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BRIEF COMMUNICATION

Drug Pretreatment Effects in Drug Induced Taste Aversions: Effects of Dose and Duration of Pretreatment

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GOUDIE, A. J., E. W. THORNTON AND T. J. WHEELER. *Drug pretreatment effects in drug induced taste aversions: effects of drug dose and duration of pretreatment.* PHARMAC. BIOCHEM. BEHAV. 4(5) 629–633, 1976. – The effectiveness of a dose of 3.0 mg/kg methamphetamine in inducing a conditioned taste aversion to saccharin was found to be reduced by chronic pretreatment with the same dose of the drug. The degree of attenuation of the aversive properties of the drug was found to be directly proportional to the duration of pretreatment, a pretreatment regime of 9 or more daily injections completely abolishing the aversive properties of the drug. However, such a regime was only slightly effective in attenuating the aversive properties of a higher dose of methamphetamine (10 mg/kg) and failed to attenuate the aversive properties of a number of other drugs (p-chloramphetamine at 5.0 mg/kg, fenfluramine at 5.0 mg/kg and morphine at 20 mg/kg). Interpretations of these data are considered and it is suggested that the most parsimonious explanation of the effectiveness of chronic drug pretreatment in attenuating the aversive properties of a drug is that the effect is due to the development of tolerance to the drug administered.

Conditioned taste aversion Methamphetamine p-Chloramphetamine Fenfluramine Morphine Tolerance Drug experience

DRUG induced taste aversions have recently attracted interest because of their possible significance in the study of drug abuse [4, 5, 8–11]. Interest has centered in particular on the effect of drug experience on the ability of a drug to induce a conditioned taste aversion (C.T.A.). A number of authors have reported that the aversive effects of drugs can be attenuated by drug experience prior to drug/taste pairing [1–9, 11, 13]. Such findings are of interest since they demonstrate that the aversive hedonistic properties of drugs can be modified by drug experience. The interpretation of this pretreatment effect remains unclear [4, 5, 8, 9, 11]. Early studies attributed the effect to alleviation of a drug induced unnatural need state [13], or to reduction of drug novelty during pretreatment, novelty per se being considered to be aversive [7]. These interpretations have since been shown to be invalid [4, 5, 9, 11].

Alternative explanations of the phenomenon have included suggestions that the effects are due to the development of tolerance [4, 5, 8, 9, 11]; that habituation to illness develops during pretreatment [2]; and that prior exposure to the aversive agent (UCS) somehow interferes with UCS–CS (taste) association on subsequent pairing [5,11].

The work reported here elucidates some of the critical variables in pretreatment studies, specifically those of drug dose and duration of pretreatment; and investigates the generality of previously reported cross-drug pretreatment effects.

EXPERIMENT 1

This experiment examined the effect of varying the number of pretreatment injections of methamphetamine at 3.0 mg/kg on the establishment of a C.T.A. by subsequent repeated pairings of the same dose of the drug with saccharin.

METHOD

Animals and Pretreatment

Female albino rats housed individually were allocated at

random to one of seven groups (n = 9). Five groups received varying numbers (2, 4, 6, 9 and 14) of daily methamphetamine pretreatments prior to pairing of the drug with saccharin. A further group (Naive group) received 14 daily saline injections prior to pairing of saccharin ingestion with the drug. The final group (Naive control) received 14 daily saline injections followed by pairing of saccharin ingestion with saline injection. Pretreatment was administered for 14 days according to a schedule designed so that the last day of pretreatment for each group was on Day 14 of the study. During pretreatment animals were maintained on ad lib food and water. DL-Methamphetamine hydrochloride was administered daily at a dose of 3.0 mg/kg. The drug was made up in 0.9% saline as the salt, and injected IP at a volume equal to 2 mls per kg body weight of rat. A dose of 3.0 mg/kg was chosen on the basis of previous reports of the aversive properties of the drug [12]. The range of pretreatments (0 14 daily injections) was chosen on the basis of previous studies of the attenuation of the aversive properties of amphetamine [8, 9, 11].

Procedure

Following pretreatment animals were immediately water deprived and an experimental cycle of 3 days duration initiated. On the first day of this cycle animals received access to water for 30 min at 1100 hr, on the second day animals received access to 0.1% saccharin for 30 min at the same time, followed by injections of drug (6 groups) or saline (Naive control group only). On the third day of the cycle animals received access to water for 30 min at 1100 hr. On this day maintenance injections of methamphetamine were also administered at 1500 hr to animals in all groups except the Naive control group. This cycle was repeated 4 times. The overall design of the study was such that access to saccharin occured 44 hours after the preceeding injection of methamphetamine (whether following pretreatment or maintenance). This procedure was adopted in order to prevent the results being confounded by possible adipsic and anorectic effects of the drug.

On saccharin access days (Trials 1–4), amounts drunk by each animal were recorded by weighing individual water bottles to the nearest 0.1 g. The relevant Treatment (drug or saline) was administered within 10 min of the end of the saccharin access period, dl-methamphetamine hydrochloride being administered with the same parameters as in the pretreatment period.

RESULTS

Figure 1 shows the mean amounts drunk by subjects in each group on succeeding days of access to saccharin (Trials 1–4).

Analysis of these data by a two-way ANOVA for repeated measures indicated that there were highly significant effects of groups, $F(6,244) = 36.69$, $p<0.001$, Trials, $F(3,244) = 5.60$, $p<0.001$, and an interaction, $F(18,224) = 9.68$, $p<0.001$. Lower levels ANOVAs for each trial indicated that although there was no significant effect of groups on Trial 1 ($F = 0.99$); there were significant effects on all other trials (smallest $F = 9.27$, $df = 6,56$, $p<0.001$ on all trials). The group effect was analysed further by comparisons between means with the Tukey (a) test [15], a criterion of $\alpha = 0.01$ being adopted for rejection of the null hypothesis. The analysis showed that a significant degree of attenuation of the aversive effects of the drug observed in the naive group was produced by 4, 6, 9 and 14 pretreatments on various trials. There were significant differences between groups receiving 2, 4 and 6 pretreatments on various trials, although on no trials was there a significant difference between groups receiving 6, 9 and 14 pretreatments, nor did animals in these groups ever differ significantly from the Naive control group. These results indicated that the degree of attenuation of the aversive effects of the drug was proportional to the amount of pretreatment, but that the pretreatment effects reached an assymptote between 6 and 14 pretreatments.

DISCUSSION

The results clearly demonstrate that pretreatment with dl-methamphetamine hydrochloride at a dose of 3.0 mg/kg attenuated the aversive effects of the same dose of the drug to an extent directly proportional to the amount of pretreatment, reaching an asymptote after approximately 9 daily injections. The increase in mean saccharin intake in the Naive control group over trials (Fig. 1) is due to reduction in neophobia on succeeding days of access to saccharin, an effect which is well documented [14].

EXPERIMENT 2

Experiment 1 indicated that the aversive properties of a dose of 3.0 mg/kg of dl-methamphetamine were totally abolished by 14 daily pretreatments with the same dose of the drug. In Experiment 2 the effects of this pretreatment regime on the aversive effects of a higher dose of the same drug were studied. It has been reported [11] that drug dose is not an important variable in pretreatment studies. This conclusion was drawn from a study in which the pretreatment doses were always higher than the effective aversive dose. It is possible that drug dosage is an important variable if the pretreatment dose is lower than the aversion inducing dose. The effect of 14 daily pretreatments with 3.0 mg/kg dl-methamphetamine on the aversive properties of 10.0 mg/kg of the drug was consequently examined. In addition the effects of this chronic pretreatment regime on the aversive properties of three other drugs, p-chloramphetamine, morphine and fenfluramine were evaluated to determine the generality of previously reported cross-drug pretreatment effects [4, 5, 9].

METHOD

Animals

Female albino rats, housed as described above, were allocated at random to one of nine groups (n = 8). The overall design of the study was such that each of the four aversion inducing Treatments was administered to both drug naive and drug experienced (i.e. methamphetamine pretreated) animals. In addition a Naive control (Saline pretreated, Saline Treated) group was included in the study in order to access the aversive effects of each Treatment, since as noted above, such effects are evaluated against a changing baseline of saccharin due to the effects of neophobia.

Procedure

Animals received the relevant pretreatment (drug or saline) for 14 days at 1100 hr. DL-Methamphetamine

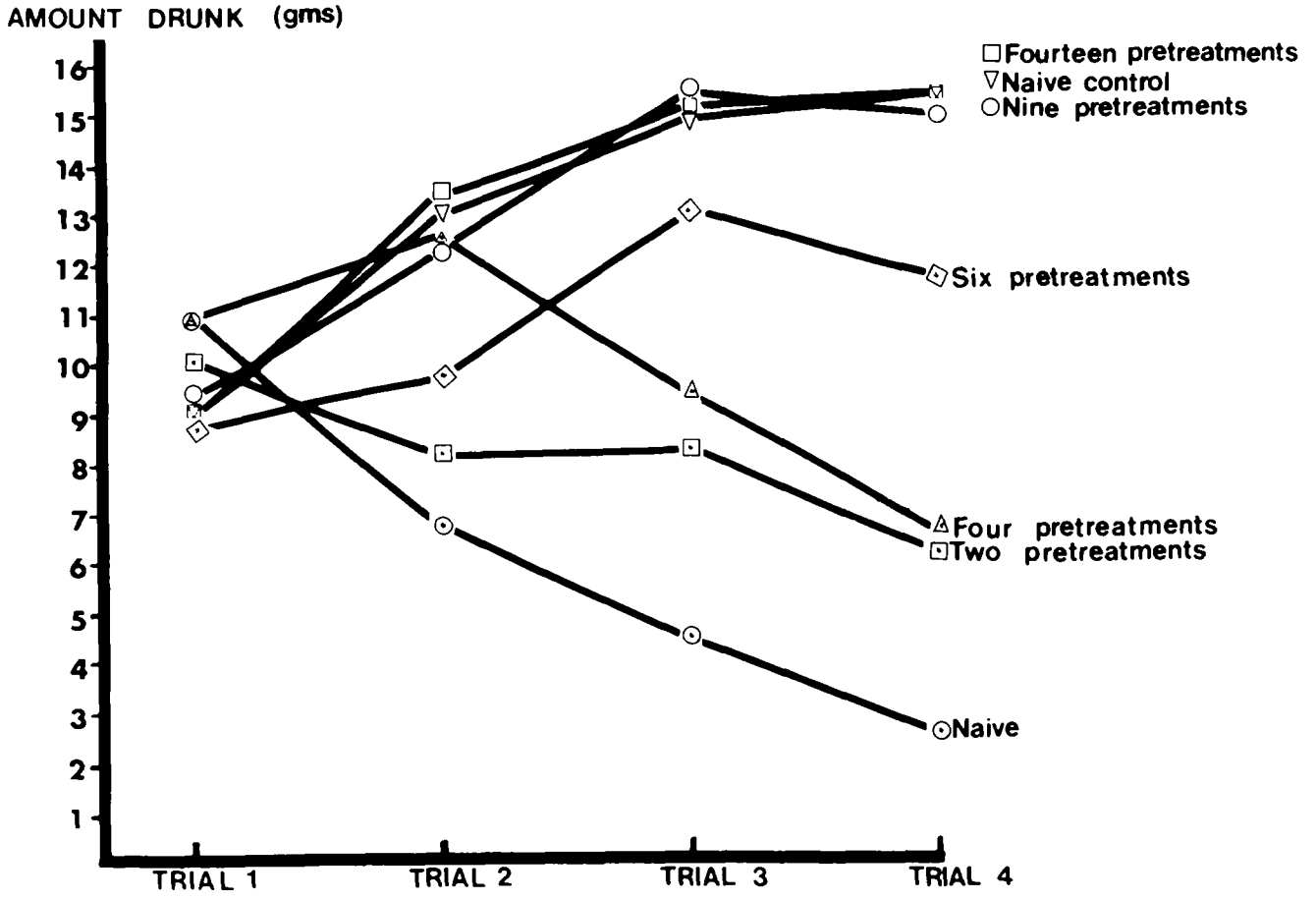

FIG. 1. Mean amounts drunk (g) by animals on each group on succeeding days of access to 0.1% sodium saccharin (Trials 1–4).

hydrochloride at 3.0 mg/kg was administered exactly as in Experiment 1. Immediately after the last pretreatment (Day 14) animals were water deprived. They received 30 min access to water on Day 15 at 1100 hr, and an experimental cycle of three days duration was then initiated (Day 16). This cycle was similar to that described in Experiment 1 (Table 2). However, in this experiment the first saccharin/drug pairing occurred on Day 17 (72 hr after the last methamphetamine pretreatment rather than 44 hours after as in Experiment 1). In addition, this study differed from Experiment 1 in that maintenance injections were not administered during the experimental period on the third day of the cycle. These slight procedural changes were considered necessary in order to prevent the results being confounded by possible interactions between drugs in the cross-drug groups. dl-fenfluramine hydrochloride dl-p-chloramphetamine hydrochloride and dl-morphine sulfate were administered at doses of 5,5, and 20 mg/kg respectively. Drugs were injected IP, at a volume equal to 2 ml/kg body weight of rat; all solutions being made up as the salt in 0.9% saline.

RESULTS

Table 1 shows the mean (± S.E.) amounts drunk by animals in each group on succeeding days of access to saccharin (Trials 1–4)

For the purpose of analysis Experiment 2 was considered as four separate studies, each study conformed to a design in which there were three groups, a Naive control group (saline pretreated and saline treated), a drug naive group (saline pretreated, drug treated), and a drug experienced group (methamphetamine pretreated, drug treated). The results of each study were analysed separately although the same Naive control group was included in all studies.

Effects of Treatment with Methamphetamine at 10 mg/kg

A two way ANOVA for repeated measures indicated that there were highly significant effects of groups, $F(2,84) = 131.90$, $p<0.001$, Trials, $F(3,84) = 6.20$, $p<0.001$, and the interaction, $F(6,84 = 18.26$, $p<0.001$. Lower levels ANOVAs indicated that although there was no effect of groups on Trial 1, $F(2,21) = 0.20$, there were significant effects of groups on all other trials (smallest $F = 41.93$, $df = 2,21$, $p<0.001$ on all trials). Comparisons between groups (two tailed t tests) indicated that on Trial 2 both drug naive and drug experienced animals differed significantly from Naive Controls ($t = 9.05$ and 6.07 respectively, $df = 14$, $p<0.001$) in both cases). Furthermore, the drug naive group differed from the drug experienced group on this Trial ($t = 3.57$, $df = 14$, $p<0.01$). However, on Trials 3 and 4 the difference between the drug naive and drug experienced groups did not reach significance ($t = 1.39$ on Trial 3, 0.4 on Trial 4). The results clearly demonstrate that methamphetamine at 10 mg/kg induced a pronounced C.T.A. and

TABLE 1

MEAN (± SE) AMOUNTS OF 0.1% SACCHARIN DRUNK BY SUBJECTS IN THE EXPERIMENTAL GROUPS ON TRIALS 1–4

Group	Trial 1	Trial 2	Trial 3	Trial 4
Naive control	9.16 ± 1.15	15.65 ± 1.17	15.07 ± 1.40	17.12 ± 0.95
Experienced Methamphetamine	8.81 ± 0.77	8.07 ± 0.96	3.07 ± 0.81	2.05 ± 0.29
Naive Methamphetamine	9.80 ± 1.34	3.61 ± 0.60*	1.54 ± 0.39	2.44 ± 0.71
Experienced fenfluramine	9.60 ± 0.90	4.02 ± 0.58	3.92 ± 1.54	2.34 ± 0.70
Naive fenfluramine	7.90 ± 1.04	2.92 ± 0.70	1.72 ± 0.29	1.62 ± 0.33
Experienced p-chloramphetamine	8.65 ± 0.74	3.17 ± 0.62	2.66 ± 0.39	1.81 ± 0.22
Naive p-chloramphetamine	8.84 ± 0.93	5.20 ± 0.63	2.60 ± 0.31	1.82 ± 0.45
Experienced morphine	9.67 ± 1.34	8.54 ± 1.03	7.09 ± 1.14	8.66 ± 1.87
Naive morphine	8.45 ± 0.78	8.21 ± 0.65	7.95 ± 1.12	8.76 ± 1.32

*Significantly different from Experienced Group ($p<0.01$ two-tailed t-test).

that pretreatment with 3.0 mg/kg of the same drug provided partial, but not complete, protection against the aversive properties of this drug dose on the first taste/drug pairing, but not on subsequent pairings.

Effects of Treatment with Fenfluramine at 5 mg/kg, P-Chloramphetamine at 5 mg/kg and Morphine at 20 mg/kg

For all these studies two way ANOVAs for repeated measures indicated that there were highly significant effects of groups (smallest F = 31.49, df = 2,84, $p<0.001$), Trials (smallest F = 2.24, df = 3,84, $p<0.05$) and interactions (smallest F = 3.56, df = 6,84, $p<0.001$). Lower level ANOVAs indicated that although in none of the three studies was there an effect of groups on Trial 1 (largest F = 0.72, df = 2,21), there were significant group effects on all other trials (smallest F = 11.50, df = 2,21, $p<0.001$ on all trials). No significant differences were noted between drug naive and drug experienced (pretreated) animals on any Trial with any kind of Treatment (two tailed t tests).

DISCUSSION

The results demonstrate that chronic pretreatment with 3.0 mg/kg methamphetamine was effective in attenuating, but not abolishing the aversive properties of 10.0 mg/kg of the drug, and completely uneffective against the aversive properties of the three other drugs studied, at the doses administered.

GENERAL DISCUSSION

Comparison between the results of Experiments 1 and 2 indicates that drug dose is an important variable in pretreatment studies, since a chronic pretreatment regime which was completely effective in abolishing the aversive properties of 3.0 mg/kg methamphetamine (Experiment 1), was only partially effective in attenuating the aversive properties of a higher (10.0 mg/kg dose of the drug (Experiment 2). (Following completion of this study and submission for publication an independent report [3] confirmed the finding that drug dosage is an important determinant of pretreatment effects.) The experiments reported consequently demonstrate that both dose and duration of pretreatment are important determinants of the effectiveness of drug pretreatment in attenuating C.T.A.s by drugs. Since these variables are critical, it is possible that cross-drug pretreatment effects could be obtained in Experiment 2 with different pretreatment parameters. In fact, the results suggest that it is difficult to specify a general negative cross-drug pretreatment condition such that it can be stated unequivocally that pretreatment with Drug X never effects the ability of Drug Y to induce a taste aversion.

The data reported here cannot be explained in terms of habituation to the aversive effects of Treatments in general, nor even in terms of habituation to the aversive effects of one particular Treatment (drug), since a pretreatment regime which was effective in abolishing the aversive properties of the drug dose used during pretreatment was relatively ineffective against a higher dose of the same drug. If the results are interpreted in terms of habituation to aversiveness [2], it appears necessary to postulate that such habituation is specific to the UCS stimulus parameters administered during pretreatment. However, the hypothesis that pretreatment effects are explicable in terms of specific habituation to the aversiveness of the pretreating agent fails to account for the finding that it is possible to attenuate the aversive properties of one drug by pretreatment with another [4, 5, 9]. The habituation to aversiveness hypothesis would consequently seem inadequate as a general explanation of the pretreatment effect.

Similar arguments are applicable to the suggestion that the effect can be accounted for by interference with the UCS (drug) → CS (taste) association. It is not clear how this hypothesis can account for the finding that prior exposure to an effective UCS can *fail* to attenuate the aversiveness of another UCS other than by assuming that the interference with UCS–CS association is specific to the UCS used in

pretreatment, rather than generalized to all UCSs. However, if one assumes that interference with UCS–CS association is stimulus specific, it is difficult to explain reported cross-drug pretreatment effects. There would seem to be a further reason for rejecting the two hypotheses considered above, in that there is a more parsimonious explanation of the data available in terms of development of aversive tolerance [8].

Two criteria are commonly accepted as evidence for the development of tolerance to the behavioural effects of a drug [4]. Either it must be shown that following chronic treatment a particular drug dose has a reduced effect; or it must be shown that following chronic treatment with a particular drug dose a specified effect on behaviour is only obtained when the dose administered is increased. Both these criteria are met in the present report. The Tolerance hypothesis is consequently compatible with the data obtained in the present study. This hypothesis also explains why pretreatment drug dose is not important in pretreatment studies when the pretreatment dose is higher than the effective aversive dose [11], but is when it is lower (present study), since in the former case tolerance may have developed to the effective UCS, whilst it will not in the latter. Furthermore, the Tolerance hypothesis may be able to account for observed cross-drug pretreatment effects in terms of pharmacological cross-tolerance.

The data reported here are consequently considered to support the Tolerance explanation of the pretreatment effect. It should however, be noted that cross-drug pretreatment effects have been obtained with drugs that do not generally show cross-tolerance [4,5] consequently, a complete explanation of the effect appears to require further parametric studies.

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