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Greater Abundance of Serotonin$_{1A}$ Receptor in Some Brain Areas of Alcohol-Preferring (P) Rats Compared to Nonpreferring (NP) Rats

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WONG, D. T., L. R. REID, T.-K. LI AND L. LUMENG. *Greater abundance of serotonin$_{1A}$ receptor in some brain areas of alcohol-preferring (P) rats compared to nonpreferring (NP) rats.* PHARMACOL BIOCHEM BEHAV **46**(1) 173–177, 1993.—Saturable [^{3}H]8-OHDPAT binding to recognition sites of 5-HT$_{1A}$ receptors was shown to be higher in cortical membranes of alcohol-preferring (P) than in membranes of alcohol-nonpreferring (NP) rats. Neither the P nor the NP lines had been previously exposed to ethanol. The increase in binding was mainly due to 40–56% higher density or maximum of binding sites (B_{max}) without significant change in affinity or dissociation constant (K_d) for the radioligand. Although B_{max} values were also consistently higher in membranes isolated from other brain areas of P rats, including hypothalamus, striatum, and hippocampus, the differences did not reach statistical significance. Similar to the previously reported lack of difference in [^{3}H]ketanserin binding to 5-HT$_2$ receptors in cortical membranes from P and NP rats, there were also no significant differences in saturable binding of [^{3}H]mesulergine and [^{3}H]LY278584 to recognition sites of 5-HT$_{1C}$ and 5-HT$_3$ receptors, respectively. Thus, an upregulation of 5-HT$_{1A}$ receptors in cerebral cortex and possibly in other brain areas of ethanol-naive P rats appears to have occurred as a consequence of the lower 5-HT innervation in this selected line of rats (13,15,27,28).

5-HT$_{1A}$ Receptors Alcohol-preferring Alcohol-nonpreferring Rats

SEROTONIN (5-hydroxytryptamine, 5-HT) transmission in the central nervous system has an inverse relation with alcohol intake in rodents (10,13,15,16). Alcohol-preferring P rats have been selectively bred and developed as a model of human alcoholism (7,8,10). Based on radioligand binding studies, recognition sites of 5-HT$_1$ receptors labeled with [^{3}H]5-HT have previously been shown to have a higher density in the cortical membranes of P rats than those of the opposite alcohol-nonpreferring (NP) rats (22). The higher density of the 5-HT$_1$ receptor class might be an indication of receptor upregulation resulting from the lower levels of 5-HT in specific brain areas of the P rats compared with the NP rats (13). Within the 5-HT$_1$ class of receptors, subtypes 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$ have now been identified (5,6). We have reported earlier that there is a higher density of 5-HT$_{1A}$ receptors labeled with [^{3}H]8-OHDPAT in membranes isolated from cerebral cortex and hippocampus of P than from those of NP rats (25). However, in that report, the animals were tested first for alcohol preference, and the observed differences in 5-HT$_{1A}$ receptor density conceivably might be caused by tolerance that could have developed in the P rats from chronic exposure to ethanol (2,12). In the present study, animals of both the P and NP lines from the 30th generation, and naive to ethanol exposure, were employed. Again, higher densities of [^{3}H]8-OHDPAT binding sites were found in membranes isolated from brain areas of P rats than of NP rats.

METHOD

Male P and NP rats, selectively bred for 30 or more generations (7,8), about 6 months of age (weighing 450–500 g) and naive to ethanol, were sacrificed by decapitation. In each experiment, eight to 10 matching pairs of P and NP lines were used. Brains were rapidly removed and brain areas were dissected at 4°C according to the published procedure (18). Brain regions from eight to 10 animals were pooled, weighed, and homogenized in 9 vol. of a medium containing 0.32 M sucrose and 10 mM glucose.

From the tissue homogenate, synaptosomal preparations (P2 fractions) of brain areas were isolated by differential centrifugation (23,25). The final pellets of membranes were frozen at −70°C until use.

Saturable [^{3}H]8-OHDPAT binding was conducted as follows (25): synaptosomal membranes in triplicate samples were incubated at 25°C for 30 min in 2 ml of medium containing 50 mM Tris-HCl (pH 7.4); 10 mM pargyline; 0.6 mM ascorbic acid; 5 mM $CaCl_2$; and [^{3}H]8-OHDPAT at the indicated concentrations. Binding was stopped by filtration through GFB

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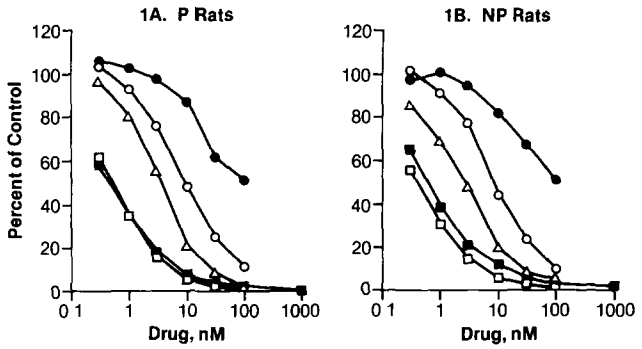

FIG. 1. Binding of [^{3}H]8-OHDPAT to the 5-HT$_{1A}$ receptor in cortical membranes of alcohol-preferring (P) and nonpreferring (NP) rats. Cortical membranes (equivalent to 800 mg protein) of P (A) or NP (B) rats in triplicate samples were incubated in medium containing six concentrations of 5-HT (filled square), ipsapirone (open square), buspirone (open circle), spiperone (filled circle), and metergoline (triangle) as indicated. Other conditions were as described in the Method section.

filters and three washes with ice-cold buffer. Radioactivity was measured by liquid scintillation technique. 5-HT at 10 mM was included in separate samples to determine specific binding (90–70% of total binding). [^{3}H]Mesulergine and [^{3}H]LY278584 binding to 5-HT$_{1C}$ and 5-HT$_3$ recognition sites was conducted as previously described (6,24).

Scatchard analysis was conducted by a computer-assisted linear regression analysis. Statistical analysis of the data, obtained from three or more separate determinations, was conducted by Student's t-test, and p value less than 0.05 was considered significant.

RESULTS

Binding of [^{3}H]8-OHDPAT was inhibited by increasing concentrations of the agonist 5-HT, which lowered binding 50% in cortical membranes of P (Fig. 1A) and NP (Fig. 1B) rats at comparable concentrations of 0.5 and 0.6 nM (IC_{50}), respectively. Comparable potencies in inhibiting [^{3}H]8-OHDPAT binding to cortical membranes of P and NP rats were observed (Fig. 1) with the two partial agonists ipsapirone (IC_{50} of 0.5 and 0.4 nM) and buspirone (IC_{50} of 10 and 9 nM), and the two antagonists metergoline (IC_{50} of 4 and 3 nM) and spiperone (IC_{50} of 86 and 96 nM). The relative potencies of the five serotonergic agents are therefore similar in the membranes derived from the P and NP rats.

Concentration-dependent (0.2–3 nM) binding of [^{3}H]8-OHDPAT was saturable in membranes isolated from frontal cerebral cortex of both P and NP rats (Fig. 2A). At each concentration of [^{3}H]8-OHDPAT, binding in the cortical membranes of the P rats was higher than in the NP rats. Scatchard analysis provided a dissociation constant (K_d) of 0.86 ± 0.03 nM and a maximum number of sites (B_{max}) or density of 206.4 ± 3.7 fmol/mg protein for the P rats, and the corresponding values of 1.01 ± 0.04 nM and 141.2 ± 2.8 fmol/mg protein for the NP rats. The difference in the B_{max} values was statistically significant ($p < 0.001$), as well as the difference in K_d values ($p < 0.025$). In four separate determinations, the B_{max} values were consistently higher in P rats than in NP rats ($p < 0.005$). The differences averaged about 40% (Table 1).

Similarly, saturable [^{3}H]8-OHDPAT binding in the mem-

	K_d(nM)	B_{max} (fmol/mg) Protein)
P	0 86 ± 0 03	206 4 ± 3 7
NP	1 01 ± 0 04	141 2 ± 2 8
	p < 0 025	p < 0 001

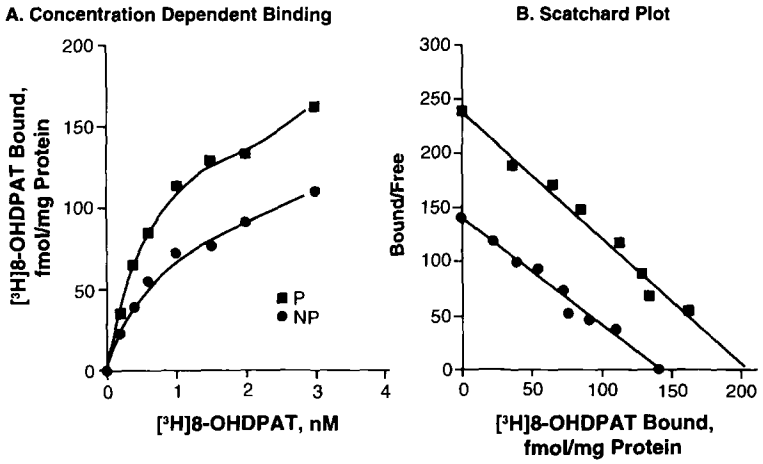

FIG. 2. Saturable [^{3}H]8-OHDPAT binding to membranes isolated from frontal cerebral cortex of P and NP rats. Membranes (equivalent to 300 mg protein) isolated from frontal cerebral cortex of P (square) or NP (circle) rats in triplicate samples were incubated in a medium containing seven concentrations (0.2–3 nM) of [^{3}H]8-OHDPAT as indicated. Other conditions were as described in the Method section. (A) Concentration-dependent binding, and (B) Scatchard plots of the same data.

TABLE 1

SATURABLE BINDING OF [^{3}H]8-OHDPAT IN MEMBRANES ISOLATED FROM BRAIN AREAS OF ALCOHOL-PREFERRING (P) AND ALCOHOL-NONPREFERRING (NP) RATS

	K_d, nM		B_{max}, fmol/mg protein		$p <$
Brain Area	NP	P	NP	P	
Frontal cortex $N = 4$	1.64 ± 0.50	1.90 ± 0.90 (116)	154.1 ± 5.52	216.4 ± 12.1 (140)	0.005
Cerebral cortex (-frontal) $N = 4$	0.90 ± 0.21	0.81 ± 0.18 (90)	155.1 ± 5.3	241.5 ± 4.1 (156)	0.001
Hypothalamus $N = 3$	0.84 ± 0.27	1.05 ± 0.31 (125)	83.7 ± 24.3	122.0 ± 33.3 (146)	NS
Striatum $N = 3$	1.22 ± 0.25	0.99 ± 0.10 (81)	43.6 ± 10.8	57.1 ± 13.5 (131)	NS
Hippocampus $N = 3$	2.17 ± 1.70	1.52 ± 1.12 (70)	473.1 ± 47.9	499.13 ± 55.2 (105)	NS
Brain stem $N = 1$	1.13	1.00 (88)	164.1	188.4 (115)	–

N = number of separate experiments using different matching groups of P and NP lines. NS = not significantly different; percent of NP rats is shown in parentheses.

branes of the remaining cerebral cortex of the P and NP rats was observed even over a wider range of concentration (0.2–10 nM) of [^{3}H]8-OHDPAT (Fig. 3A). Scatchard analysis resolved data points into straight lines, suggesting that homogenous populations of sites were found in cortical membranes of the P and NP rats (Fig. 3B). The mean B_{max} values derived from four separate groups of P rats were 56% ($p < 0.001$) higher than those of NP rats (Table 1), while no difference was observed in the K_d values of P and NP rats.

In three separate determinations, saturable [^{3}H]8-OHDPAT binding in membranes from the hypothalamus, striatum, and hippocampus of P rats showed consistently higher B_{max} values (46%, 31%, and 5%) than those of NP rats. The difference did not reach statistical significance because of the large variability among experiments, possibly due to variations in dissection (Table 1). The K_d values also showed no difference in these three brain areas of the P and NP rats.

Binding studies of [^{3}H]mesulergine, [^{3}H]ketanserin, and

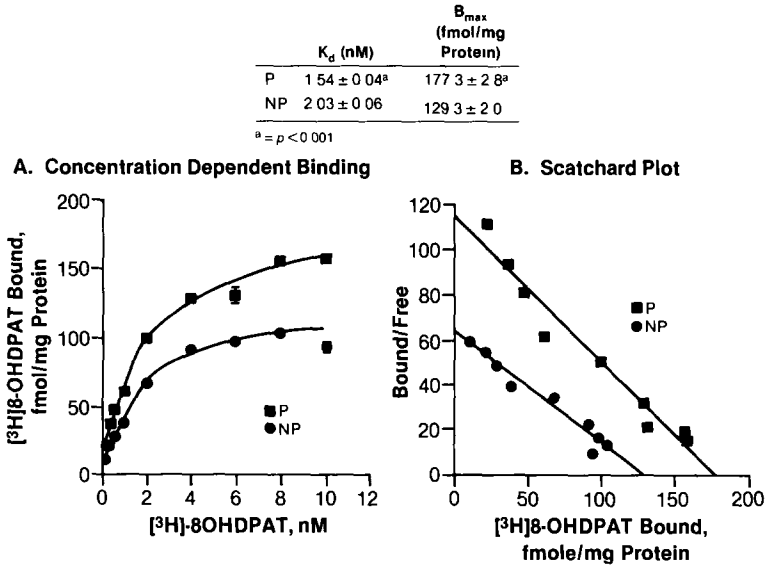

FIG. 3. Saturable [^{3}H]8-OHDPAT binding to membranes of cerebral cortex (minus frontal cortex) in P and NP rats. Membranes isolated from cerebral cortex (minus frontal cortex) (equivalent to 380 mg protein) of P (square) or NP (circle) rats in triplicate samples were incubated in a medium containing nine concentrations (0.2–10 nM) [^{3}H]8-OHDPAT as indicated. Other conditions were as described in the Method section. (A) Concentration-dependent binding, and (B) Scatchard plots of the same data.

TABLE 2

THE LACK OF DIFFERENCE IN RECOGNITION SITES OF 5-HT_{1C}, 5-HT_2, AND 5-HT_3 RECEPTORS IN CORTICAL MEMBRANES OF P AND NP RATS

Receptor (radioligand)	Animals	K_d (nM)	B_{max} (fmol/mg protein)
5-H_{1C}*	P	1.8 ± 0.1	81.2 ± 2.0
([^{3}H]mesulergine)	NP	1.9 ± 0.1	84.6 ± 2.1
5-HT_2	P	1.4 ± 0.1	579.0 ± 18.4
([^{3}H]ketanserin)	NP	1.3 ± 0.1	537.2 ± 21.8
5-HT_3	P	0.9 ± 0.0	19.8 ± 0.3
([^{3}H]LY278584)	NP	0.9 ± 0.0	19.6 ± 0.4

*Binding of [^{3}H]mesulergine was conducted in the presence of 30 nM spiperone to mask the 5-HT_2 receptors.

[^{3}H]LY278584 to 5-HT_{1C}, 5-HT_2, and 5-HT_3 receptors, respectively, were also conducted in cortical membranes from P and NP rats. Scatchard analysis yielded K_d and B_{max} values from the saturable binding measurements and did not reveal significant differences between P and NP rats in these binding parameters (Table 2).

DISCUSSION

Using P and NP rats naive to ethanol exposure, the present study confirms that densities or B_{max} values of the [^{3}H]8-OHDPAT-labeled recognition sites of 5-HT_{1A} receptors in cortical membranes of P rats are higher than those of NP rats. This finding does not differ from our earlier report that used animals that had been tested for alcohol preference before cortical membrane preparation and binding assay (25). The similarly higher densities of the [^{3}H]8-OHDPAT-labeled sites of 5-HT_{1A} receptors are consistently observed in membranes derived from the frontal cortex and the remaining cerebral cortex of ethanol-naive P rats in comparison with NP rats. However, there were no consistent and significant differences in the affinities or K_d values for [^{3}H]8-OHDPAT observed between P and NP rats. In agreement with these findings, ligands for 5-HT_{1A} receptors, including 5-HT, ipsapirone, buspirone, metergoline, and spiperone, displaced [^{3}H]8-OHDPAT from its binding site in cortical membranes of P and NP rats with comparable potencies. Thus, the greater abundance of recognition sites of 5-HT_{1A} receptors is indicative of an upregulation resulting from lower 5-HT levels in the brain areas of P rats than NP rats (13). It has also been shown that P rats exhibit lower immunostained 5-HT fiber densities in selected brain areas (27) and lower immunostained 5-HT neurons in the dorsal and median raphe nuclei (28) than NP rats.

Although densities of [^{3}H]8-OHDPAT-labeled sites of 5-HT_{1A} receptors in membranes of hypothalamus, striatum, and hippocampus were consistently observed in multiple determinations to be higher in the P rats than in the NP rats, the large variability of the B_{max} values from group to group of animals rendered the differences statistically insignificant. Quantitative receptor autoradiography might better resolve the differences in discrete brain areas. Indeed, autoradiographic techniques had clearly established that densities of 5-HT_{1A} recognition sites labeled by [^{3}H]8-OHDPAT in cortical areas and lateral nucleus accumbens were higher in P than in NP rats, but no significant difference in medial nucleus accumbens was detected (9).

In contrast to the present findings, the previously observed difference in [^{3}H]8-OHDPAT binding in hippocampal membranes of P and NP rats was much larger in magnitude (25) when the animal had been pretested for alcohol preference (i.e., exposed to ethanol before membrane preparation and binding studies). It is conceivable that the discrepancy is due to the use of tested high alcohol-preferring P and low alcohol-nonpreferring NP rats in the earlier study as compared with untested animals in the present study. On the other hand, it would be of interest to know if the biochemical parameters of [^{3}H]8-OHDPAT binding could be altered in the animals that have developed tolerance with chronic ethanol exposure, as P rats do during testing for alcohol preference (2,12).

Among animal models of alcoholism (10), an inverse relationship between alcohol intake and levels of serotonin has been observed in the alcohol-preferring P rats (13,15), the alcohol-preferring HAD rats (3), the N/Nih high alcohol-drinking rats (14), and in several inbred strains of mice that exhibit high voluntary alcohol drinking (26). The studies of serotonin receptors, thus far, have been mainly conducted with the alcohol-preferring P rats. Quantitative data on 5-HT_{1A} receptors in human postmortem brain tissues are very limited. However, 5-HT_{1A} receptors had been reported to be higher in postmortem cortical specimens of suicide victims (1,11) and in cortical areas of schizophrenic patients (4) in comparison with brains of victims from other causes of death. Thus, the alcohol-preferring rats might represent an animal model of psychiatric disorders associated with serotonergic dysfunction. Comparisons of P and NP rats in various tests of depressive behaviors or stress (18,21,22) might be informative with regard to the centrality of serotonin in abnormal behaviors and psychiatric disorders.

Involvement of 5-HT_{1C} and 5-HT_3 receptors in the control of voluntary alcohol intake has been implicated because of the suppressive effects of arylpiperazine MK212, an agonist of 5-$HT_{1C,2}$ receptors [see review (24)], and ondansetron, an antagonist of 5-HT_3 receptors (17,19). The present preliminary studies did not discern differences in the recognition sites of these two receptors in synaptosomal membranes isolated from P and NP rats. In addition, we have previously reported the absence of significant difference in binding of [^{3}H]ketanserin to 5-HT_2 receptors in cortical membranes of the P and NP rats (23). However, autoradiographic techniques may be able to uncover smaller and more localized differences involving these receptors in small discrete brain regions between P and NP rats.

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