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Regional Serotonin$_{1A}$ Receptors in the CNS of Alcohol-Preferring and -Nonpreferring Rats

W. J. McBRIDE,[1] X.-M. GUAN, E. CHERNET, L. LUMENG AND T.-K. LI

Departments of Psychiatry, Medicine and Biochemistry and Molecular Biology, Indiana University School of Medicine, Institute of Psychiatric Research, and VA Medical Center, Indianapolis, IN 46202-4887

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McBRIDE, W. J., X.-M. GUAN, E. CHERNET, L. LUMENG AND T.-K. LI. *Regional serotonin$_{1A}$ receptors in the CNS of alcohol-preferring and -nonpreferring rats.* PHARMACOL BIOCHEM BEHAV **49**(1) 7-12, 1994. – The densities of serotonin$_{1A}$ (5-HT$_{1A}$) receptors, labeled with [^{3}H]8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), were examined in the CNS of alcohol-naive adult male alcohol-preferring (P) and -nonpreferring (NP) rats using quantitative autoradiography. The densities of sites labeled with 2 nM [^{3}H]8-OH-DPAT were a) 20–30% higher in the medial prefrontal, frontal (layers 1, 2, and layers 3–6), parietal (layers 3–6), and cingulate cortex; b) 35–40% higher in the retrosplenial, occipital (all layers), temporal (all layers) cortex; and c) 15% higher in the entorhinal cortex of the P compared with the NP rat. Within the hippocampus, significant differences between the rat lines were observed only in the posterior portion where the densities of [^{3}H]8-OH-DPAT labeled sites were a) 10–15% higher in the dorsal dentate gyrus, dorsal CA1, and dorsal CA3 regions; and b) 15–25% higher in the anterior ventral hippocampal area and ventral dentate gyrus of the P relative to the NP line. In contrast to the above results, the densities of [^{3}H]8-OH-DPAT labeled sites were 15–20% lower in the dorsal, paradorsal, and median raphe nuclei of the P compared with the NP rat. No differences in [^{3}H]8-OH-DPAT binding between the rat lines were found in several basal ganglia, limbic, and brain stem regions. The data indicate that there are greater numbers of postsynaptic 5-HT$_{1A}$ receptors in certain parts of the cerebral cortex and hippocampus of the P compared with the NP rat. In addition, the lower densities of 5-HT$_{1A}$ cell body autoreceptors in the raphe nuclei suggest that there are fewer 5-HT neurons in the raphe nuclei of the P than of the NP rat.

Serotonin$_{1A}$ receptors Alcohol preference Autoradiography 8-Hydroxy-2-(di-*n*-propylamino)tetralin

THE alcohol-preferring (P) and alcohol-nonpreferring (NP) lines of rats have been selectively bred for their disparate alcohol drinking characteristics (13). Several studies have indicated that there are differences in the serotonin (5-HT) system between the P and NP rats and that administration of agents that alter the activity of the 5-HT system can have an effect on alcohol intake of the P rat.

The contents of 5-HT and/or its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) are significantly lower in several CNS regions (e.g., cerebral cortex, hippocampus, nucleus accumbens, etc.) of alcohol-naive P compared with NP rats (18,19). The lower levels of 5-HT and/or 5-HIAA in the P line may be due to lower 5-HT innervation because there are fewer immunostained 5-HT fibers in several of the same brain regions of the P relative to the NP rats (29). Neuropharmacological studies provide evidence that the lower 5-HT innervation may be an important factor contributing to the high alcohol drinking characteristics of the P rats because agents that increase the synaptic levels of 5-HT or mimic the actions of 5-HT can reduce alcohol self-administration by the P rats (16,17).

Higher densities (higher B_{max} values) of 5-HT$_{1A}$ receptors, labeled with [^{3}H]8-OH-DPAT, in membrane preparations from the cerebral cortex and hippocampus have been reported for the P compared with the NP line (28). However, because these rats had been exposed to alcohol during their preference

[1] Requests for reprints should be addressed to Dr. W. J. McBride, Institute of Psychiatric Research, 791 Union Drive, Indianapolis, IN 46202-4887.

testing phase, the study was repeated using alcohol-naive P and NP rats. In the repeated study, higher densities of 5-HT_{1A} receptors, labeled with [^{3}H]8-OH-DPAT, were found in membrane preparations from the cerebral cortex of P compared with NP rats although no differences were observed between the lines in membrane preparations from the hippocampus, striatum, or hypothalamus (27). The difference between the two studies in the results with the hippocampal membranes was attributed to prior chronic ethanol exposure of the rats in the first experiment (27).

The results of Wong et al. (27,28) do not indicate if the differences in the densities of 5-HT_{1A} sites between the rat lines exists throughout all regions and layers of the cerebral cortex or are localized to certain areas within the cortex. In addition, it is possible that there may be small localized differences within the hippocampus, hypothalamus, and/or striatum between the P and NP rats that may be masked when using membrane preparations from the whole tissue. Therefore, the present study was undertaken to determine the densities of 5-HT_{1A} sites, labeled with [^{3}H]8-OH-DPAT, within precisely defined CNS regions of alcohol-naive P and NP rats using quantitative autoradiography.

METHOD

Alcohol-naive adult male P and NP rats (weighing approximately 300–350 g) were used. These lines have been selectively bred for over 30 generations and their alcohol drinking characteristics are well established (14). Animals were housed individually in a temperature-controlled room with a 12L : 12D cycle (lights on at 0600) for at least 1 week prior to killing.

Rats (n = 7 per line) were killed by decapitation; the brains were rapidly removed and placed in liquid N_2 for 8 s. Frozen brains were stored in sealed freezer bags at −70°C until they were sectioned.

Frozen brains were placed in a cryostat (set at −20°C) for approximately 1 h prior to sectioning (Reichert-Jung Cryostat Microtome, Cambridge Instruments, Buffalo, NY). Sections (20 μm thick) were prepared and mounted on subbed slides (six sections/slide). A total of 72 sections from selected regions were prepared from each brain (36 sections for total binding and 36 adjacent sections from nonspecific binding). Mounted sections were stored at −70°C until brain sections from all P and NP rats had been prepared. Generally, sections were stored for 7 to 10 days before incubating with the radioactive ligand. An additional 36 sections were prepared for histological examination following staining with cresyl violet.

The incubation procedure for labeling 5-HT_{1A} sites for autoradiographic analysis has been previously described (21). Briefly, sections were preincubated for 30 min at room temperature in 0.17 M Tris-HCl buffer, pH 7.6, containing 4 mM $CaCl_2$ and 0.01% ascorbate. Following the preincubation step, sections were transferred to a Tris-HCl buffer solution containing 2 nM [^{3}H]8-OH-DPAT (229 Ci/mmol; Amersham, Arlington Heights, IL) and incubated for 60 min at room temperature. Nonspecific binding was determined in the presence of 100 nM 5-HT under the same incubation conditions as used for total binding. Following this incubation, sections were transferred to ice-cold Tris-HCl buffer solutions and given two 10-min rinses to remove unbound [^{3}H]8-OH-DPAT. Sections were then briefly dipped in ice-cold distilled water and dried with a stream of cold, dry air. Sections (20 μm thick) from 10 frozen brain paste standards covering a wide range of radioactivity were also mounted on subbed slides and dried under a stream of cold, dry air. Brain sections from P and NP rats, along brain paste standards, were placed in standard x-ray cassettes (10 × 12 in.) and apposed to [^{3}H]Ultrofilm (LKB, Mager Scientific, Dexter, MI). Each cassette contained one complete set of standards and sections, for total and nonspecific binding, from the same brain regions of P and NP

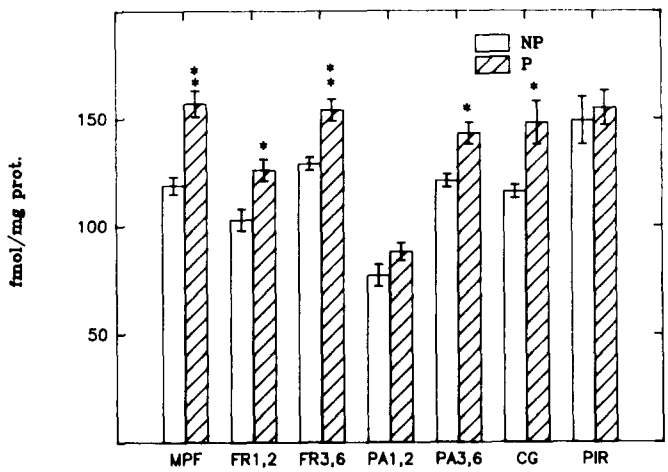

FIG. 1. Densities of 5-HT_{1A} recognition sites labeled with [^{3}H]8-OH-DPAT in subregions of the anterior cerebral cortex of P and NP rats. Data represent the means ± SEM of seven animals/line for each subregion. Statistical significance was determined with the two-tailed Student's t-test. *$p < 0.010$ and **$p < 0.001$. Abbreviations used: MPF, medial prefrontal cortex; FR, frontal cortex (layers 1 and 2 and layers 3 through 6); PA, parietal cortex; CG, cingulate cortex; PIR, piriform cortex.

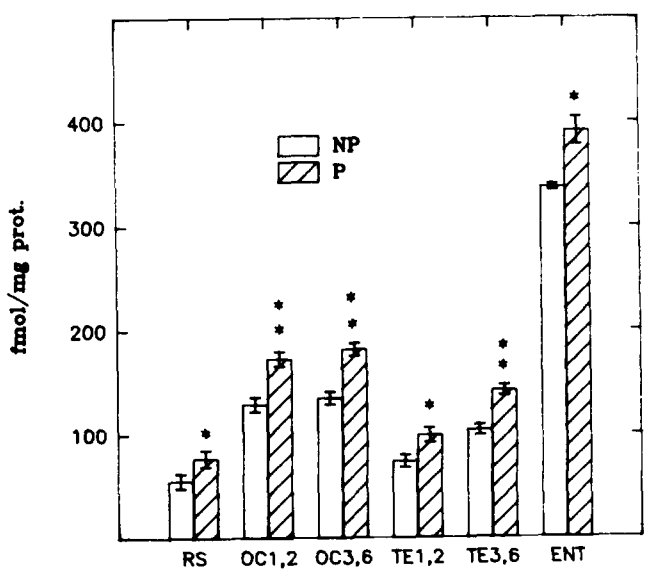

FIG. 2. Densities of 5-HT_{1A} recognition sites labeled with [^{3}H]8-OH-DPAT in subregions of the posterior cerebral cortex of P and NP rats. Data are the means ± SEM of seven animals/line for each subregion, except ENT where only three animals/line were used. *$p < 0.05$ and **$p < 0.001$ with Student's t-test. Abbreviations used: RS, retrosplenial cortex; OC, occipital cortex (layers 1 and 2 and layers 3 through 6); TE, temporal cortex; ENT, entorhinal cortex.

rats. Cassettes were stored at 4°C for 30 days prior to developing and fixing the Ultrofilm.

Quantitative autoradiography was carried out with an AIC micro-image analysis system (Analytical Imaging Concepts Inc., Atlanta, GA). Three to six bilateral measurements were made for the majority of nuclei and subregions to obtain total and nonspecific binding values. Values for specific binding were determined by subtracting nonspecific from total binding for adjacent sections. The mean of the three to six values for specific binding in each nucleus or subregion was determined and used to represent the value obtained from an individual animal. These mean values for equivalent areas in different animals were grouped according to rat line and statistically analyzed (unpaired two-tailed student *t*-test). Brain areas were identified using the atlas of Paxinos and Watson (20).

RESULTS

The densities of 5-HT$_{1A}$ sites labeled with [^{3}H]8-OH-DPAT were significantly higher in five of the seven subregions measured in the anterior portion of the cerebral cortex of P compared with NP rats (Fig. 1). The densities of 5-HT$_{1A}$ sites were 20 to 30% higher in the medial prefrontal cortex, frontal cortex (layers 1, 2, and layers 3-6), layers 3-6 of the parietal cortex and cingulate cortex of the P rats. No differences were found in layers 1 or 2 of the parietal cortex or in the piriform cortex between the two rat lines.

In the posterior portion of the cerebral cortex, the levels of 5-HT$_{1A}$ sites were 35-40% higher in the retrosplenial cortex, occipital cortex (layers 1, 2, and layers 3-6) and temporal cortex (layers 1, 2, and layers 3-6) of the P relative to the NP animals (Fig. 2). The densities of 5-HT$_{1A}$ sites were also higher in the entorhinal cortex of the P rat but the difference between the lines was smaller (15%). This smaller difference may be due to the limitations in reading films where the densities for total binding are very high and near film maximal saturation levels. In addition, many sections containing the entorhinal cortex were damaged and, as a result, reliable readings on four of the seven animals were not possible.

TABLE 1

DENSITIES OF 5-HT$_{1A}$ RECOGNITION SITES LABELED WITH [^{3}H] 8-OH DPAT IN BASAL GANGLIA AND SELECTED LIMBIC AREAS OF ALCOHOL-NAIVE P AND NP RATS

	fmol/mg prot (Mean ± SEM)	
Region	NP (n = 7)	P (n = 7)
Caudate Putamen	11 ± 1	15 ± 1*
Globus Pallidus	35 ± 6 (5)	26 ± 5 (6)
Nucleus Accumbens		
lateral	10 ± 1 (5)	15 ± 2 (6)*
medial	22 ± 2 (6)	28 ± 3 (6)
Olfactory Tubercle	61 ± 5 (6)	67 ± 3
Bed N. Stria Terminalis	121 ± 9	116 ± 7
Septal Nuclei		
lateral dorsal	359 ± 7	347 ± 17
lateral intermediate	392 ± 6	374 ± 16
medial	138 ± 5	140 ± 5
Hypothalamus		
medial preoptic area	115 ± 5	114 ± 5 (6)
lateral preoptic area	67 ± 7	66 ± 9 (6)
anterior area	104 ± 16 (4)	103 ± 16 (4)
lateral area	61 ± 6	66 ± 6
dorsomedial nucleus	119 ± 5 (5)	106 ± 8 (4)
ventromedial nucleus	145 ± 9 (6)	160 ± 6 (6)
Amygdala	152 ± 14	171 ± 19

*$p < 0.05$.

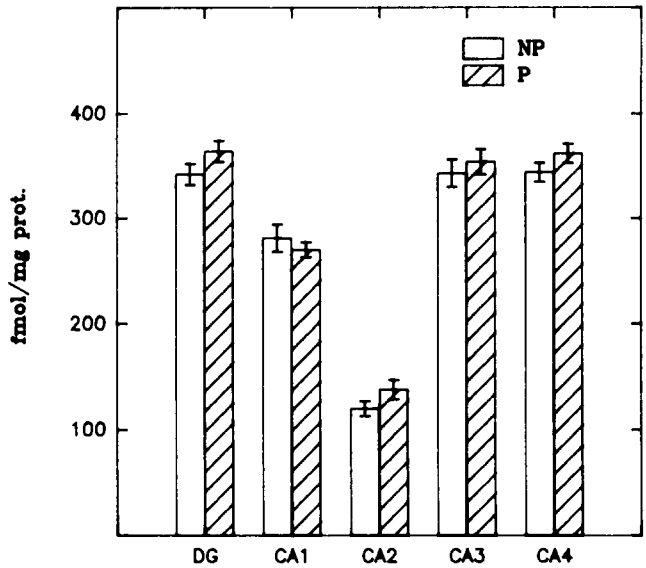

FIG. 3. Densities of [^{3}H]8-OH-DPAT labeled 5-HT$_{1A}$ sites in subregions of the anterior dorsal hippocampus of P and NP rats. Data are the means ± SEM of six animals/line for each subregion. No statistically significant differences were found between the rat lines for any subregion. Abbreviations used: DG, dentate gyrus.

Very high densities of 5-HT$_{1A}$ sites were observed in the lateral dorsal and lateral intermediate septal nuclei but there were no differences between the P and NP rats (Table 1). In addition, many other regions (i.e., hypothalamic nuclei, amygdala, etc.) which had high to medium levels of 5-HT$_{1A}$ sites, did not show a difference between the rat lines (Table 1). However, two regions (i.e., caudate-putamen and lateral nucleus accumbens), which had very low densities of 5-HT$_{1A}$ sites, did have higher values in the P compared with the NP line (Table 1). Other regions with low densities of 5-HT$_{1A}$ receptors did not have any differences between the rat lines (Table 1).

The density of 8-OH-DPAT binding was very high in the hippocampus. In the anterior dorsal portion of the hippocampus, the dentate gyrus, CA3, and CA4 regions had the highest densities, followed by the CA1 region, with the CA2 area having the lowest (Fig. 3). However, there were no significant differences between the P and NP rats in the values for any of these subregions in the anterior dorsal hippocampus (Fig. 3). On the other hand, statistically significant differences were found between the rat lines in six of the seven subregions measured in the posterior hippocampus (Fig. 4). The densities of 5-HT$_{1A}$ sites were 10-15% higher in the posterior dorsal dentate gyrus, CA1, and CA3 regions of the P compared with the NP line (Fig. 4). In the ventral portion, 15-25% higher values were observed in the anterior ventral area and ventral dentate gyrus of the P relative to the NP line (Fig. 4). No difference in the densities of 5-HT$_{1A}$ sites were found in the middle dentate gyrus between the P and NP groups (Fig. 4).

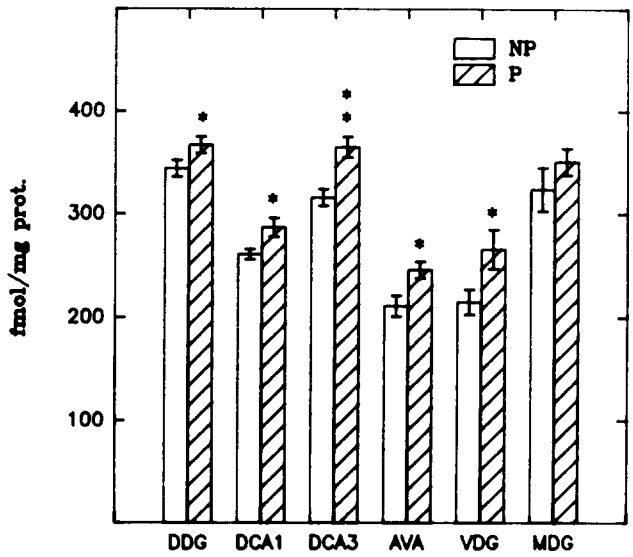

FIG. 4. Densities of [^{3}H]8-OH-DPAT labeled 5-HT$_{1A}$ sites in subregions of the posterior hippocampus of P and NP rats. Data are the means ± SEM of six animals/line for each subregion. *$p < 0.05$ and **$p < 0.010$ with Student's t-test. Abbreviations used: DDG, dorsal dentate gyrus; DCA1 and DCA3, dorsal CA1 and CA3; AVA, anterior ventral area; VDG, ventral dentate gyrus; MDG, middle dentate gyrus.

Contrary to findings observed in certain cortical and hippocampal regions, the densities of 5-HT$_{1A}$ sites were 15–20% lower in the dorsal, paradorsal, and median raphe nuclei of the P compared with the NP rats (Fig. 5). No differences were found between the rat lines in the dorsal central gray and central gray regions (Fig. 5).

DISCUSSION

The results of the present study indicate that there is higher binding of [^{3}H]8-OH-DPAT to 5-HT$_{1A}$ sites in most cerebral cortical subregions (Figs. 1 and 2), higher binding in several posterior (but not anterior) hippocampal subregions (Figs. 3 and 4), and lower binding in raphe nuclei (Fig. 5) of the P compared with the NP rats. The higher binding of [^{3}H]8-OH-DPAT in most regions of the cerebral cortex of the P compared with the NP rat is in agreement with cortical membrane binding studies (27,28). These investigators reported that the higher binding in cerebral cortical membranes from the P line was due mainly to a greater number of binding sites (B_{max}) without a significant change in the dissociation constant (K_D). Within the rat cerebral cortex, electrophysiological data (1) and results from lesioning studies indicate that 5-HT$_{1A}$ receptors are located postsynaptically on target neurons and not presynaptically on 5-HT nerve terminals (3,26). Therefore, the higher densities of [^{3}H]8-OH-DPAT observed in the cerebral cortical areas of the P relative to the NP rat indicate higher numbers of postsynaptic 5-HT$_{1A}$ receptors. The possible reasons for the higher densities of postsynaptic 5-HT$_{1A}$ receptors in the P line could be due to a) denervation supersensitivity; b) upregulation due to decreased 5-HT neuronal activity; c) loss of cellular control over the regulation of 5-HT$_{1A}$ receptor expression or degradation (i.e., increased number of 5-HT$_{1A}$ receptors/cell); and/or d) formation of more neurons expressing the 5-HT$_{1A}$ receptor.

There is evidence from neurochemical (18,19) and neuroanatomical (29) studies indicating reduced 5-HT innervation in the cerebral cortex of P relative to NP rats. The contents of 5-HT in the cerebral cortex and frontal cortex (18,19) and the densities of 5-HT immunostained fibers in the cingulate and frontal cortical areas (29) were lower in P compared with NP rats. These data are consistent with reduced 5-HT innervation in the cerebral cortex of the P line. Therefore, it is possible that as a result of this lower 5-HT innervation, an upregulation of 5-HT$_{1A}$ receptors developed as a compensatory mechanism. This may have occurred at an early developmental stage in the P rat because there is no evidence for upregulation of postsynaptic 5-HT$_{1A}$ receptors following destruction of 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT) treatment of adult Sprague–Dawley rats (8).

In a recent report, Wong et al. (27) found no statistically significant differences in the K_D or B_{max} values between the P and NP rats in the hypothalamus, striatum, or hippocampus. These results are in agreement with the autoradiographic data found for the hypothalamus (Table 1) but do not appear to agree with the findings for the caudate putamen (Table 1) and hippocampus (Fig. 4) obtained in the present study. In the case of the striatum, the apparent discrepancy may be due to measurements being taken from only part of the caudate-putamen (from 2.20 to 1.00 mm from Bregma) in the present study, whereas in the study of Wong et al. (27), membranes were prepared from the entire striatum and may also have included tissue from closely associated areas, e.g., the globus pallidus. Therefore, any small localized differences would be masked when membranes from the entire region are used. In the case of the hippocampus, small differences were localized to subregions of the posterior portion (Fig. 4), while no differences were observed in the anterior dorsal area (Fig. 3). Consequently, differences between the P and NP lines might not be seen with membrane preparations from the whole hippocampus. In addition, the relative proportion of the amount of

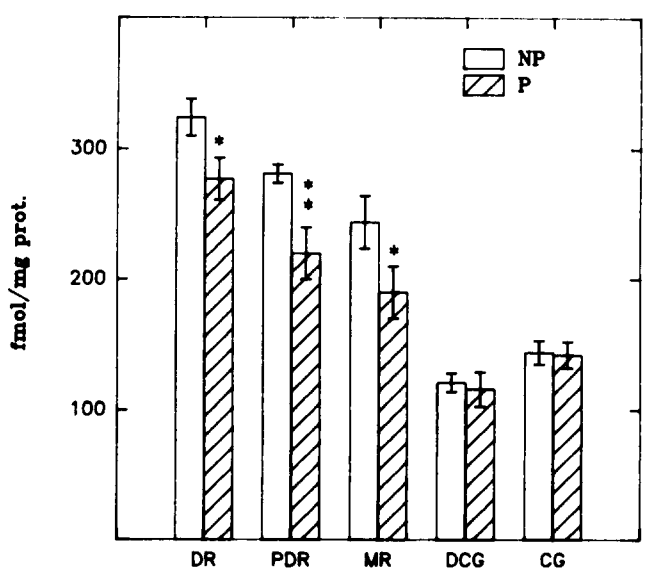

FIG. 5. Densities of [^{3}H]8-OH-DPAT labeled 5-HT$_{1A}$ sites in some brain stem nuclei of P and NP rats. Data are the means ± SEM of seven animals/line. *$p < 0.05$ and **$p < 0.025$ with Student's t-test. Abbreviations used: DR, dorsal raphe nucleus; PDR, paradorsal raphe nucleus; MR, median raphe nucleus; DCG, dorsal central gray; CG, central gray.

tissue taken from the anterior and posterior portions of the hippocampus for membrane binding assays would influence the results between the rat lines.

Within the hippocampus, 5-HT_{1A} receptors appear to be localized postsynaptically (6–8), although a small number may be located presynaptically within the CA2/CA3 region (8). The higher densities of 5-HT_{1A} receptors in subregions of the posterior hippocampus of the P rat is likely due to upregulation of the receptors resulting from reduced 5-HT innervation. Murphy et al. (18) reported lower levels of 5-HT in the hippocampus of P compared with NP rats. Zhou et al. (29) observed significantly ($p < 0.05$) lower densities of 5-HT immunostained fibers in the middle and ventral dentate gyrus of the ventral hippocampus of P compared with NP rats. The difference between the lines was not statistically significant in the anterior region of the ventral hippocampus, although the trend was in the same direction as that found for the other two subregions. The lower 5-HT innervations found in the anterior ventral area and the ventral dentate correspond well with the higher densities of 5-HT_{1A} sites observed for the P relative to the NP rat and support the hypothesis that there is an upregulation of postsynaptic 5-HT_{1A} receptors to compensate for the fewer 5-HT fibers in the P rat. However, whereas Zhou et al. (29) found a significant difference in 5-HT fiber densities in the middle dentate gyrus between the lines, there were no apparent differences in the densities of 5-HT_{1A} receptors in this subregion (Fig. 4). This could be due to the very high amount of total binding of [^{3}H]8-OH-DPAT in the middle dentate gyrus producing film densities approaching saturation level and, consequently, making detection of differences between the rat lines very difficult. The observation that no differences were found between the rat lines in the anterior dorsal part of the hippocampus could indicate that there is no reduction in 5-HT innervation in these subregions. On the other hand, it is possible that reductions in 5-HT innervation may not necessarily produce increases in the number of 5-HT_{1A} receptor sites. For example, the hypothalamus has moderate levels of 5-HT_{1A} sites in several subregions but there were no differences between the lines in any region (Table 1) even though the content of 5-HT in the hypothalamus is significantly lower in the P than NP rat (18). However, neuroanatomical studies have not been conducted to verify differences in 5-HT innervation in the hypothalamus between the rat lines.

Contrary to the results observed in the cerebral cortex and posterior hippocampus, the amount of [^{3}H]8-OH-DPAT binding was significantly lower in the dorsal, paradorsal, and median raphe nuclei of the P compared with the NP line (Fig. 5). Significant reductions in [^{3}H]8-OH-DPAT binding in the dorsal and median raphe nuclei have been reported following lesioning of 5-HT neurons with 5,7-DHT treatment (8,26). This reduction in [^{3}H]8-OH-DPAT binding is thought to be due to the destruction of 5-HT neurons (4) containing cell body 5-HT_{1A} autoreceptors (5,23–25). Therefore, the present findings for 5-HT_{1A} receptor differences in the raphe would suggest that there are fewer 5-HT neurons in the P compared with the NP rat. This conclusion is supported by the finding that there are fewer immunostained 5-HT neurons in the raphe nuclei of P compared with NP rats (30).

The results of the present study provide additional evidence that an imbalance in the serotonergic system may be involved in the innate high alcohol drinking characteristics of the P rats. The reduced population of 5-HT neurons (30) and fewer 5-HT fibers (29) in the CNS of the P rat may indicate a loss of regulation by the 5-HT system on certain neuronal circuits controlling the rewarding and/or aversive actions of alcohol. In addition to higher densities of postsynaptic 5-HT_{1A} receptors observed in the CNS of P rats in the present study, there are lower densities of 5-HT_2 receptors in several CNS regions of the P relative to the NP rat (15). One possible mechanism that might be involved in a loss of 5-HT regulation in the CNS of the P rat could be due to a net inhibitory effect on certain neuronal circuits produced by higher densities of inhibitory postsynaptic 5-HT_{1A} receptors and lower densities of excitatory postsynaptic 5-HT_2 receptors. For example, in the rat association cortex, 5-HT_2 and 5-HT_{1A} receptors mediate opposing responses on pyramidal cells (1). An imbalance in the densities of 5-HT_{1A} (higher) and 5-HT_2 (lower) receptors on these neurons could produce tonic inhibitory effects which, in turn, could alter their actions on several pathways possibly involved in mediating the rewarding actions of alcohol, e.g., medial prefrontal cortex, frontal cortex, ventral tegmental area, etc. (10). Of course, loss of 5-HT regulation could occur directly at several sites outside the cortex because higher densities of postsynaptic 5-HT_{1A} receptors or lower densities of 5-HT_2 receptors occur in several other limbic regions (e.g., hippocampus, nucleus accumbens, etc.), which are involved in mediating the reinforcing and/or neuroadaptive actions of ethanol (10). However, this imbalance in 5-HT_{1A} and 5-HT_2 receptors does not appear to be a factor involved in the disparate alcohol drinking behaviors of other selectively bred lines of rats. No difference in 5-HT_1 and 5-HT_2 receptor binding was found between the alcohol-preferring AA and alcohol-avoiding ANA rats in membranes from the brainstem, hippocampus, frontal cortex, and hypothalamus (11). In addition, Fawn-Hooded rats, which exhibit high preference for ethanol (22), have, in comparison to values for Sprague–Dawley and Wistar rats, higher numbers of 5-HT_2 binding sites and no difference in 5-HT_{1A} binding sites in membranes from the frontal cortex (9).

It is puzzling that the postsynaptic 5-HT_{1A} and 5-HT_2 receptors changed in opposite directions in the rat line with lower 5-HT innervations. Whereas the densities of 5-HT_{1A} sites increased to compensate for decreased innervation, the densities of 5-HT_2 sites decreased in response to reduced innervation. Perhaps the answer lies in the mechanisms responsible for the paradoxical response of 5-HT_2 receptors to downregulate instead of upregulate following chronic blockade with 5-HT_2 antagonists (2,12).

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