

## MODULATION OF [<sup>3</sup>H]-8-OH-DPAT BINDING TO RAT BRAIN MEMBRANES BY METAL IONS

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**Abstract.** The binding of [<sup>3</sup>H]-8-OH-DPAT to rat hippocampal and cortical membranes was specific and saturable with  $K_d = 0.87 \pm 0.18$  nM and  $K_d = 2.4 \pm 0.9$  nM, respectively. Guanine nucleotides decreased the [<sup>3</sup>H]-8-OH-DPAT binding affinity without significant influence on the number of binding sites. The radioligand binding affinity was only slightly affected by the pH in the interval from 6 to 9. The 5-HT<sub>1A</sub> receptors in cortical membranes were considerably more stable than in hippocampus, indicating that the lipid environment determines the stability of the receptor in these brain regions. All chlorides of monovalent metals studied at concentrations above 30 mM decreased the [<sup>3</sup>H]-8-OH-DPAT binding. A significant increase in 2 nM [<sup>3</sup>H]-8-OH-DPAT binding to hippocampal membranes was found in the presence of millimolar concentrations of MgCl<sub>2</sub>, CaCl<sub>2</sub>, BaCl<sub>2</sub>, MnCl<sub>2</sub>, CoCl<sub>2</sub>, and NiCl<sub>2</sub>, while the radioligand binding to cortical membranes was inhibited. It is proposed that different G proteins are coupled to 5-HT<sub>1A</sub> receptors in rat hippocampus and cerebral cortex.

**Key words:** 5-HT<sub>1A</sub> receptors, [<sup>3</sup>H]-8-OH-DPAT, rat hippocampus, rat cerebral cortex, stability, heavy metals.

### INTRODUCTION

The serotonin (5-HT) receptor family is the largest (over 30 receptors have been cloned) biogenic amine receptor family [1]. With the exception of 5-HT<sub>3</sub> receptors, which form an ion channel, all the other known subtypes belong to the superfamily of receptors coupled to G proteins [2]. Of these, the 5-HT<sub>1A</sub> receptor is of particular interest, because it has been implicated in depression, anxiety, panic disorder, and alcohol abuse [3]. This subtype has been also most extensively investigated and characterized in many pharmacological, biochemical, and electrophysiological experiments, especially since the introduction

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of the 5-HT<sub>1A</sub>-specific agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) [4, 5]. Both tritiated and iodinated derivatives of this ligand have been used to localize 5-HT<sub>1A</sub> receptors within the central nervous system of various mammalian species [6], and this is in good agreement with the distribution of the receptor mRNA [7]. In spite of the introduction of new 5-HT<sub>1A</sub>-specific radioligands such as [<sup>3</sup>H]WAY-100635 [8] and [<sup>3</sup>H]NAD-299 [9], [<sup>3</sup>H]-8-OH-DPAT has remained the only commercially available specific radioligand for the 5-HT<sub>1A</sub> receptors and it is widely used in pharmacological studies. However, the biochemical characterization of the [<sup>3</sup>H]-8-OH-DPAT binding to membranes of different brain regions has revealed different sensitivity to metal ions and guanine nucleotides [10, 11]. The obtained results indicate serious discrepancies with data of electrophysiological and behavioural experiments. In the present study we extended the comparative characterization of the [<sup>3</sup>H]-8-OH-DPAT binding to rat hippocampal and cortical membranes paying special attention to the stability and modulation by nucleotides and different ions.

## MATERIALS AND METHODS

8-Hydroxy-[<sup>3</sup>H]DPAT ([<sup>3</sup>H]-8-OH-DPAT, 220 Ci/mmol) was from Amersham Pharmacia Biotech and inorganic salts from Reakhim, Russia. Tris(hydroxymethyl)aminomethane (Tris), EDTA, Guanosine-O<sup>3</sup>-(3-thiotriphosphate) (GTPγS), N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulphonic acid) (Hepes), and 5-hydroxytryptamine (5-HT) were from Sigma Chemical Co.; methiothepine and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]-piperazine (NAN-190) were from RBI; and scintillation cocktail OptiPhase "HiSafe 3" from Wallac.

Rat hippocampi and cerebral cortices were homogenized in 20 vol (wet weight/volume) of ice-cold homogenization buffer (HB) containing 50 mM Tris-HCl (pH = 7.4) using a glass-teflon homogenizer, and centrifuged at 20 000 × g for 20 min at 4 °C. The membrane pellet was washed by resuspension in the HB and centrifuged under the same conditions. The obtained membranes were homogenized in 20 vol (ww/v) of the HB and incubated for 20 min at 25 °C to remove endogenous 5-HT. After the centrifugation of the solution at 20 000 × g for 20 min at 4 °C, the membrane pellet was washed once more as described above. The final pellet was homogenized in the incubation buffer containing 20 mM K-Hepes, 30 mM NaCl, 5 mM MgCl<sub>2</sub>, and 1 mM EDTA (pH = 7.4), if not otherwise stated, at a concentration of 25 mg wet tissue/mL, and used in the following experiments. For the equilibrium binding studies, the crude membrane homogenates (~2.5 mg wet tissue per assay) were incubated with 0.4–10 nM [<sup>3</sup>H]-8-OH-DPAT or, in the case of displacement experiments, with 2 nM [<sup>3</sup>H]-8-OH-DPAT and other ligands for 60 min at 25 °C, and the free ligand was removed by fast filtration through a glass-fibre filter (GF/B, Whatman International Ltd., Madistone, UK), which was pretreated with 0.3% (w/v)

polyethylenimine before use. The filters were washed three times with 3 ml of ice-cold washing buffer (20 mM KPBS, 100 mM NaCl, pH = 7.4), and the bound [<sup>3</sup>H]-8-OH-DPAT, trapped on the filter, was counted with a liquid scintillation counter. The specific binding was defined as the difference between total and nonspecific binding, which were measured in the absence and presence of 10 μM 5-HT, respectively.

In the experiments where the influence of inorganic salts was studied the membranes were homogenized in the buffer containing 20 mM K-Hepes and 1 mM EDTA (pH = 7.4).

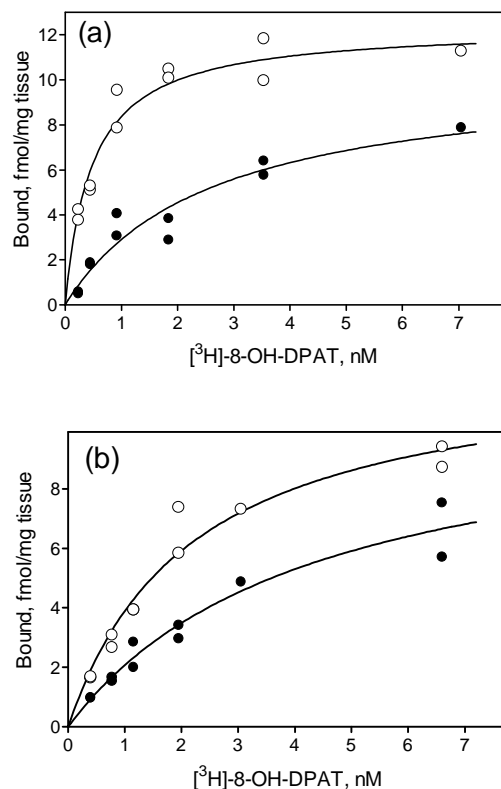
All binding data were analysed by nonlinear least-squares regression analysis by using a commercial program GraphPad PRISM™ (GraphPad Software, San Diego, CA, USA). The results are presented as mean ± SEM.

## RESULTS

### Affinity of the [<sup>3</sup>H]-8-OH-DPAT binding to rat brain membranes

The curve of the [<sup>3</sup>H]-8-OH-DPAT binding to rat hippocampal membranes was with a high affinity and saturable (Fig. 1a). The straight line obtained by Scatchard analysis indicates the presence of a single class of [<sup>3</sup>H]-8-OH-DPAT binding sites. The dissociation constant ( $K_d$ ) and maximal binding capacity ( $B_{max}$ ) were estimated to be  $0.87 \pm 0.18$  nM and  $14.3 \pm 1.4$  fmol/mg tissue, respectively. The affinity of the [<sup>3</sup>H]-8-OH-DPAT binding to cortical membranes was lower and with a smaller number of binding sites having  $K_d$  and  $B_{max}$  values of  $2.4 \pm 0.9$  nM and  $8.8 \pm 1.7$  fmol/mg tissue, respectively (Fig. 1b). The addition of the nonhydrolyzable GTP analogue GTPγS to the reaction medium caused a decrease in the affinity of [<sup>3</sup>H]-8-OH-DPAT to the level of  $K_d$  values of  $4.3 \pm 1.8$  nM in the hippocampal and  $5.1 \pm 1.3$  nM in the cortical membranes without significant influence on the number of binding sites. The studies of the [<sup>3</sup>H]-8-OH-DPAT binding were carried out at room temperature in the reaction medium that contained 20 mM Na-Hepes, 5 mM MgCl<sub>2</sub>, 1 mM EDTA, and 30 mM NaCl (pH = 7.4), proposed to be optimal for studies of agonist binding to G protein-coupled receptors [12].

Specific serotonergic ligands displaced the [<sup>3</sup>H]-8-OH-DPAT binding in concentration-dependent manner. As the Hill coefficients of the displacement curves were significantly below one, the nonlinear least squares analysis gave better approximation for the two-site model with corresponding binding constants (Table 1).



**Fig. 1.** Equilibrium of specific binding of [<sup>3</sup>H]-8-OH-DPAT to rat hippocampal (a) and cortical membranes (b) in the absence (o) or presence of 30 μM GTPγS (●). Rat brain membranes were incubated with different concentrations of [<sup>3</sup>H]-8-OH-DPAT in the absence (total binding) and presence (nonspecific binding) of 10 μM 5-HT for 60 min at 25 °C. Specific binding was defined as the difference between total and nonspecific binding.

**Table 1.** Affinities of serotonergic ligands in the displacement of the [<sup>3</sup>H]-8-OH-DPAT binding to rat hippocampal and cortical membranes

Ligand	Hippocampus			Cortex		
	$K_H$ , nM	$K_L$ , μM	$\alpha_H$	$K_H$ , nM	$K_L$ , μM	$\alpha_H$
Serotonin	8.5	13.1	$0.86 \pm 0.03$	15.1	6.2	$0.70 \pm 0.05$
NAN-190	75.4	67.7	$0.90 \pm 0.03$	26.1	4.5	$0.54 \pm 0.13$
Methiothepine	15.7	–	–	15.7	15.6	$0.18 \pm 0.05$

High- ( $K_H$ ) and low- ( $K_L$ ) affinity binding constants of ligands were calculated from the displacement curves against 2 nM [<sup>3</sup>H]-8-OH-DPAT according to the two-site binding model with corrections by the equation of Cheng–Prusoff [13];  $\alpha_H$  indicates the proportion of high affinity binding sites.

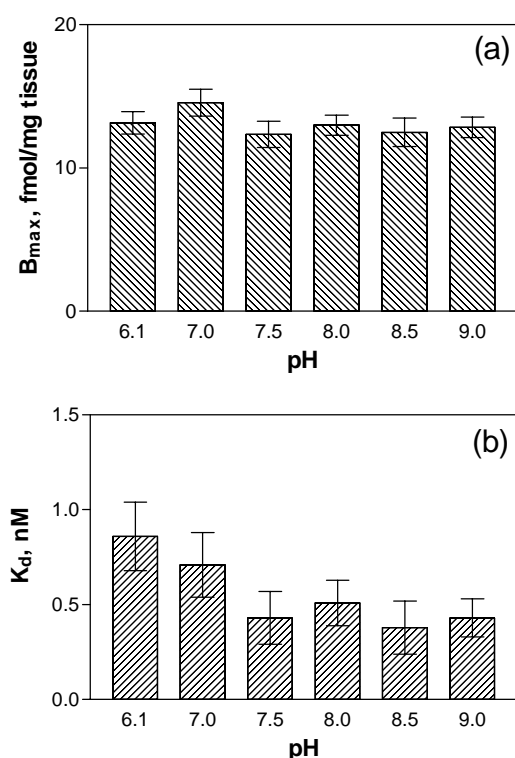
– no heterogeneity was detected in the displacement curve.

### Influence of pH on the [<sup>3</sup>H]-8-OH-DPAT binding

The influence of the pH value on the binding properties of the [<sup>3</sup>H]-8-OH-DPAT binding was studied at the interval from 6.1 to 9.0. There was no significant influence on the number of binding sites (Fig. 2a), but the affinity of the [<sup>3</sup>H]-8-OH-DPAT binding increased with the increase in the pH from 6.1 to 7.5. The additional increase of the pH up to 9.0 had no significant influence on the affinity of the [<sup>3</sup>H]-8-OH-DPAT binding (Fig. 2b). The pH value had no significant influence on the nonspecific binding of [<sup>3</sup>H]-8-OH-DPAT (data not shown) either.

### Stability of [<sup>3</sup>H]-8-OH-DPAT binding sites

The stability of the [<sup>3</sup>H]-8-OH-DPAT binding activity to rat brain membranes was measured at temperatures from 4°C to 37°C. The decrease in the specific binding of [<sup>3</sup>H]-8-OH-DPAT to hippocampal membranes was fast and depended on the incubation temperature. At 37°C the half-life of this process was



**Fig. 2.** Influence of pH on the parameters  $B_{max}$  (a) and  $K_d$  (b) of the [<sup>3</sup>H]-8-OH-DPAT binding to rat hippocampal membranes. Rat hippocampal membranes in 20 mM HEPES with 30 mM NaCl, 5 mM MgCl<sub>2</sub>, 1 mM EDTA at different pH values were incubated with different concentrations of [<sup>3</sup>H]-8-OH-DPAT in the absence (total binding) and presence (nonspecific binding) of 10  $\mu$ M serotonin for 60 min at 25°C. Specific binding was defined as the difference between total and nonspecific binding. Data are presented as mean  $\pm$  SEM.

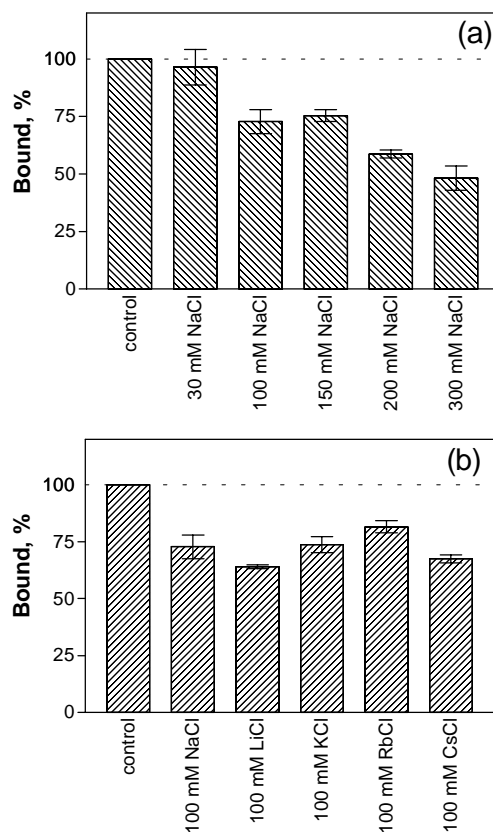
estimated to be  $\tau_{1/2} = 5.3$  min, while at  $25^\circ\text{C}$   $\tau_{1/2} = 29$  min, and at  $4^\circ\text{C}$   $\tau_{1/2} = 3.6$  h. The 5-HT receptors in rat cortical membranes were considerably more stable as the half-lives of the loss of the [ $^3\text{H}$ ]-8-OH-DPAT binding were estimated to be  $\tau_{1/2} = 3.4$  h at  $37^\circ\text{C}$ ,  $\tau_{1/2} = 11$  h at  $25^\circ\text{C}$ , and  $\tau_{1/2} = 10$  days at  $4^\circ\text{C}$ . The use of ascorbic acid as an antioxidant in the reaction medium [14] stabilized considerably the [ $^3\text{H}$ ]-8-OH-DPAT binding to rat hippocampal membranes to the values of half-lives  $\tau_{1/2} = 1.8$  h at  $37^\circ\text{C}$  and  $\tau_{1/2} = 41$  h at  $4^\circ\text{C}$ , but had no significant influence on the stability of cortical membranes. However, it has been reported that ascorbic acid inhibits considerably the [ $^3\text{H}$ ]-8-OH-DPAT binding to the membranes of hippocampus, cerebral cortex, and striatum [10], and it is not suggested to be used for the characterization of 5-HT<sub>1A</sub> receptors. The very short half-life of hippocampal 5-HT receptors at  $37^\circ\text{C}$  complicated the use of this temperature for the characterization of [ $^3\text{H}$ ]-8-OH-DPAT binding properties, and therefore  $25^\circ\text{C}$  was selected as the incubation temperature in this study.

The Arrhenius plot of the inactivation rate constants of the [ $^3\text{H}$ ]-8-OH-DPAT binding to rat hippocampal as well as to cortical membranes revealed straight lines with similar slopes, indicating a single mechanism of the inactivation process at all temperatures studied in both sources, despite the differences in rates. The activation energy was estimated to be  $77 \pm 9$  and  $92 \pm 9$  kJ/mol for membranes from rat hippocampus and cortex, respectively. These values are lower than expected for the inactivation of solubilized proteins and than found for brain cholecystokinin receptors and solubilized muscarinic receptors [15, 16], but in good agreement with the activation energy of the inactivation of muscarinic receptors in rat cortical membranes, in which case the determining role of surrounding lipids has been proposed [15].

### **Regulation of the [ $^3\text{H}$ ]-8-OH-DPAT binding to rat brain membranes by different cations of metals**

The influence of ionic strength on the binding of [ $^3\text{H}$ ]-8-OH-DPAT was studied in the buffer containing 20 mM K-Hepes and 5 mM  $\text{MgCl}_2$ . Sodium chloride at concentrations up to 30 mM had no significant influence on the number of specific binding sites on the rat hippocampal membranes labelled with 3 nM [ $^3\text{H}$ ]-8-OH-DPAT, but an increase in the salt concentration led to a decrease in the radioligand binding (Fig. 3a). This influence of NaCl seems to be nonspecific and caused by the ionic strength of the salt as all chloride salts of the first subgroup – LiCl, NaCl, KCl, RbCl, and CsCl – caused a similar decrease in the [ $^3\text{H}$ ]-8-OH-DPAT binding (Fig. 3b). The influence of sodium chloride on the [ $^3\text{H}$ ]-8-OH-DPAT binding to rat cortical membranes had a similar character, 100 mM NaCl decreasing the specific binding to the level of  $73 \pm 4\%$ .

Of two- and three-valent metals, the influence of the chlorides of  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Co}^{2+}$  on the binding of [ $^3\text{H}$ ]-8-OH-DPAT was studied.  $\text{CrCl}_3$ ,  $\text{FeCl}_3$ ,  $\text{ZnCl}_2$ , and  $\text{CuCl}_2$  inhibited the [ $^3\text{H}$ ]-8-OH-DPAT binding and at concentrations above 3 mM more than 90% of the specific binding was lost. A moderate effect was found for  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ , and  $\text{BaCl}_2$ ,



**Fig. 3.** Influence of monovalent cations on the specific binding of  $[^3\text{H}]\text{-8-OH-DPAT}$ . Rat hippocampal membranes in 20 mM K-Hepes with 5 mM  $\text{MgCl}_2$ , 1 mM EDTA were incubated with 3 nM  $[^3\text{H}]\text{-8-OH-DPAT}$  in the presence of different concentrations of NaCl (a) or 100 mM of different salts (b) for 60 min at 25 °C. Specific binding of  $[^3\text{H}]\text{-8-OH-DPAT}$  is presented as the percentage of specific binding in the absence of the added salt (control). Data are presented as mean  $\pm$  SEM.

which increased the  $[^3\text{H}]\text{-8-OH-DPAT}$  binding to hippocampal membranes at concentrations up to 2 mM, but when the concentration was further increased the binding returned to the control level (Table 2). The effect of  $\text{MnCl}_2$ ,  $\text{CoCl}_2$ , and  $\text{NiCl}_2$  was more dramatic, causing almost a doubling of the  $[^3\text{H}]\text{-8-OH-DPAT}$  binding to hippocampal membranes at millimolar concentrations of the salts (Table 2). None of the salts studied increased the specific binding of 3 nM  $[^3\text{H}]\text{-8-OH-DPAT}$  to rat cortical membranes and millimolar concentrations of  $\text{MgCl}_2$  caused a significant decrease in the radioligand binding (Table 2).

**Table 2.** Influence of salts on the binding of 3 nM [<sup>3</sup>H]-8-OH-DPAT to rat hippocampal and cortical membranes

Salt	Concentration, mM	Hippocampus, %	Cerebral cortex, %
MgCl <sub>2</sub>	1	116 ± 18	71 ± 26
	3	103 ± 1	51 ± 5
	5	100 ± 3	41 ± 7
	15	103 ± 5	43 ± 6
CaCl <sub>2</sub>	1.5	148 ± 15	ND
	3	ND	64 ± 1
	5	109 ± 12	53 ± 16
	15	78 ± 1	ND
BaCl <sub>2</sub>	1.5	147 ± 5	ND
	5	102 ± 28	ND
MnCl <sub>2</sub>	1	169 ± 11	68 ± 9
	2	194 ± 3	ND
	3	ND	20 ± 7
CoCl <sub>2</sub>	1	178 ± 5	ND
	2	147 ± 18	ND
NiCl <sub>2</sub>	1	132 ± 6	111 ± 3
	1.5	149 ± 28	ND
	3	127 ± 17	23 ± 7

Rat hippocampal or cerebral cortical membranes in 20 mM K-Hepes were incubated with 3 nM [<sup>3</sup>H]-8-OH-DPAT in the presence of salts in different concentrations for 60 min at 25 °C. Specific binding of [<sup>3</sup>H]-8-OH-DPAT is presented as the percentage of specific binding in the absence of the added salt (control). Data are presented as mean ± SEM. ND, not determined.

## DISCUSSION

For the characterization of G protein coupled receptors it is important to have specific and high-affinity radioligands. For the 5-HT<sub>1A</sub> receptors, [<sup>3</sup>H]-8-OH-DPAT has remained the only commercially available radioligand and it is therefore widely used in pharmacological studies. In the present study we paid attention to the regulation of the [<sup>3</sup>H]-8-OH-DPAT binding by different factors in *in vitro* studies. Comparison of binding parameters between two brain regions revealed considerably higher affinity and number of binding sites in hippocampal membranes than in cortical membranes. The addition of GTPγS, a nonhydrolyzable GTP analogue, caused a decrease in the affinity of the [<sup>3</sup>H]-8-OH-DPAT binding in both regions of the brain and the difference in the affinities disappeared. Thus it can be predicted that [<sup>3</sup>H]-8-OH-DPAT labels high- as well as low-affinity binding sites of 5-HT<sub>1A</sub> receptors and in hippocampal membranes more receptors are able



to couple with nucleotide-free G proteins to generate high-affinity binding [17]. The involvement of both high- and low-affinity binding sites is suggested also by the heterogeneity of displacement curves (Table 1).

The binding studies at different pH levels indicated that within a quite wide pH range the number of binding sites was not affected. Of course, some influence was found for the  $K_d$  values, suggesting that the pH should be kept above 7 in the experimental medium. However, in general terms we can conclude that the binding of [ $^3$ H]-8-OH-DPAT is effective at a very wide pH interval. This allowed us to keep the pH at 7.4 for the experiments to be comparable with the data available in the literature. Such relative insensitivity toward the pH level is quite common among G protein-coupled receptors, indicating the lack of weak ionic centres in the ligand binding pocket [18, 19].

Stability studies revealed a very fast and temperature dependent inactivation of the [ $^3$ H]-8-OH-DPAT binding sites in the hippocampal membranes, where half of the bindings were lost within 5.3 min at 37°C. The receptors in cortical membranes were considerably more stable, with 3.4 h half-life under the same conditions. The binding to hippocampal membranes could be stabilized by ascorbic acid, which led the half-life of the receptors to be 1.8 h, but had no significant influence on the stability of the cortical receptors. Thus, there seem to be essential differences between the factors determining the binding abilities of the 5-HT<sub>1A</sub> receptors in these two regions of the brain. The temperature dependence of the stability of the [ $^3$ H]-8-OH-DPAT binding sites revealed relatively low activation energies of the inactivation process, proposing that an important role in keeping active conformation of the receptors is played by the surrounding lipids [15]. It can be suggested that these lipids in the hippocampus can be easily oxidized, causing so loss of binding activity. In cortical membranes the fatty acids of coupled lipids are more saturated, thus giving better stability to the receptors, and binding does not require the stabilizing effect of ascorbic acid.

Like in the case of temperature, the influence of metal cations on the [ $^3$ H]-8-OH-DPAT binding to hippocampal and cortical membranes was very different. Thus, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, and Ni<sup>2+</sup> increased the [ $^3$ H]-8-OH-DPAT binding to hippocampal membranes in concentration-dependent manner, but inhibited the binding to cortical membranes at all concentrations studied. Mg<sup>2+</sup> is usually required for the high-affinity agonist binding to the G<sub>i</sub> protein-coupled receptors [20, 21]. It is proposed that 5-HT<sub>1A</sub> receptors are coupled to G<sub>o/i</sub> proteins and in reconstituted systems there is a proposed rank of preference G<sub>iα3</sub>>G<sub>iα2</sub>>G<sub>iα1</sub>>>G<sub>oα</sub>>>G<sub>sα</sub> [22]. However, the subtypes of G proteins coupled in different brain regions are not known and it can be only speculated that the very different sensitivity to metal ions of the [ $^3$ H]-8-OH-DPAT binding to hippocampal and cortical membranes is connected to different G proteins coupled to 5-HT<sub>1A</sub> receptors in these regions.

In summary we can conclude that the [ $^3$ H]-8-OH-DPAT binding to hippocampal and cortical membranes is similarly specific and with high affinity, but very differently regulated by the surrounding membranes and G proteins.

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## METALLIOONIDE MÕJU [<sup>3</sup>H]-8-OH-DPAT-i SIDUMISELE ROTI AJU MEMBRAANIDELE

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Serotoniin 1A retseptori spetsiifiline agonist [<sup>3</sup>H]-8-OH-DPAT seondub nii roti ajukoore kui ka hipokambi membraanidele kõrge afiinsusega, mida iseloomustavad vastavad dissotsiatsioonikonstandid  $K_d = 2,4 \pm 0,9$  nM ja  $K_d = 0,87 \pm 0,18$  nM. Guanüülnukleotiidid vähendasid [<sup>3</sup>H]-8-OH-DPAT sidumise afiinsust, kuid mitte sidumiskohtade arvu. Inkubatsioonipuhvri pH varieerimine vahemikus 6–9 ei mõjutanud oluliselt selle radioligandi ajumembraanidele sidumise omadusi. Serotoniin 1A retseptorid ajukoores olid oluliselt stabiilsemad kui hipokambis ja võib oletada, et erinevus on põhjustatud erinevatest lipiididest, mis stabiliseerivad retseptorit aju eri osades. Kõik uuritud leelismetallide kloriidid kontsentratsioonil üle 30 mM põhjustasid [<sup>3</sup>H]-8-OH-DPAT sidumise vähenemist, kuid olulisi erinevusi ei olnud metallide ega ka ajuosade vahel. Millimolaarses kontsentratsioonis suurendasid MgCl<sub>2</sub>, CaCl<sub>2</sub>, BaCl<sub>2</sub>, MnCl<sub>2</sub>, NiCl<sub>2</sub> ja CoCl<sub>2</sub> 2 nM [<sup>3</sup>H]-8-OH-DPAT sidumist hipokambi membraanidele, kuid inhibeerisid seda sidumist ajukoore membraanidele. Eeldatakse, et serotoniin 1A retseptorid ajukoore ja hipokambi membraanides on seotud erinevate G-valkudega, mis põhjustab ka erineva tundlikkuse metalliioonide suhtes.