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Differential Involvement of Central Cholinergic Mechanisms in the Aversive Stimulus Properties of Morphine and Amphetamine

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HUNT, T , R SEGAL AND Z AMIT *Differential involvement of central cholinergic mechanisms in the aversive stimulus properties of morphine and amphetamine* PHARMACOL BIOCHEM BEHAV **28**(3) 335–339, 1987 — Previously, it was reported that pretreatment with the centrally-acting cholinergic antagonist atropine, but not the peripherally-acting antagonist, methyl-atropine, may serve to attenute the positive reinforcing properties of morphine and conversely, to enhance those of amphetamine as evidenced within a drug self-administration paradigm in rats In parallel, evidence from several sources would suggest that there may be a functional relationship between the neurochemical mechanisms mediating these drugs' positive reinforcing properties and their seemingly paradoxical capacity to act as aversive stimuli, as evidenced within a conditioned taste aversion (CTA) paradigm Accordingly, the present study undertook to examine whether a similar differential involvement of central cholinergic mechanisms established for these drugs' positive reinforcing effects may be obtained for morphine and amphetamine-induced CTA Using a conventional CTA paradigm, animals were pretreated with either intraperitoneal (IP) atropine or methyl-atropine (0.6 mg/kg) 40 minutes prior to consuming a novel 0 1% saccharin solution This taste stimulus was paired with IP injection of 15 mg/kg morphine or vehicle Results showed that atropine (but not methyl-atropine) pretreatment served to attenuate the morphine CTA In a second experiment, atropine pretreatment failed to attenuate, and may have slightly potentiated, a CTA induced by 1 mg/kg amphetamine Atropine pretreatment did not affect a CTA induced by the emetic agent, lithium chloride Pretreatment with the peripherally-acting methyl-atropine had no effect on the amphetamine CTA and served, if anything, to slightly attenuate the lithium chloride CTA These findings are discussed in relation to the seeming commonality of neurochemical mechanisms (observed within but not between self-administered drugs) which would appear, somewhat paradoxically, to underlie both the positive reinforcing and CTA-inducing properties of specific drugs of abuse

Conditioned taste aversion Acetylcholine Morphine Amphetamine

AN apparent enigma in the study of the motivational properties of psychoactive drugs such as morphine and amphetamine is the finding that these drugs can act both as positive reinforcers (in that animals will readily perform an operant response resulting in their self-administration [17,23]) and as aversive stimuli (as evidenced by their capacity to induce CTA [2–4, 21]). Of particular significance in this regard is the finding that the same administration of each of these drugs can act simultaneously both as a positive reinforcer and as a CTA-inducing agent [20, 25, 26] in the same animal. Moreover, it is reported that the same pharmacological manipulations which serve to block the positive reinforcing properties of these drugs [5, 24, 27] also act to attenuate their capacity to induce CTA [10, 11, 14, 22]. For instance, pretreatment with alpha-methyl-para-tyrosine (AMPT; an inhibitor of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines) serves both to block morphine or amphetamine self-administration [5] and CTA induced by morphine or amphetamine [10,18] Administration of pimozide, a dopamine receptor blocker, both attenuates amphetamine-induced CTA [11] and also disrupts self-administration of this drug [27] Naloxone, an opiate antagonist, acts both to alter opiate self-administration [24] and to block morphine-induced CTA [14,22]. It is on the basis of such evidence that it has been proposed that the neurochemical mechanisms mediating the positive reinforcing and CTA-inducing effects of each of these self-administered drugs may be functionally related [13,18]

In the present study, the potential involvement of cholinergic systems in mediating the CTA-inducing properties of morphine and of amphetamine was investigated. In a previous study, Davis and Smith [6] reported that pretreat-

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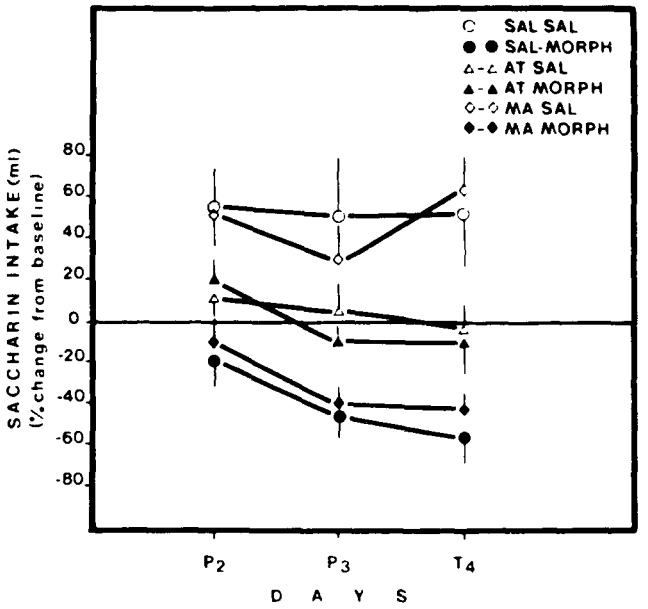

FIG 1 Saccharin intake (ml) expressed as percent change ±SEM from baseline consumption (on P1) of animals pretreated with either saline (SAL), atropine (AT), or methyl-atropine (MA), and conditioned with either saline (SAL) or morphine (MORPH) over two subsequent pairings (on P2 and P3) and final saccharin presentation day (T4)

ment with atropine, a centrally acting muscarinic receptor antagonist, served both to attenuate self-administration of morphine, and to enhance amphetamine self-administration in rats Additionally, these investigators found that administration of the peripherally-acting cholinergic antagonist, methyl-atropine, did not serve to alter the self-administration of these drugs. Thus it would appear that central, but not peripheral cholinergic systems are involved in the neurochemical mediation of morphine and amphetamine positive reinforcement In accordance with the previously described proposal that the drug-specific neurochemical mechanisms mediating both the positive reinforcing and CTA-inducing properties of particular self-administered drugs may be functionally related, the present investigation examined the potential involvement of central cholinergic systems in the mediation of morphine and amphetamine CTA It was predicted that if such a functional relationship was to exist, then a similar contrasting pattern of effects of atropine and of methyl-atropine pretreatment on morphine and amphetamine induced CTA should be observed as was observed in the study by Davis and Smith [6]. Therefore, in Experiment 1 it was hypothesized that atropine pretreatment should serve to block a morphine-induced CTA in a manner similar to the previously reported blockade of morphine positive reinforcement [6] Moreover, methyl-atropine pretreatment should not attenuate the morphine CTA In parallel, in Experiment 2, it was hypothesized that atropine pretreatment should serve to enhance an amphetamine-induced CTA, while pretreatment with methyl-atropine should not have any significant modulating effect An additional experimental group conditioned with lithium chloride (LiCl), an emetic agent widely used in CTA studies was also run, to provide a comparison to an earlier study evaluating atropine effects on the acquisition of a LiCl-induced CTA [7] In this study by Deutsch and colleagues, atropine pretreatment served to interfere with CTA acquisition

EXPERIMENT 1

In this experiment, the potential effects of atropine and methyl-atropine pretreatment on a CTA induced by morphine were examined

METHOD

Subjects

Subjects were 42 male Sprague Dawley rats weighing 300–350 g at the start of the experiment The animals were individually housed in stainless steel cages with free access to laboratory chow and tap water prior to the onset of the experiment and maintained on a 12 12 hr light.dark cycle with lights on at 08 00 hr

Drugs

Morphine hydrochloride (Merck, Sharp and Dohme Canada Ltd) was dissolved in physiological (0 9%) saline. Atropine sulphate and atropine methyl-bromide (Sigma Chemical Company) were similarly dissolved in physiological saline An injection volume of 1 ml/kg body weight was used

Procedure

Following a 1 week period of adaptation to laboratory housing conditions, animals were placed on a daily 23 hr 40 min water deprivation schedule. On Day 8 (Pairing Day P1) animals were given intraperitoneal (IP) injections of either atropine (0.6 mg/kg), methyl-atropine (0 6 mg/kg) or saline, 40 min prior to presentation of a novel 0.1% saccharin solution given in place of their normal drinking water Immediately following termination of the 20 min drinking period, animals were given IP injections of either morphine (15 mg/kg) or saline. A 3×2 factorial design (with unequal sample size) was used such that 5 animals in each pretreatment group (saline, atropine or methyl-atropine) received conditioning injections of saline (groups SAL-SAL, AT-SAL, and MA-SAL) The remaining 9 animals in each pretreatment group received injections of morphine on each conditioning day (groups SAL-MORPH, AT-MORPH, and MA-MORPH). On Days 14 and 20 (Pairing Days P2 and P3) drug treatments and saccharin presentation were given as on the first conditioning day On Day 26 (Test Day T4), a final saccharin presentation was given without drug treatments

RESULTS AND DISCUSSION

A one way ANOVA performed on initial saccharin intake (ml) of the treatment groups (observed on Day P1) showed no significant differences in baseline saccharin consumption, $F(5,41)=0\ 93$, $p<0.5$. The means (with associated standard errors) for each group were: SAL-SAL, 16 6 (2 8); SAL-MORPH, 15.4 (1 5), MA-SAL, 13.0 (2.9), MA-MORPH, 12 9 (1 3), AT-SAL, 17 2 (1 0) and AT-MORPH, 13 7 (1 7). Accordingly, the saccharin intake data of each animal were expressed as percentage change from baseline consumption level A three way (3×2×3) ANOVA, with repeated measures was subsequently performed on the transformed data (see Fig 1). This analysis revealed significant main effects of Conditioning, $F(1,36)=28.02$, $p<0\ 01$, and of Days, $F(2,72)=8\ 42$, $p<0.01$, and significant interactions of Pre-

exposure × Conditioning, $F(2,36)=5.95$, $p<0.01$, and of Conditioning × Days, $F(2,72)=4.62$, $p<0.05$. Dunnett's tests ($p<0.05$) indicated that whereas both saline conditioned groups (SAL-SAL and MA-SAL) exhibited an increase from baseline levels of saccharin intake, no such change in saccharin intake was found in atropine pretreated animals conditioned with saline (AT-SAL). Although atropine is known to suppress drinking [19], this cannot account for the present data, in which no suppressive effect of atropine was found on initial saccharin presentation. As well, the saccharin intake of the AT-SAL, saline conditioned animals remained near baseline levels even on the final (Test Day T4) saccharin presentation, when no atropine pretreatment was given. It may be speculated that the failure to observe an increase (maintenance of neophobia) in saccharin intake in the atropine pretreated animals (AT-SAL) is consistent with reports within classical conditioning paradigms that atropine but not methyl-atropine, may interfere with habituation to the conditioning stimuli [8]

In animals conditioned with morphine, Dunnett's tests revealed that whereas both saline and methyl-atropine pretreated groups (SAL-MORPH and MA-MORPH) exhibited a significant decrease in saccharin intake (indicating a morphine-induced CTA) no significant change from baseline saccharin intake levels was observed in groups pretreated with atropine prior to morphine conditioning (AT-MORPH) Thus, pretreatment with atropine, but not methyl-atropine, served to block the formation of a morphine-induced CTA In contrast to a recent report [1] that morphine-induced CTA reflects primarily a peripheral action of the drug (as indicated by a failure of vagotimized rats to exhibit a morphine CTA), the present results suggest that a central action of morphine is necessary for the establishment of such a CTA. Moreover, the pattern of effects reported in the present study is consistent with the findings of Davis and Smith [6] where atropine, but not methyl-atropine, was found to block morphine self-administration These data, therefore, support the hypothesis that a functional relationship exists between the CTA-inducing and positive reinforcing properties of morphine

EXPERIMENT 2

This experiment examined the potential effects of atropine or methyl-atropine pretreatment on CTAs induced by amphetamine, and by LiCl

METHOD

Subjects

Subjects were 74 male Sprague Dawley rats weighing 300–350 g at the start of the experiment Housing conditions were identical to those used in the previous experiment

Drugs

D-amphetamine sulphate (Smith, Kline & French, Canada, Ltd) was dissolved in physiological saline, as were atropine sulphate and atropine methyl-bromide (Sigma Chemical Company). The injection volume of these drugs was 1 ml/kg body weight. LiCl was dissolved in distilled water to make a final 0.15 molar solution and was injected in a volume of 3 ml/kg body weight.

Procedure

An identical procedure to that used in the preceding experiment was followed here. On Day 8 (Pairing Day P1) of a daily 23 hr 40 min water deprivation schedule, animals were pretreated with IP injections of atropine (AT, 0.6 mg/kg) or methyl-atropine (MA, 0.6 mg/kg), or saline (SAL). Forty minutes later, a novel 0 1% saccharin solution was presented in place of the normal drinking water. Immediately following the 20 min drinking period, animals were given IP injections of either distilled water (3 ml/kg), amphetamine (1 mg/kg) or LiCl (3 ml/kg of a 0.15 M solution) A 3×3 factorial design was used such that each pretreatment group (AT, MA or SAL) received conditioning injections of either distilled water (AT-VEH, n=8; MA-VEH, n=8, SAL-VEH, n=8), amphetamine (AT-AMPHET, n=9, MA-AMPHET, n=9; SAL-AMPHET, n=9) or LiCl (AT-LiCl, n=8; MA-LiCl, n=8, SAL-LiCl, n=7). Three conditioning days (P1, P2, P3) and a final test day (T4) were given, as in Experiment 1.

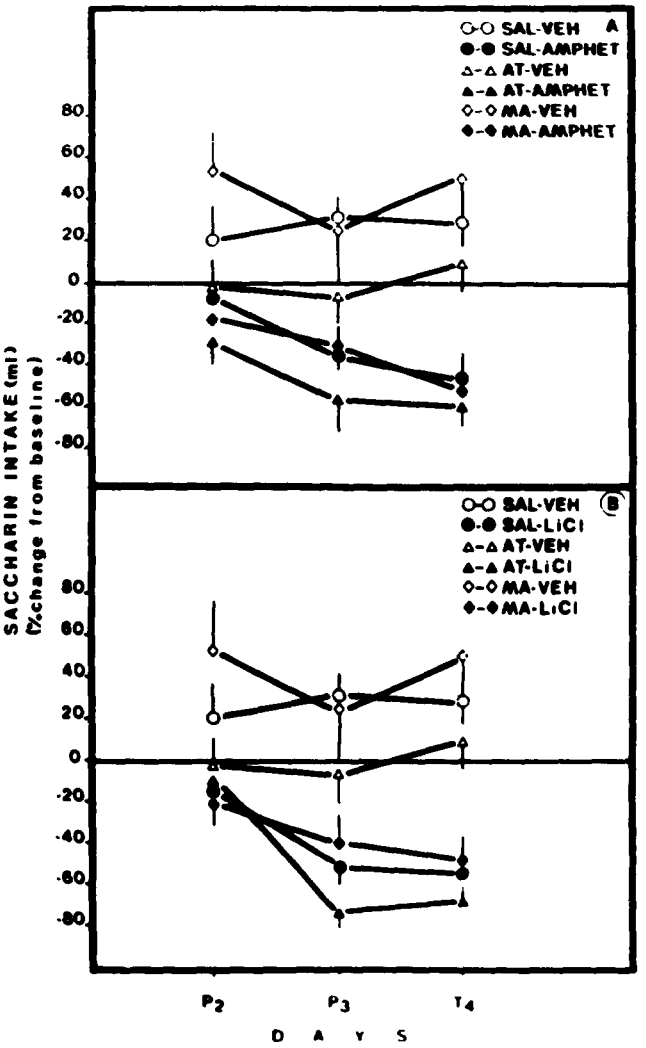

FIG 2 Saccharin intake (ml) expressed as percent change ±SEM from baseline consumption (on P1) of animals pretreated with either saline (SAL), atropine (AT), or methyl-atropine (MA) and conditioned with either vehicle (VEH) or amphetamine (AMPHET, see Panel A) or lithium chloride (LiCl, see Panel B)

RESULTS AND DISCUSSION

Separate statistical analyses were performed on the data

of the amphetamine and LiCl treated animals with the vehicle conditioning groups serving as controls for both analyses One way ANOVAs performed on the initial saccharin intake data (ml) observed on Day P1 for the various treatment groups indicated no significant differences in baseline saccharin intake among treatment groups on the first conditioning day both for the amphetamine, $F(5,45)=1\ 37$, $p>0\ 7$, and LiCl, $F(5,46)=1\ 43$, $p>0.7$) analyses The mean levels of saccharin intake (and associated standard errors) for each group were. SAL-VEH, 18.1 (1.4), AT-VEH, 14 8 (1 0), MA-VEH, 13.5 (1 8), SAL-AMPHET, 15.4 (1 0), AT-AMPHET, 15.3 (0 5); MA-AMPHET, 14.8 (1 1), SAL-LiCl, 15 0 (1 5); AT-LiCl, 13.4 (1.6) and MA-LiCl, 15 5 (1.4). The data were accordingly expressed as percent change from baseline scores as in the preceding experiment Separate three way (3×2×3) ANOVAs with repeated measures were performed on data of the amphetamine and LiCl groups as mentioned above

The ANOVA for the amphetamine conditioned animals (and appropriate vehicle control groups, see Fig 2, panel A) revealed significant main effects of Pretreatment, $F(2,45)=3\ 26$, $p<0.01$, of Conditioning, $F(1,45)=36\ 51$, $p<0\ 01$, and of Days, $F(2,90)=6.87$, $p<0.01$. A significant Conditioning × Days interaction was also evident, $F(2,90)=7\ 98$, $p<0.01$. Examination of the data of the vehicle conditioned animals suggests a similar pattern of effects as was observed in Experiment 1 Dunnett's tests indicated that only the MA-VEH group showed an increased saccharin consumption (on Days P2 and T4). In amphetamine conditioned animals, a significant decrease from baseline intake levels was evident for the SAL-AMPHET and MA-AMPHET groups only on the final Test day (T4). In contrast, the atropine pretreated, AT-AMPHET group exhibited a significant reduction in saccharin consumption on Days P3 and T4. Thus, while all amphetamine conditioned animals exhibited an amphetamine-induced CTA, the atropine pretreatment would appear to have, if anything, served to enhance this CTA. When considered together with the previous atropine blockade of a morphine CTA observed in Experiment 1, a clear disassociation of morphine's and of amphetamine's aversive stimulus properties is evident. Furthermore, these data would seem to confirm the prediction based on the Davis and Smith [6] study, in which atropine blocked morphine but enhanced amphetamine self-administration The suggestion of a similar pattern in the present taste aversion study adds to the accumulating evidence in support of an hypothesized functional relationship between these drugs' positive reinforcing and CTA-inducing properties (see [12])

A three way (3×2×3) ANOVA, with repeated measures performed on the LiCl conditioned and vehicle control groups (see Fig. 2, panel B) revealed significant main effects only of Conditioning, $F(1,41)=38.08$, $p<0\ 01$, and of Days, $F(2,82)=11\ 47$, $p<0\ 01$, and a significant Conditioning × Days interaction, $F(2,82)=9.72$, $p<0.01$ Dunnett's tests indicated that both the SAL-LiCl and AT-LiCl groups exhibited a significant reduction in saccharin intake on Days P3 and T4, while the MA-LiCl group exhibited this reduction only on the final test day, T4. It is not clear, at present, how to account for this apparent attenuative effect of methylatropine pretreatment. Additionally, the present data are in conflict with an earlier report by Deutsch [7], indicating that atropine pretreatment served to block a LiCl-induced CTA However, in the study by Deutsch, an atropine dose of 100 mg/kg was used in comparsion to the 0 6 mg/kg atropine dose used here (and in the Davis and Smith [6] study) Such a contrast in dose level clearly provides a means to explain the apparent discrepancy between the two LiCl studies Also, an earlier investigation by Samples and colleagues reporting significant cholinergic involvement in LiCl toxicity is noteworthy in this regard [16]. In contrast to the data of the present paper, atropine was found in the study by Samples *et al* to reverse the potentiation of LiCl toxicity induced by pretreatment with the cholinesterase inhibitor, physostigmine The present data may be taken to add to the evidence dissociating LiCl-induced CTA from this drug's potential toxic effects (see [12]) An investigation of the effect of physostigmine pretreatment upon a LiCl CTA may more directly address this question

In conclusion, the results of Experiments 1 and 2 would appear to strengthen the empirical support for the hypothesis that the drug-specific neurochemical mechanisms involved in the positive reinforcing and CTA-inducing properties of the self-administered drugs morphine and amphetamine are, in each case, functionally related The present data would also appear to add to the evidence suggesting clear differences in the neurochemical mediation of morphine and amphetamine CTAs Roberts and Fibiger [15] found that neurotoxic lesions of the dorsal noradrenergic bundle served to disrupt a morphine but not an amphetamine CTA Also, while naloxone pretreatment serves to attenuate a morphine CTA [22], a similar naloxone pretreatment was found not to alter an amphetamine-induced CTA [9] The present findings, indicating an atropine pretreatment blockade of a morphine CTA, compared to a possible potentiation of an amphetamine CTA, would therefore appear consistent with the evidence suggesting differential involvement of neurochemical systems mediating both the aversive (CTA-inducing) and positive reinforcing properties of these two well-established drugs of abuse.

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