etonitazene or morphine. The rigidity produced by etonitazene was antagonized by naloxone (10 mg/kg), whereas all doses of naloxone up to 160 mg/kg failed to attenuate the explosive motor behavior produced by morphine. In the final experiment, intraventriuclar infusions of levorphanol produced rigidity and not EMB. When pretreated with naloxone, rats showed EMB after intraventricular infusions of heroin but not after infusions of levorphanol. The four experiments together, indicate that the mechanisms underlying EMB and rigidity are anatomically distinct and qualitatively different. The mechanism participating in rigidity appears to be mediated by the much studied opiate receptor, whereas this receptor is not involved in the production of EMB.

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GENERAL INTRODUCTION

Man has used the opiate, morphine for its medicinal and euphoric properties for over a century. Morphine produces a wide spectrum of effects such as narcosis, constipation, nausea and gross behavioral excitation, to mention a few (Jaffe, 1970). Recently, attention has been given to an effect of morphine that is not mimicked by opioids which are synthetic, opiate-like drugs. Several investigators (Jacquet & Lajtha, 1974; Shizgal, Brown, Amit & Sklar, 1977) have reported that morphine infused intracerebrally into rats produced gross motor excitation, whereas, intracerebral infusions of opioids (e.g. etonitazene) caused marked rigidity of the body. The research presented in this thesis was aimed at investigating gross motor excitation and rigidity, with regards to site of drug action and the types of mechanisms involved.

Explosive Motor Behavior

There have been early reports that morphine administered intracerebrally produces motor excitation and convulsions in a wide variety of experimental animals such as cats, dogs, rabbits and guinea pigs (Stern & Gautier, 1921; Tanaka &

Kadowaki, 1964). More recently, several investigators have also observed gross motor excitation following an injection of morphine directly into the PAG (Jacquet & Lajtha, 1974; Sharpe, Garnett & Cicero, 1974) or the lateral cerebral ventricle of rats (Shizgal et al., 1977). For purpose of nomenclature in this thesis, the motor excitation described by these more recent authors will be termed explosive motor behavior (EMB).

EMB is characterized by violent jumps preceeded by running, rotations about the rostral-caudal axis, and is usually accompanied by vocalizations (Jacquet & Lajtha, 1974; Shizgal et al., 1977). These behaviors are usually elicited by tactile, auditory or visual stimuli. However, it has yet to be determined whether EMB is solely elicited by external stimulation or can occur spontaneously.

Several of these behaviors appear however, to be partly dependent on the physical dimensions of the environment in which the animal is tested. Shizgal et al. (1977) reported that when the testing area was an open field, animals usually displayed excessive running and sometimes

rotated about the rostral-caudal axis. In contrast, when the testing chamber was spatially restricted, such as a standard Skinner box, violent jumps were observed during which the animal frequently came in contact with more than one surface, including the ceiling of the box (Shizgal et al., 1977). When the testing area is a compromise between an open field and a Skinner box, as is the chamber used in the series of experiments contained in this thesis (15" x 15" x 20"), violent jumps, running and rotations are observed (unpublished data).

The Site of Morphine's Action in EMB

The most probable candidate for the site of morphine's action in EMB appears to be the PAG. An intraventricular infusion of morphine produced EMB (Shizgal et al., 1977). Yet, it must be noted that morphine infused intraventricularly, does not penetrate neural tissue easily (Cube, Teschemacher, Herz & Hess, 1970). This suggests that the structure involved in EMB must be situated near the ventricular system. The PAG fulfills this prerequisite since it is located adjacent to the Aqueduct of Sylvius. In addition to its proximity to the ventricular

system, the PAG is reported to be the second highest opiate binding area in the brain (Snyder & Matthysee, 1975).

There have been several studies that substantiate the role of the PAG in EMB. Schubert, Teschemacher, Kreutzberg and Herz (1970) reported that radioactively labelled morphine infused intraventricularly crosses the ventricular wall and penetrates the PAG substantially. Other studies (Jacquet & Lajtha, 1974; Sharpe et al., 1974) have shown that morphine injected directly into the PAG produced EMB. In contrast, when morphine was injected 1 mm from the PAG, EMB did not occur (Sharpe et al., 1974). Furthermore, infusions of naloxone, the opiate antagonist, directly into the PAG attenuated the occurrence of EMB for a period of 10 minutes (Jacquet & Lajtha, 1974).

EMB, Peripheral Morphine and the Opioids

EMB has not been observed in animals given a systemic injection of morphine. Instead, it has been shown that after systemic dosages of morphine that are greater than 80 mg/kg, animals display a marked rigidity of the body (Mavrojannis, 1903; Wilcox, Levitt, McCoy &

Bozarth, 1976). The production of rigidity has also been reported to occur following intracerebral or systemic injections of opioids, such as etonitazene and levorphanol (Jacquet & Lajtha, 1974; Shizgal et al., 1977).

The failure of systemic morphine to produce EMB has been accounted for in terms of morphine's inefficacy in entering the brain (Jacquet & Lajtha, 1973). Morphine is poorly soluble in lipids, and thus, presumably does not cross the Blood-Brain Barrier readily. The ineffectiveness of systemically injected morphine to produce EMB may reflect the failure of morphine to reach sufficient concentration in the brain at sub-lethal doses. This proposition however, has yet to be demonstrated.

The failure of the opioids administered intracerebrally to produce EMB is somewhat puzzling. Opioids, such as levorphanol and etorphine are very lipid soluble, and therefore should readily reach the brain sites involved in EMB when injected centrally (Cube et al., 1970). Moreover, Snyder and Matthysee (1975) have suggested that morphine, levorphanol and etor-

phine act on the same "opiate receptor" to produce their effects. Three explanations can be proposed to account for the failure to observe EMB following an opioid injection. First, it is possible that the studies to date, may not have used sufficient concentrations of the opioids. Hence, the intracerebral doses used in previous studies may not have permitted the opioids to become adequately concentrated in the brain to cause EMB. Second, the other effects produced by the opioids, such as rigidity may in some manner be masking EMB. This could result from a competition for the motor apparatus between the processes underlying EMB and rigidity, with the more dominant process being manifested as rigidity. Third, it is possible that the concept of only one type of opiate receptor subserving all of the actions of opiates and opioids is inaccurate. There may exist more than one type of receptor mechanism such that the one involved in EMB is activated by morphine alone, whereas, there exists another type of mechanism involving the much studied "opiate receptor" (Snyder & Matthysee, 1975) that is activated by morphine

and the opioids.

The present series of studies were concerned with the investigation of EMB produced by morphine and the failure of certain opioids to cause this behavior. In the first two experiments, an attempt was made to define the site which mediates morphine's action in the production of EMB, and whether or not the neural substrates underlying EMB and rigidity are anatomically distinct. In the third study, the nature of the receptors underlying EMB and rigidity was investigated to determine if they were qualitatively different. Finally, the fourth experiment investigated whether or not the failure of certain opiates and opioids to produce EMB at sub-lethal dosages resulted from 1) a masking effect, 2) an insufficient concentration of the drug in the brain, or 3) the failure of these drugs to activate the mechanism involved in EMB.

EXPERIMENT I

As mentioned previously, the PAG has been implicated in the production of EMB. Morphine injected directly into the PAG caused EMB, whereas similar doses of morphine injected 1 mm from the PAG did not produce EMB (Jacquet & Lajtha, 1974; Sharpe et al., 1974). Furthermore, other studies have demonstrated that morphine administered intraventricularly does reach and penetrate the PAG (Schubert et al., 1970). In addition to its involvement in EMB, it has been suggested that the PAG participates in the production of rigidity. Jacquet and Lajtha (1974) reported that the rigidity resulting from a systemic injection of levorphanol, was blocked by an injection of naloxone directly into the PAG. In the following experiment, the role of the PAG in EMB and rigidity was investigated by comparing the effects of intraventricular infusions of morphine and etonitazene on PAG-lesioned and non-lesioned animals.

METHOD

Subjects

The subjects were 61 male Wistar rats (Canadian Breeding Laboratories) weighing approximately 284 grams at the beginning of the experiment. Animals were housed in stainless steel cages with free access to food and water. The animal colony was illuminated on a 12 hour day/night schedule.

Drugs and Injections

Morphine hydrochloride (May and Baker of Canada Co.) was dissolved in injectable Ringer's solution (pH = 5.6). Etonitazene hydrochloride was also dissolved in Ringer's solution (pH = 4.9). Ringer's solution (pH = 5.6) was used for control purposes.

The intraventricular infusions were delivered via a Harvard Apparatus infusion pump (Dover, Mass.) at a rate of 0.34 ul/sec. The volumes of infusion were as follows: morphine - 12.4 ul; etonitazene - 8.2 ul; vehicle - 12.4 ul.

Based on pilot work, the smallest dose of morphine (248 ug) that produced EMB reliably in 90% of the animals was selected. The etonitazene dose (1.64 ug) was the minimum necessary to reliably produce rigidity without impairing the animal's ability to emit locomotor responses to the auditory stimulation produced by the jingling of keys. The

ratio of morphine to etonitazene doses used in the present study is different from the 1000 to 1 potency ratio reported previously (Wikler, Martin, Pescor & Eades, 1963). This 1000 to 1 potency ratio is based on studies of analgesia, self-administration, and physical dependence and thus, may not be relevant to rigidity and EMB.

Procedure

Subjects were anaesthetized with intraperitoneal injections of sodium pentobarbital (Abbot Co.) at a dose of 60 mg/kg and were given ether supplements when necessary. Fifteen control subjects received cannulae stereotaxically implanted into either lateral ventricle (1.0 posterior to bregma, 1.5 lateral, 3.6 ventral, incisor bar set at 0.0). The 22 gauge stainless steel cannula (Plastic Products Co.) was secured in position by dental cement anchored to the skull by jeweller's screws. Forty-six subjects received an electrolytic lesion in the PAG (2 mm x 35 seconds). The tip of the lesioning electrode (0.25 mm in diameter) was stereotaxically aimed at the center of the aqueduct (6.0 mm posterior to bregma, 0.0 lateral, 5.0 ventral, incisor bar at + 5). The PAG-lesioned animals received chronic cannula implants into either lateral ventricle immediately after the lesion was made.

Since previous pilot observations showed that PAG lesions alone may produce EMB within six hours of surgery

without any morphine infusions, all animals with a PAG lesion were tested at 15 minute intervals for EMB and rigidity following surgery. The test for EMB assessed the responsiveness of the animal to the auditory stimulation of jingling keys. The criterion used for EMB was the occurrence of violent jumps. Rotations about the rostral-caudal axis, running or circling without violent jumps were categorized as hyperactivity and not EMB. Rigidity was determined by laying the animal, dorsal side down, horizontally across two parallel pieces of wood 9.1 cm apart. In order to reach criterion for rigidity, the animal had to hold its upside-down position for 30 seconds without righting or falling between the parallel pieces of wood. It should be noted that this test assesses rigidity as well as the righting reflex. The animal however, has to be rigid and not merely lacking the righting reflex, in order to maintain the upside down position. The post-surgery testing was terminated when (1) an animal displayed EMB or (2) failed to show EMB by the sixth hour after surgery. The first criterion was used to minimize the fatality rate since pilot work had indicated that repeated episodes of violent EMB were usually fatal. The second criterion was based on pilot observations which revealed that some animals do not display EMB as a result of a lesion through an electrode aimed at the PAG. Even in those animals that showed only some hyperreactivity to the jingling

of keys, the symptoms decreased in frequency or disappeared completely by the sixth hour after surgery.

Twenty-four hours post-surgery, all lesioned and non-lesioned animals were tested in a plexiglass chamber (15" x 15" x 20") after a single intraventricular infusion of either morphine, etonitazene or the vehicle. Since some lesioned animals did show EMB as a result of a lesion in the PAG and others did not, both types were distributed across the two drug groups and the vehicle group. Following the intraventricular infusion, all animals were tested for EMB and rigidity at 1,3,6,10 and 15 minute intervals. Testing for rigidity was continued periodically up to two hours post-infusion.

At the end of the experiment, the lesioned animals were killed with an overdose of sodium pentobarbital and were perfused with saline followed by formal-saline. The brains were removed and fixed in a formal-saline solution. They were then frozen, cut in 40 u coronal sections and stained with thionin for verification of the cannula placement and the size and location of the lesion. The same histological procedures were employed in determining the cannula placements in non-lesioned animals.

RESULTS

As displayed in Table 1, intraventricular infusions of etonitazene produced rigidity in non-lesioned animals one to three minutes post-infusion. Non-lesioned animals receiving infusions of morphine (n=5) into the ventricle showed EMB between three and 15 minutes post-infusion with some animals displaying more than one episode of EMB. Signs of hyperreactivity, spontaneous rotations, and circling in response to the jingling of keys usually preceeded episodes of violent jumps (20 cm) induced by the jingling of keys. At the end of an episode of violent jumps, animals often displayed a concave arching of the back and tail accompanied by forelimb extension and tremor. No rigidity was observed in these animals during the 15 minute testing period. However, these animals did become rigid 35-40 minutes after the infusions. The rigidity persisted for at least two hours post-infusion. Non-lesioned animals receiving an infusion of the vehicle did not show EMB or rigidity. These animals became less responsive to the auditory stimulation and consistently reacted strongly to the tail pinch.

TABLE 1

Effects produced in non-lesioned animals following an intraventricular infusion of each drug

Morphine (Total n = 5)	Etonitazene (Total n = 5)	Vehicle (Total n = 5)
EMB (n = 5, 3-15 minutes post-infusion)	No EMB (n = 5)	No EMB (n = 5)
Rigidity (n = 5, 35-40 minutes post-infusion)	Rigidity (n = 5, 1-3 minutes post-infusion)	No rigidity (n = 5)

PAG-lesioned animals, when recovering from the anesthetic, were hyperactive and hyperreactive. One example of this was a lesioned pilot animal which when permitted to run along a corridor, traversed a distance of 200 meters in response to periodic jingling of keys.

All animals with a PAG lesion, when tested after surgery, were not rigid and usually righted immediately after being placed on the parallel pieces of wood. Those animals (n=39) which displayed episodes of EMB did so between three and six hours after the lesion was made. Twenty animals died shortly after displaying violent EMB. The animals which did not show episodes of EMB (n=7), either showed hyperreactivity or responded normally to the jingling of keys.

Twenty-four hours after the induction of the lesion, EMB and rigidity were not observed in any of the lesioned animals. When tested at this time following intraventricular etonitazene infusion, PAG-lesioned animals (regardless of whether they did (n=6) or did not (n=3) show EMB on the previous day) displayed rigidity and became less responsive to keys. These results are shown in Tables 2 and 3. After an

TABLE 2

Results for PAG-lesioned animals that displayed EMB from the lesion

Morphine (Total n = 6)	Etonitazene (Total n = 6)	Vehicle (Total n = 6)
No EMB (n = 6)	No EMB (n = 6)	No EMB (n = 6)
Rigidity (n = 3, 120 minutes post-infusion)	Rigidity (n = 6, 1 - 3 minutes post-infusion)	No rigidity (n = 6)

TABLE 3

Results for PAG-lesioned animals

that did not show EMB from the lesion

Morphine (Total n = 3)	Etonitazene (Total n = 3)	Vehicle (Total n = 3)
EMB (n = 3) Rigidity (n = 3, 120 minutes post-infusion)	No EMB (n = 3) Rigidity (n = 3)	No EMB No rigidity

intraventricular infusion of morphine, PAG-lesioned animals that showed EMB post-surgery (n=6), did not produce EMB during the 15 minutes of testing. These animals usually displayed spontaneous periodic arching of the back accompanied with forelimb extension with tremor. Those animals (n=3) that did not develop episodes of EMB post-surgery did display EMB 6 to 15 minutes following an intraventricular infusion of morphine. In all of the PAG-lesioned animals, rigidity was not observed during the 15 minute testing session. Rigidity did, however, develop two hours after the infusion. These data are displayed in Table 3.

All lesioned control animals, following intraventricular infusions of the vehicle, failed to show EMB or rigidity.

Histological examination revealed that all cannulae were placed in the lateral cerebral ventricle. Figure 1 illustrates a schematic section representing a typical PAG lesioned animal that displayed EMB during the six hour post-surgery testing. As can be seen in Figure 1, the lesion damaged the PAG tissue that surrounds the Aqueduct of Sylvius. The histological examination revealed no discernible difference

in location between lesions in the PAG that were or were not effective in producing EMB.

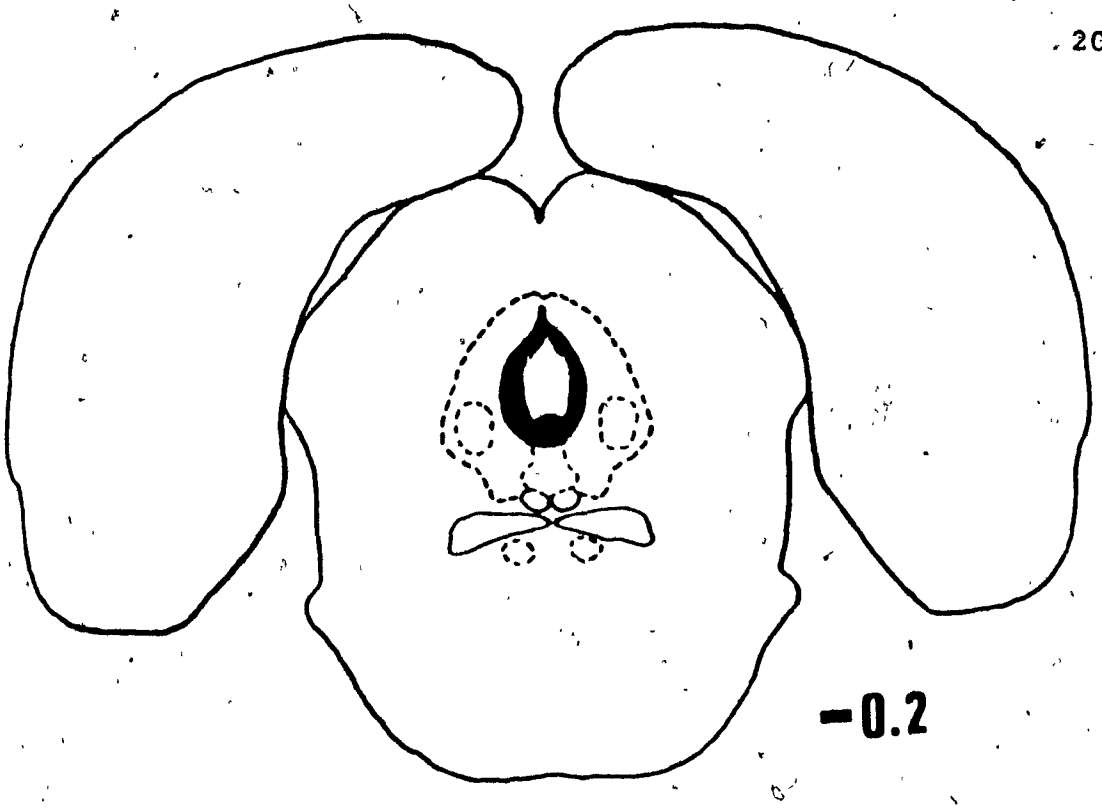

Figure 1: A representation of a typical lesion in the Periaqueductal Gray.

DISCUSSION

In non-lesioned animals, intraventricular infusions of morphine produced EMB, and later rigidity, once EMB had subsided. Intraventricular infusions of etonitazene produced only rigidity. The finding that intraventricular infusions of morphine eventually produces rigidity is also of interest because of the marked difference between the latencies for the onset of rigidity produced by morphine and etonitazene. This suggests that more time is required for morphine to travel from the ventricles to the neural structures responsible for rigidity.

In agreement with the work of Jacquet and Lajtha (1974), and Sharpe et al. (1974), the results of the present experiment suggest that the PAG is involved in EMB. A lesion of the PAG that damages tissue around the aqueduct can produce EMB by itself. Furthermore, animals that displayed EMB following lesion induction did not exhibit the usual EMB after an intraventricular infusion of morphine 24 hours post-surgery. In contrast, animals that did not display EMB from a lesion did exhibit EMB following an intraventricular infusion

of morphine.

The finding that lesions aimed at the PAG produced EMB only in some subjects suggests that the location and/or size of the "successful" lesions differed from the lesions in subjects that did not show EMB. The histological findings however, failed to bear out this inference. It is possible that such differences do in fact exist but were beyond the acuity of the histological technique.

Contrary to the previous report of Jacquet and Lajtha (1974), it appears that the PAG is not necessarily involved in the production of rigidity since intraventricular infusions of etonitazene continued to cause rigidity in PAG-lesioned animals. These discrepancies between the present results and those of Jacquet and Lajtha may be reconciled in terms of the different midbrain central gray regions investigated. In the present study, the lesion damaged tissue adjacent to the Aqueduct of Sylvius, whereas the location of the infusion sites used by Jacquet and Lajtha (1974) are in general, outside of this area. It appears then, that the production of EMB and rigidity may depend on the action of opiates and opioids in two

different and at least partially independent neural systems.

EXPERIMENT II

In Experiment I, it was demonstrated that a lesion in the PAG was effective in producing EMB, and that animals that have displayed EMB from a PAG lesion failed to show the usually observed EMB following an intraventricular infusion of morphine 24 hours later. However, on the basis of Experiment I, it is not known to what extent morphine-produced EMB is blocked by a lesion of the PAG. It is possible that the lesions only elevated slightly the dose threshold for the behavior. Since a small effect of this type might be due to a disruptive side-effect of the lesion and not to direct damage of the neural substrate for EMB, it seemed of interest to assess the degree to which morphine-produced EMB is blocked by PAG lesions. In this experiment, intraventricular infusions of morphine were repeatedly administered to rats with PAG lesions in an attempt to determine whether there was any dose of morphine that could produce EMB in these subjects.

METHOD

Subjects

The subjects were 26 male Wistar rats weighing approximately 380 grams at the beginning of the experiment. They were housed in the same manner as in the previous experiment.

Drugs and Infusions

Morphine hydrochloride was dissolved in injectable Ringer's solution. The initial dosage of morphine was 248 ug infused in a volume of 12.4 ul at a rate of 0.34 ul/sec. The subsequent infusions of morphine were doses of 400 ug each in volumes of 10.3 ul infused at a rate of 0.34 ul/sec. The dose was selected on the basis of pilot work such that it would cause death in a PAG-lesioned animal within 14 infusions. Ringer's solution was used for control purposes and was administered with the same volumes and flow rate as the morphine infusions.

Procedure

Twenty-six animals were lesioned through electrodes aimed at the PAG, and received a cannula implant in either lateral cerebral ventricle, as described in Experiment I.

All animals were tested at 15 minute intervals following surgery for EMB and rigidity, as previously described.

Post-surgery testing was terminated when one of the criteria given in Experiment I was met.

Twenty-four hours after surgery, surviving animals were randomly assigned to either the morphine or vehicle group. Six animals that had displayed EMB from the PAG lesion on the previous day received an initial intraventricular infusion of morphine (248 ug). They were then tested 1,3,6,10 and 15 minutes post-infusion for EMB and rigidity. At the end of the 15 minute testing session these animals received one infusion of morphine (400 ug) every five minutes. Three minutes after each infusion, animals were tested for EMB and rigidity. The morphine infusions were terminated after 14 infusions or when an animal manifested respiratory failure.

Each of the six control animals with a lesion in the PAG received intraventricular infusions of the vehicle which were individually matched in number to one of the experimental animals. The pairing of control animals to experimental animals was performed randomly. The animals that received vehicle infusions followed the same schedule of infusions and tests for EMB, and rigidity as the intraventricular morphine group.

Following death, the brains of the animals receiving intraventricular morphine were removed and fixed in a formal-saline solution. The brains were frozen, cut into 40 u sections and stained with thionin to verify the cannula placement and the size and location of the lesion. The control animals

were killed by carbon dioxide inhalation and the histological verification for these animals followed the same procedure as that used for the experimental group.

RESULTS

All animals except one[1] displayed EMB three to six hours post-surgery. Rigidity was not observed in any animals during this time. Fourteen animals died after an episode of violent EMB. When tested 24 hours post-surgery, prior to the intraventricular infusions, EMB and rigidity were absent.

Animals that had displayed EMB from the lesion in the PAG on the previous day did not show EMB following repeated intraventricular infusions of morphine. After the first infusion of morphine, these animals usually displayed hyperreactivity and forelimb extension with tremor. Hyperreactivity and forelimb extension subsided on the average by the fifth infusion, and at this time the animals were lying on their sides and exhibiting rapid spontaneous jerks of the body. Although all animals did not meet the criteria for rigidity, one animal tested after the fifth infusion remained on the parallel pieces of wood for three seconds. In five animals, respiratory failure was observed after receiving six infusions of morphine (in total 2648 ug). The sixth animal died one and one-half hours after the final, fourteenth infusion was administered.

Animals receiving intraventricular infusions of the

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[1] When animals were randomly assigned to groups, this animal was assigned to the vehicle group.

vehicle did not develop EMB or rigidity. These animals remained responsive to the jingling of keys, although their responsiveness decreased over tests. When tested for rigidity on the parallel pieces of wood these animals righted readily.

Histological examination revealed that all cannulae were placed in the lateral cerebral ventricle. All lesions which produced EMB post-surgery were found to have damaged the tissue around the Aqueduct of Sylvius. The histology from the one animal which did not show EMB did not reveal any discernible difference in size and location from lesions that produced EMB.

DISCUSSION

The failure of intraventricular morphine to produce EMB in PAG-lesioned animals during the first experiment could have been attributed to a small elevation in the dose threshold. If this were true, then it would be expected that EMB would develop in PAG-lesioned animals given higher doses of morphine. The results of the second experiment suggest that this is not the case since repeated intraventricular infusions of morphine up to LD_{100} failed to produce EMB.

As mentioned previously, the most likely interpretation of these results is that the PAG lesion damaged the site at which morphine acts to produce EMB. This view is supported by the interstitial infusion studies of Jacquet and Lajtha (1974) and Sharpe et al. (1974). It is unlikely that the failure to manifest EMB was due to disruption in motor performance caused by the lesion. The subjects were capable of locomotion and did show some hyperreactivity following the first few morphine infusions.

EXPERIMENT III

The results of the first two experiments indicate that the production of EMB and rigidity may depend upon the action of morphine and opioids in two discrete and at least, partially independent neural systems. In the next experiment, it seemed of interest to investigate whether the "opiate receptor" proposed by Snyder and Matthysee (1975) was involved in EMB or rigidity. The rigidity produced by levorphanol administered peripherally has been reported (Jacquet & Lajtha, 1974) to be blocked by naloxone, whereas EMB produced by intracerebral infusions of morphine is reported to be blocked for only 10 minutes by this opiate antagonist (Jacquet & Lajtha, 1974). These results suggest that the opiate receptor is involved in EMB and rigidity. Naloxone's blockade of levorphanol's effects and its attenuation of morphine-produced EMB however, is somewhat of a puzzle. The strengths of the relative binding potency of naloxone, levorphanol and morphine decline in that order respectively (Snyder & Matthysee, 1975). One would expect that naloxone would be able to antagonize the effects

of morphine much more easily than those of levorphanol, given the drug doses employed in these studies and the mobility of these compounds in tissue. Paradoxically, the behavioral evidence cited suggests that the reverse is true.

In the present study, the ability of naloxone to antagonize EMB and rigidity was investigated. It was hoped that this could shed some light on whether EMB and rigidity result from the action of opiates and opioids at the naloxone-blocked opiate receptor or via a different mechanism.

METHOD

Subjects

The experimental subjects were 81 male Wistar rats (Canadian Breeding Laboratories) weighing approximately 290 grams at the beginning of the experiment. Animals were housed in stainless steel cages with free access to food and water. The animal colony was illuminated on a 12 hour day/night schedule.

Drugs and Injections

Morphine hydrochloride (pH = 5.6; May and Baker of Canada Co.), etonitazene hydrochloride (pH = 4.9) and naloxone hydrochloride (pH = 4.8; Endo Laboratories) were dissolved in Ringer's solution (pH = 5.6) which was also used for control purposes.

The intraventricular infusions were delivered via a Harvard Apparatus infusion pump (Dover, Mass) at a rate of 0.34 ul/sec. The volumes of infusions were as follows: Morphine - 12.4 ul, and etonitazene - 8.2 ul. On the basis of pilot work, the intraventricular dose of morphine (248 ug) was selected such that it produced EMB reliably in 90% of the animals. The etonitazene dose (1.64 ug) was the minimum necessary to produce rigidity consistently.

Naloxone was injected intraperitoneally (I.P.) at doses of either 10, 20, 40, 60, 80, 100 or 160 mg/kg. All

I.P. injections were administered in volumes of 1 ml/kg.

Procedure

The animals undergoing surgery were anaesthetized with I.P. injections of sodium pentobarbital (Abbott Co.) at a dose of 60 mg/kg with supplements of 6 mg sodium pentobarbital when necessary. Sixty-three subjects received a cannula stereotaxically implanted into either lateral ventricle as described previously.

Eighteen control animals were anaesthetized with an I.P. injection of sodium pentobarbital (60 mg/kg) and did not receive a cannula implant.

All animals were tested approximately 24 hours after surgery in a plexiglass chamber with the dimensions of 15" x 15" x 20". Forty-two animals with cannula implants were divided equally into seven groups. Each group was injected I.P. with either 10, 20, 40, 60, 80, 100 or 160 mg/kg of naloxone followed five minutes later by an intraventricular infusion of morphine. An additional group of six animals with cannulae implants received an I.P. injection of naloxone (10 mg/kg) five minutes prior to an intraventricular infusion of etonitazene. Eighteen control animals

that received an I.P. injection of the anaesthetic on the preceeding day without a cannula implant were divided into three groups. Each group received only an I.P. injection of naloxone (10, 80 or 160 mg/kg) five minutes prior to testing. Another 16 control animals with cannula implants received an infusion of the vehicle (n=6), etonitazene (n=5) or morphine (n=5) but did not receive an injection of naloxone. Animals with cannulae were tested for EMB and rigidity, as described previously, at 1, 3, 6, 10 and 15 minutes following the time of the infusion. Control animals without cannula implants were tested according to the same schedule.

At the end of the experiment; implanted animals were killed with sodium pentobarbital, perfused with saline followed by formal-saline solution. The brains were removed and fixed in a formal-saline solution, then frozen, cut in 40 micron coronal sections, and stained with thionin for verification of cannula placement.

RESULTS

Animals receiving intraventricular infusions of etonitazene without a naloxone injection became rigid within one to three minutes after the infusion. Following an intraventricular infusion of morphine, animals without naloxone pretreatment showed EMB between three and fifteen minutes post-infusion with some animals manifesting more than one episode of EMB. Spontaneous rotations about the rostral-caudal axis, circling and running in response to the auditory stimulation usually preceeded episodes of EMB. Subjects that received either an intraventricular infusion of the vehicle or only an I.P. injection of naloxone (10 mg, 80 mg, or 160 mg/kg), did not show EMB or rigidity. These animals became less responsive to keys during the 15 minutes testing session.

The animals (n = 6) injected with 10 mg/kg of naloxone five minutes prior to an intraventricular infusion of etonitazene failed to manifest EMB or rigidity. Similar to the control groups, these animals showed a decline in responsiveness to the auditory stimulation.

Thirty-eight animals receiving an intraventricular infusion of morphine five minutes after an I.P. injection of naloxone (10, 20, 40, 60, 80, 100 or 160 mg/kg) developed EMB, on the average, by the sixth minute post-infusion. Fourteen of these animals died immediately after a violent

episode of EMB. Rigidity was not observed during the 15 minute testing session in any of these animals. Animals pretreated with 10, 20, 40 or 60 mg/kg of naloxone did, however, become rigid between one and two hours post-infusion. Animals injected with 80, 100 or 160 mg/kg of naloxone did not display rigidity for up to two hours post-infusion.

Four animals pretreated with naloxone did not show EMB or rigidity during the 15 minute testing session after an intraventricular infusion of morphine.

Histological examination revealed that all cannulae were placed in the lateral cerebral ventricle.

DISCUSSION

In agreement with a previous report (Shizgal et al., 1977) the results of the present study showed that an intraventricular infusion of etonitazene produces rigidity but not EMB. Similar to the findings of Jacquet and Lajtha (1974), it was found that opioid-produced rigidity can be blocked by naloxone.

As expected from the results of prior studies (Jacquet & Lajtha, 1974; Sharpe et al., 1974; Shizgal et al., 1977), animals without naloxone pretreatment displayed EMB following an intracerebral infusion of morphine. In addition, the present study demonstrated that rigidity developed 35 - 40 minutes post-infusion after EMB had subsided. The marked differences in the latencies for the onset of rigidity between etonitazene and morphine (1-3 minutes versus 35-40 minutes) is of interest. Morphine is less lipid soluble than opioids such as levorphanol and etorphine (Cube et al., 1970) and thus morphine is presumably less mobile in neural tissue. The longer latency for the rigidity produced by morphine may represent the time needed for morphine to travel from the ventricular system to the critical structures involved in rigidity.

The latency for the onset of rigidity following intraventricular infusions of morphine was increased by 10, 20, 40, 60 mg/kg of naloxone from the usual 35-40 minutes to

60-120 minutes post infusion. Furthermore, rigidity was not observed after an infusion of morphine when animals were injected with higher doses of naloxone (80, 100, 160 mg/kg). It appears then, that naloxone is capable of postponing the onset of rigidity produced by an intraventricular infusion of morphine, and that the duration of naloxone's effectiveness is dose related.

The results show that EMB is not attenuated by naloxone even when exceptionally large doses are administered. Intraventricular infusions of morphine produced EMB in 90% of the animals pretreated with naloxone. The failure of the remaining four animals to show the usually observed EMB may not be the result of naloxone's antagonism of morphine's action. Shizgal et al. (1977) have reported that some animals do not show EMB following repeated intraventricular infusions of morphine (in total 1000 ug).

The results of this experiment imply the existence of two qualitatively different types of sites at which morphine and opioids may act. It appears that the opiate receptor is involved in the production of rigidity since it can be occupied

by morphine, etonitazene and naloxone. In contrast, EMB does not seem to involve the opiate receptor. The exact site where morphine produces EMB however, has yet to be determined.

EXPERIMENT IV

It was speculated in this thesis that other effects produced by morphine and the opiates may mask EMB. Evidence supporting this notion is the observation that animals displaying EMB from a lesion in the PAG, when injected with morphine or etonitazene I.P., became rigid and did not show further episodes of EMB. In contrast, PAG-lesioned animals that received an I.P. injection of the vehicle, continued to display EMB (unpublished data). The existence of a masking effect is of interest since certain opioids, such as etonitazene and etorphine, infused intracerebrally, have been reported to cause rigidity and not EMB (Jacquet & Lajtha, 1974; Shizgal et al., 1977). Similarly, heroin injected intraventricularly produces rigidity and not EMB (unpublished data). The opioids and heroin may not be producing EMB because certain effects caused by these drugs (i.e. rigidity) may be masking the occurrence of EMB.

In addition to the masking effect, these drugs may not be producing EMB because they have not been administered in large enough doses in

previous studies (Jacquet & Lajtha, 1974; Shizgal et al., 1977). Perhaps EMB may occur if larger doses of these drugs are used.

The following experiment was concerned with investigating the possibilities of 1) an insufficient drug concentration or 2) a masking effect being responsible for the absence of EMB, following intracerebral infusions of heroin and the opioids. This was achieved by repeatedly infusing levorphanol or heroin into the ventricles of animals with and without naloxone pretreatment. There are several reasons for using naloxone as a pretreatment. Naloxone antagonizes the lethal effects of opiates (Jaffe, 1970) and thus, permits one to explore a higher range of doses. Since EMB is not antagonized by naloxone, another reason is that a naloxone pretreatment also permits the investigation of a possible masking effect. If the masking effect does exist and is mediated by the opiate receptor, the use of naloxone should antagonize the masking effect and allow the occurrence of EMB.

METHOD

Subjects

The subjects were 44 male Wistar rats weighing 250-310 grams. The animals were housed in stainless steel cages with free access to food and water. The colony room was illuminated on a 12 hour day/night schedule.

Drugs and Injections

N-levorphanol tartrate (pH = 3.55, Hoffman-La Roche Ltd.), naloxone hydrochloride (pH = 4.8, Endo Laboratories), and heroin (diacetyl-morphine, pH = 4.35, Macfarlon Smith Co.), were dissolved in Ringer's solution (pH = 5.6) which was also used for control purposes.

The intraventricular infusions were delivered via a Harvard Apparatus infusion pump (Dover, Mass.) at a rate of 0.34 ul/sec. The volume of each infusion was 11.0 ul. The naloxone was injected in a volume of 1 ml/kg.

On the basis of pilot work, the dose for each of the drugs was chosen such that the entire dose range would be explored. The dose of levorphanol (104 ug) for each infusion was the minimum

necessary to cause death in at least 80% of the animals between four and 10 injections. Each injection was administered at five minute intervals. The doses of heroin were selected such that the lower dose (106 mg) injected intraventricularly did not cause death in 80% of the animals after 10 infusions administered at five minute intervals. On the other hand, the higher dose (186 ug) was chosen such that it would cause death in 80% of the animals, before the sixth infusion. These two doses were used to explore the dose range for heroin, since pilot work had indicated that it was not possible to locate the LD_{80} dose for heroin within the repeated infusion paradigm used. Pilot work had revealed that for doses ranging from 100-250 ug death usually followed the first infusion or did not occur after 10 infusions. Moreover, the proportion of deaths to survivals had a positive correlation to the dose of each infusion. The dose of naloxone was selected using the antagonism of rigidity produced by an intraperitoneal (I.P.) injection of morphine (75 mg/kg) as a crude index of naloxone's duration of action. The dose of naloxone selected

(20 mg/kg) was the minimum necessary to antagonize rigidity for at least for a period of one hour.

Procedure

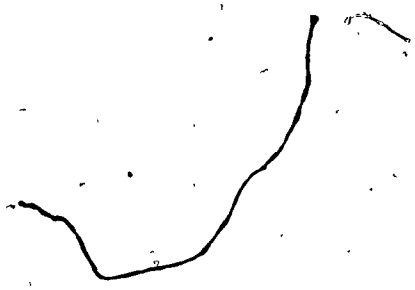
Subjects undergoing surgery were anaesthetized with intraperitoneal injections of sodium pentobarbital (Abbot Co.) at a dose of 60 mg/kg and were given ether supplements when necessary. Forty-nine subjects received a cannula stereotaxically as previously described in Experiment 1. A group of six control animals received only an I.P. of the anaesthetic.

Between twenty-four and seventy-two hours after surgery 22 animals with cannula implants received an infusion of either heroin (n=6) at 106 ug, n=5 at 186 ug), levorphanol (n=5) or the vehicle (n=5), once every five minutes for a total of ten infusions. An additional group of 17 animals with a cannula implant received an I.P. injection of naloxone five minutes prior to an infusion of either diacetyl-morphine (n=6 at 106 ug, n=5 at 186 ug) or levorphanol (n=6). The six animals which were anaesthetized without surgical manipulation were injected with naloxone only.

All animals, prior to the first intraventricular infusion and two minutes after each subsequent

infusion, were tested for EMB and rigidity. The six control animals injected with naloxone followed the same schedule of tests.

At the end of the experiment, surviving animals with cannulae were killed by carbon dioxide inhalation. The brains were removed and fixed in a formal-saline solution, then frozen and cut in 40 micron, coronal sections for verification of cannula placements.



RESULTS

Animals receiving repeated intraventricular infusions of levorphanol did not display any hyperactivity or EMB and usually became less responsive to the auditory stimulus over the testings. Only one animal receiving infusions of levorphanol displayed rigidity. An additional three animals showed some rigidity, but failed to remain on the parallel pieces of wood for the entire 30 seconds. All animals, on the average died by the fifth infusion of levorphanol (in total 520 ug). Animals given an I.P. injection of naloxone prior to the onset of the repeated, intraventricular infusions of levorphanol did not show any signs of EMB or rigidity. These animals usually became less responsive to keys and were motionless between testings. All animals pre-treated with naloxone survived the 10 infusions of levorphanol.

Repeated intraventricular infusions (106 ug each) of heroin produced rigidity by the second infusion in all animals. Although EMB was not observed, hyperactivity in the form of rotations were observed in two animals. All animals except one, (which died after the first infusion) survived the 10 infusions of heroin. When pretreated with naloxone, animals receiving repeated infusions of heroin (106 ug) did not display rigidity. Except for one, all animals displayed hyperactivity, of which two also showed episodes of EMB.

The larger dose of heroin (186 ug) produced

hyperactivity after the first infusion and later rigidity, prior to the second infusion in two animals. Both rigidity and hyperactivity disappeared prior to death. After the initial infusion, the remainder of the group were on their sides and displayed labored respiration and spontaneous jerks of the body. Death occurred in all animals on the average by the second infusion except for one which survived the 10 infusions. This animal did not display hyperactivity or rigidity. In animals pre-treated with naloxone, bellying (i.e. the animal lying on the floor) was observed after the initial infusions of heroin and the animals' response to the auditory stimulation decreased over testings. At the time of the sixth infusion, four out of five animals displayed EMB. Rigidity was absent throughout the testing sessions. All animals except for one survived the 10 infusions, and the death of this animal occurred shortly after a violent episode of EMB.

Control animals injected with Ringer's intraventricularly or just naloxone I.P. did not display EMB or rigidity. Over the testings, these animals decreased their responsiveness to auditory stimulation and usually became motionless between testings.

Histological examination revealed that all cannulae were implanted in the lateral cerebral ventricle.

DISCUSSION

The repeated infusions of heroin alone, at both dose levels, caused rigidity but not EMB. When preceeded by naloxone, heroin produced EMB at both doses with the higher dose causing episodes of EMB more frequently. Since morphine is stated to be responsible for the pharmacologically actions of heroin (Jaffe, 1970), these results suggest that the naloxone antagonized effects of morphine are masking the expression of EMB. This could involve the processes underlying both EMB and those participating in the naloxone antagonized effects competing for the motor apparatus, where the more dominant process is manifested as rigidity. Conversely, when rigidity is antagonized, EMB occurs.

In light of previous results (Jacquet & Lajtha, 1974), levorphanol unexpectedly, did not produce a profound rigidity. A possible explanation for this may be the large dose of levorphanol used. As evident from the results obtained from the heroin treated animals, it seems that rigidity disappears as the dose approaches lethality. Perhaps levorphanol did not produce a profound rigidity because the dose bordered on the lethal dose.

Surprisingly, levorphanol up to the lethal dose did not produce EMB, although it has been reported to have a greater affinity for the opiate receptor than morphine (Snyder & Matthysee, 1975). In spite of the toxic effects observed in animals without naloxone pretreatment, it does not seem possible to argue that motor impairment was responsible for the failure of levorphanol to produce EMB in naloxone treated animals. When tested, these animals were able to respond to auditory stimulation throughout the testing session.

The finding that heroin produces EMB only in animals pretreated with naloxone suggests that some, naloxone antagonized effects of morphine are preventing the occurrence of EMB. This masking effect, however, cannot account for levorphanol's failure to produce EMB when animals are pretreated with naloxone. It appears that levorphanol is not able to activate the mechanism underlying EMB.

GENERAL SUMMARY

The results of the first two experiments point to a region of the PAG which is adjacent to the Aqueduct of Sylvius as the site at which morphine produces EMB but not rigidity. A lesion of the PAG that damages tissue around the aqueduct can produce EMB by itself. Furthermore, animals that displayed EMB following lesion induction did not exhibit EMB after intraventricular infusions of morphine 24 hours post-surgery. In contrast, animals that did not display EMB from a lesion did exhibit EMB following an intraventricular infusion of morphine. It appears that the tissue of the PAG adjacent to the aqueduct is not necessarily involved in the production of rigidity since intraventricular infusions of etonitazene continued to cause rigidity in PAG-lesioned animals. This suggests that the production of EMB and rigidity may depend on the action of opiates and opioids in two different and at least partially independent neural systems.

The differences in the anatomical location of the sites responsible for EMB and rigidity may account for the differences in the behavioral effects of morphine when administered intraventricularly. Morphine has a very low lipid solubility (Cube et al., 1970) and thus has a low mobility in neural tissue. Intraventricularly infused morphine presumably has access to the critical sites in the PAG for EMB, since

Schubert et al. (1970) have reported that labelled morphine, infused intraventricularly does penetrate this area. It appears that rigidity is observed only 30-40 minutes after intraventricular morphine infusions because of the time required for morphine to reach the critical region. This site is presumably farther from the ventricular system than the site at which morphine produces EMB.

Opioids, such as etorphine and levorphanol which produce rigidity (Jacquet & Lajtha, 1974), are more lipid soluble than morphine (Cube et al., 1970). Therefore, they should be more mobile in tissue and able to reach the critical site for producing rigidity when they are injected intraventricularly. The failure of levorphanol and etorphine to produce EMB (Jacquet & Lajtha, 1974), indicates that these drugs are not able to activate the mechanism underlying EMB. In the third experiment, the role of the much studied opiate receptor in EMB and rigidity was explored.

The third experiment demonstrated that naloxone blocked the rigidity produced by an intraventricular infusion of etonitazene.

However, naloxone up to 160 mg/kg failed to antagonize EMB produced by an intraventricular infusion of morphine. It appears that the opiate receptor is involved in the mechanism underlying rigidity, and as expected, can be occupied by morphine, etonitazene and naloxone. In contrast, the opiate receptor does not appear to participate in EMB. The exact nature in which morphine produces EMB however, has yet to be determined.

While the results of the third experiment indicate that the mechanism underlying EMB is not antagonized by naloxone, it does not exclude the possibility that the opioids can elicit EMB. In the fourth experiment, the masking of EMB by the naloxone antagonized effects of opiates and opioids was investigated. In addition, repeated doses up to the LD_{80} were used to explore the dose response curve. The finding that heroin produced EMB only when the animals were pre-treated with naloxone suggests that EMB can be masked by the naloxone antagonized effects of morphine. As previously described the masking of EMB may result from the processes underlying EMB and rigidity competing for the motor

apparatus, where rigidity, the more dominant process is expressed. The masking produced by naloxone antagonized effects, however, cannot explain the failure of levorphanol to produce EMB since animals pretreated with naloxone did not display EMB. It seems unlikely that levorphanol did not become adequately concentrated in the PAG. The doses used were sufficient to cause death in 80% of the animals not treated with naloxone. This indiredtly suggests that the doses of levorphanol were not given in an ineffective quantity. Moreover, heroin was able to produce EMB at sub-lethal doses. It appears then, that the most likely explanation of levorphanol's inability to produce EMB is that it cannot activate the mechanism underlying this behavior.

In summary, the research contained in this thesis can provide some explanation as to why morphine and opioids produce differential motor effects. It is suggested that the neural substrates for EMB and rigidity are anatomically distinct and are produced by opiates and opioids acting on different types of mechanisms. The mechanism involved in rigidity appears to be mediated by the much studied opiate receptor

(Snyder & Matthysee, 1975) and hence, morphine and the opioids act as agaonist of this mechanism, while haloxone produces its antagonist effects. The results indicate that the opiate receptor however, does not participate in the production of EMB. This suggests that morphine is acting via a different means than the opiate receptor to produce EMB. The possibility of multiple opiate receptors have been argued for by Martin, Eades, Thompson, Huppler and Gilbert (1976). In their study, they presented evidence which suggests that multiple spinal receptors mediate the actions of opiates. The results of the present studies are consistent with this and indicate that morphine may produce some of its effects via other mechanisms than the opiate receptor.

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