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SHORT COMMUNICATIONS

Changes in Serotonin, but Not Catecholamine, Receptor Binding in the Brain of Morphine-Dependent Rats

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SUMMARY

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Chronic morphine administration causes a 40% reduction of ^{3}H-labeled 5-hydroxytryptamine (5-HT) binding in the brain stems of dependent rats, with little or no effect in other brain regions. Binding of [^{3}H]dihydroalprenolol, 2,2,6-[^{3}H]dimethoxyphenoxy-(ethylaminomethyl)benzodioxan, and [^{3}H]spiroperidol is not affected by this treatment. The specificity of the reduction in 5-HT receptor number in the brain stem is discussed in terms of persistent activation of central 5-HT neurons by long-term treatment with morphine.

A reduction in the number of 5-HT[1] receptors has recently been reported in the brain stems of morphine-dependent rats (1), whereas *beta*-adrenergic and dopamine receptors have been reported to be slightly increased in the rat cortex (2) and mouse striatum (3) after chronic morphine administration. Since these experiments used different treatment schedules, methodology, and animal species, in the present study we compared the binding of [^{3}H]5-HT, [^{3}H]WB4101, [^{3}H]DHA, and [^{3}H]spiroperidol in various brain areas of morphine-dependent rats.

Male CD-COBS rats (Charles River Laboratories, Calco, Italy) weighing about 200 g at the beginning of the experiments were used. Each animal received two i.p. injections of morphine sulfate (10 mg/kg) the 1st day, after which the dose of morphine was doubled every other day to reach a total daily dose of 160 mg/kg on the 7th day. This regimen was continued for 3 days. On day 11 the animals received the last injection of morphine. This regimen has been shown to cause measurable physical dependence in rats (1, 4).

Control animals were treated similarly but received 0.9% NaCl solution instead of morphine. All of the animals were killed by decapitation 4 hr after the last injection (morphine or 0.9 NaCl solution); their brains were rapidly removed and dissected on ice, according to the method of Glowinski and Iversen (5), into the following regions: cortex, hippocampus, striatum, diencephalon, and brain stem (midbrain + pons + medulla oblongata). Brain regions were stored at −80° before membrane preparation.

Crude membrane preparations for [^{3}H]5-HT binding studies were obtained as described by Bennett and Snyder (6) and Nelson *et al.* (7). The brain regions were twice homogenized in 50 volumes of cold Tris-HCl buffer (0.05 M, pH 7.4) using an Ultra-Turrax TP 18-10 (20 sec) and centrifuged at 50,000 × *g* for 10 min. The pellets were resuspended in cold Tris buffer and incubated at 37° for 10 min; they were then centrifuged, resuspended in cold Tris buffer, and centrifuged as above. The final pellets were resuspended in 50 volumes of 0.05 M Tris-HCl buffer containing 0.1% ascorbic acid, 10 μM pargyline, and 4 mM $CaCl_2$ (incubation buffer, pH 7.4). For [^{3}H]5-HT binding, a semiautomatic filtration technique (8) was used: membrane preparation samples (0.25 ml) were placed in 8 × 12 titration plates containing [^{3}H]5HT (specific activity 26.2 Ci/mmole; New England Nuclear Corporation, Boston, Mass.) in concentrations ranging from 1.5 to 15 nM. Nonspecific binding was determined in the presence of 10 μM unlabeled 5-HT and averaged 20 to 40% of total binding. Plates were incubated at 37° for 20 min and filtered using a Titertek cell harvester (Labtek srl, Milan, Italy) through Whatman GF/B filters. Samples were washed with cold Tris buffer (pH 7.4) for 15 sec, giving a washing volume of approximately 5 ml/sample. Filters were counted in 5 ml of dioxane scintillator (Supelchem) in a Packard Tri-Carb liquid scintillation spectrometer (Model 2450) with a counting efficiency of about 40%.

The membrane preparation procedure for [^{3}H]WB4101

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[1] The abbreviations used are: 5-HT, 5-hydroxytryptamine (serotonin); WB4101, 2,2,6-dimethoxyphenoxy-(ethylaminomethyl)-benzodioxan; DHA, dihydroalprenolol.

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binding studies was the same as that for [^{3}H]5-HT binding, with the exception of incubation at 37° for 10 min and successive washing. The washed pellet was resuspended in 50 volumes of 0.05 M Tris-HCl buffer (pH 8) containing 0.1% ascorbic acid and 10 μM pargyline (incubation buffer). A portion (1 ml) of the membrane preparation was added to 1 ml of incubation buffer containing [^{3}H]WB4101 (specific activity 24.4 Ci/mmole; New England Nuclear Corporation) at final concentrations ranging from 0.125 to 2 nM and incubated for 20 min at 25°. Incubation was stopped by the addition of 5 ml of ice-chilled Tris buffer followed by rapid filtration through Whatman GF/B glass fiber filters under vacuum and three additional 5-ml washes of Tris buffer. Filters were counted in 10 ml of dioxane scintillator as described for [^{3}H]5-HT binding. The amount of nonspecific binding was determined in the presence of 100 μM norepinephrine and averaged 30% of total [^{3}H]WB4101 binding.

For [^{3}H]DHA binding studies, membranes prepared as described for [^{3}H]WB4101 were resuspended in 100 volumes of Tris buffer. A portion (1 ml) of the membrane preparation was added to 1 ml of incubation buffer containing [^{3}H]DHA (specific activity 45 Ci/mmole; New England Nuclear Corporation) at final concentrations ranging from 0.5 to 6 nM and incubated for 20 min at 25°. Filtration and counting of samples were as described above. The amount of nonspecific binding was determined in the presence of 1 μM (−)-propranolol and averaged 40% of total [^{3}H]DHA binding.

For [^{3}H]Spiroperidol binding studies, the membrane preparation procedure was the same as that described for [^{3}H]WB4101. The washed pellet was resuspended in 200 volumes of 0.05 M Tris-HCl buffer (pH 7.1) containing 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (incubation buffer). A portion (0.5 ml) of membrane preparation was added to 0.5 ml of incubation buffer containing [^{3}H]spiroperidol (specific activity 23.0 Ci/mmole; New England Nuclear Corporation) at final concentrations ranging from 0.05 to 2 nM and incubated for 15 min at 37°. Filtration and counting of samples were as described for [^{3}H]WB4101. The amount of nonspecific binding was determined in the presence of 1 μM (+)-butaclamol and averaged 10% of total [^{3}H]spiroperidol binding.

Specific binding was determined for each ligand as described. Student's t-test was used for comparison of controls and morphine-treated animals, when a single concentration of ^{3}H-labeled ligand was used. Saturation curves were plotted for each ligand based on at least eight concentrations, using an Eadie-Hofstee plot of B versus B/F (9), and 95% confidence limits for B_{max} and K_d values were calculated. Unless otherwise specified, no significant differences were found in K_d and B_{max} between controls and morphine, indicating that the results could not have arisen from compensatory changes in kinetic parameters.

Table 1 shows the effect of chronic morphine administration on monoamine receptors in different rat brain regions. The specific binding of [^{3}H]WB4101, used to label $alpha_1$-receptors (10), did not differ in morphine- and vehicle-treated rats. [^{3}H]DHA binding was slightly, but not significantly, increased (17%) in the cortex of morphine-dependent animals. Saturation analysis indicated that this effect was due to an increase in the number of binding sites (9.82 ± 0.46 and 11.53 ± 0.78 pmoles/g of tissue for controls and morphine-treated rats, not statistically different) without any apparent modification for the K_d values (0.77 ± 0.04 and 0.77 ± 0.03 nM for controls and morphine-treated rats, respectively). [^{3}H]Spiroperidol binding in the striatum, reported to involve primarily dopamine receptors (11), was not affected by chronic morphine treatment. [^{3}H]5-HT binding was unchanged in the cortex, hippocampus, and striata of morphine-dependent rats but was reduced to 78% of controls in the diencephalon (not statistically significant) and to 65% of controls ($p < 0.05$) in the brain stems of morphine-treated rats.

These decreases were due to changes in the maximal

TABLE 1

5-HT and catecholamine receptors in morphine-dependent rats

Data represent means ± standard deviation for four animals. Experiments were repeated two or three times. The concentrations of ligand used were as follows: [^{3}H]5-HT, 6 nM; [^{3}H]DHA, 1 nM; [^{3}H]WB4101, 0.22 nM; [^{3}H]Spiroperidol, 0.2 nM. Saturation analyses were carried out for each ligand to ascertain that negative data were not the result of compensatory changes in K_d and B_{max}.

Ligand and treatment	Binding sites				
	Cortex	Hippocampus	Brain stem	Diencephalon	Striatum
	pmoles bound/g tissue				
[^{3}H]5-HT					
Vehicle	7.5 ± 1.0	12.9 ± 0.4	6.8 ± 0.9	4.0 ± 0.2	8.6 ± 0.9
Morphine	8.7 ± 1.3	13.8 ± 2.5	4.4 ± 0.7[a]	3.2 ± 0.9	8.0 ± 1.2
[^{3}H]DHA					
Vehicle	5.4 ± 0.3	2.3 ± 0.3	1.5 ± 0.1	3.7 ± 0.1	—
Morphine	6.3 ± 0.8	2.5 ± 0.3	1.5 ± 0.3	3.5 ± 0.5	—
[^{3}H]WB4101					
Vehicle	2.7 ± 0.3	2.9 ± 0.2	2.5 ± 0.3	1.6 ± 0.2	—
Morphine	2.6 ± 0.5	3.0 ± 0.1	2.3 ± 0.4	1.6 ± 0.2	—
[^{3}H]Spiroperidol					
Vehicle	—	—	—	—	12.4 ± 0.8
Morphine	—	—	—	—	11.8 ± 2.2

[a] $p < 0.05$, Student's t-test.

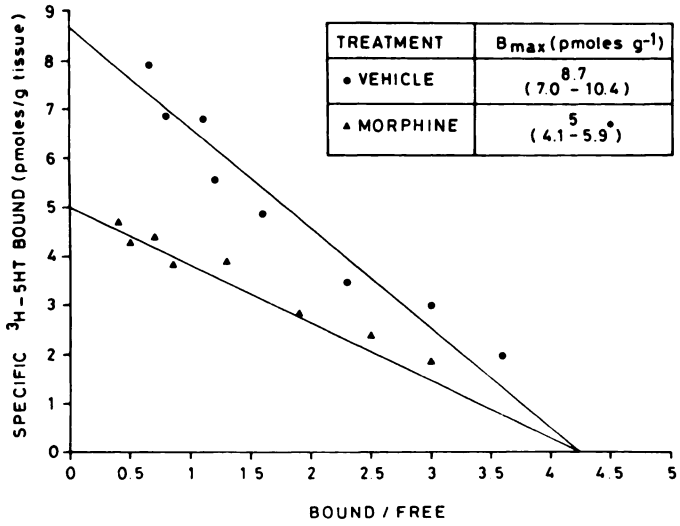

FIG. 1. *Eadie-Hofstee plot of B versus B/F with data from saturation experiments, using brain stem from vehicle (●) or morphine-treated (▲) animals*

Specific [^{3}H]5-HT binding was determined as described in the text with eight concentrations of [^{3}H]5-HT. Each *point* is the mean of four replications. The experiment was repeated twice. The K_d values were: 2.3 (1.4–3.1) for vehicle-treated rats and 1.1 (0.5–1.6) for morphine-treated rats. When morphine was added *in vitro*, nonsignificant effects were found: K_d = 2.4 (1.6–3.1), B_{max} = 7.4 (6.0–8.7).

number of binding sites, without effect on K_d values, as indicated by saturation analysis. Data for the diencephalon were as follows: B_{max} = 12.2 ± 1.2 and 9.0 ± 0.5 pmoles/g of tissue for vehicle-treated and morphine-dependent rats, respectively, not statistically different; K_d = 11.7 ± 1.5 and 11..1 ± 2.5 nM. The results for brain stem are reported in Fig. 1. To exclude the possibility that the observed effects were due to the residual morphine present in the tissue at the time of assay, we added in *vitro* a concentration of morphine comparable to that found after *in vivo* treatment (about 0.6 $\mu g/g^2$) before homogenization. No significant effects were found in [^{3}H]5-HT binding in all of the brain areas considered (see Fig. 1 for brain stem). However, it has been reported that morphine, at concentrations well above those present under our conditions, fails to displace binding of either ^{3}H-labeled ligand for 5-HT and catecholamines (6, 10, 12, 13).

The data show that chronic treatment with morphine, reported to cause marked physical dependence (4), significantly reduces the number of [^{3}H]5-HT binding sites in the brain stems of rats but has no significant effect on [^{3}H]5-HT binding in the other brain areas examined in this study. The reasons for this regional specificity of the effect of morphine are not clear. *In vitro*, morphine does not affect serotoninergic mechanisms such as membrane uptake, release (14), or postsynaptic receptors (6). Chronic treatment with *d*-fenfluramine, a direct releaser of 5-HT (15), has recently been found to reduce [^{3}H]5-HT binding in some forebrain regions but not in the brain stem (16).

[2] T. Mennini, S. Caccia, and L. Cervo, unpublished data.

It is therefore likely that morphine preferentially affects serotoninergic function in the brain stem through an indirect mechanism. These data agree with the fact that low doses of morphine cause a selective increase of 5-HT metabolism in this brain area (17). Moreover, it has been recently suggested that naloxone-precipitated jumping in morphine-dependent rats may be causally related to the decrease of [^{3}H]5-HT binding in the brain stem (1).

As regards catecholamines, no significant effect was found on [^{3}H]WB4101 or [^{3}H]DHA binding in cortex, hippocampus, diencephalon, and brain stem, or on [^{3}H]-spiroperidol binding in striatum, suggesting that in morphine-dependent rats a selective decrease of [^{3}H]5-HT binding in the brain stem occurs with little or no change in catecholamines receptor binding. Whether this applies to morphine dependence induced by schedules or in species other than those used in the present study remains to be elucidated.

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