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Anti-Conflict and Depressant Effects by GABA Agonists and Antagonists, Benzodiazepines and Non-Gabergic Anticonvulsants on Self-Stimulation and Locomotor Activity[1]

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HERBERG, L. J. AND S. F. WILLIAMS. *Anti-conflict and depressant effects by GABA agonists and antagonists, benzodiazepines, and non-gabergic anticonvulsants on self-stimulation and locomotor activity.* PHARMACOL BIOCHEM BEHAV **19**(4) 625–633, 1983.—Rats were injected systemically with different classes of gabergic agent in order to investigate gabergic involvement in limbic output. Agonists differed one from another in their effects on variable-interval self-stimulation: clonazepam (in repeatedly-tested rats), chlordiazepoxide and pentobarbitone had a strongly biphasic action, low doses being facilitatory and high doses depressant, whereas other agonists including valproate and 3-APS (homotaurine) were uniformly depressant. The facilitatory effects of the benzodiazepines were dramatically enhanced by GABA antagonists (picrotoxin or pentylenetetrazol) even though antagonists on their own produced a dose-dependent depression that was not reversible by other anticonvulsant drugs. Ventral tegmental electrode placements yielded generally similar results. Depression of self-stimulation observed on initial exposure to clonazepam was reversed by repeated self-stimulation testing in the drugged state but not by repeated daily injections without testing. Locomotor activity (under conflict-free conditions) was unaffected or was depressed both by agonists and by antagonists. Thus, the facilitation of self-stimulation by chlordiazepoxide, pentobarbitone and clonazepam appears to be accounted for in terms of non-gabergic anti-conflict activity by these agents. Self-stimulation and locomotor changes following systemic administration did not disclose facilitatory effects attributable to gabergic efferents from limbic dopamine areas.

Acetazolamide Anti-conflict Barbiturate Benzodiazepine Chlordiazepoxide Clonazepam GABA Homotaurine Locomotion Metrazol Pentobarbitone Phenytoin Picrotoxin Self-stimulation State-dependent learning Valproate 3-APS

GABA in the upper brain stem exerts a well-established inhibitory control over dopamine-containing pathways [36, 44, 52], thereby offering a possible explanation for certain behavioural effects of gabergic drugs. More recently, however, gabergic pathways have also been shown to constitute the essential output path from dopamine-terminal areas in the striatum [14, 28, 54] and nucleus accumbens [58]. Thus drugs affecting gabergic transmission (including almost all the anxiolytics, hypnotics and anticonvulsants in clinical use) may act not only to inhibit the dopaminergic pathways, but also to facilitate their output; and there is a prospect that some agents may produce one or other of these effects selectively since GABA receptors at different sites are known to be differentially sensitive to different ligands [29,63].

In the present report we have examined this possibility by investigating the effects of several classes of gabergic agents on two behavioural measures: spontaneous locomotor activity and hypothalamic self-stimulation. Spontaneous locomotor activity in the rat is related to mesolimbic dopamine [30] and is regarded as an indication of motivational arousal [24,55]. The precise role of dopamine in self-stimulation remains exceedingly obscure [67], but responding at most or all subcortical reward sites is critically dependent on it [9,33], and drugs that selectively impair dopaminergic transmission depress responding in a dose-dependent manner [65] at doses lower than are necessary to produce simple motor incapacity [16, 20, 65]. Inhibition of dopaminergic transmission by gabergic agents would thus be expected to depress either or both self-stimulation and locomotor activity, whereas facilitation of dopaminergic output would be expected to do the opposite.

Gabergic agonists and antagonists were administered both separately and in combination in order to determine

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whether the partial or selective blockade of their actions that might be achieved in this way would unmask behavioural effects that might otherwise be overshadowed [5,29]. The same objective was sought (in Experiment 3) by retesting certain of the agonists after repeated daily administrations, since rapid tolerance has been shown to develop in these circumstances to some of their behavioural effects but not to others [19,66]. In addition, it was necessary to control for the known anticonvulsant activity of certain of the agonists since self-stimulation may be disrupted by seizure activity induced by the stimulating current [3,27], and protection from this disruption, by agents with anticonvulsant properties, could result in an improved performance [56] that might be difficult to distinguish from the facilitatory effect being sought. Procedural steps taken to minimise the role of seizures in the present study are described below; in addition, two non-gabergic anticonvulsants were included in Experiment 1 as controls for changes in responding due to anticonvulsant activity per se.

METHOD

Subjects

Male Wistar rats weighing 300–350 g at the time of surgery were housed individually with free access to food and water. Twisted bipolar stainless steel electrodes (Plastic Products) were implanted in the mid-lateral hypothalamus (de Groot coordinates: A4.8, 1.3, 9.0) or in the ventral tegmental area (A10) (de Groot A2.8, 1.0, 9.0) under pentobarbital anaesthesia. Electrode placements were verified in enlarged photographic projections of unstained frozen sections at the end of the experiment.

Self-Stimulation

The rats were trained to operate a pedal for a 0.5-sec 50-Hz sinewave reinforcing impulse available at randomly varied intervals of 10 sec mean duration; use of this type of reinforcement schedule (VI 10 sec) results in a steady seizure-free rate of responding on which stimulant and depressant effects can be imposed without appreciable change in the relatively low rate (about 5 per min) at which reinforcing shocks are received. The stimulating current for each rat was fixed at the lowest intensity that elicited sustained responding. At test-sessions self-stimulation was allowed for approximately 45 min, of which the last 30 min provided a pre-injection baseline; after injection, the rat was allowed to self-stimulate for a further 60 min. If the rat stopped responding it was encouraged to restart by taps on the lever; if this failed, by administration of priming shocks, and finally by the experimenter placing the rat bodily on the lever. Response rates were recorded automatically at 5-min intervals in a digital printout, and drug effects were determined from the rate recorded during the second 15-min period after injection expressed as a percentage of the pre-injection baseline, and compared to the corresponding rate after saline.

Locomotor Activity

Locomotor activity was measured in covered circular bowls 35 cm in diameter and 24 cm high, supported by a pivot at the centre and by six microswitch actuating levers spaced about the circumference [2]. Movement of the rat from one part of the bowl to another caused the bowl to tilt on the pivot and depress one or more microswitches, each closure being automatically cumulated on printout counters. Small postural movements without locomotion did not usually actuate the microswitches or affect the count. Rats were familiarised with the bowls in four 0.5-hr test sessions before testing began; tests were started 20 min after drug administration, and lasted 20 min. Activity was scored in blocks of 5 min and expressed as log (score+1).

Drugs

Agents affecting gabergic transmission have been classified according to whether they act predominantly on the postsynaptic receptor, on the Cl− ionophore to which the receptor may be linked, or on a benzodiazepine-sensitive peptide by which the sensitivity of the GABA receptor may be modulated [1,15]. The antagonists selected for the present investigation accordingly included one compound, bicuculline, acting on the gaba receptor [61], and two, picrotoxin and pentylenetetrazol (Metrazol), acting on the Cl− ionophore [61,62]. The selected agonists included 3-amino-1-propanesulphonic acid (homotaurine, 3-APS), a structural analogue of GABA [17]; sodium valproate, which raises synaptic concentrations of GABA by slowing its metabolic degradation [26]; sodium pentobarbitone, thought to act directly on the Cl− ionophore [46]; and two benzodiazepines: chlordiazepoxide, used clinically as an anxiolytic [25], and clonazepam, ordinarily prescribed as an anticonvulsant [50] but known also to possess anti-conflict properties [8].

Phenytoin and acetazolamide are established anticonvulsants [35] which act respectively to inhibit the influx of Na+ in the stimulated cell [12], and to inhibit intracellular carbonic anhydrase [68], and are believed to depress neural excitability other than via the GABA-receptor/Cl− ionophore complex [68,69].

Picrotoxin (Sigma), pentylenetetrazol (Sigma), 3-APS (Aldrich) and sodium valproate (Epilim, Reckitt and Colman) were dissolved in physiological saline; (+)bicuculline (Sigma) was dissolved in 0.01-M HCl and brought to pH 4.5 with NaOH; sodium acetazolamide (Diamox, Cyanamide) was dissolved in distilled water; chlordiazepoxide HCl (Librium, Roche), clonazepam (Rivotril, Roche), phenytoin (diphenylhydantoin; Epanutin, Parke-Davis) and sodium pentobarbitone (Sagatal, May and Baker) were obtained from pharmaceutical ampoules and diluted with saline as necessary. All drugs apart from pentylenetetrazol were administered by intraperitoneal injection in volumes of 0.2–1.0 ml at intervals of not less than 48 hr. Pentylenetetrazol was given by subcutaneous injection in a similar volume.

Procedure

Experiment 1. Dose-response data were obtained for the effect on spontaneous locomotor activity and self-stimulation rate of five GABA agonists (valproate, 3-APS, pentobarbitone, chlordiazepoxide and clonazepam) and two antagonists (picrotoxin and pentylenetetrazol). The rats were each used to test up to two different drugs, but rats tested with clonazepam or chlordiazepoxide had not received any previous treatment. Doses were administered in a predetermined random order except where noted, and details of doses are given in the Results section.

Experiment 2. Rats with electrodes implanted in the ventral tegmentum were tested for self-stimulation after single doses of each of four agonists (clonazepam 0.3 mg/kg, chlordiazepoxide 5.0 mg/kg, 3-APS 30 mg/kg and pentobar-

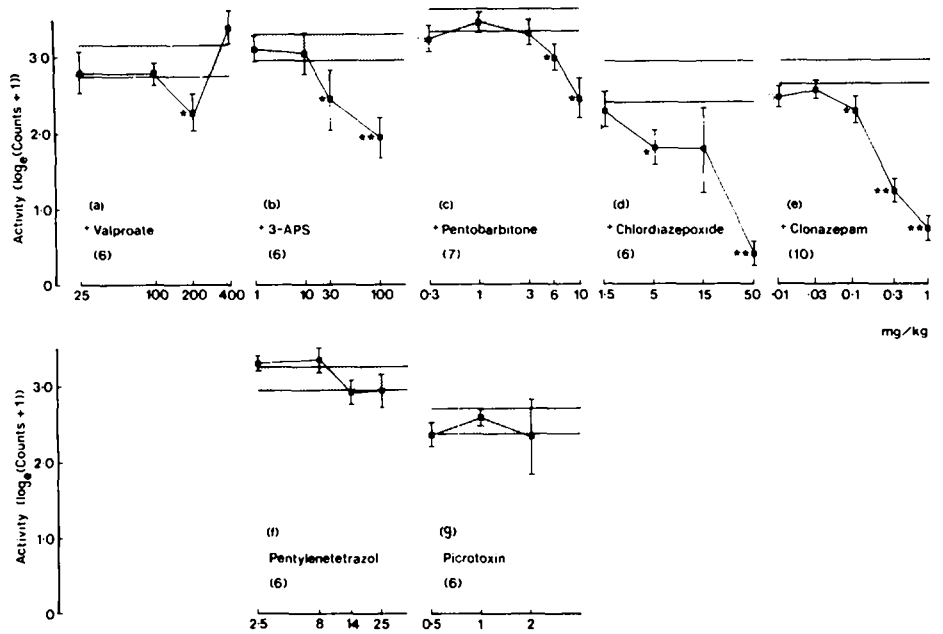

FIG. 1. Dose-response curves (and standard errors) for locomotor activity, expressed in arbitrary counts, in the second 20-min period after injection of gabergic agonists or antagonists. Absolute count values are not comparable across tests owing to adjustments to the recording apparatus between tests. Doses (mg/kg) are scaled logarithmically, and their order of administration was randomised except where noted. Shaded areas indicate NaCl control scores. Numerals in parenthesis indicate sample size (n). : Dose(s) administered out of order, at end of test series; △ Sample size=(n−1); + Drug effects were significantly dose-related over the dose-range tested (Friedman ANOVA $p<0.05$). *$p<0.05$ (compared to NaCl); **$p<0.01$ (compared to NaCl).

bitone 3.0 mg/kg), and two antagonists (picrotoxin 2.0 mg/kg and pentylenetetrazol 25 mg/kg). The selected doses corresponded to the approximate ID 50 doses identified in Experiment 1, or, in the case of chlordiazepoxide and pentobarbitone, the dose with the maximal stimulant effect. All drugs were tested in random order on every rat. Effects on self-stimulation were compared by *t*-test with the effects obtained at hypothalamic sites in Experiment 1.

Experiment 3. Investigated the development of tolerance after repeated administrations. Rats without previous exposure to drugs were tested for self-stimulation after single injections of clonazepam (0.3 mg/kg) and chlordiazepoxide (15 mg/kg) given 48 hr apart. Half the rats were then given once-daily injections of clonazepam (0.3 mg/kg) on the succeeding seven days without further self-stimulation testing, and the other half were given injections of saline; at 48 hr after the last injection both groups were retested with single injections of clonazepam and chlordiazepoxide as before. Three weeks later the procedure was repeated with the drug- and saline-groups reversed. All subjects were then given another four 1-hr sessions with clonazepam to ensure maximal familiarisation with self-stimulation in the drugged state. The effects of clonazepam were now reassessed in a dose-response study as in Experiment 1. Groups were compared by *t*-test, and the effects of repeated treatments were assessed with Page's Trend test for related samples [42].

Experiment 4. Investigated the effects on self-stimulation of GABA agonists combined with a GABA antagonist, or antagonists combined with a nongabergic anticonvulsant. The selected dose of each agent was one which had produced a moderate depression when given on its own in Experiment 1, and, in the case of chlordiazepoxide, the dose which had elicited the maximal stimulant effect. The particular combinations tested were: (a) picrotoxin with clonazepam, (b) picrotoxin with chlordiazepoxide, (c) pentylenetetrazol with clonazepam, (d) pentylenetetrazol with chlordiazepoxide, (e) picrotoxin with phenytoin, and (f) picrotoxin with acetazolamide. The agonist or anticonvulsant was generally administered first, followed immediately by the antagonist. Different rats were used for each drug combination, but half the rats tested on clonazepam with picrotoxin had previously received clonazepam in Experiment 1, and their results were compared to those from the rats which had not previously been treated with benzodiazepines.

RESULTS

Experiment 1

Locomotor activity. Dose-response curves for drug effects on locomotor activity are given in Fig. 1. All the agonists tested produced a significant depression of locomotor activity, and this effect was dose-related over the dose range tested, except in the case of valproate. No dose of any agonist produced significant stimulation. The antagonists, picrotoxin and pentylenetetrazol, in doses which significantly depressed self-stimulation, did not significantly affect locomotor activity. The effects of bicuculline were sometimes severely disruptive, but were too short-lived for valid measurement.

Self-stimulation. Dose-response data for self-stimulation are summarised in Fig. 2. All agonists tested produced a significant depression of responding, but chlordiazepoxide

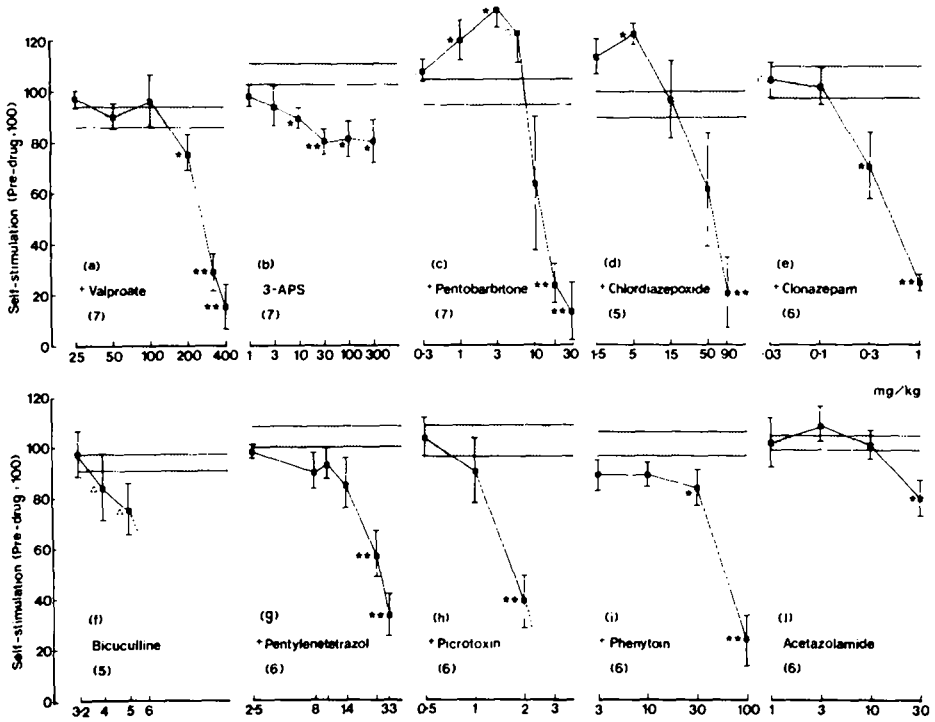

FIG. 2. Dose-response curves for self-stimulation rate (VI 10 sec) in the second 15-min period after injection of gabergic agonists or antagonists or non-gabergic anticonvulsant agents. See Figure 1 for explanation of symbols.

(Fig. 2d) and pentobarbitone (Fig. 2c) also produced a clear and significant enhancement at low doses. No enhancement was seen with clonazepam (Fig. 2e). The highest doses of all agonists, except 3-APS, induced flaccidity and ataxia, which may have affected performance. 3-APS (Fig. 2b) produced a small but significant depression of responding which was not dose-related.

The GABA antagonists, picrotoxin (Fig. 2h) and pentylenetetrazol (Fig. 2g), produced a dose-dependent depression of self-stimulation spanning a range of subconvulsant doses. Intraperitoneal injections of bicuculline (Fig. 2f) did not give consistent results: doses sufficient to produce short-lived depression of responding in some rats caused lethal convulsions in others, and testing of this agent was abandoned.

Of the non-gabergic anticonvulsants, phenytoin (Fig. 2i) produced a dose-dependent depression of self-stimulation, but acetazolamide (Fig. 2j) had relatively little effect on response rates even at dose-levels very much higher than anticonvulsant doses effective against maximal electroshock [34].

Experiment 2

Table 1 shows that the effects of GABA agonists and antagonists on ventral tegmental self-stimulation were generally similar to the effects obtained with hypothalamic electrodes; the single exception was for clonazepam, which lacked the depressant action seen in Experiment 1.

Experiment 3

Figure 3 shows that seven daily injections of clonazepam, without exposure to self-stimulation testing, did not induce tolerance to the depressant effect of this drug: repeatedly injected rats did not differ significantly from controls when retested with clonazepam, $t(16)=0.03$, or with chlordiazepoxide, $t(15)=0.6$.

But a significant upward trend in performance was apparent if one considered only those injections of clonazepam which were followed by exposure to the test situation. Figure 4a shows that four self-stimulation tests, given at 2- or 4-week intervals as part of the foregoing procedure showed a progressive diminution of clonazepam's depressant effect (Page's Trend test $p<0.05$), and the increments in response rates between successive tests were not significantly affected by whether intervening treatment was with clonazepam (mean increment=16%) or NaCl (mean increment=24%). After further self-stimulation practice in the drugged state the depressant action of clonazepam was abolished, or even reversed, giving a biphasic dose-response curve (Fig. 4b) similar to that produced by chlordiazepoxide (Fig. 2d) and pentobarbitone (Fig. 2c).

Experiment 4

Figure 5a shows that doses of clonazepam (0.3 mg/kg) and picrotoxin (2.0 mg/kg), both of which had depressed self-stimulation when given alone, produced a striking enhancement when given in combination. This occurred equally in rats which had or had not previously been treated with clonazepam injections in Experiment 1 (130% ± 11, n=5 versus 129% ± 16, n=6), and the results were pooled. Figure 5b shows that an equally clear-cut reversal of action was produced by clonazepam when combined with pentylenetetrazol (25 mg/kg).

TABLE 1

EFFECT OF GABA AGONISTS AND ANTAGONISTS ON SELF-STIMULATION OF VENTRAL TEGMENTAL AREA (A10) AND OF HYPOTHALAMIC MEDIAN FOREBRAIN BUNDLE (MFB)

Probe dose (mg/kg)	A 10	MFB	A 10 vs. MFB
Picrotoxin 2	44 ± 14 (7)	38 ± 10 (6)	$t_{11}=1.4$
Pentylenetetrazol 250	43 ± 6 (7)	57 ± 9 (6)	$t_{11}=0.7$
3-APS 30	69 ± 9 (7)	80 ± 5 (7)	$t_{12}=0.8$
Chlordiazepoxide 5.0	119 ± 8 (7)	122 ± 4 (5)	$t_{10}=0.2$
Pentobarb 30	118 ± 8 (7)	133 ± 8 (7)	$t_{12}=1.3$
Clonazepam 0.3	111 ± 6 (7)	70 ± 14 (6) (acute)	$t_{11}=2.9^*$
		109 ± 13 (6) (habituated)	$t_{11}=0.9$

Scores were recorded in the second 15-min period after injection and are expressed as percentages of pre-injection response rates.

Figures in parenthesis indicate the number of rats tested.

*$p<0.02$.

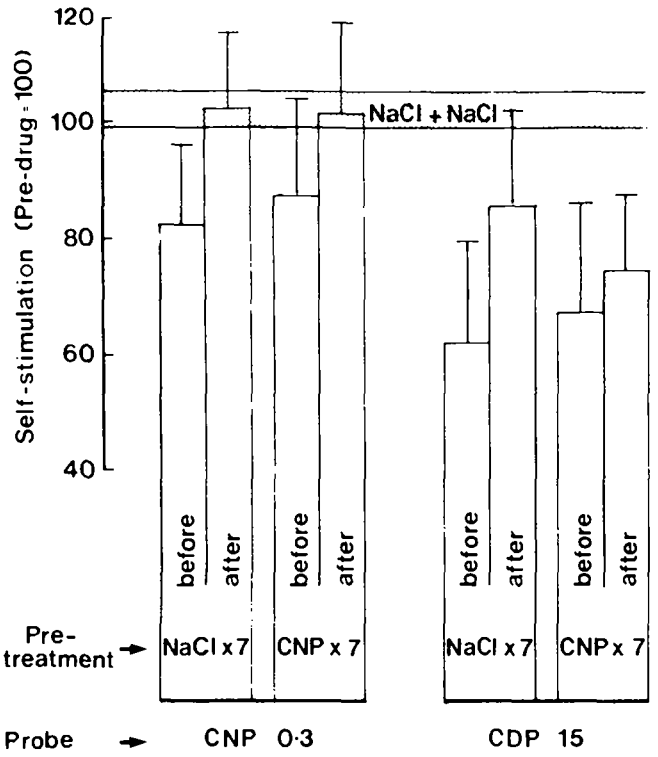

FIG. 3. Effect of a single injection of clonazepam (CNP, 0.3 mg/kg) or chlordiazepoxide (CDP, 15 mg/kg) on variable-interval self-stimulation in rats which had been pretreated with seven daily injections of either clonazepam (0.3 mg/kg) or NaCl without exposure to self-stimulation.

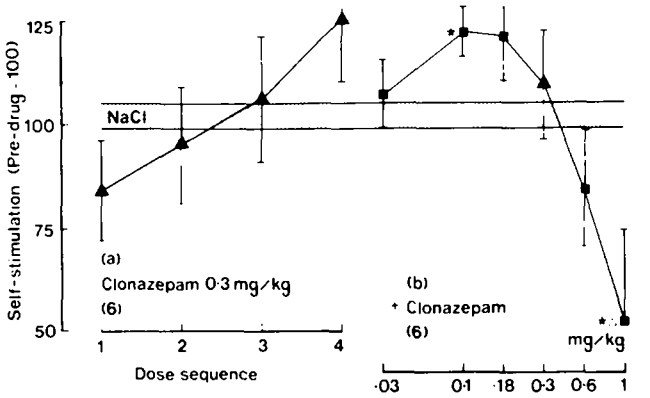

FIG. 4. (a) Variable-interval self-stimulation rate after each of four injections of clonazepam (0.3 mg/kg) given at 2- to 4-week intervals. (b) Dose-response curve for the effect of clonazepam on variable-interval self-stimulation in six rats previously given eight injections of clonazepam (0.3 mg/kg), each injection followed by exposure to self-stimulation. See Fig. 1 for explanation of symbols.

Stimulant doses of chlordiazepoxide (5 mg/kg) were no less stimulant when combined with picrotoxin (Fig. 5c), while higher doses (15 mg/kg), which had been without significant effect (Fig. 2d), became strongly stimulant when combined with pentylenetetrazol (Fig. 5d), $t(7)=2.4$, $p<0.05$, compared with NaCl.

There was no sign of any interaction between picrotoxin and non-gabergic anticonvulsants: no lessening of their respective depressant actions was seen when picrotoxin (2.0 mg/kg) was combined with phenytoin (50 mg/kg) (Fig. 5e), or with acetazolamide (10 mg/kg) (Fig. 5f).

DISCUSSION

The depression of locomotor activity in Experiment 1 confirms previous results obtained with gabergic agonists in familiar and non-stressful test-environments [7,19].

Results with self-stimulation were more complex: all agonists depressed responding at higher dose levels, but low doses of chlordiazepoxide (Fig. 2d) and pentobarbitone (Fig. 2c) caused facilitation. Previous self-stimulation studies of gabergic agents have differed in critical features such as electrode site, reinforcement parameters or the type of response measured, and results have been contradictory [45, 51, 70]. But response rates obtained with near-threshold current strengths have commonly shown facilitation by benzodiazepines similar to that found here [6, 23, 41, 47], particularly in "mixed" electrode sites where stimulation was partly aversive [49]. The question at issue in the present

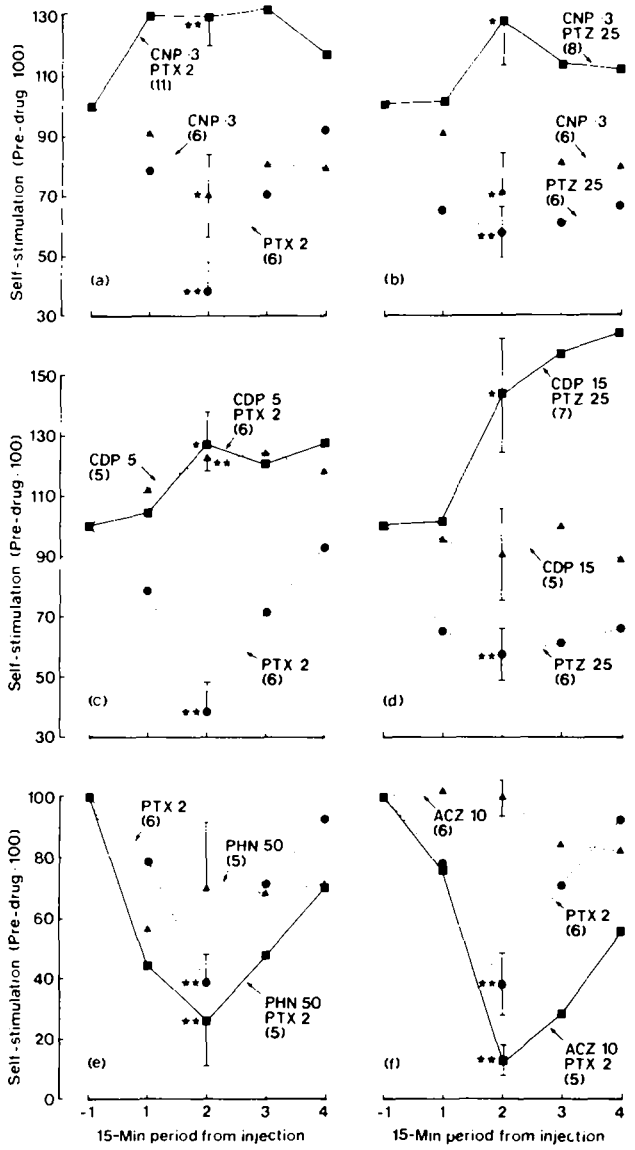

FIG. 5. Mean self-stimulation rate (VI 10 sec) in successive 15-min periods before and after administration of gabergic agonists and antagonists, or non-gabergic anticonvulsant agents, either separately or in combination. NaCl control scores have been omitted for the sake of clarity. Suffixed numerals indicate dose (mg/kg). Numerals in parenthesis indicate sample size. See Fig. 1 for explanation of symbols. ACZ=acetazolamide; CDP=chlordiazepoxide; CNP=clonazepam; PHN=phenytoin; PTX=picrotoxin; PTZ=pentylenetetrazol.

investigation was whether the effects obtained were dependent on GABA-sensitive mechanisms, acting downstream from the dopamine terminals. This possibility, and some alternative considerations, were examined in the remaining experiments.

One alternative was that chlordiazepoxide and pentobarbitone may have enhanced self-stimulation by virtue of their anticonvulsant properties [53,69], rather than by direct action on mechanisms concerned with motivation or reinforcement. The depression of self-stimulation in Experiment 1 by pentylenetetrazol (Fig. 2g) and picrotoxin (Fig. 2h) in doses that were without effect on locomotor scores (Figs. 1f and 1g) points to the possible influence of seizure activity, shown previously to depress performance on high-density reinforcement schedules [3, 27, 31]. In the present study, however, the scheduled rewarding shocks were infrequent and of near-threshold intensity so that overt seizures were seldom seen. Moreover, it was shown that phenytoin, a non-gabergic anticonvulsant agent [11,12], more effective than benzodiazepines or barbiturates in preventing electroshock-induced seizures [35], did not enhance self-stimulation performance but depressed it (Fig. 2i). Similarly, acetazolamide (another non-gabergic anticonvulsant agent [68]) and clonazepam (an anticonvulsant benzodiazepine [50]), both failed at one stage to enhance self-stimulation (Figs. 2j and 2e); thus anticonvulsant activity per se is unlikely to have contributed appreciably to the facilitation of self-stimulation by chlordiazepoxide or pentobarbitone.

Another possible interpretation of the facilitative effects of chlordiazepoxide and pentobarbital is suggested by a proposal that GABA agonists may act differentially on nigrostriatal and on limbic output [57,58]. Systemic injections of muscimol were reported to enhance methylphenidate- or cocaine-induced stereotypy (attributable to nigrostriatal activity [30]) but to suppress locomotor hyperactivity (attributable to mesolimbic activity [30]). Thus the selective enhancement of self-stimulation—and not locomotion—by chlordiazepoxide and pentobarbitone in the present study may have depended on our placement of the electrodes in hypothalamic sites involving a high proportion of nigrostriatal (extrapyramidal) pathways [39]. Improved performance could thus have been a primarily executive phenomenon, rather than a motivational one. To investigate this possibility we examined the effects of benzodiazepines at self-stimulation sites related to the ventral tegmental nucleus (A 10). This structure projects predominantly to the limbic forebrain [39], so that if the improved performance had been mostly due to facilitation of stimulation-evoked nigrostriatal activity, it would now have been less marked or absent. Table 1 shows, however, that tegmental response rates were not significantly less sensitive to the stimulant effects of pentobarbitone or chlordiazepoxide, while the stimulant action of clonazepam was now detected for the first time (a feature seen again in Experiment 3). Thus the results do not support the suggestion that gabergic agents may selectively facilitate nigrostriatal and not mesolimbic output. Recent studies in fact suggest that both these divisions of the dopamine system relay via a GABA-dependent synapse [13,59].

Another property of the benzodiazepines that might contribute to their effects on self-stimulation is their tendency to produce sedative side-effects [25]. Rapid tolerance (after only two or three doses) develops to their sedative, but not to their anxiolytic effects [19,66], suggesting that their behavioural effects are mediated by at least two different target systems [60]. Thus apparent differences between agonists could either signify differential affinities for different target systems, or simply reflect transient pharmacokinetic differences. The first part of Experiment 3 (see Fig. 3) at first suggested that the latter was unlikely to be the case, since there was no significant change in the facilitative or depressant effects of chlordiazepoxide or clonazepam attributable to seven preceding doses of clonazepam, showing that the effects on self-stimulation were largely uninfluenced by pharmacological tolerance as ordinarily conceived. There

was, however, a significant attenuation of the depressant action of clonazepam over the four test doses of the series, regardless of intervening treatment with saline or with clonazepam, and despite the fact that consecutive tests were spaced more than a week apart, thus excluding drug cumulation as a likely explanation. This puzzling phenomenon has also been observed with chlordiazepoxide in food-reinforced operant tasks [8], and seems best accounted for as an instance of state-dependent learning—in which responses acquired by an undrugged subject fail to appear at full strength when first tested in the drugged state, and vice versa [48]. After further self-stimulation practice in the drugged state the initially depressant effect of clonazepam was replaced by a significant stimulant effect, giving a biphasic dose-response curve (Fig. 4b) similar to that of chlordiazepoxide (Fig. 2d) and pentobarbitone (Fig. 2c). These results showed, first, that the facilitatory effects of chlordiazepoxide are relatively immune to pharmacological or behavioural tolerance, and second, that similar facilitation can be obtained with clonazepam after repeated practice in the drugged state, or with electrodes sited in the ventral tegmentum.

But are these facilitatory effects GABA-induced? Various lines of evidence indicate that the gabergic properties of the benzodiazepines may be separable from their anti-conflict properties [8, 18, 32, 38, 60] (but cf. [37] and [64] for a contrary view), so that the enhanced self-stimulation performance with these drugs could in principle be entirely accounted for by a nongabergic anti-conflict mechanism. There were at least two possible sources of conflict: first, an aversive component of the reinforcing shock itself, as is commonly present in brain-stimulation reward [4,43], and, second, an element of frustrative non-reward, engendered by the variable-interval reinforcement schedule on which the rats were tested [10]. Both factors would tend to depress response rates, and this depression would be reversible by the anti-conflict properties of the benzodiazepines and barbiturates [10, 21, 22].

To examine this possibility we investigated the effects of chlordiazepoxide and clonazepam when given in combination with subconvulsant doses of two non-competitive non-reversible antagonists of gabergic transmission, picrotoxin and pentylenetetrazol [1,16]; if the enhancement of self-stimulation by benzodiazepines were GABA-dependent, it should have been eliminated, or at least attenuated by these antagonists. Figures 5a to 5d clearly show the opposite: doses of chlordiazepoxide and clonazepam which had previously been depressant (Fig. 2e) or without significant effect (Figs. 2d and 4b), now brought about a striking enhancement of self-stimulation. This result was unlikely to be due to a selective cancellation of the epileptogenic properties of the antagonists, since their combination with phenytoin (Fig. 5e) or acetazolamide (Fig. 5f) did not reverse or lessen their depressant effects. Nor was this result affected to any marked degree by an adaptation of the type demonstrated in Experiment 3, since facilitation of self-stimulation was no less marked in rats receiving benzodiazepines for the first time than in rats which had had several previous doses. The key to these findings seems to be the suggestion of Lippa and colleages [40], that gabergic antagonists may eliminate the depressant effects of the benzodiazepines but not their anticonflict properties. Thus the paradoxical enhancement of self-stimulation by clonazepam combined with depressant doses of picrotoxin or pentylenetetrazol (Figs. 5a and 5b) can be ascribed to the neutralisation of the gabergic properties of these drugs and the consequent unmasking of clonazepam's anticonflict activity. This interpretation of the stimulant effects of the benzodiazepines is supported by the absence of any such effects in the locomotor tests, which were carried out in dark and featureless bowls with which the rats were fully familiar and which therefore lacked the conflict-inducing and frustrative features of the self-stimulation procedure.

Thus the behavioural effects attributable to gabergic activity in this investigation were uniformly depressant in character; systemic administration of agonists did not elicit the excitant behavioural effects that might have been expected from the stimulation of gabergic receptors immediately downstream from the dopamine terminal areas [14, 28, 54, 59]. The benzodiazepine-induced enhancement of variable-interval self-stimulation (and possibly the similar effect of pentobarbitone) seems most plausibly accounted for by the known anticonflict properties of these agents, mediated by a picrotoxin- and pentylenetetrazol-resistant, nongabergic pathway.

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