Dopamine Receptors and Their Behavioral Correlates
Pharmacology Biochemistry & Behavior, Vol. 17, Suppl. 1, pp. 37–41, 1982. Printed in the U.S.A.

Comparative Neuropharmacology of Antianxiety Drugs

STEVEN M. PAUL AND PHIL SKOLNICK

Neuroscience Branch, NIMH and Laboratory of Bioorganic Chemistry, NIADDK
National Institutes of Health, Bethesda, MD 20205

PAUL, S. M. AND P. SKOLNICK. *Comparative neuropharmacology of antianxiety drugs.* PHARMAC. BIOCHEM. BEHAV. 17: Suppl. 1, 37–41, 1982.—Over the past five years, the mechanisms of action of several classes of antianxiety drugs have been clarified. Benzodiazepines, triazolopyridazines, and barbiturates seem to produce their anxiolytic effects by interacting with a specific high affinity receptor (viz. benzodiazepine receptor) in the brain. The benzodiazepine receptor is functionally and perhaps structurally coupled to a receptor for the major inhibitory neurotransmitter GABA as well as a chloride channel or ionophore. Taken together, this receptor "complex" may mediate the behavioral effects of a number of chemically diverse antianxiety agents. Evidence for the role of the benzodiazepine-GABA receptor-chloride ionophore complex in the mechanism of action of minor tranquilizers and a possible interaction with the novel anxiolytic buspirone is discussed.

Buspirone Comparative neuropharmacology Antianxiety drugs

ANXIETY has long been recognized as an intrinsic component of the human condition. Although Freud [44] considered anxiety as having a heritable (i.e. genetic) origin, the strong influence of both existential and psychodynamic concepts of anxiety in contemporary psychiatry have hindered efforts to develop biological hypotheses of the pathogenesis of anxiety. Nonetheless, within the past five years it has been generally recognized that anxiety, like other psychopathological states, may involve a discrete set of neurochemical processes, and that antianxiety agents may exert their therapeutic actions by affecting these processes rather than in a relatively nonspecific fashion (i.e. by a combination of muscle relaxation and sedation cf. [27]).

The discovery of high affinity, saturable, and stereospecific recognition sites (receptors) for benzodiazepines in the mammalian central nervous system (CNS) [23,40] marked a dramatic change in current concepts both of the molecular mechanisms through which antianxiety agents exert their actions and of the existence of discrete neuronal systems (including specific neurotransmitters) mediating anxiety-related behaviors. Furthermore, the discovery of these receptor sites has stimulated the development of new, nonbenzodiazepine anxiolytics which may lack some of the undesirable side effects (e.g. sedation) of many currently used agents. This paper will summarize current concepts of the mechanisms of action of antianxiety agents, with particular emphasis on the molecular membrane "targets" of these compounds.

THE BENZODIAZEPINE RECEPTOR AS PART OF A SUPRAMOLECULAR COMPLEX

Shortly after the discovery of CNS benzodiazepine receptors, it was recognized that the apparent affinity of this receptor could be increased by the major inhibitory neurotransmitter of the mammalian CNS, γ-aminobutyric acid (GABA). Furthermore, this enhancement of apparent receptor affinity (reflected in an increased affinity of benzodiazepines for this site) could be mimicked by the GABAmimetic muscimol, and stereospecifically antagonized by the GABA antagonist, bicuculline [41]. These observations have now been confirmed by numerous laboratories (cf. [42]) and represents the first demonstration on a molecular level of a functional link between recognition sites (receptors) for GABA and benzodiazepines. These findings support earlier electrophysiological and pharmacologic evidence [6,15] of a functional link between benzodiazepines and GABA, although the precise role(s) of GABA in the antianxiety actions of benzodiazepines (and other anxiolytics) is unknown. The apparent affinities of other chemically unrelated compounds which bind to benzodiazepine receptors (*in vitro*) and have anxiolytic actions are also increased in the presence of GABA. This has been demonstrated by direct binding studies with [^{3}H] radioligand (analogous to studies using [^{3}H] benzodiazepines [46], and, indirectly, by using radiolabeled benzodiazepine antagonists [24,37].

A functional link between recognition sites for benzodiazepines, GABA, and the third component of this supramolecular complex, a chloride ionophore, has also been firmly established. Costa *et al.* [7] demonstrated that small permeable anions such as chloride, bromide, and iodide (but not larger, impermeable anions such as maleate and citrate) enhanced the binding of [^{3}H] benzodiazepines (such as diazepam) through an increase in receptor affinity. In addition, several groups have demonstrated that pyrazolopyridines (such as SQ 20009 (etazolate), SQ 65,396 (cartazolate) and ICI 136,753 (tracazolate)) which have antianxiety actions in rodents, also enhanced benzodiazepine receptor affinity, and that this effect was dependent on

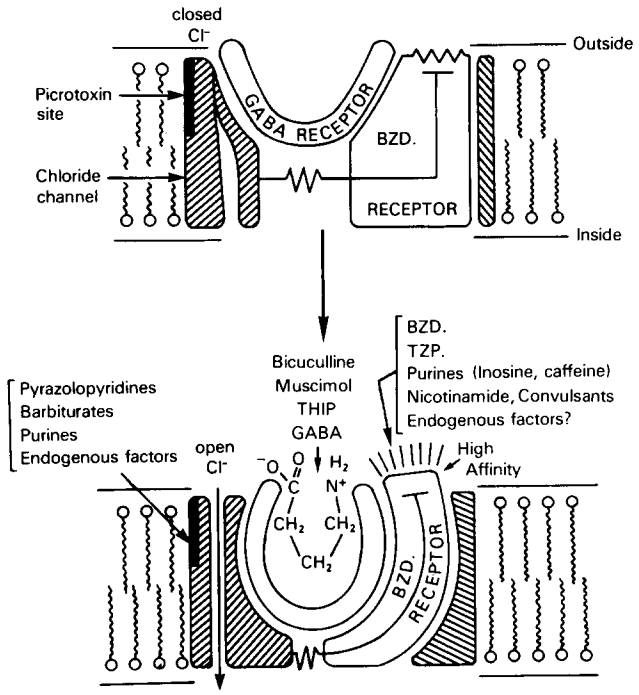

FIG. 1. The benzodiazepine-GABA receptor-chloride ionophore complex [28].

the presence of chloride ion [3, 22, 45]. The observations of Leeb-Lundberg *et al.* [18], demonstrating that the affinity of pyrazolopyridines for [^{3}H] dihydropicrotoxinin binding sites (an analog of picrotoxin that presumably binds to chloride channels) correlates well with the concentrations needed to enhance [^{3}H] benzodiazepine binding, coupled with the chloride dependence of this effect, suggested a functional link of this site to benzodiazepine receptors. Furthermore, it has been reported that pyrazolopyridines also increase [^{3}H] GABA binding [22,30] further strengthening the association between recognition sites for GABA and a chloride ionophore.

Barbiturates such as pentobarbital have been reported to enhance the apparent affinity of benzodiazepines and potentiate the actions of submaximum concentrations of GABA in enhancing benzodiazepine receptor affinity [17, 33, 36]. These actions of pentobarbital are also presumably mediated at a chloride ionophore site, since barbiturates displace [^{3}H] dihydropicrotoxinin from these sites, and the stimulatory action of pentobarbital is blocked by both picrotoxinin and bicuculline (further demonstrating the functional nature of this relationship). These observations have provided the framework of a model consisting of a chloride ionophore which is functionally linked to recognition sites for both GABA and benzodiazepines (Fig. 1).

It is currently hypothesized that many antianxiety drugs exert their pharmacologic actions by directly or indirectly perturbing one or more of the regulatory units of this supramolecular complex, and that the pathophysiologic expression of anxiety may also be mediated through this system. Table 1 summarizes possible molecular "targets" of anxiolytic action which will be discussed in detail in the following sections:

TABLE 1

PERTURBATION OF THE GABA-BENZODIAZEPINE RECEPTOR-CHLORIDE IONOPHORE COMPLEX AS A MECHANISM OF ANXIOLYTIC ACTION

(A) Direct (competitive) occupation of the benzodiazepine receptor
(B) Enhancement of benzodiazepine receptor affinity
  (1) at the GABA recognition site
  (2) at the chloride ionophore
(C) Increasing benzodiazepine receptor number
(D) Altering the turnover (i.e. synthesis, degradation, or release) of an endogenous ligand with benzodiazepine-like properties
(E) Effecting a benzodiazepine receptor linked "second messenger"

Direct Occupation of the Benzodiazepine Receptor

This mechanism of drug action is the most obvious, and probably best documented. Several chemically disparate classes of anxiolytic such as the benzodiazepines (e.g. diazepam), triazolopyridazines (e.g. CL 218,872), [20], quinolines (e.g. PK 9084) [19], and the pyrrolopyrazine, zopiclone [4] have been demonstrated to competitively inhibit the binding of [^{3}H] benzodiazepines at this site with relatively high affinities (K_i ~ 1–1000 nM). Furthermore, there is an excellent correlation between the abilities of a series of benzodiazepines to displace [^{3}H] diazepam from this site and their potencies *in vivo* [40]. We [26] have demonstrated an excellent correlation between the number of benzodiazepine receptors occupied and the anticonvulsant actions of diazepam against pentylenetetrazole-induced seizures while Lippa *et al.* [20] have reported a similar correlation for the anticonflict actions of diazepam. In both studies, the maximum number of receptors occupied for an optimum pharmacologic action did not exceed 30%. These studies were the first indications that benzodiazepines may exert specific behavioral actions through a subpopulation of receptors. Finally, the recent development of compounds such as Ro 15-1788 and CGS-8216 which bind to benzodiazepine receptors with high affinities *in vitro* and antagonize the anxiolytic actions of benzodiazepines *in vivo* (cf. [38]) supports the contention that at least some anxiolytic compounds may exert their effects via the benzodiazepine receptor.

Enhancement of Benzodiazepine Receptor Affinity

The affinity of benzodiazepine receptors has been reported to be enhanced at two loci, a recognition site for GABA (which is qualitatively different from the "classical" $GABA_A$ receptor [12]) and a chloride ionophore. Pyrazolopyridines (such as tracazolate) and barbiturates (such as pentobarbital) which possess anxiolytic action [21,25] may both interact at the latter site. Recently, ethanol has also been reported [9] to enhance receptor affinity; however, the locus of this enhancement is not well defined. Muscimol has recently been reported to have an anxiolytic action in a thirsty rat conflict model, although it must be administered intraventricularly, and the dose range is quite narrow for this action [5]. Nonetheless, this compound could also act through enhancing benzodiazepine receptor affinity at a GABA site. Enhancement of receptor affinity as a mechanism of drug action implies that the binding of an endoge-

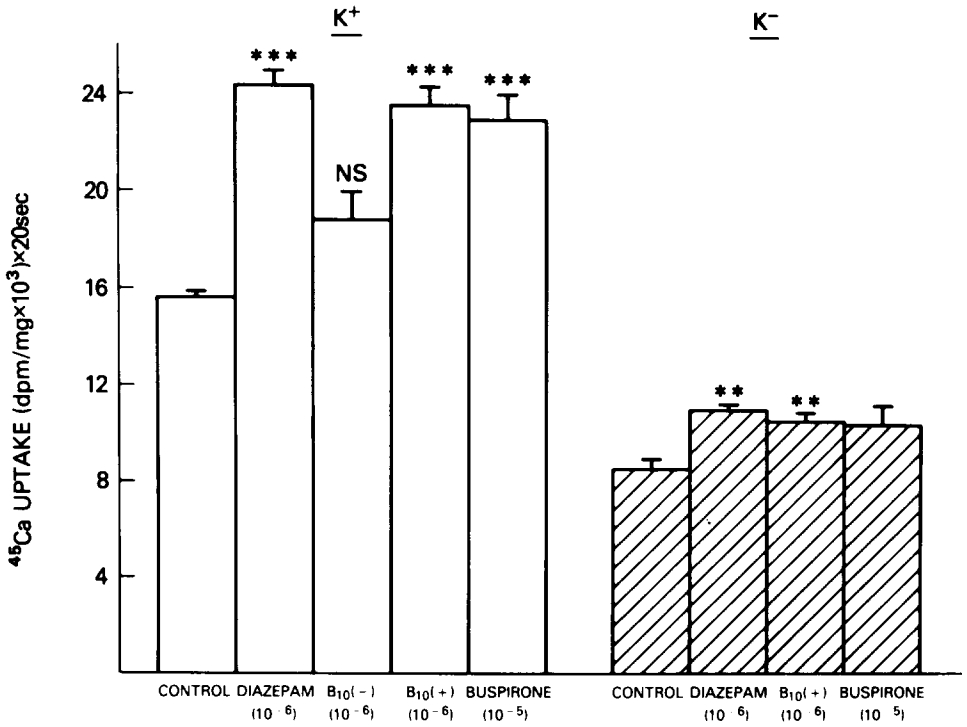

FIG. 2. The effects of diazepam, (+) B_{10}, (−) B_{10}, and buspirone on $^{45}Ca^{++}$ uptake in synaptosomes. Synaptosomes were prepared from rat forebrain (whole brain less cerebellum and brain stem) by the method of Gray and Whittaker using discontinuous sucrose density gradients. Synaptosomes were then washed twice in isotonic medium at 0–4°. Synaptosomal pellets were suspended in calcium-free medium containing either KCl (25 mM) (open bars) or an equivalent concentration of NaCl (hatched bars). The effects of various drugs on $^{45}Ca^{++}$ uptake were examined by adding the agents immediately prior to the equilibration period. Synaptosomes were preincubated (equilibrated) for 30 min at 37° prior to the addition of $^{45}Ca^{++}$. Uptake studies were initiated by addition of 0.8 ml of synaptosomes to 0.2 ml of 1 mM $CaCl_2$ and 0.6–1.2 μCi $^{45}CaCl_2$ (39 mCi/mg) in buffer. Uptake studies were terminated after 20 seconds by rapid filtration under vacuum through GF/C filters that were washed three times with 5 ml of a solution containing 145 mM KCl, 1.2 mM $CaCl_2$, and 1.4 mM $MgCl_2$. The final protein concentration in the incubations was 1–2 mg/ml. The data depicted are from a typical experiment. Symbols: ***$p<0.001$; **$p<0.01$ compared with addition of vehicle.

nous ligand with benzodiazepine-like properties may be involved in this action. At least one endogenous compound, although not fully characterized or identified, has been isolated from rat brain and plasma extracts and possess anticonflict activity in rats [8].

Increasing Benzodiazepine Receptor Number

Several compounds that have anxiolytic properties have been demonstrated to increase benzodiazepine receptor number after *in vivo* administration. Methaqualone, which competitively inhibits [^{3}H] diazepam binding with a relatively low affinity *in vitro* (IC_{50} ~ 220 μM [1] has also been reported to increase receptor number *ex vivo* when administered at a dose of 100 μmol/kg. The increases in receptor number were reported to be in the range of 20–30% [16]. Similarly, PK 9084 and PK 8165, which bind to benzodiazepine receptors with moderate affinity (IC_{50} ~ 500 nM), [19] increase benzodiazepine receptor number when administered *in vivo* (G. LeFur, personal communication). EMD 28422 (an N^6-substituted adenosine derivative) is active in the thirsty rat conflict test [34] and increases benzodiazepine receptor number both *in vivo* and *in vitro* [35]. The increases in receptor number observed following *in vivo* administration of these compounds usually occurs within 15–30 min of administration, and the increase in receptor number is usually no more than 30% [34]. The mechanism by which these compounds elicit the increases in receptor number is unknown, but the rapid time course for this increase and the observation that EMD 28422 will increase receptor number *in vitro* [35] suggests that new protein (viz. receptor) synthesis is not responsible for this phenomenon. An "unmasking" of receptor by removal of an endogenous (noncompetitive) inhibitor of benzodiazepine receptors or an increase in the affinity of low affinity of receptors to a higher affinity form are possible explanations for this phenomenon. Other reports have shown that under appropriate conditions both pyrazolopyridines and barbiturates elicit an increase in GABA receptor number *in vitro* [2,30], suggesting that other components of this supramolecular complex share a similar type of regulatory mechanism. The increases in benzodiazepine receptor number, observed with at least three chemically unrelated compounds having anxiolytic actions, suggest that this could be an important molecular mechanism for exerting a pharmacologic action.

Altering the Synthesis, Release, Reuptake or Degradation of an Endogenous Ligand

Both the existence and nature of an endogenous ligand(s) for the benzodiazepine receptor remains an area of active investigation. Nonetheless, by analogy with "classical" neurotransmitter systems, the potential exists for compounds to exert an antianxiety action by altering the synthesis, storage, or release of such a compound.

Within the last two years, at least two groups have reported the presence of ligand candidates which have peptide-like qualities. One of these candidates had benzodiazepine-like actions in a thirsty rat conflict test [8], while the other substance antagonized the actions of diazepam in the same paradigm [14]. Following the definitive identification of an endogenous ligand(s) of the benzodiazepine receptor, it should be possible to design agents which exert pharmacologic actions by altering the disposition of this ligand(s).

Effecting a Benzodiazepine Receptor-linked "Second Messenger"

Although the exact mechanism(s) by which benzodiazepine receptors transduce a biological signal to neurons is still unclear, several lines of evidence suggest the involvement of Ca^{++} linked events. Studies in our laboratories [29] have demonstrated a significant enhancement by benzodiazepines of the K^+-depolarized uptake of $^{45}Ca^{++}$ into synaptosomes. This effect was stereospecific, occurred at pharmacologically relevant concentrations of benzodiazepines, and was antagonized by CGS 8216, a potent benzodiazepine receptor antagonist. In preliminary experiments (Fig. 2) we have observed a similar enhancement of presynaptic K^+-stimulated Ca^{++} uptake by buspirone. Furthermore, this effect was not observed with the neuroleptics haloperidol or chlorpromazine. Although further studies will be necessary to confirm the role of presynaptic Ca^{++} mobilization in the mechanisms of action of anxiolytics, these results support the notion that buspirone may act "downstream" from the benzodiazepine recognition site. Other benzodiazepine-sensitive Ca^{++} mediated events such as calmodulin-dependent protein phosphorylation [10] may also be candidates for buspirone's site of action.

CONCLUSION

Experimental evidence accumulated during the past five years has resulted in the formulation of a molecular model which is consistent with the neurochemical actions of many antianxiety compounds. This model, however, cannot account for the actions of all clinically effective antianxiety agents. For example, phenobarbital, long used as an anxiolytic (cf. [21]), does not appear to enhance benzodiazepine receptor affinity, and will antagonize the actions of pentobarbital to increase receptor affinity [39]. This observation suggests that phenobarbital may act at the same locus as pentibarbital, but fails to transduce an identical signal. Furthermore, the potency of a series of barbiturates to enhance benzodiazepine receptor affinity *in vitro* appears best correlated with the anesthetic actions of these compounds [17], although there are a number of exceptions (cf. [39]). Meprobamate and related compounds inhibit [^{3}H] diazepam binding with IC_{50} values of approximately 800 μM (i.e. weakly active) [28]. Nonetheless, brain levels of ^{14}C meprobamate following pharmacologically relevant doses in rats (100 mg/kg) have been estimated to be 230 μmol/kg [11], sufficient to occupy 10–30% of benzodiazepine receptors. It has been previously demonstrated that only a small percentage of receptors need be occupied to fully manifest either an antianxiety or anticonvulsant action of diazepam [20,26]. However, *in toto,* the evidence that meprobamate exerts a pharmacologic action through the benzodiazepine receptor complex is far from overwhelming.

Finally, a novel series of dibasic substituted azaspirodecadiones, represented by buspirone (Buspar™), are anxioselective in experimental animals and man [13,32], but do not appear to act through this complex [31]. Recent results from our laboratories have shown that buspirone has benzodiazepinelike effects on presynaptic Ca^{++} uptake. It is possible that this action may account for the anxiolytic properties of buspirone. Buspirone appears to have a relatively high affinity for central dopamine receptors [31] and it has been postulated that buspirone may exert its anxioselective action through dopamine receptors. Nonetheless, a small modification of this molecule eliminates central dopamine binding, yet retains an anxiolytic profile [43]. This observation, coupled with other data suggesting that active metabolite(s) may be present, suggests that the antianxiety actions of buspirone and related compounds may not be due to an action at central dopaminergic receptors.

The somatic and psychological manifestations of anxiety are complex and varied. Therefore, it may be a gross oversimplification to attribute the anxiolytic action of all agents to a single system. Most certainly, an anxiolytic action could result either from an action distal to this system (e.g. the benzodiazepine-GABA receptor chloride ionophore complex) or from an action on an entirely unrelated system(s). Further electrophysiologic, neurochemical, and behavioral studies will be needed to better define the molecular "targets" of such new and interesting antianxiety agents as buspirone, and to refine current neurochemical models of anxiolytic action.

REFERENCES

1. Antonidas, A., W. Muller and U. Wollert. Central nervous system stimulating and depressing drugs as possible ligands of the benzodiazepine receptor. *Neuropharmacology* **19:** 121–124, 1980.
2. Asano, T. and N. Ogasawara. Stimulation of GABA receptor binding by barbiturates. *Eur. J. Pharmac.* **77:** 355–357, 1982.
3. Beer, B., C. Klepner, A. Lippa and R. Squires. Enhancement of [^{3}H]diazepam binding by SQ 65,396: A novel anti-anxiety agent. *Pharmac. Biochem. Behav.* **9:** 849–851, 1978.
4. Blanchard, J., A. Boireau, C. Carret and L. Julou. *In vitro* and *in vivo* inhibition by zopiclone of benzodiazepine binding to rodent brain receptors. *Life Sci.* **24:** 2417–2420, 1979.
5. Cananzi, A., E. Costa and A. Guidotti. Potentiation by intraventricular muscimol of the anticonflict effect of benzodiazepines. *Brain Res.* **196:** 447–453, 1980.

6. Costa, E., A. Guidotti and C. Mao. Evidence for the involvement of GABA in the action of benzodiazepines: Studies in rat cerebellum. In: *Advances in Biochemical Psychopharmacology*, edited by E. Costa and P. Greengard. New York: Raven Press, 1975, pp. 113–130.
7. Costa, T., D. Rodbard and C. Pert. Is the benzodiazepine receptor coupled to a chloride ion channel? *Nature* **277:** 315–317, 1979.
8. Davis, L. Is a peptide the natural ligand for the benzodiazepine receptor? In: *Pharmacology of Benzodiazepines*, edited by S. Paul, P. Skolnick, J. Tallman and E. Usdin. London: MacMillan Publishing Co., in press.
9. Davis, W. and M. Ticku. Ethanol enhances [^{3}H] diazepam binding at the benzodiazepine-GABA receptor-ionophore complex. *Molec. Pharmacol.* **20:** 287–294, 1981.
10. DeLorenzo, R., S. Burdette and J. Holdernes. Benzodiazepine inhibition of the calcium-calmodulin protein kinase system in brain membranes. *Science* **213:** 546–549, 1981.
11. Emmersen, J., T. Miya and G. Yim. The distribution and metabolic state of ^{14}C meprobamate in rat brain. *J. Pharmac. exp. Ther.* **129:** 89–97, 1960.
12. Enna, S. and T. Andree. GABA receptor heterogeneity: Relationship to benzodiazepines. In: *Pharmacology of Benzodiazepines*, edited by S. Paul, P. Skolnick, J. Tallman and E. Usdin. London: MacMillan Publishing Co., 1982, in press.
13. Geller, I. and R. Hartmann. Effects of buspirone on operant behavior of laboratory rats and cynomolgus monkeys. *J. clin. Psychiat.*, in press, 1982.
14. Guidotti, A., C. Forchetti, B. Ebstein and E. Costa. Purification and characterization of an endogenous peptide putative effector for the benzodiazepine recognition site. In: *Pharmacology of Benzodiazepines*, edited by S. Paul, P. Skolnick, J. Tallman and E. Usdin. London: MacMillan Publishing Co., 1982, in press.
15. Haefely, W., R. Kulcsar, H. Mohler, L. Pieri, P. Polc and R. Schaffner. Possible involvement of GABA in the central actions of benzodiazepines. *Adv. Biochem. Psychopharmac.* **14:** 131–151, 1975.
16. Koe, K. Facilitation of benzodiazepine receptor binding: A mechanism of action for methaqualone? *Soc. Neurosci. Abstr.* **7:** Abstr. No. 281.2, 1981.
17. Leeb-Lundberg, F., A. Snowman and R. Olsen. Barbiturate receptor sites are coupled to benzodiazepine receptors. *Proc. natn Acad. Sci. U.S.A.* **77:** 7468–7472, 1980.
18. Leeb-Lundberg, F., A. Snowman and R. Olsen. Perturbation of benzodiazepine receptor binding by pyrazolopyridines involves picrotoxin/barbiturate receptor sites. *J. Neurosci.* **1:** 471–477, 1981.
19. LeFur, G., J. Mizoule, M. Burgevin, O. Ferris, M. Heaulme, A. Gautheir, C. Gueremy and A. Uzan. Multiple benzodiazepine receptors: Evidence of a dissociation between anticonflict and anticonvulsant properties of PK 8165 and PK 9084 (two quinoline derivatives). *Life Sci.* **28:** 1439–1448, 1981.
20. Lippa, A., D. Critchett, M. Sano, C. Klepner, E. Greenblatt, J. Coupet and B. Beer. Benzodiazepine receptors: Cellular and behavioral characteristics. *Pharmac. Biochem. Behav.* **10:** 831–843, 1979.
21. Lippa, A., P. Nash and E. Greenblatt. Preclinical neuropsychopharmacological testing procedures for anxiolytic drugs. In: *Anxiolytics*, edited by S. Fielding and H. Lal. Mt. Kisco, NY: Futura Publishing Co., 1979, pp. 41–81.
22. Meiners, B. and A. Salama. Enhancement of benzodiazepine and GABA binding by the novel anxiolytic, tracazolate. *Eur. J. Pharmac.* **78:** 315–322, 1982.
23. Mohler, H. and T. Okada. Benzodiazepine receptor: Demonstration in the central nervous system. *Science* **209:** 507–509, 1980.
24. Mohler, H. and J. Richards. Agonist and antagonist benzodiazepine receptor interaction *in vitro*. *Nature* **294:** 763–765, 1981.
25. Patel, J. and J. Malick. Pharmacological properties of tracazolate: A new non-benzodiazepine anxiolytic agent. *Eur. J. Pharmac.* **78:** 323–333, 1982.
26. Paul, S., P. Syapin, B. Paugh, V. Moncada and P. Skolnick. CNS benzodiazepine receptors: Correlation between receptor occupation and anticonvulsant effects of diazepam. *Nature* **281:** 588–589, 1979.
27. Paul, S. and P. Skolnick. Benzodiazepine receptors and psychopathological states: Towards a neurobiology of anxiety. In: *Anxiety: New Research and Changing Concepts*, edited by D. Klein and J. Rabkin. New York: Raven Press, 1981, pp. 215–230.
28. Paul, S., P. Marangos and P. Skolnick. The benzodiazepine-GABA-chloride-ionophore receptor complex: Common site of minor tranquilizer action. *Biol. Psychiat.* **16:** 213–229, 1981.
29. Paul, S., M. D. Luu and P. Skolnick. The effects of benzodiazepines on presynaptic calcium transport. In: *Pharmacology of Benzodiazepines*, edited by S. Paul, P. Skolnick, J. Tallman and E. Usdin. London: MacMillan Publishing Co., 1982, in press.
30. Placheta, P. and M. Karobath. *In vitro* modulation by SQ 20,009 and SQ 65,396 of GABA receptor binding in rat CNS membranes. *Eur. J. Pharmac.* **62:** 225–228, 1980.
31. Riblet, L., D. Taylor, M. Eison and H. Stanton. Buspirone: Pharmacology and neurochemistry. *J. clin. Psychiat.*, in press, 1982.
32. Rickels, K. Buspirone, diazepam and placebo in anxious outpatients: A controlled study. *J. clin. Psychiat.*, in press, 1982.
33. Skolnick, P., S. Paul and J. Barker. Pentobarbital potentiates GABA-enhanced [^{3}H] diazepam binding to benzodiazepine receptors. *Eur. J. Pharmac.* **65:** 125–127, 1980.
34. Skolnick, P., K. L. Lock, B. Paugh, P. Marangos, R. Windsor and S. Paul. Pharmacologic and behavioral effects of EMD 28422: A novel purine which enhances [^{3}H] diazepam binding to brain benzodiazepine receptors. *Pharmac. Biochem. Behav.* **12:** 685–689, 1980.
35. Skolnick, P., K. L. Lock, S. Paul, P. Marangos, R. Jonas and K. Irmscher. Increased benzodiazepine receptor number elicited *in vitro* by a novel purine, EMD 28422. *Eur. J. Pharmac.* **67:** 179–186, 1980.
36. Skolnick, P., V. Moncada, J. Barker and S. Paul. Pentobarbital has dual actions to increase brain benzodiazepine receptor affinity. *Science* **211:** 1448–1450, 1981.
37. Skolnick, P., M. Schweri, E. Williams, V. Moncada and S. Paul. An *in vitro* test which differentiates benzodiazepine "agonists" and "antagonists". *Eur. J. Pharmac.* **78:** 133–136, 1982.
38. Skolnick, P, D. Hommer and S. Paul. Benzodiazepine antagonists. In: *Pharmacology of Benzodiazepines*, edited by S. Paul, P. Skolnick, J. Tallman and E. Usdin. London: MacMillan Publishing Co., 1982, in press.
39. Skolnick, P., K. Rice, J. Barker and S. Paul. Interaction of barbiturates with benzodiazepine receptors in the central nervous system. *Brain Res.* **233:** 143–156, 1982.
40. Squires, R. and C. Braestrup. Benzodiazepine receptors in rat brain. *Nature* **266:** 732–734, 1977.
41. Tallman, J., J. Thomas and D. Gallager. GABAergic modulation of benzodiazepine binding site sensitivity. *Nature* **274:** 383–385, 1978.
42. Tallman, J., S. Paul, P. Skolnick and D. Gallager. Receptors for the age of anxiety: Molecular pharmacology of the benzodiazepines. *Science* **207:** 274–281, 1980.
43. Temple, D., J. Yevich and J. New. Buspirone: Chemical profile of a new class of anxioselective agent. *J. clin. Psychiat.*, in press, 1982.
44. Villeneuve, A. Differential psychopharmacology of anxiolytics and sedatives. *Mod. Probl. Pharmacopsychiat.* **14:** 1–12, 1979.
45. Williams, M. and E. Risley. Enhancement of the binding of [^{3}H] diazepam to rat brain membranes *in vitro* by SQ 20,0009, a novel anxiolytic, GABA, and muscimol. *Life Sci.* **24:** 833–842, 1979.
46. Yamamura, H., T. Mimaki, S. Yamamura, W. Horst, M. Morelli, G. Bautz and R. O'Brien. [^{3}H] CL 281,872, a novel triazolopyridazine which labels the benzodiazepine receptor in rat brain. *Eur. J. Pharmac.* **77:** 351–354, 1982.