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Benzodiazepine Receptors: Cellular and Behavioral Characteristics

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LIPPA, A. S., D. CRITCHETT, M. C. SANO, C. A. KLEPNER, E. N. GREENBLATT, J. COUPET AND B. BEER. *Benzodiazepine receptors: Cellular and behavioral characteristics.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 831–843, 1979.—Brain specific benzodiazepine receptors appear to mediate the pharmacological properties of benzodiazepines. A neuronal localization for these receptors is suggested by the parallel decrease in the number of benzodiazepine receptors and cerebellar Purkinje cells in "nervous" mutant mice. Electrophysiological results are compatible with an action of benzodiazepines on neuronally localized, physiological receptors. Biochemical, electrophysiological and behavioral experiments highlight the possible importance of frontal cortex in mediating the anxiolytic properties of the benzodiazepines. Triazolopyridazines act upon benzodiazepine receptors, increase punished responding and protect against pentylenetetrazole-induced convulsions, but do not produce the side effects associated with benzodiazepines or affect classical neurotransmitter systems. The structural similarities between triazolopyridazines, purines and the indole portion of certain peptides may provide insights into the nature of the endogenous ligand.

Cellular characteristics Behavioral characteristics

AN understanding of the basic mechanisms by which the benzodiazepines exert their anxiolytic effects might provide tools for investigating those neural substrates involved in anxiety. Recently, several investigators have reported the existence of binding sites for ^{3}H-diazepam and ^{3}H-flunitrazepam in several species including humans [6, 8, 13, 29, 30, 31, 40, 41]. These binding sites conform to many of the criteria established for identifying physiological receptors [47]. Brain-specific ^{3}H-diazepam and ^{3}H-flunitrazepam binding has a high affinity, is saturable and stereo-specific [6, 8, 13, 29, 30, 31, 40, 41, 46]. Significant correlations have been obtained between the *in vivo* and *in vitro* ability of benzodiazepines to inhibit ^{3}H-benzodiazepine binding and the clinical usefulness of these drugs in humans [9, 30, 31, 40, 41]. Significant correlations have also been reported between the ability of benzodiazepines to inhibit ^{3}H-diazepam binding and their ability to inhibit pentylenetetrazole (PTZ)-induced convulsions [9, 30, 41] and to increase punished responding in a conflict situation [14,26], procedures which are highly correlated with the anxiolytic activity of benzodiazepines. For these reasons, it has been suggested that these binding sites represent a common receptor mechanism through which benzodiazepines produce their anxiolytic actions. Because of the potential importance of these findings, research in this area has greatly increased. This paper descibes our attempts to delineate some of the cellular and behavioral characteristics of benzodiazepine receptors.

CELLULAR LOCALIZATION OF BENZODIAZEPINE RECEPTORS

To study the cellular localization of benzodiazepine receptors, we have investigated ^{3}H-diazepam binding in brains from nervous mutant mice, an autosomal recessive mutation which results in a selective degeneration of cerebellar Purkinje cells [25]. Purkinje cells are present in normal numbers until the mice are approximately 23 days of age, at which time the Purkinje cells begin to degenerate, so that by 60 days of age, an estimated 90% of the Purkinje cells in the cerebellar hemispheres and more than 50% in the vermis have disappeared [22].

At 15–21 days of age, ^{3}H-diazepam binding in cerebella of mutant mice did not differ from that observed in normal littermates and approximated adult levels (see Table 1). At 60–70 days of age, however, binding was reduced in mutant mice to 17% of normal littermate control values (Table 1). These results demonstrate an age-related loss of benzodiazepine receptors in the cerebellum of mutant mice parallel to that observed for the degeneration of cerebellar Purkinje cells.

Additional experiments in 60–70 day old mice revealed that the decreased ^{3}H-diazepam binding only occurred in cerebellum and was due to a decrease in the maximal number of binding sites (B_{max}) and not to any change in affinity (K_D) (see Fig. 1). Since the age-related, regionally specific loss of ^{3}H-diazepam binding sites in nervous mutant mice paralleled the loss of cerebellar Purkinje cells, these results suggest that benzodiazepine receptors reside on Purkinje cells.

In additional experiments we have also attempted to localize benzodiazepine receptors in frontal cortex. Stereotaxically placed, unilateral coronal knife cuts (hemitransections) were made in anesthetized albino rats at the anterior border of the caudate nucleus. Three weeks after surgery, all animals were decapitated and ^{3}H-diazepam binding in frontal cortex was determined in the operated and

TABLE 1

BENZODIAZEPINE BINDING SITES IN CEREBELLA OF NORMAL AND NERVOUS MUTANT MICE

Age	Specifically Bound ^{3}H-Diazepam* (p moles/mg protein) Normal Littermates	Nervous Mutants
15–21 days	0.533 ± 0.022	0.597 ± 0.180
60–70 days	0.530 ± 0.015	0.089 ± 0.005†

*Data are expressed as mean ± standard error of mean, n=4–5 mice per group.

†$p<0.01$, t-test.

intact sides. We reasoned that knife cuts would reduce ^{3}H-diazepam binding if benzodiazepine receptors were located either presynaptically on fibers afferent to the frontal cortex or postsynaptically on the cell bodies of fibers efferent from the frontal cortex. In fact, ^{3}H-diazepam binding was significantly higher ($p<0.05$, t-test) on the cut side than on the intact side (0.598 ± 0.030 p moles/mg protein for the cut side vs. 0.467 ± 0.020 p moles/mg protein for the intact side). While not providing direct evidence for a cellular localization of benzodiazepine receptors in frontal cortex, these results could be interpreted as suggesting that benzodiazepine receptors are located on cells intrinsic to frontal cortex. According to this hypothesis, the increased binding on the cut side might represent some supersensitivity phenomenon resulting from the removal of afferent inputs.

ELECTROPHYSIOLOGICAL EFFECTS OF BENZODIAZEPINES

In order to understand the electrophysiological actions of any drugs, it is imperative to record from cells which contain

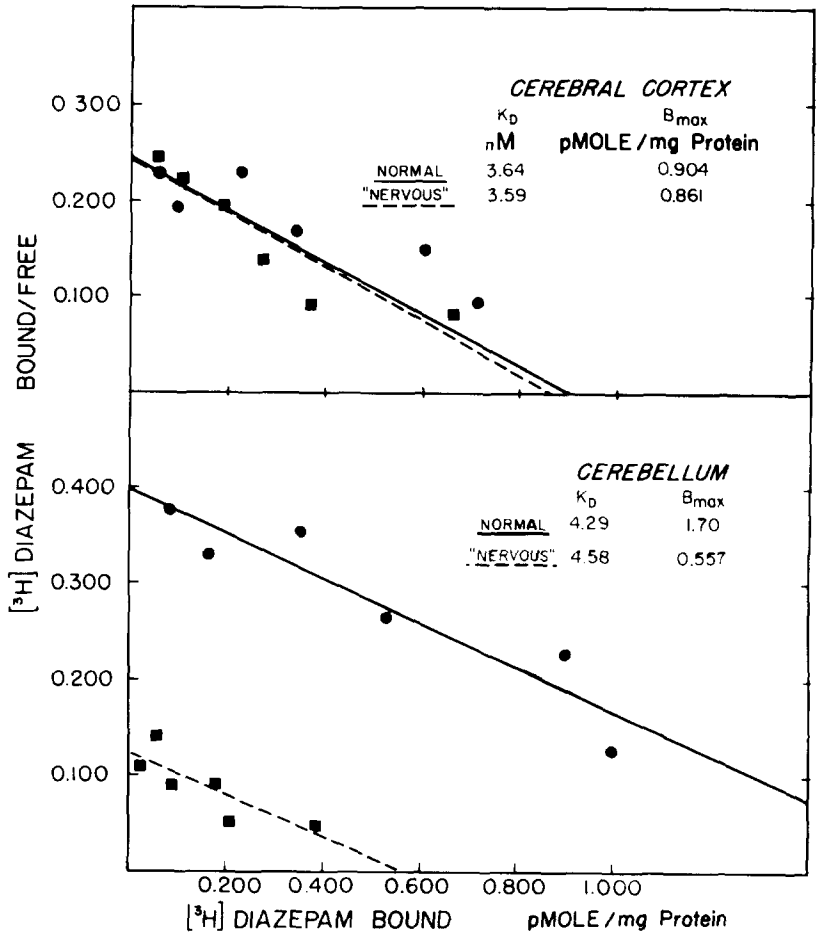


FIG. 1. Incubations were performed as described in methods, in the presence of 0.25 to 8.0 nM ^{3}H-diazepam. Top and bottom graphs represent the lines of regression of the Scatchard plot for the data in the cerebral cortex and the cerebellum; respectively. ○, normal mice; ■, nervous mutants. Selective loss of Purkinje cells in the cerebellum of nervous mutant mice corresponds to a relative decrease in ^{3}H-diazepam receptor density (B_{max}) in these animals. No apparent changes in either the affinity constant (K_d) or the B_{max} were observed in the cerebral cortex between normals and nervous mutants.

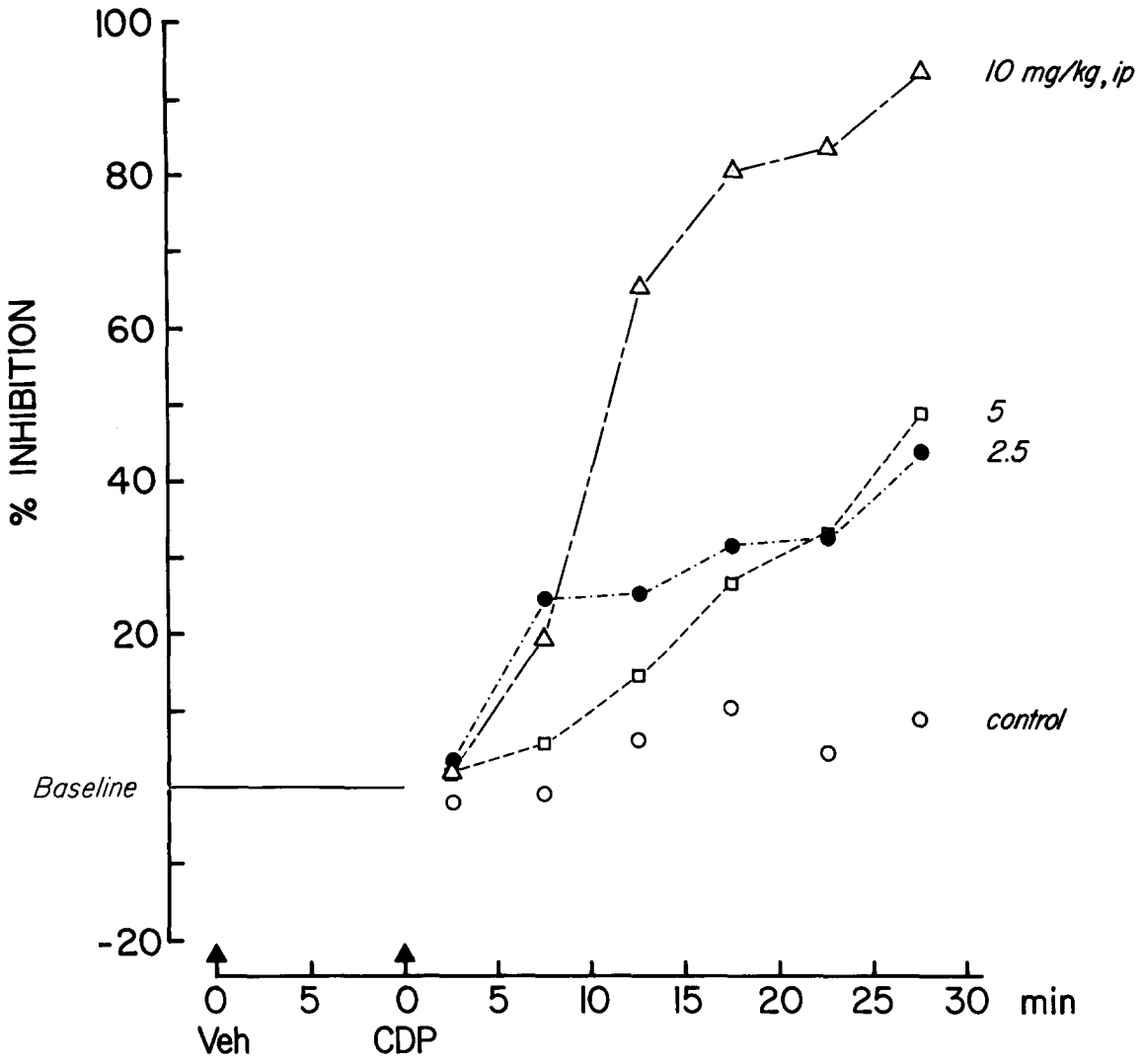


FIG. 2. The effects of chlordiazepoxide on cerebellar Purkinje cells. Only one cell was recorded in each animal, and each group consists of 6–8 rats. Baseline firing rates were determined after an injection of vehicle and inhibition was determined from this baseline. Data represent mean percent inhibition per group.

the relevant receptors [4]. Since prior experiments in nervous mutant mice suggested that benzodiazepine receptors were located on cerebellar Purkinje cells [25,39], we investigated the effects of both peripheral and microiontophoretic administration of benzodiazepines on the firing of these cells [18, 24, 27, 36]. Single and multi-barrel glass micropipettes were stereotaxically placed into the cerebellar vermis of male, albino rats anesthetized with chloral hydrate (400 mg/kg, intraperitoneally (IP)) or urethane (1 g/kg, IP). Single cerebellar Purkinje cells were identified by their amplitude, characteristic firing pattern and/or ability to be inhibited by microiontophoretically applied gamma aminobutyric acid (GABA).

Intraperitoneal administration of chlordiazepoxide produced a dose-related suppression of Purkinje cell firing rate with a latency of 3–15 minutes (see Fig. 2). No effects were produced by isovolumetric injections of the vehicle solution. This inhibition lasted for as long as the recording session (>1 hour). Similar effects with other benzodiazepines have also been reported [19,34].

Like the effects observed after peripheral injection of chlordiazepoxide, microiontophoretic administration of flurazepam (a soluble benzodiazepine) consistently inhibited firing in the majority of Purkinje cells tested [18, 24, 27]. Increasing amounts of ejection current produced increasing amounts of inhibition (see Fig. 3).

Since Purkinje cells also receive a GABA innervation from cerebellar interneurons [35], we investigated GABA-benzodiazepine interactions by comparing the abilities of bicuculline and picrotoxin to antagonize the electrophysiological effects of iontophoretically administered flurazepam and GABA on cerebellar Purkinje cells. Bicuculline is believed to antagonize GABA by a direct action at the GABA recognition site, while picrotoxin is believed to antagonize GABA by interfering with a chloride conductance channel distinct from the GABA recognition site [32,33]. Be-

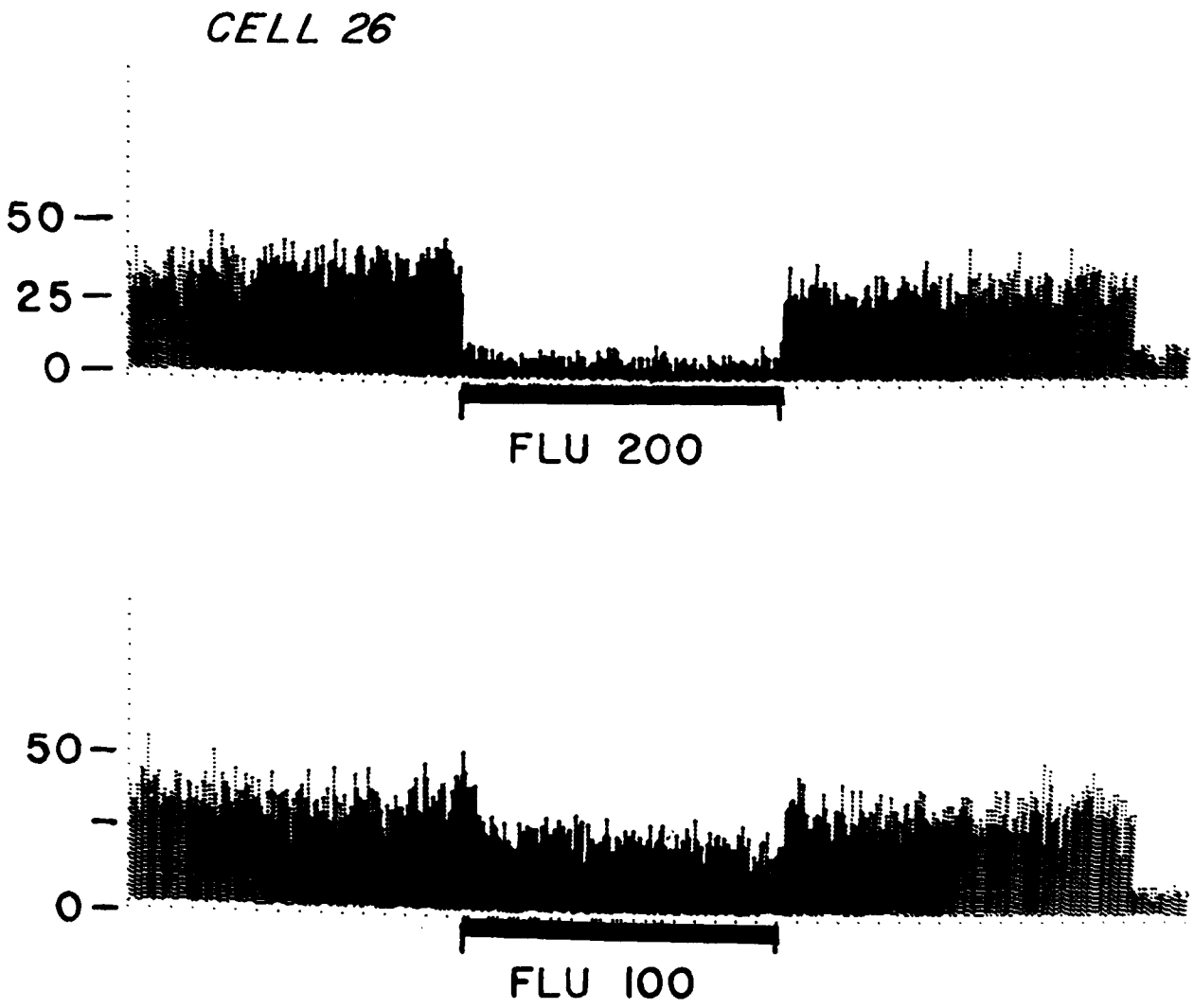


FIG. 3 The effect of flurazepam on a cerebellar Purkinje cell. Each computer generated histogram is comprised of the total number of counts per bin over 5 consecutive, triggered sweeps where bin time equals 128 msec and length of sweep equals one min. Solid horizontal bars indicate ejection periods and numbers denote ejection current (nA) levels.

fore testing their ability to antagonize flurazepam, ejection currents for picrotoxin and bicuculline were first adjusted so that these drugs antagonized the inhibitory actions of GABA by at least 75%.

Iontophoretic administration of GABA (1–30 nA) inhibited the firing of cerebellar Purkinje cells (Figs. 4 and 5). This effect was antagonized by picrotoxin (10–100 nA) in 32 out of 39 cells tested (see Fig. 4). Similarly, bicuculline (50–100 nA) antagonized GABA in 31 out of 37 cells tested (see Fig. 5). Iontophoretic administration of flurazepam (10–120 nA) reduced the firing rate of 50 out of 52 Purkinje cells (see Fig. 5). Picrotoxin antagonized these depressant action in 13 out of 23 cells (Fig. 4). However, as illustrated in Fig. 5, bicuculline was unable to antagonize flurazepam in 19 out of 22 cells where a clear antagonism of GABA was observed, suggesting that these effects of flurazepam are not mediated through a direct action on the GABA recognition site.

While picrotoxin may affect several nuerotransmitter systems in the brain, its primary action is assumed to be at the level of a chloride ionophore distinct from the GABA recognition site [32, 33]. Because picrotoxin blocked the depressant effects of flurazepam, the present studies suggest that the benzodiazepine receptor, like the GABA recognition site, may be intimately associated with chloride ion conductance channels. This hypothesis receives support from the recent observations of Costa *et al.* [17] that chloride and several other small anions, known to penetrate specific chloride channels (ionophores) associated with injibitory receptors on the membranes of cat motoneurones, increase ^{3}H-Diazepam binding.

If benzodiazepine receptors are associated with chloride ionophores, why did picrotoxin fail to antagonize flurazepam in almost 50% of the cells tested, while antagonizing the inhibitory effects of GABA on these same cells? The answer to this question may lie in the recent demonstration of two similar, but distinct types of benzodiazepine receptors in rat brain. Benzodiazepines and triazolopyridazines (see below) have a high affinity for Type I receptors, but only benzodiazepines have a high affinity for Type II receptors [42,43]. The cerebellum contains both types of receptors

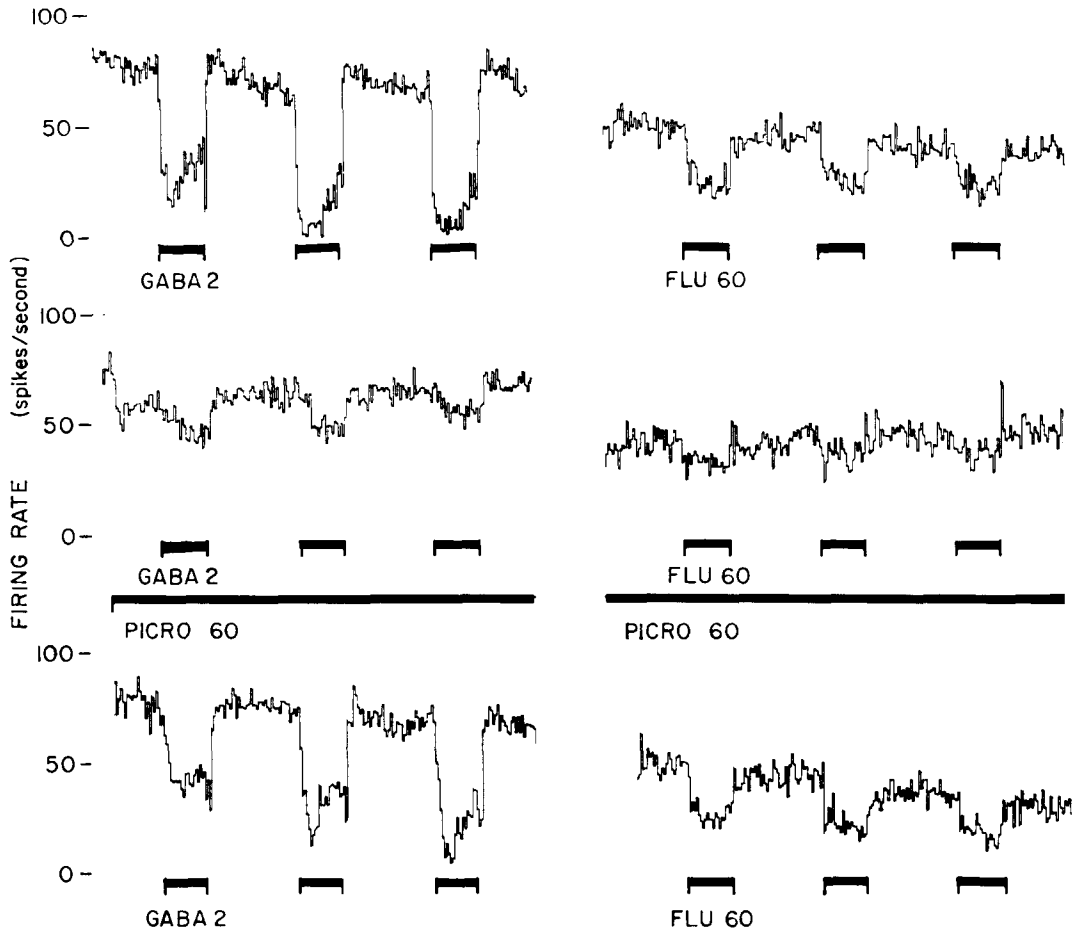


Fig. 4 The effects of GABA and flurazepam on the firing rate of a single cerebellar Purkinje cell. Data are from polygraphic rate meter recordings. Each segment shows three consecutive 20 second ejections of GABA or flurazepam. Bars indicate ejection periods and numbers indicate ejection current in nanoamperes. Top traces demonstrate inhibitory actions of GABA and flurazepam before picrotoxin. Middle traces demonstrate antagonistic effects of concomitant ejection of picrotoxin. Bottom traces show recovery after picrotoxin.

(unpublished observations). The present results are explainable if only one type of receptor utilizes a chloride ionophore.

In preliminary studies, microintrophoretic administration of flurazepam also suppressed the firing of 45 out of 58 unidentified cells in rat frontal cortex (see Fig. 6). In 11 out of 13 cells, the depressant effects of flurazepam were antagonized by the concurrent microiontophoretic administration of picrotoxin. The neuro-anatomical location of these cells are dipicted in Fig. 7. While the physiological relevance of these preliminary results must be viewed cautiously (since it is not known whether benzodiazepine receptors were located on the cells from which we recorded), they are certainly compatible with the hypothesis that benzodiazepine receptors are somehow linked to chloride ion conductance channels.

Additional support for this hypothesis has been obtained in other experiments. Therefore, picrotoxin (0.75–3 mg/kg IP), in a dose dependent manner, antagonized the rod-walking deficits produced by chlordiazepoxide (10 mg/kg, IP). However, bicuculline, in doses as high as 2 mg/kg, IP, was unable to affect the chlordiazepoxide-induced rod-walking deficits (see Fig. 8). Higher doses of bicuculline produced convulsions and so were not tested.

FUNCTIONAL LOCALIZATION

Benzodiazepine receptors are unevenly distributed throughout the mammalian brain, with the highest concentrations found in the cerebral cortex. [6, 7, 9, 13, 29, 30, 40, 41]. Although available data strongly suggest that actions on benzodiazepine receptors mediate the anxiolytic effects of these drugs, the precise brain regions where these anxiolytic actions take place are still unknown. Our initial attempts to answer this question havs focused on the frontal cortex.

We have investigated the relationship between ^{3}H-diazepam binding in frontal cortex and a measure of situational anxiety as reflected by the ability of electric shock to suppress behaviour in a conflict situation. Numerous studies in animals and humans have demonstrated that drugs used in the treatment of anxiety are able to selectively release behavior previously suppressed by a punishing stimulus (see [25] for review.) For these reasons, it has been suggested that conflict procedures may serve as a model for situational anxiety [2, 3].

We first sought to determine if the *in vivo* actions of diazepam on frontal cortex benzodiazepine receptors were related to the ability of diazepam to increase punished responses in a conflict test [26]. Various groups of food and

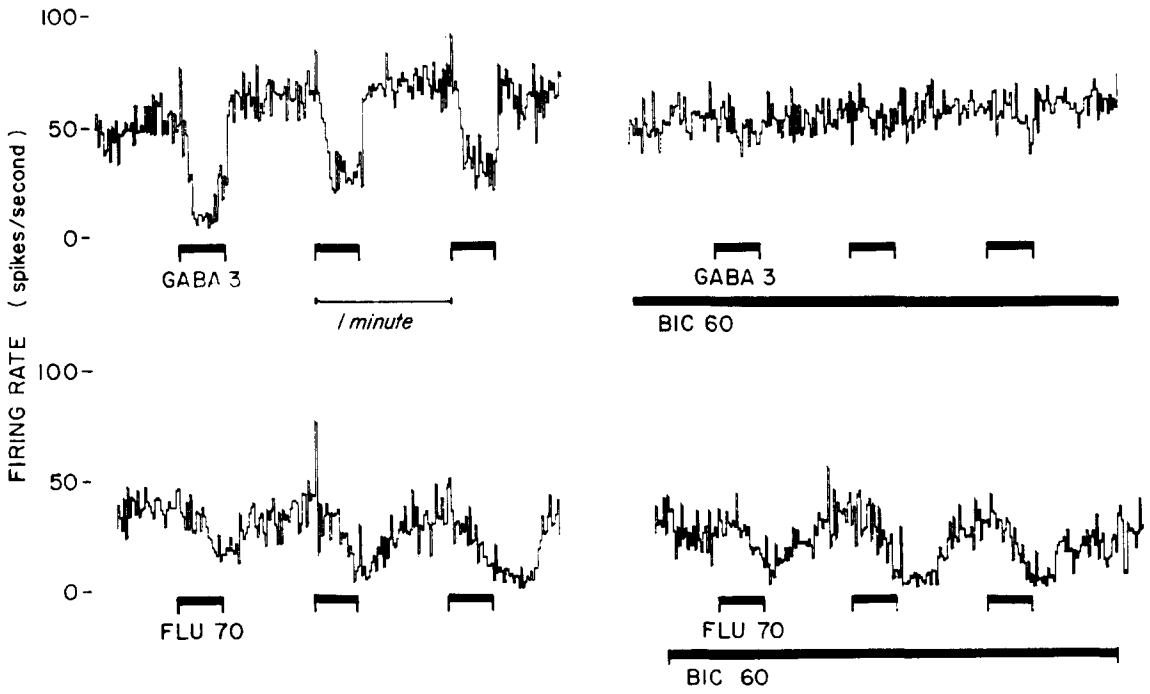


FIG. 5 Inhibitory effects of GABA and flurazepam on a single cerebellar Purkinje cell. Traces on the left demonstrate inhibitory actions before bicuculline. Note the ability of bicuculline to antagonize the depressant actions of GABA while not affecting the depressant actions of flurazepam.

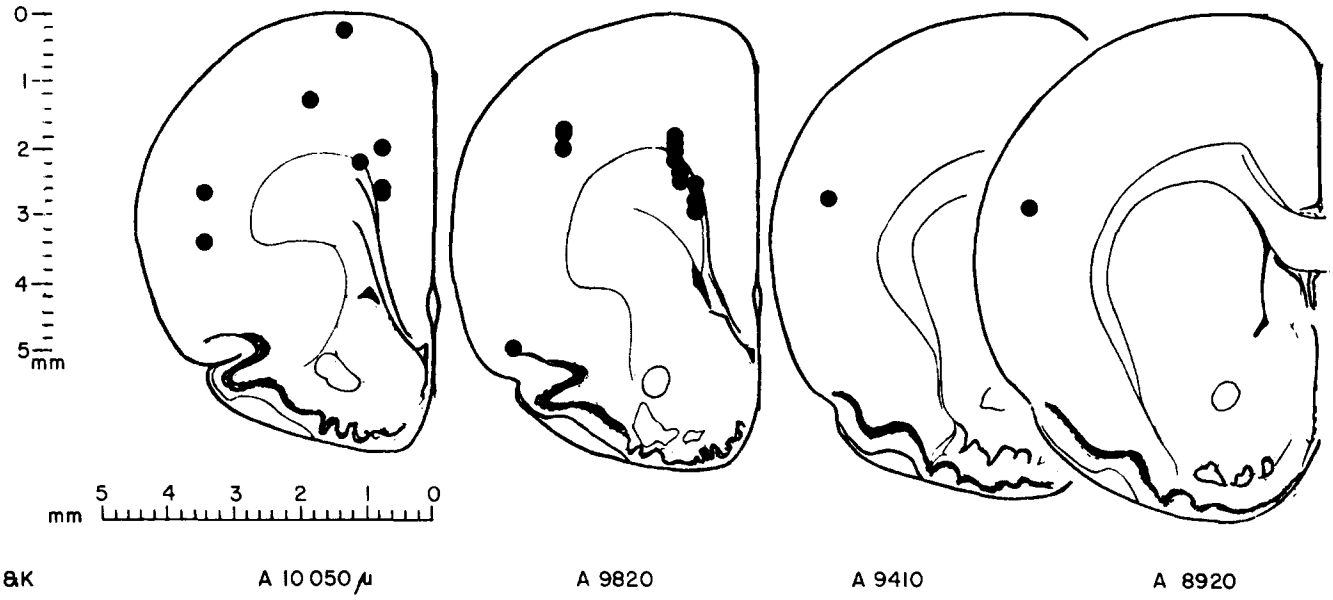


FIG. 6. Anatomical localization of flurazepam sensitive neurons. Solid circles represent electrode tips. Plates are from König and Klippel (1963).

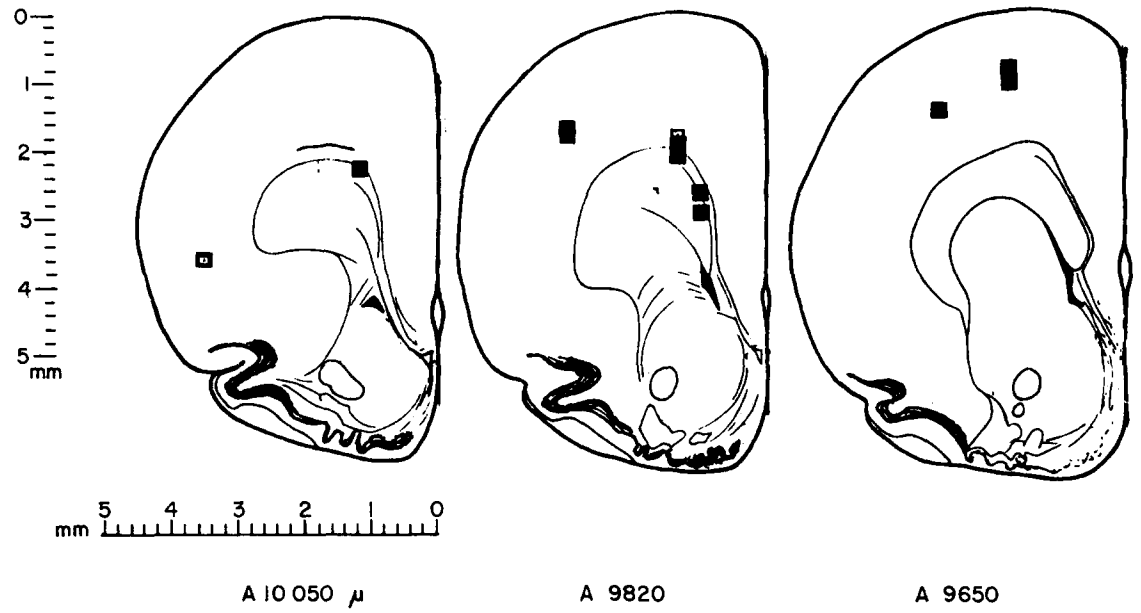


FIG. 7. Anatomical localization of flurazepam sensitive neurons. Solid squares represent cells in which picrotoxin antagonized the affects of flurazepam; open squares represent cells in which picrotoxin did not antagonize flurazepam.

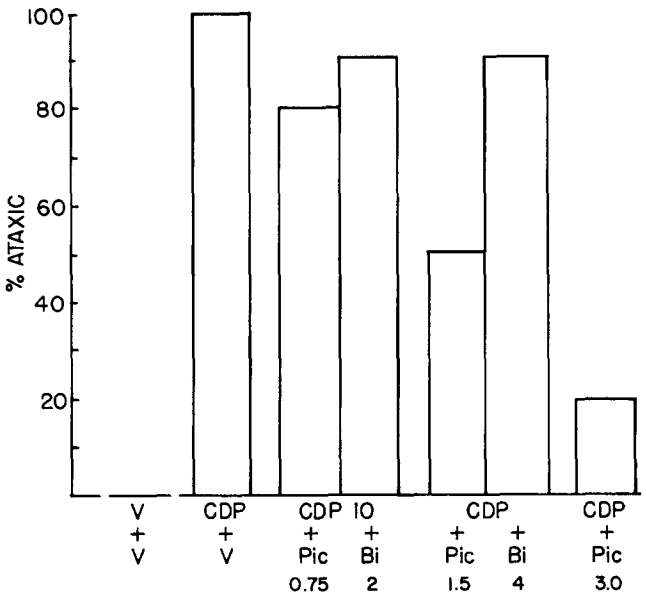


FIG. 8. Effects of bicuculline and picrotoxin on ataxia produced by chlordiazepoxide. Chlordiazepoxide (10 mg/kg,IP) was administered 10 minutes before testing. Bicuculline (1 and 2 mg/kg IP) and picrotoxin (0.75, 1 and 2 mg/kg, IP) were administered 20 minutes after chlordiazepoxide.

water deprived rats were injected with diazepam (2.5, 5 and 10 mg/kg IP; 3, 6, 12 and 18 mg/kg, p.o.) or isovolumetric amounts of the vehicle solution. Thirty minutes after IP administration and 60 minutes after oral administration, approximately half of these rats were placed into drinking chambers, and the number of punished responses (shocks) were recorded. The remaining naive animals were decapitated and frontal cortex was removed for the *in vitro* determination of ^{3}H-diazepam binding. As can be seen in Fig. 9, both IP and p.o. administration of diazepam produced a dose-related inhibition of the subsequently measured specific ^{3}H-diazepam binding, with the first statistically significant ($p<0.05$, t-test) effects observed at 2.5 mg/kg, IP, and 6 mg/kg, p.o. Diazepam administration also produced the expected increase in punished responding with the minimally effective anticonflict doses inhibiting ^{3}H-diazepam binding (see Fig. 9). It should be pointed out that only a small number of benzodiazepine receptors (approximately 15–20%) need be affected to obtain a significant anti-conflict response. These data demonstrate that *in vivo* administered diazepam can affect frontal cortex benzodiazepine receptors in parallel with its ability to overcome the effects of punishment.

We next sought to determine whether exposure to anxiety-provoking situations would alter binding at frontal cortex receptors. Food (24 hour) and water (48 hour) deprived rats were placed into the conflict procedure for a standard 5 minute test. These animals received an average of 20 shocks. Immediately after testing, animals were sacrificed by decapitation and frontal cortex was removed to determine ^{3}H-diazepam binding. An equal number of deprived animals, which were not exposed to the conflict procedure, were used as controls. Exposure to the conflict procedure produced an approximately 25% decrease ($p<0.01$, t-test) in ^{3}H-diazepam specifically bound (Table 2).

In another experiment, deprived rats were placed into a small test chamber (9×8×8 in) with a stainless steel grid floor. The experimental group was placed into the test chamber and electric foot shock (100 msec of 300 μA scrambeled sine wave current) was applied every 15 seconds for 5 minutes, but electric shock was not administered. At the end of the 5 minute period, all rats were decapitated and frontal cortex removed for the determination of ^{3}H-diazepam binding. The application of electric foot shock produced a smaller (relative to the conflict procedure) but still significant ($p<0.05$, t-test) decrease in specifically bound ^{3}H-diazepam (Table 2.).

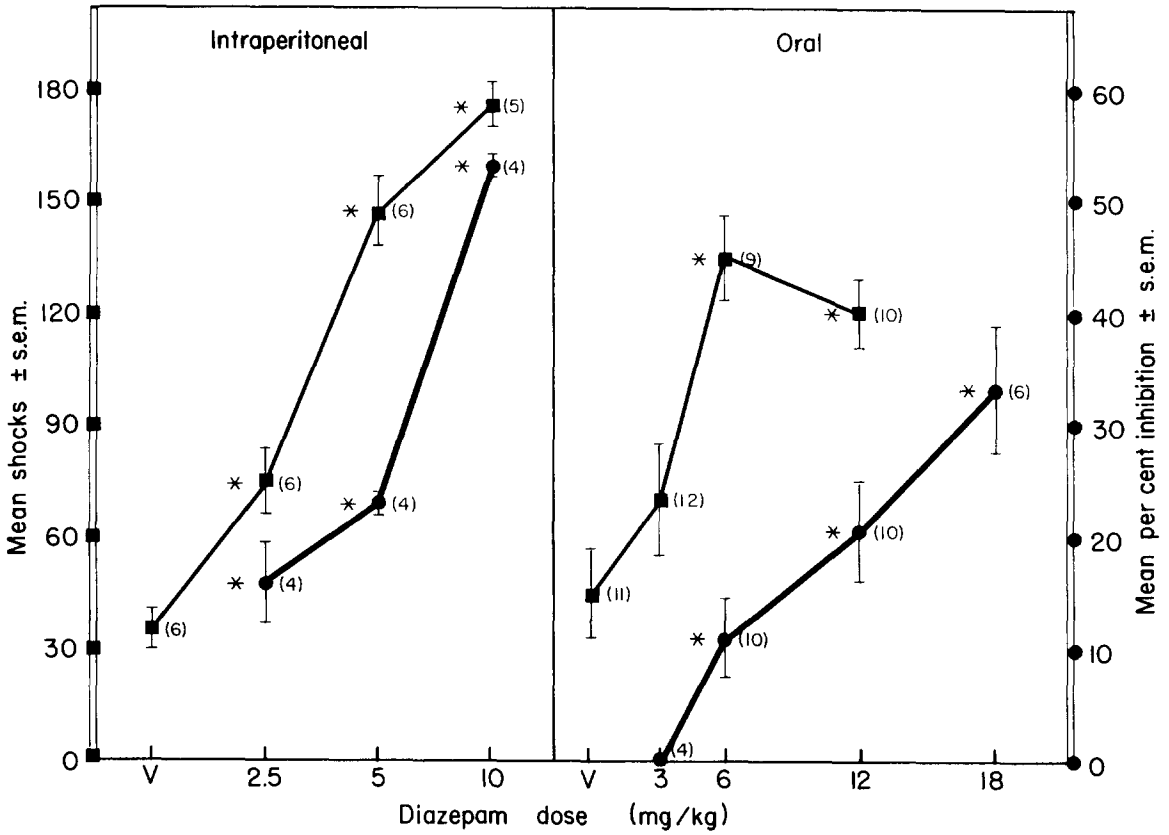


FIG. 9. Effects of *in vivo* adminstration of diazepam on conflict responding and *in vitro* ^{3}H-diazepam binding. Solid squares represent the mean shocks received during a 5 minute conflict test. Solid circles represent the mean percent inhibition of specific ^{3}H-diazepam binding. Percent inhibition was determined by the following formula:

Percent inhibition = (pmoles ^{3}H-diazepam/mg protein in vehicle controls) — (pmoles ^{3}H-diazepam/mg protein in drugged animals)/pmoles ^{3}H-diazepam in vehicle controls × 100

Mean ^{3}H-diazepam binding in IP treated controls was 0.504 ±0.017 ρmoles/mg protein, n=20. Numbers in parenthesis represent the number of animals per group. *$p<0.05$, t-test.

TABLE 2

EFFECTS OF AVERSIVE STIMULI ON ^{3}H-DIAZEPAM BINDING

Treatment	N	^{3}H-Diazepam Binding (p moles/mg protein)*
Deprived Controls	6	0.535 ± 0.060
Conflict	6	0.408 ± 0.036‡
Deprived Controls	8	0.580 ± 0.035
Foot Shock	8	0.501 ± 0.031†

*Data expressed as mean ± SEM.
†$p<0.05$, t-test.
‡$p<0.01$, t-test.

In the final series of experiments, the frontal cortices of anesthetized male, albino rats were either removed by aspiration or surgically isolated by stereotaxically placed, bilateral coronal knife cuts (bilateral hemitransections) at the anterior border of the caudate nucleus. Sham controls were treated identically to lesioned animals except for the actual placement of the lesions. Approximatley three weeks after surgery, all animals were food (24 hour) and water (48 hour) deprived and tested for conflict activity. As can be seen in Table 3, both surgically treated groups took significantly ($p<0.05$, Mann-Whitney U Test) more shocks than their respective sham controls.

According to the results of these studies, the following model may be generated. The frontal cortex (through some as yet unknown mechanism) inhibits ongoing behavioral responses. After removal or surgical isolation of frontal cortex, infibition is lost and behavior is released. Benzodiazepines may similarly disinhibit behavior by inhibiting the firing of certain benzodiazepine receptor-containing cells in frontal cortex (see previous section). We further hypothesize that an endogenous ligand is normally released when an organism is

TABLE 3
EFFECTS OF LESIONS ON CONFLICT ACTIVITY

Surgical Treatment	Mean Shocks ± SEM
Sham Controls (n=16)	26 ± 6
Bilateral Frontal Cortex Aspiration (n=9)	44 ± 9*
Sham Controls (n=9)	49 ± 18
Bilateral Hemitransection (n=7)	143 ± 24*

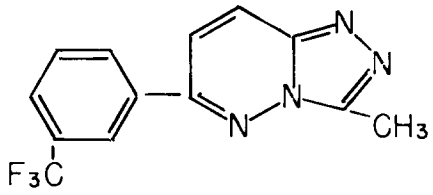
$p<0.05$, Mann-Whitney U Test.

exposed to anixiety-provoking stimuli and interacts at those same receptor sites which selectively bind benzodiazepines. According to this model, the observed decrease in ^{3}H-diazepam binding after exposure to anxiety-provoking stimuli (conflict and electric shock) may reflect the increased release and occupation of receptor sites by an endogenous ligand. It should be pointed out that this hypothesis is still highly speculative and should not be viewed as excluding or minimizing any possible contributions from other neuronal regions.

RECEPTOR SPECIFICITY AND THE SEARCH FOR AN ENDOGENOUS LIGAND

Since a large number of drugs with agonist and antagonist actions on known neurotransmitters and hormones have very low affinity for ^{3}H-diazepam binding sites [7, 10, 41], reports of brain extracts containing endogenous, competitive inhibitors of benzodiazepine binding [21, 28] raise the possibility that these binding sites represent receptors for endogenous ligand(s), which may act as neurotransmitter(s). Evidence for a neuronal localization of benzodiazepine receptors strongly supports such an hypothesis [11, 12, 25, 39], as does data presented in the previous sections. Quite recently, the purines, inosine and hypoxanthine, have been isolated from these brain extracts and suggested to be responsible for at least some of the activity of brain extracts [37]. These two substances are very weak, however, in their ability to displace ^{3}H-diazepam (with Ki values of approximatley 1 mM), but intraventricular administration of inosine does protect somewhat against pentylenetetrazole (PTZ)-induced conculsions [38], a property also seen with benzodiazepines and highly correlated with their clinical efficacy [25].

We have recently discovered a new class of pharmacologically unique substances with highly specific actions on benzodiazepine receptors. CL 218,872 (3-methyl-6-[3-(trifluoromethyl)]-1,2,4-triazolo [4,3-b]pyridazine) is the first of these substance to be reported (see Fig. 10). Cl 218,872 produced dose-related decreases in ^{3}H-diazepam binding with a calculated Ki of 94.0 nM compared to 4.8 nM and 196.0 nM for diazepam and chlordiazepoxide, respectively. By contrast, CL 218,872 did not significantly ($p<0.05$, t-test) alter ^{3}H-QNB or ^{3}H-spiroperidol binding even in concentrations as high as 1 μM. This is the first non-benzodiazepine substance which seletively inhibits ^{3}H-diazepam binding with a potency approximating that of the benzodiazepines themselves.



CL 218,872
3-methyl-6-[3-(trifluoromethyl) phenyl]-
1,2,4-triazolo [4,3-b] pyridazine

FIG. 10. Chemical structure of CL 218,872.

Like the benzodiazepines, CL 218,872 also increases punished responding in the rat conflict procedure (see Fig. 11) and protects against PTZ-induced convulsions (see Table 4). However, CL 218,872 was very weak (both relative to the benzodiazepines and to its own potency in the PTZ and conflict tests) in producing motor depression and inclined screen deficits (see Table 5), common side effects produced by the benzodiazepines [25]

As can be seen in Fig. 10, certain geometrical features of the CL 218,872 structure are surprisingly similar to the general structure of purines. Why then is 218,872 approximately 10^4 times more potent than inosine and hypoxanthine in its ability to act at the benzodiazepine receptor? One possibility may be that while the endogenous ligand is purine-like, it is neither inosine nor hypoxanthine, but these substances bear enough similarity to the ligand that they have still retained some, albeit, weak activity. Other purine-like substances such as cyclic AMP can inhibit ^{3}H-diazepam binding (unpublished observations) and have also been reported to display anti-conflict activity like benzodiazepines [1].

INTERACTIONS WITH NEUROTRANSMITTER SYSTEMS

Considerable controversy has centered around the possibility that indirect benzodiazepine actions on neurotransmitter systems utilizing serotonin (5HT) [44, 45], gamma aminobutyric acid (GABA) [15, 16, 19, 20], or glycine

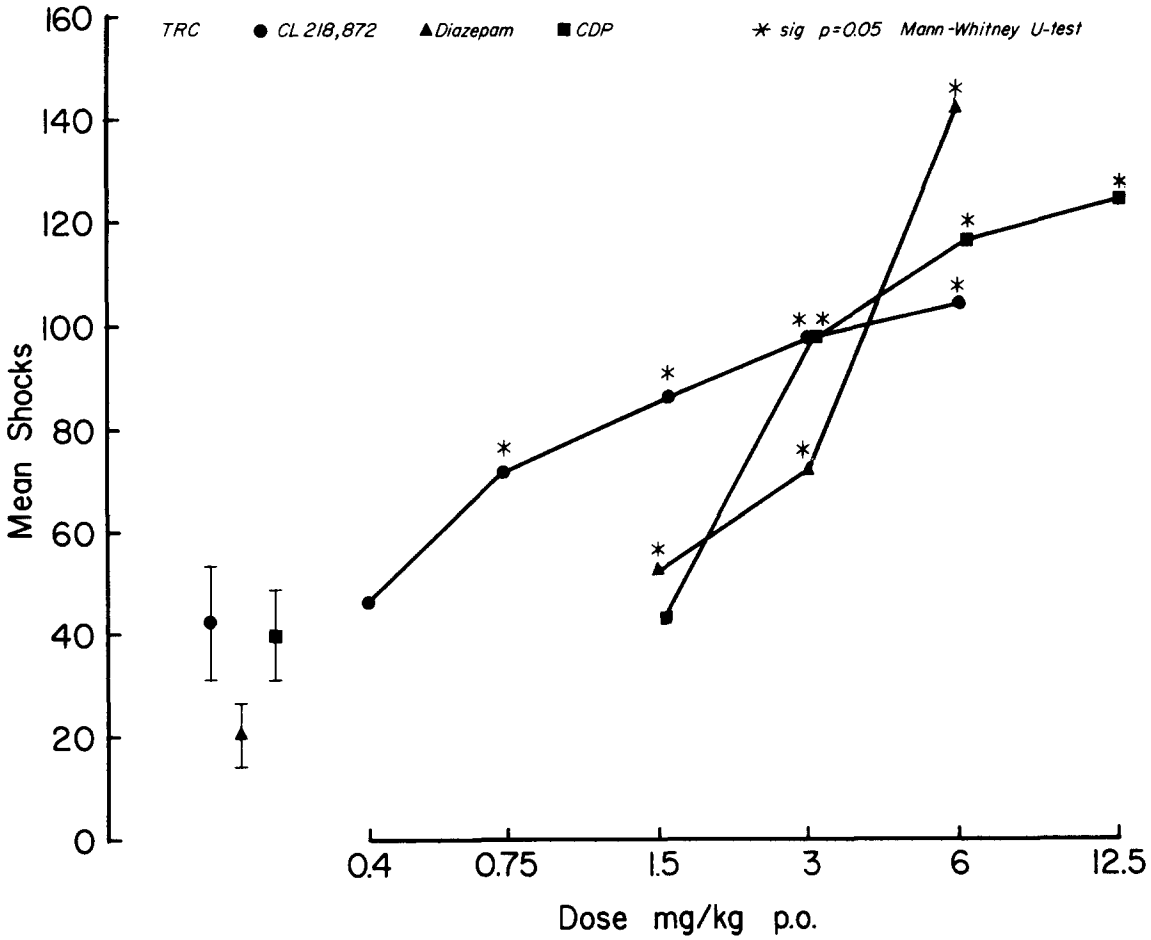


FIG. 11. Effects of CL 218,872, diazepam and chlordiazepoxide on punished responding.

TABLE 4

EFFECTS ON PENTYLENETETRAZOLE (PTZ) SEIZURES IN RATS AND MICE

Treatment	Species	Median Effective Dose mg/kg (95% Confidence Limits)
CL 218,872	Rat	1.7 (1.3–2.2)
	Mouse	3.8 (3.0–4.9)
Diazepam	Rat	1.6 (1.3–2.1)
	Mouse	.82 (0.63–1.05)
Chlordiazepoxide	Rat	2.4 (1.8–3.2)

All drugs were orally administered.

[48] may somehow mediate the anxiolytic activity of these drugs. It has recently been demonstrated that the benzodiazepines affect 5HT, GABA and glycine systems within the same dose range that they affect animal procedures which are highly correlated with the anxiolytic properties of the benzodiazepines. Because the benzodiazepines also produce various side effects unrelated to their anxiolytic actions (i.e., sedation, muscular incoordination), but within the same dose range that they produce their axiolytic actions, it has not been possible to determine which neurotransmitter actions, if any, are responsible for the anxiolytic actions and which are responsible for producing side effects [23, 24, 25]. Because CL 218,872 was reasonably devoid of side effects (see previous section) it would seem to be an excellent tool for assessing the importance of neurotransmitter systems in contributing to anxiolytic actions.

Since benzodiazepines antagonize the convulsions produced by bicuculline [23], isoniazid [15, 16] and strychnine [23], we have utilized these procedures to estimate the possible GABA-ergic and glycine-ergic properties of CL 218,872. CL 218,872 was very weak (both relative to the benzodiazepines and its own potency in inhibiting PTZ-induced convulsions) in its ablilty to inhibit the convulsions produced by bicuculline, isoniazid and strychnine. As can be see in Table 6, chloridazepoxide inhibited isoniazid-induced

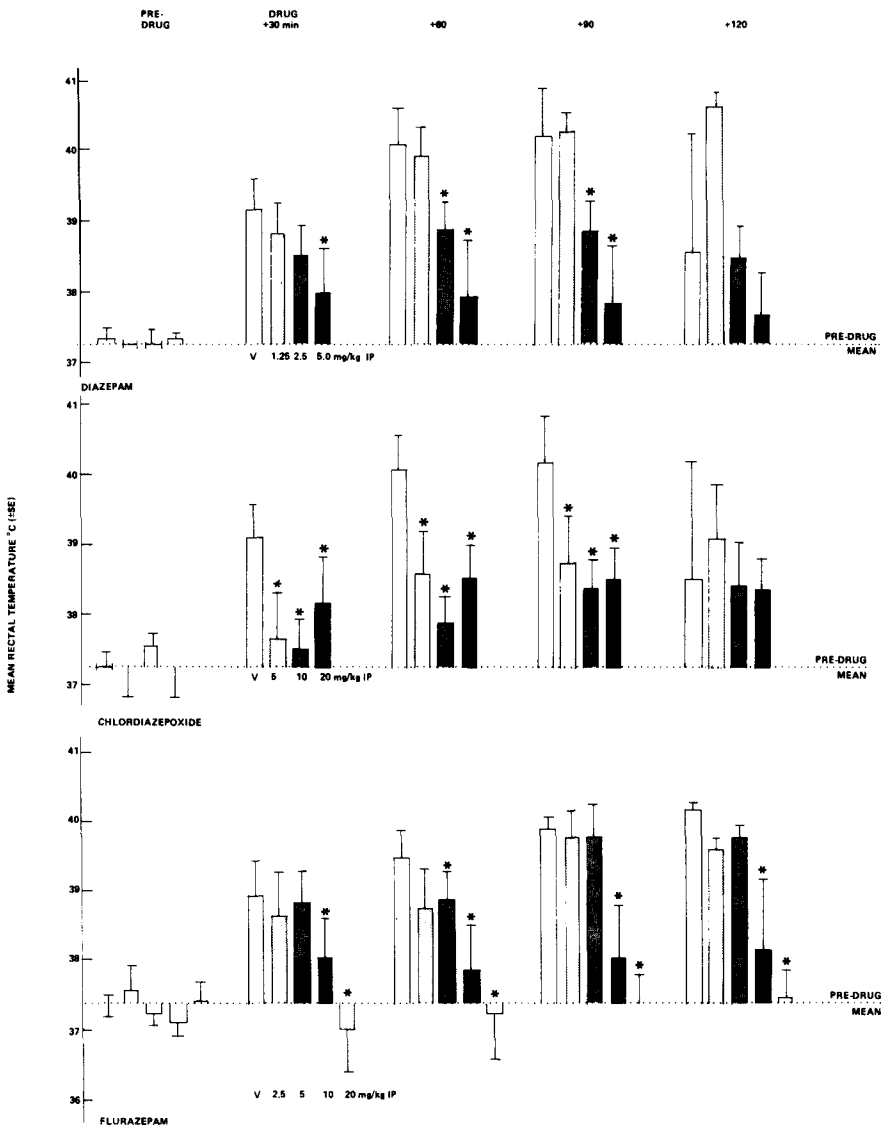


FIG. 12. Effects of diazepam, chlordiazepoxide and flurazepam on *l*-tryptophan-induced hyperthermia. Data are presented as mean rectal temperature ±S.E.M. $^{*}p<0.05$.

TABLE 5
EFFECTS ON MOTOR FUNCTIONING

Drug	Inclined Screen Deficits Median Effective Dose - mg/kg, p.o. (95% Confidence Limits)	Motor Depression MD_{50} mg/kg, p.o.
CL 218,872	396 (72–2165)	223
Diazepam	22 (12–38)	22
Chlordiazepoxide	34 (23–51)	52

TABLE 6
EFFECTS ON CONVULSIONS PRODUCED BY BICUCULLINE, STRYCHNINE OR ISONIAZID

Treatment	Bicuculline ED_{50} (95% C.L.)	Strychnine ED_{50} (95% C.L.)	Isoniazid ED_{50} (95% C.L.)
CL 218,872	31.2 (25.7–37.9)	161.2 (76.7–339.0)	25.0 (16.0–39.1)
Diazepam	1.63 (1.14–2.33)	1.03 (0.77–1.39)	—
Chlordiazepoxide	—	—	4.0 (3.0–5.3)

convulsions with an ED_{50} of 4 mg/kg. CL 218,872 was approximatley 15 times more potent in its ability to protect against PTZ than against isoniazid. Likewise, CL 218,872 was also weak in its ability to protect against bicuculline-induced convulsions (ED_{50}=31.2 mg/kg for CL 218,872 vs.1.03 mg/kg for diazepam). These results suggest that CL 218,872 has very weak actions, if any, on the GABA and glycine transmitter systems.

The adminstation of *l*-tryptophan in combination with a monoamine oxidase inhibitor produces a measurable hyperthermia which appears to be 5HT mediated [28]. All anxiolytics tested to date inhibit this 5HT-mediated hyperthermia [28]. We. therefore, utilized this procedure in an attempt to measure the anti-5HT properties of CL 218,872. As can be seen in Fig. 12, diazepam, chlordiazepoxide and flurazepam produced dose-related reversals of the *l*-tryptophan and tranylcypromine-induced hyperthermia, with minimal effective doses being 2.5, 5.0 and 10.0 mg/kg, respectively. CL 218,872 did not alter the hyperthermia with doses as high as 20 mg/kg.

Since CL 218,872 was quite potent in the PTZ and conflict tests, its relative inactivity in tests designed to measure actions of GABA, glycine and 5HT questions the necessity of these transmitters in mediating anxiolytic actions.

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