CHANGES IN ALPHA 2 ADRENORECEPTORS IN VARIOUS AREAS OF THE RAT BRAIN AFTER LONG-TERM ADMINISTRATION OF "MU" AND "KAPPA" OPIATE AGONISTS

Charles B. Smith, Peggie J. Hollingsworth, Joann J. Geer, Hylan C. Moises

Departments of Pharmacology and Physiology, The University of Michigan Medical School, Ann Arbor, Michigan 48109

(Received in final form June 26, 1983)

Summary

Clonidine, an alpha 2 adrenoreceptor agonist, is used to treat opiate dependent individuals who are experiencing the signs and symptoms of withdrawal. Changes in the apparent number of alpha 2 adrenoreceptors in specific areas of the rat brain have been observed after chronic morphine administration. In the present study, the effects of chronically administered morphine sulfate upon alpha 2 adrenoreceptors were compared to those of UM-1072, (+)-5,9-alpha-dimethyl-2-hydroxy-2-tetrahydro-furfuryl-6,7-benzomorphin HCl, a "kappa" agonist which does not produce typical morphine-like dependence. The maximum number of specific binding sites (Bmax) and dissociation constants (Kd's) for 3H-clonidine were measured with neural membranes isolated from saline or drug-treated rats. Rats were injected with saline, morphine or UM-1072, i.p., every 8 hr for 14 days. Doses of morphine ranged from 10 mg/kg, t.i.d., on the first three days to 100 mg/kg, t.i.d., on the last two days. Doses of UM-1072 covered a similar range. In control experiments, the Bmax's for specific binding of 3H-clonidine were (in fmoles/mg protein): hypothalamus, 142 ± 7; amygdala, 141 ± 3; brainstem, 70 ± 2; parietal cortex, 130 ± 4; hippocampus, 94 ± 2; and caudate nucleus, 62 ± 3. After chronic morphine treatment, the Bmax's were decreased significantly in all areas except the hippocampus. After chronic UM-1072 treatment, the Bmax's were decreased significantly in all areas studied. Neither treatment altered appreciably the Kd's for 3H-clonidine. This study suggests that "mu" and "kappa" agonists might have similar actions upon noradrenergic systems in the brain.

Neuronal release of norepinephrine is regulated in part by stimulation of the alpha 2 adrenoreceptor (1,2). Stimulation of this receptor results in inhibition of further norepinephrine release which provides for autoregulation of the amount of norepinephrine in the synaptic cleft. Morphine increases the turnover of catecholamines in the rat (3) and mouse brain (4). Tolerance develops to this effect of morphine, and during withdrawal, catecholamine turnover is reduced in the brains of dependent animals (5). These changes in catecholamine turnover might be related to changes in the function of alpha 2 adrenoreceptors.

Recently, implantation of morphine pellets for three days was reported to increase the apparent number of alpha 2 adrenoreceptors in rat cerebral cortex and brainstem (6). In addition, clonidine, a drug which acts upon
alpha2 adrenoreceptors, has been used to treat dependent patients who are undergoing opiate withdrawal (7,8). Such findings have been interpreted as evidence for a role of alpha2 adrenoreceptors in opiate dependence. The purpose of the present study was to compare the effects of chronically administered morphine sulfate to those of UM-1072, a "kappa" agonist, upon alpha2 adrenoreceptors in specific areas of the rat brain. UM-1072 produces a form of dependence when administered chronically to experimental animals which is not like that produced by morphine.

Materials and Methods

Homogenates were made from the following brain areas isolated from male Sprague-Dawley rats (220-240 g): amygdala, hippocampus, hypothalamus, brainstem, parietal cortex and caudate nucleus. The rats were injected with saline, morphine sulfate, or UM-1072, i.p., every 8 hr for 14 days. Morphine sulfate was injected i.p. every 8 hr according to the following schedule (doses are given per injection): day 1-3, 10 mg/kg; day 4-6, 20 mg/kg; day 7-9, 40 mg/kg; day 10-12, 70 mg/kg; day 13-14, 100 mg/kg. UM-1072 was injected according to the same schedule except that the dose was not increased above 40 mg/kg per injection after day 9. The rats were killed by decapitation 8 hr after the last injection. The various areas of the rat brain were identified histologically. The area which is referred to as the brainstem consisted of a section of brainstem 1 mm thick which was medial and inferior to the superior cerebellar peduncle, superior to the nucleus of the Vth cranial nerve, and which contained the mesencephalic nucleus of the trigeminal nerve as well as the locus coeruleus. The brain areas isolated from six rats were pooled for each experiment. The brain areas were homogenized in 5 ml of ice-cold Tris-sucrose buffer which consisted of 5 mM Tris (hydroxymethyl) aminomethane, 0.25 M sucrose and 1 mM MgCl2.6H2O adjusted to pH 7.4 with 1 N HCl. The homogenates were centrifuged at 1000 x g for 1 min and the supernates were saved and re-centrifuged at 40,000 x g. The crude membrane pellet was washed twice with ice-cold Tris-incubation buffer, and recentrifuged at 40,000 x g. The incubation buffer was composed of 50 mM Tris (hydroxymethyl) aminomethane and 10 mM MgCl2.6H2O adjusted to pH 7.5 with 6 N HCl. The final pellet was resuspended in Tris-incubation buffer. To measure total binding, 1 ml aliquots of the neural membranes were incubated in duplicate for 30 min at 25°C with concentrations of 3H-clonidine (sp. act. 22.2 Ci/mmol, NEN, Boston, Mass.) which ranged from 10^-9 M to 6.4 x 10^-8 M. Nonspecific binding was determined by adding unlabeled clonidine, 10^-5 M, as well as 3H-clonidine to a second pair of incubates. The incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters and by washing with two 10 ml aliquots of Tris-incubation buffer at 25°C. Results are expressed as fmoles of 3H-clonidine specifically bound per mg of protein.

Results

The distribution of specific binding of 3H-clonidine to neural membranes isolated from various areas of the rat brain was uneven (Table I). The greatest number of binding sites was found with membranes from the hypothalamus and amygdala, and the lowest number was found with brainstem and caudate nucleus membranes. Nonspecific binding ranged from 6% in the amygdala to 18% in the caudate nucleus. Neither morphine nor UM-1072 altered nonspecific binding to membranes isolated from any of the brain areas. Scatchard analyses indicated that only a single population of binding sites was present upon the isolated membranes (Figure 1). The dissociation constants (Kd's) did not differ significantly among the various areas.
Effects of chronically administered morphine sulfate and UM-1072 upon alpha2 adrenoreceptors on neural membranes isolated from rat parietal cortex. Left graph. Saturation isotherms: ordinate, 3H-clonidine specifically bound; abscissa, concentration of free 3H-clonidine. Right graph. Scatchard plots: ordinate, 3H-clonidine specifically bound/free 3H-clonidine in incubate; abscissa, 3H-clonidine specifically bound. Solid circles, saline controls; solid triangles, morphine-treated rats; solid squares, UM-1072-treated rats. Shown are representative experiments.

TABLE I

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Control B&lt;sub&gt;max&lt;/sub&gt; (fmol/mg protein)</th>
<th>Morphine B&lt;sub&gt;max&lt;/sub&gt; (fmol/mg protein)</th>
<th>UM-1072 B&lt;sub&gt;max&lt;/sub&gt; (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>142.1 ± 7.3*</td>
<td>112.3 ± 14.8</td>
<td>102.2 ± 6.7</td>
</tr>
<tr>
<td>Amygdala</td>
<td>140.6 ± 2.8</td>
<td>111.2 ± 6.5</td>
<td>108.2 ± 15.4</td>
</tr>
<tr>
<td>Parietal Cortex</td>
<td>129.8 ± 4.2</td>
<td>103.4 ± 0.7</td>
<td>95.8 ± 8.6</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>93.7 ± 2.1</td>
<td>71.4 ± 7.2</td>
<td>69.2 ± 3.2</td>
</tr>
<tr>
<td>Brainstem</td>
<td>70.1 ± 1.5</td>
<td>61.2 ± 3.8</td>
<td>62.9 ± 3.6</td>
</tr>
<tr>
<td>Caudate Nucleus</td>
<td>62.4 ± 3.0</td>
<td>31.7 ± 2.5</td>
<td>44.9 ± 6.8</td>
</tr>
</tbody>
</table>

* Each value represents the mean of 3-6 determinations ± S.E.M.

Administration of morphine sulfate for two weeks resulted in significant decreases in the maximum number of specific binding sites for 3H-clonidine to neural membranes isolated from the hypothalamus (21%, P < 0.05); amygdala (21%, P < 0.025); parietal cortex (20%, P < 0.025); brainstem (13%, P < 0.05) and caudate nucleus (49%, P < 0.005). UM-1072 caused decreases in the number of binding sites in all areas of the brain studied. These decreases were: hypothalamus (28%, P < 0.01); amygdala (23%, P < 0.025); parietal cortex (26%, P < 0.005); hippocampus (26%, P < 0.005); brainstem (10%, P < 0.025) and caudate nucleus (28%, P < 0.05). Neither drug caused appreciable changes in the K<sub>D</sub>'s for the specific binding of 3H-clonidine to neural membranes isolated from the various brain areas.
Discussion

Long-term administration of both morphine sulfate, a "mu" agonist, and UM-1072, a "kappa" agonist, decreased the maximum number of alpha_2 adrenoreceptors in specific areas of the rat brain. The present study is not in agreement with that of Hamburg and Tallman (6); however, there were significant procedural differences between the two studies which include route and duration of drug administration. A number of studies have shown that opiates increase the turnover of catecholamines in the brain (3-5). Thus, the observed changes in alpha_2 adrenoreceptors might be secondary to increases in neuronal norepinephrine turnover. Other drugs and procedures which are thought to increase synaptic norepinephrine levels also cause decreases in the number of alpha_2 adrenoreceptors on neural membranes isolated from certain areas of the rat brain. These include chronically administered amitryptiline (9), a tricyclic antidepressant, chronically administered chlorgyline (10), a monoamine oxidase inhibitor, and repeated electroshock treatment (11). Changes in catecholamine turnover in brain have been related to the dependence produced by opiates (12). If the changes in receptor number in the present study reflect changes in catecholamine turnover, the fact that both a "mu" and "kappa" agonist cause the same changes in receptor number might lead to a reassessment of the relationship of noradrenergic mechanisms to opiate-induced dependence.

Acknowledgement

These studies were supported in part by USPHS grants DA-00254, DA-03365 and MH-36336. H.C. Moises is a recipient of a career development award from The Chicago Community Trust/Searle Scholars Program.

References