

Full Paper

Failure of Repeated Electroconvulsive Shock Treatment on 5-HT₄-Receptor-Mediated Depolarization Due To Protein Kinase A System in Young Rat Hippocampal CA1 NeuronsKumatoshi Ishihara^{1,*} and Masashi Sasa^{2,#}¹Department of Pharmacotherapy, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734-8551, Japan²Department of Pharmacology, Hiroshima University School of Medicine, Hiroshima 734-8551, Japan

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Abstract. We previously demonstrated that repeated electroconvulsive shock (ECS) treatment enhanced serotonin (5-HT)_{1A}- and 5-HT₃-receptor-mediated responses in hippocampal CA1 pyramidal neurons. The electrophysiological studies were performed to elucidate the effects of ECS treatment on depolarization, which was an additional response induced by 5-HT, and the second messenger system involved in this depolarization of hippocampal CA1 neurons. Bath application of 5-HT (100 μ M) induced depolarization of the membrane potential in the presence of 5-HT_{1A}-receptor antagonists. This depolarization was mimicked by 5-HT₄-receptor agonists, RS 67506 (1–30 μ M) and RS 67333 (0.1–30 μ M), in a concentration-dependent manner. 5-HT- and RS 67333-induced depolarization was attenuated by concomitant application of RS 39604, a 5-HT₄-receptor antagonist. H-89, a protein kinase A (PKA) inhibitor, inhibited 5-HT-, RS 67506-, and RS 67333-induced depolarizations, while forskolin (10 μ M), an activator of adenylate cyclase, induced depolarization. Furthermore, RS 67333-induced depolarization was not significantly different between hippocampal slices prepared from rats administered ECS once a day for 14 days and those from sham-treated rats. These findings suggest that 5-HT₄-receptor-mediated depolarization is caused via the cAMP-PKA system. In addition, repeated ECS-treatment did not modify 5-HT₄-receptor functions in contrast to 5-HT_{1A}- and 5-HT₃-receptor functions.

Keywords: serotonin (5-HT), 5-HT₄ receptor, depolarization, hippocampal CA1 region, electroconvulsive shock

Introduction

Serotonin (5-HT) in the central nervous system plays important roles in the regulation of mood (1). The hippocampus which is considered to be involved in mood control, receives dense serotonergic innervation originating in the medial raphe nucleus, and contains almost all subtypes of 5-HT receptors (2). Therefore,

when 5-HT is applied to a bath, where a hippocampal slice was placed, hippocampal CA1 neurons show complex responses such as hyperpolarization, depolarization, and/or increased spontaneous postsynaptic potentials (sPSP), as previously reported (3–5). In hippocampal CA1 neurons, hyperpolarization induced by 5-HT is mediated by 5-HT_{1A}-receptor activation (3, 6), and an increase in frequency of sPSP is due to an increase in GABA release from the terminal of interneurons where 5-HT₃ receptors are located (7, 8). 5-HT₄-receptor activation has been reported to decrease afterhyperpolarization and depolarize the membrane potential (9). 5-HT₄-receptor-mediated decrease in afterhyperpolarization is mediated by the cAMP-protein kinase A system (10), but the second messenger system

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involved in this depolarization of the resting membrane potentials remains to be determined. In the present study, the second messenger system involved in this depolarization was investigated using hippocampal slices with intracellular recording techniques.

In addition, electroconvulsive therapy (ECT) is effective in treating drug-resistant major depression (11, 12). We previously reported, in animal experiments, that repeated electroconvulsive shock (ECS) treatment increased 5-HT_{1A}-receptor-mediated hyperpolarization and the frequency of sPSP in hippocampal CA1 neurons via 5-HT₃ receptors located on interneurons (3, 5). However, there have been few studies on the ECS effects on 5-HT-induced depolarization. Therefore, we performed an electrophysiological study to determine whether or not repeated ECS treatment affects 5-HT-induced depolarization.

Materials and Methods

Animals

Fifty male Wistar rats obtained from Charles River Japan (Tokyo), between 4- and 7-week-old, were used. For the slice preparations in the ECS experiments, 5- to 6-week-old rats were used. Rats were housed in a shoe box cage in a 12-h light-and-dark cycle at the Animal Facility at the Hiroshima University School of Medicine until preparing hippocampal slices. The animal room was maintained at $23 \pm 2^\circ\text{C}$ and $55 \pm 5\%$ humidity. All experiments were approved by the Ethical Committee of Hiroshima University for animal experiments.

ECS-treatments

ECS (100-V intensity and 1-s duration) was administered to each animal through ear clips once daily for 14 days. Rats in the control group were handled and treated in a similar manner to the ECS group except for electroshock application. ECS or sham-treatments were started at the age of 3 weeks. Additional details were described previously (3).

Intracellular recording

Hippocampal slices (thickness: 450 μm) were made with a microslicer (DTK-1000; Dosaka EM, Kyoto) in ice-chilled artificial cerebrospinal fluid (ACSF). Slices were incubated at 34°C in ACSF for 1 h and then kept at room temperature until use. Each slice was transferred to the recording chamber into which ACSF was continuously perfused at a rate of 1.5–3 ml/min at room temperature. Intracellular recording was performed from the cells in the hippocampal CA1 pyramidal cell layer with a glass microelectrode filled

with 3 M KCl. Drugs dissolved in ACSF at final concentrations were applied via the bath perfusion system. The composition of ACSF was as follows: 113 mM NaCl, 3 mM KCl, 1 mM NaH₂PO₄, 2 mM CaCl₂, 1 mM MgCl₂, 25 mM NaHCO₃, and 11 mM D-glucose, aerated with 95% O₂ and 5% CO₂ mixture.

Drugs

5-HT creatinine sulfate (Research Biochemical International (RBI), Natick, MA, USA) was dissolved in ACSF. RS 67506 {1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-(2-methylsulphonylamino)ethyl-4-piperidinyl]-1-propanone HCl} (Tocris, Avonmouth, UK), RS 67333 {1-(4-amino-5-chloro-2-methoxyphenyl)-3-[1-butyl-4-piperidinyl]-1-propanone HCl} (Tocris), RS 39604 {1-[4-amino-5-chloro-2-(3,5-dimethoxyphenyl)methoxy]-3-[1-[2-methylsulphonylamino]ethyl]piperidin-4-yl]propan-1-one HCl} (Tocris), WAY-100,635 {N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexane-carboxamide maleate} (RBI), and LY 278,584 {1-methyl-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1H-indazole-3-carboxamide maleate} (RBI) were dissolved in distilled water at 10 mM and added to ACSF as final concentrations. H-89 {N-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide · 2HCl} (Biomol Res. Lab., Plymouth Meeting, PA, USA) and forskolin (Sigma-RBI, St. Louis, MO, USA) were dissolved in dimethylsulfoxide (DMSO). The final amount of DMSO in ACSF did not exceed 0.1%. The agonists and stimulant were applied to the bath for 5 or 10 min.

Statistics

Values are shown as means \pm S.E.M. Statistical significance was evaluated with Student's *t*-test.

Results

5-HT- and 5-HT₄-receptor agonists-induced depolarization

5-HT (100 μM) depolarized the membrane potential in the presence of WAY-100,635 (10 μM), a 5-HT_{1A}-receptor antagonist [in some cases LY278,584 (10 μM), a 5-HT₃-receptor antagonist, was also added]. RS 67506 (1–30 μM) and RS 67333 (0.1–30 μM), both 5-HT₄-receptor agonists (13), induced depolarization in a concentration-dependent manner (Figs. 1A and 2). 5-HT-induced depolarization in the presence of WAY-100,635 (10 μM) was 9.3 ± 1.5 mV ($n = 8$) (Fig. 2). This depolarization was antagonized by the concomitant application of RS 39604 (10 μM), a 5-HT₄-receptor antagonist (14) (Fig. 2). RS 67333-induced depolarization was also antagonized by concomitant application

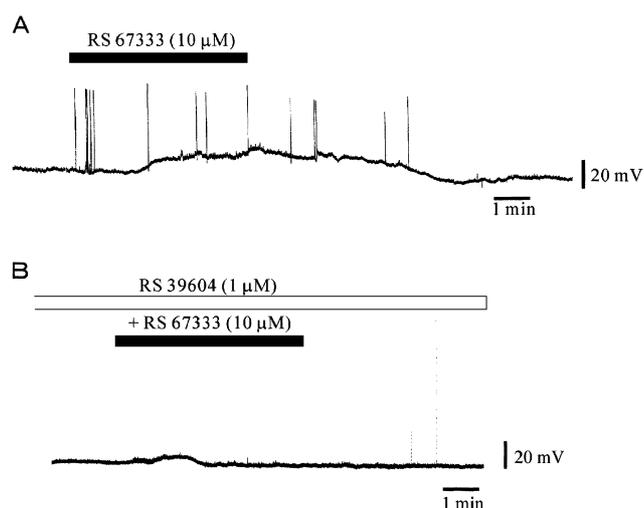


Fig. 1. 5-HT₄-receptor agonist-induced depolarization in hippocampal CA1 neurons. Depolarization was induced by RS 67333, a 5-HT₄-receptor agonist (A). RS 39604, a 5-HT₄-receptor antagonist, antagonized against RS 67333-induced depolarization (B). Drugs were applied during the period indicated with bars. Traces of A and B were obtained from different neurons.

of RS 39604 at 1 μ M (Figs. 1B and 2).

Second messenger systems involved in 5-HT₄-receptor-mediated depolarization

RS 67506-induced depolarization was attenuated by simultaneous application of H-89 (1 μ M) (Figs. 3A and 4), a protein kinase A inhibitor (15). 5-HT- and RS 67333-induced depolarization was also attenuated by H-89 (1 μ M) (Fig. 4). Forskolin (10 μ M), a stimulator of adenylate cyclase (16), caused similar depolarization of 12.7 ± 3.4 mV ($n = 12$) to 5-HT₄-receptor stimulation (Figs. 3B and 4), although DMSO (0.1%)

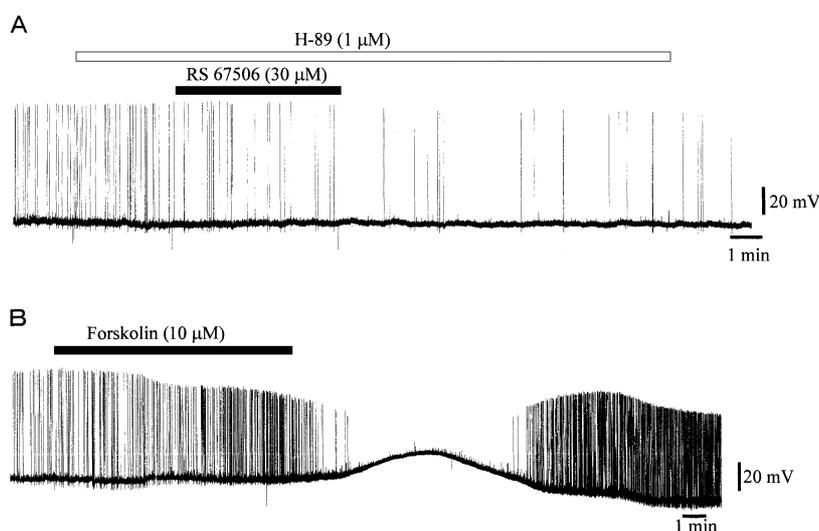


Fig. 3. Effects of H-89 (a protein kinase A inhibitor) on 5-HT₄-agonist-induced depolarization and effects of forskolin (an activator of adenylate cyclase). In the presence of H-89, RS 67506-induced depolarization was diminished (A). Forskolin (10 μ M)-induced depolarization in a hippocampal CA1 neuron (B). Traces of A and B were obtained from different neurons.

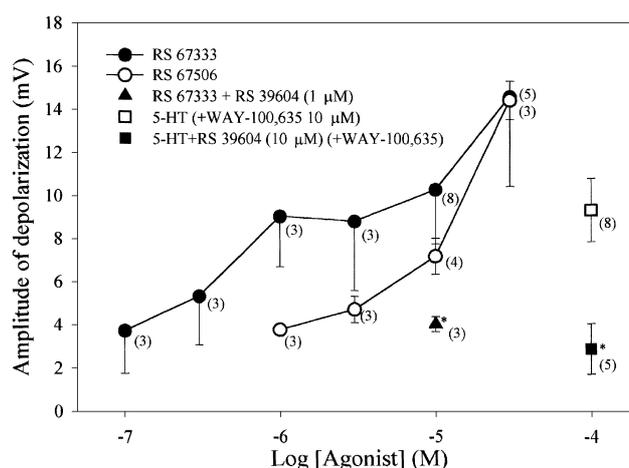


Fig. 2. Depolarization of hippocampal CA1 neurons induced by 5-HT and 5-HT₄-receptor agonists. The abscissa and ordinate represent the concentration of the agonist and the induced depolarization, respectively. Each symbol and bar shows the mean \pm S.E.M., respectively. The number in parentheses was the number of neurons tested. * $P < 0.05$, significantly different from values in the absence of RS 39604.

alone caused only a small depolarization of 4.0 ± 0.8 mV ($n = 5$) (Fig. 4).

Effects of ECS on 5-HT₄-receptor-mediated depolarization

There was no significant difference in the resting membrane potential of the neurons between the sham-operated and ECS-treated group: the resting membrane potentials were -56.3 ± 1.3 ($n = 3$) and -59.3 ± 2.0 mV ($n = 6$), respectively. The RS 67333 (10 μ M)-induced depolarization was 9.4 ± 4.6 mV ($n = 3$) in the slices prepared from rats from the sham-treated group. In the

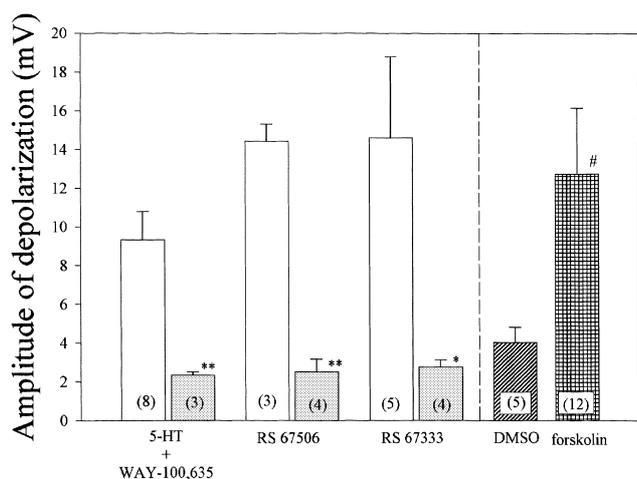


Fig. 4. Inhibitory effects of H-89 on 5-HT₄-agonist (5-HT, RS 67506, RS 67333)-induced depolarization and depolarizing effects of forskolin. Ordinate indicates the amplitude of depolarization induced by application of 5-HT (100 μ M), RS 67506 (30 μ M), RS 67333 (30 μ M), DMSO (0.1%), or forskolin (10 μ M). Open and shaded columns are the effects of 5-HT₄-agonists in the absence and presence of H-89 (1 μ M), respectively. Values for drug treatment without H-89 are the same as those in Fig. 2. Numbers in parentheses are the numbers of neurons tested. * P <0.05 and ** P <0.01, significantly different from the value in the absence of H-89. # P <0.05, significantly different from DMSO.

ECS treated rats, the RS 67333-induced depolarization was 7.8 ± 2.5 mV ($n=6$). There was no significant difference in 5-HT₄-receptor-mediated depolarization between the ECS and sham-treated groups.

Discussion

Hyperpolarization in CA1 neurons is usually observed during application of 5-HT and is followed by depolarization after termination of the application as demonstrated previously (4). In the presence of a 5-HT_{1A}-receptor antagonist (WAY-100,635), depolarization was evident during application of 5-HT in the hippocampal CA1 neurons, probably due to blockade of hyperpolarization mediated by 5-HT_{1A} receptors. This depolarization was antagonized by the 5-HT₄-receptor antagonist (RS 39604), suggesting that 5-HT-induced depolarization is mediated by 5-HT₄ receptors as reported previously (9, 17). In addition, the findings that 5-HT₄-receptor agonists, RS 67506 and RS 67333, induced RS 39604-reversible depolarization also suggests the existence of a 5-HT₄ receptor that mediates depolarization in the CA1 neurons. The 5-HT₄-receptor-mediated depolarization was suggested to be induced by activation of protein kinase A since the depolarization was blocked by H-89, a protein kinase A

inhibitor. This conclusion is supported by the additional finding that forskolin, a stimulator of adenylate cyclase, induced depolarization of CA1 neurons as observed with 5-HT₄ agonist. This finding is in line with biochemical observations that 5-HT₄ receptors are positively coupled to adenylate cyclase and the receptor stimulation increases cAMP in cytosol that activates protein kinase A (18–21). In addition, another 5-HT₄-mediated response, a decrease in the calcium-activated potassium current resulting in afterhyperpolarization, was also due to an activation of the protein kinase A system (9, 10). Thus, 5-HT₄-receptor activation could be suggested to cause two different excitatory responses through activation of protein kinase A system, resulting in a long-lasting depolarization. Recently, it was reported that 5-HT₄-receptor-mediated depolarization was not mediated by protein kinase A (22). This discrepancy from our results may be due to the possibility that 5-HT simultaneously acted on additional 5-HT-receptor subtypes such as 5-HT₆ and 5-HT₇, which can cause excitatory responses (23), although modulation of single neuronal activity through 5-HT₆ and 5-HT₇ receptors remains to be determined. In addition, the possibility that the depolarization pulse used by Chapin et al. (22) for simultaneous measurement of afterhyperpolarization may have affected a second messenger system in 5-HT₄-induced depolarization could not completely be excluded.

Repeated ECS treatment enhanced the 5-HT_{1A}-receptor-mediated hyperpolarization response (3) and the 5-HT₃-receptor-mediated increase in the frequency of spontaneous postsynaptic potentials probably due to release of GABA (5) in rat hippocampal CA1. However, 5-HT₄-receptor-mediated depolarization was found not to be altered following repeated ECS treatment in the present study. It seems unlikely that this result is due to the young rats (aged 5 weeks) used here since the 5-HT₄-receptor-mediated depolarization was not different from that obtained in age-matched sham-operated animal and the same treatment of the same aged animals potentiated 5-HT_{1A}- and 5-HT₃-receptor-mediated responses (3, 5). This negative finding suggests that ECS does not always cause general activation or sensitization of all 5-HT-receptor subtypes and/or the related second messenger system. Recently, Bijak et al. (24) reported that zacopride, a 5-HT₄-receptor agonist, induced a small depolarization, which was not changed by repeated ECS treatment, although the afterhyperpolarization was attenuated by the treatment (24). This observation is partly in line with our results that 5-HT₄-receptor-mediated depolarization with other agonist RS 67333 was not affected by repeated ECS treatment. However, the depolarization obtained by Bijak et al.

using zacopride was only 2 mV, which appears to be too small to evaluate the changes of the membrane potential by repeated ECS. In contrast, the depolarization obtained herein using RS 67333 was 8–10 mV, which was large enough to evaluate the change of membrane potential following repeated ECS. Thus, in more dominant depolarization, ECS was confirmed to not affect the 5-HT₄-receptor-mediated responses in our study. In addition, responses mediated by the same cAMP second messenger system due to activation by 5-HT₄ receptor are probably given different modulations by ECS treatment. This difference is considered to be due to the difference of final target ion channels affected by 5-HT₄-receptor activation. However, the reports on the final target ion channel mechanism to induce depolarization via 5-HT₄ receptors are still controversial and it remains to be determined (22, 25). The previous findings that repeated ECS enhances 5-HT_{1A}- and 5-HT₃-receptor-mediated responses, which are involved in the inhibition of excitability of CA1 pyramidal neurons, and lack of effects of ECS on depolarization mediated by 5-HT₄ receptor suggest that repeated ECS enhances the inhibitory system in the hippocampal CA1 region. Although in the physiological condition, the role of depolarization induced by 5-HT₄-receptor activation in hippocampal CA1 region need to be elucidated, it appears to be difficult at present to discuss such point because of coexistence of many 5-HT receptors including 5-HT_{1A}, 5-HT₂, 5-HT₃, and 5-HT₄ in the CA1 region and lack of data on 5-HT₄-receptor-mediated physiological responses except for depolarization. In conclusion, the significant finding here is that inhibitory regulation of neuronal activity by 5-HT is enhanced by ECS treatment, but not excitatory effects. This conclusion is supported by previous findings that repeated administration of some anti-depressants such as imipramine, citalopram, fluvoxamine, paroxetine, and repeated ECS treatment reduced 5-HT₄-receptor-mediated enhancement of population spikes in the hippocampal CA1 region (24, 26, 27).

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References

- Murphy DL. Neuropsychiatric disorder and the multiple human brain serotonin receptor subtypes and subsystems. *Neuropsychopharmacology*. 1990;3:457–471.
- Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev*. 1994;46:157–203.
- Ishihara K, Amano T, Hayakawa H, Yamawaki S, Sasa M. Enhancement of serotonin_{1A} receptor function following repeated electroconvulsive shock in young rat hippocampal neurons in vitro. *Int J Neuropsychopharmacol*. 1999;2:101–104.
- Ishihara K, Sasa M. 5-HT₄ receptor-mediated depolarization in hippocampal CA1 neurons. *Jpn J Pharmacol*. 2000;82 Suppl I:180P.
- Ishihara K, Sasa M. Potentiation of 5-HT₃ receptor functions in the hippocampal CA1 region of rats following repeated electroconvulsive shock treatments. *Neurosci Lett*. 2001;307:37–40.
- Andrade R, Nicoll RA. Pharmacologically distinct actions of serotonin on single pyramidal neurons of the rat hippocampus recorded in vitro. *J Physiol (Lond)*. 1987;394:99–124.
- Ropert N, Guy N. Serotonin facilitates GABAergic transmission in the CA1 region of rat hippocampus in vitro. *J Physiol (Lond)*. 1991;441:121–136.
- McMahon LL, Kauer JA. Hippocampal interneurons are excited via serotonin-gated ion channels. *J Neurophysiol*. 1997;78:2493–2502.
- Andrade R, Chaput Y. 5-Hydroxytryptamine₄-like receptors mediate the slow excitatory response to serotonin in the rat hippocampus. *J Pharmacol Exp Ther*. 1991;257:930–937.
- Torres GE, Chaput Y, Andrade R. Cyclic AMP and protein kinase A mediate 5-hydroxytryptamine type 4 receptor regulation of calcium-activated potassium current in adult hippocampal neurons. *Mol Pharmacol*. 1995;47:191–197.
- Avery D, Winokur G. The efficacy of electroconvulsive therapy and antidepressants in depression. *Biol Psychiatry*. 1977;12:507–523.
- Strober M, Rao U, DeAntonio M, Liston E, State M, Amaya-Jackson L, et al. Effects of electroconvulsive therapy in adolescents with severe endogenous depression resistant to pharmacotherapy. *Biol Psychiatry*. 1998;43:335–338.
- Eglen RM, Bonhaus DW, Johnson LG, Leung E, Clark RD. Pharmacological characterization of two novel and potent 5-HT₄ receptor agonists, RS 67333 and RS 67506, in vitro and in vivo. *Br J Pharmacol*. 1995;115:1387–1392.
- Hegde SS, Bonhaus DW, Johnson LG, Leung E, Clark RD, Eglen RM. RS 39604: a potent, selective and orally active 5-HT₄ receptor antagonist. *Br J Pharmacol*. 1995;115:1087–1095.
- Chijiwa T, Mishima A, Hagiwara M, Sano M, Hayashi K, Inoue T, et al. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. *J Biol Chem*. 1990;265:5267–5272.
- Seamon KB, Padgett W, Daly JW. Forskolin: unique diterpene activator of adenylate cyclase in membrane and in intact cells. *Proc Natl Acad Sci USA*. 1981;78:3363–3367.
- Torres GE, Holt IL, Andrade R. Antagonist of 5-HT₄ receptor-mediated responses in adult hippocampal neurons. *J Pharmacol Exp Ther*. 1994;271:255–261.
- Bockaert J, Sebben M, Dumuis A. Pharmacological characterization of 5-hydroxytryptamine₄ (5-HT₄) receptors positively

- coupled to adenylate cyclase in adult guinea pig hippocampal membranes: effect of substituted benzamide derivatives. *Mol Pharmacol.* 1990;37:408–411.
- 19 Dumuis A, Bouhelal R, Sebben M, Bockaert J. A 5-HT receptor in the central nervous system, positively coupled with adenylate cyclase, is antagonized by ICS 205 930. *Eur J Pharmacol.* 1988;146:187–188.
 - 20 Dumuis A, Bouhelal R, Sebben M, Cory R, Bockaert J. A nonclassical 5-hydroxytryptamine receptor positively coupled with adenylate cyclase in the central nervous system. *Mol Pharmacol.* 1988;34:880–887.
 - 21 Shenker A, Maayani S, Weinstein H, Green JP. Pharmacological characterization of two 5-hydroxytryptamine receptors coupled to adenylate cyclase in guinea pig hippocampal membranes. *Mol Pharmacol.* 1987;31:357–367.
 - 22 Chapin EM, Haj-Dahmane S, Torres G, Andrade R. The 5-HT₄ receptor-induced depolarization in rat hippocampal neurons is mediated by cAMP but is independent of I_h. *Neurosci Lett.* 2002;324:1–4.
 - 23 Chapin EM, Andrade R. A 5-HT₇ receptor-mediated depolarization in the anterodorsal thalamus. I. Pharmacological characterization. *J Pharmacol Exp Ther.* 2001;297:395–402.
 - 24 Bijak M, Zahorodna A, Tokarski K. Opposite effects of antidepressants and corticosterone on the sensitivity of hippocampal CA1 neurons to 5-HT_{1A} and 5-HT₄ receptor activation. *Naunyn Schmiedeberg Arch Pharmacol.* 2001;363:491–498.
 - 25 Gasparini S, DiFrancesco D. Action of serotonin on the hyperpolarization-activated cation current (I_h) in rat CA1 hippocampal neurons. *Eur J Neurosci.* 1999;11:3093–3100.
 - 26 Bijak M. Imipramine-induced subsensitivity to the 5-HT₄ receptor activation, a possible mediation via an alteration in the postreceptor transduction mechanism involving adenylate cyclase. *Pol J Pharmacol.* 1997;49:345–350.
 - 27 Bijak M, Tokarski K, Maj J. Repeated treatment with antidepressant drugs induces subsensitivity to the excitatory effects of 5-HT₄ receptor activation in the rat hippocampus. *Naunyn Schmiedeberg Arch Pharmacol.* 1997;355:14–19.