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Selective decline of 5-HT$_{1A}$ receptor binding sites in rat cortex, hippocampus and cholinergic basal forebrain nuclei during aging

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Abstract

The effect of aging on 5-HT$_{1A}$ receptor binding in several forebrain areas associated with the basal forebrain cholinergic system was investigated in rats of 3-, 24- and 30-months-old by receptor autoradiography and biochemical binding assay using [3H]8-OH-DPAT as a ligand. Autoradiographic measurements demonstrated a marked region-specific decline of ligand binding in: (i) regions of the basal forebrain cholinergic cell groups, i.e. the medial septum, diagonal band nuclei and magnocellular nucleus basalis, (ii) the frontal and parietal neocortex and (iii) the dentate gyrus of the hippocampus. No change or only a slight decrease of the 5-HT$_{1A}$ receptor density was found in other areas investigated: the CA1 and CA3 sectors of hippocampus, the cingular and perirhinal cerebral cortex and the lateral septum. The autoradiographic findings were substantiated by the biochemical binding assay, which revealed a comparable loss of 5-HT$_{1A}$ receptor in the hippocampus and neocortex at the age of 30 months. The results clearly show that with increasing age the decrement of 5-HT$_{1A}$ receptor binding in the rat forebrain is remarkably region-selective and particularly affects the cholinergic cell groups that innervate cortex and hippocampus. This phenomenon appears to be especially significant in relation to the neuronal substrates underlying the age-related alterations of mood and cognition. © 1997 Elsevier Science B.V.

Keywords: 5-HT$_{1A}$ receptor; Aging; Rat forebrain; Autoradiography; Binding assay

1. Introduction

The functional decline of the forebrain serotonergic (5-HT) system has been implicated in several disturbances that become manifest with aging and in aging-related diseases, including cognitive deficits and affective disorders (Mann and Yates, 1983; Gottfries, 1990; K ahn et al., 1990; Luine et al., 1990; Westenberg and den Boer, 1994), disturbances in food intake, sleep, and sexual behavior (Curzon, 1990; Glennon, 1990). The physiological effects of the neurotransmitter 5-HT are mediated by an impressive number of post- and pre-synaptic receptor types (Boess and Martin, 1994). For several reasons, the 5-HT$_{1A}$ subtype appears to be of special importance in view of its presumed role in...
affective disorders and cognition. The 5-HT$_{1A}$ receptor has been proposed as the neural substrate underlying mood and emotional state (McEntee and Crook, 1992; Artigas et al., 1996). Secondly, 5-HT probably via its 1A receptor, being dense in entorhinal cortex and hippocampus, has been implicated in rhythmic slow activity, which is associated with learning and memory processes (VanderWolf and Baker, 1986; McEntee and Crook, 1992; Pompeiano et al., 1992).

The 5-HT$_{1A}$ receptor appears to be particularly relevant to neuropathology because of the generally hyperpolarizing and inhibitory neuronal effects induced by 5-HT$_{1A}$ agonists (Sprouse and Aghajanian, 1987; Khateb et al., 1993). It is this receptor characteristic that has implicated the 5-HT$_{1A}$ receptor as a target for neuroprotective strategies in excitotoxic brain damage (Oosterink et al., 1966; Prehn et al., 1993; Strosznajder et al., 1996). It is anticipated that 5-HT$_{1A}$ drugs in a similar fashion may be relevant to progressive neuronal decline as in aging and symptoms of anxiety and depression which are common in Alzheimer’s disease (A.D.) (McEntee and Crook, 1992; Eastwood and Riesberg, 1996).

It is, however, not well known how in humans the 5-HT$_{1A}$ receptor is affected by aging and A.D. Some studies reported a reduction of this receptor subtype in postmortem A.D. brain (Middlemiss et al., 1986; Cross et al., 1988). In the rat there is only a limited number of studies on aging of 5-HT$_{1A}$ receptor systems, and none extending its attention to the aging process up to senescence.

Not only the breakdown of cholinergic neurotransmission (Whitehouse et al., 1981) but also the concurrent reduction of 5-HT function has been associated with the pathogenesis of A.D. (Curcio and Kemper, 1984; Yamamoto and Hirano, 1985). A number of behavioral and pharmacological studies point to a functional interaction between these two neurotransmitter systems in learning (Nilsson et al., 1988; Normile et al., 1990; Riekkinen et al., 1990; Cassel and Jeltsch, 1995). For those reasons our primary interest was directed towards the 5-HT$_{1A}$ receptor in the forebrain cholinergic cell groups in addition to hippocampus and cerebral cortex being the brain regions that are of pivotal in learning and memory functions.

To assess age-related changes in 5-HT$_{1A}$ receptor density the selective ligand 8-hydroxy-2-(di-n-propylamino)tetralin ([3H]8-OH-DPAT) (Sijbesma et al., 1991) was used in autoradiographic and biochemical binding studies, comparing young rats (3 months) with old (24 months) and senescent (30 months) animals. A marked region-selective decline in binding was found in aged rats especially in the areas of forebrain cholinergic cell nuclei, neocortex and dentate gyrus of the hippocampus.

2. Materials and methods

2.1. Animals

Male Wistar rats of three different ages, i.e. 3, 24, and 30 months were used in the present study. The animals were housed five per cage in an air conditioned room with an artificial 12:12 light-dark cycle (lights on from 07:00 h). Standard laboratory chow and water were available ad libitum. The rats were decapitated and the brains removed rapidly from the skull, immediately frozen on powdered dry ice and stored at $-80^\circ$C.

In case of biochemical determinations the hippocampus and the frontoparietal cortex of 3- and 30-month-old Wistar rats were dissected from the brain, and the dissected tissues frozen and stored at $-80^\circ$C until further processing.

2.2. Quantitative autoradiography

[3H]8-OH-DPAT binding to brain sections was performed according to published methods (Sijbesma et al., 1991) with some minor modifications. Briefly, coronal sections of 20 $\mu$m were cut on a cryostat microtome and thaw-mounted onto gelatin-coated glass slides. The mounted sections, after 30 min pre-incubation at room temperature (RT), were incubated in 0.17 M Tris–HCl, pH 7.6, containing 4 mM CaCl$_2$, 0.01% ascorbic acid and 10 $\mu$M pargyline in the presence of 1.5 nM [3H]8-OH-DPAT (2(N,N-di[2,3-(n)-$^3$H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene, s.a. 221 Ci/mmol, Amersham TRK 850) for 60 min at RT. Following incubation the slides were washed in incubation buffer (2 x 15 min) at 4°C and dried in a stream of cold air. Non-specific binding was determined in the presence of 1 $\mu$M 5-HT. Sections were exposed to $^3$H-sensitive film (Hyperfilm, Amersham) and exposed at RT for 2 months.

2.3. Densitometric analysis

 Autoradiograms were quantified double blind by using an automatic image analysis system (Quantimet 600, Leica, Cambridge). After shading and background correction the optical density of [3H]8-OH-DPAT binding was determined and expressed as tissue equivalent (nCi/mg brain tissue) according to the Autoradiographic [3H]Micro-scales of Amersham (RPA 506). Specific binding was calculated by subtracting tissue equivalent values of non-specific binding from those of specific binding in each selected region separately. Every sixth case was processed for non-specific binding measurements. The final results are expressed in fmol [3H]8-OH-DPAT bound to a mg tissue. The brain regions were selected for measurement according to the atlas of Paxinos and Watson (1982): (1) cornu
Ammonis (CA1–CA 3) regions and dentate gyrus (DG) of the dorsal hippocampus 3.30 mm posterior to bregma (−3.30), (2) lateral septum (LS) 0.30 mm anterior to bregma (0.30), (3) cholinergic cell groups in medial septum (MS) and ventral diagonal band of Broca (VDB, 0.30), horizontal diagonal band of Broca (HDB) and the anterior region of magnocellular nucleus basalis-substantia innominata (MNB-SI, −1.00), (4) frontal (FC) and parietal neocortex (PC, 0.70 and −3.30, respectively), and (6) cingulate (CC) and perirhinal cortical (PR) areas (0.70 and −3.30, respectively). Within the hippocampus the optical densities were measured for the hippocampus proper in stratum oriens and radiatum-molecularare, and for the dentate gyrus in the outer (oml) and inner (iml) molecular layers as well as in the hilus. The receptor density in the CA2 area was very low and therefore this region was not included in the present study but served as a border to delineate CA1 and CA3 sectors. Similarly to CA2, the pyramidal cell bodies, which were lightly labeled served to indicate the border between the stratum oriens and stratum radiatum-molecularare (rad+) mol in CA1 and CA3. At each brain region two sections were measured bilaterally without knowledge of the case condition. The mathematical average of the four measurements were used for the statistical analysis of the individual data. For documentation of individual autoradiograms, computerized images were prepared on the hippocampal and septal areas of the brain with the Quantimet 600 software ‘TIF’ package, which were printed there after with the aid of a Polaroid CI-5000S diaprint instrument.

2.4. Biochemical binding assay

Determination of [3H]8-OH-DPAT binding to 5-HT1A receptors in membrane fragments was carried out with a previously published method (Hall et al., 1985) with some modifications. Tissue samples were homogenized in 20 volumes of ice-cold 0.32 M sucrose in 50 mM Tris–HCl buffer, pH 7.4, and centrifuged at 10,000 × g for 10 min. The pellet was resuspended in Tris buffer containing 4 mM CaCl2 and pre-incubated at 37°C for 10 min in order to eliminate the endogenous 5-HT. This suspension was centrifuged again at 30,000 × g for 15 min and the final pellet was resuspended in 10 volumes Tris buffer containing 4 mM CaCl2, 10 μM pargyline and 0.1% ascobic acid. The number of 5-HT1A binding sites was measured in a 500 μl incubation mixture containing 8 nM [3H]8-OH-DPAT for 10 min at 37°C in a temperature-controlled shaking apparatus. The reaction was stopped by 10 ml ice-cold Tris buffer. The samples were rapidly filtered on Whatman GF/B glass fiber papers which were subsequently washed twice with 5.5 ml buffer. Bound radioactivity retained on the filter was measured in 5 ml Bray cocktail. The protein concentration of the membrane samples was measured from 10-μl aliquots using a Bio-Rad protein micro-assay. Non-specific binding was determined in the presence of 0.5 μM 8-OH-DPAT.

2.5. Statistics

Statistical analysis of data was performed with one-way ANOVA which was followed by paired comparisons of groups with the post-hoc t-test applying the STATS statistical package (Statsoft, 1985). For the biochemical binding data the Student’s t-test was used. Results are expressed as means ± SEMs.

3. Results

3.1. Regional distribution of autoradiographic [3H]8-OH-DPAT binding as a function of age

The age dependent and regional differences in the distribution of [3H]8-OH-DPAT binding sites are shown in Table 1. The number of 5-HT1A receptor sites diminished with age only in selected forebrain areas. In the hippocampus the decline of [3H]8-OH-DPAT binding was restricted to the dentate gyrus. Both in the outer and inner molecular layers (oml and iml) significant decrements were measured. In the ‘oml’ the decrease reached significance only at the age of 30 months. At the ‘iml’ the decrease was present at both old ages but was more pronounced in senescence (30 months) as compared to the 24-month-old animals (t = 2.81, P < 0.02, post-hoc t-test after ANOVA). In the hilar region, which largely corresponds to the CA4 area of the hippocampus proper, there was also a marked loss of 5-HT1A binding at both 24- and 30-months of age. In the CA3 area a slight decrease was visible at the age of 24 months, but this change did not reach the statistically significant level. No sign of any change in binding sites was found in CA1, the largest hippocampal CA sector having the highest receptor density within the hippocampus proper. The numerical results obtained in the hippocampus, presented in Table 1, is well illustrated by the randomly selected examples of autoradiograms comparing the three ages (Fig. 1). The most prominent decline in binding activity is present indeed in the ‘iml’ of DG, followed by the hilar region. The density in CA1 remains high even in the senescent rat. CA2 shows very limited DPAT binding and the density of label in CA3 is also far less intense as compared to CA1 or DG. The highest contrast in change of ligand binding in the hippocampus, therefore, could be observed between CA1 and the ‘iml’ of DG, which points a region-specific loss of 5-HT1A receptor binding within the hippocampus.
Table 1
Age dependency of 5-HT<sub>1A</sub> receptor binding in different brain regions measured with quantitative autoradiography

<table>
<thead>
<tr>
<th>Region</th>
<th>Age (months)</th>
<th>% of young age</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 (n = 7)</td>
<td>24 (n = 5)</td>
<td>30 (n = 7)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornu Ammonis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1 rad + mol</td>
<td>78.73 ± 2.53</td>
<td>70.59 ± 4.39</td>
<td>75.70 ± 3.12</td>
</tr>
<tr>
<td>CA1 oriens</td>
<td>66.92 ± 2.94</td>
<td>60.41 ± 4.12</td>
<td>66.20 ± 2.35</td>
</tr>
<tr>
<td>CA3 rad + mol</td>
<td>22.58 ± 2.71</td>
<td>16.56 ± 0.77</td>
<td>20.81 ± 2.22</td>
</tr>
<tr>
<td>CA3 oriens</td>
<td>22.31 ± 3.35</td>
<td>17.74 ± 1.22</td>
<td>22.08 ± 2.40</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>omi</td>
<td>104.89 ± 6.74</td>
<td>89.05 ± 4.34</td>
<td>78.73 ± 4.62**</td>
</tr>
<tr>
<td>imi</td>
<td>93.85 ± 4.34</td>
<td>70.50 ± 4.80**</td>
<td>56.06 ± 2.26**</td>
</tr>
<tr>
<td>hilus</td>
<td>50.54 ± 4.66</td>
<td>36.11 ± 2.49*</td>
<td>32.08 ± 2.35**</td>
</tr>
<tr>
<td>Lateral septum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>101.95 ± 6.15</td>
<td>96.52 ± 9.46</td>
<td>86.11 ± 7.96</td>
</tr>
<tr>
<td>LS I</td>
<td>72.31 ± 4.71</td>
<td>53.85 ± 2.49*</td>
<td>49.50 ± 4.07**</td>
</tr>
<tr>
<td>Cholinergic nuclei</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M S</td>
<td>24.89 ± 3.21</td>
<td>15.38 ± 2.17**</td>
<td>10.36 ± 0.95**</td>
</tr>
<tr>
<td>VDB</td>
<td>25.16 ± 4.43</td>
<td>17.69 ± 2.90</td>
<td>15.43 ± 1.49*</td>
</tr>
<tr>
<td>HDB</td>
<td>22.71 ± 2.40</td>
<td>16.70 ± 3.53*</td>
<td>16.20 ± 1.00*</td>
</tr>
<tr>
<td>MNB-SI ant</td>
<td>10.45 ± 1.18</td>
<td>4.71 ± 0.54**</td>
<td>5.07 ± 0.63**</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neocortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC (M o) deep</td>
<td>33.52 ± 2.13</td>
<td>24.62 ± 0.90**</td>
<td>24.30 ± 1.31**</td>
</tr>
<tr>
<td>FC (M o) sup</td>
<td>17.15 ± 1.99</td>
<td>12.90 ± 0.81*</td>
<td>11.99 ± 0.54**</td>
</tr>
<tr>
<td>PC (SS) deep</td>
<td>21.04 ± 1.58</td>
<td>10.14 ± 2.40**</td>
<td>9.91 ± 0.81**</td>
</tr>
<tr>
<td>PC (SS) sup</td>
<td>6.70 ± 1.18</td>
<td>3.76 ± 1.67</td>
<td>4.07 ± 1.04</td>
</tr>
<tr>
<td>Limbic cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC deep</td>
<td>31.90 ± 3.44</td>
<td>23.17 ± 2.08</td>
<td>29.05 ± 2.40</td>
</tr>
<tr>
<td>CC sup</td>
<td>18.10 ± 3.03</td>
<td>14.07 ± 1.72</td>
<td>18.96 ± 2.13</td>
</tr>
<tr>
<td>Paleocortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR deep</td>
<td>25.02 ± 2.08</td>
<td>22.71 ± 2.35</td>
<td>23.98 ± 1.63</td>
</tr>
<tr>
<td>PR sup</td>
<td>10.14 ± 0.54</td>
<td>7.67 ± 2.35</td>
<td>8.69 ± 1.09</td>
</tr>
</tbody>
</table>

Values are fmol [³H]8-OH-DPAT bound to 1 mg wet tissue (Means ± SEM).

*P < 0.05; **P < 0.01 vs. 3-month-old rats (post-hoc t-test after ANOVA).

The 'F' distribution values were calculated by ANOVA (STATS program).

The lateral septum (LS), and especially its dorsal subdivision (LSD) revealed an extremely dense [³H]8-OH-DPAT binding (Table 1, see also Fig. 2 and Fig. 3). Of the two labeled regions of the LS, the age-dependent decline of radioactive label was only significant in the intermediate part (LSI), which was already present at the age of 24 months.

Regions of the cholinergic forebrain nuclei (Fig. 2) showed a substantial degree of 5-HT<sub>1A</sub> agonist binding. The density of 5-HT<sub>1A</sub> receptor sites was comparable in the regions of medial septum (MS), the vertical and horizontal diagonal bands of Broca (VDB and HDB), and became more diluted posterior to these nuclei at the level of magnocellularis nucleus basalis (MNB-SI) (Table 1). In all four regions a marked and significant loss of receptor binding was observed in the old and senescent rats. In the region of MS there was a further decline in the binding sites from the old to the senescent age (t = 2.70, P < 0.02). In fact, among the brain regions analyzed, the largest decrease in [³H]8-OH-DPAT binding was present in MS, i.e. 38% in the old and 58% in the senescent rats. The massive decrease in labeling in both MS and VDB regions is clearly demonstrated at the ages of 24- and 30 months compared to young animals (Fig. 3). The darkly labeled LS, however, seems to resist the aging process.

The effect of aging on 5-HT<sub>1A</sub> receptors in the developmentally distinct regions of the cerebral cortex, the frontal and parietal neocortex and the phylogenetically older cingulate and perirhinal cortex revealed a striking contrast (Table 1). In the autoradiograms a differentiation in deep and superficial bands could be easily distinguished corresponding approximately to the cellular layers of V and VI, and I-IV, respectively. In the frontal cortex (FC, primary motor cortex), the decrease in labeling varied between 25-30% in the old and senescent rats. In the parietal cortex (PC, primary somatosensory cortex), the loss of 5-HT<sub>1A</sub> receptors appeared even more marked and varied between 39-53%. The superficial layers in PC showed a very low level of...
Fig. 1. Photomicrographs of autoradiograms at the level of dorsal hippocampus (3.30 mm posterior to bregma) are shown from rats of 3-, 24- and 30-months of age. Scale bar = 1 mm.

Fig. 2. Position and extent of areas measured autoradiographically to quantify [3H]8-OH-DPAT binding sites on the fields covered by cholinergic cell bodies (black dots). The densities of cholinergic cell bodies were determined as described previously (Luiten et al., 1987; Nyakas et al.). Panel A is 0.30 mm anterior to bregma, panel B is positioned 1.00 mm posterior to bregma.

Fig. 3. Photomicrograms, taken at the level of septal nuclei, show a marked decrease in grain density in MS and VDB regions on autoradiograms of [3H]8-OH-DPAT binding in 24- and 30-month-old rats compared to the 3-month-old young adult control. Scale bar = 1 mm.
labeling as compared to the deeper layers. This might be the reason that, although there was 46 and 39% percent decrease in old ages, this change was statistically not significant. The reduction of \[^3H\]8-OH-DPAT binding in FC and PC can also be appreciated from Fig. 1, although the neocortical areas which were measured do not correspond to the fragments shown by the figure (see Section 2). In any case, in both FC and PC there is a marked decline in 5-HT1A receptor density with advancing age. In the phylogenetically older cortical areas, perirhinal and cingular cortices (PR and CC), there was no significant decrease found in the older rats compared to the young group (Table 1).

### 3.2. \[^3H\]8-OH-DPAT binding to brain homogenates

The number of \[^3H\]8-OH-DPAT binding sites measured in the presence of a saturating concentration of the labeled ligand was lower in both the hippocampus and frontoparietal neocortex of senescent rats (Table 2). The reductions were 23 and 37% respectively, and statistically significant (hippocampus: \(t = 2.81, P < 0.02\); neocortex: \(t = 2.31, P < 0.05\)).

### 4. Discussion

The novel and most striking findings of the present investigation are a marked region-selective and age-dependent decrease in \[^3H\]8-OH-DPAT binding and a probable loss of 5-HT1A receptors in several forebrain areas associated with the basal forebrain cholinergic system, and in the dentate region of the hippocampus. These results, which were substantiated by both autoradiographic and biochemical binding experiments, indicate that the expression of 5-HT1A receptors is likely to be subject to different age-related factors and a sensitive measure of the action of such factors.

Previous experimental data on age-related changes in serotonergic receptors in rats are very scarce. In an earlier autoradiographic study (Gozlan et al., 1990) no changes were found in \[^3H\]8-OH-DPAT binding in the dentate gyrus, frontoparietal cortex and raphe nuclei in 10- and 22-month-old rats. As is indicated by the present findings, it may well be concluded that the decline of 5-HT1A receptor expression is manifested relatively late in the life of laboratory rats and over the age of 22 months. This conclusion is further supported by a previous biochemical binding study claiming a reduction of the maximal binding of \[^3H\]8-OH-DPAT in the cerebral cortex of 24-month-old rats (Huguet et al., 1994).

Within the two major subdivisions of the hippocampus, the dentate gyrus and the cornu Ammonis, a consistent decrease in receptor binding could only be observed in the DG, whereas the CA regions were unaffected by the aging process. In both ages studied, i.e. 24- and 30 months, a loss of 5-HT1A receptor binding was observed in the dentate ‘iml’ and the hilus, which comprises also the CA4 sector. By the age of 30 months the decrease was also significantly extended to the outer molecular layer. The CA1 and CA3 sectors maintained their 5-HT1A receptor density at the level of young controls up to the senescent age of 30 months. These results are interpreted as a selective loss of 5-HT1A receptor sites in DG during aging. Such selective receptor changes in the dentate gyrus agree well with the unique regulation of hippocampal 5-HT1A receptors by corticosterone in rats. Chronic corticosterone treatment suppresses the expression of 5-HT1A receptor mRNA selectively in the dentate gyrus (M ejer and De K loet, 1994) and induces a reduction of \[^3H\]8-OH-DPAT binding that is confined to the dentate gyrus and the oriens and lacunosum moleculare layers of CA4 (M endelson and M Ce won, 1992). It may be an obvious conclusion that the higher basal plasma corticosterone level and the prolonged plasma corticosterone stress response during aging (H ess and R eggle, 1970; Brett et al., 1983; Sapolsky et al., 1983; Van E eken et al., 1991; K ort et al., 1992, 1996) may be a major factor underlying the age-dependent selective loss of 5-HT1A receptors in the dentate gyrus.

One of the main questions of the present investigation was the effect of high age on 5-HT1A receptor expression in the areas of cell groups of the cholinergic basal forebrain that are the origin of the cholinergic innervation of cortex and hippocampus (Mesulam et al., 1983; Luiten et al., 1987; N yakas et al., 1987; G aykema et al., 1990). A large reduction of \[^3H\]8-OH-DPAT binding was found in all four areas explored: MS, VDB and HDB and the MNB-SI (Fig. 2). Compared to the of 5-HT1A receptors in the lateral septal nuclei and espe-
cially its dorsal subdivision, the loss of the serotonin receptors was much more pronounced in the MS-VDB region (see also Fig. 3). It was recently reported that approximately a quarter of the cholinergic cells in MS and VDB are immunoreactive to 5-HT$_{1A}$ receptor antibodies (Kia et al., 1996). Also the mRNA coding for 5-HT$_{1A}$ receptors is clearly expressed in MS and VDB regions even more so than in the lateral septal nuclei (Pompeiano et al., 1992). The anatomical data summarized above indicate that the presence of 5-HT$_{1A}$ receptors on basal forebrain cholinergic neurons will be of direct importance for the regulation of cholinergic projections to hippocampus and neocortex. Such a direct impact of serotonergic influence on cholinergic forebrain activity may be, at least partly, responsible for the remarkable functional interaction of the two transmitter systems in the organization of cognitive behaviour (Nilsson et al., 1988; Normile et al., 1990; Riekkinen et al., 1990; Cassel and Jeltsch, 1995). The generalized decrease of 5-HT$_{1A}$ receptor binding in the frontal and parietal neocortex and the selective decline in the dentate gyrus as found in the present study, might influence ACh release locally contributing to the learning and memory decline found in aged rats. In summary, cholinergic neurons are regulated by 5-HT$_{1A}$ receptor sites twofold: directly through somatodendritic synapses at the level of the cholinergic nuclei (Sprouse and Aghajanian, 1987; K hateb et al., 1993), and probably indirectly at the level of cortical target areas (Bianchi et al., 1990; Izumi et al., 1994). What the characteristic decline of 5-HT$_{1A}$ receptor binding at these two levels mean for the functional properties of cholinergic neurons during aging is only partly understood and will certainly require further study.

There is some recent biochemical evidence suggesting that $[^3H]$B-OH-DPAT exclusively binds to G-protein coupled receptors (Emerit et al., 1990; Gozlan et al., 1995). Although the regional distribution of specific $[^3H]$B-OH-DPAT binding sites in the rat’s brain, measured autoradiographically, precisely matches that of another 5-HT$_{1A}$ ligand, the antagonist $[^3H]$WAY-100635 which binds to both G-protein coupled and uncoupled receptors (Gozlan et al., 1995), it can not be excluded at the moment that during aging there is a selective decline of 5-HT$_{1A}$ receptors coupled to G-protein. To resolve this question, however, further study is required comparing autoradiographic binding of $[^3H]$B-OH-DPAT to that of other selective 5-HT$_{1A}$ antagonists or agonists recognizing the different forms of 5-HT$_{1A}$ receptors.

Previous data on the fate of 5-HT$_{1A}$ receptors in aged humans and AD is rather limited. Some investigations have shown a decline in 5-HT$_{1A}$ receptor densities in certain neocortical and hippocampal areas. Dillon et al. (1991) with autoradiographic binding of $[^3H]$B-OH-DPAT in postmortem brain, found a decrease with age in a number of areas within the frontoparietal cortex and in the dentate gyrus. Interestingly, in this human study there was also no change found in the CA fields during aging similar to in our present findings in rats. However, both 5-HT$_{1A}$ receptor binding and 5-HT$_{1A}$ receptor mRNA level were found to be decreased in the hippocampus of old patients in another study (Burnet et al., 1994). The number of $[^3H]$B-OH-DPAT binding sites were reported to decrease in the temporal cortex (Middelmiss et al., 1986) and in precentral and postcentral cortical gyrus homogenates (Dillon et al., 1991) in aged humans compared to young individuals.

In conclusion, we found that 5-HT$_{1A}$ receptor binding decreases with age in rats in a region selective way. The specific loss of receptors found in the dentate gyrus within the hippocampus raises the possibility that exogenous factors, like an increased corticosterone production, may play a role in this age-related receptor down-regulation. Regarding the anatomical substrates for learning and memory it is remarkable that 5-HT$_{1A}$ receptors strongly decreased in the neocortex and in the basal forebrain cholinergic nuclei, but were preserved in the limbic- and paleocortical areas and in the dorso-lateral septal area. It may be concluded that the striking decline of 5-HT$_{1A}$ receptor binding on cholinergic neurons may have significant consequences for the regulation of cognitive and affective behaviours during aging. The availability of a suitable 5HT$_{1A}$ ligand for PET now provide a way to study the fate of this receptor in vivo in humans, which is subject to our present investigation.

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References


Strosznajder, J., Chalimoniuk, M., Samochoki, M., 1996. Activation of serotonergic 5-HT$_{1A}$ receptor reduces Ca$^{2+}$- and glutamatergic receptor-evoked arachidonic acid and NO/cGMP release in adult hippocampus. Neurochem. Int. 28, 439–444.


