Naunyn-Schmiedeberg's Arch Pharmacol (1987) 335:588–592

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Short communication

Piperazine derivatives including the putative anxiolytic drugs, buspirone and ipsapirone, are agonists at 5-HT_{1A} receptors negatively coupled with adenylate cyclase in hippocampal neurons*

Joël Bockaert, Aline Dumuis, Rochdi Bouhelal, Michèle Sebben, and Robert N. Cory

Centre CNRS, INSERM de Pharmacologie-Endocrinologie, Rue de la Cardonille, F-34094 Montpellier Cedex, France

Summary. Two putative anxiolytic drugs [ipsapirone (TVXQ 7821) and buspirone], structurally unrelated to benzodiazepines, have negligible ataxic and sedative side effects. These drugs are piperazine analogs which interact at 5-HT_1 binding sites. It is demonstrated here that these drugs and two other piperazine derivatives, trifluoromethylphenylpiperazine (TFMPP) and m-chlorophenylpiperazine (mCPP), are agonists at 5-HT_{1A} receptors, a subclass of the 5-HT_1 receptor, mediating inhibition of forskolin (100 μM) stimulated adenylate cyclase in particulate fractions of guinea pig hippocampus as well as inhibition of the formation of cyclic AMP promoted by vasoactive intestinal polypeptide (0.1 μM) plus forskolin (1 μM) in mouse hippocampal neurons in primary culture. This study demonstrates that these piperazine based drugs act in both brain homogenate preparations and in intact neurons in a similar manner. The biochemical models described here may aid in the development of even more active drugs in this class.

Key words: Anxiolytics – Serotonin – 5-HT_{1A} receptors – Piperazine-derived anxiolytic drugs – Hippocampus

Introduction

Two long chain substituted piperazines, buspirone (Goldberg and Finnerty 1979) and ipsapirone (TVXQ 7821) (Traber et al. 1984), are able to reduce anxiety related behaviour in humans (Goldberg and Finnerty 1979) and rodents (Traber et al. 1984) without producing the sedative and ataxic effects of the classic anxiolytics, the benzodiazepines. It was recently shown that these piperazines displace (^{3}H)-5-HT (Traber et al. 1984; Glaser and Traber 1985) and (^{3}H)-8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)-tetralin; Gozlan et al. 1983] from their respective binding sites in mammalian brain, suggesting an interaction at 5-HT receptors. At least two classes of 5-HT receptors, 5-HT_1 and 5-HT_2 (Peroutka and Snyder 1979), exist in brain. The former possesses a higher affinity for agonists than for antagonists, whereas for the latter, the reverse is usually true. 5-HT_1 receptors can thus be labelled with (^{3}H)-5-HT (Gozlan et al. 1983; Peroutka and Snyder 1979) whereas 5-HT_2 receptors are labelled with antagonists such as (^{3}H)-ketanserin (Leysen et al. 1982). Heterogeneity within the 5-HT_1 subtype was proposed based on the finding that spiperone inhibits (^{3}H)-5-HT in a biphasic manner (Pedigo et al. 1981). Binding sites displaying high and low affinity for spiperone were designated as 5-HT_{1A} and 5-HT_{1B} respectively. (^{3}H)-8-OH-DPAT was shown to specifically label the 5-HT_{1A} subtype (Gozlan et al. 1983). An additional subtype (5-HT_{1C}) has been proposed to account for the labelling by mesulergine, 5-HT and LSD of a site with low affinity for ligands reported as selective for 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 binding sites (Cortes et al. 1984; Pazos et al. 1985). Several observations indicate that short or long chain substituted piperazines interact with 5-HT_1 binding sites. Studies showing the activity of trifluoromethylphenylpiperazine (TFMPP) and m-chlorophenylpiperazine (mCPP) in behavioural experiments imply that there is a functional role for these 5-HT_1 sites (Cunningham and Appel 1986). Binding data also indicate a possible site of action for buspirone and ipsapirone at 5-HT_1 receptors (Traber et al. 1984; Glaser and Traber 1985). A recent study by De Vivo and Maayani (1986) showed that buspirone causes inhibition of adenylate cyclase via a 5-HT_{1A} receptor in hippocampal membranes prepared from rat and guinea pig. In this study we confirm this observation for buspirone and show that this is a general property of a class of piperazine analogs. To characterize the agonist properties of these compounds at brain 5-HT_1 receptors, we studied their action in two recently described models in which biochemical responses to 5-HT_1 receptors can be measured. One was described by us and consists of measuring the inhibition of cyclic AMP production in primary cultures of neurons by a 5-HT_1 receptor (Weiss et al. 1986a), the other was described by De Vivo and Maayani (1985) and measures the inhibition of adenylate cyclase activity in hippocampal membranes by a 5-HT_{1A} receptor.

Methods

Adenylate cyclase assay. Male guinea pigs were killed by decapitation. The hippocampi were removed and homogenized in 10 ml of a solution containing 300 mM sucrose, 20 mM Tris-HCl pH 7.4, 1 mM ethylene glycol bis (beta-aminoethylether),-N,N-tetraacetic acid (EGTA), 5 mM EDTA, 5 mM dithiothreitol. The homogenate was diluted in 50 ml of the same medium and centrifuged at 40,000 × *g*

* This work was supported by grants from the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé et de la Recherche Médicale (INSERM), the Direction des Recherches Etudes et Techniques (DRET, N°85/188) of the French Defense Ministry and by the Direction and Ministère de la Recherche Scientifique

Send offprint requests to J. Bockaert at the above address

for 10 min at 0° C. The pellet from this centrifugation was resuspended in 4 ml of the medium. To start the assay, aliquots of the resuspended medium (40 µl) were added to 100 µl of assay medium. The final composition of the medium was: 80 mM Tris HCl, 0.1 mM ATP, 2 mM $MgCl_2$, 10 µM GTP, 1 mM cyclic AMP, 5 mM creatine phosphate, 0.2 mg/ml creatine-kinase, 100 mM NaCl, 2 mM theopylline, 0.25 mM ascorbic acid, 100 µM forskolin, 1–2 µCi of alpha-^{32}P-ATP (20–30 Ci/mmole; DuPont NEN, France) and $1-2\times10^{-2}$ µCi of (^{3}H)-cyclic AMP (29 Ci/mmole; Amersham, UK). (^{3}H)-cyclic AMP was added for determination of cyclic AMP recovery during the purification procedure. The incubations were for 5 min at 30° C and stopped by addition of 900 µl of 5.5 mM Tris-HCl (pH 7.6), 0.4 mM ATP, 0.6 mM cyclic AMP, 10 mM $CaCl_2$, and 0.1 N HCl. The tubes were centrifuged at 5000 × *g* for 5 min. (^{32}P)-cyclic AMP formed and (^{3}H)-cyclic AMP were isolated according to Salomon et al. (1974).

Culture of hippocampal neurons. Cultures of mouse hippocampal neurons were prepared according to the technique of Weiss et al. (1986b) originally described for the culture of striatal neurons. Briefly, hippocampi were removed from 16–17-day-old Swiss albino mouse embryos (Iffa Credo; Lyon, France) and mechanically dissociated with a fire-narrowed Pasteur pipette in serum-free medium. Cells were plated in 12-well Costar (Cambridge, MA, USA) culture dishes previously coated with poly(L-ornithine). Cells were seeded in serum-free medium composed of a 1:1 mixture of Dulbecco's modified Eagle's medium and F-12 nutrient (GIBCO Europe; Paris, France). In the place of serum, a defined hormone and salt mixture that included insulin (25 µg/ml), transferrin (100 µg/ml), progesterone (20 nM), putrescine (60 µM), and selenium salt (30 nM) (all from Sigma) were added. These cultures of mouse hippocampal neurons devoid of non-neuronal cell types were grown for 6 days.

Formation of cyclic AMP. Intracellular cyclic AMP formation was determined by measuring the conversion of (^{3}H)-ATP (formed in the neurons incubated with 2 µCi/ml (^{3}H)-adenine (24 Ci/mmole; Amersham, UK) to (^{3}H)-cyclic AMP. On the sixth day in culture, neurons were washed and incubated at 37° C (5% CO_2-air mixture) with culture media containing 2 µCi/ml (^{3}H)-adenine. After 2–3 h, the cultures were washed and incubated with 0.75–1.0 mM isobutylmethylxanthine and test agents (all prepared in culture medium) in a volume of 1 ml for 5 min at 37° C. The reaction was terminated by aspiration of the media and addition of 1 ml ice-cold 5% trichloroacetic acid. Cells were scraped with the aid of a rubber stick and to the mixture was added 100 µl of cold 5 mM (ATP + cyclic AMP). Cellular protein was centrifuged at 5000 × *g*. (^{3}H)-ATP and (^{3}H)-cyclic AMP were separated by sequential chromatography on Dowex and alumina columns as described by Salomon et al. (1974). Cyclic AMP formation is expressed as percent conversion of (^{3}H)-ATP to (^{3}H)-cyclic AMP.

The IC_{50} values for the agonists and antagonists effects are reported as the mean ± SEM for (*n*) experiments.

Drugs. The following drugs were generously donated: buspirone (F. D. Yocca of Bristol-Myers Company), ipsapirone (TVXQ 7821) (J. Traber, Troponwerke GmbH and Co., Cologne, FRG), 5-carboxamidotryptamine (5-CT) (P. P. A. Humphrey, Glaxo Group Research, Hertfordshire, UK). Drugs purchased were 5-HT (Sigma Chemical Corp., St. Louis, MO, USA); TFMPP (Aldrich Chemical Corp., Milwaukee, WI, USA); mCPP (EGA Chemie, Steinheim, GDR); and 8-OH-DPAT (Research Biochemical Inc., Wayland, MA, USA).

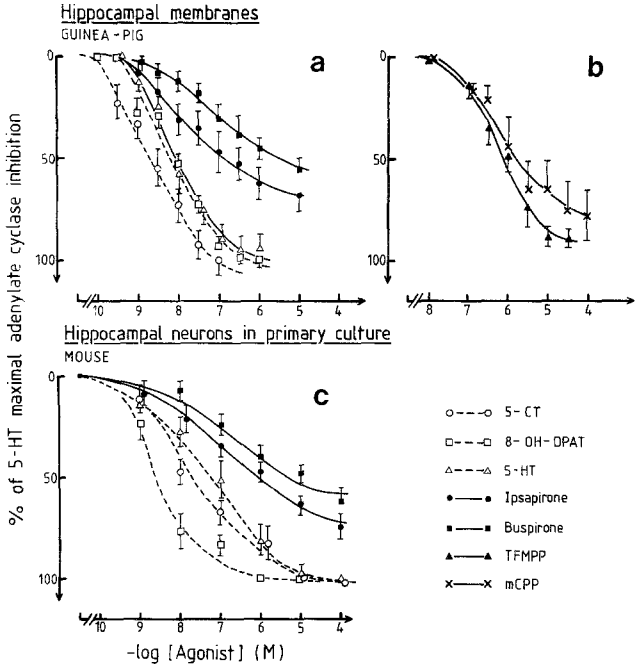


Fig. 1. Agonist mediated inhibition of forskolin-stimulated adenylate cyclase in hippocampal membranes and intact neurons. **a** Effect of 5-HT, 8-OH-DPAT, 5-CT, ipsapirone and buspirone on forskolin (100 µM) stimulated adenylate cyclase activity in guinea pig hippocampal membranes. The inhibition of adenylate cyclase activity is expressed as a percentage of the maximal inhibitory effect of 5-HT (23.5% ± 7.3% inhibition, $n = 10$). In the absence and in the presence of 5-HT (1 µM) adenylate cyclase activities were 1.2 ± 0.25 and 0.92 ± 0.18 nmoles cyclic AMP/5 min/mg protein, respectively. **b** Effects of the piperazines, TFMPP and mCPP on forskolin (100 µM) stimulated adenylate cyclase activity in guinea pig hippocampal membranes. **c** Effects of 5-HT, 8-OH-DPAT, 5-CT, ipsapirone and buspirone on VIP + forskolin stimulated cAMP formation in murine hippocampal neurons in primary culture. Neurons were incubated in the presence of 0.1 µM VIP and 1 µM forskolin and increased concentrations of agonists. Conversion of (^{3}H)-ATP to (^{3}H)-cyclic AMP was determined after 5 min at 37° C (see Methods). In the absence of 5-HT, the percent conversion was 4.0% ± 0.1% ($n = 10$) whereas in the presence of 10^{-5} M 5-HT, the percent conversion was 3.0% ± 0.1% ($n = 10$). The inhibition of cyclic AMP formation is expressed as a percentage of the maximal inhibitory effect of 5-HT. In Fig. 1, the values are the means ± SEM of 3–10 experiments (see Fig. 3 for the value of *n* for each agonist), each performed with triplicate determinations

Results

In hippocampal membranes, 5-HT as well as 8-OH-DPAT, a 5-HT_{1A} selective compound, were inhibitors of forskolin-stimulated adenylate cyclase activity (Figs. 1a, 3). The most potent among these full agonists was 5-carboxamidotryptamine (5-CT) which in binding studies shows a higher affinity than 5-HT for 5-HT_1 sites (Engel et al. 1983). As shown in Fig. 1a the piperazine related compounds with

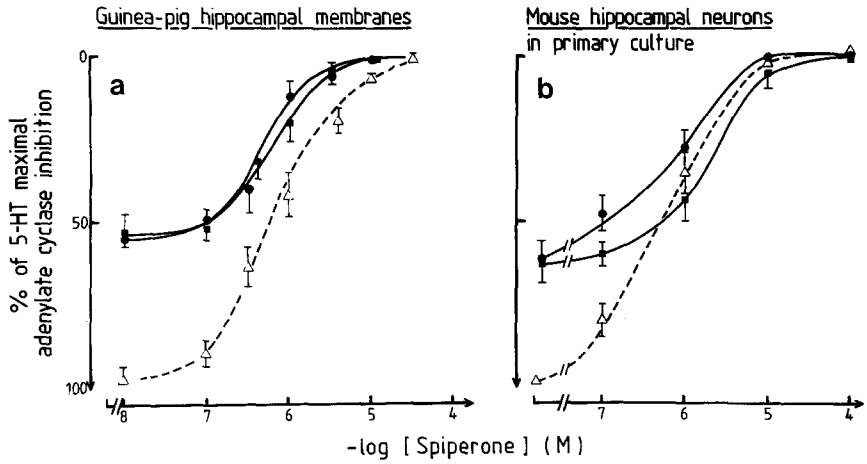


Fig. 2. Reversal of the effects of 5-HT, ipsapirone and buspirone by the antagonist spiperone. **a** Reversal by spiperone of the effects of 5-HT (1 μM) (△——△), ipsapirone (1 μM) (●——●), and buspirone (10 μM) (■——■). The reversal of the agonists' effect by spiperone is expressed as the diminution of their ability to inhibit 100 μM forskolin- stimulated adenylate cyclase activity [relative to the inhibitory action of 1 μM 5-HT (100%)]. The data show cumulative results from three experiments, each performed with triplicate determinations. **a** Reversal by spiperone of the effects of 5-HT (1 μM), ipsapirone (10 μM), and buspirone (10 μM) (same symbols as in Fig. 2a). The reversal of the agonists' effect by spiperone is expressed as the diminution of their ability to inhibit 0.1 μM VIP + 1 μM forskolin-stimulated adenylate cyclase activity [relative to the inhibitory action of 1 μM 5-HT (100%)]. The data shows the cumulative results from three experiments, each performed with triplicate determinations

anxiolytic properties (ipsapirone and buspirone) potently inhibited the adenylate cyclase in guinea pig hippocampal membranes. However, the maximal inhibitions produced by ipsapirone and buspirone were lower than those produced by 5-HT, 5-CT and 8-OH-DPAT (Figs. 1a, 3). Other piperazine related 5-HT compounds such as TFMPP and mCPP were less potent partial agonists (Figs. 1b, 3). Nevertheless, TFMPP produced a maximal response similar to that of 5-HT (intrinsic activity = 0.87) in this system. The inhibitory effects of a maximally stimulating concentration of 5-CT (1 μM), 8-OH-DPAT (1 μM) and the piperazine TFMPP (10 μM) were not additive (in four experiments) with those of 5-HT suggesting that these agonists and 5-HT act through the same population of receptors. We observed, as did De Vivo and Maayani (1986), that spiperone is a potent antagonist of the 5-HT_{1A} receptor in guinea pig hippocampus. The adenylate cyclase inhibition produced by 1 μM 5-HT was entirely reversed by spiperone (Fig. 2a; the IC_{50} of the antagonist effect was 610 nM ± 200 nM, $n = 3$). From these data the apparent affinity constant of spiperone at this neuronal receptor was estimated, using the Cheng and Prusoff (1973) equation, to be 9.6 nM ± 3.2 nM ($n = 3$). Similarly, the apparent affinity constants of spiperone determined from its antagonism of the ipsapirone and buspirone effects (Fig. 2a) were 25.8 nM + 15 nM and 12 nM ± 10.2 nM ($n = 3$), respectively.

In order to verify that the piperazines also interact with the 5-HT receptor in functional intact neurons, we studied their effects in a primary culture of hippocampal neurons. In this system the maximal stimulation of adenylate cyclase was not produced by high concentrations of forskolin but rather with a mixture of vasoactive intestinal polypeptide (VIP) (0.1 μM) and forskolin (1 μM). We have previously shown that when cyclic AMP is stimulated by VIP (0.1 μM) plus forskolin (1 μM), no additional receptor-mediated stimulation can be obtained. Thus, under these conditions, in which the stimulatory components of adenylate cyclase are maximally activated, the agonists dopamine (Weiss et al. 1985) and 5-HT (Weiss et al. 1986 a) can produce only an inhibition of adenylate cyclase activity. As seen in Figs. 1c and 3, the rank order of agonist IC_{50} values for the 5-HT receptors which inhibit cyclic AMP production in the neurons (8-OH-DPAT > 5-CT > 5-HT > ipsapirone > buspirone) was similar to that observed for the same agonists acting at the 5-HT_{1A} receptors that inhibit adenylate cyclase in adult guinea pig hippocampus membranes (5-CT ≥ 8-OH-DPAT > 5-HT > ipsapirone > buspirone). In particular, in mouse neurons, 8-OH-DPAT was a full agonist with a higher potency than 5-HT; and 5-CT was slightly less active than 8-OH-DPAT but more active than 5-HT. Additionally, both buspirone and ipsapirone were partial agonists at this receptor. The effects of the two drugs were not additive nor were they additive with the effects of 10 μM 5-HT ($n = 3$). However, a small reduction (10%) of the maximal response to 5-HT was observed in the presence of either piperazine. In mouse hippocampal neurons, the response to 1 μM 5-HT was entirely reversed by spiperone (Fig. 2b, the IC_{50} of the antagonist effect was 457 nM ± 220 nM, $n = 3$). From these data, the apparent affinity constant of spiperone at this neuronal receptor was estimated to be 22.6 nM ± 10.8 nM, a value similar to that observed in hippocampal membranes. The apparent affinity constants of spiperone determined from its antagonism of the ipsapirone and buspirone effects (Fig. 2b) were 6.9 nM ± 4.5 nM and 25 nM ± 12 nM ($n = 3$), respectively.

Discussion

A commonly accepted hypothesis for the mechanism of action of the benzodiazepine anxiolytics is that they facilitate GABA mediated neuronal inhibition (Stein et al. 1977). GABA receptors are widely distributed in the brain. This may explain the diffuse behavioral side effects caused by these drugs. However, the anxiolytic properties of benzodiazepines are more likely due to their facilitation of GABA

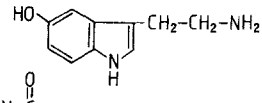
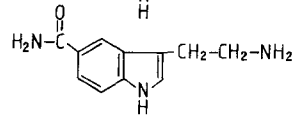
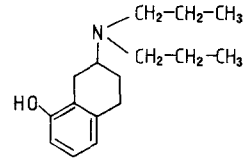
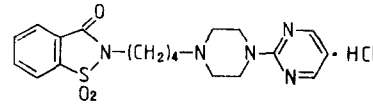
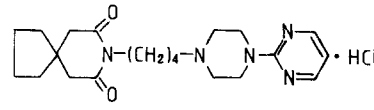
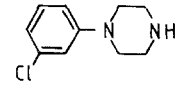
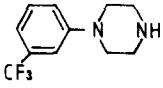
	Mouse — Cultured Hippocampal Neurons			Guinea Pig — Hippocampal Membranes			
	IC_{50} (nM)	Intrinsic Activity	(n)	IC_{50} (nM)	Intrinsic Activity	(n)	
5-HT	52 ± 18	1.00	(4)	16.1 ± 1.7	1.00	(10)	
5-CT	18 ± 4	1.00 ± 0.02	(3)	4.2 ± 2.2	1.00 ± 0.03	(6)	
8-OH-DPAT	7.1 ± 3.0	1.00 ± 0.04	(3)	7.4 ± 1.5	1.00 ± 0.06	(7)	
Ipsapirone	82 ± 16	0.77 ± 0.07	(4)	70 ± 35	0.65 ± 0.08	(7)	
BUSPIRONE	128 ± 25	0.64 ± 0.06	(4)	140 ± 39	0.63 ± 0.05	(7)	
m CPP	—	—		1798 ± 472	0.78 ± 0.16	(3)	
TFMPP	—	—		1016 ± 253	0.87 ± 0.02	(4)	

Fig. 3. The structural formulae and IC_{50} values of several 5-HT agonists whose abilities to inhibit cyclic AMP formation in embryonic murine hippocampal neurons and adenylate cyclase activity in guinea pig hippocampal membranes were assessed. Values given are means ± SEM for (*n*) experiments

induced inhibition of a particular class of neurons, e.g., the 5-HT neurons of the raphé nucleus (Stein et al. 1977; Soubrie et al. 1983; Gallager 1978; Gray 1982). The present report, showing that putative anxiolytic drugs such as ipsapirone and buspirone are agonists at the 5-HT_{1A} receptors in hippocampus, an area receiving a dense innervation from 5-HT neurons of the raphé nucleus, is of particular interest. One may speculate that these new drugs exert their anxiolytic effects in a manner similar to the benzodiazepines, that is via inhibition of activity of 5-HT neurons. This speculation is corroborated by a report showing that ipsapirone and other piperazine analogs inhibit the firing of 5-HT neurons in the raphé nucleus (Sprouse and Aghajanian 1987). The 5-HT_{1A} receptors on which these drugs act in hippocampus are not likely to be the 5-HT terminals of 5-HT neurons since we show that they are present in hippocampal cultures devoid of 5-HT innervation. They may, however, be located on interneurons that recurrently inhibit 5-HT neurons.

The profile of agonist activity and the high affinity antagonism of this activity by spiperone lead us to conclude that the 5-HT receptor in guinea pig hippocampal membranes and in mouse hippocampal neurons are nearly the same if not identical subtypes of 5-HT receptors. We therefore tentatively classify the murine hippocampal receptor as a 5-HT_{1A} receptor as is the case with the guinea pig hippocampal receptor (De Vivo and Maayani 1986).

In two recent reports it was shown that 5-HT stimulates adenylate cyclase activity in guinea pig (Shenker and Maayani 1985) and rat (Markstein et al. 1986) hippocampal homogenates. The stimulation was partly (in guinea pig) and entirely (in rat) due to the stimulation of a 5-HT_{1A} receptor subtype. The question therefore arises whether or not, in hippocampal tissue, the 5-HT_{1A} receptors which stimulate adenylate cyclase activity in the absence of forskolin are identical to the 5-HT_{1A} receptors which inhibit adenylate cyclase activity in the presence of forskolin. To answer this question, and extensive pharmacological comparison between the two receptors would be required. The effects of piperazine derivatives have not been tested on the stimulatory 5-HT_{1A} receptors. However, whereas 5-CT is a full agonist at both the stimulatory and inhibitory 5-HT_{1A} receptors, 8-OH-DPAT is a partial agonist at the 5-HT_{1A} stimulatory receptor (Markstein et al. 1986) but a full agonist at the 5-HT_{1A} inhibitory receptor (Figs. 1, 3). The divergent relationship between these agonist effects may indicate that the receptors mediating adenylate cyclase stimulation and inhibition are not pharmacologically identical.

In summary, the experiments described here clearly indicate that the two novel anxiolytic drugs (ipsapirone and buspirone), both with long chain substituted piperazines, as well as two other piperazines, TFMPP, and mCPP are

agonists at the 5-HT_{1A} receptors inhibiting cyclic AMP production in hippocampal homogenates and in hippocampal neurons in primary culture.

Acknowledgements. We wish to thank Mrs. A. Turner-Madeuf, Mrs. C. Bunoust and Mrs. M. Passama for their gracious assistance in the preparation of this manuscript.

References

Cheng Y, Prusoff WH (1973) Relationship between the inhibition constant (K_i) and the concentration of an inhibitor that causes a 50% inhibition (I_{50}) of an enzymatic reaction. Biochem Pharmacol 22:3099–3108

Cortes R, Palacios JM, Pazos A (1984) Visualization of multiple serotonin receptors in the rat brain by autoradiography. Br J Pharmacol 82:202–209

Cunningham KA, Appel JB (1986) Possible 5-hydroxytryptamine (5-HT_1) receptor involvement in the stimulus properties of 1-(n-trifluoromethylphenyl) piperazine (TFMPP). J Pharmacol Exp Ther 237:369–377

De Vivo M, Maayani S (1985) Inhibition of forskolin-stimulated adenylate cyclase by 5-HT receptor agonists. Eur J Pharmacol 119:231–234

De Vivo M, Maayani S (1986) Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J Pharmacol Exp Ther 238:248–253

Engel G, Gothert M, Müller-Schweinitzer E, Schicken E, Sistonen L, Stadler PA (1983) Evidence for common pharmacological properties of (^{3}H)-5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. Naunyn-Schmiedeberg's Arch Pharmacol 324:116–124

Gallager DW (1978) Benzodiazepines: Potentiation of a gaba inhibitory response in the dorsal raphe nucleus. Eur J Pharmacol 49:133–143

Glaser T, Traber J (1985) Binding of the putative anxiolytic TVXQ 7821 to hippocampal 5-hydroxytryptamine (5-HT) recognition sites. Naunyn-Schmiedeberg's Arch Pharmacol 329:211–215

Goldberg HL, Finnerty RJ (1979) The comparative efficacy of buspirone and diazepam in the treatment of anxiety. Am J Psychiatry 136:1184–1187

Gozlan H, El Mestikawy S, Pichat L, Glowinski J, Hamon M (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: (^{3}H)-PAT. Nature (Lond) 305:140–142

Gray JA (1982) Precis of neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system. Behav Brain Sci 5:469–534

Leysen JE, Niemegeers CJE, Van Nueten JM, Laduron P (1982) (^{3}H)-ketanserin (R 41468) a selective (^{3}H)-ligand for serotonin$_2$ receptor binding sites. Mol Pharmacol 21:301–314

Markstein R, Hoyer D, Engel G (1986) 5-HT_{1A}-receptors mediate stimulation of adenylate cyclase in rat hippocampus. Naunyn-Schmiedeberg's Arch Pharmacol 333:335–341

Pazos A, Hoyer D, Palacios JM (1985) The binding of serotoninergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. Eur J Pharmacol 106:539–546

Pedigo NN, Yamamura HJ, Nelson KL (1981) Discrimination of multiple (^{3}H)-5-hydroxytryptamine binding sites by neuroleptic spiperone in the rat brain. J Neurochem 36:220–226

Peroutka SJ, Snyder SH (1979) Multiple serotonin receptors: differential binding of (^{3}H)-5-hydroxytryptamine, (^{3}H)-lysergic acid diethylamide and ^{3}H-spiroperidol. Mol Pharmacol 16:687–699

Salomon Y, Londos C, Rodbell M (1974) A highly sensitive adenylate cyclase assay method. Anal Biochem 58:541–548

Shenker A, Maayani S, Weinstein H, Green JP (1985) Two 5-HT receptors linked to adenylate cyclase in guinea pig hippocampus are discriminated by 5-carboxamidotryptamine and spiperone. Eur J Pharmacol 109:427–429

Soubrie P, Blas C, Ferron A, Glowinski J (1983) Chlordiazepoxide reduces in vivo serotonin release in the basal ganglia of encephale isole but not anesthetized cats: Evidence for a dorsal raphe site of action. J Pharmacol Exp Ther 226:526–532

Sprouse FS, Aghajanian GK (1987) Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists. Synapse 1:3–9

Stein L, Bellyzi JD, Wise CD (1977) Benzodiazepines: Behavioral and neurochemical mechanisms. Am J Psychiatry 174:665–669

Traber J, Davies MA, Dompert WU, Glaser T, Schurman T, Seidel TR (1984) Brain serotonin receptors as a target cell for the putative anxiolygic TVXQ 7821. Brain Res Bull 12:741–744

Weiss S, Sebben M, Garcia-Sainz JA, Bockaert J (1985) D_2 dopamine receptor mediated inhibition of cyclic AMP formation in striatal neurons in primary culture. Mol Pharmacol 27:595–599

Weiss S, Sebben M, Kemp D, Bockaert J (1986a) Serotonin 5-HT_1 receptors mediate inhibition of cyclic AMP production in neurons. Eur J Pharmacol 120:227–230

Weiss S, Pin JP, Sebben M, Kemp DE, Sladeczek F, Gabrion G, Bockaert J (1986b) Synaptogenesis of cultured striatal neurons in serum-free medium: A morphological and biochemical study. Proc Natl Acad Sci USA 83:2238–2242

Received November 10, 1986/Accepted January 2, 1987