Effects of pentazocine and concomitant clonidine on opioid receptors in the rat brain.

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Abstract

The changes in opioid receptors (Op-R) caused by repeated administration of pentazocine and the effect of concomitant clonidine were investigated. Binding of [3H] naloxone was markedly decreased in the absence of Na+, but was increased in the presence of Na+ in the diencephalon-mesencephalon of chronic pentazocine-treated rats. No significant changes were observed in the cerebral cortex of pentazocine-treated rats. The pentazocine-induced changes in Op-R were abolished by the concurrent use of clonidine, an alpha-adrenergic agonist, which has been shown to relieve the withdrawal symptoms of morphine. This result indicated that the behavioral action of clonidine can also be observed at the Op-R level.

KEYWORDS: opioid receptors, pentazocine, clonidine, naloxone binding, sodium effect

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EFFECTS OF PENTAZOCINE AND CONCOMITANT CLONIDINE ON OPIOID RECEPTORS IN THE RAT BRAIN

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Abstract. The changes in opioid receptors (Op-R) caused by repeated administration of pentazocine and the effect of concomitant clonidine were investigated. Binding of [\(^{3}H\)] naloxone was markedly decreased in the absence of Na\(^+\), but was increased in the presence of Na\(^+\) in the diencephalon-mesencephalon of chronic pentazocine-treated rats. No significant changes were observed in the cerebral cortex of pentazocine-treated rats. The pentazocine-induced changes in Op-R were abolished by the concurrent use of clonidine, an \(\alpha\)-adrenergic agonist, which has been shown to relieve the withdrawal symptoms of morphine. This result indicated that the behavioral action of clonidine can also be observed at the Op-R level.

Key words: opioid receptors, pentazocine, clonidine, naloxone binding, sodium effect.

Opioid receptors (Op-R) have been discovered in the central nervous system (1, 2), followed by the discovery of endorphins which are bound to Op-R (3, 4). The study of endorphins, their precursors and the biochemical features of Op-R has since progressed remarkably. Receptors in general are known to be regulated by a variety of factors, and Op-R are regulated by opiates themselves. Pentazocine, rather than morphine, is the current choice of drug for analgesia. While there are many reports on the changes in Op-R due to morphine and withdrawal from it (5), little information is available on the changes due to pentazocine. Recently, animal and clinical studies have shown that clonidine, an \(\alpha\)-adrenergic agonist, relieves morphine-induced withdrawal symptoms (6-9). In the present study, we investigated the changes in Op-R caused by daily administration of pentazocine and the effect of concomitant clonidine.

MATERIALS AND METHODS

Male Sprague-Dawley rats were divided into four groups (Fig. 1). Groups A and B received daily intraperitoneal (i.p.) injections of pentazocine (20 mg/kg) for seven days, and Groups C and D received physiological saline solution for seven days. Clonidine at a dose of 50 \(\mu\)g/kg/day was simultaneously injected i.p. into the rats in Groups B and C for three days from day 5 of the pentazocine or saline injections. Three hours after the final injection,
Fig. 1. Injection schedules for investigation of the effects of chronic pentazocine administration and concomitant clonidine on opioid receptor

the rats were sacrificed by decapitation, and the forebrain was immediately removed and divided into the cerebral cortex and diencephalon-mesencephalon. The tissue blocks were homogenated by the previously reported method (10) and subjected to radiolabeled receptor assay (RRA) with $[^3H]$ naltroxone (11). In brief, the tissue regions were homogenized in a glass homogenizer in 35 vol. of ice-cold Tris buffer (50 mM Tris-HCl buffer, pH 7.6 at 25°C). The homogenate was centrifuged at 50,000 × g for 20 min at 4°C and the pellet was resuspended in the original volume of Tris buffer and re-centrifuged. The pellet was then resuspended in the original volume of Tris buffer and used for RRA. The receptor preparations (500 µl, containing 800 µg protein) were incubated in ice for 120 min with 500 µl of Tris buffer containing 4 nM $[^3H]$ naltroxone (New England Nuclear, specific activity 20 Ci/mmol). The reaction was terminated by rapid filtration on Whatman GF/C glass fiber filters. The filters were washed twice with 3 ml of cold Tris buffer and then placed in scintillation vials with 10 ml of scintillation fluor for counting. Specific binding was the difference between radioactivity bound to the receptor in the presence of 2 µM naltroxone (Endo lab., New York) and that bound in its absence.

RESULTS AND DISCUSSION

When naltroxone, an opiate antagonist, is used as a tracer, the number of high affinity binding sites in the presence of 100 mM of NaCl is about twice that in the absence of Na$^+$ (11, 12). In contrast, no detectable high affinity binding sites are observed when an opiate agonist is used as a tracer (11, 12). Previously, we have reported that it is possible to differentiate antagonist dominant sites and agonist dominant sites of Op-R by using NaCl (13). Therefore, we determined simultaneously specific binding of $[^3H]$ naltroxone in the presence of 100 mM of NaCl and in its absence.

None of the groups chronically treated with pentazocine (Group A), a combination of pentazocine and clonidine (Group B), or clonidine alone (Group C)
showed significant differences from the control group given physiological saline (Group D) with respect to the specific binding sites for naloxone in the cerebral cortex (Table 1). In the presence of Na$^+$ 100 mM, every group showed an increase in the specific naloxone binding sites. The ratio of the specific binding with Na$^+$ to that without Na$^+$ (+Na$^+$/−Na$^+$) was about 2. There were no significant differences among the experimental groups.

In the diencephalon-mesencephalon (Table 2), however, specific binding of $[^{3}$H] naloxone in the absence of Na$^+$ was markedly decreased, with increased binding in the presence of Na$^+$. In the chronic pentazocine group (Group A), the +Na$^+$/−Na$^+$ ratio was about twice that of the other groups. These changes in naloxone binding were due to changes in Op-R capacity, not in affinity (data not shown). These pentazocine-induced changes in Op-R are probably related to dependence on and tolerance to that agent. Membrane receptors are known to show down regulation as a result of chronic agonist administration (14, 15). The present study suggests that pentazocine was a potent agonist effect on receptor regulation. Pentazocine is clinically used as an analgesic because of its potent agonist effect, despite its exhibiting mixed agonistic-antagonistic activity (11, 12). It is of interest to note that the pentazocine-induced changes in Op-R were abolished by the concurrent use of clonidine (Table 2, Group B), which reportedly relieves withdrawal

**Table 1. Effects of pentazocine and clonidine on opioid receptors in the cerebral cortex**

<table>
<thead>
<tr>
<th></th>
<th>Specfic naloxone binding (fmol/mg protein)</th>
<th>Ratio of +Na$^+$/−Na$^+$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>−Na$^+$</td>
<td>+Na$^+$</td>
</tr>
<tr>
<td>Group A</td>
<td>154.2 ± 8.6</td>
<td>295.5 ± 18.9</td>
</tr>
<tr>
<td>Group B</td>
<td>155.2 ± 4.6</td>
<td>293.0 ± 6.0</td>
</tr>
<tr>
<td>Group C</td>
<td>142.7 ± 7.8</td>
<td>292.5 ± 23.4</td>
</tr>
<tr>
<td>Group D</td>
<td>165.9 ± 6.1</td>
<td>304.0 ± 29.6</td>
</tr>
</tbody>
</table>

The data are shown as the mean ± SEM of 5 rats.

**Table 2. Effects of pentazocine and clonidine on opioid receptors in the diencephalon-mesencephalon**

<table>
<thead>
<tr>
<th></th>
<th>Specfic naloxone binding (fmol/mg protein)</th>
<th>Ratio of +Na$^+$/−Na$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−Na$^+$</td>
<td>+Na$^+$</td>
</tr>
<tr>
<td>Group A</td>
<td>44.9 ± 2.0**</td>
<td>203.8 ± 18.1*</td>
</tr>
<tr>
<td>Group B</td>
<td>68.4 ± 5.9</td>
<td>176.7 ± 7.1</td>
</tr>
<tr>
<td>Group C</td>
<td>60.8 ± 7.6</td>
<td>180.4 ± 16.1</td>
</tr>
<tr>
<td>Group D</td>
<td>75.3 ± 11.7</td>
<td>162.3 ± 11.7</td>
</tr>
</tbody>
</table>

The data are shown as the mean ± SEM of 5 rats. ** p < 0.01, * p < 0.05 (one-way ANOVA)
symptoms of morphine (6-9). This result indicates that the behavioral action of clonidine can also be observed at the Op-R level. Examination of Op-R, as well as the dosage and method of administration, may be necessary when clonidine is used concurrently for the treatment of narcotic analgesic dependence and tolerance.

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REFERENCES