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Chlordiazepoxide and Valproate Enhancement of Saline Drinking by Nondeprived Rats: Effects of Bicuculline, Picrotoxin and RO15-1788

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SHEPHARD, R. A. AND M. S. HAMILTON. *Chlordiazepoxide and valproate enhancement of saline drinking by nondeprived rats: Effects of bicuculline, picrotoxin and RO15-1788.* PHARMACOL BIOCHEM BEHAV **33**(2) 285–290, 1989.—Drinking of 0.85% saline by nondeprived rats was significantly enhanced by chlordiazepoxide (5 or 10 mg/kg) and by valproate (100 or 300 mg/kg), drug effects being strongest in the earlier parts of a 30-minute test. When given alone, both bicuculline and picrotoxin significantly reduced saline drinking at 2.5 mg/kg, but not 1.5 mg/kg. Administration of valproate at either dose or of chlordiazepoxide (10 mg/kg) completely prevented bicuculline action and 5 mg/kg chlordiazepoxide reduced it. Picrotoxin, however, largely prevented the actions of both chlordiazepoxide and valproate. The increase in saline drinking induced by valproate (300 mg/kg) was also blocked by RO15-1788 (10 or 25 mg/kg). These findings are discussed in the context of the three-site model of the GABA/benzodiazepine receptor complex. It is concluded that drugs acting at the benzodiazepine site or the chloride ion channel affect saline drinking, but that there is little evidence of an important functional role for the $GABA_a$ site at present.

Chlordiazepoxide Valproate Picrotoxin RO15-1788 Bicuculline GABA Rats Saline drinking

IT is now established that benzodiazepine anxiolytics can enhance fluid consumption by rats, even in situations where aversive components such as novelty or punishment are absent (1, 5, 6, 20, 24, 43). Enhanced drinking with benzodiazepines is especially evident in situations of high fluid palatability, for instance when subjects are water deprived, or when consumption of highly-preferred solutions of condensed milk, sucrose, saccharin or sodium chloride is measured (6,35). These effects on fluid consumption probably depend upon interaction with brain benzodiazepine receptors since, in addition to the effects of anxiolytic benzodiazepines, essentially opposite effects of the inverse agonist CGS 8216 have been reported (9,18). Moreover, RO15-1788 antagonises both enhanced saline drinking induced by chlordiazepoxide (43) and attenuated condensed milk drinking induced by CGS 8216 (9). Beyond this, however, there has been little analysis of the mechanisms by which benzodiazepines enhance drinking or of how this relates to other effects of these drugs, e.g., anxiolytic or anticonvulsant actions.

A number of lines of evidence derived from biochemical, neurophysiological and behavioral studies suggest that many actions of benzodiazepines are mediated by GABA systems [e.g., (8, 13, 33, 34)]. More specifically, it has been proposed that there is a macromolecular GABA/benzodiazepine receptor complex, the main elements of which are a $GABA_a$ receptor, a benzodiazepine binding site and a chloride ion permeability channel which functions as a final common pathway at the complex mediating subsequent neural events (8, 12, 13, 25). The prototypical antagonists at these sites are, respectively, bicuculline, RO15-1788 and picrotoxin.

The GABA agonist valproate has consistently been shown to simulate anxiolytic actions of benzodiazepines in a variety of behavioral test procedures (19, 22, 28, 30, 37, 39). Antagonism of the antineophobic effects of valproate by RO15-1788 as well as by picrotoxin (39) suggests a relationship of this action to the benzodiazepine receptor complex (8), but valproate does not potentiate the antineophobic effects of chlordiazepoxide but rather attenuates them (37). Increases in brain GABA levels by inhibition of GABA transaminase does not appear to explain behavioral effects of valproate (30, 33, 34). In order to assess the generality of these interactions one should compare the effects of valproate with benzodiazepines in procedures not directly related to anxiety, such as enhanced drinking.

The present paper reports results of four experiments on chlordiazepoxide and valproate enhancement of saline drinking by rats. Experiments One and Two examine effects of chlordiazepoxide (0, 5 or 10 mg/kg) alone and in combination with bicuculline (0, 1.5 or 2.5 mg/kg) or picrotoxin (0, 1.5 or 2.5 mg/kg), respectively, in 3×3 design experiments. The third assesses valproate (0, 100 or 300) mg/kg alone and in combination with bicuculline (0, 1.5 or 2.5 mg/kg) in a 3×3 design and the fourth examines 300 mg/kg valproate alone and combined with picrotoxin (1.5 mg/kg) or RO15-1788 (10 or 25 mg/kg).

METHOD

Subjects were 45 experimentally-naive adult male Wistar rats, bred in our laboratory, caged individually and weighing 350–450 g throughout the study. They were maintained on a 12-hr light/dark cycle at 22°C. Groups of 10 rats were used for Experiments One, Two and Three and 15 for Experiment Four. Within these groups, rats were randomly allocated to blocks which received the drug treatments in fully randomised and balanced repeated measures designs.

The animals were tested in their home cages and prior to testing were thoroughly acclimatised to all aspects of the procedure (including IP injection of vehicle). Their drinking water bottles were replaced by pipettes with 0.1 ml calibrations attached to drinking tubes containing 0.85% saline for one hour per day for 14 days prior to beginning observations. Apart from these and test periods, animals had free access to food and water at all times. On test days fluid consumption was assessed 6, 12, 18, 24 and 30 minutes after the start and is expressed in ml. Test days were separated by three to five days during which control injections were given. Fluid intake invariably returned to control levels on the days between drug tests. Results were analysed by ANOVA with further comparisons based on the error terms.

All drug injections or appropriate vehicle controls were given IP 30 minutes prior to making saline available and beginning observations. The experiments used chlordiazepoxide hydrochloride (Roche), sodium valproate (Labaz Sanofi), bicuculline (Sigma), picrotoxin (Sigma) and RO15-1788 (Roche). Chlordiazepoxide and valproate doses are expressed as salt. Drug dosages and the group sizes needed to produce statistically-reliable results were chosen on the basis of pilot work. Where combinations of drugs were given, these were as single injections. One percent Tween 80 was used to suspend RO15-1788.

RESULTS

Experiment One

Analysis of variance showed significant main effects of chlordiazepoxide (C), $F(2,18) = 136.5$, $p<0.001$, of bicuculline (B), $F(2,18) = 19.5$, $p<0.001$, and of observation time, (T), $F(4,36) = 59.9$, $p<0.001$. Significant interactions also occurred between CB, $F(4,36) = 3.4$, $p<0.05$, and CT, $F(8,72) = 5.7$, $p<0.001$. The CT interaction can be understood by reference to Fig. 1, which shows the time course of the effects of chlordiazepoxide only.

As can be seen, chlordiazepoxide increased saline drinking throughout the test at both doses, but the size and statistical significance of this effect declined somewhat over time, and was nonsignificant for 5 mg/kg in the last period. The limited effects of bicuculline in this study are shown in Fig. 2 which depicts drug effects on saline drinking in the whole 30-min period. Given alone, bicuculline significantly reduced saline drinking at 2.5 mg/kg, but not at 1.5 mg/kg. However, its efficacy was reduced by 5 mg/kg chlordiazepoxide and it was completely inactive in the presence of 10 mg/kg of the benzodiazepine. Variance measures are omitted from Figs. 1 and 2 for clarity because they were fairly constant and small. The average standard deviation for the 45 drug conditions/observation periods was 0.063.

Experiment Two

Analysis of variance showed significant main effects of chlordiazepoxide (C), $F(2,18) = 125.0$, $p<0.001$, of picrotoxin (P), $F(2,18) = 78.3$, $p<0.001$, and of observation time (T), $F(4,36) = 25.5$, $p<0.001$. Significant interactions also occurred between

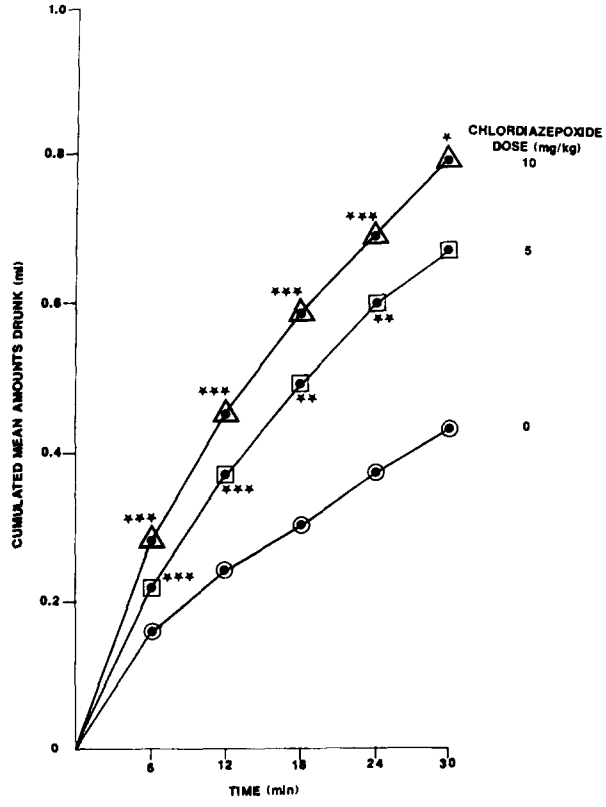

FIG. 1. The figure shows the effects of chlordiazepoxide (5 mg/kg or 10 mg/kg) on saline drinking across time. Data shown are cumulated means but significance tests are based on six-minute observation periods, e.g., 18–24 min. Significance of the differences from control are shown by stars: Significant beyond ★ 5% level; ★★ 1% level; ★★★ 0.1% level.

CP, $F(4,36) = 7.6$, $p<0.001$, and CT, $F(8,72) = 3.8$, $p<0.01$. The CT interaction was similar to that observed in Experiment One in that chlordiazepoxide selectively increased drinking in the earlier observation periods (data not shown). The effects of the drug treatments on drinking in the full 30 min of the test are shown in Fig. 3 which, like Fig. 1, shows highly significant enhancement of saline drinking with chlordiazepoxide alone at both 5 mg/kg and 10 mg/kg dose levels. Like bicuculline, picrotoxin alone inhibited saline drinking at 2.5 mg/kg, but not at 1.5 mg/kg. Picrotoxin, however, reduced saline drinking in the presence of chlordiazepoxide, this effect being highly significant in all four conditions where two drugs were given. Indeed, Fig. 3 shows that the efficacy of chlordiazepoxide was greatly attenuated by the presence of picrotoxin, especially by the 1.5 mg/kg dose. Variance measures are omitted from Fig. 3 for clarity because they were fairly constant and small. The average standard deviation for the 45 drug conditions/observation periods was 0.068.

Experiment Three

Analysis of variance showed significant main effects of valproate (V), $F(2,18) = 26.6$, $p<0.001$, of bicuculline (B), $F(2,18) = 10.5$, $p<0.01$, and of observation period (P), $F(4,36) = 17.0$, $p<0.001$. Significant interactions also occur between VB, $F(4,36) = 7.2$, $p<0.001$, and VP, $F(8.72) = 2.7$, $p<0.05$. The VP interaction can be understood by reference to Fig. 1, which shows the time course of the effects of valproate only. As can be seen, 300

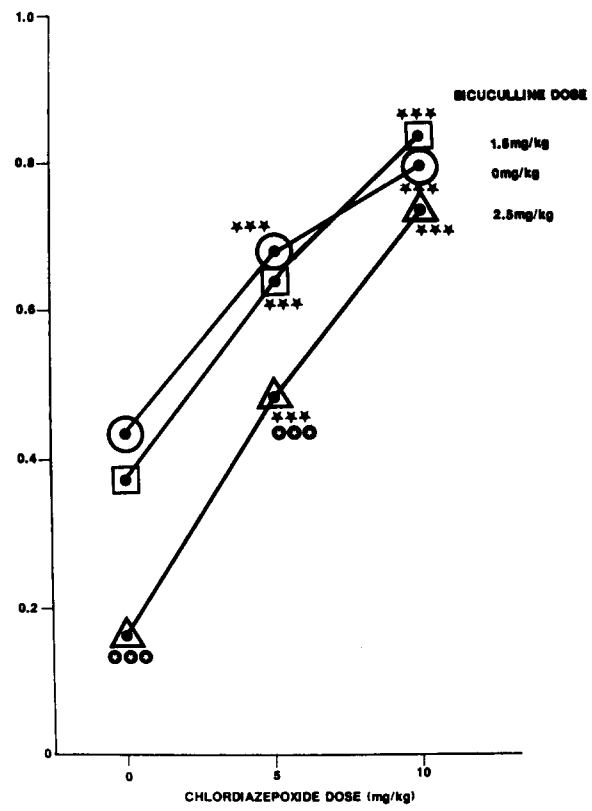

FIG. 2. The figure shows the effects of nine combinations of chlordiazepoxide (0, 5 or 10 mg/kg) with bicuculline (0, 1.5 or 2.5 mg/kg) on saline drinking (ml) in 30 min. Three stars denote significantly different from no chlordiazepoxide control beyond 0.1% level. Three white stars on black denote significantly different from no bicuculline control beyond 0.1% level.

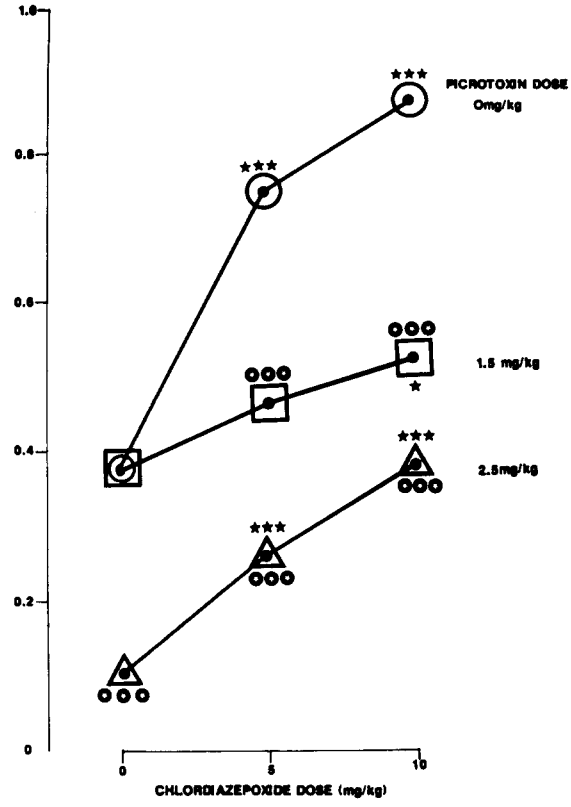

FIG. 3. The figure shows the effects of nine combinations of chlordiazepoxide (0, 5 or 10 mg/kg) with picrotoxin (0, 1.5 or 2.5 mg/kg) on saline drinking (ml) in 30 min. Stars denote significantly different from no chlordiazepoxide control (one, 5% level; three, 0.1% level). Three white stars on black denote significantly different from no picrotoxin control beyond 0.1% level.

mg/kg of valproate increased saline drinking throughout the test, but the size and statistical significance of this effect declined somewhat over time, and was nonsignificant in the 18–24-min period. The lower dose of valproate (100 mg/kg), however, only significantly increased saline drinking in the first two six-min periods. The limited effects of bicuculline in this study are shown in Fig. 5 which depicts drug effects on saline drinking in the whole 30-min period. Given alone, bicuculline significantly reduced saline drinking at 2.5 mg/kg, but not at 1.5 mg/kg. However, it was completely inactive in the presence of either valproate dose. The 100 mg/kg dose of valproate only significantly enhanced drinking over the 30-min period in the 2.5 mg/kg bicuculline condition. The higher dose of valproate (300 mg/kg) significantly enhanced saline drinking in all bicuculline conditions. The interdependence of drug effects on the dose of the other compound gave rise to the significant VB interaction. Since bicuculline was inactive in the presence of valproate, curves for the combinations were omitted from Fig. 4 for clarity. As can be adduced from Fig. 5 and from the nonsignificance of the BP and VBP interactions they would lie close to those shown, save for the 2.5 mg/kg bicuculline, no valproate condition. Variance measures are omitted from Figs. 4 and 5 for clarity because they were fairly constant and small. The average standard deviation for the 45 drug conditions/observation periods was 0.072 ml.

Experiment Four

Analysis of variance showed significant effects of the drugs used (D), $F(4,56) = 10.9$, $p<0.001$, of the observation period (P), $F(4,56) = 32.4$, $p<0.001$, and of the DP interaction, $F(16,224) = 1.8$, $p<0.05$. As for Experiment Three, there was a tendency for rats to drink more saline early in the session and this was exaggerated by 300 mg/kg valproate, giving rise to the P and DP effects. The drug effects are shown in Fig. 3, which clearly illustrates that valproate (300 mg/kg) significantly increased drinking over 30 min and that drinking was reduced back to control levels by addition of RO15-1788 (10 or 25 mg/kg) and also by picrotoxin (1.5 mg/kg). Variance measures are omitted from Fig. 6 for clarity because they were fairly constant and small. The average standard deviation for the 25 drug conditions/observation periods was 0.081 ml.

DISCUSSION

Experiments One and Two clearly show enhancement of drinking of 0.85% saline solutions by chlordiazepoxide. This accords with a number of previous reports of enhanced drinking with benzodiazepines reviewed earlier. In previous studies of benzodiazepines and saline drinking, water-deprived rats have been used (6,43). The published procedure has some advantages over the present one in that it generates much higher levels of saline drinking by control subjects and drug effects which are arithmetically larger (though smaller as a proportion of the baseline). However, the use of water-deprived rats produces a complicated

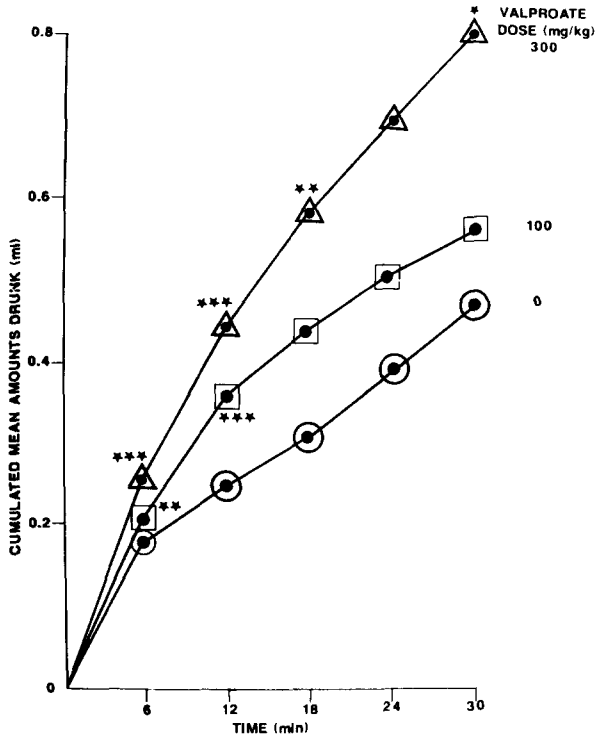

FIG. 4. The figure shows the effects of valproate (100 mg/kg or 300 mg/kg) on saline drinking across time. Data shown are cumulated means but significance tests are based on six-minute observation periods, e.g., 18–24 min. Significance of the differences from control are shown by stars: Significant beyond ★ 5% level; ★★ 1% level; ★★★ 0.1% level.

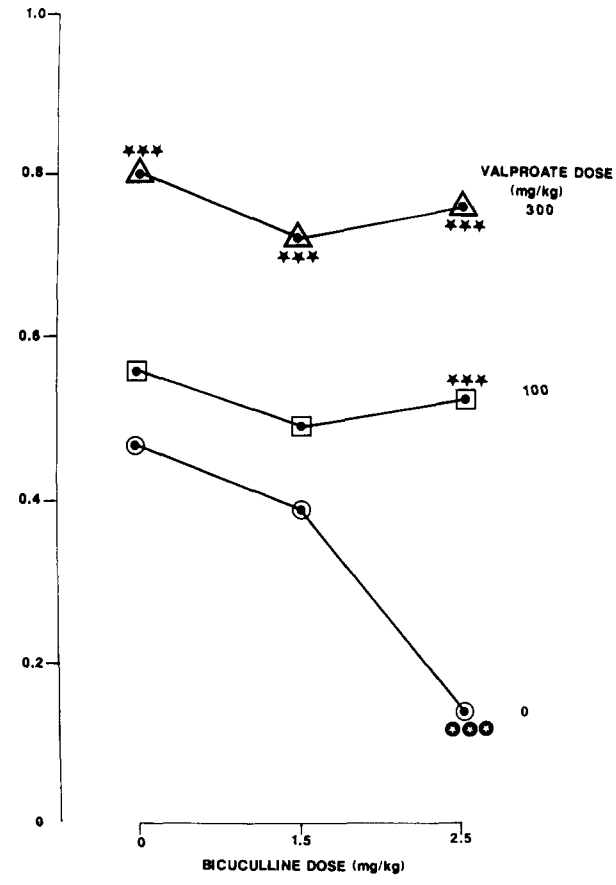

FIG. 5. The figure shows the effects of the nine combinations of valproate (0, 100 or 300 mg/kg) with bicuculline (0, 1.5 or 2.5 mg/kg) on saline drinking (ml) in 30 minutes. Three stars denote significantly different from appropriate no valproate control beyond 0.1% level. Three white stars on black significantly different from no bicuculline control beyond 0.1% level.

time-course of drinking and drug action. It also necessitates consideration of the possibility that drug-induced increases in plain water consumption by deprived rats (10, 20, 43) might contribute to the effects on saline drinking. Since 0.85% lies within a highly palatable range of sodium chloride concentrations (14,44) and subjects were familiarised with it before drug testing, no component of aversiveness of neophobia is evident in this procedure. Therefore, anxiolytic actions of chlordiazepoxide cannot readily explain the present behavioral effects. It may be, however, that the brain mechanisms by which benzodiazepines enhance drinking are similar to those involved in attenuation of anxiety.

Experiments Three and Four clearly show the drinking of saline by rats to be consistently and significantly elevated by valproate (300 mg/kg), and, in the early part of the sessions of Experiment Three by 100 mg/kg valproate. This finding extends the parallel between effects of valproate and of benzodiazepines, already known to share anticonvulsant, muscle relaxant and apparent anxiolytic properties in animal model procedures (19, 29, 34) by showing similarity in a behavioral procedure not related to anxiety systems (6).

Bicuculline, however, was virtually inactive in these studies apart from a suppression of drinking induced by 2.5 mg/kg alone. This treatment induced minor convulsive phenomena in 10/20 subjects during the preobservation period and the 2.5 mg/kg dose is clearly at the upper extreme of the range suitable for behavioral experimentation. Even this dose, however, was completely without effect on saline drinking when given with chlordiazepoxide (10 mg/kg) or valproate (100 mg/kg or 300 mg/kg); coadministration of valproate also eliminated convulsive effects of bicuculline but 1/10 rats receiving 5 mg/kg chlordiazepoxide plus 2.5 mg/kg bicuculline showed some evidence of these. We have already seen that convulsant effects of bicuculline were confined to the preobservation period and this drug is known to have a short half-life in rats. Even though effects of 2.5 mg/kg bicuculline alone were evident using the present methods it could be argued that our preinjection times were too long for antagonism of chlordiazepoxide and valproate effects to occur. For this reason a replication was attempted using five-minute preinjection times and lower bicuculline doses (Shephard, unpublished). With this modification it became impossible to establish a dose of bicuculline which reduced saline drinking (with or without chlordiazepoxide or valproate), which did not also induce convulsive phenomena and therefore no publishable data were gathered.

Picrotoxin alone also reduced saline drinking and induced minor convulsive reactions (4/10 subjects) at 2.5 mg/kg in Experiment Two. No convulsant effects of any other picrotoxin regime used were noted. The interaction of picrotoxin with chlordiazepoxide was clearly very different from the bicuculline/chlordiazepoxide interaction. Although the 1.5 mg/kg dose of picrotoxin lacked effect on saline drinking alone it greatly attenuated the effects of chlordiazepoxide, to nonsignificance in the case of the 5 mg/kg dose. Chlordiazepoxide at both dose levels, did however significantly increase drinking when given with 2.5 mg/kg picrotoxin. The apparently greater effectiveness of the lower picrotoxin dose in antagonising chlordiazepoxide may seem paradoxical but at least two explanations should be considered.

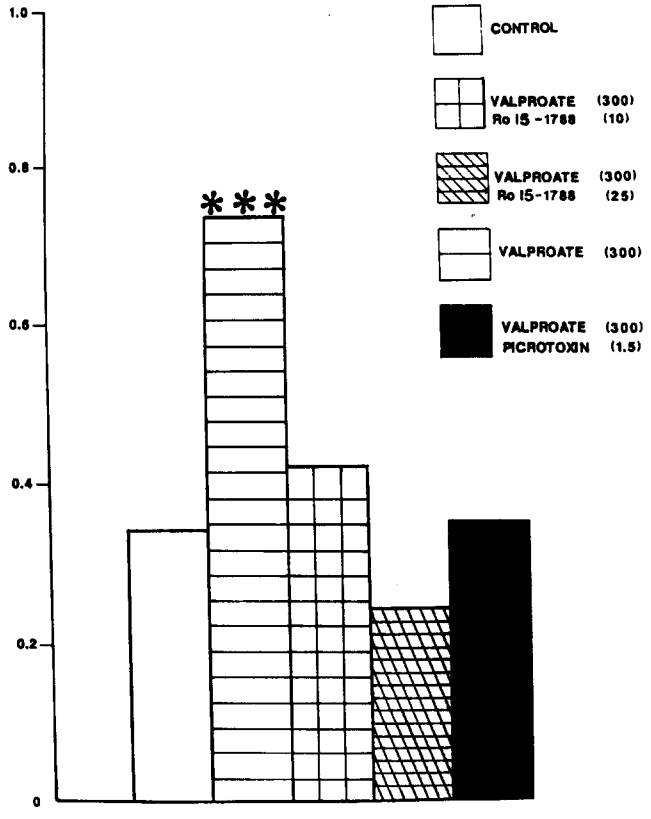

FIG. 6. The figure shows the effects of drug conditions shown on saline drinking (ml) in 30 minutes. Three asterisks denote significantly different from vehicle control beyond 0.1% level.

Firstly, since 2.5 mg/kg picrotoxin produced overt toxic signs which were antagonised by chlordiazepoxide, it may be that at least some of the enhancement in drinking induced by chlordiazepoxide here is a by-product of this reduced toxicity. Secondly, and perhaps not unrelatedly, 2.5 mg/kg picrotoxin produced large reductions in drinking alone and it is generally easier to increase behavior from a low baseline. It should be noted that although chlordiazepoxide increased drinking in the presence of 2.5 mg/kg picrotoxin, it barely returned it to the control level shown by undrugged rats even at 10 mg/kg. Effects of 1.5 mg/kg picrotoxin which greatly attenuated chlordiazepoxide action whilst being inactive alone, should be regarded as more useful for analytical purposes, and this dose prevented the effects of valproate (300 mg/kg), in Experiment Four. RO15-1788 (10 or 25 mg/kg) prevented the effects of valproate on saline drinking (Fig. 6). This agent also prevents actions of chlordiazepoxide (43) and CGS 8216 (9) in this paradigm but is inactive alone [(9,43); Shephard, unpublished].

The most striking finding of the present investigations is the contrast between the effects of bicuculline and picrotoxin in antagonising both chlordiazepoxide and valproate-induced enhancement of saline drinking. Whereas bicuculline fails to do this even at a rather toxic dose, picrotoxin is effective at a dose which is too low to affect the behavior alone. In order to assess the implications of these interactions, they should be compared with those observed in other behavioral procedures. The literature on interactions between benzodiazepines and bicuculline or picrotoxin in procedures regarded as anxiety models is somewhat diffuse and contradictory (32–34). However, whilst picrotoxin has generally proved effective in antagonising apparent anxiolytic actions of benzodiazepines (2, 16, 36, 40), studies of bicuculline/benzodiazepine interactions have shown antagonism (3), no effect of bicuculline in the presence of diazepam (7) and even potentiation of some effects of chlordiazepoxide by bicuculline (4). Reduction of conditioned suppression induced by chlordiazepoxide is antagonised by picrotoxin, but not bicuculline (21). Similarly, in drug discrimination procedures, the stimulus properties of various benzodiazepines are antagonised by picrotoxin (17,27) but not by bicuculline (15, 23, 26).

These studies would seem to clarify the mechanism of action of valproate at the GABA/benzodiazepine receptor complex. The emerging view of the latter (8, 12, 13, 25) is that the three main elements of the complex are a GABA binding site of the $GABA_a$ receptor type, a benzodiazepine binding site and a chloride ion permeability channel which functions as a final common pathway at the complex mediating subsequent neural events. The prototypical antagonists at these sites are, respectively, bicuculline, RO15-1788 and picrotoxin, the drugs investigated in the present experiments. As bicuculline failed to alter valproate action even at high doses, stimulation of $GABA_a$ receptors would not seem to be the mechanism of action of valproate. Antagonism at either the benzodiazepine receptor or the chloride ion channel prevented the effects of valproate, as had been shown in some studies of other behavioral actions (22, 30, 39). Of these two sites, the displacement of receptor-bound tritiated dihydropicrotoxinin but not tritiated diazepam, by valproate (42) would suggest the chloride ion channel as the more probable site of action of valproate. Moreover, picrotoxin appears to be a competitive antagonist of the effects of valproate in enhancing food intake suppressed by novelty (39), suggesting a common site of action. Further work would be needed to determine why behavioral effects of valproate can be disrupted by both antagonists [(22,39), Experiment Four] and agonists (37) at the benzodiazepine site, whilst being insensitive to actions at the $GABA_a$ site [(22), Experiment Three]. Since benzodiazepines and related drugs are known to affect other neurotransmitter systems, such as serotonin and norepinephrine (33,40), it would also be of interest to test drugs affecting these systems in the saline drinking paradigm. Methysergide, clonidine and propranolol might be especially worth examining as these have been shown to have anxiolytic activity.

It may be that the different sites of action of bicuculline and picrotoxin at the benzodiazepine receptor complex explain the differences in efficacy between them. Since picrotoxin seems to act at the chloride ion channel, the "effector" of the receptor complex, it might be expected that picrotoxin would be an effective antagonist of the actions of benzodiazepines and related drugs. By contrast, bicuculline seems relatively inactive in behavioral studies which may be due to a comparatively marginal role of the $GABA_a$ receptor site in mediating these functional activities of the benzodiazepine receptor complex. Such an hypothesis would also explain why the $GABA_a$ agonist muscimol so persistently fails to elicit benzodiazepine-like behavioral effects (4, 11, 12, 31, 38, 41) despite evidence that it enters the brain and produces neurophysiological effects following peripheral injections of low doses (12).

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REFERENCES

1. Berridge, K. T.; Treit, D. C. Chlordiazepoxide directly enhances positive ingestive responses in rats. Pharmacol. Biochem. Behav. 24:217–221; 1986.
2. Billingsley, M. L.; Kubena, R. K. Effects of naloxone and picrotoxin on the sedative and anti-conflict effects of benzodiazepines. Life Sci. 22:897–906; 1978.
3. Birk, J.; Nobel, R. C. Bicuculline blocks diazepam-induced feeding in the Syrian hamster. Life Sci. 30:321–325; 1982.
4. Buckland, C.; Mellanby, J.; Gray, J. A. The effects of compounds related to γ-aminobutyrate and benzodiazepine receptors on behavioural responses to anxiogenic stimuli in the rats: Extinction and successive discrimination. Psychopharmacology (Berlin) 88:285–295; 1986.
5. Cooper, S. J. Benzodiazepine mechanisms and drinking in the water-deprived rat. Neuropharmacology 21:775–780; 1982.
6. Cooper, S. J.; Estall, L. B. Behavioural pharmacology of food, water and salt intake in relation to drug action at benzodiazepine receptors. Neurosci. Biobehav. Rev. 9:5–19; 1985.
7. Corda, M. G.; Biggio, G. Proconflict effect of GABA receptor complex antagonists: Reversal by diazepam. Neuropharmacology 25:541–544; 1986.
8. Costa, E. Benzodiazepines and neurotransmitters. Arzneimittelforschung 30:858–861; 1980.
9. Estall, L. B.; Cooper, S. J. Benzodiazepine receptor mediated effects of CGS 8216 on milk consumption in the non-deprived rat. Psychopharmacology (Berlin) 89:477–479; 1986.
10. Falk, J. L.; Burnidge, G. K. Fluid intake and punishment-attenuating drugs. Physiol. Behav. 5:199–202; 1970.
11. Gardner, C. R.; Piper, D. C. Effects of agents which enhance GABA-mediated neurotransmission on licking conflict in rats and exploration in mice. Eur. J. Pharmacol. 83:25–33; 1982.
12. Gray, J. A.; Quintero, S.; Mellanby, J.; Buckland, C.; Fillenz, M.; Fung, S. C. Some biochemical, behavioural and electrophysiological tests of the GABA hypothesis of anti-anxiety drug action. In: Bowery, N. G., ed. Actions and interactions of GABA and benzodiazepines. New York: Raven Press; 1984:239–262.
13. Haefely, W.; Pieri, L.; Polc, P.; Schaffner, R. General pharmacology and neuropharmacology of benzodiazepine. In: Hoffmeister, F.; Stille, G., eds. Handbook of experimental pharmacology. vol. 55. Berlin: Springer-Verlag, 1981:13–262.
14. Hall, W.; Blass, E. Orogastric, hydrational and behavioral controls of drinking following water deprivation in rats. J. Comp. Physiol. Psychol. 89:939–954; 1975.
15. Haug, T. Neuropharmacological specificity of the diazepam stimulus complex: Effects of agonists and antagonists. Eur. J. Pharmacol. 93:221–227; 1983.
16. Hodges, H. M.; Green, S. E. Evidence for the involvement of brain GABA and serotonin systems in the anticonflict effects of chlordiazepoxide in rats. Behav. Neural Biol. 40:127–154; 1984.
17. Jarbe, T. U. C. Characteristics of pentobarbital discrimination in the gerbil: Transfer and antagonism. Psychopharmacology (Berlin) 49: 33–40; 1976.
18. Kirkham, T. C.; Cooper, S. J. CGS 8216, a novel anorectic agent, selectively reduces saccharin solution consumption in the rat. Pharmacol. Biochem. Behav. 25:341–345; 1986.
19. Lal, H.; Shearman, G. T.; Fielding, S.; Dunn, R.; Kruse, H.; Theurer, K. Evidence that GABA mechanisms mediate the anxiolytic action of benzodiazepines: A study with valproic acid. Neuropharmacology 19:785–789; 1980.
20. Leander, J. D. Effects of punishment-attenuating drugs on deprivation-induced drinking: Implications for conflict procedures. Drug Dev. Res. 3:185–191; 1983.
21. Leslie, J. C.; Shephard, R. A.; Toal, L. Conditioned suppression of operant behaviour in rats: Effects of chronic administration of chlordiazepoxide and sodium valproate. Paper presented at the second European meeting on the Experimental Analysis of Behaviour, Liege, 1988.
22. Liljequist, S.; Engel, J. A. Reversal of the anti-conflict action of valproate by various GABA and benzodiazepine antagonists. Life Sci. 34:2525–2533; 1984.
23. McElroy, J. F.; Feldman, R. S. Generalization between benzodiazepine- and triazolopyridazine-elicited discriminative cues. Pharmacol. Biochem. Behav. 17:709–713; 1982.
24. Maikel, R. P.; Maloney, G. J. Taste phenomena influences on stimulation of deprivation-induced fluid consumption in rats. Neuropharmacology 13:763–767; 1974.
25. Meldrum, B. Classification of GABA and benzodiazepine receptors. J. Psychopharmacol. 1:1–5; 1987.
26. Nielsen, E. B.; Valentine, J. D.; Holohean, A. M.; Appel, J. B. Benzodiazepine receptor mediated discriminative cues: Effects of GABA ergic drugs and inverse agonists. Life Sci. 33:2213–2220; 1983.
27. Oka, M.; Yamada, K.; Yosieda, K.; Shimizu, M. Avoidance enhancement and discriminative response control by anxiolytics with drugs acting on the GABA system. Jpn. J. Pharmacol. 30:325–336; 1980.
28. Petersen, E. N.; Lassen, J. B. A water lick conflict paradigm using drug experienced rats. Psychopharmacology (Berlin) 75:236–239; 1981.
29. Pinder, R. M.; Brogden, R. N.; Speight, T. M.; Avery, G. S. Sodium valproate: A review of its pharmacological properties and therapeutic efficacy in epilepsy. Drugs 13:81–123; 1977.
30. Rasmussen, K. J.; Schneider, H. H.; Petersen, E. N. Sodium valproate exerts anti-conflict activity in rats without any concomitant rise in forebrain GABA level. Life Sci. 29:2163–2170; 1981.
31. Sanger, D. J. Chlordiazepoxide induced hyperphagia in rats: lack of effect of GABA agonists and antagonists. Psychopharmacology (Berlin) 84:388–392; 1984.
32. Sanger, D. J. GABA and the behavioral effects of anxiolytic drugs. Life Sci. 36:1503–1511; 1985.
33. Shephard, R. A. Neurotransmitters, anxiety and benzodiazepines: A behavioral review. Neurosci. Biobehav. Rev. 10:449–461; 1986.
34. Shephard, R. A. Behavioral effects of GABA agonists in relation to anxiety and benzodiazepine action. Life Sci. 40:2429–2436; 1987.
35. Shephard, R. A. Chlordiazepoxide and the drinking of water by rats: Effects of shock and other suppressive measures. Pharmacol. Biochem. Behav. 31:313–316; 1988.
36. Shephard, R. A.; Broadhurst, P. L. Effects of diazepam and picrotoxin on hyponeophagia in rats. Neuropharmacology 21:771–773; 1982.
37. Shephard, R. A.; Estall, L. B. Effects of chlordiazepoxide and of valproate on hyponeophagia in rats: Evidence for a mutual antagonism between their anxiolytic properties. Neuropharmacology 23:677–681; 1984.
38. Shephard, R. A.; Leslie, J. C.; Toal, L. Conditioned suppression of operant behaviour in rats: Effects of agonists and antagonists at the GABA/benzodiazepine receptor complex. Paper presented at the second European Meeting on the Experimental Analysis of Behaviour, Liege, 1988.
39. Shephard, R. A.; Stevenson, D.; Jenkinson, S. Effects of valproate on hyponeophagia in rats: Competitive antagonism with picrotoxin and non-competitive antagonism with RO15-1788. Psychopharmacology (Berlin) 86:313–317; 1985.
40. Stein, L.; Wise, C. D.; Belluzzi, J. D. Effects of benzodiazepines on central serotonergic mechanisms. Adv. Biochem. Psychopharmacol. 14:29–44; 1975.
41. Thiébot, M. H.; Jobert, A.; Soubrié, Effets comparés du muscimol et du diazépam sur les inhibitions du comportement induites chez le rat par la nouveauté, la punition et le non-renforcement. Psychopharmacology (Berlin) 61:88–89; 1979.
42. Ticku, M. K.; Davis, W. C. Effect of valproic acid on [^{3}H] diazepam and [^{3}H] dihydropicrotoxinin binding at the benzodiazepine-GABA receptor-ionophore complex. Brain Res. 223:218–222; 1981.
43. Turkish, S.; Cooper, S. J. Enhancement of saline drinking by chlordiazepoxide in thirsty rats: Antagonism by RO 15-1788. Pharmacol. Biochem. Behav. 20:869–873; 1984.
44. Weiner, I. H.; Stellar, E. Salt preference of the rat determined by a single stimulus method. J. Comp. Physiol. Psychol. 44:394–401; 1951.

circadian oscillator: the suprachiasmatic hypothalamic nucleus. Fed. Proc. 42:2783–2789; 1983.
30. Mrosovsky, N. Phase response curves for social entrainment. J. Comp. Physiol. 162:35–46; 1988.
31. Nishino, H.; Koizumi, K. Responses of neurons in the suprachiasmatic nuclei of the hypothalamus to putative neurotransmitters. Brain Res. 120:167–172; 1977.
32. Ohde, T. W.; Post, R. M.; Siever, L. Clonidine: effects on mood, anxiety, and pain. Psychopharmacol. Bull. 17:125–126; 1981.
33. Pratt, B. L.; Goldman, B. D. Environmental influences upon circadian periodicity in Syrian hamsters. Physiol. Behav. 36:91–95; 1986.
34. Raiteri, M.; Maura, G.; Versace, P. Functional evidence for two stereochemically different alpha-2 adrenoreceptors regulating central norepinephrine and serotonin rlease. J. Pharmacol. Exp. Ther. 224: 679–684; 1983.
35. Raiteri, M.; Bonnano, G.; Maura, G. Changes of sensitivity of presynaptic alpha-2-adrenoceptors induced by chronic clonidine in rat brain. J. Hypertens. 4:S122–S124; 1986.
36. Rietveld, W. J.; Korving, J.; Boon, M. E.; Wirz-Justice, A. The circadian control of behavior in the rat affected by the chronic application of methamphetamine. In: Pauly, J. E.; Scheving, L. E., eds. Advances in chronobiology B. New York: A. R. Liss; 1987: 513–517.
37. Rosenwasser, A. M.; Adler, N. T. Structure and function in circadian timing systems: evidence for multiple coupled circadian oscillators. Neurosci. Biobehav. Rev. 10:431–448; 1986.
38. Sandyk, R.; Gillman, M. A.; Iacono, R. P.; Bamford, C. R. Clonidine in neuropsychiatric disorders: a review. Int. J. Neurosci. 35:205–215; 1987.,
39. Shibata, S.; Liou, S. Y.; Ueki, S. Different effects of amino acids, acetylcholine and monoamines on neuronal activity of suprachiasmatic nucleus in rat pups and adults. Neurosci. Lett. 39:187–192; 1983.
40. Stewart, K. T.; Rosenwasser, A. M.; Adler, N. T.; Volpicelli, J. R. Circadian activity rhythms in an animal model of depression. Soc. Neurosci. Abstr. 12:597; 1986,.
41. Stewart, K. T.; Rosenwasser, A. M.; Volpicelli, J. R.; Hauser, H. H.; Adler, N. T. Circadian rhythms and escape learning in rats exposed to shock. Soc. Neurosci. Abstr. 13:607; 1987.
42. Teicher, M. H.; Lawrence, J. M.; Barber, N. I.; Finklestein, S. P.; Lieberman, H. R.; Baldessarini, R. J. Increased activity and phase delay in circadian motility rhythms in geriatric depression. Arch. Gen. Psychiatry 45:913–917; 1988.
43. Turek, F. W. Circadian neural rhythms in mammals. Annu. Rev. Physiol. 47:49–64; 1985.
44. Turek, F. W.; Gwinner, E. Role of hormones in the circadian organization of vertebrates. In: Aschoff, J.; Daan, S.; Groos, G., eds. Vertebrate circadian systems: Structure and physiology. Berlin: Springer-Verlag; 1982:173–182.
45. Wehr, T. A.; Gillin, J. C.; Goodwin, F. K. Sleep and circadian rhythms in depression. In: Chase, M.; Weitzman, E. D., eds. Sleep disorders: Basic and clinical research. New York: Spectrum Pubs.; 1983:195–226.
46. Wehr, T. A. Biological rhythms and manic-depressive illness. In: Post, R. M.; Ballenger, J. C., eds. Neurobiology of mood disorders. Baltimore: Williams and Wilkins; 1984:190–206.
47. Wehr, T. A.; Wirz-Justice, A.; Goodwin, F. K.; Duncan, W.; Gillin, J. C. Phase-advance of the circadian sleep-wake cycle as an antidepressant. Science 206:710–713; 1979.
48. Whybrow, P. C.; Akiskal, H. S.; McKinney, W. T., Jr. Mood disorders: Toward a new psychobiology. New York: Plenum Press; 1984.
49. Wirz-Justice, A.; Groos, G. A.; Wehr, T. A. The neuropharmacology of circadian timekeeping in mammals. In: Aschoff, J.; Groos, G., eds. Vertebrate circadian systems: Structure and physiology. Berlin: Springer-Verlag; 1982.
50. Wirz-Justice, A.; Campbell, I. C. Antidepressant drugs can slow or dissociate circadian rhythms. Experientia 38:1301–1309; 1982.
51. Yamada, N.; Shimoda, K.; Ohi, K.; Takahashi, S.; Takahashi, K. Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. Physiol. Behav. 42:87–91; 1988.
52. Zubenko, G. S.; Cohen, B. M.; Lipinski, J. F.; Jones, J. M. Clonidine in the treatment of a mania and mixed bipolar disorder. Am. J. Psychiatry 141: 1617–1618; 1984.