Pharmacology Biochemistry & Behavior, Vol. 20, pp. 507–511, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Pharmacologically Specific Pretreatment Effects on Apomorphine-Mediated Conditioned Taste Aversions in Rats

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Received 3 September 1983

PRATT, J. A. AND I. P. STOLERMAN. *Pharmacologically specific pretreatment effects on apomorphine-mediated conditioned taste aversions in rats.* PHARMACOL BIOCHEM BEHAV **20**(4) 507–511, 1984.—Pretreatment with pimozide (0.2–1.2 mg/kg) reduced a conditioned taste aversion produced by apomorphine (0.4 mg/kg) in a dose-related manner. This pretreatment effect was pharmacologically specific as shown by the inability of pimozide to prevent a conditioned taste aversion produced by nicotine (0.4 mg/kg). The results argue against the hypothesis that "proximal pre-exposure" effects are always non-specific and indicate that further pharmacological characterisation of drug-induced conditioned taste aversion may be possible. Pretreatment with a peripherally-acting antiemetic compound, domperidone, did not prevent apomorphine producing conditioned taste aversions. These data suggest that conditioned taste aversions produced by apomorphine are mediated through central dopamine receptors unrelated to the emetic properties of apomorphine and are not a result of conditoned nausea.

Conditioned taste aversion Apomorphine Nicotine Pimozide Domperidone

PSYCHOTROPIC drugs from a wide range of pharmacological classes share the ability to produce conditioned taste aversions (CTA) [5]. There have been a number of approaches aimed at characterising the mechanisms underlying this phenomenon. Interest has centered in particular on the effect of drug experience on the ability of drugs to induce CTA. Domjan [12] has partly reviewed work on such "proximal pre-exposure" (pretreatment) effects and suggests that they are due mainly to the pre-exposure drug treatment creating some malaise which reduces the associability of the taste stimulus and the subsequent conditioning drug treatment. In support of this view Domjan [12] has cited studies which demonstrated that pretreatment with atropine disrupts taste-aversions produced by lithium or radiation exposure [10,18]. Similarly, Brown *et al.* [3] reported that pretreatment with diazepam blocked CTA induced by both itself and morphine. There are also many studies showing that pretreatment "distal" to an aversion-inducing agent attenuates CTA produced by either the same or different drugs [6,17].

In contrast, however, a number of pretreatment agents attenuate CTA induced by some compounds but not those produced by other drugs. These effects have been explained in terms of neurochemical mechanisms since only those treatments commonly used to block other behavioural and pharmacological effects of drugs can block CTA. For example, alpha-methylparatyrosine attenuates amphetamine but not lithium CTA [14,16]. Similarly, 6-hydroxydopamine lesions abolish CTA induced by amphetamine and methylamphetamine but not those mediated by lithium or fenfluramine [21, 27, 29]. We have demonstrated that mecamylamine can prevent nicotine taste aversions but not those produced by apomorphine [20]. Clearly, there is some indication that pharmacologically specific pretreatment effects can be shown with drug-induced taste aversions.

Another approach to the study of CTA has concentrated on the involvement of mechanisms involving conditioned nausea. The results of studies with antiemetic drugs have not shown consistently that such drugs attenuate CTA [8, 15, 24]. Investigations involving lesions of the area postrema (an area which is functionally involved in vomiting, in species that are capable of doing so) have, however, produced equivocal data. While CTA produced by lithium or methylscopolamine can be reduced by area postrema lesions, amphetamine aversions are unaffected [1,26].

In this investigation we have been concerned mainly with the question of whether pharmacologically specific "proximal" pretreatment effects can be shown with CTA produced by apomorphine. In other procedures stereotyped behaviour induced by apomorphine in rats can be attenuated by the dopamine antagonist pimozide but not by domperidone (which penetrates the blood-brain barrier poorly). The latter compound however is more potent than pimozide in blocking apomorphine-induced emesis in the dog [22]. In view of the demonstration of catecholamine-containing neurones in the area postrema, [13] an area which lacks a blood brain barrier, we have tested the ability of domperidone and pimozide to block CTA produced by apomorphine. Such investigations should help to resolve the role of emetic mechanisms in mediating apomorphine CTA. In

order to determine whether the pretreatment effects have any pharmacological specificity, pimozide was also tested against a CTA produced by nicotine.

METHOD

Male Lister hooded rats (Olac, Bicester) weighing 220–230 g at the start of the experiment were used. Animals were housed individually in a temperature- (22°C) and light- (12-hr cycle) controlled room. Food was available throughout the study.

Conditioning Procedure

Full details of the two-trial conditioning procedure used were given by Kumar *et al.* [20]. Access to water was limited to 1 hr/day for 8 days before any flavoured solutions were presented and on all days between flavour presentation. Following the adaptation to restricted access to water, one of two flavoured solutions (sodium saccharin 0.1% or sodium chloride 0.9%) were presented for 15 min on every second day. The two flavours were presented alternately. Immediately after the flavours were removed animals were injected with apomorphine (or nicotine) or appropriate vehicle (flavour-injection "pairing"). For half of the rats in which each drug was tested, one flavour was paired twice with drug whilst the other flavour was paired twice with vehicle. The flavour-injection pairings were reversed in the remaining rats, thus ensuring that the effects of the inherent palatabilities of the flavours were balanced out.

After two flavour-drug and two flavour-vehicle pairings (1-stimulus tests) injections were stopped. Two days later, drug- and solvent-paired flavoured solutions were presented simultaneously for 15 min (2-stimulus test). On the next day, the positions of the two stimuli (flavours) were reversed. The mean scores for the two days of 2-stimulus tests are presented.

Drug Pretreatments

Prior to each conditioning trial, different groups of rats were pretreated with a range of doses of pimozide (0.2–1.2 mg/kg SC), domperidone (0.12–12 mg/kg SC) or vehicle. These groups were then compared with respect to the degree of CTA (assessed during the two-stimulus test) produced by a dose of 0.4 mg/kg SC of apomorphine (or nicotine). The intervals between injections of pretreatment agent and apomorphine (or nicotine) were chosen from a previous study, [22]. These were 3.25 hr for pimozide and 0.5 hr for domperidone.

Statistical Analyses

Results from the two stimulus tests were analysed as follows to determine the presence or absence of CTA. For each rat the amount of fluid consumed of the flavour paired with apomorphine (or nicotine) injections was calculated as a percentage of the total fluid intake. These percentage scores were subjected to arc-sine transformation to normalise their distributions [30] and then *t*-tests were performed to determine whether the means differed from 50%. A mean score of 50% would indicate that a drug produced neither conditioned aversion nor conditioned preference. Scores significantly below 50% indicate CTA.

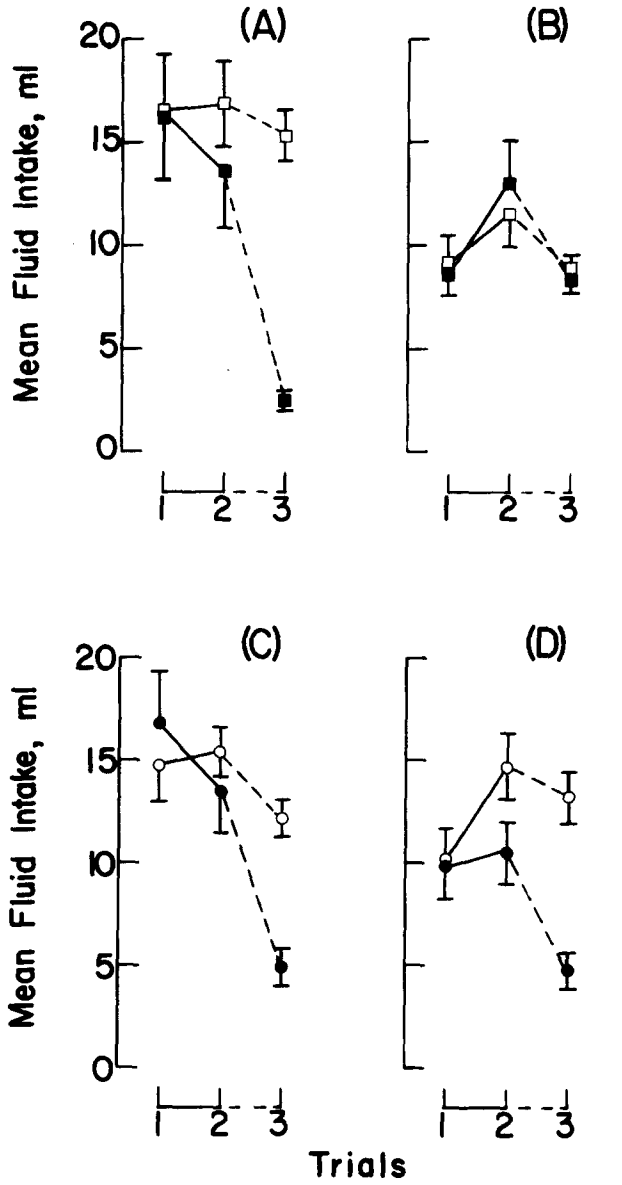

FIG. 1. Conditioned taste aversions in four groups of rats (n=6–8). Trials 1 and 2 were conditioning sessions and trial 3 was two-stimulus test with simultaneous presentation of both flavoured solutions. Vertical bars indicate S.E.M. In (A) apomorphine (■; 0.4 mg/kg) produced CTA which was blocked by pretreatment with pimozide (1.2 mg/kg) in (B). In (C) nicotine (●; 0.4 mg/kg) produced CTA which was not blocked by the same dose of pimozide (D). Intakes of control flavoured solutions for each group of rats provided baselines for assessing degree of CTA (□ and ○).

Drugs

Apomorphine hydrochloride (Macfarlan Smith) was dissolved in a solution of ascorbic acid (0.2 mg/ml) in distilled water. (−)-Nicotine bitartrate (BDH) was dissolved in 0.9% NaCl and the pH adjusted to 7 with 0.5 N NaOH. Pimozide (Janssen) plus 1.5× the amount of tartaric acid were dissolved in warmed distilled water and stored in the dark at room temperature until used. Domperidone (Janssen) was

TABLE 1

EFFECT OF DOPAMINE ANTAGONISTS ON DEVELOPMENT OF CONDITIONED TASTE AVERSIONS (CTA) IN RATS

Pretreatment (mg/kg)	n	Drug-Paired Flavour Intake (Mean % ±SEM)
Apomorphine (0.4 mg/kg) CTA		
Vehicle	22	16.2 ± 1.8***
Pimozide (0.2)	6	32.8 ± 10.2
Pimozide (0.4)	8	32.6 ± 5.7*
Pimozide (0.8)	8	40.9 ± 7.4
Pimozide (1.2)	8	49.4 ± 4.0
Domperidone (0.12)	8	10.9 ± 3.7***
Domperidone (1.2)	6	11.9 ± 2.3***
Domperidone (12.0)	8	27.4 ± 8.1*
Nicotine (0.4 mg/kg) CTA		
Vehicle	8	28.0 ± 4.8**
Pimozide (1.2)	8	26.8 ± 5.1**

Data from two-stimulus tests (trial 3). Pimozide and domperidone were administered 3 hr and 15 min respectively prior to presentation of flavoured solutions on previous conditioning trials 1 and 2.

*$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with mean scores of 50% indicating significant taste aversion. A 50% score indicates no preference or aversion to the flavoured solutions.

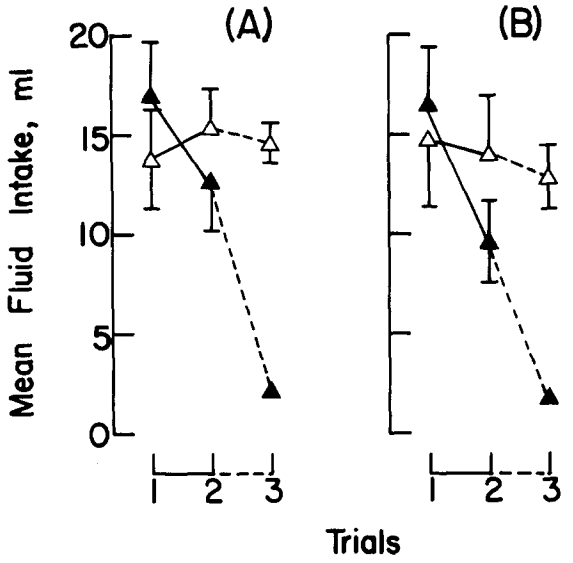

FIG. 2. Conditioned taste aversions to apomorphine in two groups of rats. In (A) apomorphine (▲; 0.4 mg/kg, n=8) produced CTA which was not blocked by pretreatment with domperidone (1.2 mg/kg; n=6) in (B). Intakes of control flavoured solutions for each group of rats provided baselines for assessing degree of CTA (△).

dissolved in the minimum of glacial acetic acid and the pH adjusted to 4.5 with 0.5 N NaOH.

RESULTS

Effect of Pretreatment With Pimozide on CTA Produced by Apomorphine and Nicotine

In animals receiving pimozide (1.2 mg/kg) before each conditioning trial, apomorphine (0.4 mg/kg) did not produce a CTA (Fig. 1B). Thus the mean fluid intakes (±SEM) of the apomorphine-paired and vehicle-paired flavour were almost identical at trial 3 (8.4±0.6 ml and 8.7±0.7 ml respectively). In contrast, control animals pretreated with vehicle instead of pimozide showed marked CTA at trial 3 (Fig. 1A).

The baseline consumption of flavoured solutions was reduced following pimozide administration (1.2 mg/kg). Figure 1 shows that control animals consumed 15–17 ml of the flavoured solutions at trial 1 whereas animals receiving pimozide drank an average of 9.5 ml. Such reductions of fluid intake in animals pretreated with pimozide did not prevent CTA developing to nicotine (Fig. 1D). Control animals in this instance displayed CTA of similar magnitude to that for animals pretreated with pimozide (Fig. 1C).

The results for all doses of pimozide tested are summarised in Table 1. Analysis of variance of the pooled results for experiments concerned with the effects of pimozide on apomorphine CTA showed a difference between groups; $F(4,39)=5.45$, $p<0.01$. The blocking effect of pimozide on apomorphine CTA seemed to be dose-related. Doses of pimozide less than 0.8 mg/kg had no significant effect on the percentage of drug-paired flavour intake when compared with controls. At a dose of pimozide of 0.8 mg/kg there was a partial block of the apomorphine CTA, $t(39)=2.85$, $p<0.05$, while a larger dose of pimozide (1.2 mg/kg) completely blocked the CTA, $t(39)=3.88$, $p<0.01$.

Effect of Pretreatment with Domperidone on Apomorphine CTA

Pretreatment with domperidone (0.12–12.0 mg/kg) did not prevent the development of apomorphine CTA as shown in Table 1. The scores for the controls were very similar to those obtained in the previous experiments and so have been pooled for clarity of presentation. Analysis of variance on the percentage scores indicated there were no differences between groups; $F(3,26)=2.71$, $p>0.05$, indicating that in all cases, pretreatment with domperidone produced similar degrees of CTA to that observed with apomorphine alone. The mean fluid intakes during the two conditioning trials and the 2-stimulus test (trial 3) in animals receiving apomorphine and pretreated with domperidone (1.2 mg/kg) are shown in Fig. 2. The baseline intake of fluid for the domperidone pretreated group was similar to that in controls. In control rats pretreated with vehicle, apomorphine CTA was well developed at trial 3. In rats pretreated with domperidone, apomorphine produced CTA of similar magnitude as shown in Fig. 2.

DISCUSSION

The results indicate that pharmacologically specific pretreatment effects can be obtained in CTA procedures. In this study pretreatment with pimozide attenuated apomorphine CTA but not a CTA of similar magnitude produced by nicotine. These data complement earlier findings that mecamylamine prevents nicotine but not apomorphine CTA [20]. The present results are also consistent with the known ability of the dopamine antagonist pimozide to block other behavioural effects of apomorphine [23]. The discriminative stimulus effect of apomorphine was blocked by doses of pimozide [9] similar to those which prevented apomorphine CTA, whereas pimozide was more potent in antagonising

stereotyped behaviours produced by apomorphine [22]. Pimozide also partly blocked a CTA produced by amphetamine although the pharmacological specificity of this effect was not determined [19].

Some investigators have interpreted drug pretreatment effects in terms of learning processes without consideration of pharmacological interactions. Domjan [12] for example suggested that prior exposure to drugs induces a malaise which prevents the subsequent association between taste experience and drug treatment. Others have shown that state-dependent learning effects can exist in CTA pretreatment studies [25]. It is difficult to explain the pharmacological specificity of our pretreatment effects through such learning mechanisms. The pretreatment drugs used in this and our previous study may have exhibited some inhibitory effects on fluid consumption in the largest doses tested, but despite this CTA developed when given in combination with a drug from a different pharmacological class. Reducing fluid consumption by manipulating deprivation also failed to attenuate CTA [11].

In the present studies we also investigated the effect of the dopamine antagonist haloperidol on CTA produced by apomorphine (Pratt and Stolerman, unpublishd data). At trial 3 the percentages of drug-paired flavour intake were similar in the control group and in rats pretreated with haloperidol at a dose of 0.06 mg/kg (16.4±4% and 22.5±0.6% respectively). The lack of attenuating effect of haloperidol would argue against involvement of dopamine receptors in apomorphine CTA. Alternatively, the dose of haloperidol used may have been too small. Haloperidol is 2–3 fold more potent than pimozide in blocking stereotyped behaviours produced by apomorphine [22]. By applying the same arguement to our results only doses of haloperidol greater than 0.27 mg/kg would be expected to be active. Such doses could not be tested because of their inhibitory effects on fluid intake. The influence of other dopamine antagonists on apomorphine CTA has not been tested.

The results from the domperidone experiment argue against an apomorphine CTA being mediated through peripheral dopamine receptors. Thus domperidone did not attenuate apomorphine taste aversions even at doses larger than those known to be active at peripheral dopamine receptors [7]. Colpaert was also unable to demonstrate a blocking effect of domperidone on apomorphine CTA (Colpaert, Personal communication).

One of the best known properties of apomorphine is its ability to produce nausea and emesis. Emesis is mediated through the area postrema which lacks a blood brain barrier [2]. Certain CTA can be abolished following lesions of this structure [1,26] and this supports the proposal that emetic mechanisms also may be important in mediating CTA. Both pimozide and domperidone might be expected to antagonise the effects of apomorphine by acting on dopamine receptors thought to be present in this brain area [13,28]. In species that vomit domperidone is generally slightly more potent than pimozide against apomorphine-induced emesis [22]. In this investigation, however, a dose of domperidone 15 times an effective dose of pimozide did not prevent taste aversions produced by apomorphine. These data would suggest that emetic mechanisms are unlikely to be important in the production of apomorphine taste aversions. Other recent studies also argue against a general involvement of emetic mechanisms in the mediation of CTA [4, 15, 20, 24].

In summary, apomorphine CTA can be blocked by pretreatment with the dopamine antagonist pimozide which does not block nicotine CTA. Pharmacologically specific pretreatment effects have only rarely been demonstrated with taste aversion procedures; that they can occur indicates that further pharmacological characterisation of drug-induced CTA may be possible. Taken together, the data from the domperidone and pimozide experiments suggest that apomorphine CTA is largely a centrally-mediated effect unrelated to emetic mechanisms. These findings may have implications for the use of apomorphine in aversion therapy for alcoholism or other conditions.

ACKNOWLEDGEMENTS

We thank the Medical Research Council for financial support and Janssen Pharmaceutica for donating pimozide and domperidone.

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