AN INVESTIGATION OF THE INVOLVEMENT OF ADRENOCORTICOTROPHIC HORMONE IN MEDIATING THE AVERSIVE PROPERTIES OF MORPHINE

David Sinyor

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ABSTRACT

AN INVESTIGATION OF THE INVOLVEMENT OF ADRENOCORTICOTROPHIC HORMONE IN MEDIATING THE AVERSIVE PROPERTIES OF MORPHINE

David Sinyor

The possible involvement of adrenocorticotrophic hormone (ACTH) in mediating the conditioned taste aversion (CTA) produced by morphine, in rats, was investigated. In the first experiment, it was shown that while exogenous ACTH did not manifest any aversive properties, it potentiated an aversion when administered with morphine during conditioning. In the second experiment, the contribution of morphine-induced ACTH release to the aversive properties of morphine, was examined. It was found that pharmacological blockade of this release, at the time of conditioning, did not attenuate the morphine-induced CTA. In the third experiment, it was shown that prior exposure to a dose of ACTH twice that used in the first experiment, did not attenuate the morphine-induced CTA. This dose of ACTH was shown not to possess any aversive properties, in the fourth experiment. The results of these experiments, although obtained using a limited range of parameters, do not

support a major involvement of pituitary-released ACTH in mediating the aversive properties of morphine.

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In addition to its well-documented positive reinforcing properties (e.g. Woods & Shuster, 1968; Deneau, Yanagita & Seevers, 1969), the opiate drug morphine also possesses aversive properties (e.g. Cappell, LeBlanc & Endrenyi, 1973). Furthermore, these aversive and positive properties appear to be functionally related, and may, in fact, be mediated by the same physiological systems (Sklar & Amit, 1977; Cappell & LeBlanc, 1977). Morphine has, for some time, been known to cause a release of adrenocorticotropin (ACTH) from the pituitary (Selye, 1936; Briggs & Munson, 1955), and since this release has been implicated in the drug's aversive properties (Riley, Jacobs & Lolordo, 1976, 1978; Braveman, 1977), the present investigation was aimed at evaluating the possible involvement of ACTH in mediating the aversive properties of morphine.

In the following sections, the stimulus properties of morphine will be briefly reviewed by describing behavioural consequences of the administration of the drug, as well as possible common mechanisms mediating these behaviours. Following this, a general description of ACTH effects exerted centrally will be presented. Finally, a summary of the literature implicating ACTH as a mediator of the aversive effects of morphine, among other compounds, will be discussed.

It has been apparent for some time, that morphine possesses positive reinforcing properties. In addition, these properties appear to be independent of the ability of the drug to produce physical dependence. In 1957, Beach reported that rats who had been repeatedly treated with morphine, exhibited a preference for stimuli associated with drug injections, even when not undergoing withdrawal. This initial finding suggested that it was not necessary to invoke a "need state" notion to account for all morphine-oriented responding. The intrinsic positive reinforcing properties of morphine have since been confirmed in numerous self-administration studies. Drug-naive laboratory animals learned to perform operants for infusions of morphine delivered intravenously (Deneau, Yanagita & Seevers, 1969; Weeks & Collins, 1964; Woods & Shuster, 1968), intragastrically (Smith, Werner & Davis, 1975) and intraventricularly (Amit, Brown & Sklar, 1976), as well as consuming morphine solutions orally (Stolerman & Kumar, 1960; Nichols, 1968). Withdrawal signs, which are a necessary criteria for physical dependence, were not evident when morphine ceased to be available intravenously (Woods & Shuster, 1968), nor were they evident following administration of the opiate antagonist naloxone in the intraventricular study (Amit et al., 1976). Thus, it appears that the intrinsically

positive reinforcing properties of morphine are sufficient to support the acquisition and maintenance of morphine self-administration.

In the light of these positive reinforcing properties, the demonstration that morphine can be seen to possess aversive properties in the conditioned taste aversion (CTA) paradigm (Cappell et al., 1973; Jacquet, 1973) seems paradoxical. Specifically, it was discovered that when morphine was administered to rats immediately following exposure to a novel-tasting fluid, consumption of this fluid upon subsequent exposure was diminished. This diminished consumption suggested that the drug experience was somehow aversive (Cappell et al., 1973). Consequently, morphine, as well as the majority of other psychoactive drugs (e.g. ethanol, Berman & Cannon (1974); amphetamine, mescaline, Cappell & LeBlanc (1971)), have been added to a lengthy list of stimulus events (e.g. lithium chloride, radiation, Garcia & Koelling (1966); cf. Riley & Clarke (1977)) which are known to function as aversive UCSs in the CTA paradigm.

However, in contrast to the toxicity effects which are clearly operating with, for example, lithium chloride (LiCl), a drug which produces gastro-intestinal illness, the doses of morphine that are used for aversive conditioning, are apparently non-toxic. These doses are

within the range of unit doses which rats will self-administer (Weeks & Collins, 1971; Smith et al., 1975). In addition, the work of White, Sklar and Amit (1977) appears to mitigate against the morphine-induced CTA being merely a toxicity effect. Food deprived rats were trained to run down an alley for food. Once trained, the rats were then exposed to a novel-tasting food in the goal box, followed by injections of CTA-producing doses of morphine. Repeated taste-drug pairings revealed that the rats increased their running speed to reach the goal box, but once there, consumed less of the flavored food as compared to saline-treated controls. In contrast, LiCl-treated rats significantly reduced both running speed and food consumed. This demonstration of simultaneously expressed reinforcement and aversion resulting from morphine treatment, has been replicated and extended by Switzman, Amit, White and Fishman (1978). Using the same paradigm, these investigators further reported evidence of a correlation between these apparently conflicting responses, such that the amount of food eaten was inversely related to the elevation of running speed. Thus, it appears that morphine possesses properties, which can yield, depending on the response observed, either approach or avoidance behaviour. The fact that these properties can be observed

simultaneously, and that the extent to which they control behaviour is correlated, suggests that the physiological systems which translate them into behaviour are functionally related.

Information regarding the nature of these mediating mechanisms is provided by studies which examine the effects of altered neurotransmitter levels on either the approach or avoidance component. Treatment with the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine (AMPT), prior to conditioning in the CTA paradigm, blocked the morphine-induced CTA (Sklar & Amit, 1977). Administration of AMPT, which results in a depletion of central catecholamines, attenuated oral consumption of morphine (Glick, Zimmerberg & Charap, 1973) as well as intravenous morphine self-administration in rats (Davis & Smith, 1973). In addition, depletions of central noradrenaline by inhibition of the enzyme dopamine-beta--hydroxylase (DBH), blocked both the CTA produced by morphine (Sklar & Amit, 1977), as well as intravenous (Davis, Smith & Khalsa, 1975) and oral (Brown, Amit, Sinyor, Rockman & Ögren, 1978) self-administration of morphine.

Thus, there appears to be a commonality of neurophysiological systems mediating both the aversive as well as the reinforcing properties of morphine. The

CTA paradigm, as a means to elucidate morphine action, becomes even more interesting. More explicitly, any manipulation which can modify the aversive components becomes potentially more interesting, by the information it may provide on aspects of positive reinforcement.

One such manipulation which has generated considerable research interest, involves the effects of prior experience with the conditioning agent, on the ability of that agent to produce a CTA. Morphine-induced CTA is blocked by pre-exposure to the drug (LeBlanc & Cappell, 1974; Parker, Failor & Weidman, 1973). Parker et al. (1973) have suggested that prior exposure to morphine results in a physiological "need state" which is alleviated by morphine during conditioning. According to this notion, the novel food which preceded morphine injection may have come to be associated with the subsequent alleviation, and hence, was readily consumed on later exposure.

Pre-exposure effects in attenuating CTAs are seen with a variety of other psychoactive drugs (e.g. amphetamine, LeBlanc & Cappell (1974); amobarbital, Vogel & Nathan (1976)). Moreover, Goudie and Thornton (1975) have shown that prior experience with fenfluramine, a drug which is neither self-administered nor produces physical dependence, attenuates the normally observed

CTA when it is used as the conditioning agent. While this study does not address itself specifically to the issue of the CTA produced by morphine, it nevertheless calls into question the Parker et al. (1973) hypothesis, as regards pre-exposure effects in general. Accordingly, these effects have alternatively been interpreted as resulting from the reduced novelty of the drug experience (Amit & Baum, 1970; Gamzu, 1977). While the novelty hypothesis presents problems in terms of being directly assessed, it should be noted that evidence for pre-exposure of one drug attenuating the CTA produced by another (Goudie & Thornton, 1975; Vogel & Nathan, 1976) is not predicted from a strict interpretation of this hypothesis.

Another hypothesis which has been proposed to account for pre-exposure data, is the tolerance hypothesis, which, although not without its problems, appeared to emerge relatively unscathed from an analysis of pre-exposure effects (Goudie & Thornton, 1975; Cappell & LeBlanc, 1977). According to this hypothesis, it is suggested that repeated drug administrations result in progressively diminishing physiological responses to the drug. Subsequently, at the time of conditioning, the aversive UCS properties of the drug are not as salient as in a drug-naive animal. It would appear that the cross-drug

effects mentioned previously (e.g. Goudie & Thornton, 1975) would present problems for such a hypothesis. In fact, Braveman (1975) has demonstrated that the aversion induced by rotation was attenuated following pre-exposure to such diverse drugs as amphetamine, scopolamine, and LiCl. However, pre-exposure effects resulting from such cross-treatments might be accounted for by the tolerance of some physiological event, common to different drugs or treatments (e.g. rotation) which results in the reduced salience of the conditioning agent.

Even though the tolerance hypothesis can account for cross-drug effects, and at one time was thought to be tenable (Goudie & Thornton, 1975; Cappell & LeBlanc, 1977), it is now thought that physiological tolerance alone cannot account for all of the pre-exposure data. For example, it has been shown that if morphine injections during pre-exposure are paired with a distinctive taste, the subsequent CTA induced by morphine, is not attenuated (Stewart & Eikelboom, 1978). In addition, the recent demonstration of asymmetrical cross-drug effects in a pre-treatment paradigm (Brown, Amit, Smith & Rockman, 1978) presents problems for a tolerance notion. One would expect to see reciprocal effects if pre-exposure effects were to be explained through tolerance. As a result, it has been suggested that associative interference can

account for the pre-exposure data, i.e. the UCS becomes associated with some unintended cue during pre-exposure, which then interferes with subsequent conditioning (Poulos & Cappell, 1979; Brown et al., 1979; Stewart & Eikelboom, 1978).

Nevertheless, it is partly on the basis of a tolerance argument that several investigators have suggested that it is the release of ACTH from the pituitary at conditioning, and the subsequent adrenal output of glucocorticoids, that underlies CTA (Riley et al., 1976, 1978; Braveman, 1977).

ACTH is an anterior pituitary hormone which is released in response to a wide variety of environmental stimuli (Selye, 1977). The hormone, a polypeptide consisting of 39 amino acids, is named for its effects on the adrenal cortex, causing a release of glucocorticoids as well as a growth of adrenocortical cells. The metabolic and anti-inflammatory properties of glucocorticoids are known to be critical components in an organism's response to stress (Selye, 1977).

It is well-established that ACTH can also exert central effects. Murphy & Miller (1955) were the first to report on the effects of ACTH in delaying extinction, when administered prior to test sessions in an active avoidance paradigm. Since that time, a vast literature

describing diverse central effects of ACTH on learned and unlearned behaviour, has been steadily accumulating (see reviews in Beckwith & Sandman, 1978; Dunn & Gispen, 1977). DeWied and his colleagues have shown that fragments of the hormone which are devoid of any corticotrophic activity, possess similar behavioural effects, as the full chain (DeWied, 1974). Administration of ACTH or its fragments, reinstates active avoidance learning in hypophysectomized rats and delays extinction of active avoidance behaviour in intact rats (Greven & DeWied, 1973). In addition, ACTH delays extinction of appetitively (Garrud, Gray & DeWied, 1974) as well as sexually motivated (Bohus, 1975) behaviour. ACTH also produces effects in passive avoidance paradigms, delaying extinction of avoidance (Greven & DeWied, 1973), as well as alleviating amnesia induced by CO_2 in a one-trial passive avoidance situation (Rigter, Van Riezen & DeWied, 1974).

The likelihood that these effects, seen in different learning paradigms, are the result of stereospecific binding in the CNS, is suggested by evidence that the potency and even the direction of the effects, can be dramatically altered by the substitution of one or more amino acids within the ACTH fragments (see DeWied, 1974). Furthermore, there is accruing evidence suggesting that ACTH can interact with opiate receptor systems. ACTH

is one of the few non-opiate-like substances which possesses affinity for opiate receptors *in vitro* (Terenius, 1975; Terenius, Gispen & DeWied, 1975). ACTH also mimics the inhibitory action produced by morphine on contractions of the mouse vas deferens (Plomp & Van Ree, 1978). Furthermore, naloxone treatment blocks both the appearance of a stereotyped behaviour produced by central administration of ACTH, as well as the development of tolerance to a second administration (Jolles, Wiegant & Gispen, 1978). Moreover, these investigators report some evidence of cross-tolerance between ACTH and morphine, such that if morphine is infused first, the reduced behavioural response to a subsequent infusion of ACTH is seen. However, it has also been shown that ACTH can act as an opiate receptor antagonist. ACTH has been shown to possess mixed agonist/antagonist properties *in vitro* (Terenius, 1976), and this may account for the counteraction, by ACTH, of morphine-induced analgesia (Gispen, Buitelaar, Wiegant, Terenius & DeWied, 1976) as well as the reduction *in vitro* and *in vivo*, of spinal reflex activity (Zimmerman & Krivoy, 1973).

Riley et al. (1976) have proposed that since most CTA-producing drugs and treatments cause a release of ACTH, the possibility exists that it is this release and its resultant effects, which may provide the salient

aversive cues, that are expressed as CTAs. They further suggest that since ACTH release tolerates with repeated drug administrations, this may account for the attenuation of the CTAs by pre-exposure, both in cases where the pre-exposing and conditioning drugs are the same, as well as in the cross-treatment (Braveman, 1975) situation (Riley et al., 1976). Consistent with this hypothesis, there is some evidence that ACTH serves as an important component of the CTA produced by LiCl. It has been shown that treatment with dexamethasone, a synthetic corticosteroid which inhibits the secretion of ACTH, attenuates the CTA produced by LiCl, when administered prior to conditioning (Hennessy, Smotherman & Levine, 1976). It has also been shown that the administration of ACTH prior to recovery sessions, prolongs extinction from a LiCl-induced CTA (Kendler, Hennessy, Smotherman & Levine, 1976). This latter effect is apparently not attributable to the action of corticosteroids, which are released by ACTH treatment, since an ACTH fragment similarly prolongs recovery while having no steroid releasing properties (Rigter, 1975; Rigter & Popping, 1976; Smotherman & Levine, 1978).

Additional evidence for ACTH involvement in CTA, although decidedly correlational, is provided by CTA studies demonstrating corticosteroid elevations during

testing trials, following conditioning with CTA-producing agents (Ader, 1976; Smotherman, Hennessy & Levine, 1976). These conditioned corticosteroid elevations, seen after one conditioning trial, were equivalent to those elicited by the conditioning drug itself (Ader, 1976) and were not seen when animals could avoid consuming the drug in a free-choice CTA paradigm (Smotherman et al., 1976). These investigators suggest that this conditioned release of ACTH and/or corticosteroids results in a reinstatement of part of the stimulus complex which was experienced during conditioning. This, in turn, results in a retrieval of the "memory" of the illness which then results in avoidance of the paired substance (Ader, 1976; Smotherman et al., 1976).

One well established phenomenon associated with morphine administration is the accompanying pituitary release of ACTH, as indicated by increased plasma corticosteroid levels (Selye, 1936; Briggs & Munson, 1955; Kokka, Garcia & Elliot, 1973). Interestingly, morphine can also inhibit stress-induced ACTH release (Briggs & Munson, 1955). Both of these effects are blocked by prior administration of opiate antagonists (Kokka et al., 1973; Briggs & Munson, 1955). Furthermore, when morphine is repeatedly administered, the stimulant effect on ACTH release tolerates with as few as four

administrations (Kokka et al., 1973).

Thus, there are noteworthy parallels between the characteristics of morphine-induced CTA and morphine-induced ACTH release. Both the CTA (Cappell et al., 1973) and the release (Kokka et al., 1973) are seen with acute administration, and both (Parker et al., 1973; Kokka et al., 1973) are blocked by repeated administration. Furthermore, naloxone treatment attenuates both the morphine-induced CTA (LeBlanc & Cappell, 1975), as well as the morphine-induced release of ACTH (Kokka et al., 1973). This apparent correlation between the release of ACTH and the morphine-induced CTA raises the possibility that this release may mediate the aversive properties of morphine.

The purpose of the following experiments was to assess the possible involvement of ACTH in mediating the aversive properties of morphine.

EXPERIMENT 1

If, as various investigators (Riley et al., 1976, 1978; Hennessy et al., 1976; Braveman, 1977) suggest, ACTH can function as an aversive cue in mediating CTAs, it should be possible to demonstrate aversive properties of administered ACTH. With the exception of some evidence for this provided by a report describing intravenous ACTH self-administration which diminishes below the operant rate at higher doses (Jouhaneau-Bowers & LeMagnen, 1979), the only attempt, to date, to demonstrate a CTA using ACTH as the conditioning agent, was unsuccessful (Smotherman & Levine, 1978).

Furthermore, it has been shown that the administration of ACTH or ACTH fragments prior to recovery sessions prolongs the extinction from a LiCl aversion (Kendler et al., 1976; Rigter, 1975; Rigter & Popping, 1976; Smotherman & Levine, 1978). In addition, Dray and Taylor (1979), using rat pups, have recently shown that the administration of $ACTH_{4-10}$ prior to testing for the LiCl-induced CTA, results in the appearance of an aversion which is not seen without it. However, these studies do not provide any information as to the role of ACTH as an aversive cue, since ACTH is administered prior to recovery sessions in all cases. The behaviour of animals under

the influence of ACTH is confounded by hormone action on any one of several mechanisms (e.g. fear) thought to account for ACTH effects on avoidance behaviours (see Beckwith & Sandman, 1978).

In order to demonstrate that the ACTH released during conditioning is an important contributor to the stimulus complex that results in a CTA, it would be necessary to demonstrate that ACTH treatment at this time, can exert effects on the acquisition of the CTA. ACTH administered at the time of conditioning has no effect upon the magnitude of the CTA produced by LiCl (Kendler et al., 1976). However, it is difficult to demonstrate an enhancement of the LiCl-CTA, since these aversions are characterized by almost complete suppression of test day drinking, both in forced choice (Sklar & Amit, 1977) as well as in free choice (Kendler et al., 1976) paradigms. In contrast, the moderately weak morphine-induced CTA affords an opportunity of assessing ACTH effects on acquisition, when it is administered during conditioning.

In pilot work, it was seen that across a dose range of 5, 10, and 20 I.U./kg, a CTA was obtained at the 20 I.U./kg dose of ACTH (Switzman & Amir, Note 1). In this experiment, a dose of 10 I.U./kg was administered along with one of four doses of morphine. This was done to

test for a possible summation of the exogenous ACTH and the morphine-induced endogenous release of ACTH. If ACTH is a critical component in morphine-CTA, it would be expected that this summation would be reflected behaviourally in this paradigm. The effects of this non-aversive dose of ACTH, when coupled with the non-aversive doses of morphine used here, was deemed of particular interest.

Method

Subjects. The subjects were 146 male Wistar rats (Canadian Breeding Farms and Labs, Ltd.) weighing approximately 200-250 grams at the beginning of the experiment. The animals were housed individually in stainless steel cages with free access to Purina Lab Chow and water.

Drugs. Morphine hydrochloride (May & Baker Canada, Ltd.) and ACTH (ACTHAR, Armour Pharmaceuticals) were dissolved in injectable Ringer's solution (Abbott Laboratories).

Procedure. Following seven days of adaptation to laboratory housing conditions, the rats were placed on a 23 hour 40 minute water deprivation schedule. A water-filled test tube with a double ball-bearing spout was presented to each rat in the home cage for 20 minutes at the same time each day for seven consecutive

days. Fluid intake during the first ten minutes was measured to the nearest ml throughout the experiment.

On the eighth day (conditioning day) a novel tasting saccharin-water (0.1% w/v) solution was presented for ten minutes, followed within one minute by two successive intraperitoneal injections, each administered on either side of the midline. The rats had been randomly assigned to one of ten treatment conditions, in a factorial design, where ACTH or Ringer's were injected along with Ringer's or one of four doses of morphine. Morphine doses used were 2.25, 4.5, 9 or 18 mg/kg; the dose of ACTH used was 10 I.U./kg throughout. The volume of all injections was 1 ml/kg.

After conditioning, rats were returned to the water deprivation schedule, receiving water for 20 minutes a day for the next five days. On the sixth day after conditioning (test day), the saccharin solution was presented once more, and fluid intake during these 10 minutes was measured.

Results

There were no significant differences in conditioning day intake among any of the groups (one way ANOVA: ($F(9,136) = 1.90$, $p>.10$) with animals drinking a mean of 13.4 mls on conditioning day. Each rat's test day intake was expressed as a percentage of his conditioning

(baseline) day intake, and mean scores (± S.E.M.) for all groups are presented in Figure 1. As can be seen, both the Ringer's-Ringer's and ACTH-Ringer's groups drank above baseline, and these did not differ significantly from each other ($t = 1.086$, $df = 18$, $p>.10$). The individual percentage scores were transformed logarithmically, and a subsequent analysis of variance of the morphine groups revealed a significant treatment x morphine dose interaction ($F(3,118) = 3.30$, $p<.03$). Further analysis revealed a highly significant effect of treatment at the 4.5 mg/kg morphine dose (simple main effects post hoc test: $F(1,118) = 13.158$, $p<.0005$). Whereas the Ringer's-Morphine group drank above baseline, the ACTH-Morphine group manifested an aversion. No effect of treatment was seen at any of the other doses.

The raw data for this experiment can be found in the Appendix, Table 1.

Discussion

The results of this experiment indicate that a dose of ACTH which does not manifest any aversive properties in this paradigm, can, when administered with a non-aversion-producing dose of morphine, result in a substantial aversion. This aversion was equivalent in magnitude to the expected attenuations in drinking seen at the two higher doses of morphine.

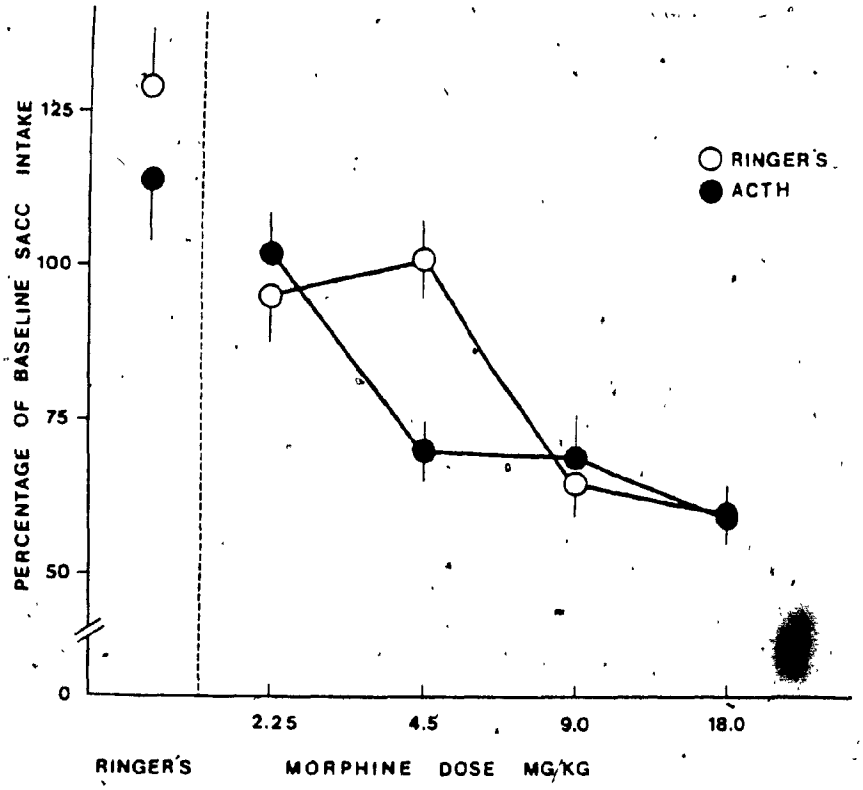

Figure 1. Test day intake for all groups expressed as a percentage (± S.E.M.) of conditioning (baseline) day intake. At conditioning, rats first received an injection of Ringer's (o-o) or ACTH 10 I.U./kg (●-●) immediately followed by another injection of either Ringer's or one of four doses of morphine, as shown on x-axis.

Whether the potentiation of ACTH at the 4.5 mg/kg dose represents a summation of morphine and ACTH on some common substrate(s) is open to question. It is possible that the aversion seen at this dose reflects the nonspecific net effect of administering subthreshold doses of these agents. If that were the case, however, an enhancement, by ACTH, of the aversions seen at the two higher doses, would be expected (i.e. if ACTH was expressing aversive properties through action on systems entirely distinct from those on which morphine is acting).

Furthermore, the fact that the CTAs seen at the two higher doses were equivalent in magnitude, suggests that at the 9 mg/kg dose, there may be a saturation of the system(s) underlying the aversion. The comparable aversion seen at the ACTH-Morphine 4.5 mg/kg treatment may be indicative of ACTH and morphine summing their effects to saturate these same systems. The nature of this summation, as well as the involvement of corticosteroids, is open to question. Nevertheless, it is possible that the effect seen at the 4.5 mg/kg dose reflects a summation of endogenous ACTH along with the exogenous ACTH, with both contributing to the internal stimulus complex which has been implicated as a mediator of CTAs (Riley et al., 1978; Braveman, 1977).

In this context, it is noteworthy that ACTH release, as measured by corticosteroid levels, is not significant at a dose of 5 mg/kg of morphine, whereas at doses of 10 and 20 mg/kg, the levels differ significantly from saline-treated controls (Kokka et al., 1973). Thus, the failure to obtain a CTA at the 4.5 mg/kg dose of morphine (Ringer's), as well as the expected CTAs at the 9 and 18 mg/kg doses, are not inconsistent with an ACTH-mediating hypothesis. It is possible then, that the increased ACTH levels which result from morphine administration at these higher doses, underlie the morphine-induced CTA as has been suggested by Riley et al. (1978). This possibility was investigated in the next experiment.

EXPERIMENT 2

Riley et al. (1976) have suggested that the drug-induced release of ACTH from the pituitary at the time of conditioning, may underlie the aversions to a variety of CTA-producing agents. In a later paper, these investigators claim that the characteristics of ACTH release induced by LiCl and by morphine, differ, and do so in a manner which correlates with the type and strength of CTA that they produce. Thus, the CTA to morphine is less potent and more variable than that obtained with LiCl, and this is paralleled by a weaker, more variable release of ACTH to morphine administration than that seen with LiCl administration (Riley et al., 1978).

Support for the hypothesis that pituitary-adrenal activation underlies LiCl-induced aversions is provided by the work of Hennessy et al. (1976). These investigators administered the synthetic corticosteroid dexamethasone prior to LiCl conditioning, and found that it both suppressed LiCl-induced release of ACTH (as measured by corticosteroid levels) as well as attenuating the LiCl CTA (Hennessy et al., 1976).

It has also been shown that morphine-induced ACTH release is completely blocked by pretreatment with

dexamethasone (Zimmerman, Branch, Taylor, Young & Pang, 1974). The following experiment employed dexamethasone pretreatment at conditioning in order to assess the contribution of this release to the morphine-induced CTA.

Method

Subjects. Subjects were 42 male Wistar rats (Canadian Breeding Farms and Labs, Ltd.) weighing approximately 200-250 grams at the beginning of the experiment. The animals were housed individually in stainless steel cages with free access to Purina Lab Chow and water.

Drugs. Morphine hydrochloride (May & Baker, Montreal) was dissolved in injectable Ringer's solution (Abbott Laboratories). Dexamethasone phosphate supplied in a 4 mg/ml solution (Decadron, Merck, Sharp & Dohme) was diluted with injectable Ringer's solution.

Procedure. The pre-conditioning procedure was identical to that used in the first experiment. On the eighth day (conditioning day) the rats were randomly assigned to treatment groups with three groups of 10 rats per group receiving subcutaneous injections of either Ringer's, dexamethasone (100 µg/kg), or dexamethasone (200 µg/kg). A fourth group of 12 rats received injections of 400 µg/kg. The volume of all

injections was 1 ml/kg. These injections were administered one hour before a 10 minute presentation of the novel-tasting saccharin-water (0.1% w/v) solution.

One minute following removal of the saccharin one-half of the rats in each treatment condition received I.P. injections of Ringer's, while the other half received I.P. injections of morphine hydrochloride (9 mg/kg). There were thus eight treatment combinations in all. The post-conditioning procedure was identical to that used in the first experiment.

Results

There were no significant differences in conditioning day intake among any of the groups (one-way ANOVA = $F(7,34) = 1.30$, $p>.20$) with animals drinking a mean of 15 mls on conditioning day. Each rat's test day intake was expressed as a percentage of his conditioning day intake, and mean scores (± S.E.M.) for all groups are presented in Figure 2. Analysis of variance revealed no significant effect of pretreatment ($F(3,34) = 1.26$, $p>.30$), a highly significant treatment effect ($F(1,34) = 76.13$, $p<.0001$) and no pretreatment-treatment interaction ($F(3,34) = .11$, $p>.90$). Thus, dexamethasone at three different doses did not have any effect on the CTA to morphine.

The raw data for this experiment can be found in the appendix, Table 2.

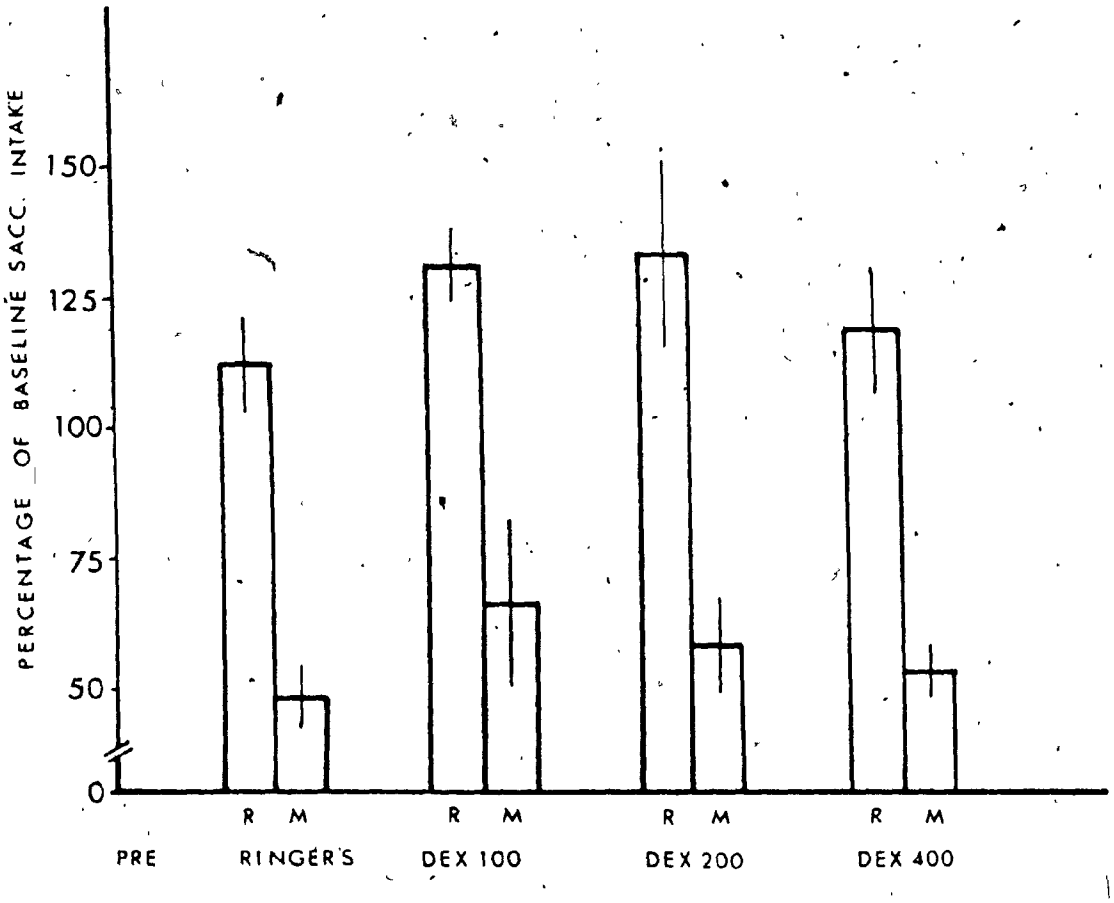

Figure 2. Test day intake for all groups expressed as a percentage (± S.E.M.) of conditioning (baseline) day intake. One hour prior to saccharin presentation on conditioning day, rats received pretreatment (PRE) injections of either Ringer's (R), Dexamethasone 100 µg/kg (DEX 100), 200 µg/kg (DEX 200) or 400 µg/kg (DEX 400). This was followed by conditioning with either Ringer's (R) or morphine 9. mg/kg (M).

Discussion

The results of this experiment indicate that the blockade of morphine-induced ACTH release at the time of conditioning, does not attenuate the morphine-induced CTA. According to a report by Zimmerman et al.(1974), 100 or 400 µg/kg dexamethasone administered prior to morphine (30 mg/kg) administration in rats, completely blocked the corticosteroid increase, and presumably the ACTH release, that normally accompanies morphine administration. Therefore, these results suggest that the morphine-induced release of ACTH does not measurably contribute to the morphine-induced CTA.

These data are not consistent with the results of Hennessy et al. (1976), regarding the suppression of LiCl-induced CTA by dexamethasone pretreatment. It should be mentioned that these investigators performed a biochemical assay to ensure that the dexamethasone treatment in fact suppressed the LiCl-induced corticosteroid release. No such assay was performed here, this work being predicated upon the previously mentioned assay work (Zimmerman et al., 1974). Furthermore, although any comparison between studies mentioned previously (Kendler et al., 1976; Hennessy et al., 1976; Smotherman & Levine, 1978) and the experiments reported here must be cautious, given the

different conditioning agents used, there is also a procedural difference between paradigms which may be of particular importance here. The report describing suppression of LiCl-induced CTA employed a free choice situation where animals were not water-deprived. It has been shown that water-deprivation in rats, results in an altered adrenocortical rhythm, such that the peak in corticosteroid levels occurs just prior to water presentation (Johnson & Levine, 1973). There is thus the added complication in this experiment of high circulating levels of ACTH and of steroids, just prior to conditioning. It is assumed that dexamethasone treatment blocked the morphine-induced release of ACTH (Zimmerman et al., 1974). However, the levels of ACTH being elevated regardless (Johnson & Levine, 1973), may have interacted with the stimulus properties of morphine to produce the CTAs seen. ACTH has been suggested to act on memory, attentional, or motivational processes (Beckwith & Sandman, 1978); it is possible that the high levels of ACTH here may have, through one of these processes, directed attention to, or heightened the salience of, the stimulus properties of morphine. A free-choice situation might have perhaps allowed us to see an effect of dexamethasone suppression on the morphine-induced CTA. This confound notwithstanding,

the results reported here do not support an involvement of ACTH in the morphine-induced CTA. The following experiment was undertaken to further examine this suggested involvement.

EXPERIMENT 3

While the attenuation of the CTA produced by a drug, by prior exposure to that drug, can be understood in terms of physiological tolerance, the cross-treatment effects reported by Braveman (1975) suggests that cross-tolerance of different drugs can be accounted for by actions of these drugs on common physiological substrates. However, the asymmetry of these cross-drug pre-exposure effects (Goudie & Thornton, 1975) suggests not a simple overlap of the systems that mediate aversive drug effects.

Nevertheless, the assumption seems warranted that if drugs can be shown, by pre-exposure, to attenuate each other's CTA, the possibility exists that they may be acting on common systems to produce their aversive properties. As previously mentioned, ACTH is one of the few non-opiate like substances which possesses an affinity for opiate receptors *in vitro* (Terenius, 1975). ACTH fragments mimic morphine effects on the contractions of the mouse vas deferens (Plomp & Van Ree, 1978). Intraventricular infusions of ACTH in the rat result in excessive grooming, a behaviour which is blocked by pre-treatment with naloxone (Gispen & Wiegant, 1976). Furthermore, as regards this

behaviour, there is evidence of cross-tolerance between intraventricularly infused morphine and ACTH (Jolles, Wiegant & Gispen, 1978).

The following experiment was conducted in order to examine the possibility that ACTH could exert tolerating effects on systems subserving morphine-induced CTA. Specifically, the effects of prior experience with ACTH on morphine's ability to establish a CTA, was examined.

Method

Subjects. Subjects were 24 male Wistar rats (Canadian Breeding Farms and Labs, Ltd.) weighing approximately 200-250 grams at the beginning of the experiment. The animals were housed individually in stainless steel cages with free access to Purina Lab Chow and water.

Drugs. Morphine hydrochloride (May & Baker, Montreal) and ACTH (ACTHAR, Armour Pharmaceuticals) were dissolved in injectable Ringer's solution (Abbott Laboratories).

Procedures. The pre-conditioning procedure was identical to that used in the first experiment, with the changes in procedure noted.

Approximately two hours following water presentation on the second day, the rats were randomly assigned to one of two treatment conditions, with 12 rats receiving

intraperitoneal injections of ACTH (20 I.U./kg) while the other 12 received intraperitoneal injections of Ringer's. This procedure was repeated on the fourth day and the sixth day of water presentation. There were thus three pre-exposures to either Ringer's or ACTH.

On the eighth day (conditioning day), a novel-tasting saccharin-water (0.1% w/v) solution was presented for 10 minutes. One minute following removal of the tubes, 6 rats from each of the treatment groups received intraperitoneal injections of morphine hydrochloride (9 mg/kg), while the other 6 rats from each group received intraperitoneal injections of Ringer's.

The post-conditioning procedure was identical to that used in the first experiment.

Results

There were no significant differences in conditioning day intake among any of the groups (one-way ANOVA = $F(3,20) = 1.47, p>.20$) with animals drinking a mean of 12.0 mls. Each rat's test day intake was expressed as a percentage of his conditioning day intake, and mean scores (± S.E.M.) for all groups are presented in Figure 3. Analysis of variance revealed no significant effect of pre-exposure ($F(1,20) = .703, p>.50$), a highly significant effect of treatment on conditioning day ($F(1,20) = 63.9, p<.0001$), and a non-significant pre-exposure x treatment

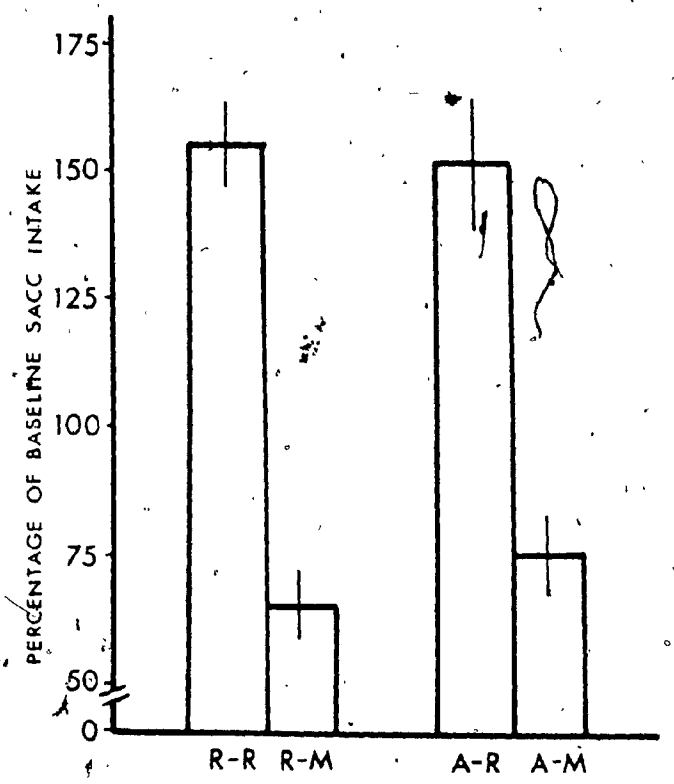

Figure 3. Test day intake for all groups expressed as a percentage (± S.E.M.) of conditioning (baseline) day intake. Pre-exposure injections of either Ringer's (R), or ACTH 20 I.U./kg (A) were given on days 2, 4 and 6 of the water-deprivation schedule. On conditioning days, rats received either Ringer's (R) or morphine 9 mg/kg (M). First letter of pair refers to pre-exposure agent, second, to conditioning agent.

interaction ($F(1,20) = .47$, $p>.50$). Thus, pre-exposure with ACTH failed to attenuate the morphine CTA.

The raw data for this experiment can be found in the appendix, Table 3.

Discussion

Pre-exposure to ACTH did not have any attenuating effect on the morphine-induced CTA. This finding further supports the possibility that ACTH is not the mediating mechanism in the morphine-induced CTA. Alternatively, it is possible that the dose of ACTH used, was insufficient to produce tolerance to the extent afforded by morphine-induced ACTH release, in the morphine pre-exposure situation.

Consistent with this possibility, Goudie, Taylor and Wheeler (1975) have shown the pre-exposure effects are dependent upon the relation of the pre-exposure dose to that used at conditioning. Pre-exposure to an agent at a lower dose than that used for conditioning, resulted in only a mild attenuation of the CTA. This can be explained in terms of incomplete tolerance (Goudie et al., 1975; Riley et al., 1976). However, the dose of ACTH used here was based on the pilot study (Note 1) in which was seen a CTA induced by ACTH which was equal in magnitude to that seen with morphine at 9 mg/kg. It was on the basis of this, that this combination of

pre-exposure and conditioning doses were used.

It was decided in the next experiment to confirm the pilot work demonstrating aversive properties of ACTH. It also seemed possible that these aversive properties may have been expressed through ACTH action on systems separate from those upon which morphine exerts its effects. As such, the tolerance that presumably developed to ACTH pre-exposure may have been irrelevant to the morphine drug experience. This would account for the results seen here, and this possibility was examined in the next experiment.

EXPERIMENT 4

The purpose of this experiment was two-fold. First, to examine the aversive properties of ACTH at the dose used in the previous experiment. Second, to examine the effects of naloxone pre-treatment on the expected ACTH-induced CTA. Naloxone was used here in a dose known to attenuate morphine-induced CTA (LeBlanc & Cappell, 1975). If, in fact, the aversive properties of ACTH could be antagonized by naloxone, then the possibility would exist that ACTH is expressing its aversive properties through action on those systems (i.e. opiate receptors) which appear to mediate morphine's aversive properties (LeBlanc & Cappell, 1975).

Method

Subjects. Subjects were 19 male Wistar rats (Canadian Breeding Farms and Labs, Ltd.) weighing approximately 200-250 grams at the beginning of the experiment. The animals were housed individually in stainless steel cages with free access to Purina Lab Chow and water.

Drugs. Naloxone hydrochloride (Endo Labs) and ACTH (ACTHAR, Armour Pharmaceuticals) were dissolved in injectable Ringer's solution (Abbott Laboratories).

Procedure. The pre-conditioning procedure was identical to that used in the first experiment. On the eighth day (conditioning day), a novel tasting saccharin-water solution was presented for 10 minutes. One minute following removal of the tubes, the rats were randomly assigned to one of two treatment conditions, with 9 rats receiving intraperitoneal injections of Ringer's, while the other 10 received intraperitoneal injections of naloxone hydrochloride (10 mg/kg). Five minutes following these injections, 4 of the rats in the first treatment condition and 5 in the second received intraperitoneal injections of Ringer's while the remaining 5 rats in each treatment condition received ACTH (20 I.U./kg). It was necessary to pretreat the rats following the drinking since naloxone affects drinking in this paradigm (Eikelboom & Stewart, Note 2). The post-conditioning procedure was identical to that used in the first experiment.

Results

There were no significant differences in conditioning day intake among any of the groups (one-way ANOVA: $F(3,15) = .87$, $p > .50$) with animals drinking a mean of 12.0 mls. Each rat's test day intake was expressed as a percentage of his conditioning day intake, and mean scores (± S.E.M.) for all groups are presented in

Figure 4. Analysis of variance revealed no significant effect of pretreatment ($F(1,15) = 2.57$, $p>.10$), no significant effect of conditioning day treatment ($F(1,15) = 1.83$, $p>.10$) and no pretreatment-treatment interaction ($F(1,15) = 2.26$, $p>.10$). Thus, ACTH did not manifest any aversive properties. Indeed, visual inspection reveals what appears to be a conditioned taste preference in the R/A group but this finding was not significant.

The raw data for this experiment can be found in the appendix, Table 4.

Discussion

ACTH, when used as the conditioning agent, failed to produce a CTA, even though the dose used was seen to possess aversive properties in a pilot study (Note 1). It is unclear why this discrepancy occurred. However, the work reported here differs from the pilot study in that a pretreatment injection preceded ACTH treatment; this injection may have interfered with the salience of the subsequent ACTH injection. Alternatively, the temporal distancing (5 minutes) of the saccharin drinking from the ACTH injection may have impaired conditioning, although this appears unlikely, given previous work on gustatory conditioning (Garcia & Koelling, 1966). Nevertheless, these findings would

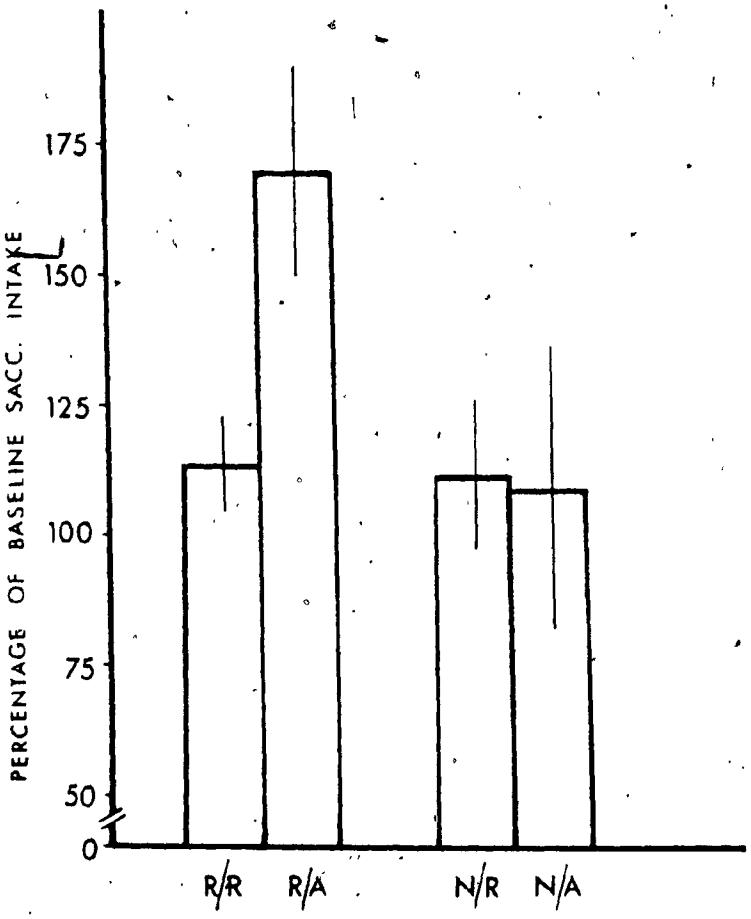

Figure 4. Test day intake for all groups expressed as a percentage (± S.E.M.) of conditioning (baseline) day intake. One minute following saccharin removal on conditioning day, rats were pre-treated with either Ringer's (R) or naloxone 10 mg/kg (N). Five minutes later, rats received injections of either Ringer's (R) or ACTH 20 I.U./kg (A). First letter of pair refers to pre-treatment agent, second, to conditioning agent.

seem to corroborate the absence of aversive properties of exogenous ACTH, seen in the first experiment, as well as that described in a previous report (Smotherman & Levine, 1978).

An alternative possibility is that ACTH does possess aversive properties, but that its rapid systemic degradation (Hudson, Ambler, Bennett & McMartin, 1979) precludes the expression of these properties. Duration of action has been suggested to be an important correlate of the ability of a drug to produce a CTA (Goudie, Dickens & Thornton, 1978), and the rapid metabolism of the hormone may account for the results seen here.

It is interesting to note that the Ringer's-ACTH group consistently increased their intake on test day relative to conditioning day. It is possible that ACTH treatment enhanced or aided recall of the reinforcing properties of the saccharin solution, through action on one or more of the processes upon which ACTH is hypothesized to exert its effects in various learning paradigms (Beckwith & Sandman, 1978). Naloxone appeared to antagonize this effect, and the mechanism(s) involved in this is open to question.

GENERAL DISCUSSION

Given the parameters employed in the present investigation, the results of these experiments do not support a major involvement of ACTH released from the pituitary, in mediating the aversive properties of morphine. In the first experiment, it was shown that ACTH did not manifest any aversive properties. However, when this same dose was administered along with a similarly ineffective dose of morphine, a substantial aversion resulted. The involvement of the endogenous release of ACTH in mediating the morphine-induced CTA, was examined in the second experiment. It was found that the blockade of ACTH release at the time of conditioning, failed to attenuate the CTA produced by morphine. In the third experiment, the possibility that ACTH could act on the same systems that mediate the aversive properties of morphine, was examined. It was expected that prior experience with a dose of ACTH, twice that used in the first experiment, would attenuate the CTA produced by morphine, but this failed to occur. In the fourth experiment, it was shown that this higher dose of ACTH, when used as the conditioning agent, did not produce a CTA. Thus, while these findings are not entirely conclusive, the data do not support a major

contribution, by ACTH, to the morphine-induced CTA, as has been suggested by Riley et al. (1978).

There is one qualification to this conclusion. These experiments do not address the possible role of ACTH synthesized and present in the brain (Watson, Richards & Barchas, 1978) in mediating these properties.

The first experiment provides the only suggestion of any significant effect of ACTH in the CTA paradigm. The potentiation seen here is difficult to interpret, given the absence of any effect of ACTH or manipulation of ACTH, in the subsequent three experiments. However, the possibility exists that while ACTH is not inherently aversive, an aversion results when it is administered in conjunction with another interoceptive cue. This effect may be a function of the enhancement of the discriminative stimulus properties of the injected morphine, or may reflect an effect on the retention or retrieval of a memory trace, as has been suggested by various investigators (Hennessy et al., 1976; Kendler et al., 1976; Rigter et al., 1974). At the higher doses of morphine, no further enhancement of the aversions by ACTH was seen. It is unclear whether a floor effect in drinking is masking an enhanced aversion in these cases, or whether ACTH treatment can no longer further enhance the discriminative properties of an already potent

morphine dose. Additional work, looking at this effect in a two-bottle choice paradigm should be conducted in order to further examine this question. As well, the effects of ACTH treatment on extinction of the aversion would provide additional information in this regard. An enhanced aversion would be expected to be reflected in delayed extinction.

ACTH possesses in vitro (Terenius, Gispen & DeWied, 1975) as well as in vivo (Gispen, Buitelaar, Wiegant, Terenius & DeWied, 1976) affinity for opiate receptor systems, although these do not appear to be involved in the effects of ACTH in the different learning paradigms. It has been shown, for example, that $ACTH_{4-10}$ interferes with the discrimination by rats of the narcotic fentanyl, and this does not appear to be mediated at the level of the opiate receptor (Colpaert, Niemegeers, Janssen, Van Ree & DeWied, 1978). Furthermore, the effect shown here is analogous to that reported recently where LiCl-induced CTA was potentiated by ACTH treatment (Dray & Taylor, 1979). For these reasons, it is unlikely that the effect seen here is due to the action of ACTH on opiate receptor systems, but rather may reflect altered attention to particular components of the narcotic cue, as has already been suggested (Colpaert et al., 1978).

It is difficult to generalize these findings to

CTAs induced by other drugs, notably LiCl, which has been predominantly used in examining hormonal involvement in CTA. Aside from the fact that morphine and LiCl belong to different drug classes, there are methodological differences between the present experiments and those previously reported, e.g. free vs forced choice, timing of manipulation (Kendler et al., 1976; Hennessy et al., 1976; Rigter, 1975), which preclude such a generalization.

Nevertheless, in light of the results obtained here, it appears that the ACTH-mediating hypothesis should be re-examined with regards to supporting empirical evidence. As mentioned earlier, the basis for the hypothesis appears to derive largely from the correlational evidence of, ACTH release at conditioning (Riley et al., 1976), ACTH release at testing (Smotherman et al., 1976; Ader, 1976), tolerance of ACTH release paralleled by attenuation of CTA (Riley et al., 1976), and delay of extinction of the CTA by administration of ACTH prior to testing (Kendler et al., 1976; Rigter, 1975; Smotherman & Levine, 1978). The delay of extinction effect provides the only evidence of a non-correlational nature. However, it is not unique to the CTA paradigm, having been seen in other passive avoidance paradigms (e.g. Greven & DeWied, 1973). Thus, ACTH may be exerting effects in the CTA paradigm, but is not necessarily involved as a mediator.

Hence, these studies in themselves, do not warrant the conclusion that ACTH mediates CTA. It would appear the only study which directly supports an ACTH-mediating hypothesis, is the one describing suppression of the LiCl-induced CTA by dexamethasone (Hennessy et al., 1976). It can be argued that the effect shown by these investigators is not due to levels of ACTH being attenuated, but rather may be due to associative interference by the pretreatment injection as described by other investigators (Brown et al., 1979; Poulos & Cappell, 1979). That possibility notwithstanding, the second experiment of the present study failed to demonstrate any suppression of the morphine-induced CTA by dexamethasone, and, as previously mentioned, procedural differences present problems for comparing these results with those of Hennessy et al. (1976). Nevertheless, some attenuation would have been expected if the ACTH release which accompanies morphine was contributing to the morphine-induced CTA. Furthermore, it would be requisite to demonstrate a CTA with ACTH used as the conditioning agent, if in fact, ACTH were involved in CTAs. This was not apparent with the two doses used in the first and fourth experiments. However, the possibility exists that with different doses and different routes of administration, a CTA may be obtained.

It could be argued that the short half-life of the hormone prevented the exogenous ACTH from exerting an aversive effect, whereas in fact, the morphine-induced release would be qualitatively different so that endogenous ACTH could underlie morphine-induced CTA. However, there is evidence that there is rapid uptake and degradation of the endogenous hormone, when it is released by acute morphine administration (Riley, Note 3). The short duration notion would be equally unable to account for the absence of an effect with ACTH pre-exposure. In this context, it is interesting that pre-exposures to one hour of restraint, with the same procedure used as in the third experiment, failed to attenuate morphine-induced CTA (Sinyor & Amit, Note 4). Since restraint has been shown to elicit a massive release of ACTH (see reviews in Anisman, 1978; Yuwiler, 1978), it would have been expected that pre-exposure would block the morphine-induced CTA. This, of course, would apply only if the functional mechanism mediating the CTA was morphine-induced ACTH release.

In fact, when restraint was used as the UCS in the CTA paradigm, no CTA was obtained (Smith, Galina & Amit, Note 5). These findings, taken together, are consistent with the present results, and support the notion that ACTH does not play a substantial role in the mediation

of CTAs.

The present experiments still leave unanswered the significance of the correlation which exists between morphine administration and ACTH release. It seems likely that this parallel is simply due to the stress effects of drug injection. A variety of stressors, such as restraint, footshock and hypothermic stress, have been shown to cause a release of ACTH. Moreover, as in the case of morphine injections, repeated exposure to these stressors results in an adaptation to the stress and hence, a tolerance of ACTH release (see reviews in Anisman, 1978; Yuwiler, 1976). Thus, it seems possible that ACTH release induced by morphine is simply due to the stress effects of morphine injections.

Recent evidence for a retrograde transport of pituitary peptides into the brain (Mezey, Palkovitz, de Kloet, Verhoef & DeWied, 1978; Oliver, Mical & Porter, 1977) as well as evidence for ACTH localized in brain areas (Watson et al., 1978) raises the intriguing possibility that central ACTH may be involved in mediating morphine actions.

Experiments aimed at further elucidating the role of ACTH in mediating aversive properties of morphine would have to employ a wider range of parameters than those employed here, in order to adequately evaluate

this role. In addition, it appears that an investigation of the role of brain ACTH in this context, would further clarify this issue.

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1. Switzman, L. and Amir, S. Unpublished observations.

2. Eikelboom, R. and Stewart, J. Unpublished observations.

3. Riley, A.J. Personal communication to N.S. Braveman, in Learning Mechanisms in Food Selection, Baylor University Press, 1977.

4. Sinyor, D. and Amit, Z. Unpublished observations.

5. Smith, B.R., Galina, Z.H. and Amit, Z. Unpublished observations.

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APPENDIX

The following appendix contains the saccharin consumption in mls on conditioning and test days, for all experiments.

TABLE 1

Experiment 1: Saccharin consumption on conditioning and test days

R-R		A-R		$R\text{-}M_{2.25}$		$A\text{-}M_{2.25}$		$R\text{-}M_{4.5}$		$A\text{-}M_{4.5}$	
C	T	C	T	C	T	C	T	C	T	C	T
16	20	19	20	18	19	13	6	16	15	19	16
14	21	9	7	17	11	13	15	9	13	13	13
19	14	17	15	11	13	12	11	14	15	16	12
20	22	10	14	13	10	13	16	12	14	15	10
17	19	13	18	10	7	12	11	10	6	13	12
14	19	14	15	11	10	11	14	7	7	16	15
13	16	17	19	13	18	10	12	14	17	16	8
11	16	11	7	15	20	11	13	13	14	15	5
12	16	12	19	11	18	10	7	9	10	15	9
11	21	13	19	19	16	11	20	12	16	14	6
				11	6	15	14	16	9	10	6
				12	11	16	14	14	12	14	10
n = 10		n = 10		14	13	20	17	12	17	16	10
				12	14	15	18	11	6	8	8
				15	17	15	9	9	12	12	3
				10	6	15	9	13	11	9	8
				14	12	15	18	15	15	16	12
				12	6	13	18	11	9	16	15
				18	19	17	13			10	7
				n = 19		n = 19		n = 18		n = 19	

TABLE 1 (Cont'd)

$R\text{-}M_9$		$A\text{-}M_9$		$R\text{-}M_{18}$		$A\text{-}M_{18}$	
C	T	C	T	C	T	C	T
14	7	13	9	10	5	14	8
8	9	16	11	16	9	16	9
13	6	15	15	10	7	18	9
12	10	16	14	13	9	16	10
11	9	18	14	14	9	14	11
16	15	17	12	14	6	18	9
18	8	15	18	11	8	16	6
14	8	15	10	12	11	6	11
14	10	14	9	10	4	18	10
10	3	20	7	11	9	10	8
19	11	7	7	10	5	14	10
9	5	18	8	14	7	12	9
		11	7	9	5	10	4
n = 12		n = 13		n = 13		n = 13	

TABLE 2

Experiment 2: Saccharin consumption on conditioning and test days

R-R		R-M		$DEX_{100\mu g}$-R		$DEX_{100\mu g}$-M	
C	T	C	T	C	T	C	T
15	18	16	11	12	18	14	12
14	15	15	6	12	17	10	3
16	13	16	9	16	19	11	8
16	20	20	7	13	16	18	6
16	20	18	9	17	20	15	17
n = 5		n = 5		n = 5		n = 5	

$DEX_{200\mu g}$-R		$DEX_{200\mu g}$-M		$DEX_{400\mu g}$-R		$DEX_{400\mu g}$-M	
C	T	C	T	C	T	C	T
15	20	17	9	17	18	16	9
17	13	17	8	18	18	14	8
17	21	14	5	19	19	11	5
14	20	17	12	13	19	14	10
10	19	16	13	18	17	16	8
				11	17	15	7
n = 5		n = 5		n = 6		n = 6	

TABLE 3

Experiment 3: Saccharin consumption on conditioning and test days

R-R		R-M		A-R		A-M	
C	T	C	T	C	T	C	T
13	19	14	7	15	21	17	9
11	18	12	8	11	19	11	9
15	17	10	8	7	14	11	8
11	19	9	5	12	18	16	12
10	18	11	9	14	19	14	16
10	14	9	5	16	17	13	7
n = 6		n = 6		n = 6		n = 6	

TABLE 4

Experiment 4: Saccharin consumption on conditioning and test days

R-R		N-R		R-A		N-A	
C	T	C	T	C	T	C	T
12	15	13	15	15	20	11	7
14	13	7	11	13	17	16	9
13	17	9	7	8	16	8	17
17	17	14	17	8	17	13	15
		12	10	14	21	11	13
n = 4		n = 5		n = 5		n = 5	