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Disruption of Taste Aversion Learning by Pretreatment with Diazepam and Morphine

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BROWN, Z. W., Z. AMIT, B. SMITH AND G. ROCKMAN. *Disruption of taste aversion learning by pretreatment with diazepam and morphine*. PHARMAC. BIOCHEM. BEHAV. **10**(1) 17–20, 1979.—Laboratory rats were pretreated with either morphine (9 mg/kg IP), diazepam (4 mg/kg IP) or Ringer's solution 2, 3½, and 2 hr, respectively, prior to ingestion of a novel tasting saccharin solution followed immediately by a single injection of one of these agents. Animals pretreated with Ringer's solution followed by an injection of either morphine or diazepam showed a conditioned taste averstion (CTA) as determined by a significant reduction in the mean saccharin intake on a subsequent test trial. Although the drug pretreatments alone produced no conditioned avoidance behavior, the diazepam pretreatment completely blocked the development of both diazepam and morphine-evoked CTAs while the morphine pretreatment prevented a CTA induced by itself but not by diazepam. The results were discussed in terms of the attenuating effects of the pretreatments on the relative saliency of the subsequent conditioning drug injection.

Conditioned taste aversion Morphine Diazepam Laboratory rats

ANIMALS readily learn to associate a novel taste with a subsequent aversive internal state. Originally, conditioned taste aversion (CTA) learning was demonstrated with a variety of emetic agents and toxins [14, 15, 21, 22]. Paradoxically psychoactive drugs which are self-administered by laboratory animals presumably for their positive reinforcing properties, also exhibit conditioned aversive effects [3, 7, 9, 20, 30, 32, 33].

A number of investigators have recently utilized the CTA paradigm in an attempt to elucidate the mechanisms underlying some of the pharmacological actions of drugs. For example, it has been shown that with a variety of drugs, pre-exposure reduces the strength of the conditioned aversion [4, 8, 13, 19, 31]. It has been suggested that this attenuation of a CTA by prior exposure to a drug may be the result of development of an "artificial need state" [24], tolerance to the aversive effects [8,19] and/or the loss of novelty of the pharmacological consequences of the drug [1,13]. These proposed explanations have been unable to adequately account for the results of recent studies which demonstrated the elimination of CTAs by prior exposure to drugs which do not develop tolerance and physical dependence, or which are pharmacologically different from the agents used in the conditioning trials [5, 6, 10, 19, 31]. Another approach used to examine the possible mechanisms of drug action has been to determine the effects of neurochemical manipulations on the induction of CTAs by different drugs. It has been shown that specific neural destruction [28], inhibition of neurotransmitter synthesis [12, 16, 29] or receptor blockade [17,29] differentially affect aversive conditioning to a number of drugs, presumably as a result of the alteration or elimination of their pharmacological effects.

More generally, it has been proposed that the association between a conditioned stimulus (CS) and an unconditioned stimulus (US) may be interfered with by reducing the saliency of the stimuli [18, 26, 27]. It is conceivable that prior exposure to a drug or pretreatment with neurochemical altering agents may reduce the salience of the pharmacological effects of a subsequent drug injection (US) and thereby disrupt its association with a novel flavor (CS). The present study examined the effect of an acute injection of a drug just prior to conditioning on the development of a CTA induced by the same or a different drug.

METHOD

Animals

A total of 84 male Wistar rats (Canadian Breeding Farms Ltd.) each weighing 225–250 g served in the experiment and were exposed to the experimental manipulations in 2 consecutive groups of 30 and 54 animals. The animals were individually maintained in stainless steel cages throughout the experiment in a room regulated for constant temperature and humidity and a 12 hr light-dark cycle. Purina rat chow was available ad lib at all times.

Procedure

Following 3 or 4 days of acclimatization to the housing conditions, the animals were allowed restricted access to tap water for 20 min daily. The drinking fluids were presented in a single glass tube fitted with a metal ball-bearing spout mounted through the front of each home cage. The volumes of fluid consumed in each session were measured to the

nearest ml. On the ninth day (Conditioning Day) of the water deprivation schedule, the animals were randomly pretreated with intraperitoneal injections of either morphine hydrochloride (9 mg/kg; May and Baker Ltd.; n=30), diazepam (4 mg/kg; Valium®; Roche Ltd.; n=30) or Ringer's solution (1 ml/kg; n=24). The sequence of drug injections and the order of cage positions were randomized in order to avoid possible systematic effects of these variables. Based on previously determined times for recovery from the observable sedative effects of morphine and diazepam, 2 and 3½ hr intervals, respectively, were allowed before presentation of the test solutions. The Ringer's animals had a post-injection interval of 2 hr. Each of the animals was then presented with a 0.1% (w/v) sodium saccharin solution to drink for 10 min. One min later 1/3 of the animals in each of the pretreatment groups were injected with either morphine, diazepam or Ringer's solutions in the same doses used previously. After 5 intervening days of restricted days of restricted availability of water, all the animals were given a final 10 min session (Test Day) with their drinking tubes filled with saccharin solution. For purposes of reference, the pretreatment/treatment conditions will be denoted by the initial letter of the injected drugs.

RESULTS

Figure 1 shows the pooled mean saccharin consumption on Conditioning Day and on Test Day in groups of animals injected with the different combinations of morphine, diazepam and Ringer's in the pretreatment/treatment conditions. A two-way analysis of variance with repeated measures on the days factor, yielded a significant groups×days interaction effect ($F(8,75)=10.186$, $p<0.001$). Subsequent Neuman-Keuls tests for multiple comparisons ($\alpha=.05$) revealed that on conditioning day there were no significant differences in saccharin intake between the groups. Within group comparisons showed that whereas for most of the groups there was either no change or an increase in saccharin consumption, the R/M, R/D and M/D groups significantly reduced their intake from conditioning day to test day. Although the test day saccharin consumption for these latter groups was significantly lower than for the remaining groups, there were no differences among them.

Following the pretreatment injections, the animals were periodically observed and the duration of sedation (sleeptime and ataxia) was recorded. The diazepam-pretreated rats were sedated for approximately 2½ hr and appeared to be fully recovered 3½ hr after the injection. The sedative effects of the morphine pretreatment endured for approximately 1-1½ with normal activity resuming by 2 hr. It was also noted that the extent of the sedative effects of the subsequent conditioning treatments with the drugs did not vary systematically with the pretreatment condition. Furthermore, there did not appear to be any relationship between sedation and the development of a CTA.

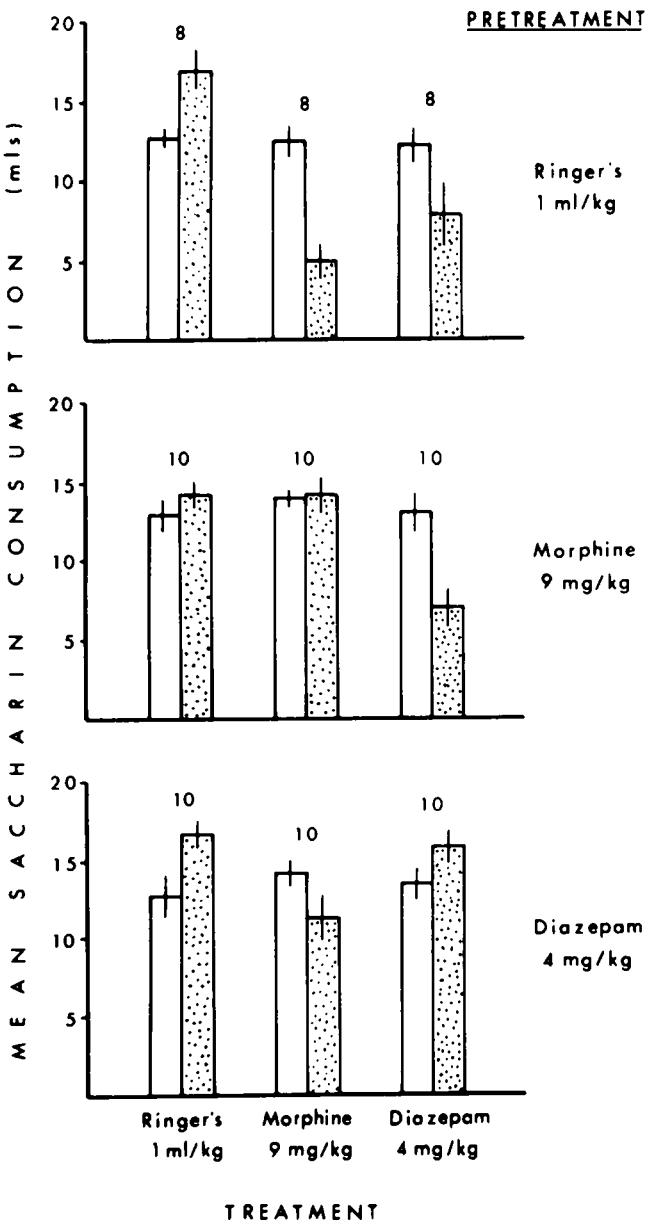

FIG. 1. Conditioning day (empty bars) and test day (stippled bars) consumption of a saccharin solution by groups of animals pretreated with Ringer's solution, morphine or Valium and subsequently injected with one of the agents. Numbers above the bars indicate group size. Vertical lines represent SEM.

DISCUSSION

The present experiment showed that an acute injection of either morphine or diazepam, 2 or 3½ hr, respectively, prior to the conditioning trial, disrupted the learning of associations between a saccharin flavor and a subsequent treatment with morphine or diazepam. The morphine and diazepam pretreatments alone did not produce any conditioning effects in the "backward" US-CS paradigm [2,25]. Nonetheless, the diazepam pretreatment interfered with the induction of a CTA by both diazepam and morphine while the morphine pretreatment was only effective in blocking a conditioned aversion normally induced by itself. Because of these asymmetrical results, the pretreatment interference effects on CTA learning cannot simply be attributed to some nonspecific toxicosis that may have been produced by the drugs. Furthermore, the relative magnitude of aversion between the pretreatments and the conditioning treatments cannot account for the present results since diazepam, which pro-

duced a somewhat weaker CTA than morphine was nevertheless effective in completely blocking a morphine-induced CTA. It would seem therefore that the comparative effects of the pretreatment and conditioning injections that modify the learned associations are qualitative rather than quantitative in nature. The possibility that the data may be interpreted in terms of state-dependent learning [23] can be ruled out by the fact that the morphine pretreatment, which blocked a CTA to itself, did not interfere with the learning of a diazepam-induced CTA.

Consistent with other reports [22,31] no relationship was found between the sedative effects of the drugs and the manifestation of a CTA. It was also observed that the pretreatments did not systematically alter the susceptibility to sedation by the conditioning injections suggesting that there was no rapid development of pharmacological tolerance or cross-tolerance. Consequently, the tolerance hypothesis which has been proposed as an explanation for the effects of repeated pre-exposures to a drug on subsequent aversive conditioning [8,19] cannot account for the present findings. Similarly, the suggestion that chronic pre-exposure to a drug may produce an "artificial need state" [24] thereby altering its effects as a US in a CTA paradigm is inadequate in this case since it is doubtful that the single preinjection could have induced a state of physical dependence. Finally, habituation [31] or loss of novelty [1,13] to the US effects cannot satisfactorily explain the present results since the diazepam pretreatment prevented a CTA induced not only by itself but also by morphine.

It has also been shown that CTAs induced by a variety of psychoactive drugs can be blocked by disruption of the neurochemical systems that presumably mediate their pharmacological effects [16, 17, 28, 29]. Similarly, in the present experiment it is possible that the diazepam and morphine pretreatments may have differentially altered neurochemical functioning so as to attenuate the pharmacological impact of the subsequent conditioning injections.

An alternative more general explanation which can account for the results of the present as well as some of the previous reports is based on the hypothesis that associations between a CS and a US may be interfered with if the saliency of either stimulus is diminished [18, 26, 27]. Relative to the animals' prevailing state resulting from the drug preinjection, the saliency of the effects of the conditioning injection may have been diminished thereby interfering with the associative process. Therefore, in the case of the M/M or D/D groups, the residual effects from the drug pretreatment may have attenuated the relative magnitude of pharmacological change normally elicited by subsequent treatment with the same drug. However, it is more difficult to understand the asymmetrical results of the D/M and M/D groups. It has been suggested that the pharmacological effects of a drug constitute a multidimensional discriminative stimulus complex [11], parts of which may be common to the stimulus complex elicited by another drug. Consequently, the internal state induced by the diazepam pretreatment may have masked the aversive pharmacological effects of the subsequent morphine injection. On the other hand, diazepam may possess additional pharmacologically aversive characteristics which are not common to morphine so that the morphine pretreatment could not effectively alter the saliency of the diazepam treatment. In light of the present results, one must consider the possibility that the attenuation of drug-induced CTAs by neurochemical or drug pretreatments may in part be due to a general interference of the perceived saliency of the US and/or CS.

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