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Effects of Agonists and Antagonists at the GABA/Benzodiazepine Receptor on Conditioned Suppression in Rats

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SHEPHARD, R. A., L. TOAL AND J. C. LESLIE. *Effects of agonists and antagonists at the GABA/benzodiazepine receptor on conditioned suppression in rats.* PHARMACOL BIOCHEM BEHAV **36**(1) 39–43, 1990.—Certain drugs generally regarded as GABA agonists, such as valproate and combinations of muscimol and baclofen, have been reported to produce apparent anxiolytic effects in various animal behavioral tests. The present paper reports two experiments on the effects of these agents on conditioned suppression in rats. In the first study, muscimol (0, 1.25 μg/kg or 1 mg/kg), baclofen (0, 1 mg/kg) and combinations of these treatments failed to alleviate conditioned suppression. Experiment Two showed that valproate (200 mg/kg) did attenuate conditioned suppression, and that its effects were antagonised by picrotoxin (1.5 mg/kg), but not by bicuculline (1.5 mg/kg), Ro 15-1788 (10 mg/kg) or by δ-amino-n-valeric acid (10 mg/kg). The findings are discussed in the context of the proposed GABA/benzodiazepine receptor complex, with the conclusion that there is little evidence for a mediating role of GABAa or GABAb receptors in such drug actions, and that the site of valproate action is probably the chloride ion channel associated with the receptor complex.

GABA Muscimol Baclofen Valproate Picrotoxin Bicuculline Ro 15-1788
δ-Amino-n-valeric acid Conditioned suppression

FOR some time it has been argued that anxiolytic effects of certain drugs, including benzodiazepines and barbiturates, are mediated through a receptor complex which is related to GABA neurotransmission. Specifically, the existence of a macromolecular GABA/benzodiazepine receptor complex, the main elements of which are a GABA receptor of the "A" subtype, a benzodiazepine binding site and a chloride ion permeability channel which functions as a final common pathway for the complex mediating subsequent neural events, is now widely accepted (6, 9, 10, 22). Although there is considerable biochemical and neurophysiological evidence of such a complex, attempts to produce behavioral evidence of reduced anxiety with indirectly acting GABA agonists have been generally unsuccessful (31, 33, 42). Perhaps more surprising is the inactivity of the directly acting GABAa agonists muscimol (3, 8, 30, 31, 40), THIP (29,31) and progabide (30) in a variety of animal tests of anxiolytic actions since, in view of the model, this type of neuropharmacological action would seem likely to result in benzodiazepine-like effects. Moreover, muscimol enters the brain and produces neurophysiological effects following peripheral injection of even very low doses (9). These are, however, two studies reporting anxiolytic effects of muscimol. Firstly, intracerebral administration of muscimol attenuates experimental anxiety (6), although as GABA has widespread inhibitory functions in the brain results from such doses probably depend heavily on sites of administration and distribution characteristics and may be difficult to interpret (33). Secondly, peripheral administration of both the GABAa agonist baclofen (1 mg/kg) and muscimol (1.25 μg/kg), has been reported to increase operant behavior suppressed by response-contingent punishment although neither component was effective alone (9).

The anticonvulsant drug valproate, which is generally thought to act through GABA systems (29,33), differs from most GABAergic drugs in that it has been shown to reproduce most of the behavioral effects of benzodiazepines. Thus, valproate releases both operant (17) and drinking (19, 28, 29, 34) behaviors suppressed by punishment, has antineophobic activity (34, 36, 38) antagonises pentylenetetrazole effects in drug-discrimination experiments (16) and enhances saline drinking by nondeprived rats (37). When contrasted with the reports of inactivity of other GABA agonists in such procedures, this renders the elucidation of the mechanism of these behavioral effects of valproate an important question.

The present experiments examine the effects of putative agonists and antagonists at the GABA/benzodiazepine receptor complex on the conditioned suppression of operant behavior by shock which is not contingent upon the rats' behavior. This procedure has high face validity as an animal anxiety model because, unlike punishment procedures based on response-contingent shock, it cannot readily be argued that the inhibition of behavior which occurs is an adaptive response; such inhibition reduces neither the number or intensity of shocks. Benzodiazepine anxiolytics generally alleviate the suppression of operant responding during stimuli associated with noncontingent shock (18,24). The first experiment assessed the effects of six combinations of muscimol (0, 1.25 μg/kg and 1 mg/kg) with baclofen (0, 1 mg/kg). In the second experiment, which used two cohorts of rats, we examined the

effects of valproate (200 mg/kg) alone and in combination with some putative antagonists at the GABA/benzodiazepine receptor complex. These were picrotoxin (1.5 mg/kg), bicuculline (1.5 mg/kg), Ro 15-1788 (10 mg/kg) and δ-amino-n-valeric acid or DANVA (10 mg/kg). The last is a GABAb antagonist (15,25), which modifies the anticonvulsant effects of other drugs following peripheral administration at this dose (21), but which has not been assessed for activity in anxiety models.

METHOD

Subjects

Fifteen experimentally naive male Sprague-Dawley rats, approximately 100 days old at the start of the experiments, were used. Rats were housed two to a cage with water freely available and were maintained at close to 85% of their free-feeding weight. Eight rats were used for Experiment One and seven for Experiment Two.

Apparatus

Four two-lever Campden Instruments rat test chambers (Model CI 410) were used. Only the left lever was operative. A 2.8-W stimulus light was situated 4 cm above each lever and a third was 15 cm above the floor and midway between the two levers. During sessions the chamber was lit by a 2.8-W houselight. The reinforcer was 5-sec access to a 5% sucrose solution (by weight) that was delivered by a motor driven dipper into an aperture in the floor of a recessed tray situated between the two levers at floor level. The tray was covered by a lightly hinged clear plastic flap and was illuminated by a 2.8-W bulb during reinforcer delivery. Scrambled shock could be delivered to the grid floor, made of stainless steel rods 1.3 cm apart, from a constant current shock source (Campden Instruments Model 521C). Each test chamber was encased in a sound-attenuating housing that was fitted with a ventilating fan. A Data General NOVA 2/10 minicomputer programmed in ACT (23) controlled the experiment and collected data.

Procedure

Rats were feeder trained and shaped to press a lever. After this training period, lever pressing was reinforced according to a VI 48-sec schedule. Each daily session (there were seven test days per week) lasted 30 minutes. After 10 sessions of the VI 48-sec schedule, a stimulus was presented for 60-sec at irregular intervals on three occasions during each session. The stimulus consisted of the three stimulus lights flashing at 2.5 Hz (ontime = offtime). After two sessions of stimulus presentations, the rats were exposed to a further seven sessions during which a 0.5-sec duration electric shock (US) was delivered contiguous with termination of the stimulus. Under these conditions all rats suppressed responding during the sitmulus (CS). The intensity of the US was initially 0.1 mA but was increased to 0.2 mA for six rats and 0.25 mA for nine in the first in the first six sessions of CS-US pairings and was maintained at these levels throughout the remainder of the experiments.

Drug Administrations

Muscimol (Sigma), (±)baclofen (Research Biochemicals Inc.), sodium valproate (Labaz Sanofi), (+)bicuculline (Sigma), picrotoxin (Sigma), Ro 15-1788 (Roche) and DANVA hydrochloride (Sigma) were all dissolved or suspended in 0.9% saline/1% Tween 80. All drugs were given by intraperitoneal injection 20 minutes before experimental sessions in a volume of 1 ml/kg. All drug sessions were separated by at least two control days on which only vehicle injections were given. In Experiment One, the six drug treatments were given in a random sequence to the subjects, then this was repeated twice with different random sequences, so that each animal received all drug combinations thrice. In Experiment Two, four animals received valproate, valproate plus picrotoxin, valproate plus bicuculline and valproate plus DANVA; three received valproate, valproate plus picrotoxin, valproate plus bicuculline and valproate plus Ro 15-1788. Randomisation was analogous to Experiment One. All doses are expressed as salt and were chosen on the basis of pilot and published work.

Data Analysis

Although means are shown for illustrative purposes in this paper, there is a tendency for response rates (especially those not during the CS) to increase steadily over the course of such experiments (18). This phenomenon, together with between-rat variations, inflates variability and means that the best statistical comparison is with the most proximal control session. Therefore, for Experiment One, effects of muscimol and baclofen combinations were compared with behavior on the previous days (always vehicle ones) by two-tailed sign tests. For Experiment Two the effects of valproate alone were assessed likewise and the combinations of valproate with putative antagonists compared with the valproate only session in the same sequence. Values of N in the sign tests reflect the number of pairs of sessions (summated across subjects) were the two response rates differed. For the comparison between valproate and vehicle, the values of X are the number of pairs within which valproate response rate was higher. Where the putative antagonists were also given, values of X are the number of pairs within which the valproate plus antagonist response rate was lower than in the corresponding vaproate-only session. In Fig. 2, numerators and denominators of the proportions shown are, respectively, X and N. All results from the first series of drug administrations were discarded since many drugs, including valproate and benzodiazepines, tend to induce weak and variable effects on their first administrations in this (and some other) procedures.

RESULTS

Experiment One

The effects of baclofen and muscimol combinations on average response rats during the CS presentations and in the 60-second periods immediately preceding the CS presentations (pre-CS rates) are shown in Fig. 1. This shows operant behaviour to be suppressed markedly by the stimulus associated with shock but there is no suggestion that these CS rates are elevated by any of the combinations of muscimol and baclofen, which would be expected of an anxiolytic treatment and no sign test on CS rates approaches significance. None of the effects on pre-CS rates suggested by Fig. 1 are significant either.

Experiment Two

Effects of valproate (200 mg/kg) alone and in combination with bicuculline (1.5 mg/kg), picrotoxin (1.5 mg/kg), Ro 15-1788 (10 mg/kg) and DANVA (10 mg/kg) are shown in Fig. 2. As can be seen, valproate increases CS responding more than three-fold, the comparison with saline being highly significant (X = 14, N = 14,

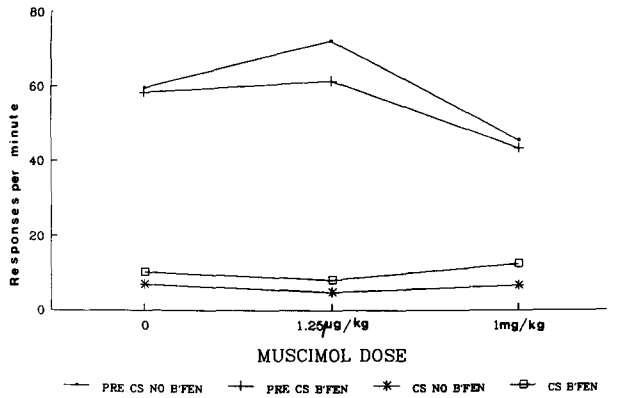

FIG. 1. Lack of effect of muscimol and baclofen on conditioned suppression. The figure shows mean response rates for eight rats × two replications during CS presentations (lower two lines) and for 60-sec periods before CS presentations (upper two lines). Six combinations of muscimol at 0, 1.25 µg/kg and 1 mg/kg (see abscissa) and baclofen at 0 and 1 mg/kg (see symbol key) were given IP 20 minutes prior to test sessions. None of the drug regimes produced results significantly different from vehicle control ($p>0.05$).

$p<0.001$). Picrotoxin significantly antagonised this effect (X = 12, N = 14, $p=0.012$), but none of the other putative antagonists significantly reduced the effect of valproate. None of the drug effects on pre-CS responding were significant.

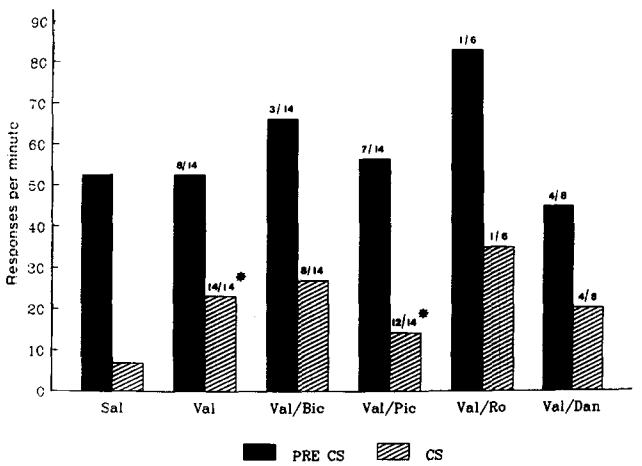

FIG. 2. Effects of valproate and GABA/benzodiazepine antagonists on conditioned suppression. The figure shows effects of the drug regimes of experiment two on mean response rates during CS presentations (solid bars) and for 60-sec periods before CS presentations (hatched bars). Sal = vehicle control, Val = valproate (200 mg/kg), Bic = bicuculline (1.5 mg/kg), Pic = picrotoxin (1.5 mg/kg), Ro = Ro 15-1788 (10 mg/kg) and Dan = DANVA (10 mg/kg). Drugs were given IP 20 minutes prior to test sessions. The vehicle bars show means for seven rats × two replications, other ns are given by the denominators of the proportions shown. Numerators for the valproate bars are the number of comparisons with vehicle showing increased rate of responding; numerators for the other bars are the number of comparisons with valproate showing reduced rate of responding. Asterisks indicate significant comparisons by two-tailed sign test (see text for method and probability values).

DISCUSSION

The results of Experiment One, together with the inactivity of bicuculline and DANVA in Experiment Two, do not suggest a major role for either GABAa or GABAb receptors in conditioned suppression. Although an apparent anxiolytic effect of combined administration of baclofen (1 mg/kg) and a low dose of muscimol (1.25 µg/kg) has been reported (9), we did not detect this using either the same dose of the latter or a more conventional dose of 1 mg/kg. Moreover, the GABAa and GABAb antagonists used, bicuculline and DANVA, failed to attenuate valproate-induced increases in CS response rates. In the case of bicuculline, it is tempting to speculate that use of higher doses or shorter intervals between injection and test might have produced some antagonism. However, such modifications produce overt toxicity (37) and there are several other instances of bicuculline failing to antagonise effects of anxiolytics including benzodiazepines (3, 5, 11, 18, 20, 26) and valproate (37). Regarding DANVA, we have investigated effects of higher doses on conditioned suppression (Toal *et al.*, unpublished) and of a range of doses on shock-suppressed drinking (Wilson *et al.*, unpublished), alone and with valproate or chlordiazepoxide without detecting activity. The most parsimonious conclusion from the present studies is therefore that neither GABAa or GABAb sites have a major role in conditioned suppression. Moreover, it does not seem that the attenuation of conditioned suppression induced by valproate depends upon activation of GABAa or GABAb receptors either directly or by facilitating the neurotransmitter function of endogenous GABA. It remains, however, at least a theoretical possibility that another subtype of GABA receptor insensitive to muscimol, baclofen, bicuculline and DANVA is involved.

Antagonism at the benzodiazepine site with Ro 15-1788 also failed to attenuate the increase in CS response rate induced by valproate. Although not formally significant, inspection of Fig. 2 suggests that Ro 15-1788 actually enhances the valproate effect. However, we have not observed any such tendency in other conditioned suppression experiments with this combination, in which Ro 15-1788 failed to modify valproate action in either direction (18). Effects of valproate on shock-suppressed drinking (19) on neophobia (38) and on saline drinking (37) are antagonised by Ro 15-1788 and therefore the selective effect of Ro 15-1788 in antagonising chlordiazepoxide (18), but not valproate (Experiment Two), effects on conditioned suppression may be regarded as evidence of greater pharmacological specificity of the latter procedure.

Picrotoxin, however, attenuated the increases in CS response rates included by valproate, but did not affect pre-CS response rates. Picrotoxin also antagonises valproate effects on shock-suppressed drinking (19), neophobia (38) and saline drinking (37); as well as the effects of benzodiazepines in a variety of behavioral tests (2, 12, 14, 27, 35, 39). Regarding conditioned suppression, picrotoxin also antagonises increases in CS responding induced by chlordiazepoxide (18). All of these interactions are consistent with picrotoxin having antagonistic properties based on an action at the final common pathway or effector of the GABA/benzodiazepine receptor complex, that is, the chloride ion channel. Investigations of receptor binding (41), as well as the inactivity of the other antagonists reported here and the apparent competitive antagonism between valproate and picrotoxin observed in neophobia studies (38), would suggest that the chloride ion channel is the site of action of valproate.

These results also have implications for the utility of conditioned suppression as a technique for investigating anxiolytic and related behavioral pharmacological effects. Although this proce-

dure has high face validity as discussed earlier and a long history in behavioral research (24), its status as a useful anxiety model has been questioned (4,7). The main grounds of such criticism are that attenuation of conditioned suppression with benzodiazepines is subject to rapid tolerance and that the procedure lacks pharmacological specificity. The first is directly contrary to our experiments in which the effects of chlordiazepoxide (and also valproate) on conditioned suppression remain, and even increase, over weeks of daily administrations (18). The second is based largely on studies showing attenuation of conditioned suppression with reserpine and certain phenothiazines. However, since reserpine depletes brain serotonin, most phenothiazines are antagonists at serotonin receptors (1) and serotonin is clearly involved in the physiology of anxiety (13, 32, 39), this apparent nonspecificity of conditioned suppression becomes less disturbing. Moreover, at least in the context of interactions between valproate and Ro 15-1788, the present experiments suggest that conditioned suppression is more specific than some other procedures. Further work is required to examine the extent, mechanisms and significance of differences between the behavioral pharmacology of conditioned suppression and of other procedures used in the analysis of anxiolytic and related drug effects.

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