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

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ABSTRACT

The effects of chronic treatment with desimipramine (a tricyclic antidepressant), fluoxetine (a specific 5-hydroxytryptamine (5-HT) uptake inhibitor), clorglyline (a specific monoamine oxidase 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 clorglyline 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 (Shopein 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-I-s of the A type, such as clorglyline 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

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; MAO-I, monoamine oxidase 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-HT₁₅ receptors during antidepressant therapies.

It has recently been shown that one of the mechanisms of action of postsynaptic hippocampal 5-HT₁₅ receptors is its negative coupling to adenylyl cyclase (De Vivo and Maayan, 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-HT₁₅ receptor coupling to adenylyl cyclase in rats.

We have also investigated the effects of intensive stimulation of 5-HT₁₅ 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, 10⁶ 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 mixture. Cellular protein was centrifuged at 39,000 x g.

**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% CO₂/95% air mixture) with 2 tCi/ml of [³H]adenine (24 Ci/mol; Amer sham, United Kingdom). After 2 hr, the cultures were washed and the following drugs were generously donated: ipsapirone (TVXQ 7821; J. Treber, 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 0.1 and 0.4 µM, indicating a good reproducibility between experiments. The Eadie-Hoftree’s plot of the concentration response curve (fig. 1B) indicates a single 5-HT₁₅-adenyl cyclase system is involved (r = 0.992). It also provides a way of accurately calculating both the maximal inhibition induced by 5-HT (I₅₀ = 7.6 ± 1.2%, mean ± S.E.M., n = 28) and the EC₅₀ (EC₅₀ = 55 ± 6 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-HT₁₅ inhibited adenylyl cyclase of hippocampus. Rats...
The treatment was carried out for 21 days, with ECT (50 mA). 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 DM1 on the inhibition by 5-HT of forskolin-stimulated adenylyl cyclase. Groups of 4 rats were given either saline or DM1 (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 DM1 (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 DM1 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$_{1A}$-sensitive adenylyl 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-

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 serotonergic 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 Maayan, 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, 1990; 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 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 $\mu$M), ipsapirone (10 $\mu$M) or 8-OH-DPAT (10 $\mu$M) for 1 and 2 hr. After extensive washing (see “Materials and Methods”), the inhibition of cAMP production stimulated by vasoactive intestinal polypeptide (0.1 $\mu$M) plus forskolin (1 $\mu$M) was studied. None of the treatments modified the 5-HT$_{1A}$-induced adenylyl cyclase inhibition (fig. 5). Basal cAMP production was not modified by the treatments (see legend to fig. 5).
TABLE 1

Effect of chronic treatments of rats with antidepressants, electro convulsive shocks and 5-HT1A agonist on the forskolin-stimulated adenylyl cyclase activity and its inhibition, by 5-HT, in hippocampus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 (nM) Control</th>
<th>Treated</th>
<th>I50 % Control</th>
<th>Treated</th>
<th>Forskolin-Stimulated Adenylyl Cyclase Activity pmol/mg/min Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI 15 mg/kg, 16 days, tested 48 hr after*</td>
<td>39 ± 9 0</td>
<td>34 ± 3</td>
<td>37.3 ± 2.5</td>
<td>38.0 ± 0.7</td>
<td>181 ± 6</td>
<td>187 ± 11</td>
</tr>
<tr>
<td>15 mg/kg, 23 days, tested 24 hr after*</td>
<td>51 ± 4</td>
<td>42 ± 4</td>
<td>37.8 ± 1.3</td>
<td>37.3 ± 0.8</td>
<td>175 ± 6</td>
<td>181 ± 2</td>
</tr>
<tr>
<td>Clorgyline 1 mg/kg, 21 days, tested 2 hr after*</td>
<td>83 ± 4</td>
<td>86 ± 3</td>
<td>31.3 ± 1.2</td>
<td>26.1 ± 1.1*</td>
<td>210 ± 12</td>
<td>193 ± 8</td>
</tr>
<tr>
<td>Fluoxetine 10 mg/kg, 21 days, tested 48 hr after*</td>
<td>62 ± 6</td>
<td>68 ± 6</td>
<td>38.6 ± 1.7</td>
<td>36.0 ± 1.9</td>
<td>203 ± 6</td>
<td>192 ± 4</td>
</tr>
<tr>
<td>Ipsapirone 10 mg/kg, 20 days*</td>
<td>58 ± 7</td>
<td>60 ± 6</td>
<td>34.5 ± 1.6</td>
<td>36.4 ± 1.5</td>
<td>209 ± 7</td>
<td>193 ± 2</td>
</tr>
<tr>
<td>Tested 4 hr after</td>
<td>47 ± 7</td>
<td>40 ± 5</td>
<td>43.1 ± 2.0</td>
<td>42.1 ± 1.4</td>
<td>171 ± 4</td>
<td>189 ± 4</td>
</tr>
<tr>
<td>Tested 48 hr after</td>
<td>2.5 38.0</td>
<td>21 0</td>
<td>21 0</td>
<td>21 0</td>
<td>21 0</td>
<td>21 0</td>
</tr>
<tr>
<td>ECT 50 mA, 10 days, tested 48 hr after*</td>
<td>53 ± 3</td>
<td>59 ± 3</td>
<td>39.2 ± 0.8</td>
<td>34.5 ± 1*</td>
<td>194 ± 6</td>
<td>182 ± 7</td>
</tr>
</tbody>
</table>

* P < .05
# Values are the means ± S.E.M. Comparisons were made by a Student’s t test.

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-HT1A receptor-induced adenylyl cyclase, we do not confirm the results given by Newman and Lerer (1988), showing that ECT completely suppressed the 5-HT1A 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-HT1A receptor adenylyl cyclase system is not easily desensitized during an in vitro-induced receptor hyperstimulation. In vivo, we have confirmed this relative resistance to desensitization by directly stimulating the 5-HT1A receptor adenylyl cyclase system in primary cultures of hippocampal neurons. Incubating these neurons for 1 or 2 hr with 5-HT (10^{-6} M) did not desensitize the 5-HT1A 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-HT1A 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-HT1A receptors to desensitization is in accordance with the absence of effect of antidepressant treatment on 5-HT1 or 5-HT1A binding in several brain areas including hippocampus (Maggi and Enna, 1980;
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 0. 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 0. 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 0. 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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inhibition of the Electrical Activity of Hippocampus Neurons by 5-HT</th>
<th>Inhibition by 5-HT of Hippocampus Adenylyl Cyclase Observed in This Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5-HT1A agonists</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clorglyline</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ECT</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* De Montigny and Aghajanian, 1978; Gallager and Bunney, 1979.
* Blier et al., 1987
* Blier and De Montigny, 1987.
* Blier et al., 1986.
* De Montigny, 1984.

References


Glaser, T. and Traber, J.: Binding of the putative anxiolytic TVQX 7821 to

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-HT1A receptors (Andrade and Nicoll, 1987). As summarized in table 2, the effects of antidepressant treatments observed on 5-HT1A 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-HT1A 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-HT1A receptor transduction mechanisms, this may explain the differences in responses described in table 2. The second possibility is that the 5-HT1A-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-HT1A receptor level.

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