EFFECT OF MORPHINE ADMINISTRATION ON ADENYL CYCLASE AND 3', 5'-CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITIES IN THE BRAIN

Katsumi NAITO and Kinya KURIYAMA

Department of Pharmacology, Kyoto Prefectural University of Medicine, Kamikyo-ku, Kyoto, Japan

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Adenosine 3', 5'-monophosphate (cyclic AMP) has been established as an intracellular mediator of the action of a number of amines and polypeptide hormones (1) and may well play an important role in the function of the central nervous system (2, 3). Although it has been shown that morphine induces alterations in the function of the endocrine system (4) and the metabolism of biogenic amines in the brain (5), little information is available concerning morphine induced changes in the metabolism of cyclic AMP in the brain.

In this study, the effect of acute and chronic morphine administration on activities of brain adenyl cyclase and 3', 5'-cyclic nucleotide phosphodiesterase (phosphodiesterase), enzymes catalyzing respectively the synthesis and degradation of cyclic AMP have been examined.

Swiss albino male mice weighing 25–28 g were used. For chronic experiments morphine HCl was administered continuously by the liquid diet method as previously described by Shuster et al. (6). The daily dose of morphine HCl was 55–98 mg per kg body wt. Control animals were given an equivalent amount of liquid diet without the morphine HCl. Adenyl cyclase activity in the cerebral cortex of the mouse was measured by the chromatographic separation of cyclic 3H-AMP formed from 3H-ATP as described by Krishna et al. (7). The basic incubation medium (1.8 ml in a final volume) contained tris-HCl (pH 7.3): 40 mM, MgSO₄: 3.3 mM, theophylline: 10 mM, tris ATP: 1 mM (including 0.015 mM 3H-ATP (S.A. 26 C/m mole) and an enzyme preparation (3–5 mg of crude mitochondrial (P₂) protein). Incubations were carried out for 5 min at 30°C and terminated, after the addition of 10 μ moles of carrier cyclic AMP, by immersion in a boiling bath for 2 min. Recoveries of the carrier cyclic AMP in all incubations were determined spectrophotometrically after the chromatographic separation and ZnSO₄-Ba(OH)₂ precipitation (7), and used to correct the experimental value of cyclic 3H-AMP formed in each of these fractions. For the measurement of radioactivity, an aliquot of sample was added to 12 ml of Bray's solution (8) and the radioactivity was measured in a Packard 3375 liquid scintillation spectrometer. Phosphodiesterase activity in a homogenate of cerebral cortex prepared in 0.32 M sucrose was assayed according to the procedure of Weiss (9) and the
inorganic phosphate liberated from cyclic AMP was determined spectrophotometrically by the method of Swanson et al. (10). Protein content was determined by the method of Lowry et al. (11).

Following continuous morphine administration from 1 to 4 week, adenyl cyclase activity in the cerebral cortex showed an increase of 15–29% (Table 1 A). Increases observed in the 2 and 4 week groups respectively were statistically significant. On the other hand, no alteration in phosphodiesterase activity was detected following chronic administration of morphine (Table 1 B). Neither adenyl cyclase nor phosphodiesterase activity in cerebral cortex was altered following the administration of an analgesic dose of morphine (25 mg/kg body wt.) (Table 2 A, B).

The present study clearly demonstrates that while long-term administration of morphine induces an increase of adenyl cyclase activity in the cerebral cortex, acute administration does not. It has been reported that chronic administration of ethanol, a drug

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**TABLE 1. Effect of chronic morphine administration on adenyl cyclase and phosphodiesterase activities in mouse cerebral cortex.**

<table>
<thead>
<tr>
<th>Morphine administration</th>
<th>7 days</th>
<th>14 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>182±19 (4)</td>
<td>186±8 (7)</td>
<td>166±5 (7)</td>
</tr>
<tr>
<td>Morphine treated</td>
<td>227±24 (4)</td>
<td>240±12 (7)*</td>
<td>187±5 (7)**</td>
</tr>
</tbody>
</table>

**B) Phosphodiesterase activity**

<table>
<thead>
<tr>
<th>Morphine administration</th>
<th>7 days</th>
<th>14 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.5±2.6 (4)</td>
<td>37.1±3.3 (4)</td>
<td>36.7±2.7 (4)</td>
</tr>
<tr>
<td>Morphine treated</td>
<td>43.8±1.4 (4)</td>
<td>37.3±4.2 (4)</td>
<td>33.2±4.6 (4)</td>
</tr>
</tbody>
</table>

a) pmole cyclic AMP formed/mg prot./min±S.E.
b) nmole cyclic AMP hydrolyzed/mg prot./min±S.E.
Numbers in parenthesis represent the number of experiments.
*P<0.001, **P<0.05

**TABLE 2. Effect of acute morphine administration on adenyl cyclase and phosphodiesterase activities in mouse cerebral cortex.**

<table>
<thead>
<tr>
<th>Time after administration (min)</th>
<th>Per cent change in analgesic response</th>
<th>Adenyl cyclase activitya)</th>
<th>Phosphodiesterase activityb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Morphine treated</td>
</tr>
<tr>
<td>0</td>
<td>-2</td>
<td>160±13</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>+2</td>
<td>162±8</td>
<td>172±12</td>
</tr>
<tr>
<td>60</td>
<td>+12</td>
<td>153±16</td>
<td>155±12</td>
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<tr>
<td>90</td>
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<td>157±13</td>
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</tr>
<tr>
<td>150</td>
<td>-4</td>
<td>155±20</td>
<td>148±22</td>
</tr>
</tbody>
</table>

a) pmole cyclic AMP formed/mg prot./min±S.E.
b) nmole cyclic AMP hydrolyzed/mg prot./min±S.E.
Analgesic response was measured by a hot plate method (13). Morphine HCl (25mg/kg body wt.) was injected i.p. Control mice were given an equal volume of saline only. Each value is the mean±S.E. obtained from five separate experiments.
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capable of inducing dependence or habituation, produces a similar change in adenylyl cyclase
activity in the brain (12). These facts suggest that the observed increase in adenylyl cyclase
activity following chronic morphine administration is not directly related to the develop-
ment of morphine dependence, but may be an important factor involved in the develop-
ment of drug dependence.

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