Effects of Spiradoline Mesylate, a Selective \( \kappa \)-Opioid-Receptor Agonist, on the Central Dopamine System with Relation to Mouse Locomotor Activity and Analgesia

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Received December 21, 1992 Accepted April 6, 1993

ABSTRACT—Neurochemical and behavioral investigations were made to assess the role of central dopaminergic systems in mouse locomotor activity and analgesia by spiradoline mesylate. Analgesic activities of the \( \kappa \)-opioid-receptor agonists spiradoline and U-50488H were not altered by haloperidol or L-dopa, whereas morphine analgesia was enhanced by haloperidol but attenuated by L-dopa. Spiradoline decreased spontaneous locomotor activity in mice and inhibited methamphetamine- or morphine-induced locomotor activity. In contrast, morphine given alone increased locomotor activity and enhanced methamphetamine-induced locomotor activity. In a neurochemical study, spiradoline decreased the amounts of dopamine metabolites in the striatum, but did not alter them in the brainstem and cerebral cortex. Morphine increased the dopamine metabolite contents in all three brain regions tested. These results suggest that inhibition of the dopaminergic pathway in the brain by spiradoline may be involved in its suppression of locomotor activity, but not in its analgesia; whereas, stimulation of the dopaminergic pathway by morphine seems to function in both behaviors: enhancement of locomotor activity and inhibition of analgesia.

Keywords: Spiradoline, \( \kappa \)-Opioid-receptor agonist, Dopaminergic system, Analgesia, Locomotor activity

Spiradoline mesylate, \((5a,7a,8;3)-(\pm)-3,4\text{-dichloro-N-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]}\text{methansulfonyl, an analogue of U-50488H, is a selective \( \kappa \)-opioid receptor agonist. The analgesic activities of spiradoline found in various antinociceptive tests were higher than those of morphine and U-50488H (1, 2). Spiradoline is a mixture of the \((-)\) and \((+)\) enantiomers, the former enantiomer being a \( \kappa \)-opioid receptor agonist and the latter, a \( \mu \)-opioid receptor agonist (1). However, spiradoline has the same pharmacological properties as U-50488H in terms of \( pA_2 \) for naloxone antagonism, cross-tolerance, and physical dependence (1, 2). Receptor-binding studies have shown the selectivity of spiradoline for \( \kappa \)-receptors to be 84 times that for \( \mu \)-receptors and 100 times that for \( \delta \)-receptors (3). These reports indicate that spiradoline, like U-50488H, is a highly selective \( \kappa \)-opioid-receptor agonist.

Many reports based on results of behavioral, neurochemical, and electrophysiological studies show that the actions of opioids on dopamine (DA) neurons in the brain are primarily related to \( \mu \)-opioid receptors. Little is, however, known about the actions of \( \kappa \)-opioid-receptor agonists on DA neuronal systems in the brain. Early results of pharmacological studies of various compounds with \( \kappa \)-opioid-receptor agonists, such as pentazocine, cyclazocine, ethylcyclazocine, and ketazocine, were inconclusive because of the lack of specificity towards \( \kappa \)-opioid receptors. The development of U-50488H has made possible the unequivocal examination of the role of \( \kappa \)-opioid receptors in modulating central DA neuron activity. U-50488H did not alter the activity of nigrostriatal DA neurons in normal rat brain, but did decrease the DA neuron activities in haloperidol-treated rats, in which the nigrostriatal DA neuron activities had been stimulated (4). In contrast, the putative \( \mu \)-opioid-receptor agonist morphine stimulated both nigrostriatal and mesolimbic DA neuron activities (5).

In many cases, however, the neurochemical and behavioral investigations have been performed independently, without attempting to correlate them to each other. Therefore, we first assessed the effects of a new \( \kappa \)-opioid-receptor agonist, spiradoline, on analgesia and locomotor activity; and we measured DA and its metabolites to clarify the relationship between DA metabolism and behavioral func-
tions. We also compared the neurochemical and behavioral effects of spiradoline with those of the \( \mu \)-agonist morphine and the \( \kappa \)-agonist U-50488H.

MATERIALS AND METHODS

Animals

Four-week-old male ICR strain mice, purchased from Charles River Japan Co. (Atsugi), were given free access to commercial solid food (MF: Oriental Yeast Co., Tokyo) and city drinking water in an animal room in which conditions were controlled at 22±2°C, 55±5% relative humidity, and a 12-h light-dark cycle (lights on 07:00–19:00). The mice were housed in a holding room for 4 or 5 days before the tests for analgesic activity and were denied food on the morning of the test day.

Measurement of locomotor activity

The locomotor activity of each mouse was measured with a mouse ambulometer (Ohara Medical Co., Tokyo) by the tilting cage method (6). The mouse was placed in an open round cage, 25 cm in diameter. Whenever the mouse moved, the cage tilted. The number of tilts was recorded as locomotor activity through three electromagnetic counters set at 0.5-cm intervals around the open cage.

In the case of spiradoline, locomotor activity was measured for 30 min immediately after an intraperitoneal injection of the compound in order to elucidate its inhibitory activity. In the case of morphine, the intraperitoneal injection was done when the exploratory behavior of the mouse had ceased, after which locomotor activity was measured for 180 min. In the combination study done with the DA agonist methamphetamine and the analgesic, 10 mg/kg of spiradoline or morphine was first given intraperitoneally, and then methamphetamine at 2 mg/kg was immediately injected subcutaneously. Locomotor activity was thereafter measured for 240 min at intervals of 10 min. In the combination study done with the two analgesics, 3, 10 or 30 mg/kg of spiradoline was first given intraperitoneally, and then 10 mg/kg of morphine was injected subcutaneously. Locomotor activity was thereafter measured for 180 min.

Assay of analgesic activity

The mouse tail-pin method was used. A commercial arterial clamp (Natsume Industrial Co., Tokyo) was clipped to the base of the tail, and the time that elapsed until the mouse turned and bit the clamp was recorded as the analgesic latency time. The clamp test was repeated 2 to 3 times with resting periods between tests before administration of the test drug in order to establish the base-line of the analgesic response. At the final trial, mice that showed latency times of more than 2 sec were dropped from the studies. The maximal cut off time was 15 sec.

Spiradoline and U-50488H were injected at 15 min, and morphine at 30 min, before the analgesic test in order to obtain the maximal response. Intraperitoneal injection of the DA agonist L-dopa (100 mg/kg) or subcutaneous injection of the DA antagonist haloperidol (1 mg/kg) was made 45 min before the analgesic test; i.e., morphine was administered at 15 min or spiradoline at 30 min after the DA agonist or antagonist.

Measurement of brain dopamine and its metabolites

Brain enzymes were inactivated by giving 5-kW microwave irradiation per mouse for 0.6 sec with a microwave applicator (L-2000, Shinnihonmusen, Tokyo). Thereafter, the whole brain was removed immediately and placed on aluminum foil chilled to 0°C with crushed ice. Dissection using a modification of the method of Glowinski and Iversen (7) gave 5 regions: the cerebrum, brainstem, hippocampus, striatum and cortex. To homogenize the brainstem, cortex, and striatum, each of those tissues was sonicated in 0.5 ml of 0.05 N perchloric acid containing 1.25 mM EDTA with 50 ng/ml deoxyepinephrine as the internal standard. Thirty minutes later, the homogenate was centrifuged for 15 min at 12,000 rpm and 4°C. A 250 \( \mu l \) portion of the supernatant was then added to the same volume of chloroform, and the whole mixture mixed for 30 sec. After a 15-min centrifugation at 12,000 rpm and 4°C, a 10-\( \mu l \) sample of the upper phase was analyzed by high performance liquid chromatography (HPLC).

DA, DOPAC (dihydroxyphenylacetic acid) and HVA (homovanillic acid) were assayed by the BAS HPLC system (LCEC II) equipped with an electrochemical detector. The HPLC system consisted of an ODS reverse phase analytical column (30 mm in diameter, 15-cm long), a temperature controller (37°C), and a glassy carbon detector (700-mV loaded potential). One liter of the mobile phase contained 21 g citrate anhydrate, 10 ml 5 mM EDTA, 3 ml 10 N NaOH, 80 mg sodium octyl sulphate, and 60 ml acetonitrile. The flow rate was 0.8 ml/min.

Drugs

The doses of spiradoline mesylate (Upjohn, Kalamazoo, MI, USA), morphine HCl (Sankyo Co., Tokyo), and U-50488H (Upjohn) were expressed as the weights of the free bases. These analgesics were dissolved in 0.9% NaCl solution (saline) and then injected to the mice intramuscularly at 2 ml/kg or intraperitoneally at 5 ml/kg. Methamphetamine (Philopon\( ^{\text{TM}} \), Dainippon Pharmaceutical Co., Osaka) was dissolved in saline at 2 mg/5 ml, and then injected at 5 ml/kg.
Statistical analyses

A non-parametric method of statistical analysis was used for the behavioral analgesic studies. The Kruskal-Wallis test was first done to show the significances of the differences among the groups (P < 0.05), and this was followed by Dunnett's multiple test. For the neurochemical studies, a parametric method was used, Dunnett's test being utilized only when the one way analysis of variances (ANOVA) was significant (P < 0.05).

RESULTS

Effects of morphine and spiradoline on locomotor activity

The effect of morphine on locomotor activity was measured for 3 hr after exploratory behavior (locomotor, rearing, and grooming activity) had ended in order to evaluate the increasing effect clearly. Morphine caused a dose-dependent, statistically significant increase in locomotor activity at 10 and 30 mg/kg, i.p. (Table 1). In particular, the 30 mg/kg dose produced 1859 counts/3 hr for locomotor activity, as compared to the 68 counts/3 hr of the vehicle control.

Because a preliminary study showed that locomotor activity was inhibited by spiradoline, this activity was measured for 30 min during the enhancement of exploratory behavior to determine the decreasing effect clearly. Spiradoline had no effect on locomotor activity at 3 and 10 mg/kg, i.p., but decreased it significantly at 30 mg/kg.

Table 1. Effects of spiradoline and morphine on locomotor activity in mice

<table>
<thead>
<tr>
<th>Drugs (mg/kg, i.p.)</th>
<th>Locomotor activity (counts/3 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>68 ± 9.4</td>
</tr>
<tr>
<td>Morphine</td>
<td>91 ± 11.8</td>
</tr>
<tr>
<td>3</td>
<td>185 ± 34.3</td>
</tr>
<tr>
<td>10</td>
<td>712 ± 112.8*</td>
</tr>
<tr>
<td>30</td>
<td>1859 ± 347.1**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drugs (mg/kg, i.p.)</th>
<th>Locomotor activity (counts/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>101 ± 11.3</td>
</tr>
<tr>
<td>Spiradoline</td>
<td>96 ± 24.6</td>
</tr>
<tr>
<td>10</td>
<td>81 ± 18.7</td>
</tr>
<tr>
<td>30</td>
<td>18 ± 3.1**</td>
</tr>
</tbody>
</table>

Locomotor activity was measured for 0.5 hr immediately after the intraperitoneal injection of spiradoline and for 3 hr after the injection of morphine. Data shown are means ± S.E.M. for 9–10 animals. *: **: Significantly different from saline at P < 0.05 and P < 0.01, respectively.

Fig. 1. Effects of morphine (left) and spiradoline (right) on methamphetamine-induced locomotor activity in mice. A 10 mg/kg dose of morphine or spiradoline was given intraperitoneally to mice after exploratory behavior ceased. Immediately after 2 mg/kg of methamphetamine was injected subcutaneously, locomotor activity was measured for 240 min at intervals of 10 min. Data are shown as means ± S.E.M. of 10 animals.
(18 counts/30 min) as compared to the effect of the saline vehicle control (101 counts/30 min).

Effects of morphine and spiradoline on methamphetamine-induced locomotor activity

Locomotor activity was markedly increased by 2 mg/kg, s.c. of methamphetamine alone (1657 counts/3 hr, P < 0.001) and by 10 mg/kg, i.p. of morphine alone (718 counts/3 hr, P < 0.01), as compared to the value for the saline vehicle control (55 counts/3 hr). When morphine was given to mice together with methamphetamine, there was a remarkable increase in locomotor activity (6930 counts/3 hr, P < 0.01 vs. methamphetamine alone and P < 0.001 vs. morphine alone). This increase was more than additive (Fig. 1, left).

The effect of spiradoline alone at 10 mg/kg, i.p. on locomotor activity was similar to that of the vehicle control (111 counts/3 hr), but spiradoline inhibited methamphetamine-induced locomotor activity completely (Fig. 1, right).

**Effect of spiradoline on morphine-induced locomotor activity**

As shown in Fig. 2, locomotor activity was markedly increased by 10 mg/kg, s.c. of morphine (1776 counts/3 hr). Pretreatment of mice with 3 and 10 mg/kg, i.p. of spiradoline produced no significant changes in morphine-induced locomotor activity, the respective activities being 2015 and 1279 counts/3 hr, whereas 30 mg/kg blocked morphine-induced locomotor activity significantly (284 counts/3 hr, P < 0.001).

Effects of L-dopa and haloperidol on morphine-, spiradoline- and U-50488H-induced analgesia

Neither the DA agonist L-dopa nor the DA antagonist haloperidol alone produced significant changes in the latency time following nociceptive stimulation by the mouse tail-pinch test (Fig. 3). Morphine at up to 6 mg/kg, i.m. prolonged the latency in a dose-dependent manner. The latency time of 6 mg/kg morphine was significantly shortened by pretreatment with L-dopa, but that of 3 mg/kg was not. In contrast, the latency time of 3 mg/kg morphine was much more enhanced by pretreatment with haloperidol, and that of 6 mg/kg was near the maximal cut-off time, 15 sec.

Spiradoline at 0.3 and 0.6 mg/kg significantly prolonged latency (Fig. 4), but neither L-dopa nor haloperidol affected the prolongation produced by spiradoline.

![Fig. 2. Effects of spiradoline on morphine-induced locomotor activity in mice. A 10 mg/kg dose of morphine was given subcutaneously to mice 30 min after an intraperitoneal injection of 3, 10 or 30 mg/kg of spiradoline. Locomotor activity was measured for 180 min at intervals of 10 min. The open circle, closed circle, closed triangle and closed square indicate the respective locomotor activities of the vehicle and 3, 10, and 30 mg/kg of spiradoline after morphine administration. Data shown are means ± S.E.M. for 9–10 animals.](image)

![Fig. 3. Effects of L-dopa and haloperidol on morphine-induced analgesia in the mouse tail-pinch test. Morphine at 3 or 6 mg/kg was given intramuscularly to mice 15 min after 100 mg/kg, i.p. of L-dopa or 1 mg/kg, s.c. of haloperidol. The tail-pinch test was done 30 min later. The open, hatched and dotted columns show the respective latency times of the vehicle, L-dopa, and haloperidol in combination with morphine. Data shown are means ± S.E.M. for 10–15 animals. *: Significantly different from the latency time of morphine alone at P < 0.05; **: significantly different from the latency time of morphine alone at P < 0.01.](image)
Fig. 4. Effects of L-dopa and haloperidol on spiradoline-induced analgesia in the mouse tail-pinch test. Spiradoline at 0.3 or 0.6 mg/kg was given intramuscularly to mice 30 min after 100 mg/kg, i.p. of L-dopa or 1 mg/kg, s.c. of haloperidol. The tail-pinch test was done 15 min later. The open, hatched and dotted columns show the respective latency times of the vehicle, L-dopa and haloperidol in combination with morphine. Data shown are means ± S.E.M. for 10–15 animals.

Fig. 5. Effects of L-dopa and haloperidol on U-50488H-induced analgesia in the mouse tail-pinching test. U-50488H at 6 and 12 mg/kg was given intramuscularly to mice 30 min after 100 mg/kg, i.p. of L-dopa or 1 mg/kg, s.c. of haloperidol. The tail-pinching test was done 15 min later. The open, hatched and dotted columns show the respective latency times of the vehicle, L-dopa and haloperidol in combination with morphine. Data shown are means ± S.E.M. for 10–15 animals.

**Table 2.** DA, DOPAC and HVA contents in the brainstem, cerebral cortex and striatum after the intramuscular injection of morphine

<table>
<thead>
<tr>
<th>Region</th>
<th>Dose (mg/kg)</th>
<th>Time (min)</th>
<th>DA (ng/g of wet tissue)</th>
<th>DOPAC (ng/g of wet tissue)</th>
<th>HVA (ng/g of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brainstem</td>
<td>—</td>
<td>0</td>
<td>259 ± 20.4</td>
<td>67 ± 9.4</td>
<td>145 ± 19.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td>316 ± 40.9</td>
<td>95 ± 15.7</td>
<td>188 ± 13.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>364 ± 55.7</td>
<td>97 ± 10.7</td>
<td>191 ± 19.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>278 ± 28.1</td>
<td>90 ± 11.4</td>
<td>174 ± 22.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>310 ± 60.8</td>
<td>110 ± 9.7*</td>
<td>195 ± 20.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>359 ± 60.0</td>
<td>106 ± 15.4</td>
<td>199 ± 20.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>376 ± 26.0</td>
<td>110 ± 10.0*</td>
<td>224 ± 19.9*</td>
</tr>
<tr>
<td>Cortex</td>
<td>—</td>
<td>0</td>
<td>1253 ± 153.9</td>
<td>94 ± 15.1</td>
<td>240 ± 26.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td>1100 ± 53.7</td>
<td>110 ± 14.4</td>
<td>284 ± 20.0</td>
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<td></td>
<td></td>
<td>30</td>
<td>1249 ± 85.4</td>
<td>129 ± 14.0</td>
<td>266 ± 18.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>1326 ± 180.6</td>
<td>127 ± 13.3</td>
<td>316 ± 31.7</td>
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<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>1159 ± 81.5</td>
<td>158 ± 22.7</td>
<td>265 ± 18.4</td>
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<tr>
<td></td>
<td></td>
<td>30</td>
<td>1250 ± 121.0</td>
<td>172 ± 23.1*</td>
<td>325 ± 20.9*</td>
</tr>
<tr>
<td></td>
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<td>60</td>
<td>1270 ± 83.6</td>
<td>168 ± 35.8</td>
<td>361 ± 26.3*</td>
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<tr>
<td>Striatum</td>
<td>—</td>
<td>0</td>
<td>9261 ± 474.0</td>
<td>515 ± 63.4</td>
<td>947 ± 48.8</td>
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<tr>
<td></td>
<td>3</td>
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<td>9569 ± 610.1</td>
<td>761 ± 95.4*</td>
<td>1090 ± 73.8</td>
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<td></td>
<td></td>
<td>30</td>
<td>10347 ± 420.6</td>
<td>754 ± 89.0*</td>
<td>1073 ± 43.1*</td>
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<td></td>
<td></td>
<td>60</td>
<td>9590 ± 720.8</td>
<td>722 ± 76.8*</td>
<td>1174 ± 87.1</td>
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<tr>
<td></td>
<td>6</td>
<td>15</td>
<td>10793 ± 746.3</td>
<td>747 ± 75.6*</td>
<td>1197 ± 99.4</td>
</tr>
<tr>
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<td></td>
<td>30</td>
<td>10586 ± 782.0</td>
<td>781 ± 103.0*</td>
<td>1075 ± 50.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>10489 ± 819.1</td>
<td>921 ± 182.0*</td>
<td>1356 ± 43.7*</td>
</tr>
</tbody>
</table>

Mice were killed by microwave irradiation 15, 30 or 60 min after the injection. Data shown are means ± S.E.M for 5–6 animals. *: Significantly different from 0 min at P < 0.05.
The prolonged latency by 6 and 12 mg/kg of U-50488H, a reputed typical \(\kappa\)-opioid-receptor agonist, was also not altered by L-dopa or haloperidol. Haloperidol rather tended to shorten the latency time of 12 mg/kg U-50488H (Fig. 5).

Effects of morphine and spiradoline on DA, DOPAC, and HVA contents in the brainstem, cerebral cortex and striatum

Morphine at 3 and 6 mg/kg, i.m. caused no significant changes in DA contents of the brainstem, cerebral cortex, and striatum (Table 2). A 3 mg/kg dose of morphine significantly increased the amounts of DOPAC and HVA in the striatum, but there was a tendency to increase both DA metabolites in the other two brain regions. A 6 mg/kg dose of morphine increased the two DA metabolite contents markedly in all three regions.

The DA, DOPAC, and HVA contents in the brainstem and cerebral cortex were not changed significantly 15 or 30 min after injection of 0.3 or 0.6 mg/kg of spiradoline (Table 3). The striatal DA, DOPAC, and HVA contents tended to decrease 30 min after the injection of 0.3 or 0.6 mg/kg spiradoline and showed significant decreases 30 min after the larger dose.

DISCUSSION

The results reported here demonstrate clearly that the putative \(\mu\)-opioid receptor agonist morphine increased locomotor activity and evoked analgesia in response to tail-pinch nociception, whereas the \(\kappa\)-opioid-receptor agonist spiradoline decreased locomotor activity and evoked analgesia. This suggests that the \(\mu\) and \(\kappa\)-opioid receptor agonists have different pharmacological actions. In particular, the two non-peptide opioids appear to have opposite actions on locomotor activity. Indeed, we found marked, dose-dependent inhibition of morphine-induced locomotor activity by spiradoline in mice. In contrast, both morphine and spiradoline had analgesic action in response to pressure nociception by the mouse tail-pinch; the analgesic potency of the \(\kappa\)-opioid receptor agonist was about 10 times that of the \(\mu\)-opioid receptor agonist. However, there are distinct profiles even in both analgesic actions: 1) Spiradoline analgesia is blocked by much higher amounts of naloxone than is morphine analgesia (1). 2) There is no cross-tolerance between these analgesics (1, 2). 3) Spiradoline analgesia observed in the mouse tail-pinch test is potentiated by re-uptake inhibitors of noradrenaline (NE) such as imipramine and desipramine, but morphine analgesia is not (8). The actions of spiradoline on the central nervous system including analgesia and locomotor activity are therefore distinct from those of morphine, presumably because they work through different opioid receptors. Some reports also have shown that \(\kappa\)-opioid-receptor agonists evoke potent diuretic activity and sedation (9–11), thereby differing
from the "-opioid-receptor agonists.

In our present study, morphine potentiated the locomotor activity of the DA agonist methamphetamine in a more than additive manner. Moreover, the morphine analgesia was inhibited by the DA agonist L-dopa, but potentiated by the DA receptor antagonist haloperidol. These behavioral results suggest that the activation of DA neurons stimulates the motor neuron system and depresses the analgesic neuron system. In fact, morphine increased the amounts of the DA metabolites DOPAC and HVA significantly in the brain stem, cerebral cortex, and striatum. That is, the activation of DA neurons by morphine in the neurochemical study may be explained by its increasing effect on locomotor activity and inhibitory effect on analgesia. However, there are conflicting reports on DA agents and morphine analgesia (12–14). Treatment with the DA agonist apomorphine has been reported to cause inhibition of, enhancement of, or to have no effect on morphine analgesia depending on the dose of the DA agonist; the low doses (0.1 mg/kg or less) of apomorphine inhibit presynaptic "autoreceptors", thereby depressing DA release in rats, whereas the higher doses (more than 0.3 mg/kg) stimulate postsynaptic DA receptors, thereby enhancing DA neuron activity (15). In addition, spiperone, a putative DA receptor antagonist, has been reported to potentiate morphine analgesia at 0.1 mg/kg, but this DA receptor antagonist neither potentiates morphine-induced analgesia nor impairs morphine-induced rotarod performance at 0.03 mg/kg, a dose sufficient to block postsynaptic DA receptors. It was concluded that 0.1 mg/kg of spiperone caused sedation/ataxia by blocking DA postsynaptic receptors, and the sedation/ataxia appears to enhance morphine analgesia (16). Much effort will be needed to characterize morphine analgesia with regard to central DA neurotransmission.

On the basis of reports (17, 18) on dosage, we used 100 mg/kg, s.c. of L-dopa and 1 mg/kg, i.p. of haloperidol, respectively administered at 25 and 15 min before the analgesic test. Our preliminary experiments using the mice treated with these DA agents apparently showed sedation and ataxia with this administration schedule. The DA agents, therefore, were given to mice 45 min before the analgesic test in order to avoid sedation and ataxia, with the result that L-dopa and haloperidol alone did not change the latency times. However, because mice treated with a DA agent, in particular haloperidol, showed slight sedation even under the revised administration schedule here, we cannot say definitely that slight sedation produced by haloperidol does not contribute to morphine analgesia. In contrast, enhancement of locomotor activity by the -opioid agonist morphine is correlated to the enhancement of DA metabolism, possibly by the stimulation of DA release in the central nervous system as stated previously.

In our study, spiradoline inhibited methamphetamine-and morphine-induced locomotor activity, and neither L-dopa nor haloperidol altered spiradoline analgesia. Our behavioral results suggest that DA mechanisms are involved in the locomotor inhibition produced by spiradoline, but not in the analgesic activity of this compound. The DA agents used also had no significant effects on U-50488H analgesia, although haloperidol inhibited it somewhat. Thus, the antinociceptive response to -opioid agonists may not be related to DA neuron activity. Our neurochemical study showed that spiradoline tended to decrease the DOPAC content in the striatum and also significantly decreased the HVA content, although there was no dose-dependency. It is therefore suggested that striatal inhibition of DA metabolism by spiradoline may contribute to its inhibition of locomotion. We previously reported that spiradoline analgesia reached a maximum 15 min after its intramuscular administration, the drug being eliminated 30 min after its administration (2). Since the peak time for DA metabolism was 30 min after the spiradoline administration, this report supports the behavioral results that DA neuron activities may not be related to spiradoline analgesia. As shown elsewhere (8), spiradoline analgesia may be instead caused by NE and 5-HT neuron activations.

Less is known about the effects of -opioid-receptor agonists on the DA neuronal system in the brain than about the -opioid agonists. Results of in vitro and in vivo electrophysiological and neurotransmitter release studies (19–22) indicate that -opioid-receptor agonists inhibit the activities of nigrostriatal and mesolimbic DA neurons, but there are only a few reports that -opioid-receptor agonists stimulate (23) or have no effect on (24, 25) the activities of DA neurons. From brain dialysis experiments, Di Chiara and Imperato (5) reported that the systematic administration of U-50488H produced a dose-related decrease in the release of DA from the striatum and nucleus accumbens. With respect to DA neuron activity, there is no discrepancy between our neurochemical results on spiradoline and these reports. In the brainstem and cerebral cortex, however, spiradoline did not alter the DA, DOPAC or HVA contents, which suggests a regional difference in brain DA metabolism for spiradoline. Morphine and other -opioid-receptor agonists are known to have different actions on DA release in the brain: the -opioid receptor agonists stimulate activity in most brain regions, but the opioids have been reported to both stimulate and inhibit the hypothalamic DA neurons (5, 26, 27). Manzanares et al. (4) reported that U-50488H produced a dose- and time-related decrease in basal DA release in the tuberohypophyseal DA neurons, but did not alter the activity of the tuberoinfundibular DA neurons projecting...
from the hypothalamus to the posterior pituitary. Therefore, it may be unexpected that spiradoline shows the regional differences in DA metabolism.

In summary, inhibition of the dopaminergic pathway in the brain by spiradoline may be involved in its inhibitory action on locomotor activity, but not in its analgesia. In contrast, enhancement of the dopaminergic pathway by morphine seems to function in both its increasing effect on locomotor activity and inhibition of its analgesia.

REFERENCES


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