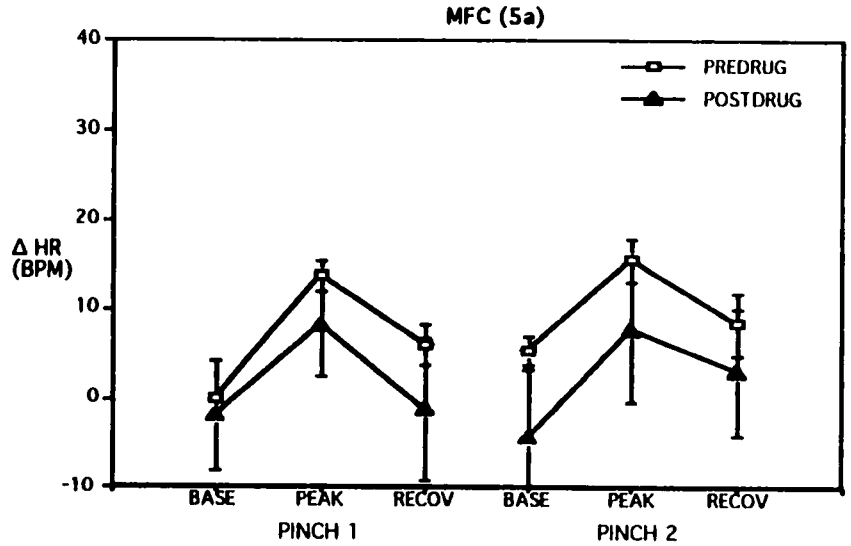
Effects of Injections of the D_2 Receptor Agonist Quinpirole

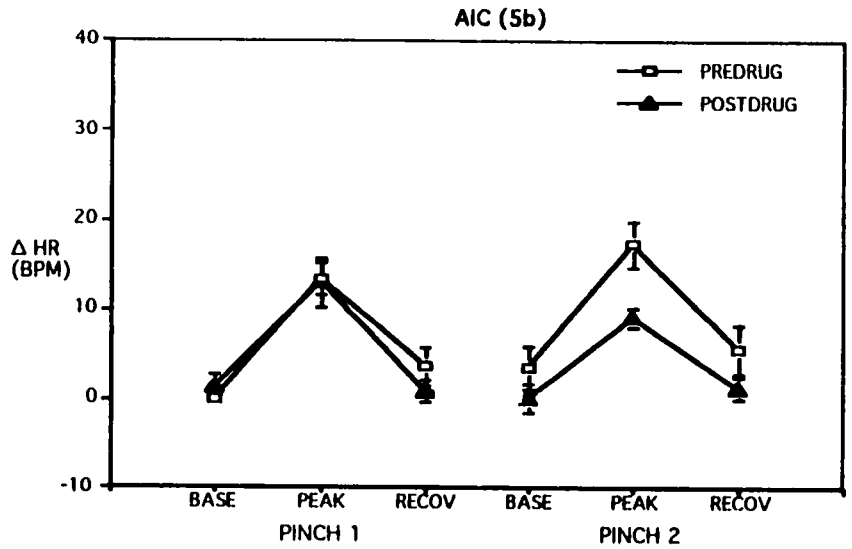
Highlights

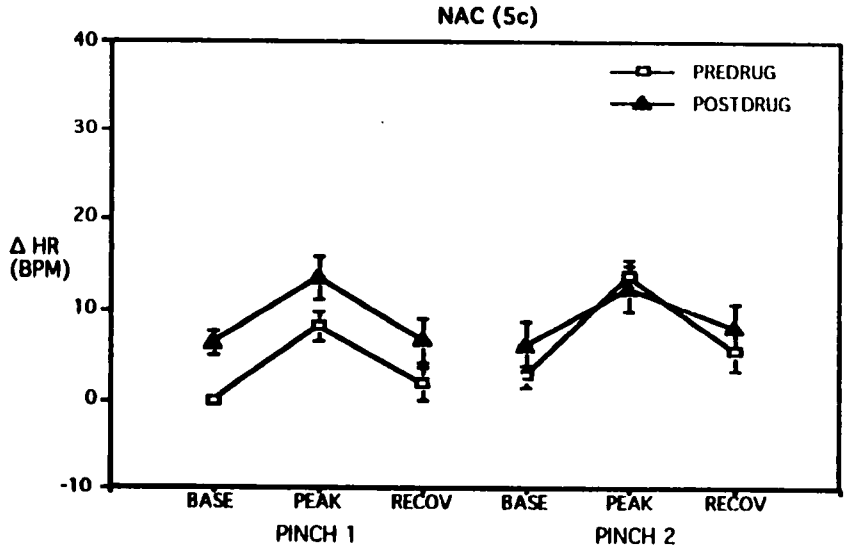
The effects of injections of 20 nmol of the D_2 receptor agonist quinpirole into the MFC (n=5), AIC (n=8), NAC (n=7) or CeA (n=3) on basal HR and the pinch response are shown in Figure 5. NAC injections of

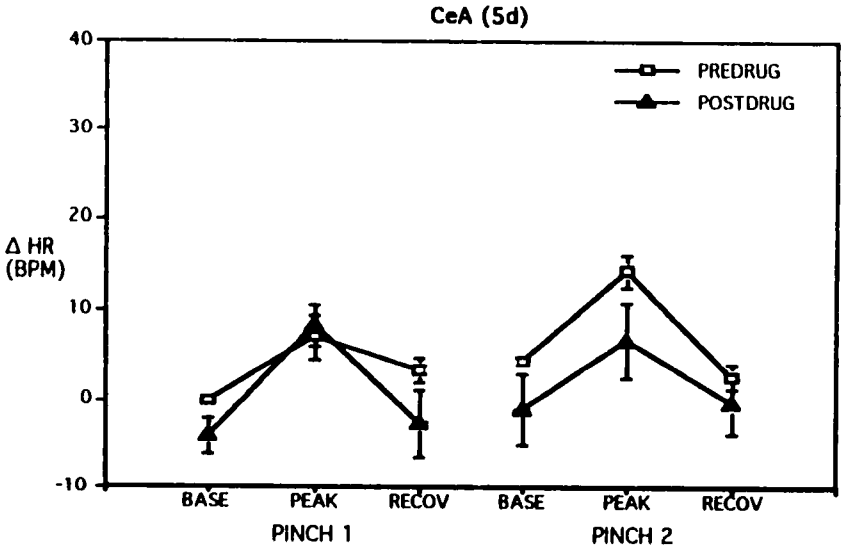
Figure 5 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 20 nmol of the D_2 receptor agonist quinpirole into the MFC (5a), AIC (5b), NAC (5c) or CeA (5d).

Quinpirole (20 nmol)









quinpirole caused small increases in baseline HR and reductions in the magnitude of the response to tail pinch. Quinpirole injections into the MFC, AIC and CeA tended to reduce baseline HR. The drug did not significantly affect the magnitude of the response to pinch when injected into the MFC, AIC or CeA, although it resulted in a slight decrease in the case of AIC injections.

Medial Frontal Cortex

Injections of 20 nmol of quinpirole into the MFC did not significantly affect baseline HR, as shown by the test of the main effect of drug [$F(1,4)=1.24$, $p > .32$] and simple effect of drug at the baseline prior to the first pinch [$F(1,24)=.11$, $p > .75$] (Figure 5a). There was, however, an overall tendency for these injections to reduce baseline HR. Quinpirole did not affect the response to tail pinch when it was injected into the MFC as shown by the result of the test of the drug x sample interaction [$F(2,8)=.09$, $p > .91$].

Agranular Insular Cortex

Figure 5b shows that quinpirole injections into the AIC did not affect baseline HR as revealed by the test result for the main effect of drug [$F(1,7)=2.62$, $p > .14$] or the simple effect of drug at the baseline prior to the first pinch [$F(1,42)=.24$, $p > .63$]. The response to tail pinch was not significantly affected by these injections, as shown by the overall drug x sample interaction result [$F(2,14)=2.39$, $p > .12$]. There was, however, a tendency for injections of quinpirole to reduce the magnitude of the response to the second pinch, as shown by the results of the test of the simple effect of the drug x sample interaction [$F(2,28)=2.55$, $p < .10$].

Nucleus Accumbens

Although the main effect of drug was not statistically significant [$F(1,6)=3.46$, $p > .11$], quinpirole injections made into the NAC increased

baseline HR as shown by the result of the test of the simple effect of drug at the baseline prior to the first pinch [$F(1,36)=7.97$, $p < .05$] (Figure 5c). Such injections significantly altered the response to tail pinch as revealed by the test of the drug x sample interaction [$F(2,12)=4.56$, $p < .05$]. Further analyses of the simple effects of the drug x sample interaction at each pinch showed that quinpirole injections significantly reduced the magnitude of the response to the second pinch [$F(2,24)=4.50$, $p < .05$].

Central Nucleus of the Amygdala

Figure 5d shows that quinpirole injections into the CeA did not significantly affect baseline HR, as demonstrated by the results of the test of the main effect of drug [$F(1,2)=3.40$, $p > .20$] and by the test of the simple effect of drug at the baseline prior to the first pinch [$F(1,12)=1.85$, $p > .22$]. There was, however, a tendency for these injections to reduce HR, an effect that occurred after the first pinch. Quinpirole injections did not affect the response to tail pinch as revealed by the result of the test of the drug x sample interaction [$F(2,4)=.19$, $p > .83$].

Effects of Injections of the D_2 Receptor Antagonist Raclopride

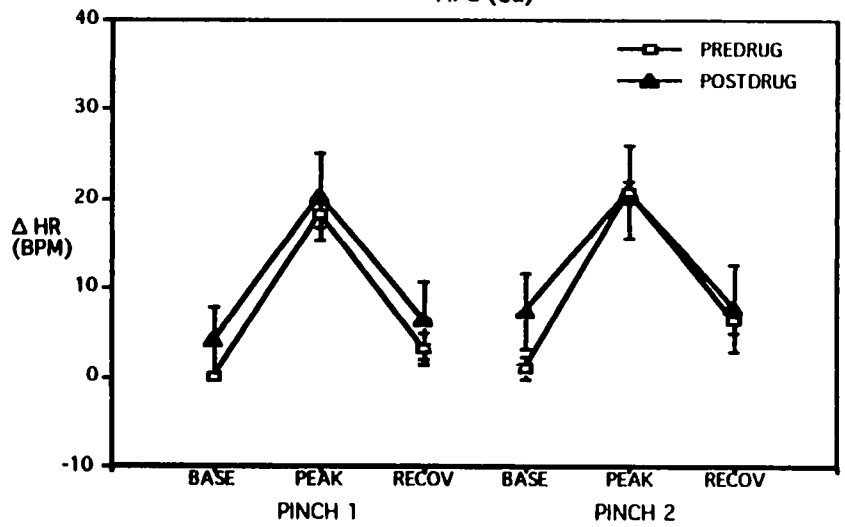
Highlights

The effects of injections of 20 nmol of the D_2 receptor antagonist raclopride into the MFC (n=5), AIC (n=4), NAC (n=5) or CeA (n=5) on baseline HR and the response to pinch are shown in Figure 6. Injections of raclopride increased baseline HR slightly when it was injected into the AIC. Injections of this drug into the CeA decreased the magnitude of the response to tail pinch. These injections caused a slight decrease in the response to tail pinch when made into the MFC.

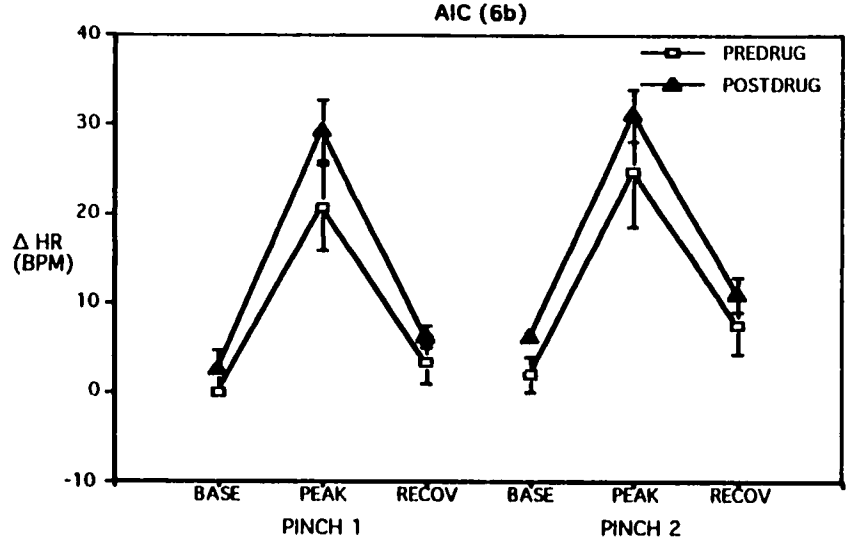
Figure 6 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 20 nmol of the D_2 receptor antagonist raclopride into the MFC (6a), AIC (6b), NAC (6c) or CeA (6d).

Raclopride (20 nmol)

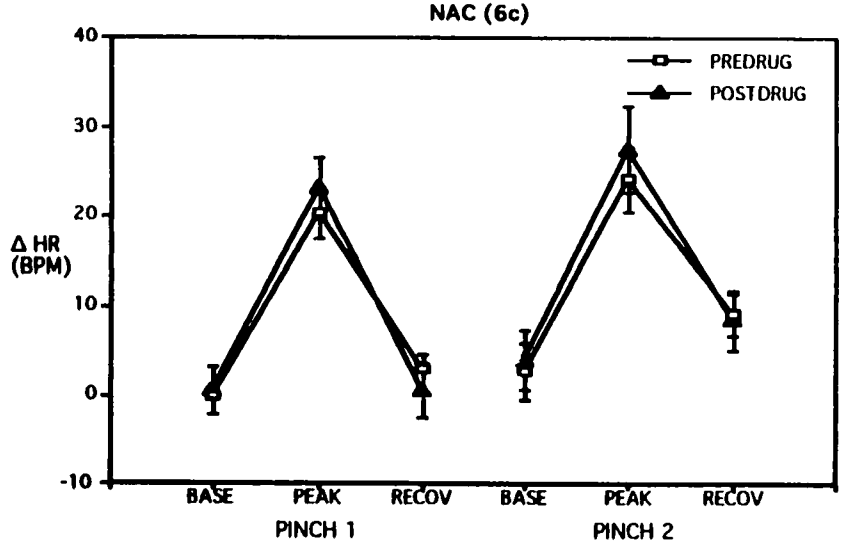
MFC (6a)



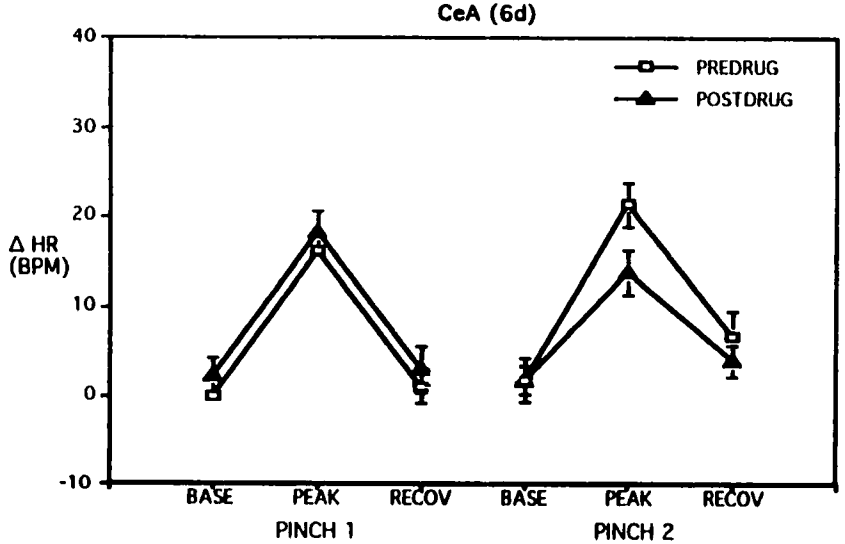
AIC (6b)



NAC (6c)



CeA (6d)



Medial Frontal Cortex

Injections of raclopride into the MFC did not significantly affect baseline HR, as shown by the results the tests of the main effect of drug [$F(1,4)=.64$, $p > .47$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,24)=.99$, $p > .35$] (Figure 6a). Raclopride did not affect the response to tail pinch as shown by the results of the test of the drug x sample interaction [$F(2,8)=1.45$, $p > .29$]. There was, however, a slight tendency for the response to the second pinch to be reduced by these injections, as revealed by the test of the drug x sample interaction [$F(2,16)=2.44$, $p=.12$].

Agranular Insular Cortex

Figure 6b shows that raclopride injections into the AIC did not significantly affect baseline HR as demonstrated by the results of the tests of the main effect of drug [$F(1,47)=5.22$, $p > .10$] and the simple effect of drug at the baseline prior to the first pinch [$F(1,18)=.45$, $p > .51$]. There was, however, a tendency towards increased baseline HR after raclopride injection. The response to tail pinch, as assessed by the test of the drug x sample interaction was not affected by injections of raclopride into the AIC [$F(2,6)=.57$, $p > .59$].

Nucleus Accumbens

Injections of raclopride into the NAC did not significantly affect baseline HR as shown by the result of the test of the main effect of drug [$F(1,4)=.17$, $p > .69$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,24)=.03$, $p > .85$] (Figure 6c). Neither was the response to pinch affected by raclopride as revealed by the test of the drug x sample interaction [$F(2,8)=.80$, $p > .48$].

Central Nucleus of the Amygdala

In Figure 6d, it can be seen that injections of raclopride into the CeA did not significantly affect baseline HR, as shown by tests of the main effect of

drug [F(1,4)=.71, p > .44] and of the simple effect of drug at the baseline prior to the first pinch [F(1,24)=1.77, p > .19]. These injections tended to affect the response to tail pinch, as demonstrated by the test result of the drug x sample interaction [F(2,8)=3.55, p < .10]. Further analysis of the simple effects of the drug x sample interaction at each pinch showed that injections of raclopride into the CeA significantly reduced the magnitude of the response to the second pinch [F(2,16)=5.89, p < .05].

Effects of Injections of a Combination of D_1 and D_2 Receptor Agonists

Highlights

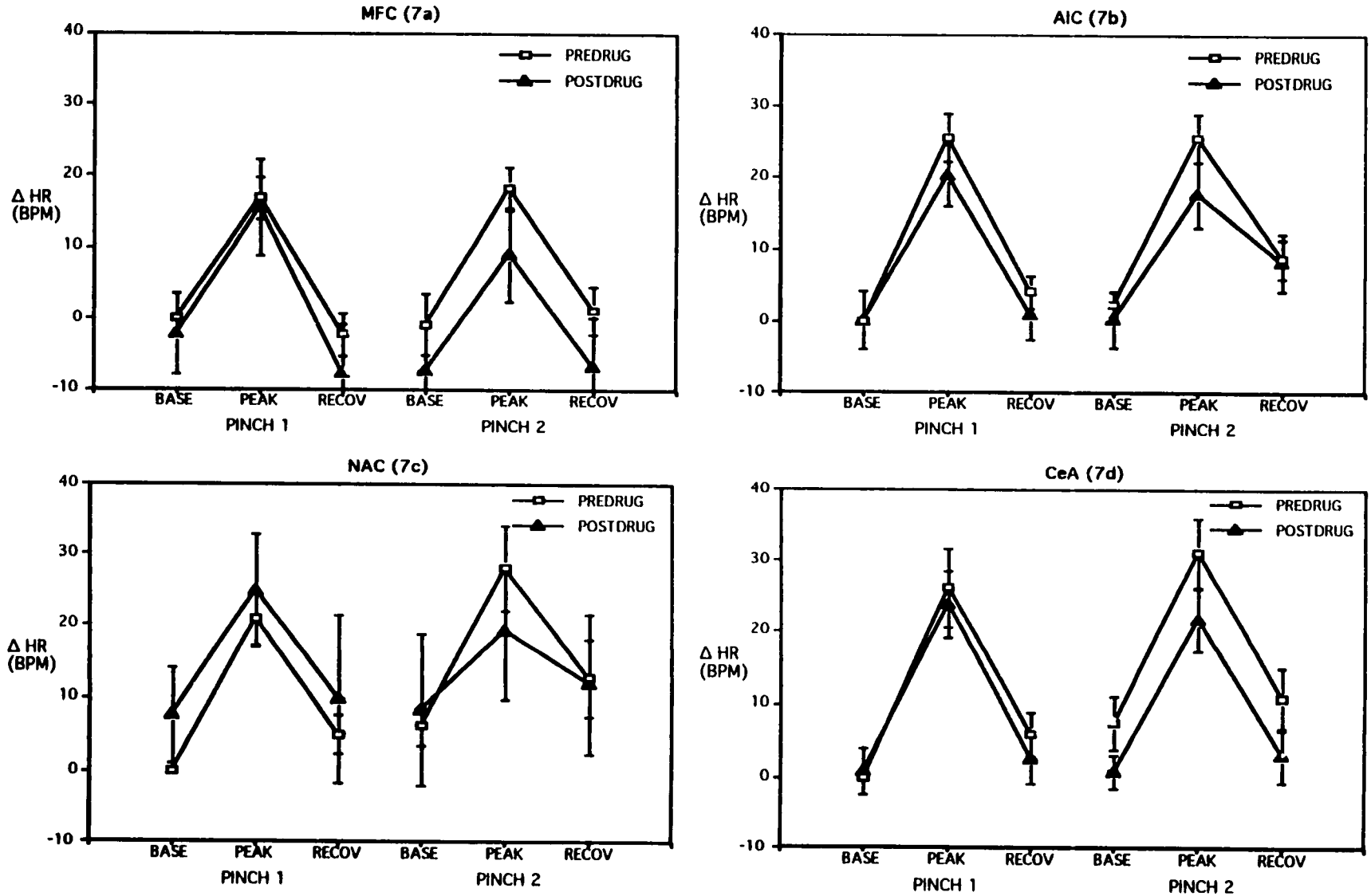
The effects of injections of a combination of 20 nmol of the D_1 receptor agonist SKF82958 and 20 nmol of the D_2 receptor agonist quinpirole into the MFC (n=6), AIC (n=7), NAC (n=5) or CeA (n=6) on baseline HR and the response to pinch are shown in Figure 7. In summary, injections of a combination of SKF82958 and quinpirole tended to decrease baseline HR when injected into the MFC and CeA. This drug combination also tended to decrease the magnitude of the response to pinch when injected into the AIC and NAC.

Medial Frontal Cortex

Injections of a combination of SKF82958 and quinpirole did not significantly affect baseline HR as shown by tests of the main effect of drug [F(1,5)=1.23, p > .31] and of the simple effect of drug at the baseline prior to the first pinch [F(1,30)=.17, p > .69] (Figure 7a). These injections did, however, tend to reduce baseline HR slightly, especially after the first pinch. This drug combination did not affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [F(2,10)=1.36, p > .29].

Figure 7 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of a combination of 20 nmol of the D_1 receptor agonist SKF82958 and 20 nmol of the D_2 receptor agonist quinpirole into the MFC (7a), AIC (7b), NAC (7c) or CeA (7d).

Quinpirole (20 nmol) + SKF82958 (20 nmol)



Agranular Insular Cortex

As seen in Figure 7b, injections of a combination of SKF82958 and quinpirole into the AIC did not significantly alter baseline HR, as demonstrated by the results of tests of the main effect of drug [$F(1,6)=.97$, $p > .36$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,36)=.00$, $p > .97$]. These injections tended to reduce the response to tail pinch as shown by the result of the test of the drug x sample interaction [$F(2,12)=3.61$, $p < .10$]. Subsequent tests of the simple effects of the drug x sample interaction showed that the magnitude of the response to the second pinch tended to be reduced after injection [$F(2,24)=2.93$, $p < .10$].

Nucleus Accumbens

Figure 7c shows that the injection of SKF82958 and quinpirole into the NAC did not significantly affect baseline HR as demonstrated by the results of the test of the main effect of drug [$F(1,4)=.03$, $p > .86$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,24)=.66$, $p > .44$]. These drug injections did not significantly affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [$F(2,8)=1.79$, $p > .22$]. Despite this fact, analyses of the simple effects of the drug x sample interaction at each pinch showed that the magnitude of the response to the second pinch tended to be reduced by these injections [$F(2,16)=2.30$, $p=.13$].

Central Nucleus of the Amygdala

Injections of the combination of SKF82958 and quinpirole into the CeA significantly affected baseline HR (Figure 7d) as revealed by the test of the main effect of drug [$F(1,5)=6.65$, $p < .05$]. The simple effect of drug at the baseline prior to the first pinch was not significant, however [$F(1,30)=.06$, $p > .80$]. This pattern of results was caused by the fact that baseline HR tended to

be reduced following the first pinch in the postdrug phase. For example, the baseline prior to the second pinch in the postdrug phase was smaller compared with the corresponding sample in the predrug phase [$F(1,30)=3.90$, $p=.06$]. Intra-CeA injections of a combination of SKF82958 and quinpirole did not affect the response to tail pinch as shown by the test of the drug x sample interaction [$F(2,10)=.39$, $p > .68$].

Effects of Injections of a Combination of a D_2 Receptor Agonist and a D_1 Receptor Antagonist

Highlights

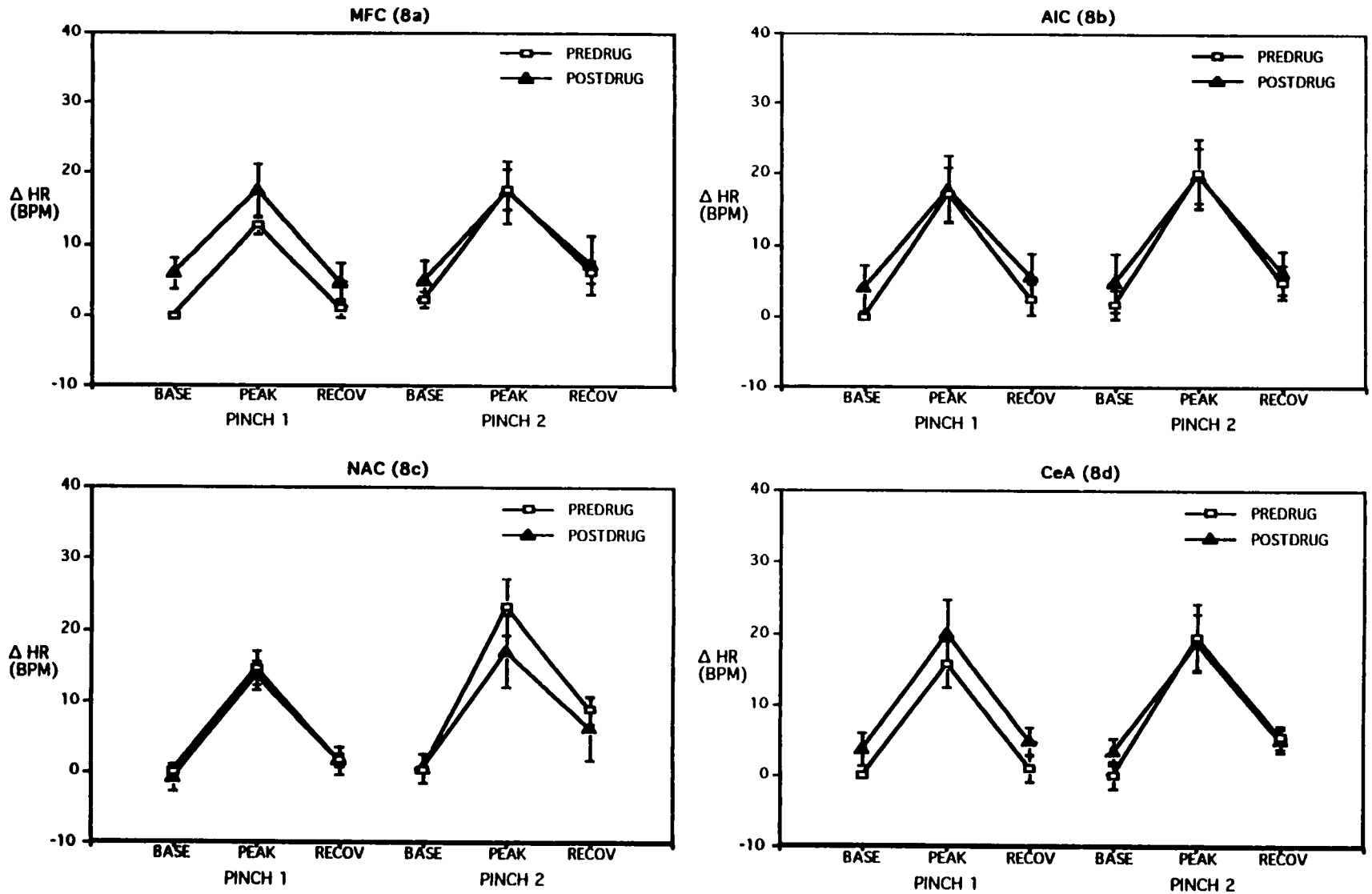
The effects of injections of a combination of 20 nmol of the D_2 receptor agonist quinpirole and 20 nmol of the D_1 receptor antagonist SKF83566 into the MFC (n=7), AIC (n=8), NAC (n=9) or CeA (n=6) on baseline HR and the response to pinch are shown in Figure 8. Injections of a combination of quinpirole and SKF83566 tended to increase baseline HR slightly when injected into the MFC or CeA. When injected into the NAC, this combination of drugs tended to reduce the magnitude of the response to pinch.

Medial Frontal Cortex

Injections of quinpirole and SKF83566 into the MFC tended to increase baseline HR as shown by the test of the simple effect of drug at the baseline prior to the first pinch [$F(1,36)=4.20$, $p < .10$] (Figure 8a). The main effect of drug was not significant [$F(1,6)=1.23$, $p > .31$]. These injections did not affect the response to tail pinch as revealed by the result of the test of the drug x sample interaction [$F(2,12)=1.42$, $p > .28$].

Figure 8 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of a combination of 20 nmol of the D_2 receptor agonist quinpirole and 20 nmol of the D_1 receptor antagonist SKF83566 into the MFC (8a), AIC (8b), NAC (8c) or CeA (8d).

Quinpirole (20 nmol) + SKF83566 (20 nmol)



Agranular Insular Cortex

Injections of this drug combination into the AIC did not affect baseline HR as shown by tests of the main effect of drug [$F(1,7)=.52$, $p > .49$] and the test of the simple effect of drug at the baseline prior to the first pinch [$F(1,42)=1.55$, $p > .23$] (Figure 8b). This drug combination did not affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [$F(2,14)=2.12$, $p > .15$].

Nucleus Accumbens

Figure 8c shows that injections of quinpirole and SKF83566 into the NAC did not significantly effect baseline HR, as demonstrated by the results of tests of the main effect of drug [$F(1,8)=.73$, $p > .41$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,48)=.10$, $p > .76$]. Although the drug x sample interaction was not statistically significant [$F(2,16)=2.31$, $p > .13$], tests of the simple effects of the drug x sample interaction at each pinch revealed that the injections did tend to reduce the magnitude of the response to the second pinch [$F(2,32)=2.72$, $p < .10$].

Central Nucleus of the Amygdala

Although the result of the test of the main effect of drug was not significant [$F(1,5)=2.53$, $p > .17$], quinpirole and SKF83566 injections into the CeA did tend to increase baseline HR prior to the first pinch [$F(1,30)=3.15$, $p < .10$] (Figure 8d). These injections did not significantly affect the response to tail pinch, as shown by the results of the test of the drug x sample interaction [$F(2,10)=.88$, $p > .44$].

Summary: The Effects of Injections of Dopaminergic Drugs

The effects of injections of D_1 and D_2 agonists and antagonists, and their combination into the MFC, AIC, NAC and CeA on baseline HR and the

response to pinch were assessed in this phase of the study. In general, it was found that injections of DAergic drugs into these nuclei did not result in marked alterations in these parameters. In some cases, increases or decreases were observed in baseline HR and the response to pinch, but these effects were small and were not found to be systematic with regards to the type of drug tested and site of injection.

Effects of Injections of Noradrenergic Drugs

Effects of Injections of the β-Adrenoceptor Agonist Isoproterenol

Highlights

The effects of injections of 10 nmol of the β-adrenoceptor agonist isoproterenol into the MFC (n=10), AIC (n=11), NAC (n=13) or CeA (n=11) on baseline HR and the response to pinch are shown in Figure 9. Injections of isoproterenol increased baseline HR significantly when injected into the MFC, AIC and NAC. This drug increased baseline HR slightly when injected into the CeA. Isoproterenol increased the magnitude of the response to tail pinch when it was injected into the NAC.

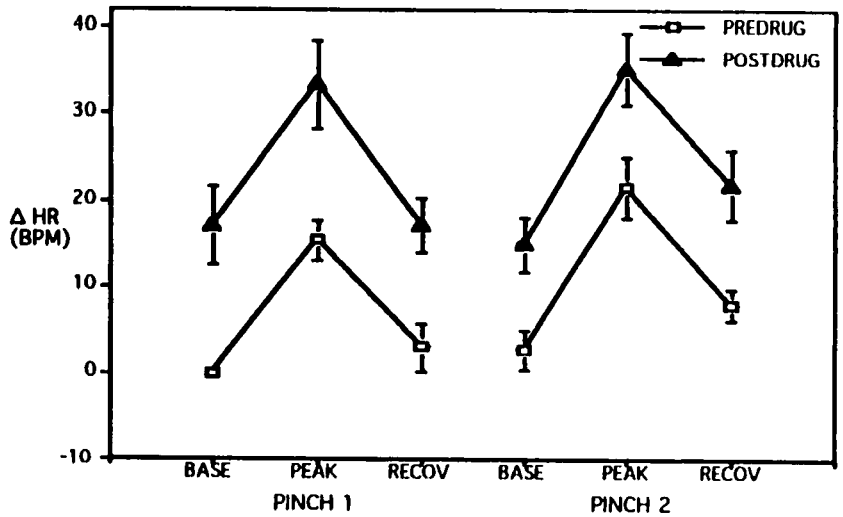
Medial Frontal Cortex

When injected into the MFC, isoproterenol increased baseline HR significantly (Figure 9a). This was reflected in the results of tests for the main effect of drug [$F(1,9)=14.78$, $p < .005$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,54)=15.20$, $p < .005$]. Isoproterenol injections into the MFC did not affect the response to tail pinch as demonstrated by the result of the test of the drug x sample interaction [$F(2,18)=.30$, $p > .74$].

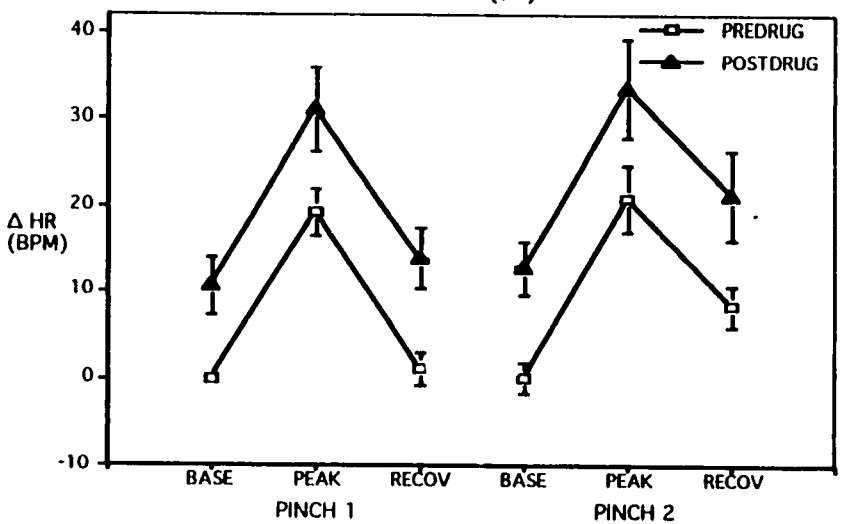
Figure 9 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 10 nmol of the β-adrenoceptor agonist isoproterenol into the MFC (9a), AIC (9b), NAC (9c) or CeA (9d).

Isoproterenol (10 nmol)

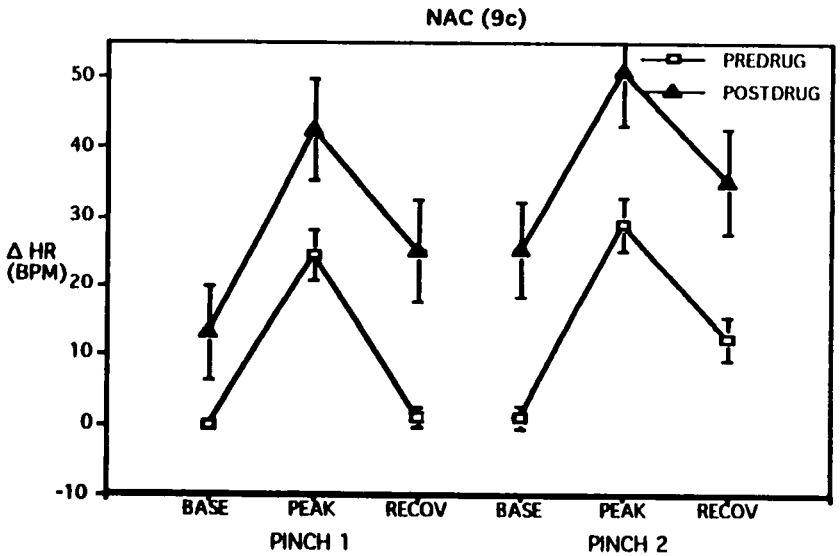
MFC (9a)



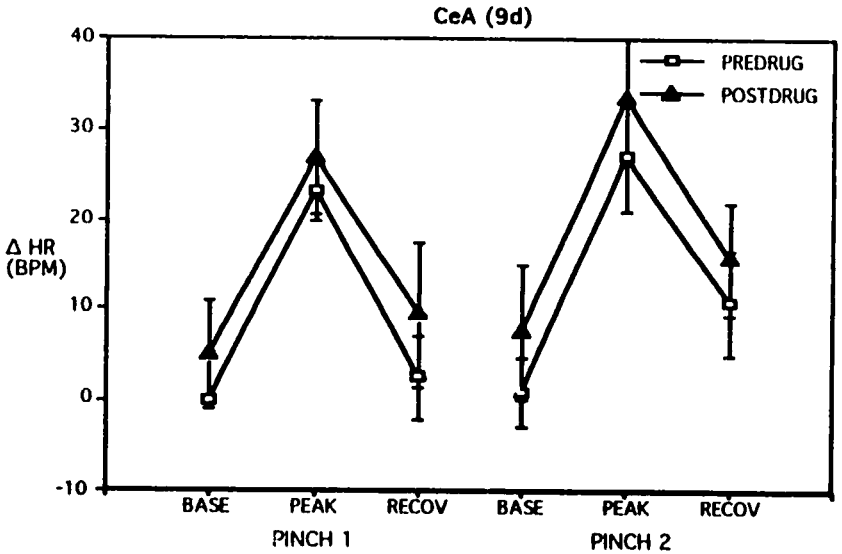
AIC (9b)



NAC (9c)



CeA (9d)



Agranular Insular Cortex

As seen in Figure 9b, isoproterenol injections into the AIC significantly increased baseline HR as demonstrated by tests of the main effect of drug [$F(1,10)=15.13$, $p < .005$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,60)=9.36$, $p < .01$]. Such injections did not affect the response to tail pinch, as shown by the result of test of the drug x sample interaction [$F(2,20)=.23$, $p > .79$].

Nucleus Accumbens

As shown in Figure 9c, isoproterenol injections into the NAC increased baseline HR, and this was reflected in the significant effect of drug [$F(1,12)=12.65$, $p < .005$]. Despite this fact, the test of the simple effect of drug at the baseline prior to the first pinch only approached statistical significance [$F(1,72)=4.11$, $p < .10$]. The drug injection also altered the response to tail pinch; although the drug x sample interaction was not statistically significant [$F(2,24)=1.04$, $p > .36$], analysis of the simple effects of this interaction showed that it was significant at the first pinch [$F(2,48)=3.45$, $p < .05$]. The drug-induced alteration of the response to the first pinch was characterized by an increase in the magnitude of the response and a slower recovery of HR.

Central Nucleus of the Amygdala

Isoproterenol did not significantly affect baseline HR when injected into the CeA, as shown by the result of the test of the main effect of drug [$F(1,10)=2.84$, $p > .12$] or of the test of the simple effect of drug at the baseline prior to the first pinch [$F(1,60)=1.32$, $p > .26$] (Figure 9d). HR did, however, tend to be slightly elevated by these injections. The response to tail pinch was not affected, as revealed by the test of the drug x sample interaction [$F(2,20)=.11$, $p > .89$].

Effects of Injections of the β-Adrenoceptor Antagonist Propranolol

Highlights

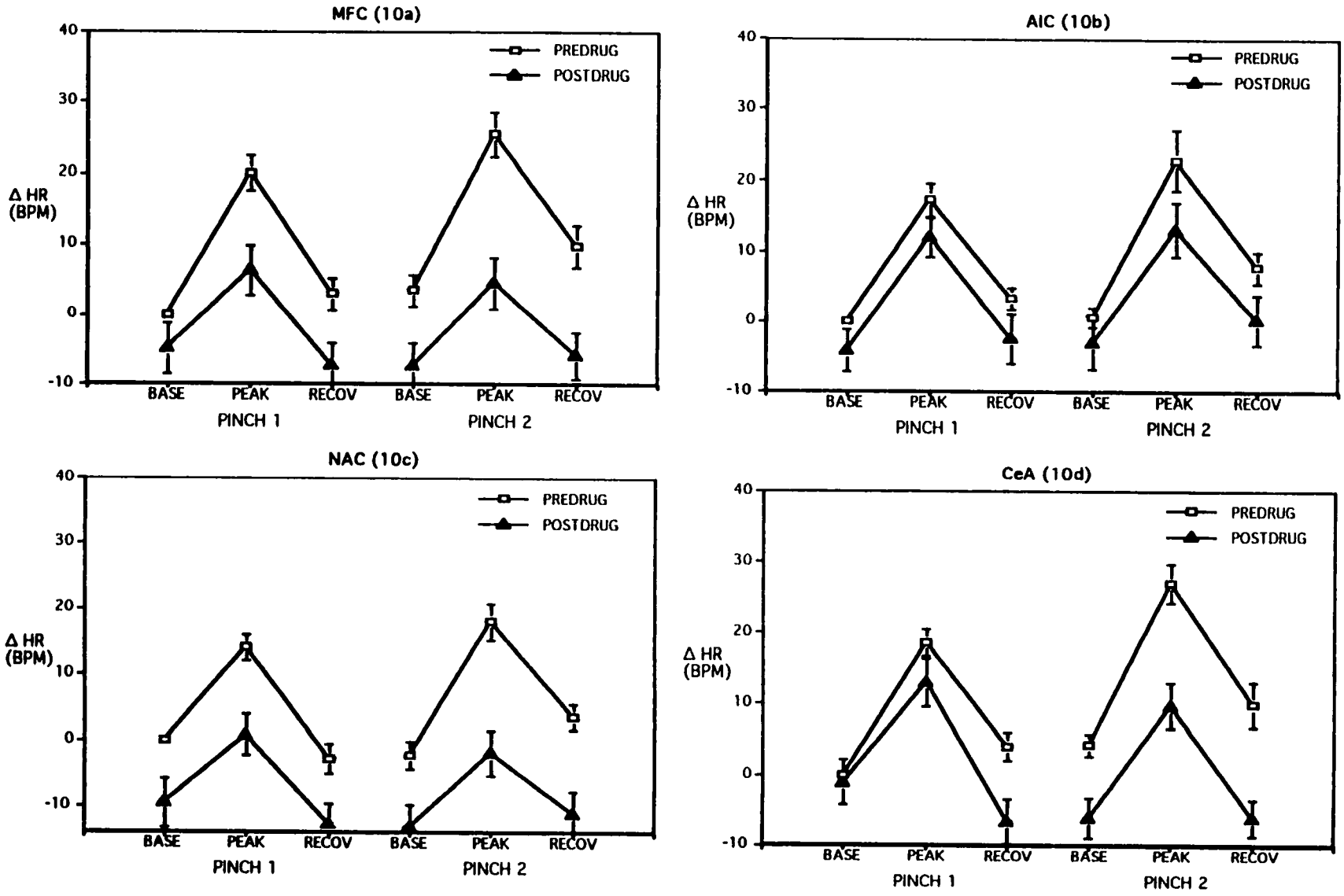
The effects of injections of 40 nmol of the β-adrenoceptor antagonist propranolol into the MFC (n=10), AIC (n=10), NAC (n=11) or CeA (n=10) on baseline HR and the response to pinch are shown in Figure 10. Injections of propranolol significantly reduced baseline HR when injected into the MFC, NAC and CeA. Propranolol injections tended to reduce HR when it was injected into the AIC. In the case of the MFC and CeA, propranolol-induced reductions in baseline HR were most marked after the first pinch in the postdrug phase. These injections significantly reduced the magnitude of the response to tail pinch when injected into any of the four sites.

Medial Frontal Cortex

Injections of propranolol into the MFC decreased baseline HR significantly [$F(1,9)=16.76$, $p < .005$] (Figure 10a). Although baseline HR was not significantly affected immediately after injection, as shown by the test result for the simple effect of drug at the baseline prior to the first pinch [$F(1,54)=1.75$. $p > .20$], these injections did decrease it significantly by the baseline prior to the second pinch [$F(1,54)=8.68$, $p < .01$]. Propranolol injections into the MFC also significantly reduced the magnitude of the response to pinch as shown by the result of the test of the drug x sample interaction [$F(2,18)=7.31$, $p < .01$]. Tests of simple effects revealed that the drug x sample interaction was significant during both the first [$F(2,36)=4.46$, $p < .05$] and the second pinch [$F(2,36)=5.68$, $p < .01$]. The decrease in the response to pinch was characterized by a reduction in the magnitude of the peak response and in a more rapid recovery after pinch.

Figure 10 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 40 nmol of the β-adrenoceptor antagonist propranolol into the MFC (10a), AIC (10b), NAC (10c) or CeA (10d).

Propranolol (40 nmol)



Agranular Insular Cortex

As shown in Figure 10b, injections of propranolol into the AIC tended to decrease baseline HR, as demonstrated by the result of the test of the main effect of drug [$F(1,9)=3.85$, $p < .10$]. Although baseline HR was lower at the sample point prior to the first pinch, the difference was not statistically significant when assessed with a test of the simple effect of drug [$F(1,54)=1.48$, $p > .24$]. These injections reduced the magnitude of the response to pinch. Although the drug x sample interaction only approached statistical significance [$F(2,18)=3.42$, $p < .10$], analysis of the simple effects of the interaction revealed that it was significant during the second pinch [$F(2,36)=4.63$, $p < .05$], reflecting the fact that the magnitude of the peak increase in HR was reduced, and the recovery of HR was more rapid after the injection of propranolol into the AIC.

Nucleus Accumbens

Figure 10c shows that propranolol injections into the NAC significantly reduced baseline HR. This is demonstrated by the results of tests of the main effect of drug [$F(1,10)=14.10$, $p < .005$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,60)=6.31$, $p < .05$]. The test of the drug x sample interaction showed that propranolol significantly altered the response to tail pinch [$F(2,20)=9.18$, $p < .005$]. Further analysis of simple effects revealed that the response to the second pinch was affected by these injections [$F(2,40)=7.17$, $p < .01$], reflecting the fact that they reduced the magnitude of the peak response, and augmented the recovery of HR after pinch.

Central Nucleus of the Amygdala

When injected into the CeA, propranolol significantly reduced baseline HR as demonstrated by results of the test of the main effect of drug

[F(1,9)=26.11, p < .001] (Figure 10d). Analysis of simple effects showed that these injections did not affect HR at the baseline prior to the first pinch [F(1,54)=.15, p > .70], but did significantly reduce it at the baseline prior to the second pinch [F(1,54)=15.58, p < .001]. These injections significantly affected the response to pinch, as revealed by the test of the drug x sample interaction [F(2,18)=1.70, p < .001]. Analyses of the simple effects of this interaction revealed that it was significant at both pinches (first pinch: [F(2,36)=9.29, p < .001]; second pinch: [F(2,36)=5.47, p < .01]). After the injection of propranolol into the CeA, the magnitude of the peak increase in HR induced by pinch was smaller, and the recovery of HR was more rapid.

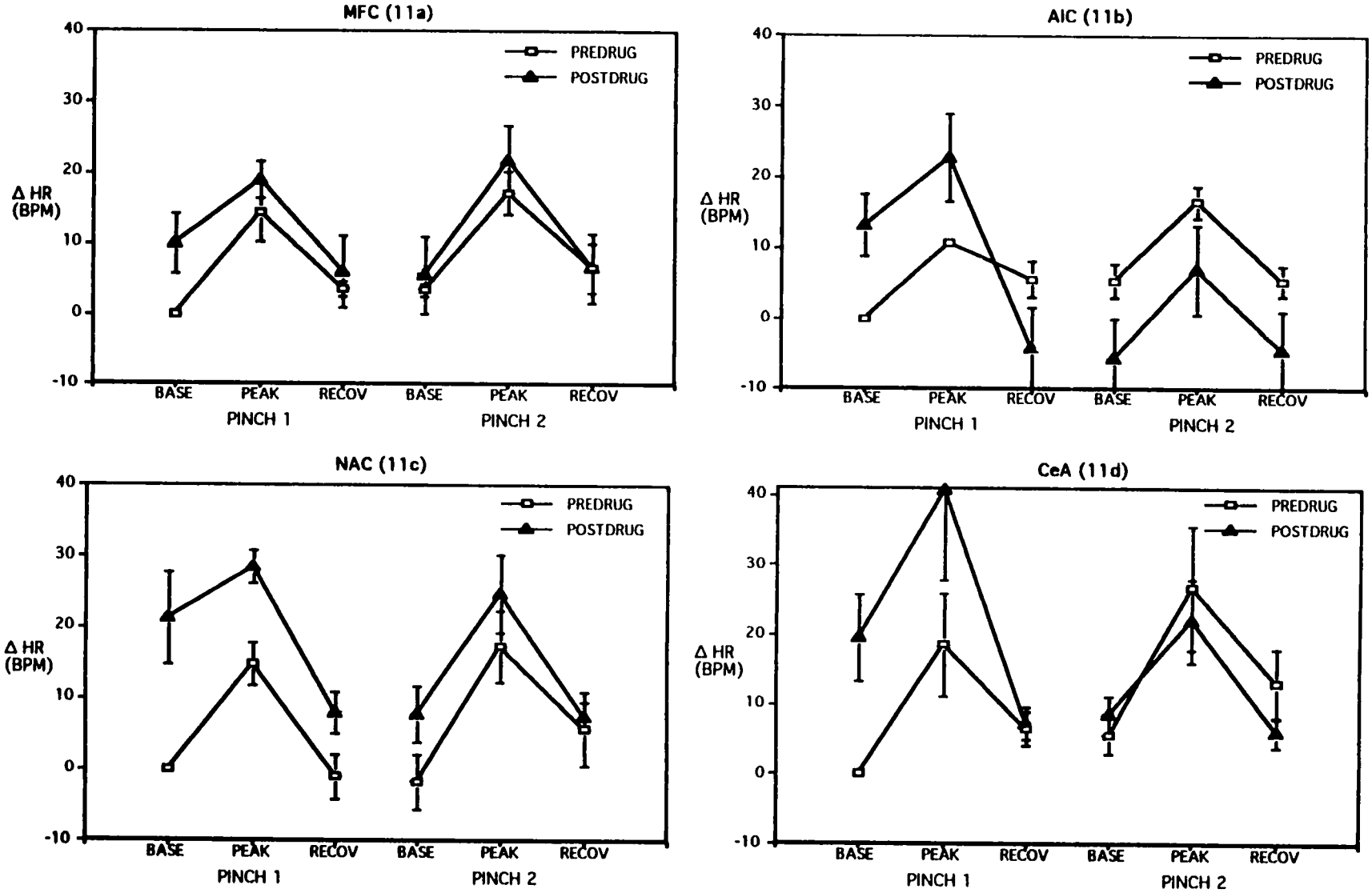
Effects of Injections of a Combination of a β-Adrenoceptor Agonist and a β-Adrenoceptor Antagonist

Highlights

The effects of injections of a combination of 10 nmol of the β-adrenoceptor agonist isoproterenol and 40 nmol of the β-adrenoceptor antagonist propranolol into the MFC (n=5), AIC (n=5), NAC (n=4) or CeA (n=4) on baseline HR and the response to pinch are shown in Figure 11. Injections of a combination of propranolol and isoproterenol increased baseline HR when injected into each of the four sites. Injections made into the MFC and CeA decreased baseline HR to a level similar to that seen in the predrug phase, and to below predrug levels in the case of the AIC. The reductions in baseline HR caused by injections of this drug combination were most marked at samples occurring later in the postdrug phase. Injections of a combination of isoproterenol and propranolol into any of the four sites did not affect the magnitude of the response to pinch.

Figure 11 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of a combination of 10 nmol of the β-adrenoceptor agonist isoproterenol and 40 nmol of the β-adrenoceptor antagonist propranolol into the MFC (11a), AIC (11b), NAC (11c) or CeA (11d).

Isoproterenol (10 nmol) + Propranolol (40 nmol)



Propranolol appeared to be effective in reducing or blocking the baseline-elevating effects of isoproterenol in the case of injections made into the MFC, AIC and NAC. This drug combination unexpectedly caused large increases in baseline HR when injected into the CeA, an effect not seen when only isoproterenol was injected. In the case of injections made into the NAC, the inclusion of propranolol also blocked the isoproterenol-induced increase in the magnitude of the response to pinch.

Medial Frontal Cortex

Figure 11a shows that injections of isoproterenol and propranolol into the MFC caused a slight elevation in baseline HR. Although the result of the test of the main effect of drug was not significant [$F(1,4)=.86$, $p > .40$], the result of the test of the simple effect of drug at the baseline prior to the first pinch approached statistical significance [$F(1,24)=4.65$, $p < .10$]. The test of the drug x sample interaction suggested that these injections tended to alter the response to pinch [$F(2,8)=4.19$, $p < .10$] and further analysis of the simple effect of this interaction revealed that this occurred due to the significant alteration of the response to the first pinch [$F(2,16)=5.54$, $p < .05$]. This appeared to result, however, from the drug-induced elevation in baseline HR, rather than from a change in the magnitude of the response to tail pinch.

Agranular Insular Cortex

Injections of the combination of isoproterenol and propranolol into the AIC increased baseline HR (Figure 11b). Although the result of the test of the main effect of drug was not significant [$F(1,4)=.27$,$p > .62$], that for the simple effect of drug at the baseline prior to the first pinch indicated a trend towards increased baseline HR [$F(1,24)=4.47$, $p < .10$]. Following the first pinch after injection, HR decreased to levels below the predrug baseline. The result of the test of the drug x sample interaction indicated that these injections

significantly altered the response to pinch [$F(2,8)=5.85$, $p < .05$]. Analysis of the simple effects of this interaction revealed that it was significant only in the first pinch [$F(2,16)=14.22$, $p < .0005$]. This significant result occurred due to the very low level of the recovery sample of the first pinch rather than from a change in the magnitude of the response to pinch.

Nucleus Accumbens

As depicted in Figure 11c, propranolol coinjected with isoproterenol increased baseline HR. The main effect of drug approached statistical significance [$F(1,3)=8.11$, $p < .10$], due to the fact that baseline HR was initially increased after the injection of the drug combination, as shown by tests of simple effects of drug at this point [$F(1,18)=14.64$, $p < .005$]. In between the first and second pinch in the postdrug phase, however, HR decreased to levels below those of the initial postdrug baseline. Although the baseline prior to the second pinch tended to be higher after injection, it did not differ significantly from that in the predrug phase as shown by the results of the test of the simple effects of drug at this point [$F(1,18)=2.93$, $p > .11$]). The injection of this drug combination did not significantly affect the response to tail pinch as revealed by the test of the drug x sample interaction [$F(2,6)=2.18$, $p > .19$].

Central Nucleus of the Amygdala

Figure 11d shows that injections of a combination of isoproterenol and propranolol into the CeA increased baseline HR. Although the main effect of drug was not statistically significant, [$F(1,3)=3.89$, $p > .14$], the result of the test of the simple effect of drug at the baseline prior to the first pinch indicated that HR was significantly higher at this point [$F(1,18)=15.76$, $p < .005$]. By the baseline of the second pinch, however, HR had decreased to a level that did not differ significantly from the corresponding sample in the predrug phase, a fact reflected in the result of the test of the simple effect of drug at this point

[F(1,18)=.37, p > .55]. The response to tail pinch was significantly altered by the administration of this drug combination, as revealed by the test of the drug x sample interaction [F(2,6)=7.43, p < .05]. Analyses of the simple effect of this interaction showed that it was significant only at the first pinch [F(2,12)=6.97, p < .05]. It is apparent, however, that this effect resulted from the large decrease in HR seen after the pinch in the postdrug phase, rather than from a change in the magnitude of the response.

Effects of Injections of the α-Adrenoceptor Agonist Phenylephrine

Highlights

The effects of injections of 20 nmol of the α adrenoceptor agonist phenylephrine into the MFC (n=5), AIC (n=8), NAC (n=5) or CeA (n=7) on baseline HR and the response to pinch are shown in Figure 12. Injections of phenylephrine caused a small but significant increase in baseline HR when injected into the CeA. These injections also tended to increase baseline HR when injected into the NAC and decrease baseline HR when injected into the MFC. Injections of phenylephrine did not affect the magnitude of the response to pinch when injected into any of the four sites.

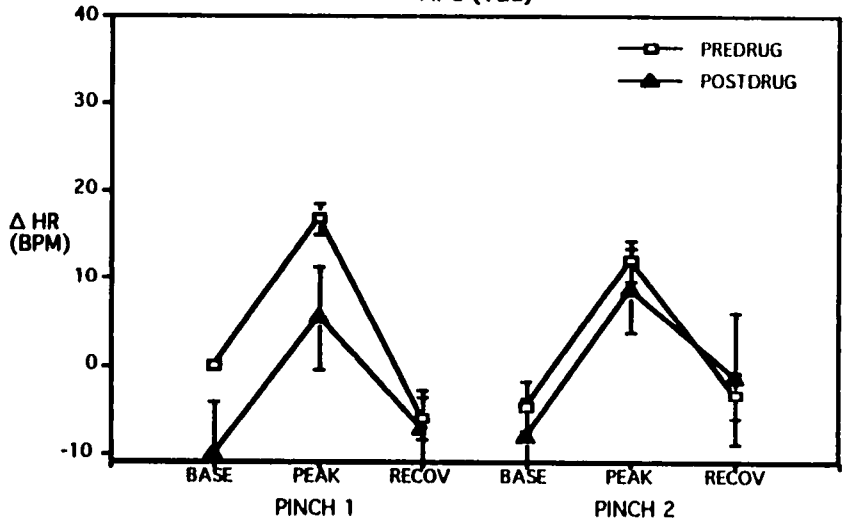
Medial Frontal Cortex

The injection of phenylephrine into the MFC tended to decrease baseline HR, as revealed by the result of the test of the simple effect of drug at the baseline prior to the first pinch [F(1,24)=2.68, p > .15] (Figure 12a). The main effect of drug was not significant, however [F(1,4)=.65, p > .46]. The result of the test of the drug x sample interaction suggested that these injections significantly altered the response to pinch [F(2,8)=7.77, p < .05] and further analysis of the simple effects of the interaction showed that it was

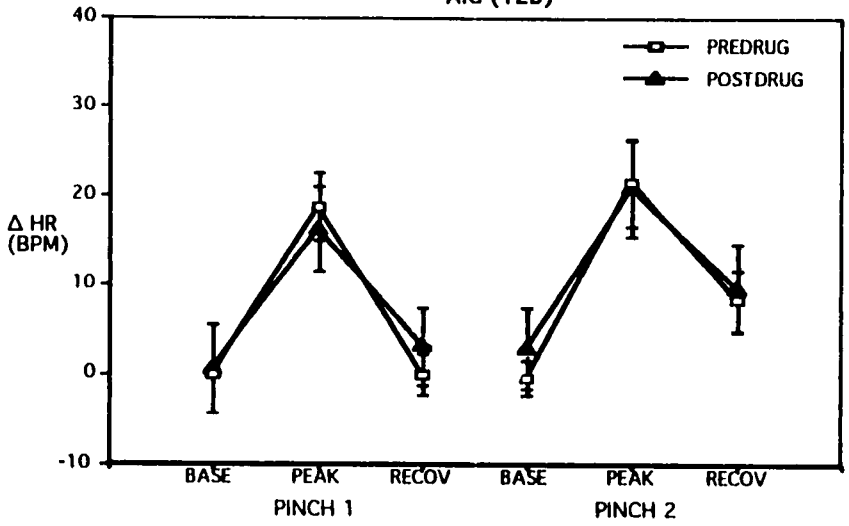
Figure 12 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 20 nmol of the α-adrenoceptor agonist phenylephrine into the MFC (12a), AIC (12b), NAC (12c) or CeA (12d).

Phenylephrine (20 nmol)

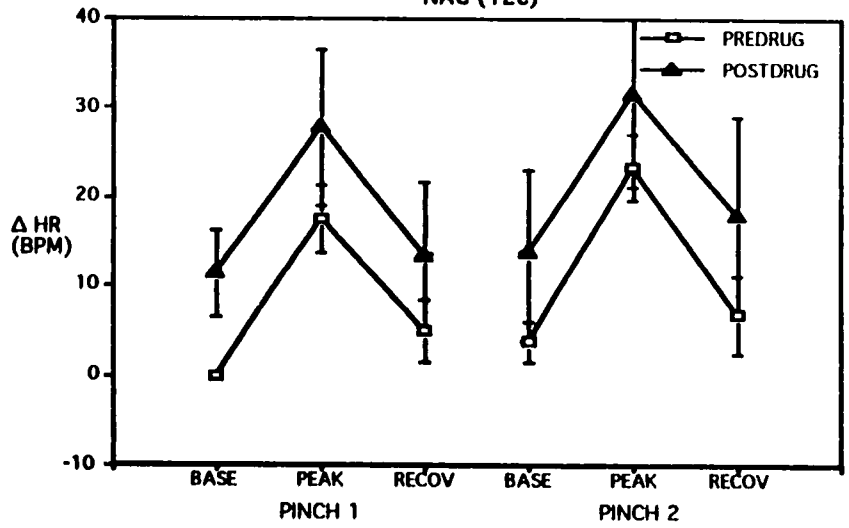
MFC (12a)



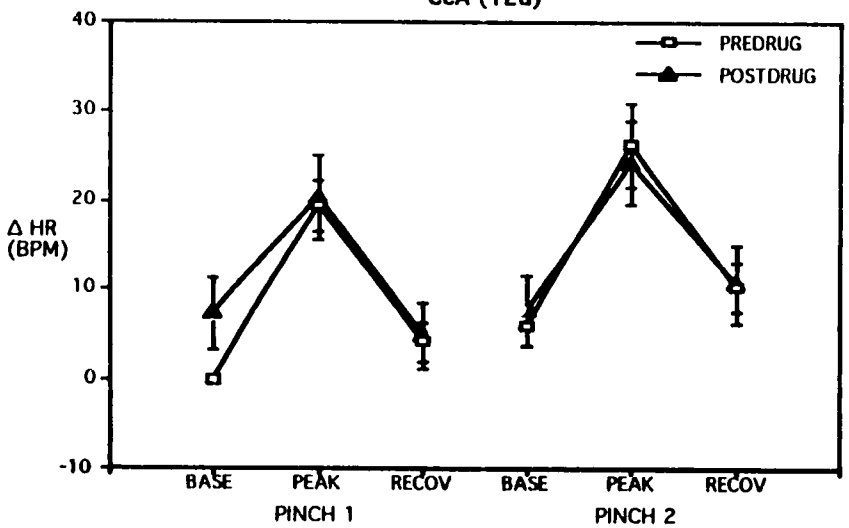
AIC (12b)



NAC (12c)



CeA (12d)



significant only at the first pinch [$F(2,16)=4.55$, $p < .05$]. It appears, however, that this pattern of results arose due to the anomalously high levels of the baseline and peak samples of the first pinch in the predrug phase, rather than from a drug-induced change in the magnitude of the response to pinch.

Agranular Insular Cortex

Injection of phenylephrine into the AIC did not significantly affect baseline HR as shown by the analysis of the main effect of drug [$F(1,7)=.08$, $p > .78$] and the simple effect of drug at the baseline prior to the first pinch [$F(1,42)=.03$, $p > .86$] (Figure 12b). The injection of phenylephrine into the AIC did not affect the response to pinch, as revealed by the test of the drug x sample interaction [$F(2,14)=2.41$, $p > .12$].

Nucleus Accumbens

As illustrated in Figure 12c, injections of phenylephrine into the NAC tended to increase baseline HR. The tests of the main effect of drug [$F(1,4)=1.75$, $p > .25$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,24)=1.97$, $p > .21$] revealed, however, that these differences were not statistically significant. The injection of phenylephrine into the NAC did not affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [$F(2,8)=.26$, $p > .77$].

Central Nucleus of the Amygdala

Figure 12d shows that injections of phenylephrine into the CeA caused a small increase in baseline HR. Although the result of the test of the main effect of drug was not significant [$F(1,6)=.54$, $p > .49$], the test of the simple effect of drug at the baseline prior to the first pinch revealed that the drug-induced increase in HR was significant [$F(1,36)=5.42$, $p < .05$]. Such injections did not affect the response to tail pinch as shown by the result of the test of the drug x sample interaction [$F(2,12)=2.22$, $p > .15$].

Effects of Injections of the α-Adrenoceptor Antagonist Phentolamine

Highlights

The effects of injections of 20 nmol of the α-adrenoceptor antagonist phentolamine into the MFC (n=6), AIC (n=5), NAC (n=6) or CeA (n=5) on baseline HR and the response to pinch are shown in Figure 13. Injections of phentolamine into any of the four sites affected neither baseline HR nor the response to pinch.

Medial Frontal Cortex

Figure 13a shows that injections of phentolamine into the MFC did not affect baseline HR a fact reflected in the results of tests of the main effect of drug [$F(1,5)=.58$, $p > .48$] and of the simple effect of drug at the baseline prior to the first pinch [$F(1,30)=.37$, $p > .55$]. The result of test of the drug x sample interaction showed that phentolamine did not affect the response to tail pinch [$F(2,10)=.60$, $p > .56$].

Agranular Insular Cortex

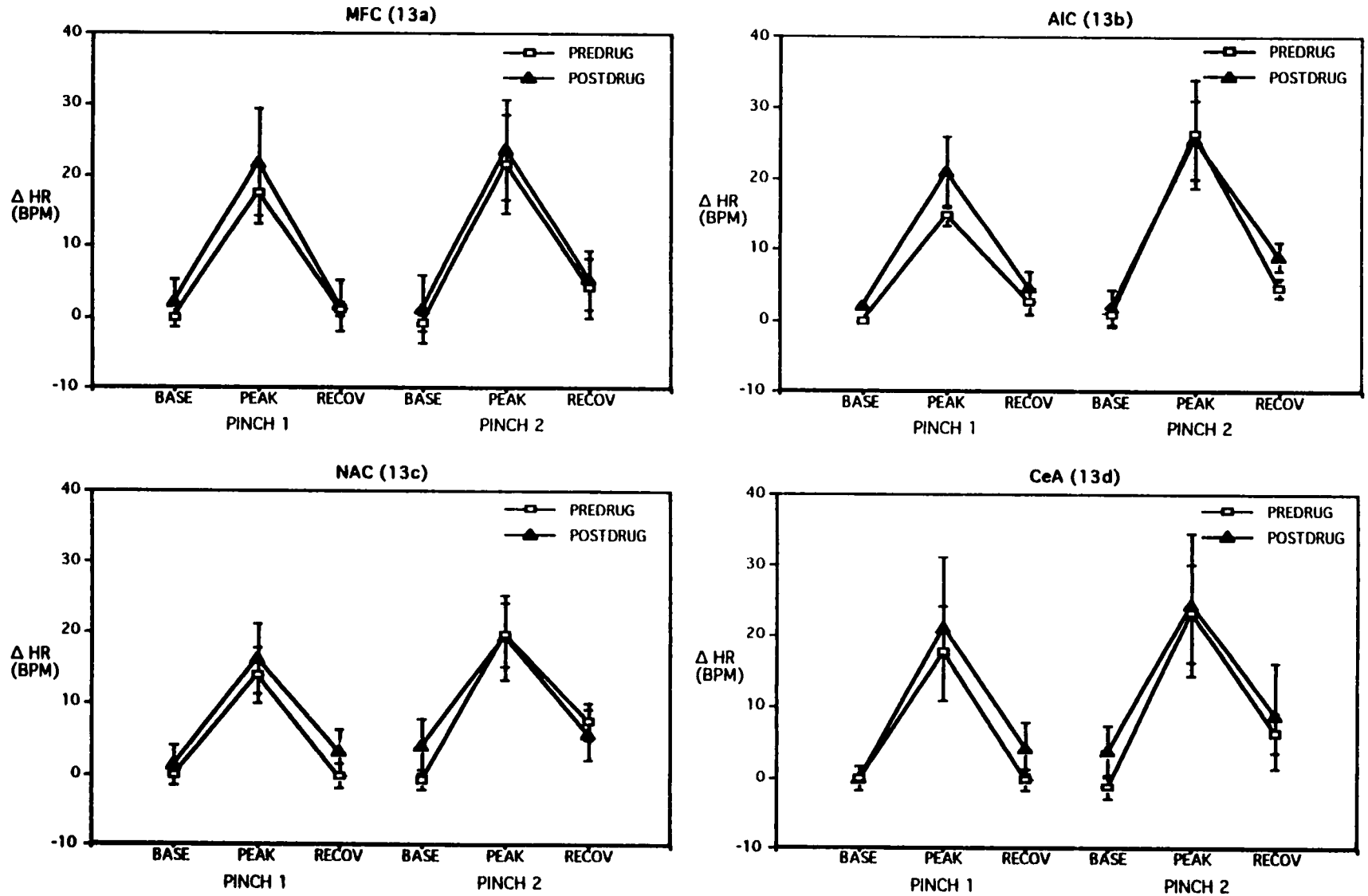
As illustrated in Figure 13b, injections of phentolamine into the AIC did not affect baseline HR, a fact reflected in the results of tests for the main effect of drug [$F(1,4)=3.21$, $p > .14$] and for the simple effect of drug at the baseline prior to the first pinch [$F(1,24)=.59$, $p > .44$]. The result of the test of the drug x sample interaction indicated that the injection of phentolamine into the AIC did not affect the response to tail pinch [$F(2,8)=.48$, $p > .63$].

Nucleus Accumbens

Injections of phentolamine into the NAC did not significantly affect baseline HR, as shown by analysis of the main effect of drug [$F(1,5)=.76$, $p > .42$] and the simple effect of drug at the baseline prior to the first pinch

Figure 13 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of 20 nmol of the α-adrenoceptor antagonist phentolamine into the MFC (13a), AIC (13b), NAC (13c) or CeA (13d).

Phentolamine (20 nmol)



[F(1,30)=.34, p > .57] (Figure 13c). These injections did not affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [F(2,10)=.83, p > .46].

Central Nucleus of the Amygdala

When injected into the CeA, phentolamine did not significantly affect baseline HR as shown by the result of the test of the main effect of drug [F(1,4)=.77, p > .42] and by the test of the simple effect of drug at the baseline prior to the first pinch [F(1,24)=.00, p > .98] (Figure 13d). The drug x sample interaction was not significant, indicating that these injections did not affect the response to tail pinch [F(2,8)=.20, p > .82].

Effects of Injections of a Combination of an α-Adrenoceptor Agonist with a β-Adrenoceptor Agonist

Highlights

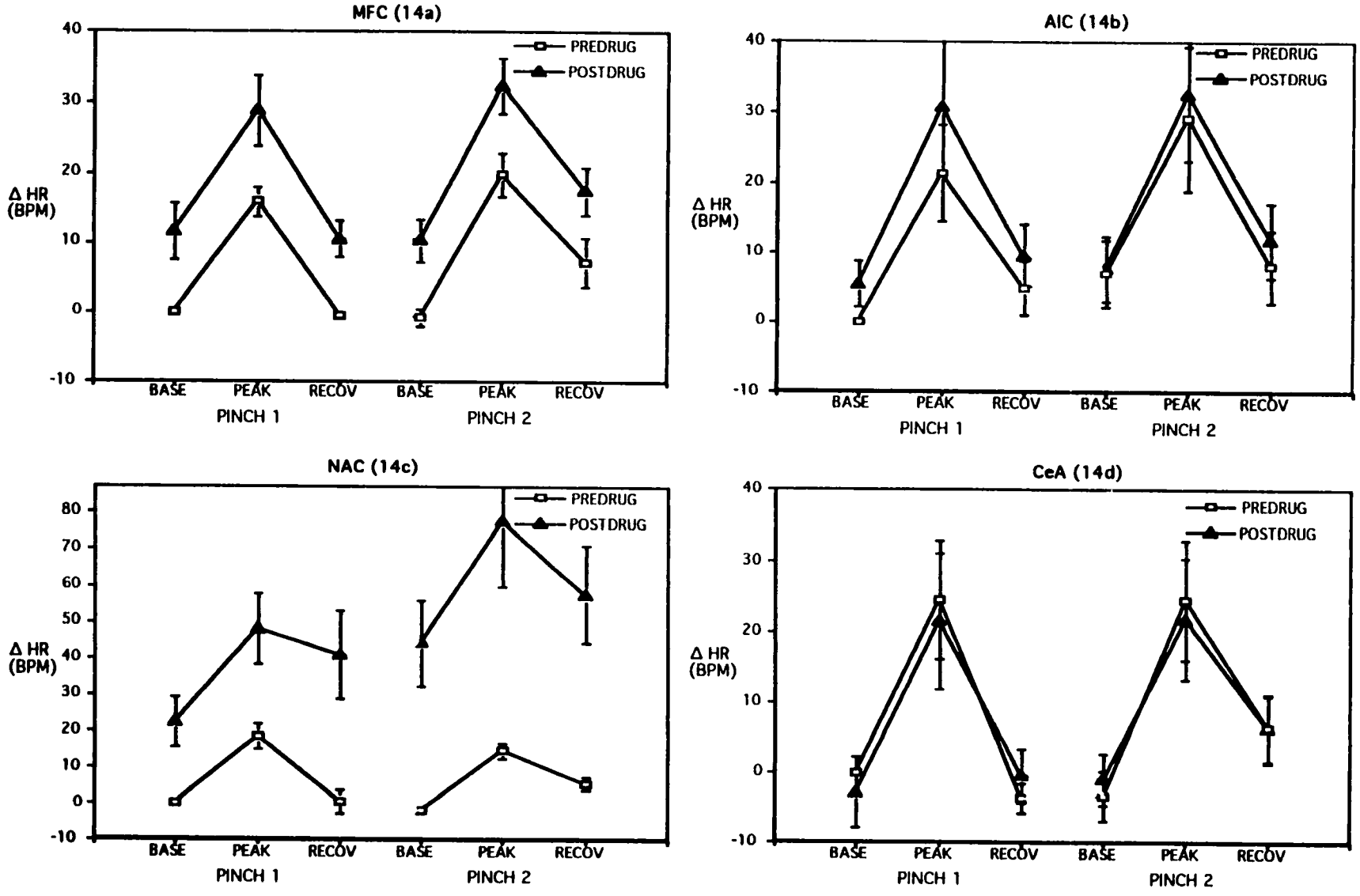
The effects of injections of a combination of 20 nmol of the α-adrenoceptor agonist phenylephrine and 10 nmol of the β-adrenoceptor agonist isoproterenol into the MFC (n=6), AIC (n=5), NAC (n=6) or CeA (n=6) on baseline HR and the response to pinch are shown in Figure 14. When injected into the MFC or NAC, a combination of phenylephrine and isoproterenol increased baseline HR. This drug combination also tended to increase baseline HR when injected into the AIC. These injections significantly increased the magnitude of the response to tail pinch when injected into the NAC.

Medial Frontal Cortex

Figure 14a shows that the injection of the combination of isoproterenol and phenylephrine into the MFC significantly increased baseline HR. This

Figure 14 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of a combination of 10 nmol of the β-adrenoceptor agonist isoproterenol and 20 nmol of the α-adrenoceptor agonist phenylephrine into the MFC (14a), AIC (14b), NAC (14c) or CeA (14d).

Isoproterenol (10 nmol) + Phenylephrine (20 nmol)



was reflected in the significant main effect of drug [$F(1,5)=22.21$, $p < .01$], and in the significant simple effect of drug at the baseline prior to the first pinch [$F(1,30)=12.99$, $p < .005$]. Such injections did not affect the response to pinch, as shown by the result of the test of the drug x sample interaction [$F(1,10)=.52$, $p > .61$].

Agranular Insular Cortex

Injections of the combination of isoproterenol and phenylephrine into the AIC did not significantly affect baseline HR, although there was a slight tendency towards increased HR (Figure 14b). Neither the main effect of drug [$F(1,4)=1.88$, $p > .24$], nor the test of the simple effect of drug at the baseline prior to the first pinch were significant [$F(1,24)=1.88$, $p > .20$]. These injections did not affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [$F(1,8)=2.80$, $p > .11$].

Nucleus Accumbens

Figure 14c shows that injections of a combination of isoproterenol and phenylephrine into the NAC increased in baseline HR. This is reflected in the significant main effect of drug [$F(1,5)=18.64$, $p < .01$]. The result of the test of the simple effect of drug at the baseline prior to the first pinch indicated a strong trend towards an increase [$F(1,30)=4.32$, $p=.07$]. Injections of this drug combination into the NAC also affected the response to tail pinch, as shown by the test result of the drug x sample interaction [$F(2,10)=4.41$, $p < .05$]. Subsequent analysis of the simple effects of this interaction revealed that it was significant at both the first [$F(2,20)=4.95$, $p < .05$] and second [$F(2,20)=4.32$, $p < .05$] pinches. The effects of these injections on the response to pinch were characterized by an increase in magnitude of the peak response and in a tendency towards a slower recovery of HR.

Central Nucleus of the Amygdala

Injections of the combination of isoproterenol and phenylephrine into the CeA did not affect baseline HR, as shown by results of tests of the main effect of drug [$F(1,5)=.05, p > .83$], and the simple effect of drug at the baseline prior to the first pinch [$F(1,20)=.85, p > .37$] (Figure 14d). Such injections did not significantly affect the response to tail pinch, although there was a slight tendency for the recovery of HR to be slower during the first pinch after drug injection. This is demonstrated by the test result of the drug x sample interaction [$F(2,10)=3.06, p > .09$].

Summary: The Effects of Injections of Noradrenergic Drugs

Injections of an agonist of the β-adrenoceptor, isoproterenol, increased baseline HR when made into the MFC, AIC and NAC, and tended to increase it when made into the CeA. Isoproterenol did not affect the magnitude of the response to pinch when injected into any of the four sites tested. When the β-adrenoceptor antagonist propranolol was injected into the MFC, NAC and CeA, baseline HR was reduced, an effect that was most prominent at sample points later in the postdrug phase. Injections of propranolol, an antagonist of β-adrenoceptors significantly reduced the magnitude of the response to pinch when made into each of the four sites. The coinjection of propranolol with isoproterenol reduced or reversed the increases in baseline HR induced by the latter drug. The baseline-increasing effects of isoproterenol were still noted at sample points immediately after injection of this drug combination, but were reduced by propranolol at sample points following the first pinch after injection.

The α-adrenoceptor agonist phenylephrine resulted in slight decreases and increases in baseline HR when injected into, respectively, the MFC and

NAC. Phenylephrine did not affect the magnitude of the response to pinch when injected into any of the four sites. Injections of an agonist of the α-adrenoceptor, phentolamine affected neither baseline HR nor the magnitude of the response to pinch when made into any of the four sites tested.

Injections of a combination of a β-adrenoceptor agonist, isoproterenol, and an α-adrenoceptor agonist, phenylephrine, into the NAC was found to markedly increase the magnitude of the response to pinch, a effect not seen when either agonist alone was injected.

Effects of Injections of Combinations of Dopaminergic and Noradrenergic Drugs

Effects of Injection of a Combination of a β-Adrenoceptor Agonist and a D_2 Receptor Antagonist

Highlights

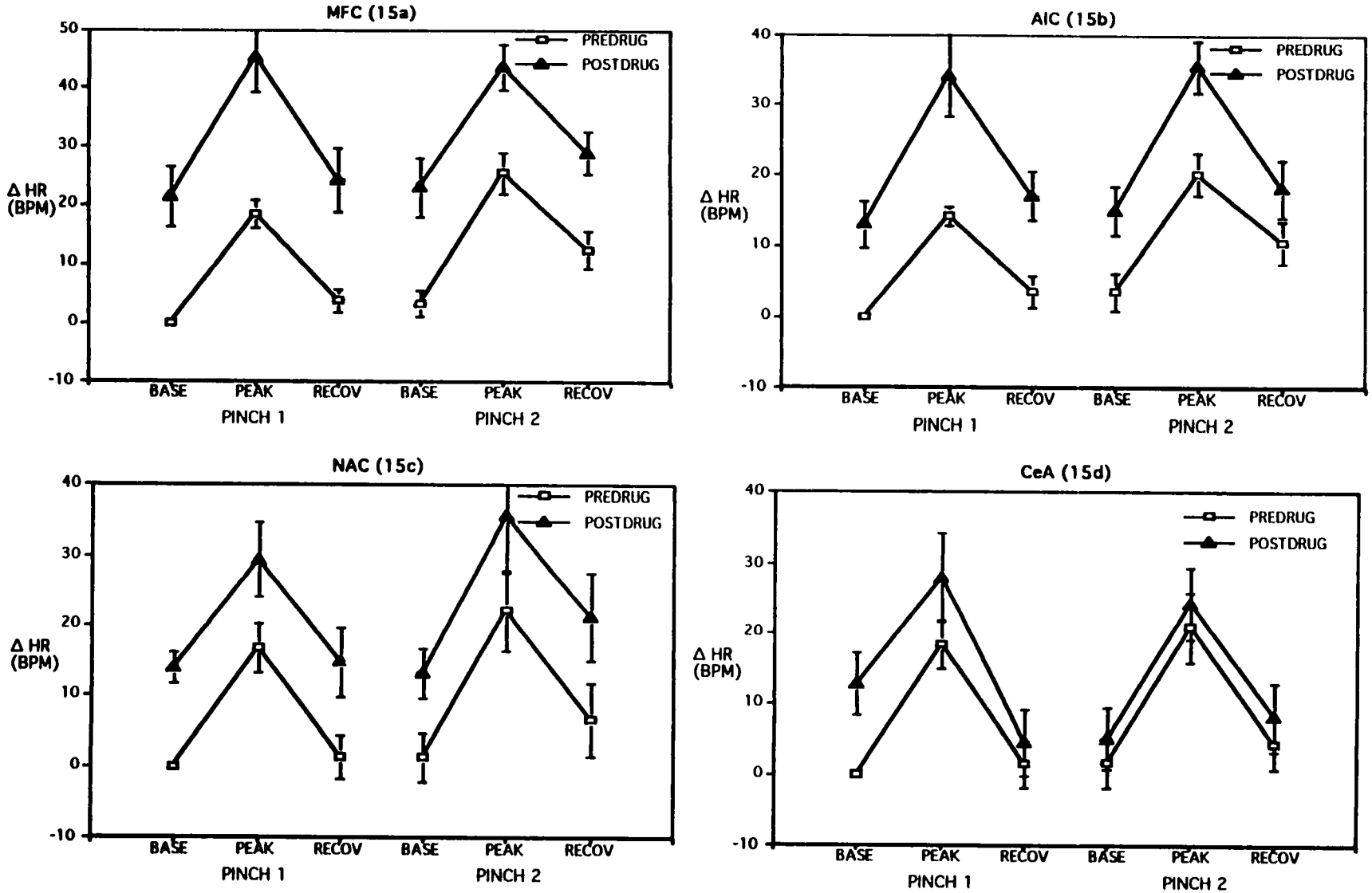
The effects of injections of a combination of 10 nmol of the β-adrenoceptor agonist isoproterenol and 20 nmol of the D_2 receptor antagonist raclopride into the MFC (n=8), AIC (n=6), NAC (n=6) or CeA (n=7) on baseline HR and the response to pinch are shown in Figure 15. The combination of isoproterenol and raclopride increased baseline HR when injected into any of the four sites. When injected into the AIC, this combination of drugs increased the magnitude of the response to tail pinch.

Medial Frontal Cortex

The injection of a combination of raclopride and isoproterenol into the MFC increased baseline HR (Figure 15a). This was reflected in the significant main effect of drug [$F(1,7)=34.80$, $p < .001$] and in the significant simple effect

Figure 15 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of a combination of 10 nmol of the β-adrenoceptor agonist isoproterenol and 20 nmol of the D_2 receptor antagonist raclopride into the MFC (15a), AIC (15b), NAC (15c) or CeA (15d).

Isoproterenol (10 nmol) + Raclopride (20 nmol)



of drug at the baseline prior to the first pinch [$F(1,42)=21.18$, $p < .0005$]. Injections of this combination of drugs did not effect the response to tail pinch, as shown by the results of the test of the drug x sample interaction [$F(2,14)=.98$, $p > .39$].

Agranular Insular Cortex

Figure 15b shows that injections of the combination of isoproterenol and raclopride into the AIC increased baseline HR. This fact is reflected in the significant main effect of drug [$F(1,5)=1.22$, $p < .05$] and in the significant simple effect of drug at the baseline prior to the first pinch [$F(1,30)=8.28$, $p < .05$]. These injections increased the magnitude of the response to tail pinch, as shown by the results of the test of the drug x sample interaction [$F(2,10)=7.43$, $p < .05$]. Analysis of the simple effects of this interaction showed that it was significant in both the first pinch [$F(2,20)=4.37$, $p < .05$] and the second pinch [$F(2,20)=4.21$, $p < .05$]. The change in the response to pinch was characterized primarily by an increase in the magnitude of the peak response.

Nucleus Accumbens

The injection of the combination of isoproterenol and raclopride into the NAC increased baseline HR significantly (Figure 15c). This is demonstrated by the significant main effect of drug [$F(1,5)=14.23$, $p < .05$], and by the significant simple effect of drug at the baseline prior to the first pinch [$F(1,30)=1.03$, $p < .01$]. Injections of this drug combination did not affect the response to tail pinch, as shown by the result of the test of the drug x sample interaction [$F(2,10)=.12$, $p > .88$].

Central Nucleus of the Amygdala

Figure 15d shows that injections of isoproterenol with raclopride into the CeA caused increases in baseline HR, as shown by the test of the simple effect of drug at the baseline prior to the first pinch [$F(1,36)=5.87$, $p < .05$]. The

main effect of drug was not significant [$F(1,6)=1.75$, $p > .23$], however, reflecting the fact that following the first pinch after drug injection, levels of HR were comparable to those seen in the predrug phase. Such injections did not alter the response to tail pinch as the drug x sample interaction was not statistically significant [$F(2,12)=1.54$, $p > .25$]. Despite this fact, analyses of the simple effects of this interaction were carried out at each pinch, which showed that it was significant at the first pinch [$F(2,24)=3.66$, $p < .05$]. This effect appeared to be caused primarily by the return of HR to near baseline levels following the first pinch after the drug injection, rather than by a change in the magnitude of the response to pinch.

Effects of Injections of a Combination of a D_2 Receptor Agonist and a β-Adrenoceptor Antagonist

Highlights

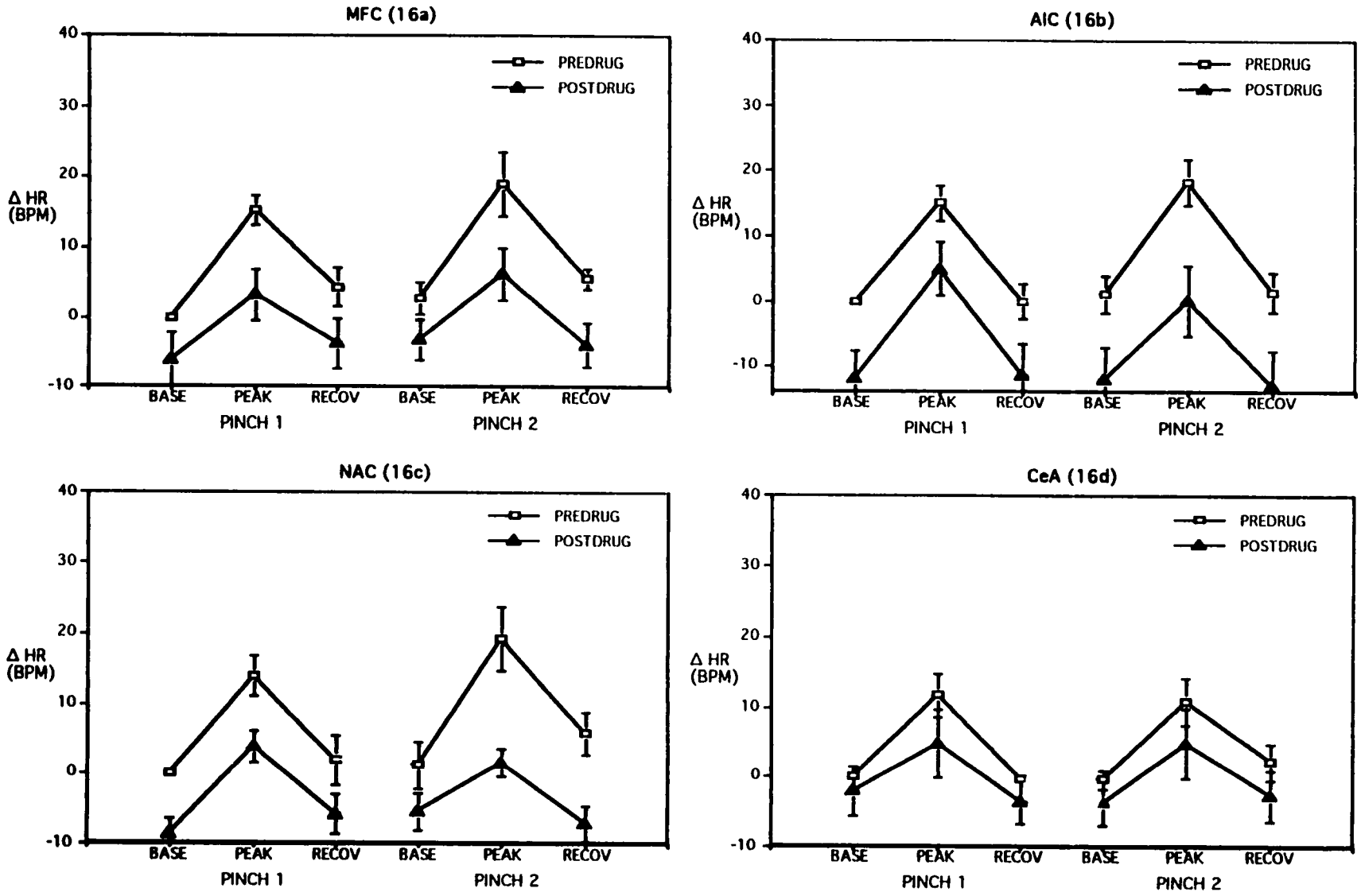
The effects of injections of a combination of 20 nmol of the D_2 receptor agonist quinpirole and 40 nmol of the β-adrenoceptor antagonist propranolol into the MFC (n=8), AIC (n=7), NAC (n=6) or CeA (n=8) on baseline HR and the response to pinch are shown in Figure 16. The combination of quinpirole and propranolol decreased baseline HR when it was injected into the MFC, AIC and NAC. When injected into the CeA, this drug combination tended to reduce baseline HR. These injections significantly reduced the magnitude of the response to pinch when injected into any of the four sites.

Medial Frontal Cortex

The injection of a combination of quinpirole and propranolol into the MFC significantly reduced baseline HR, as revealed by the test of the main effect of drug [$F(1,7)=7.80$, $p < .05$] (Figure 16a). Although there was a trend

Figure 16 Mean ΔHR (± 1 S.E.M.) shown in response to two 10 s tail pinches separated by 5 min, administered prior to (PREDRUG), and following (POSTDRUG) injections of a combination of 20 nmol of the D_2 receptor agonist quinpirole and 40 nmol of the β-adrenoceptor antagonist propranolol into the MFC (16a), AIC (16b), NAC (16c) or CeA (16d).

Quinpirole (20 nmol) + Propranolol (40 nmol)



towards reduced HR at the baseline prior to the first pinch, this difference was found not to be statistically significant in a test of the simple effect of drug at this point [$F(1,42)=2.70$, $p=.13$]. Injection of this drug combination into the MFC significantly affected the response to tail pinch, as reflected in the significant drug x sample interaction [$F(2,14)=5.72$, $p < .05$]. Subsequent analysis of the simple effect of the drug x sample interaction at each pinch revealed a tendency towards alteration of the response to both pinches, although neither of these were statistically significant (First pinch: [$F(2,28)=1.98$, $p=.16$]; Second pinch: [$F(2,28)=2.63$, $p=.09$]). The drug-induced changes in the response to pinch were characterized by reductions in the magnitude of the increase in HR caused by pinch, and a more rapid recovery of HR following each pinch.

Agranular Insular Cortex

Figure 16b shows that injections of quinpirole and propranolol into the AIC significantly decreased baseline HR. This is reflected in the significant main effect of drug [$F(1,6)=16.37$, $p < .01$] and in the significant simple effect of drug at the baseline prior to the first pinch [$F(1,36)=11.77$, $p < .01$]. Although analysis of the drug x sample interaction revealed that it was not significant [$F(2,12)=.71$, $p > .50$], subsequent analyses of the simple effect of the interaction revealed that it was significant at the second pinch [$F(2,24)=3.89$, $p < .05$] but not at the first [$F(2,24)=.65$, $p > .53$]. During the second pinch, the magnitude of the peak increase in HR was reduced compared to that seen prior to the injection of this combination of drugs.

Nucleus Accumbens

Injections of the combination of quinpirole and propranolol into the NAC decreased baseline HR, as shown by the results of tests of the main effect of drug [$F(1,5)=7.21$, $p < .05$] and of the simple effect of drug at the baseline

prior to the first pinch, although this latter effect was not statistically significant [$F(1,30)=3.45$, $p=.09$] (Figure 16c). Although the drug x sample interaction was not statistically significant [$F(2,10)=3.94$, $p=.05$], analysis of the simple effects of this interaction showed that it was significant in the second pinch [$F(2,20)=94.08$, $p < .05$], but not in the first [$F(2,20)=.26$, $p > .77$]. During the second pinch, these injections induced a large reduction in the magnitude of the peak increase in HR and enhanced the recovery of HR after the pinch.

Central Nucleus of the Amygdala

Injections of the combination of quinpirole and propranolol into the CeA did not significantly affect baseline HR, as shown by the results of the test of the main effect of drug [$F(1,7)=3.22$, $p > .11$] and of the test of the simple effect of drug at the baseline prior to the first pinch (Figure 16d) [$F(1,42)=.60$, $p > .45$]. There was, however, a slight tendency towards reduced HR after these injections. Injections of this drug combination into the CeA altered the response to tail pinch. Although the result of the test of the drug x sample interaction only approached statistical significance [$F(2,14)=3.61$, $p=.05$], further analysis of the simple effects of the interaction at each pinch showed it to be significant during the first pinch [$F(2,28)=4.16$, $p < .05$], but not the second [$F(2,28)=1.30$, $p > .28$]. During the first pinch, the primary effect of these injections was to reduce the magnitude of the peak increase in HR.

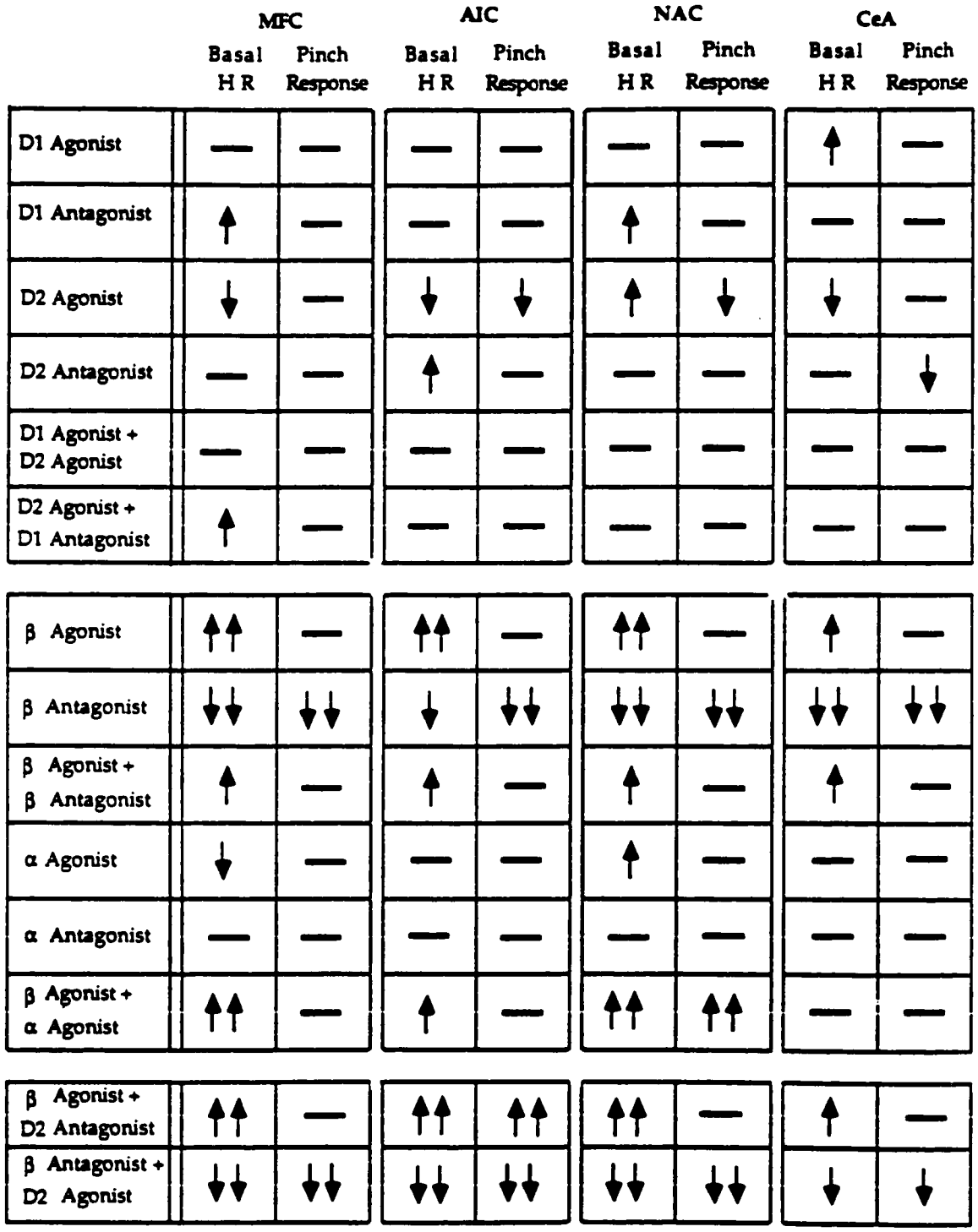
Summary: Effects of Injection of Combinations of Dopaminergic and Noradrenergic Drugs

A combination of the D_2 antagonist, raclopride with the β-adrenoceptor agonist, isoproterenol significantly increase the magnitude of the response to pinch when injected into the AIC. This potentiation was not seen when either drug was injected alone.

The effects of injections of the combination of the D_2 agonist quinpirole with the antagonist of the β-adrenoceptor propranolol were similar to those seen when propranolol was injected alone.

Figure 17 summarizes, in tabular form, the effects of injections of each drug or drug combination when made into the MFC, AIC, NAC or CeA.

Figure 17 Summary chart of the effects of injections of drugs acting at DAergic and NAergic receptors into the MFC, AIC, NAC and CeA on basal HR and on the magnitude of the response to tail pinch. Single arrows denote slight increases or decreases in basal HR or the magnitude of the response to pinch that were not statistically significant. Double arrows indicate statistically significant increases or decreases in these parameters. Dashes indicate that the drug injections had no effect.


MFC
AIC
NAC
CeA
Basal HR
Pinch Response
D1 Agonist
D1 Antagonist
D2 Agonist
D2 Antagonist
D1 Agonist + D2 Agonist
D2 Agonist + D1 Antagonist
β Agonist
β Antagonist
β Agonist + β Antagonist
α Agonist
α Antagonist
β Agonist + α Agonist
β Agonist + D2 Antagonist
β Antagonist + D2 Agonist

DISCUSSION

This investigation was carried out to assess the possible modulatory influences of the catecholaminergic projections to the forebrain on the expression of responses to noxious or stressful stimuli mediated by the ANS. Although the DA and NAergic projections are known to be activated by stressors, the notion that they modulate the autonomic responses elicited by such stimuli has not been studied.

Neuroanatomical work has demonstrated that the terminal fields of the catecholaminergic systems such as the MFC, AIC, NAC and CeA project to hypothalamic and brainstem nuclei involved in the control of autonomic output. The results of functional studies suggest that, depending on the organ system studied, the catecholaminergic projections can play facilitatory or inhibitory roles in the modulation of autonomic output during basal conditions or in response to stress. For example, injections of either DA or NA into the CeA block the development of stress-induced ulcers of the stomach, an autonomically-controlled organ. Aside from the few studies of this type, the notion that increased activity in the catecholaminergic projections could influence the basal output of the ANS or the magnitude of its responses to stress has not been evaluated in a systematic manner.

To help resolve this issue, the present investigation examined the effects of injections of agonists or antagonists of DA and NAergic receptors into the MFC, AIC, NAC or CeA on a response to stress mediated by the sympathetic division of the ANS, the increase in HR induced by tail pinch, in the anesthetized rat.

The results of this investigation provide the first clear evidence that the NAergic projections to the forebrain are important modulators of the

basal and stress-induced output of the ANS. The DAergic projections to the forebrain, in contrast, appear to play a less salient role as modulators of these autonomic parameters, although evidence was obtained indicating that DA may, in turn, modulate the actions of NA.

It was found that unilateral injections of an antagonist of the β-adrenoceptor into the MFC, AIC, NAC or CeA reduced basal HR and the magnitude of the increase in HR induced by tail pinch. The converse treatment, injections of an agonist of the β-adrenoceptor, increased basal HR when made into the MFC, AIC and NAC, but did not effect the magnitude of the response to pinch. These results show for the first time that the release of NA in individual nuclei of the forebrain has an important facilitatory action in the modulation of the output of the sympathetic nervous system in the basal condition and when it is activated by stress. These actions of NA appear to be mediated via stimulation of β-adrenoceptors in these regions. The fact that such large effects were noted with unilateral injections of β-adrenergic drugs serves to further underscore the importance of the release of NA in the forebrain in the modulation of autonomic responses to stress.

The role of α-adrenoceptors in these regions in the modulation of autonomic output was also assessed in this thesis. Although it was found that injections of drugs acting at the α adrenoceptor were without significant effect on basal HR or the response to pinch, when an agonist of the α adrenoceptor was coinjected with an agonist of the β-adrenoceptor into the NAC, the magnitude of the HR response to tail pinch was markedly potentiated, an effect not seen when either drug was injected alone. These results suggest that in the NAC, the stimulation of α-adrenoceptors by NA released during stress serves to potentiate the activation of sympathetic outflow mediated by the stimulation of β-adrenoceptors in this region.

In contrast to the NAergic projections, the DAergic projections to these nuclei appear to be less important in the control of sympathetic output. Injections of agonists or antagonists of DAergic receptors into these forebrain regions were observed to exert only small effects on basal HR and the response to pinch. Overall, the stimulation of D_2 receptors in these nuclei appear to mediate reductions in sympathetic outflow.

Although these results suggest that the DAergic projections to the forebrain play a minor role in the control of sympathetic output, evidence was obtained suggesting that DA is an important modulator of the effects of stimulation of β-adrenoceptors. When an agonist of the β-adrenoceptor was coinjected with an antagonist of the D_2 receptor into the AIC, the magnitude of the response to tail pinch was significantly increased. This potentiated response was not observed when either drug was injected alone. These results suggest that stimulation of D_2 receptors modulates, in an inhibitory manner, neurotransmission mediated by the β-adrenoceptor in the AIC in the control of sympathetic output.

The Role of Catecholaminergic Projections to the Forebrain in the Modulation of Autonomic Responses to Stress

The present results suggest that the activation of β-adrenoceptors on neurons in the MFC, AIC, NAC and CeA by NA modulates the activity of projections to brainstem centers involved in the control of sympathetic output. This facilitatory influence of NA appears to operate during basal conditions and when sympathetic output is activated by stress. The DAergic projections that also innervate these forebrain nuclei seem to play a less important role in this regard, although evidence was obtained suggesting that D_2 receptors mediate an inhibition of the effects of β-adrenergic stimulation

in one of the sites, the AIC. The implications that these findings have on the understanding of the role of the forebrain in autonomic responses to stress and how they are modulated in turn by catecholamines will be discussed.

The Dopamine Systems

The first part of this investigation examined whether the DAergic projections to the forebrain modulate the output of the sympathetic nervous system during basal conditions and in response to stress. The results obtained from experiments where DAergic drugs were injected alone suggest that DA does not play a significant role in either of these phenomena. Injections of a D_2 agonist did result in small reductions in basal HR when made into the MFC, AIC and CeA, but did not alter the magnitude of the response to pinch. Injections of agonists or antagonists of the D_1 receptor did not reliably affect basal HR or the response to pinch. Neither did injections of a combination of agonists of the D_1 and D_2 receptors exert any strong effects on basal HR or the response to pinch. Taken together, these results suggest that DA, via the stimulation of D_2 receptors in these three regions may exert a mild inhibitory effect on sympathetic output during basal conditions.

Although these results may lead to the conclusion that DA released in the forebrain plays a minimal role in the modulation of autonomic output, evidence was also obtained suggesting that DA may modulate neurotransmission mediated by the β-adrenergic receptor. Injections of a combination of an antagonist of the D_2 receptor and a β-adrenoceptor were observed to potentiate the magnitude of the response to pinch, an effect not seen when either drug was injected alone. These results suggest that at least in the AIC, DA may act via D_2 receptors to modulate in an inhibitory manner, the actions of NA on β-adrenoceptors. This interaction will be discussed in

greater detail in a later section.

Few studies have formally assessed the possibility that the activation of the DA systems modulates autonomic responses to stress. The studies which addressed this question in the most detail have dealt with the role of the DAergic projections to the CeA, and to a lesser extent, the NAC in the modulation of gastric ulceration induced by stress. As described earlier, the autonomic circuitry underlying ulceration likely differs from that involved in cardiovascular responses, making the results of these studies difficult to compare with those of the present thesis. No studies have examined the influence of forebrain DA in responses to stress mediated by the sympathetic nervous system.

The systemic or intracerebroventricular administration of DAergic agonists reduces the incidence of ulcers of the stomach, an autonomically-controlled organ, induced by exposure to a strong stressor. The CeA has been shown to be an important site of action of DA in these inhibitory effects. Injections of DA or agonists of the D_1 receptor directly into this nucleus also reduce the ulcer response. Conversely, injections of blockers of DA receptors into the CeA or 6-OHDA lesions of the DAergic innervation of the CeA exacerbate the development of these ulcers. These results suggest that neurotransmission in the DAergic projections, most notably those to the CeA, are inhibitory on the output of the ANS (Glavin et al., 1991).

Overall, the role for DA in the CeA in the control of the ANS revealed by the ulcer studies is similar to that shown by the present investigation. The results of the ulcer studies suggest that DA in the CeA exerts a strong inhibitory action on autonomic output, and the primary mediator of this effect is the D_1 receptor. The results of the present work suggest that while the DAergic projections to the CeA do inhibit autonomic output, their effect is

relatively weak, and is mediated primarily by the D_2 receptor. These apparent discrepancies may be explained by the fact that gastric ulceration is mediated parasympathetically, while the response examined in the present study, the increase in HR induced by tail pinch, is primarily mediated by the sympathetic nervous system. Taken further, it may be suggested that the stimulation of DA receptors of the D_1 subtype, in at least in the CeA, has a greater influence on the output of the parasympathetic than the sympathetic nervous system. The stimulation of D_2 receptors in the CeA, in contrast, may be more involved in the inhibitory modulation of the sympathetic projections controlling cardiovascular activity.

The DAergic projections to the NAC have been found to play a more subtle role in protecting against the gastric ulceration induced by stress. Destruction of these projections, or the coinjection of an antagonist of DA receptors was found to block the protective effects of injections of neurotensin into the NAC. Neither of these DAergic manipulations significantly influenced the ulcer response when administered in the absence of the neurotensin injections (Xing et al., 1991). As in the case of the studies on the role of DA in the CeA in gastric ulceration, they are difficult to compare with the present findings. On one level, however, the results of these studies on the NAC and gastric ulceration are consistent with those of the present investigation in suggesting that an important function of DA may be to modulate the actions of other transmitters.

The Noradrenaline Systems

In the second set of experiments in this thesis, the role of the NAergic projections to the forebrain in the modulation of sympathetic output was examined. It was found that injections of an antagonist of the β-adrenoceptor

into the MFC, AIC, NAC or CeA reduced both baseline HR and the magnitude of the increase in HR seen in response to tail pinch. Injections of an agonist of the β-adrenoceptor increased baseline HR when injected into the MFC, AIC or NAC, but did not affect the magnitude of the pinch response. These results provide the first direct evidence that NA released in these forebrain nuclei is an important modulator of the basal and stress-induced activity of the sympathetic nervous system.

As in the case of the DA systems, few studies have assessed the functions of the NAergic projections to the forebrain in the basal output of the ANS. The studies which did examine this looked at the role of the NAergic projections to the AIC and CeA in, respectively, basal metabolic and cardiovascular activity.

McGregor et al. (1991) found that injections of NA into the AIC of awake rats resulted in changes in metabolism and thermogenesis that were suggestive of decreased sympathetic output. In contrast, in the present thesis, injections of an agonist of the β-adrenoceptor increased HR, which is suggestive of increased sympathetic output. The measures chosen in the McGregor et al. (1991) study are, however much less direct measures of sympathetic output than HR. It is possible that the effects noted in that study were mediated by one of the many other pathways and systems known to influence metabolism.

Two studies showed that injections of NA into the CeA potently increase blood pressure (Leonzio et al., 1987; Ohta et al., 1991). Interestingly, these injections either did not affect or decreased HR. Overall, the results of these studies suggest that NA, at least in the CeA acts to increase sympathetic, which is reflected in at least increases in blood pressure. These results are consistent, at least in part, with the present finding that injections of an

agonist of the β-adrenoceptor failed to significantly affect HR when made into the CeA. In the present investigation, injections of the corresponding antagonist into this nucleus did, however, reduce HR, which is suggestive of a reduction in sympathetic output.

The results of these studies suggest that the NAergic projections to at least the AIC and CeA may serve to increase or reduce the basal output of the sympathetic nervous system. depending on the system studied. The finding most relevant to the present thesis is that the NAergic projections to the CeA are facilitatory on sympathetic output, as indexed by blood pressure (Leonzio et al., 1987; Ohta et al., 1991). The present results in part support these earlier findings, and also extend them in suggesting that the NA may exert an activational influence on the basal output of the sympathetic nervous system in other regions of the forebrain and that this is mediated via the stimulation of β-adrenoceptors.

The role of NAergic projections to the forebrain as possible modulators of responses to stress mediated by the ANS has received little study. The only work of this kind studied NAergic mechanisms in the CeA in the development of gastric ulcers. Ray, Henke & Sullivan (1990) found that injections of NA or an agonist of the β-adrenoceptor into the CeA greatly reduced the incidence of gastric ulcers induced by exposure to a strong stressor. These effects appear to be inconsistent with the facilitatory role for NA in this and other forebrain regions revealed by the present work. The fact that these two stress responses are mediated by different limbs of the ANS likely contributes to this inconsistency. Briefly, gastric ulceration is known to be mediated via changes in the output of the parasympathetic nervous system, while the HR response examined in the present thesis is most probably sympathetically mediated.

With this in mind, one possibility explanation of this discrepancy is that the injection of NA or agonists of the β-adrenoceptor into the CeA have differential effects on parasympathetic and sympathetic output. Another hypothesis is that, since the sympathetic and parasympathetic innervation are known to exert reciprocal effects on gastric activity, that the protective effects of these injections from ulceration may, in fact be mediated by an increase in sympathetic output.

Aside from these ulcer studies, the present investigation is the first to address the function of the NAergic projections to the forebrain in an autonomically-mediated response to stress. The results of this thesis provide the first evidence that the NAergic projections to the forebrain are important modulators of responses to stress mediated by the sympathetic nervous system. This NAergic influence is facilitatory in tone, and is mediated by the β-adrenoceptor.

The fact that these modulatory functions of NA were demonstrated in the MFC, AIC and CeA is consistent with the results of anatomical and functional studies implicating these nuclei in the control of autonomic output (Loewy, 1991). A similar activational role for the NAergic projections to the NAC was also revealed by the present investigation, which was somewhat unexpected, since there is little anatomical or functional evidence for its involvement in the control of autonomically-mediated processes. These results provide, therefore, the first direct evidence that projections from the NAC influence autonomic output. As with the other regions tested, the present work demonstrates that NA is an important modulator of this influence of the NAC on the output of the ANS.

The injection of agonists or antagonists of the α-adrenoceptor into any of the four sites tested did not significantly affect baseline HR or the

magnitude of the response to tail pinch. This suggests that α-adrenoceptors in these regions are not the primary mediators of the NAergic influence on sympathetic output. Evidence was obtained, however, pointing to a facilitatory role for α-adrenoceptors on neurotransmission mediated by β-adrenoceptors. When injected into the NAC, the combination of an α and a β-adrenoceptor agonist increased the magnitude of the HR response to pinch, an effect not seen when either drug was injected alone. This suggests that in the NAC, the stimulation of α-adrenoceptors facilitates the effects of stimulation of β-adrenoceptors. This finding is in agreement with those of experiments carried out on other regions of the brain, showing that α-adrenoceptors are positively coupled to the cellular mechanisms activated by stimulation of the β-adrenoceptor (Radisavljevik et al., 1994). The reasons why these potentiating effects of α-adrenoceptors on β-mediated responses were not seen in any of the other brain regions tested are not clear.

Interactions of DA with NA in the Forebrain in the Modulation of Autonomic Responses to Stress

The injection of agonists or antagonists of DAergic receptors into the forebrain sites tested in the present investigation were found to have minimal effects on the basal and stress-induced activity of the sympathetic nervous system. Although not fully explored in the present investigation, evidence was obtained suggesting the presence of an interaction between DAergic and NAergic receptors in the modulation of responses to tail pinch.

When injected into the AIC, the combination of a β agonist and a D_2 antagonist potentiated the response to tail pinch, an effect not seen when either drug was injected individually. This pattern of results agree with the known effects of the stimulation of these receptors on second messenger

systems. Stimulation of β-adrenoceptors activates adenylate cyclase, the enzyme responsible for the production of cAMP, while stimulation of D_2 receptors is known to inhibit it (Lohse, Strassler & Helmreich, 1993; Strange, 1993). It is possible, therefore, that the blockade of D_2 receptors while stimulating the β-adrenoceptors in the AIC resulted in a net potentiation in the activity of adenylate cyclase and production of cAMP. From this, it may be suggested that DA, through the stimulation of D_2 receptors may serve to dampen the effects of NA in at least the AIC. This finding is partially supported by the results of a study carried out in conjunction with the present one, that intra-AIC injections of amphetamine, a drug that stimulates the release of both DA and NA influenced neither basal HR nor the response to pinch (data not shown).

Interestingly, this increase in the magnitude of the response to tail pinch was not accompanied by a similar potentiation of the baseline-increasing effects of isoproterenol. This result suggests that the interaction of the postsynaptic effects of DA and NA receptor stimulation may be especially important under conditions of stress, when the occupancy of the receptors of both transmitters would be expected to be high, and when the firing of neurons in the frontal cortex is increased.

The effects of injections of this combination of drugs into the AIC would suggest that a combination of a D_2 agonist with an antagonist of the β-adrenoceptor would result in a very large reduction in basal HR and the response to pinch, when compared to the effects of injections of the β-adrenergic antagonist alone. Although the presence or absence of this effect was impossible to verify statistically due to the nature of the experimental design, it did not appear to occur. The reasons for this are not clear. One possible explanation is that further reductions in basal HR and the response

to pinch induced by the D_2 agonist would be obscured by the already large decreases in these parameters caused by the antagonist of the β-adrenoceptor. Additional support for this notion comes from the fact that the dose of the antagonist of the β-adrenoceptor used was twice that of the D_2 agonist.

Evidence for an interaction of DAergic with NAergic receptors in the production of their synaptic effects is scanty, with the most compelling evidence coming from studies on lesioned preparations. For example, it has been shown that the development of supersensitivity of DAergic receptors following a 6-OHDA lesion of the DAergic projections to the MFC is dependent on the presence of intact NAergic innervation. Interestingly, the converse also appears to be true. These results, although not providing direct evidence for such a receptor interaction in the intact animal, does suggest that at least in some circumstances, the effects of manipulations of one receptor type are dependent on the other (Herve, Trovero, Blanc, Vezina, Glowinski & Tassin, 1990; Nowak, Zak & Superata, 1991).

Another interaction that is known to occur between the NA and DA systems may also help to explain the present results. Rossetti, Pani, Portas & Gessa (1989) presented evidence that DA, via the D_2 receptor, acts to inhibits the release of NA from the NAergic terminal in the cortex. In the present investigation, it was observed that injections of an agonist of the D_2 receptor tended to reduce HR. If the D_2 agonist had inhibited the release of NA, less NA would be available to stimulate β-adrenoceptors, resulting in the mild reductions in HR that were observed.

This hypothesized function of the D_2 receptor may also explain the potentiation of the HR response to tail pinch when the combination of the β agonist and a D_2 antagonist was injected into the AIC. In this case, blockade of the inhibitory effects of the D_2 receptors on the release of NA would lead to

relatively larger amounts of NA being available to stimulate adrenoceptors, which may have served to potentiate the actions of the agonist of the β-adrenoceptor.

In summary, mechanisms through which the intra-AIC injection of the agonist of the β-adrenoceptor and the antagonist of the D_2 receptor potentiated the response to pinch are not clear. The present results are nevertheless important in that they provide the first direct evidence for an interaction between NA and DA receptors in at least one forebrain region in the modulation of physiological responses to stress.

Circuitry and Mechanisms Underlying the Modulatory Actions of Catecholamines on Autonomic Output

The present results show that the catecholaminergic projections to the forebrain modulate the output of the ANS during basal conditions and when it is activated by stress. The most important mediator of this influence is NA, which facilitates autonomic output via the stimulation of β-adrenoceptors. These results also provide evidence that an important function of DA in at least one of these forebrain regions is to modulate the actions of NA on β-adrenoceptors.

The neuronal mechanisms underlying the present effects were not assessed. It is, however, reasonable to assume that the injections of drugs acting at β-adrenoceptors altered cardiovascular output through actions on projections to autonomic centers in the brainstem.

Hypotheses regarding the mode of action of the β-adrenergic drugs can be proposed. Their development is complicated by the lack of detailed knowledge of the functional neuroanatomy of the NAergic projections at the level of the forebrain nuclei tested in the present study. Most importantly,

the identity of the neurons affected by the injections of β-adrenergic drugs are not known. These drugs may have stimulated receptors located on neurons furnishing the efferents from these nuclei, or on interneurons that, in turn, impinge on the projection neurons. Yet other work provides evidence that the effects of NA may have been mediated by the stimulation of β-adrenoceptors located on glia (Stone & Ariano, 1989).

A clear understanding of the effects of the stimulation of adrenoceptors on the activity of neurons in these regions is also lacking. The stimulation of β-adrenoceptors has been shown to potently inhibit neurons in many regions of the brain, while other studies have revealed it to be a facilitator of the actions of other neurotransmitters (Mouradian et al., 1991; Unemoto et al., 1985a).

It is therefore not clear whether the net effect of drugs acting at the β adrenoceptor is primarily excitatory or inhibitory on projections descending from these forebrain nuclei to autonomic centers. Despite these uncertainties regarding the influence of NA on forebrain neurons, a number of mechanisms underlying the present results can be proposed. Of the four brain regions tested, the MFC and AIC have been the most thoroughly studied regarding their role in the modulation of autonomic output. For this reason, the clearest picture of the neuronal mechanisms underlying the effects noted in the present thesis can be developed for these regions.

Electrophysiological studies have shown that neurons in at least the MFC region of the frontal cortex fire spontaneously during basal conditions (Thierry, Godbout, Mantz & Glowinski, 1990). These observations, taken with the finding that electrical or chemical stimulation of the MFC and AIC reduces HR and blood pressure, suggests that the projections from neurons in the frontal cortex may serve to tonically inhibit sympathetic output during

basal conditions.

In the present study, injections of an agonist of the β-adrenoceptor in the MFC or AIC increased, while injections of the antagonist decreased basal HR. Viewed with the finding that the stimulation of β-adrenoceptors inhibits the spontaneous firing of neurons in the MFC and other cortical regions, these results lead to one hypothesis about the role of NA in these sites in the modulation of cardiovascular output. It is possible that the injections of the agonist of the β-adrenoceptor increased basal HR by inhibiting the spontaneous firing of neurons in the MFC and AIC that function to tonically inhibit sympathetic output. The injections of antagonists of the β-adrenoceptor, on the other hand, may have decreased baseline HR by reducing the inhibition of these projection neurons by endogenous NA, resulting in a net increase in their basal activity, which would be reflected in an increased inhibitory tone on the basal output of the cardiovascular system.

A similar mechanism may account for the modulatory effects of the antagonist of the β-adrenoceptor on HR responses to pinch observed in the present investigation. This requires the hypothesis that the increases in the firing of frontocortical neurons in response to noxious stimulation is reflected in a net increase in their inhibitory influence on sympathetic output. If this is the case, it may be suggested that the injections of an antagonist of the β-adrenoceptor reduced the magnitude of the response to pinch by blocking the inhibitory action of NA on the pinch-induced activation of frontocortical neurons. This would lead to proportionally more firing of the cortical neurons in response to pinch and thus more activity in the inhibitory projections to sympathetic nuclei. This would result in a reduction in the magnitude of the HR response to the pinch.

The results of a number of electrophysiological studies on the role of NA in cortical neurotransmission supports the hypothesis that these inhibitory actions of NA in the frontal cortex underlie the present results. Stimulation of the locus coeruleus or the local application of either NA or agonists of the β-adrenoceptor have all been shown to inhibit the basal activity of neurons in many regions of the cerebral cortex (Thierry et al., 1990). Other studies have demonstrated that NA inhibits the evoked firing of cortical neurons. Stimulation of the locus coeruleus reduces the magnitude of the activational response shown by these neurons in the MFC to noxious stimulation, including tail pinch (Godbout et al., 1991; Thierry et al., 1990).

One problem with this hypothesized mechanism is that it is not consistent with the observed failure of intracortical injections of an agonist of the β-adrenoceptor to potentiate the response to pinch. This mechanism would have predicted that injections of the agonist would reduce the pinch-induced firing of the MFC and AIC neurons and thus reduce the activity of the inhibitory projections they send to sympathetic nuclei. This would have been reflected in increases in the magnitude of the HR response to pinch, which were not observed.

A number of factors may have contributed to this discrepancy. One possibility is that levels of NA were high in the cortex prior to injections of the agonist of the β-adrenoceptor. As a result, the agonist may have been ineffective in potentiating the response to pinch due to the high occupancy of the receptors. Tail pinch is known to elicit the release of NA in the frontal cortex, and the animals had received at least 8 pinches in the 90 min prior to the injections of the agonist, two of those in the last 15 min. This explanation is also consistent with the observed effectiveness of injections of the antagonist of the β-adrenoceptor in reducing the response to pinch. The

hypothesized occupation of β-adrenoceptors in the cortex by endogenous NA may have also led to their downregulation, which is also consistent with the failure of the agonist of the β-adrenoceptor to increase the magnitude of the response to pinch (Lohse et al., 1993).

The results from other studies on the role of NA in cortical neurotransmission are difficult to reconcile with the present results. In these studies, NA was revealed to facilitate the activation of cortical cells induced by the local application of excitatory compounds or by the stimulation of brain regions known to provide excitatory inputs to these cells. These effects of NA appear to be mediated by the stimulation of β-adrenoceptors, although at least one study found that the α-adrenoceptor is the most important mediator (Mouradian et al., 1991; Radisavljevik, 1994).

The findings from studies using the technique of c-Fos immunocytochemistry are similarly inconsistent with those of the present findings. C-Fos is an immediate early gene whose expression is widely used as an index of neuronal activity. Using this technique it was found that either lesions of the LC or the systemic administration of an antagonist of the β-adrenoceptor blocked the activation of MFC neurons by footshock (Stone et al., 1992, 1993). These results suggest that the stimulation of the β-adrenoceptor is involved in the activation of neurons in at least the MFC. It must be remembered, however, that the induction of c-Fos requires exposure of animals to stressors of relatively long duration, in the order of at least several minutes, whereas the electrophysiological effects of pinch are of a very short latency. β-adrenergic receptors may play a differential role in the modulation of firing and c-Fos production induced by stress.

This notion that the primary effects of the stimulation of β-adrenoceptors is to potentiate the evoked activity of cortical neurons is not

consistent with the present findings. If stimulation of the β-adrenoceptor facilitated the activation of neurons of the frontal cortex by pinch whose projections inhibit sympathetic outflow, the magnitude of the response to pinch would have been decreased. Conversely, the injections of the antagonist of the β-adrenoceptor would have been expected to reduce the activity of the cortical cells, leading to increased sympathetic output and increased basal HR and a potentiated response to pinch. The opposite pattern of results was observed. The present results suggest, therefore, that NA in the frontal cortex, through the stimulation of β-adrenoceptors, acts to inhibit, rather than facilitate, the basal and evoked firing of cortical neurons whose projections inhibit sympathetic output.

Hypotheses about the circuitry underlying the autonomic effects of β-adrenergic drugs in the NAC and CeA are more difficult to develop. This is due to the limited knowledge regarding the localization of adrenoceptors and their role in the activity of neurons in these regions. Two studies have demonstrated that the activation of β-adrenoceptors appears to inhibit the evoked activity of neurons in the NAC (Unemoto et al., 1985a, 1985b). The only study carried out on the CeA found that NA, via the β-adrenoceptor, serves to make neurons in this region more excitable (Rainnie et al., 1992). In none of these studies was the effect of NA on the basal activity of neurons in these regions assessed.

There is little good evidence that projections from the NAC influence HR. In a study carried out in conjunction with the present work, however, the injection of a high dose of glutamate (1000 nmol) into the NAC was found to increase basal HR, but did not affect the response to pinch (data not shown). This observation suggests that the projections from the NAC do have access to the circuitry involved in cardiovascular output and serve to

increase it. This leads to the conclusion that the net effects of the β-adrenergic agonist and antagonist were to, respectively excite or inhibit neurons of the NAC whose projections provide a tonic drive on autonomic output. This proposed mechanism conflicts with the results of studies showing that the stimulation of β-adrenoceptors on neurons of the NAC inhibits their evoked activity (Unemoto et al., 1985a, 1985b). This discrepancy may be due to the fact that the neurons tested in these electrophysiological studies may not have been projection neurons involved in autonomic output.

The projections from the CeA are known to be excitatory on HR and blood pressure (Iwata et al., 1987). In the present work, injections of an antagonist of the β-adrenoceptor were found to reduce basal HR and the magnitude of the response to pinch, while injections of the corresponding agonist did not significantly affect these parameters. The one study on the electrophysiological effects of NA in the CeA demonstrated that it is facilitatory on the activity of neurons (Rainnie et al., 1992). The results observed in the present experiment are therefore consistent with the idea that NA facilitates the activity of the projection neurons of the CeA providing an activational drive on autonomic output.

Function of the Catecholaminergic Projections to the Forebrain in Responses to Stress

The results of this thesis are the first to provide clear evidence that the catecholaminergic projections to discrete nuclei in the forebrain such as the MFC, AIC, NAC and CeA exert an important modulatory influence on responses to stress mediated by the ANS. The NAergic projections play the strongest role in this regard, and apparently facilitate the increases in autonomic output seen in response to noxious or stressful stimuli. Evidence

was also obtained indicating that NA in these regions has facilitatory influence on the basal output of the ANS. β-adrenoceptors were found to be the primary mediators of these facilitatory effects. In the NAC, however, these β-mediated effects on autonomic output were observed to be strongly potentiated by the stimulation of α-adrenoceptors.

These results show, in contrast, that the DAergic projections to the forebrain play a lesser role in the modulation of autonomic output. The stimulation of DA receptors of the D_2 subtype in these regions in general, exerts a slight inhibitory influence on the basal activity of the ANS. Additional evidence was obtained suggesting that the stimulation of D_2 receptors in at least the AIC acts to inhibit the effects of stimulation of β-adrenoceptors on autonomic responses to stress. This mechanism may also explain the baseline-reducing effects of the stimulation of D_2 receptors.

Interestingly, evidence for the operation of these receptor interactions in the modulation of autonomic output were not seen during basal conditions. Only when the ANS was activated by stress were they evident. Stressors are known to evoke large increases in the release of DA and NA and to activate the firing of neurons throughout the forebrain. Taken together with the present findings, these results suggest that the influence of these receptor interactions on autonomic output may be especially prevalent during conditions of intense stress when the receptor populations in question are stimulated maximally. In the case of the NAC, very high levels of NA may result in the potentiation of autonomic responses to stressors via the combined stimulation of β- and α-adrenoceptors. For the same reasons, the inhibitory influence of D_2 receptors on β-mediated events may be the most salient during stress.

The present findings agree with those of numerous studies in

suggesting that the catecholamines, DA and NA, have important modulatory actions on neuronal systems underlying the behavioral and physiological responses shown by animals to salient stimuli in their environment. The NAergic projections appear to be the most important in the expression of the responses evoked by stressful stimuli. For example, the NAergic projections have been shown to modulate many of the changes in attention and ongoing behavior elicited by exposure to noxious or stressful stimuli (Mohammed, Callenholm, Jarbe, Swedberg, Danysz, Robbins & Archer, 1986; Selden, Everitt & Robbins, 1991; Selden, Robbins & Everitt, 1990). An example of a physiological system in which NA has a strong modulatory role is the HPA axis. Destruction of the NAergic projections to the hypothalamus has been shown in many studies to greatly reduce the magnitude of the HPA response to stress (Maccari, Le Moal, Angellucci & Mormede, 1990; Szafarczyk, Alonso, Ixart, Malaval & Assenmacher, 1985).

The bulk of these studies used relatively non-selective techniques to manipulate the NAergic projections, making the functions of this transmitter in individual nuclei impossible to infer from their results. The present results overcame this limitation in using the technique of microinjection into discrete nuclei in the forebrain. This thesis therefore extends these previous findings in demonstrating that NA exerts an important modulatory function in the individual terminal fields of the catecholaminergic projections, the MFC, AIC, NAC and CeA, in at least those responses to stress mediated by the ANS.

The MFC, AIC, NAC and CeA have been shown to be involved in diverse cognitive and behavioral phenomena such as learning, motivation, attention, emotion and planning. Stressors are known to elicit characteristic changes in these processes that are thought to allow the animal to successfully

cope with such situations. The present results further underscore the importance of these regions of the forebrain in these responses and also go further in suggesting that the influence of these regions on responses to stress are, in turn, modulated by their catecholaminergic afferents.

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APPENDIX 1

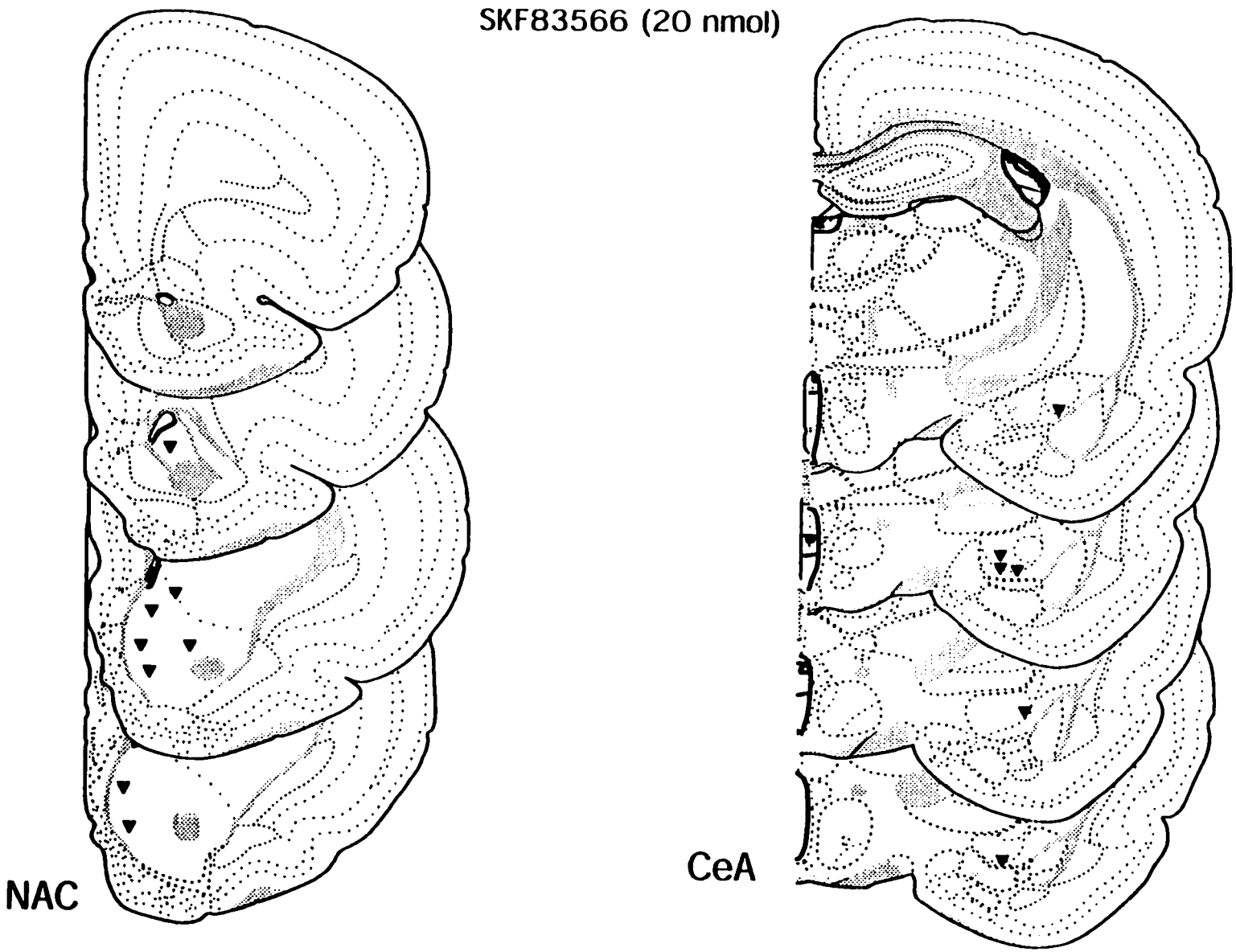
Saline
AIC
MFC

Saline
CeA
NAC

SKF82958 (20 nmol)
AIC
MFC

SKF82958 (20 nmol)
CeA
NAC

SKF83566 (20 nmol)
AIC
MFC


SKF83566 (20 nmol)
NAC
CeA

Quinpirole (20 nmol)
AIC
MFC

Quinpirole (20 nmol)
CeA
NAC

Raclopride (20 nmol)
AIC
MFC

Raclopride (20 nmol)
CeA
NAC

SKF82958 + Quinpirole
AIC
MFC

SKF82958 + Quinpirole
CeA
NAC

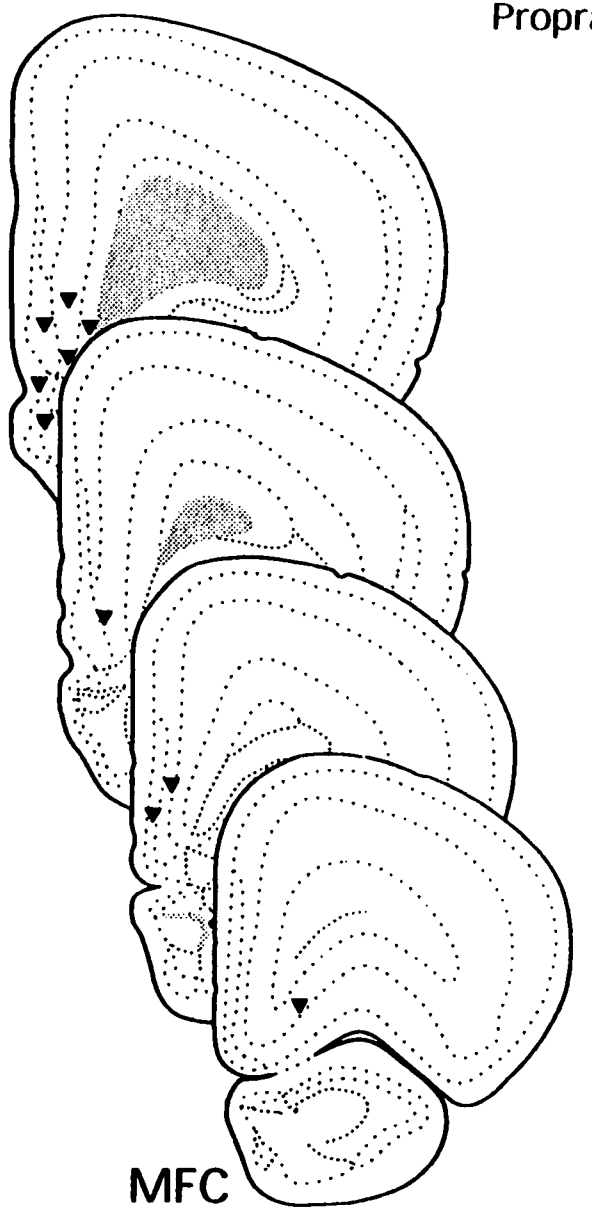
Quinpirole + SKF83566
AIC
MFC

Quinpirole + SKF83566
CeA
NAC

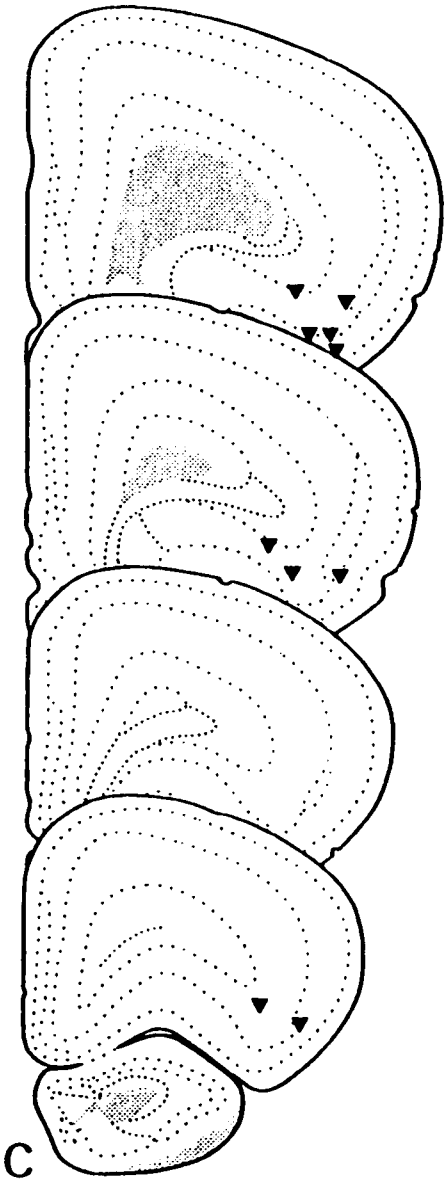
Isoproterenol (10 nmol)
AIC
MFC

Isoproterenol (10 nmol)
CeA
NAC

Propranolol (40 nmol)



MFC

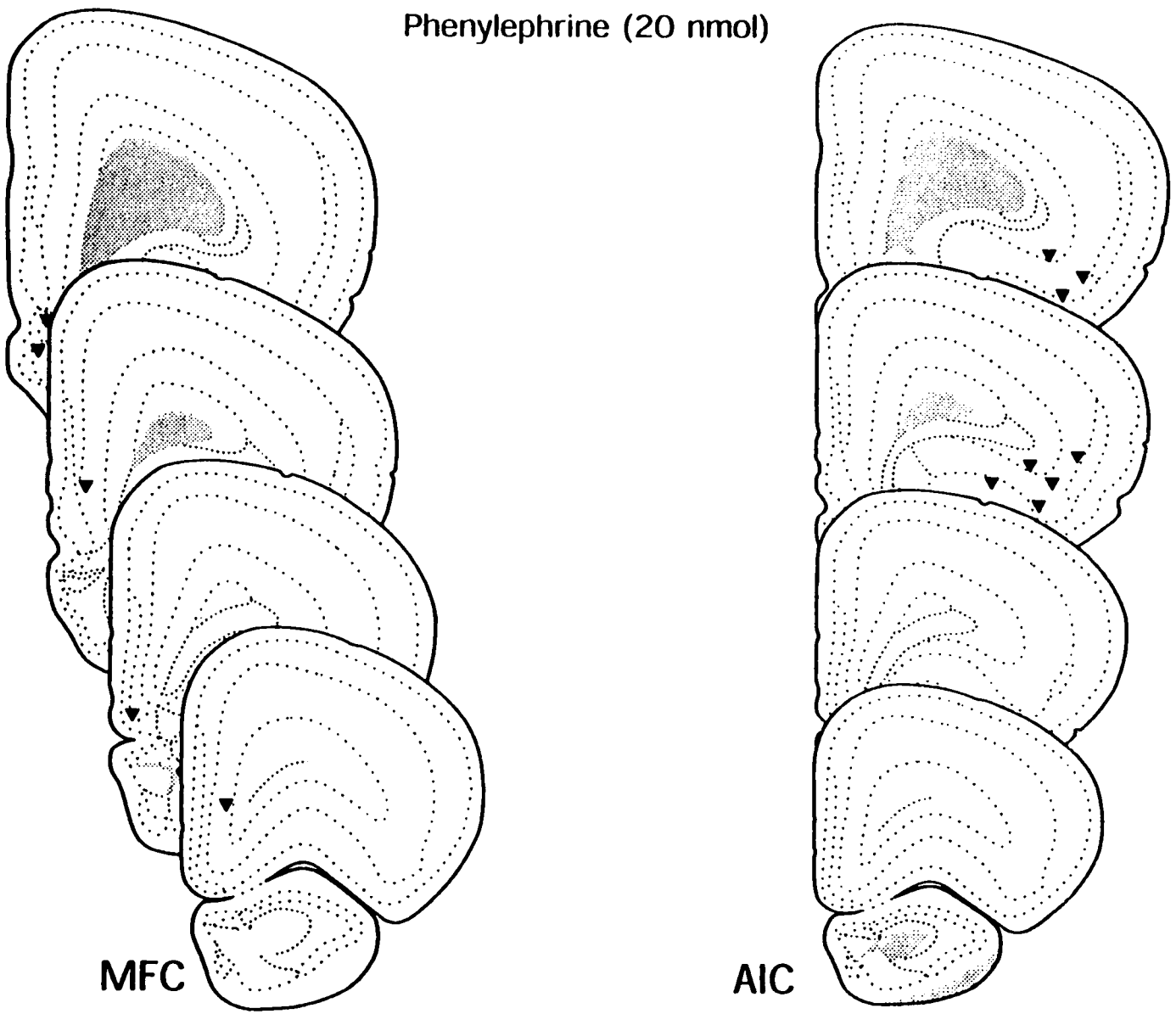


AIC

Propranolol (40 nmol)
CeA
NAC

Isoproterenol + Propranolol
AIC
MFC

Isoproterenol + Propranolol
CeA
NAC


Phenylephrine (20 nmol)
MFC
AIC

Phenylephrine (20 nmol)
CeA
NAC

Phentolamine (20 nmol)
AIC
MFC

Phentolamine (20 nmol)
CeA
NAC

Isoproterenol + Phenylephrine
AIC
MFC

Isoproterenol + Phenylephrine
CeA
NAC

Isoproterenol + Raclopride
AIC
MFC

Isoproterenol + Raclopride
CeA
NAC

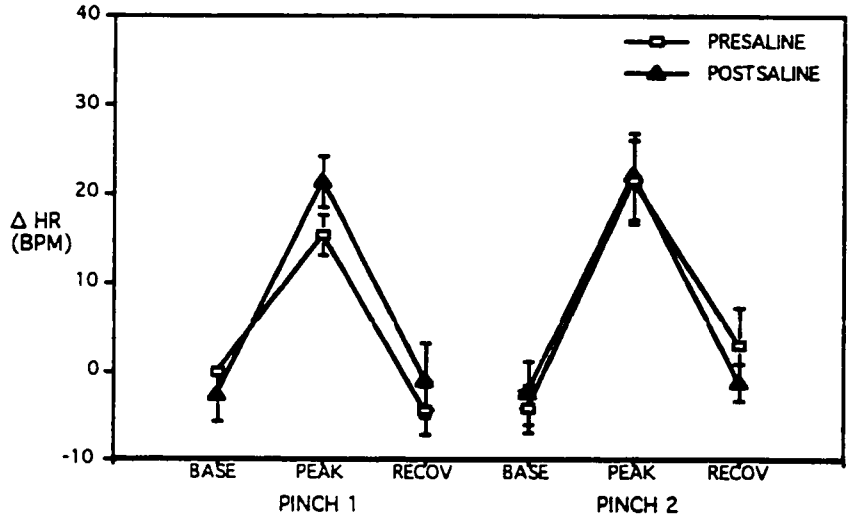
Quinpirole + Propranolol
AIC
MFC

Quinpirole + Propranolol
CeA
NAC

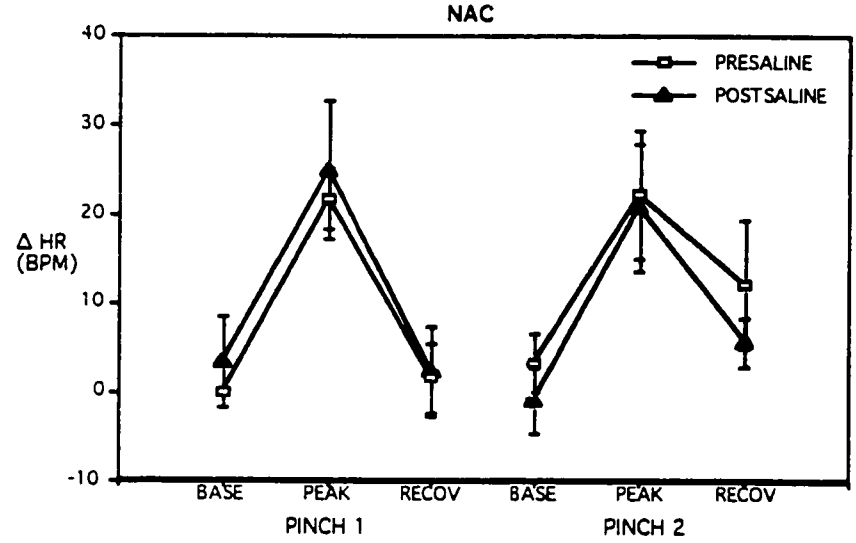
APPENDIX 2

Saline (.5 μl)

AIC



NAC



CeA