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Diazepam-induced place preference conditioning: Appetitive and antiaversive properties*

Christina Spyraki, Anna Kazandjian, and Denis Varonos
Department of Experimental Pharmacology, Medical School, University of Athens, Goudi, 11527 Athens, Greece

Abstract. The place conditioning paradigm was used to examine the reinforcing properties of diazepam. Rats were injected with diazepam (0.5–5.0 mg/kg, IP) and 30 min later were confined for 30 min to one side of a shuttle box, in which each of the two compartments had distinctive features. On alternate (control) days they received vehicle injections and were confined for 30 min to the opposite side. At almost all doses tested, diazepam produced place preference for the distinctive compartment that had been previously associated with the drug. Preference for the drug side developed regardless of whether diazepam was paired or unpaired with the least-preferred side, and regardless of whether testing was carried out in the undrugged or in the drugged state. The rats preferred the drug side over a novel compartment, but they did not change their initial preference for the side when diazepam was given after removal from the training box.

Animals injected with meprobamate (70 mg/kg, PO), a non-benzodiazepine anxiolytic, also developed conditioned preference for the drug side, comparable to that seen following cocaine hydrochloride (10 mg/kg, IP).

The diazepam (2.5 mg/kg)-induced place preference was antagonized by CGS 8216 (3 mg/kg, IP), picrotoxin (2 mg/kg, IP) and naloxone (0.8 mg/kg, SC), injected 3 min before and 15 and 20 min after diazepam respectively. Sodium valproate (200 mg/kg, IP) did not influence diazepam (1 mg/kg)-induced place preference. Sodium valproate by itself had marginal effects on place conditioning. Picrotoxin and naloxone, but not CGS 8816, produced place aversion which, in the case of picrotoxin, was due to state dependent learning. The results provide a clear indication that the place preference paradigm is valid as a test for evaluating appetitive properties of minor tranquilizers. They suggest that the rewarding effects of diazepam are mediated through central benzodiazepine receptors. Whether GABA and/or endogenous opioid peptides are involved in the reinforcing properties of diazepam remains an open question.

Key words: Place conditioning – Diazepam – Meprobamate – CGS 8216 – Picrotoxin – Sodium valproate – Naloxone – Reward – Aversion – Rat

Benzodiazepines are the most commonly prescribed psychotropic drugs, among which diazepam is the most popular. Although the incidence of abuse of benzodiazepines is low (Marks 1978; Rickels 1981), there are clinical reports of excessive use of benzodiazepines, as well as experimental reports of idiosyncratic preferences for diazepam in certain human subjects (Griffiths et al. 1980; Healey and Pickens 1983).

In animal studies, drug abuse potential has been assessed by allowing animals the opportunity to voluntarily ingest or inject drugs as reinforcers (Johanson 1978). However, it is difficult to demonstrate reliably self-administration of minor tranquilizers, especially of benzodiazepines (Findley et al. 1972; Yanagita and Takahashi 1973; Griffiths et al. 1981). These difficulties in the case of diazepam (Griffiths and Ator 1982) can be attributed to its sedative and muscle relaxant properties (Zbinden and Randal 1967), which render the animal incapable of performing the operant responses required by the self-administration procedure.

An alternative paradigm, place conditioning, capitalizes on the classical conditioning of drug effects to environmental stimuli and permits simultaneous assessment of appetitive and aversive properties of drugs, having the advantage over operant procedures of not being disrupted by drug-elicited gross behavioural changes. Place conditioning has been employed successfully to confirm the appetitive effects of a variety of drugs of abuse, within the classes of opiates and psychostimulants (Mucha et al. 1982; Sherman et al. 1980a, b; Spyraki et al. 1982a, b, 1983).

The main aim of this study was to evaluate rewarding properties of anxiolytics, in particular diazepam and meprobamate, a non-benzodiazepine anxiolytic, using place conditioning.

The demonstration that benzodiazepines facilitate GABA-ergic transmission (Costa and Guidotti 1979; Study and Barker 1982), the identification of benzodiazepine receptors in the CNS (cf Möhler and Richards 1983) and the discovery of agents that selectively block the effects of benzodiazepines, acting at their receptors (cf Haefeley et al. 1983), have led to a greater understanding of the synaptic actions of benzodiazepines (Haefeley 1983). Thus, further experiments reported here were designed to asess the pharmacological specificity of the rewarding properties of diazepam. First, the efficacy of the selective benzodiazepine antagonist CGS 8216 (Bernard et al. 1981), in antagonizing the effect of diazepam on place conditioning was evaluated. We also explored the possibility of GABA-ergic involvement in the rewarding effects of diazepam by testing the GABA antagonist picrotoxin (Andrews and Johnston 1979) and sodium valproate, which raises the synaptic concentration of GABA by retarding its metabolic degradation (Har-

Offprint requests to: C. Spyraki

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vey 1976), both separately and in combination with diazepam. Finally, the effect of the opiate antagonist naloxone (Sawynok et al. 1979), on diazepam-induced place preference was investigated, as behavioral and biochemical data show that benzodiazepine effects are reversed by opiate antagonists (cf. Cooper 1983; Millan and Duka 1981). Thus it was of interest to see if diazepam engages the activity of reward mechanisms through an interaction with endogenous opioid peptides.

Materials and methods

Animals

Experimentally naive male Wistar rats, bred in our laboratory, weighing 250–280 g, were housed in group cages ($N=8$) in a climatically-controlled colony room. Except for periods of testing, food and water were continuously available and the colony was maintained under a 12-h light-dark cycle (lights on 8 a.m.). All behavioural tests were conducted in rectangular Plexiglas shuttle boxes, with distinctive compartments differing in wall colouring and in flooring, as described elsewhere (Spyraki et al. 1982c).

General experimental procedure

Behavioural testing was conducted in the light phase of the diurnal cycle (9 a.m. – 4 p.m.). Each experiment was carried out over 12 consecutive days and each rat was run once only. The procedure, previously extensively described (Spyraki et al. 1982a, b, c, 1983), consisted of three phases and briefly was as follows. During the first, preconditioning phase, the animals were allowed to investigate the entire shuttle box, over 15 min. On the 3rd day, the time spent by each animal in each of the compartments was recorded, this providing a measure of the initial unconditioned preference between the two compartments. During the second 8-day conditioning phase, animals were injected with vehicle or drug and after a standard interval time, that varied for the different kinds of treatment, they were confined for 30 min to one side of the shuttle box. This was the initially non-preferred side, when the drug was tested for place preference and the initially preferred side, when the drug was tested for place aversion. On alternate days, rats received vehicle injections and were confined to the opposite side. On the test day, third post-conditioning phase, animals were tested, drug free, for their preference for the side, over 15 min.

The difference in the time spent in the drug-paired compartment between the final test trial and the last day of the preconditioning period represents a measure of place conditioning. Difference (+) in favour of the drug compartment reflects appetitive properties of the drug, while difference (−) in favour of the vehicle compartment reflects aversive properties of the drug. This theoretical view relies on the assumption that the appetitive effects of drugs (unconditioned stimuli) enable the environmental stimuli to become conditioned incentive stimuli by association.

Diazepam-induced place conditioning as a function of dose

The purpose of this experiment was to determine whether the place conditioning paradigm could be used to measure rewarding properties of diazepam and also to screen for possible dose-related effects in diazepam-induced place preference. For this experiment, 50 rats were selected which did not show particularly strong biases during the preconditioning tests (time in the non-preferred side – 3rd preconditioning day – 400 s ± 32). Efforts were made to include in each group equal number of rats preferring the black or white side. The 50 rats were divided into five groups, each containing ten animals. The five groups did not differ in the amount of time spent on the less preferred side, prior to conditioning [$F(4,45)=0.16$].

Training by pairing diazepam with the preferred side and training without pretest

In order to control for nonassociative changes produced by diazepam, three additional groups of animals were run. Two groups ($N=7$/group) were injected with vehicle or diazepam (1 mg/kg) following the preconditioning phase and after 30 min were confined for 30 min to the initially preferred side. On alternate days the animals received vehicle and were placed on the initially non-preferred side.

Animals in the third group ($N=9$) were not tested for initial biases. In this group, each rat was placed four times in the white compartment and an equal number of times (on alternate days) in the black compartment. One side was associated with the drug and the other with vehicle. The particular environment that was associated with the drug was counterbalanced over subjects. Following training, testing was identical for all rats.

Preference for familiar or novel place cues

During training, the rats experience the diazepam-paired cues under the influence of the drug, while during testing animals are in a drug-free state. In this state the diazepam-paired environmental stimuli were not experienced during conditioning and the animals could be approaching them because of stimulus novelty. To test this possibility, two groups of animals were treated as follows. One group ($N=6$) was injected with vehicle and the other group ($N=8$) received diazepam (1 mg/kg). Thirty minutes later the animals were placed for 30 min in the white or the black compartment. The same treatment was given on 4 consecutive days and each rat was placed each day in the same compartment. Assignment of the drug or vehicle to the salient environment was counterbalanced for the rats of each group. Following training, rats were tested as usual.

Testing and training under the same drug state

The present experiment tested a) if conditioning occurs with the drug given following exposure of the animal to the particular environment and b) whether drug-produced state dependency could be an alternative explanation of conditioned place preference.

Twenty-four animals were divided into three groups ($N=8$/group) and trained according to our standard method (see general procedure) with the following differences. The first group received diazepam or vehicle immediately after removal from the training environment. The second and third groups were tested under the influence of diazepam or vehicle respectively. Animals were administered diazepam (1 mg/kg) or vehicle 30 min before testing.

Place preference induced by meprobamate and cocaine

The procedure was identical to that employed in the first part of the experiment, except that instead of diazepam, meprobamate (70 mg/kg) was administereed orally, 30 min before the animal was put in the place conditioning apparatus. Rats in another group received cocaine (10 mg/kg, IP), immediately before they were placed in the initially non-preferred compartment of the shuttle box.

The effect of CGS 8216 on diazepam-induced place preference

Forty male Wistar rats were divided into four groups, each containing eight to ten animals. The first group was injected with vehicle and served as a control. The second and third groups were injected with vehicle or CGS 8216 (3 mg/kg, IP) 3 s prior to diazepam (2.5 mg/kg) administration. The fourth group was injected with vehicle 3 s prior to CGS 8216 administration. The procedure was identical to that described in the first experiment. Sixteen additional rats received only CGS 8216 (3 mg/kg) as a test for any direct effect of this drug on place conditioning. On drug days half of those animals were placed on the initially preferred side and the other half on the initially less preferred side. On vehicle days the animals were placed in the opposite compartment.

Diazepam-GABA interactions on place conditioning

a. The effect of sodium valproate. Subjects were 40 male Wistar rats divided into four groups ($N=10$/group). The first group (control) received only vehicle, the second group received vehicle (1 ml/kg) and diazepam (1 mg/kg) and the third group received diazepam (1 mg/kg) and sodium valproate (Depakine, Labaz, 200 mg/kg, IP). Finally, the fourth group was injected with sodium valproate. Injections of the different solutions were made IP with 3-s interval time. Animals were put in the place conditioning apparatus 30 min following injections. The procedure was identical to that described in the first experiment.

b. The effect of picrotoxin. The procedure was identical to that described in the previous experiments. Briefly, 40 Wistar rats were used. The first and second group received diazepam (2.5 mg/kg), 10 min prior to vehicle or picrotoxin (2 mg/kg, IP) administration. Twenty minutes following picrotoxin animals were confined for 30 min to the initially non-preferred compartment of the shuttle box. Rats in the third and fourth groups were injected with picrotoxin (Sigma, 2 mg/kg) only, 20 min prior to being placed in the initially preferred side of the place conditioning apparatus. On the test day (phase 3), each rat in group 3 (picrotoxin) was injected with saline 20 min before being tested for 15 min. Rats in the fourth group (picrotoxin) were injected with picrotoxin (2 mg/kg), 20 min before the test. The third group was tested for possible place aversion induced by picrotoxin. The fourth group was included in our experiment for the following reason. It was anticipated that picrotoxin might induce place aversion, i.e. the animals, on the test day, would avoid the compartment associated with picrotoxin during conditioning, preferring the compartment associated with vehicle injections. Because animals on the test day are drug free, they may at this time choose the compartment that had previously been associated with no-drug (i.e. saline), not because the drug itself is aversive but probably because it results in state dependent learning. To test this state dependent hypothesis, the fourth group was tested for post-conditioning place preference under the influence of picrotoxin.

Diazepam-naloxone interactions in place conditioning

Forty male Wistar rats were divided into four groups, each containing ten animals. The first and second group were injected with diazepam (2.5 mg/kg, IP) 15 min prior to vehicle or naloxone (0.8 mg/kg, SC) administration. Animals in the first and second groups were confined to the initially non-preferred side, 15 min following naloxone or vehicle injections. Rats in the third and fourth groups were injected with naloxone, 15 min prior to being placed in the initially preferred compartment of the shuttle box. The third and fourth groups were injected, on the post-conditioning test day, with vehicle or naloxone 15 min before the trial. These last groups served to control for possible aversive effects of naloxone, perhaps attributable to state dependent learning.

Results

Diazepam-induced place conditioning as a function of dose

The results are summarized in Fig. 1. Compared to phase I, rats injected with diazepam showed a significant [$F(1,90)=33.9$, $P<0.01$] shift in preference towards the side that had been associated with the drug (positive difference between post and preconditioning tests). The two-way ANOVA also yielded a significant dose effect [$F(4,90)=5.6$, $P<0.01$] and a significant preference × dose interaction [$F(4,90)=19.8$,

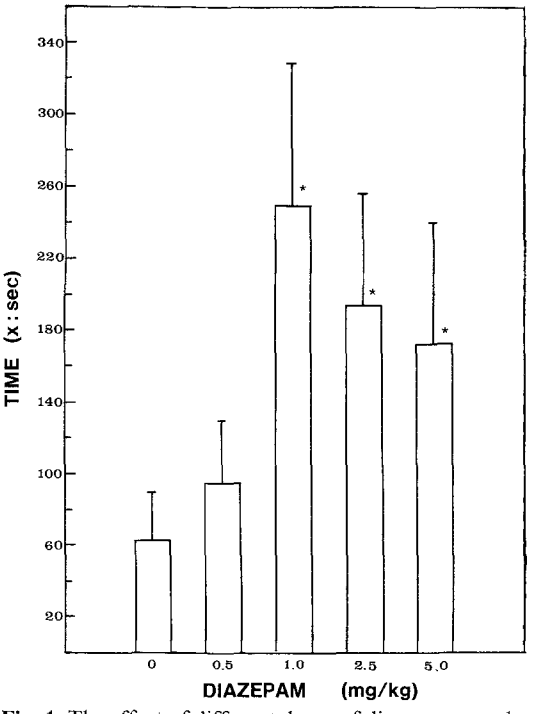

Fig. 1. The effect of different doses of diazepam on place preference conditioning: Data represent means (±SEM) of the difference in time (s) spent on the drug side between pre- and post-conditioning test sessions ($N=10$/group). * $P<0.05$ comapred to controls

$P<0.01$]. Individual post-hoc comparisons indicated that the significant interation was due to the fact that the vehicle- and 0.5 mg/kg diazepam-injected animals failed to show significant place preference conditioning. A significant effect was achieved with doses of 1 and 2.5 mg/kg ($P<0.01$). Increasing the dose did not produce greater place preference conditioning (5 mg/kg: $P<0.05$) than 1 mg/kg.

Pairing diazepam with the initially preferred side

Prior to any conditioning, vehicle- and diazepam-injected animals remained on the most preferred side (630 s ± 17 and 616 s ± 29 respectively). The mean times on the preferred side of the test apparatus following conditioning were: controls: 597 s ± 15; diazepam: 762 s ± 35. The diazepam-treated animals increased significantly ($P<0.01$) their time spent in the compartment which was previously associated with diazepam.

Conditioning without pretest

Six animals preferred the diazepam-paired side and three rats showed a preference for the saline-paired compartment. The mean times spent on the drug- and vehicle-associated side were 532 s ± 62 and 367 s ± 62 respectively. There was a significant ($P<0.05$) difference in the time spent on each side.

Preference for familiar or novel place cues

Vehicle-injected animals spent more time in the novel compartment (484 s ± 29) than in the vehicle-associated (familiar) compartment (416 s ± 29). The difference in the time spent on each side only approached significance.

Diazepam-treated animals spent significantly ($P<0.001$) more time in the drug-paired (familiar) (677 s ± 44) than in the novel (223 s ± 44) compartment.

The two groups differed significantly ($P<0.01$) in the amount of time spent on each side of the shuttle box.

Testing and training under the same drug state

Animals given diazepam after removal from the training box did not change significantly their initial preference for the side associated with post-training injection of diazepam. The mean times spent on that side before and after training were: 269 s ± 35 and 357 s ± 74 respectively.

Animals tested under the influence of diazepam showed a clear preference for the drug-associated side [Pre-conditioning time (CT) = 243 s ± 33, Post-CT = 486 s ± 32, $P<0.05$]. Similarly, animals tested following vehicle administration changed their initial preference (287 s ± 30) in favour of the diazepam-associated side (470 s ± 28). No significant differences were detected between the two groups.

The effect of meprobamate and cocaine

As expected, cocaine induced a significant place preference ($P<0.005$). Rats injected with meprobamate spent significantly ($P<0.005$) more time in the drug-paired compartment during the post-than the preconditioning trial. The results are summarized in Table 1.

Table 1. Mean (± SE) time (s) spent in the drug-paired environment before and after conditioning

Group	N	Time on drug side		
		Pre-conditioning	Post-conditioning	
Cocaine (10 mg/kg, IP)	10	317 ± 38	606 ± 100	$P<0.01$
Meprobamate (70 mg/kg, PO)	10	261 ± 19	600 ± 124	$P<0.01$

The effect of CGS 8216 on diazepam-induced place preference

The data (Fig. 2) demonstrate that diazepam-induced place preference can be significantly attenuated by pretreatment with the benzodiazepine antagonist CGS 8216. The pre- and postconditioning scores for each of the four groups were analyzed by a split-plot factorial ANOVA. This analysis confirmed a significant main effect for the group [$F(3,68)=5.76$, $P<0.01$] and trial [$F(1,68)=8.0$, $P<0.01$] factors. Post-hoc analysis revealed that the diazepam-vehicle group was significantly different from the vehicle group ($P<0.05$), the CGS 8216-diazepam group ($P<0.02$) and the vehicle-CGS 8216 group ($P<0.05$). No differences were detected between the CGS 8216-diazepam and the CGS 8216-vehicle groups.

The data in Table 2 show that CGS 8216-treated animals do not avoid the compartment associated with the drug, regardless of whether CGS 8216 was paired with the initially preferred or least-preferred side.

The effect of sodium valproate

The data are summarized in Fig. 3. Split-plot factorial ANOVA revealed significant main effect for the trial (pre- and post-conditioning) [$F(1,72)=15.53$, $P<0.01$] but not for the group [$F(3,72)=1.5$] variable. Post hoc analyses revealed no differences between the diazepam and the diazepam + sodium valproate groups, or between sodium valproate and vehicle groups. Thus, sodium valproate did not influence diazepam-induced place conditioning and failed by itself to induce place preference.

The effect of picrotoxin

Figure 4 depicts the results. It is obvious that diazepam (first group) induces place preference, while picrotoxin (third group) produces significant ($P<0.01$) place aversion (negative difference between post 485 s ± 74 and preconditioning 659 s ± 32 trials). This effect of picrotoxin was not significant for animals tested under picrotoxin (fourth group: Pre-CT = 736 s ± 21, Post-CT = 622 s ± 63). The combination of diazepam and picrotoxin (second group) had no effect on place conditioning (Pre-CT = 280 s ± 72, Post-CT = 235 s ± 40). A split-plot factorial analysis of the data over four groups revealed significant conditioning [$F(1,72)=9.5$, $P<0.01$] and group [$F(3,72)=56.7$, $P<0.01$] effects. Individual comparisons indicated that the diazepam + picrotoxin group differed significantly from both diazepam-($P<0.01$) and picrotoxin ($P<0.05$)-treated

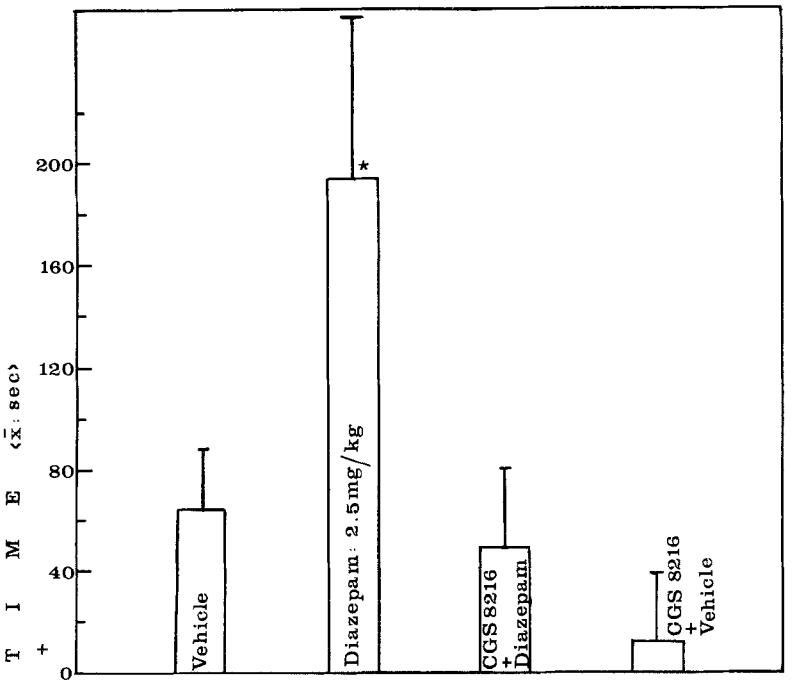

Fig. 2. Effect of CGS 8216 on diazepam-induced place preference. Data represent means (±SEM) of the difference in time (s) spent on the drug-paired side between pre- and postconditioning test session ($N=10$/group), * $P<0.05$

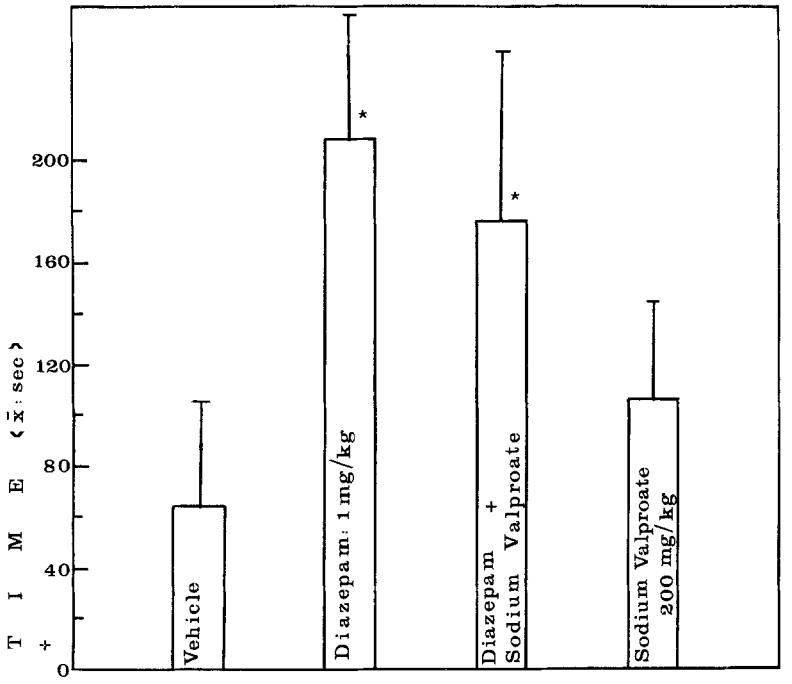

Fig. 3. the effect of sodium valproate on place conditioning and on diazepam-induced place preference. Data represent means (±SEM) of the difference in time (s) spent on the drug-paired side between pre- and postconditioning test sessions ($N=10$/group), * $P<0.05$–0.02

Table 2. Mean (±SE) time (s) spent in the drug-paired environment before and after conditioning

CGS 8216	*N*	Time on drug side		
		Pre-conditioning	Post-conditioning	
Preferred side	(10)	828±21	774±25	n.s.
Non-preferred side	(6)	214±37	210±33	n.s.

groups. It appears then, that diazepam and picrotoxin have opposite effects on place conditioning, which are cancelled out when both drugs are combined. When data from the third (picrotoxin-vehicle on the test day) and fourth (picrotoxin-picrotoxin on the test day) groups were analyzed alone, the group effect was again significant [$F(1,36)=5.2$, $P<0.05$].

The effect of naloxone

The results are summarized in Fig. 5. As was expected, diazepam (first group) induced place preference, while naloxone (third group) induced highly significant ($P<0.005$) place aversion (Pre-CT = 685 s±40, Post-CT = 361 s±93). The aversive effect of naloxone was also present ($P<0.02$) in animals (fourth group) which had received the drug before the postconditioning test (Pre-CT = 706 s±26, Post-CT = 484 s±112). Following combination of both drugs (second group) there was no significant place conditioning

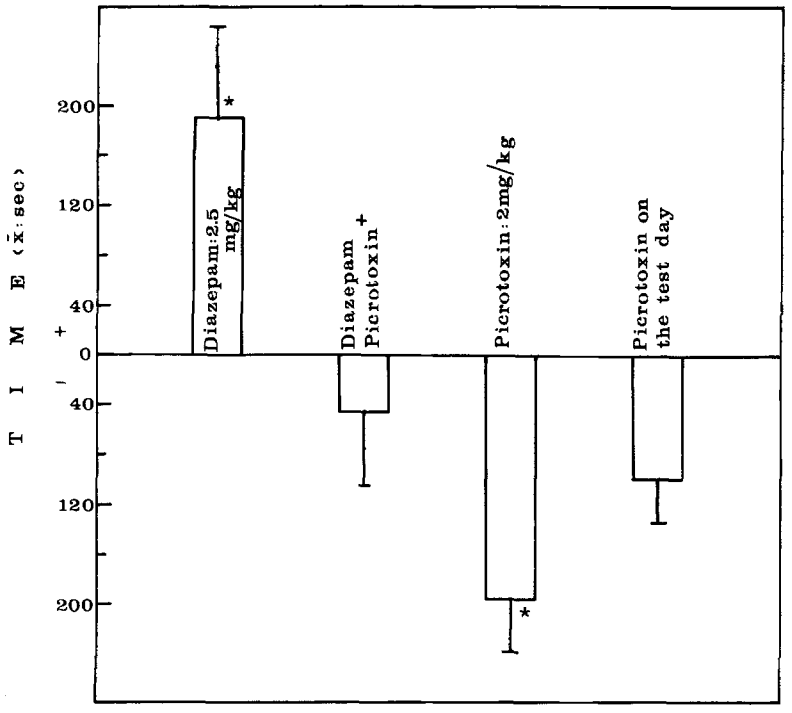

Fig. 4. The effect of picrotoxin on place conditioning and on diazepam-induced place preference. *Last column* represents data from animals tested under picrotoxin (state dependent learning: see text for details). Data are means (±SEM) of the difference in time (s) spent on the drug-paired side between pre- and postconditioning test sessions ($N=10$/group), * $P<0.02$–0.01. control values were +62 s±28

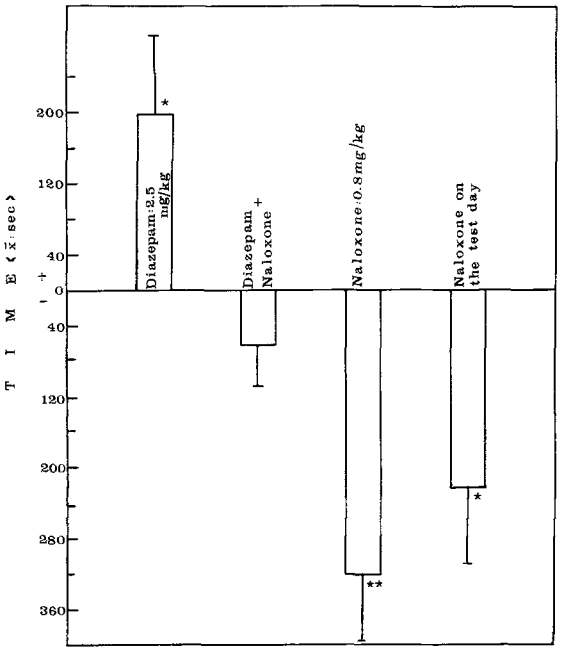

Fig. 5. The effect of naloxone on place conditioning and on diazepam-induced place preference. *Fourth column* represents data from animals tested under naloxone. Data are means (±SEM) of the difference in time (s) spent on the drug-paired side between pre- and postconditioning test sessions ($N=10$/group), * $P<0.02$, ** $P<0.05$

(Pre-CT = 258 s±40, Post-CT = 96 s±26). When data from all four groups were analyzed by a split-plot factorial ANOVA, there were significant main effects for the group [$F(3.72)=36.15$, $P<0.01$] and trial [$F(1,72)=4.9$, $P<0.02$] factors. There was also a significant group × trial interaction [$F(3,72)=30.24$, $P<0.01$], indicating a difference between specific groups over the two test sessions. Post hoc analyses revealed a significant difference between the diazepam-naloxone group and both the diazepam group ($P<0.01$) and the naloxone group ($P<0.01$). No differences were detected between the third and fourth groups (vehicle vs naloxone on test day).

Discussion

The present experiments demonstrate that diazepam (1–5 mg/kg) produces place preference in the rat, as a result of associative conditioning. They also rule out an alternative explanation of diazepam's place preference involving the notions of drug-produced state dependency and the motivational properties of novelty. Therefore, it is concluded that diazepam-induced place preference rather reflects appetitive properties of the drug. Thus, our data provide independent confirmation of the rewarding properties of diazepam in the rat, as previously indicated by drug discrimination procedures (Johanson and Jarbe 1975; Haug and Gotestam 1982; Shannon and Herling 1983) and intracranial self stimulation (ICSS) (Caudarella et al. 1982; Gerhardt et al. 1982; Olds 1966). They are also consistent with the reports that diazepam is self-administered by monkeys (Findley et al. 1972; Yanagita and Takahashi 1973; Griffiths et al. 1981) and preferred over placebo by humans (Griffiths et al. 1980).

The magnitude of the rewarding effects of diazepam appeared to reach a maximum at 1 mg/kg in the rat. Increasing the dose to 2.5 or 5.0 mg/kg did not result in a stronger effect. Similar maximum and plateau effects at approximately 1–6 mg/kg diazepam were observed in cue discrimination studies (Shannon and Herling 1983) and in rewarding brain stimulation in a shuttle box (1–2.5 mg/kg) (Gerhardt et al. 1982). In human studies, when diazepam alone was available, preference for the drug was also not observed (Healey and Pickens 1983; Griffiths et al. 1980).

The meprobamate data added to those of diazepam, along with anecdotal reports that the anxiolytic buspirone (Ortman, personal communication) induces place prefer-

ence, clearly indicate the validity of the place conditioning paradigm as a test of the appetitive effects of anxiolytics. However, the place conditioning method has also detected aversive effects of ethanol (Sherman et al. 1983) and pentobarbital (Mucha and Iversen 1984), two substances with some antianxiety properties. Although we have no explanation for this discrepancy, it should be noticed that there are differences in pharmacodynamics between the abovementioned anxiolytics.

We have shown that the selective benzodiazepine (BNZ) antagonist CGS 8216 could block the diazepam-induced place preference. This observation is in agreement with other reports indicating that this compound can specifically antagonize the pharmacological effects of BNZs (Bernard et al. 1981; Yokohama et al. 1982). Shannon and Davis (1984) have demonstrated that CGS 8216 (at the same dose used in this study) antagonized the discriminative stimulus properties of diazepam. CGS 8216, at least at the dose tested, does not appear to have primary aversive effect, as revealed in the place conditioning procedure. This observation argues against the possibility that CGS 8216 might block the diazepam-induced place preference as a result of having unspecified aversive properties, rather than by blocking the rewarding properties of diazepam. Thus, it is suggested that the diazepam-induced place preference is mediated via central BNZ receptors.

Benzodiazepines are known to facilitate GABA-ergic transmission (Costa and Guidotti 1979), but sodium valproate was unable to mimic or potentiate the effects of diazepam on place conditioning. In accordance with our findings, a variety of GABA agonists were found to be incapable of mimicking the diazepam discrimination cue (Haug 1983; Nielsen et al. 1983). Moroever, sodium valproate, unlike BNZs, has been reported to depress responding maintained by intracranial stimulation (Herberg and Williams 1983). The general lack of BNZ-like effects of GABA-ergic compounds, as well as the incapacity of those agents to potentiate the rewarding effects of BNZs, suggest that the GABA system may not be the critical link for the stimulus properties of BNZs. On the other hand, the GABA antagonist picrotoxin reversed the diazepam-induced place preference. In view of the apparent ability of picrotoxin to induce place aversion, probably through a state dependent learning process, the inability of a diazepam-picrotoxin combination to affect place conditioning cannot unreservedly be ascribed to the nullification of the GABA-ergic properties of both drugs. Conflicting reports have appeared in the literature concerning the effect of GABA antagonists in the reinforcing properties of BNZs. For example, whilst GABA antagonists were not able to abolish the diazepam discrimination cue in one study (Haug 1983), bicuculline produced significant, albeit small, attenuation of the diazepam discrimination cue in another (Nielsen et al. 1983). Moroever, contradictory results have derived from the ICSS studies using GABA blockers. For example, picrotoxin, which by itself depressed responding for ICSS, was surprisingly reported to significantly increase it when combined with BNZs (Herberg and Williams 1983). These results provide difficulties in explaining the rewarding or antiaversive effects of BNZs on the basis of GABA transmission.

Finally, in our last experiment, we observed that diazepam-induced place preference was abolished in naloxone-treated animals. Accordingly, a variety of behavioural effects of BNZs have been found to be antagonized by opiate antagonists (Billingsley and Kubena 1978; Duka et al. 1981; Soubrié et al. 1980). However, attributing the naloxone effect on diazepam-induced place preference solely to opiate receptor blockade may be unwarranted. Because naloxone by itself produced a significant place aversion (Mucha and Iversen 1984; Mucha et al. 1982; this study), the lack of effect in the diazepam-naloxone group may simply have been due to a cancellation of two independent, but opposite effects, diazepam preference and naloxone aversion on place conditioning.

In conclusion, our results do not provide unequivocal evidence for a role of central opiate systems in diazepam-induced place preference. Inasmuch as the diazepam discrimination cue seems not to be blocked by naloxone (Shearman et al. 1982), the hypothesis that the rewarding properties of diazepam in the rat are mediated via endorphinergic mechanisms remains at best speculative.

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