0022-3565/91/2571-0433$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 1991 by The American Society for Pharmacology and Experimental Therapeutics

Vol. 257, No. 1
Printed in U.S.A.

5-HT$_{1A}$-Sensitive Adenylyl Cyclase of Rodent Hippocampal Neurons: Effects of Antidepressant Treatments and Chronic Stimulation with Agonists[1]

ANNIE VARRAULT, VINCENT LEVIEL and JOËL BOCKAERT

Centre National de la Recherche Scientifique-Institut National de la Santé et de la Recherche Medicale de Pharmacologie-Endocrinologie (A.V., J.B.), Rue de la Cardonille, 34094 Montpellier cedex 5, and Laboratoire de Physiologie Nerveuse, Centre National de la Recherche Scientifique (V.L.), Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

Accepted for publication December 17, 1990

ABSTRACT

The effects of chronic treatment with desimipramine (a tricyclic antidepressant), fluoxetine [a specific 5-hydroxytryptamine (5-HT) uptake inhibitor], clorgyline (a specific monoamine oxydase inhibitor of A type), ipsapirone (a specific 5-HT$_{1A}$ receptor agonist) as well as electroconvulsive shock treatment were investigated on rat hippocampal 5-HT$_{1A}$ receptors negatively coupled to adenylyl cyclase. Drugs were injected intraperitoneally in rats for 2 or 3 weeks, and biochemical determinations were made 4 to 72 hr after the final dose. Chronic treatments with desimipramine, ipsapirone and fluoxetine did not induce any change in the 5-HT$_{1A}$-induced inhibition of the adenylyl cyclase activity. In contrast, chronic treatment with clorgyline and electroconvulsive shock treatment induced a slight but significant reduction of 5-HT's ability to inhibit hippocampal adenylyl cyclase. This indicates that, at least in hippocampal neurons, the 5-HT$_{1A}$ receptor coupled to adenylyl cyclase is not easily desensitized. This was verified *in vitro* on murine hippocampal neurons in culture, by measuring the effects of intense stimulation (1 and 2 hours), with 5-HT, ipsapirone and 8-hydroxy-2-(di-*n*-propylamino)tetralin. Indeed, such stimulations did not significantly affect the 5-HT$_{1A}$ receptor-induced inhibition of cAMP production in these hippocampal neurons in culture. Our results indicate that it is not the post-synaptic 5-HT$_{1A}$ receptor of hippocampus that is modified during antidepressant treatments, at least at the level of its coupling to adenylyl cyclase.

It is probably hazardous to reduce the development of complex affective disorders, such as depression, to the dysfunction of one neurotransmitter system. However, there has been growing evidence for the involvement of the serotonergic (5-HT) system in the pathogenesis of major depression (Coppen and Doogan, 1988).

One piece of evidence came from the observation that inhibition of 5-HT synthesis with *p*-chlorophenylalanine reverses the therapeutic effects of tranylcypromine, a MAO-I, on depression (Shopsin *et al.*, 1976). Another piece of evidence is the observation that *via* different mechanisms, major antidepressant treatments are able to increase 5-HT neurotransmission. TCAs are 5-HT as well as norepinephrine uptake inhibitors (Glowinski and Axelrod, 1964), whereas antidepressants like amitryptiline, fluoxetine, zimelidine, indalpine and citalopram are specific 5-HT uptake inhibitors. MAO-Is of the A type, such as clorgyline and meclobemide, suppress 5-HT and norepinephrine inactivation and are effective antidepressants, whereas MAO-I of the B type, such as deprenyl, specifically suppresses dopamine inactivation and is not active in major depression (Murphy *et al.*, 1981). Specific 5-HT$_{1A}$ agonists, such as buspirone, gepirone and ipsapirone, have been found to be anxiolytics, but also antidepressant drugs (Goldberg and Finnerty, 1979; Amsterdam *et al.*, 1987; Traber and Glaser, 1987). Finally, several studies have documented an increased "hyperactivity syndrome" induced by systemic administration of 5-HT agonists or precursors after repeated ECT in rats, suggesting that ECT may also enhance serotonergic neurotransmission (Evans *et al.*, 1976; Grahame-Smith *et al.*, 1978; Costain *et al.*, 1979). ECT is estimated to be superior to TCA drugs, particularly in severe and delusional depression (Avery and Lubrano, 1979). However, in all these treatments, including 5-HT$_{1A}$ agonist administration and chronic ECT, a delay is required to obtain a therapeutic effect (Avery and Lubrano, 1979; Feighner *et al.*, 1982). Therefore, in order to understand the biochemical effects of antidepressants, the modifications of

Received for publication July 17, 1990.

[1] This work was supported by Bayer (France and Federal Republic of Germany), the Centre National de la Recherche Scientifique (CNRS) and the Institut National de la Santé et de la Recherche Medicale (INSERM).

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; MAO-I, monoamine oxydase inhibitor; TCA, tricyclic antidepressant; ECT, electroconvulsive shock treatment; DMI, desmethylimipramine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; I_{max}, maximal inhibition.

5-HT neurotransmission, and in particular of 5-HT receptors after long-term treatments, have to be studied. Many studies carried out on rodents (for reviews, see Sugrue, 1987; Green, 1985; Ogren and Fuxe, 1985) have emphasized the involvement of hippocampal postsynaptic $5\text{-}HT_{1A}$ receptors during antidepressant therapies.

It has recently been shown that one of the mechanisms of action of postsynaptic hippocampal $5\text{-}HT_{1A}$ receptors is its negative coupling to adenylyl cyclase (De Vivo and Maayani, 1986; Weiss *et al.*, 1986; Bockaert *et al.*, 1987; Dumuis *et al.*, 1988). We have therefore studied the effects of several antidepressant treatments such as long-term administration of TCA, fluoxetine, ipsapirone, clorgyline and repeated ECT on hippocampal $5\text{-}HT_{1A}$ receptor coupling to adenylyl cyclase in rats.

We have also investigated the effects of intensive stimulation of $5\text{-}HT_{1A}$ receptors on their coupling to adenylyl cyclase, in primary culture of hippocampal neurons.

Materials and Methods

Primary cultures of mouse hippocampal neurons and determination of intracellular cAMP production. Neuronal cell cultures generated from hippocampi of 16- to 17-day-old Swiss mouse embryos were grown for 6 days. These cultures were prepared as described previously (Bockaert *et al.*, 1987). Briefly, 10^6 hippocampal cells were mechanically dissociated and plated in the absence of fetal calf serum, in 12-well Costar plates, previously coated with 1.5 μg/ml poly-L-ornithine. The culture medium consisted of a mixture (1:1) of Dulbecco's modified Eagle's medium and F12 nutrient (Gibco), supplemented with glucose (33 mM), glutamine (2 mM), sodium bicarbonate (3 mM) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (5 mM). In the place of serum, a defined hormone and salt mixture including insulin (25 μg/ml), transferrin (100 μg/ml), progesterone (20 nM), estradiol (10^{-12} M) and putrescine (60 μM) was added.

Formation of cAMP. The cAMP content of cells was measured by the prelabeling technique as described previously (Weiss *et al.*, 1985). On the sixth day, the cells were washed and incubated at 37°C (5% CO_2/95% air mixture) with 2 μCi/ml of [^{3}H]adenine (24 Ci/mol; Amersham, United Kingdom). After 2 hr, the cultures were washed and incubated with 0.75 mM isobutyl-methylxanthine, 0.1 μM vasoactive intestinal polypeptide and 1 μM forskolin (all prepared in culture medium) in a volume of 1 ml, for 5 min at 37°C. The reaction was stopped by aspiration of the media and addition of 1 ml of ice-cold 5% trichloroacetic acid. Cells were scraped with the aid of a rubber policeman and 100 μl of 5 mM ATP and 5 mM cAMP were added to the mixture. Cellular protein was centrifuged at 5000 × *g*. [^{3}H]ATP and [^{3}H]cAMP were separated by sequential chromatography on Dowex and alumina columns. cAMP formation is expressed as percentage conversion:

$$\frac{[^3H]cAMP}{[^3H]cAMP + [^3H]ATP} \times 100$$

Preparation of rat hippocampal membranes and measurement of adenylyl cyclase activity. Wistar rats (150–200 g) were killed by decapitation. The two hippocampi of each rat were dissected and homogeneized in 5 ml of an ice-cold solution containing 300 mM sucrose, 20 mM Tris-HCl pH 7.4, 1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N*-tetraacetic acid, 5 mM EDTA and 5 mM dithiothreitol. The homogenate was centrifuged at 39,000 × *g* for 10 min at 0°C. The pellet was resuspended in 2 ml of the same buffer and filtered over a silk screen (Nytrel TI, 150 μm).

Forskolin (10 μM final concentration) was added in the homogenate, which was kept on ice. To start the adenylyl cyclase assay, 20 μl of the homogenate was added to 100 μl (final volume) of assay medium. The final composition of the medium was: 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM cAMP, 4 mM theophylline, 5 mM creatine phosphate, 0.02 mg creatine kinase, 10 μM GTP, 2 mM $MgSO_4$, 0.2 mM ATP, 1 μCi of [α^{32}P]ATP and 1 to 2 × 10^{-2} μCi of [^{3}H]cAMP. 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 cAMP, 10 mM $CaCl_2$ and 0.1 N HCl. The tubes were centrifuged at 5000 × *g* for 5 min. [^{32}P] cAMP formed and [^{3}H]cAMP were isolated according to the method of Salomon *et al.* (1974). Protein content was measured by the method of Lowry *et al.* (1951).

***In vivo* chronic drug treatments.** All chronic drug treatments were administered intraperitoneally. For each treatment, four rats received the drug and four rats received the vehicle (NaCl 9%).

Data analysis of *in vivo* treatment. For each rat, the inhibition of forskolin-stimulated hippocampal adenylyl cyclase in the presence of seven 5-HT concentrations (10^{-8}, 2 × 10^{-8}, 4 × 10^{-8}, 8 × 10^{-8}, 1.6 × 10^{-7}, 6.4 × 10^{-7}, 1.3 × 10^{-8} M) was measured (each concentration was tested in triplicate). Although the concentration response curves were very similar for controls from one experiment to another [see the mean curve for all the controls (n = 28) in fig. 1], we always carried out the eight concentration response curves (four for controls and four for treated rats) for a single experiment. The data are presented in the following manner:

1. For each individual concentration response curve, the Eadie-Hofstee's plot was done (eight for each drug treatment). I_{max} and EC_{50} were determined. The mean values of I_{max} and EC_{50} ± S.E.M. are given in table 1.

2. In the figures (part A), the mean inhibition ± S.E.M. (n = 4) for each 5-HT concentration is presented. A Student's t test was done for each concentration between control and treated samples. In part B of the figures the Eadie-Hofstee's plot is calculated from the mean value of inhibition for each concentration.

ECT. Rats were shocked through earclip electrodes by application of a 50-mA sinusoidal current for a duration of 2 sec, as described previously (Leviel *et al.*, 1990). All shocked rats experienced generalized tonic-clonic seizures that lasted approximately 30 sec. Shocks were administered once daily for 10 consecutive days. At 48 hr after the last shock, rats were killed by decapitation.

Drugs. The following drugs were generously donated: ipsapirone (TVXQ 7821; J. Traber, Troponwerke Gmbh and Co., Cologne, Federal Republic of Germany) and fluoxetine (Dr. R. W. Fuller, Lilly Research Laboratories).

The purchased drugs were 8-OH-DPAT (Research Biochemical Inc., Wayland, MA), DMI, clorgyline and 5-HT (Sigma Chemical Co., St. Louis, MO).

Results

Effect of 5-HT on adenylyl cyclase of rat hippocampal membranes. As previously described (Bockaert *et al.*, 1987), 5-HT inhibited forskolin (10 μM) stimulated adenylyl cyclase of rat hippocampal membranes in a dose-dependent manner. Figure 1A gives the concentration response curve for 5-HT obtained after pooling individual curves. The S.E.M. on each point is between 0.7 and 1.7%, indicating a good reproducibility between experiments. The Eadie-Hofstee's plot of the concentration response curve (fig. 1B) indicates that a single $5\text{-}HT_{1A}$-adenylyl cyclase system is involved (r = 0.992). It also provides a way of accurately calculating both the maximal inhibition induced by 5-HT (I_{max} = 37.6 ± 1.2%, mean ± S.E.M., n = 28) and the EC_{50} (EC_{50} = 56 ± 4 nM, mean ± S.E.M., n = 28). We have also verified that, under both basal and 5-HT conditions, the adenylyl cyclase activity was linear as a function of time and protein concentration (data not shown).

Effect of chronic treatments of rats with DMI on $5\text{-}HT_{1A}$ inhibited adenylyl cyclase of hippocampus. Rats

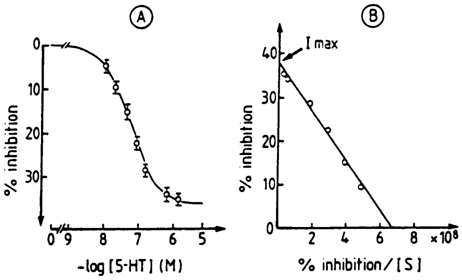

Fig. 1. 5-HT mediated inhibition of forskolin (10 μM)-stimulated adenylyl cyclase in rat hippocampal membrane. Seven groups of four rats were injected with saline buffer. A: dose-response curve. The values are the means ± S.E.M. ($n = 28$), each performed in triplicate. B: Eadie-Hofstee's plot ($r = 0.992$).

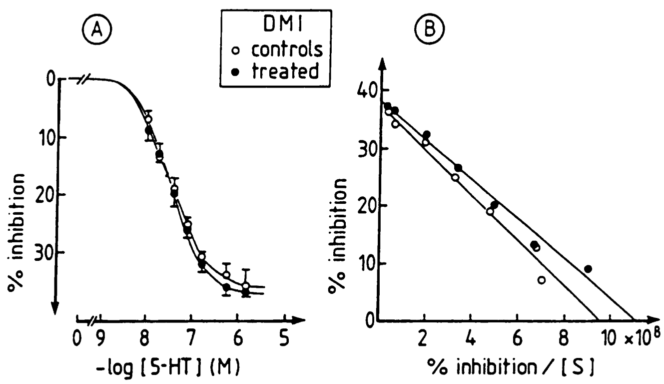

Fig. 2. Effect of chronic DMI on the inhibition by 5-HT of forskolin-stimulated adenylyl cyclase. Groups of 4 rats were given either saline or DMI (15 mg/kg i.p.) during 16 days, followed by 48 hr withdrawal. A: dose-response curves. The values are the means ± S.E.M., each performed in triplicate. B: Eadie-Hofstee's plot ($r = 0.974$ for the control group and 0.986 for the treated group).

were treated for 16 days with a daily intraperitoneal injection of DMI (15 mg/kg) or saline. Two days later, the characteristics of the 5-HT$_{1A}$ receptor-adenylyl cyclase system of hippocampus were analyzed. As seen in figure 2, there was no significant modification of either the I_{max} or the EC_{50}. Another DMI treatment was carried out: rats were treated with the same dose for 23 days, and the 5-HT$_{1A}$-adenylyl cyclase system was analyzed 24 hr later. No modification of the I_{max} or EC_{50} was observed (table 1).

Effect of chronic treatments with fluoxetine, a specific 5-HT uptake inhibitor, and with ipsapirone, a specific 5-HT$_{1A}$ agonist, on 5-HT$_{1A}$-inhibited adenylyl cyclase of hippocampus. Fluoxetine treatment (10 mg/kg per day for 21 days followed by a withdrawal period of 24 hr before analysis) as well as ipsapirone treatments (10 mg/kg for 20 days followed by withdrawal periods of 4 or 48 hr) did not induce any change in 5-HT$_{1A}$-sensitive adenylyl cyclase of hippocampus (table 1).

Effects of chronic treatments with clorgyline, a MAO-I of the A type on 5-HT$_{1A}$ inhibited adenylyl cyclase of hippocampus. Chronic treatment with clorgyline (1 mg/kg per day for 21 days) was administered; 72 hr later, the 5-HT-sensitive adenylate cyclase system was studied. As seen in figure 3 and table 1, no change in the EC_{50} was found. However, a minor decrease in the I_{max} was apparent (see table 1).

Effect of chronic ECT on 5-HT$_{1A}$-inhibited adenylyl cyclase of hippocampus. Anesthetized rats treated for 10 days with ECT (50 mA for 2 sec) and control rats that were anesthetized but not submitted to ECT were compared for 5-HT's ability to inhibit their hippocampal adenylyl cyclase. Figure 4 and table 1 indicate that no modification of the EC_{50} was observed, but that a slight but significant decrease in I_{max} was clearly apparent.

Absence of desensitization of 5-HT$_{1A}$-adenylyl cyclase system of mouse hippocampal neurons in culture. We have previously shown that mouse hippocampal neurons in culture contain a typical 5-HT$_{1A}$ receptor inhibiting adenylyl cyclase. In this system, 8-OH-DPAT was a full agonist having a better affinity for 5-HT$_{1A}$ receptors than 5-HT (EC_{50} were 7 and 52 nM, respectively), whereas ipsapirone was a partial agonist ($EC_{50} = 100$ nM; Dumuis *et al.*, 1988). In order to check whether or not 5-HT$_{1A}$ adenylyl cyclase was easily desensitized by chronic stimulation with high agonist concentrations, we treated hippocampal neurons with 5-HT (10 μM), ipsapirone (10 μM) or 8-OH-DPAT (10 μM) for 1 and 2 hr. After extensive washing (see "Materials and Methods"), the inhibition of cAMP production stimulated by vasoactive intestinal polypeptide (0.1 μM) plus forskolin (1 μM) was studied. None of the treatments modified the 5-HT-induced adenylyl cyclase inhibition (fig. 5). Basal cAMP production was not modified by the treatments (see legend to fig. 5).

Discussion

Most antidepressant treatments have a common mechanism of action, *i.e.*, an increase in the stimulation of all 5-HT receptor subtypes resulting from an inhibition of reuptake (DMI, fluoxetine), an inhibition of 5-HT degradation (clorgyline) and an increase in serotoninergic neurotransmission (ECT). Treatment with ipsapirone is the only one that results in specific stimulation of the 5-HT$_{1A}$ subtype (Glaser and Traber, 1985; Traber and Glaser, 1987). It has been reported that hippocampal 5-HT$_{1A}$ receptors can trigger their action by at least two transduction mechanisms: the adenylyl cyclase inhibition (De Vivo and Maayani, 1986; Bockaert *et al.*, 1987; Dumuis *et al.*, 1988) and an activation of K^+ channels (Andrade *et al.*, 1986). Our main goal was to determine whether or not antidepressant treatments result in desensitization of 5-HT$_{1A}$ receptor-mediated adenylyl cyclase inhibition of hippocampus. Such a study is particularly interesting since it has been proposed that desensitization of receptors, and in particular of 5-HT$_2$ and *beta* adrenergic receptors, is an important step in the action of antidepressant drugs (Peroutka and Snyder, 1980; Reisine, 1981).

Our results indicate that no modification of the 5-HT$_{1A}$-induced adenylyl cyclase inhibition can be observed after DMI, fluoxetine or ipsapirone treatments (table 1, fig. 2). We do not confirm the slight desensitization of the 5-HT$_{1A}$ inhibited adenylyl cyclase system reported by Newman *et al.* (1990) after DMI treatment. However, in this work, concentration response curves were performed with only three 5-HT concentrations. We have shown that treatments with clorgyline, a MAO-I of the A type, and ECT reduced 5-HT induced adenylyl cyclase inhibition by about 10% (figs. 3 and 4, table 1). This weak desensitization of the 5-HT$_{1A}$ response on hippocampus adenylyl cyclase system after clorgyline treatment has also been reported by Sleight *et al.* (1988). It is difficult to know whether such a small reduction of the 5-HT$_{1A}$ receptor-induced adenylyl cyclase inhibition might have a physiologic significance or not. In this context, it is interesting to note that some antidepressant treatments also induce a 20 to 30% down-regulation of

TABLE 1

Effect of chronic treatments of rats with antidepressants, electro convulsive shocks and 5-HT$_{1A}$ agonist on the forskolin-stimulated adenylyl cyclase activity and its inhibition, by 5-HT, in hippocampus

	EC_{50}		I_{max}		Forskolin-Stimulated Adenylyl Cyclase Activity	
	Control	Treated	Control	Treated	Control	Treated
	nM		%		*pmol/mg/min*	
DMI						
15 mg/kg, 16 days, tested 48 hr after[a]	39 ± 9[b]	34 ± 3	37.3 ± 2.5	38.0 ± 0.7	181 ± 6	187 ± 11
15 mg/kg, 23 days, tested 24 hr after[a]	51 ± 4	42 ± 4	37.8 ± 1.3	37.3 ± 0.8	175 ± 6	181 ± 2
Clorgyline						
1 mg/kg, 21 days, tested 2 hr after[a]	83 ± 4	86 ± 3	31.3 ± 1.2	26.1 ± 1.1[a]	210 ± 12	193 ± 8
Fluoxetine						
10 mg/kg, 21 days, tested 48 hr after[a]	62 ± 6	68 ± 6	38.6 ± 1.7	36.0 ± 1.9	203 ± 6	192 ± 4
Ipsapirone						
10 mg/kg, 20 days[a]						
Tested 4 hr after	58 ± 7	60 ± 6	34.5 ± 1.6	36.4 ± 1.5	209 ± 7	193 ± 2
Tested 48 hr after	47 ± 7	40 ± 5	43.1 ± 2.0	42.1 ± 1.4	171 ± 4	189 ± 4
ECT						
50 mA, 10 days, tested 48 hr after[a]	53 ± 3	59 ± 3	39.2 ± 0.8	34.5 ± 1[a]	194 ± 6	182 ± 7

[a] $P < .05$.
[b] Values are the means ± S.E.M. Comparisons were made by a Student's *t* test.

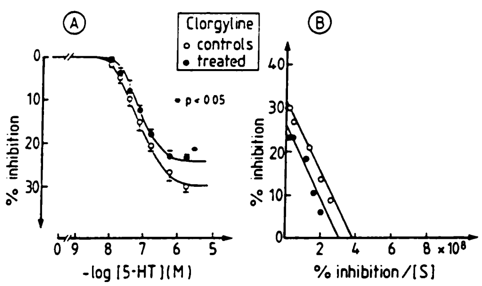

Fig. 3. Effect of chronic clorgyline on the inhibition by 5-HT of forskolin-stimulated adenylyl cyclase. Groups of 4 rats were given either saline or clorgyline (1 mg/kg i.p.) during 21 days, followed by 72 hr withdrawal. A: dose-response curves. The values are the means ± S.E.M., each performed in triplicate. B: Eadie-Hofstee's plot (r = 0.989 for the control group and 0.971 for the treated group).

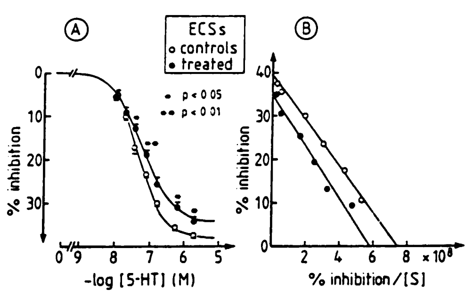

Fig. 4. Effect of chronic ECT on the inhibition by 5-HT of forskolin-stimulated adenylyl cyclase. Groups of 4 anesthetized rats were given either ECS (50 mA, 2 sec), or nothing, during 10 days, followed by 48 hr withdrawal. A: dose-response curves. The values are the means ± S.E.M., each performed in triplicate. B: Eadie-Hofstee's plot (r = 0.995 for the control group and 0.970 for the treated group).

beta adrenergic receptors. Such a reduction is generally believed to have a crucial role in the mechanisms of action of these drugs (Reisine, 1981). Although we found that ECT slightly reduced the 5-HT$_{1A}$ receptor-induced adenylyl cyclase, we do not confirm the results given by Newman and Lerer (1988), showing that ECT completely suppressed the 5-HT$_{1A}$ receptor-mediated effect on adenylyl cyclase. Their results were surprising since such a complete desensitization of neurotransmitter receptor actions had never been reported by anyone else and seems unlikely.

Our results indicated that the 5-HT$_{1A}$ receptor adenylyl cyclase system is not easily desensitized during an *in vivo*-induced receptor hyperstimulation. *In vitro*, we have confirmed this relative resistance to desensitization by directly stimulating the 5-HT$_{1A}$ receptor adenylyl cyclase system in primary cultures of hippocampal neurons. Incubating these neurons for 1 or 2 hr with 5-HT (10^{-5} M) did not desensitize the 5-HT$_{1A}$ receptor-induced adenylyl cyclase inhibition. It has been shown that phosphorylation is likely to be an important step in the desensitization of guanine nucleotide binding protein coupled receptors (Lefkowitz *et al.*, 1990). It is interesting to note that, in contrast to *beta* adrenergic receptors, 5-HT$_{1A}$ receptors have no evident protein kinase A consensus sequence in the third intracellular loop, as well as in the C-terminal domain (Kobilka *et al.*, 1987). The C-terminal domain is also devoid of serine and threonine residues, which are preferred sites for phosphorylation in transducin and *beta* adrenergic receptors by rhodopsin and *beta* adrenergic receptor kinase, respectively (Lefkowitz *et al.*, 1990). However, a consensus sequence for protein kinase C is present in the third cytoplasmic loop (Albert *et al.*, 1990). Such a resistance of the 5-HT$_{1A}$ receptors to desensitization is in accordance with the absence of effect of antidepressant treatment on 5-HT$_1$ or 5-HT$_{1A}$ binding in several brain areas including hippocampus (Maggi and Enna, 1980;

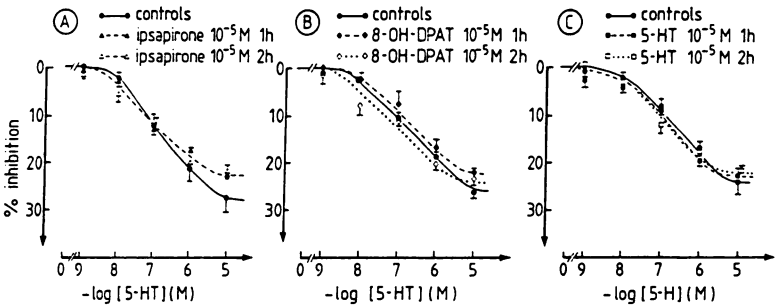

Fig. 5. 5-HT mediated inhibition of forskolin (1 μM) + VIP (0.1 μM)-stimulated cAMP formation in murine hippocampal neurons in primary culture. The values are the means ± S.E.M. (n = 3), each performed in duplicate. A: cells were treated with either ipsapirone 10 μM, 1 hr ▲ or 2 hr △, or with medium alone ●. In the absence of 5-HT, the percentage of conversion (means ± S.E.M.) were 4.9 ± 0.9, 6.2 ± 0.6 and 5.1 ± 0.7, respectively. B: cells were treated with either 8-OH-DPAT 10 μM, 1 hr ◆ or 2 hr ◇, or with medium alone ●. In the absence of 5-HT, the percentages of conversion (means ± S.E.M.) were 6.3 ± 0.3, 5.0 ± 0.5 and 5.7 ± 0.5, respectively. C: cells were treated with either 5-HT 10 μM, 1 hr ■ or 2 hr □, or with medium alone ●. In the absence of 5-HT, the percentages of conversion (means ± S.E.M.) were 6.9 ± 0.3, 6.3 ± 0.1 and 5.6 ± 0.2, respectively.

TABLE 2

Comparison of the effects of chronic antidepressant treatments on serotonin-induced activity and inhibition of adenylyl cyclase, in rat hippocampus neurons

Treatments	Inhibition of the Electrical Activity of Hippocampus Neurons by 5-HT	Inhibition by 5-HT of Hippocampus Adenylyl Cyclase Observed in This Study
DMI	↗ [a]	→
Fluotexine	→ [b]	→
$5\text{-}HT_{1A}$ agonists	→ [c]	→
Clorgyline	↘ [d]	↘
ECT	↗ [e]	↘

[a] De Montigny and Aghajanian, 1978; Gallager and Bunney, 1979.
[b] Blier *et al.*, 1987
[c] Blier and De Montigny, 1987.
[d] Blier *et al.*, 1986.
[e] De Montigny, 1984.

Peroutka and Snyder, 1980; Holta *et al.*, 1986; Welner *et al.*, 1989; Newman *et al.*, 1990).

The effects of antidepressant treatments have also been followed by studying 5-HT-mediated inhibition of electrical activity of hippocampal neurons: a 5-HT effect mediated by $5\text{-}HT_{1A}$ receptors (Andrade and Nicoll, 1987). As summarized in table 2, the effects of antidepressant treatments observed on $5\text{-}HT_{1A}$ receptor-mediated adenylyl cyclase inhibition and those observed on the inhibition of hippocampal neuron electrical activity are not always similar. Two explanations may be given to explain such differences. The $5\text{-}HT_{1A}$ receptor-mediated effect on electrical activity is probably due to an activation of K^+ channels (Andrade *et al.*, 1986) and not to an inhibition of adenylyl cyclase activity. Therefore, if the antidepressant treatments differently affect these two $5\text{-}HT_{1A}$ receptor transduction mechanisms, this may explain the differences in responses described in table 2. The second possibility is that the $5\text{-}HT_{1A}$-mediated electrical response is modulated by other 5-HT receptor subtypes (Andrade and Nicoll, 1987). Therefore, modifications of 5-HT-induced electrical responses after antidepressant treatments could be due to an integration of changes occurring at the level of several receptor subtypes and not only at the $5\text{-}HT_{1A}$ receptor level.

Acknowledgments

We would like to thank Mrs. M. Paolucci, Mrs. M. Passama and Mrs. A. Turner-Madeuf for their assistance in the preparation of the manuscript. This work was supported by Bayer-Troponwerpse (France and FRG).

References

Albert, P. R., Zhou, Q. Y., Van Tol, H. H. M., Bunzow, J. R. and Civelli, O.: Cloning, functional expression, and mRNA tissue distribution of the rat $5\text{-}HT_{1A}$ receptor gene. J. Biol. Chem. **265:** 5825–5832, 1990.

Amsterdam, J. D., Berwish, N., Potter, L. and Rickels, K.: Open trial of gepirone in the treatment of major depressive disorder. Curr. Ther. Res. Clin. Exp. **41:** 185–193, 1987.

Andrade, R., Malenka, R. L. and Nicoll, R. A.: A G protein couples serotonin and G A B A_B receptors to the same channels in hippocampus. Science **234:** 1261–1265, 1986.

Andrade, R. and Nicoll, R. A.: Pharmacologically distinct actions of serotonin on single pyramidal neurons of the rat hippocampus recorded *in vivo*. J. Physiol. (Lond.) **394:** 95–124, 1987.

Avery, D. and Lubrano, S.: Depression treated with imipramine and ECT: The De Carolis study reconsidered. Am. J. Psychiatry **136:** 559–562, 1979.

Blier, P., de Montigny, C. and Azzaro, A. J.: Modification of serotonergic and noradrenergic neurotransmission by repeated administration of monoamine oxidase inhibitors: Electrophysiological studies in the rat CNS. J. Pharmacol. Exp. Ther. **334:** 253–260, 1986.

Blier, P. and de Montigny, C.: Modification of 5-HT neuron properties by sustained administration of the $5\text{-}HT_{1A}$ agonist gepirone: Electrophysiological studies in the rat brain. Synapse **1:** 470–480, 1987.

Blier, P., de Montigny, C. and Chaput, Y.: Modifications of the serotonin system by antidepressant treatments: Implications for the therapeutic response in major depression. J. Clin. Psychopharmacol. **7:** 24S–35S, 1987.

Bockaert, J., Dumuis, A., Bouhelal, R., Sebben, M. and Cory, R. N.: Piperazine derivatives including the putative anxyolitic drugs, buspirone, are agonists at $5\text{-}HT_{1A}$ receptors negatively coupled with adenylate cyclase in hippocampal neurons. Naunyn-Schmiedeberg's Arch. Pharmacol. **335:** 588–592, 1987.

Coppen, A. J. and Doogan, D. P.: Serotonin and its role in the pathogenesis of depression. J. Clin. Psychiatry **49:** 4S–11S, 1988.

Costain, D. W., Green, A. R. and Grahame-Smith, E. G.: Enhanced 5-hydroxytryptamine-mediated behaviorial responses in rats following repeated electroconvulsive shocks: Relevance to the mechanism of the antidepressive effect of electroconvulsive therapy. Psychopharmacology **61:** 167–170, 1979.

De Montigny, C.: Electroconvulsive shock treatments enhance responsiveness of forebrain neurons to serotonin. J. Pharmacol. Exp. Ther. **228:** 230–234, 1984.

De Montigny, C. and Aghajanian, G. K.: Tricyclic antidepressants: Long term treatment responsivity of rat forebrain neurons to serotonin. Science **202:** 1303–1306, 1978.

De Vivo, M. and Maayani, S.: 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, 1986.

Dumuis, A., Sebben, M. and Bockaert, J.: Pharmacology of 5-hydroxytryptamine-1A receptors which inhibit cAMP production in hippocampal and cortical neurons in primary culture. Mol. Pharmacol. **33:** 178–186, 1988.

Evans, J. P. M., Grahame-Smith, D. G., Green, A. R. and Tordoff, A. F. C.: Electroconvulsive shocks increase the behavioral responses of rats to brain 5-hydroxytryptamine stimulation and central nervous system stimulant drugs. Br. J. Pharmacol. **56:** 193–199, 1976.

Feighner, J. P., Meredeth, C. H. and Henrickson, G. A.: A double blind comparison of buspirone and diazepam in out patients with generalized anxiety disorder. J. Clin. Psychiatry. **43(12,Sec 2):** 103–107, 1982.

Gallager, D. W. and Bunney, W. E. Jr.: Failure of chronic lithium treatment to block tricyclic antidepressant-induced 5-HT supersensitivity. Naunyn Schmeideberg's Arch. Pharmacol. **307:** 129–133, 1979.

Glaser, T. and Traber, J.: Binding of the putative anxiolytic TVXQ 7821 to hippocampal 5-hydroxytryptamine recognition site. Naunyn Schmeideberg's Arch. Pharmacol. **329:** 211–215, 1985.

Glowinski, J. and Axelrod, J.: Inhibition of uptake of tritiated noradrenaline in the intact rat brain by imipramine and structurally related compounds. Nature **204:** 1318–1319, 1964.

Goldberg, H. L. and Finnerty, R. J.: The comparative efficacy of buspirone and diazepam in the treatment of anxiety. Am. J. Psychiatry **136:** 1184–1187, 1979.

Grahame-Smith, D. G., Green, A. R. and Costain, D. W.: Mechanism of the antidepressant action of electroconvulsive therapy. Lancet **1:** 254–256, 1978.

Green, A. R.: Antidepressant treatments and serotonin receptor number and function. Acta Pharmacol. Toxicol. 56, suppl. 1, 128–137, 1985.

Holta, I., Yamawaki, S. and Segawa, T.: Long-term lithium treatment causes serotonin receptor down-regulation via serotoninergic presynapses in rat brain. J. Neuropsychobiol. **16:** 19–26, 1986.

Kobilka, B. K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T. S., Francke, U., Lefkowitz, R. J. and Caron, M. G.: An intronless gene encoding a potential member of the family of receptors coupled to guanine nucleotide regulatory proteins. Nature **329:** 75–79, 1987.

Lefkowitz, R. J., Hansdorff, W. P. and Caron, M. G.: Role of phosphorylation in desensitization of the β-adrenegic receptor. Trends Pharmacol. Sci. **11:** 190–194, 1990.

Leviel, V., Fayada, C., Guibert, B., Chaminade, M., Machek, G., Mallet, J. and Faucon Biguet, N.: Short and long-term alterations of gene expression in limbic structures by repeated electroconvulsive-induced seizures. J. Neurochem. **54:** 899–904, 1990.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275, 1951.

Maggi, A. and Enna, S. J.: Regional alterations in rat brain neurotransmitter systems following chronic lithium treatment. J. Neurochem. **34:** 888–892, 1980.

Murphy, D. L., Lipper, S., Pickar, D., Simerson, D., Cohen, R. M., Garrick, N. A., Alterman, I. S. and Campbell, I. C.: Selective inhibition of monoamine oxidase type A: Clinical antidepressant effects and metabolic changes in man. *In* Monoamine Oxidase Inhibitors. The State of the Art, ed. by M. B. H. Youdim and E. S. Paykel, John Wiley, New York pp. 189–205, 1981.

Newman, M. E. and Lerer, B.: Chronic electroconvulsive shock and desimipramine reduce the degree of inhibition by 5-HT and carbachol of forskolin-stimulated adenylate cyclase in rat hippocampal membranes. Eur. J. Pharmacol. **148:** 257–260, 1988.

Newman, M. E., Drummer, D. and Lerer, B.: Single and combined effects of desimipamine and lithium on serotoninergic receptor number and second messenger function in rat brain. J. Pharmacol. Exp. Ther. **252:** 826–831, 1990.

Ogren, S. O. and Fuxe, K.: Effects of antidepressant drugs on serotonin receptor mechanisms. In Neuropharmacology of serotonin, ed. by A. R. Green, Oxford University Press, Oxford, pp. 131–136, 1985.

Peroutka, S. J. and Snyder, S. H.: Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science **210:** 88–90, 1980.

Reisine, T.: Adaptative changes in catecholamine receptors in the central nervous system. Neuroscience **6:** 1471–1502, 1981.

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

Shopsin, B., Friedman, E. and Gershon, S.: Parachloro-phenylalanine reversal of tranylcypromine effects in depressed patients. Arch. Gen. Psychiatry **33:** 811–819, 1976.

Sleight, A. J., Marsden, C. A., Palfreyman, M. G., Mir, A. K. and Lovenberg, W.: Chronic MAO A and MAO B inhibition decreases the 5-HT_{1A} receptor mediated inhibition of forskolin-stimulated adenylate cyclase. Eur. J. Pharmacol. **154:** 255–261, 1988.

Sugrue, M. F.: Do antidepressants possess a common mechanism of action? Biochem. Pharmacol. **32:** 1811–1813, 1983.

Traber, J. and Glaser, T.: 5-HT_{1A} receptor-related anxiolytics. Trends Pharmacol. Sci. **8:** 432–437, 1987.

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

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

Welner, S. A., De Montigny, C., Desroches, J., Desjardins, P. and Suranyl-Cadotte, B. E.: Autoradiographic quantification of serotonin-1A receptors in rat brain following antidepressant drug treatment. Synapse **4:** 347–352, 1989.

Send reprint requests to: Joël Bockaert, Centre CNRS-INSERM de Pharmacologie-Endocrinologie, rue de la Cardonille, 34094 Montpellier, Cedex 5, France.