INVolvEMENT OF CENTRAL CHOLINERGIC SYSTEM IN ANTINOCESSION INDUCED BY SUMATRIPTAN IN MOUSE

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Summary: The antinociceptive effect of the antimigraine drug sumatriptan was assessed in mice (hot-plate and abdominal constricton tests). Antinociception induced by sumatriptan (10–30 mg kg$^{-1}$ i.p.) was prevented by the muscarinic antagonist atropine (5 mg kg$^{-1}$ i.p.), the ACh-depleter hemicholinium-3 (1 μg per mouse i.c.v.) and the 5-HT$_{1A}$ antagonist NAN-190 (0.5 mg kg$^{-1}$ i.p.). Naloxone, CGP-35348 and reseprine administered in doses suitable for blocking analgesia respectively induced by morphine, bacosfen and clonipramine did not modify sumatriptan antinoceception. On the basis of the above findings, we can deduce that sumatriptan was able to induce antinoceception by increasing cholinergic neurotransmission through the stimulation of 5-HT$_{1A}$ receptors.

Introduction

The 5-HT$_{1A}$ agonists: 8-OH-DPAT, buspirone and gepirone, are able to induce an antinoceception in rodents mediated by the amplification of cholinergic neurotransmission (1, 2). These observations are in agreement with the data of Bianchi et al. (3) showing an increase in ACh efflux from the cerebral cortex of freely moving guinea-pigs after administration of 8-OH-DPAT. Since the antimigraine drug sumatriptan (GR43175) is a 5-HT$_{1A}$ receptor agonist (4, 5) our aim consisted in ascertaining whether sumatriptan, like 8-OH-DPAT, buspirone and gepirone, is able to induce antinoceception by increasing ACh release.

Materials and methods

Animals. Male Swiss-Webster mice (22–28 g), from Molini (San Polo d’Enza-Italy) were used. The animals were kept at 22 ± 1°C, with a 12:12 light:dark cycle, light at 7 a.m., with food and water ad libitum. All experiments were carried out according to the guidelines of the European Community Council on animal care.

Hot-plate test. The method described by O’Callaghan & Holzman (6) was adopted, using a stainless-steel container (36 x 28 x 30 cm), thermostatically set at 52.5 ± 0.1°C, in a precision water-bath. Mice with a licking latency below 12 and over 16 s in the test before drug administration (30%) were rejected, an arbitrary cut-off time of 45 s was adopted.
Abdominal-constriction test. The test was performed in mice according to Koster et al. (7). This number of stretching movements was counted for 10 min, starting 5 min after 0.6% acetic acid injection.

Rota-rod. The integrity of motor coordination was assessed on the basis of the endurance time of the animals on the rotating rod according to Vaught et al. (6). The performance time was measured both before and 15, 30 and 45 min after treatment.

Drugs. The following drugs were used: atropine sulfate (Sigma); hemicholinium-3 hydrobromide, naloxone hydrochloride, NAN-190 hydrobromide, (RB); CGP-35348 and reserpine (Ciba-Geigy); sumatriptan succinate (Glaxo). The doses given in the text are expressed as salts. All drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use, except reserpine which was dissolved in a 20% solution of ascorbic acid. Intracerebroventricular (i.c.v.) administration was performed under short ether anaesthesia according to the method described Haley & McCormick (9).

Statistical analysis. Results are given as the mean±SE. Analysis of variance, followed by Fisher’s PLSD procedure for post-hoc comparison, was used to verify significance of differences between two means. P values of less than 0.05 were considered significant. Data were analysed with the StatView for the Macintosh computer program (1992).

Results

Effect on pain threshold

The antinociceptive effect of sumatriptan was investigated by means of the hot-plate test and the abdominal constriction test in mice. In the hot-plate test, sumatriptan injected i.p. at doses between 10 and 30 mg kg⁻¹ induced a significant increase in the pain threshold (Table I). Sumatriptan antinociception was completely prevented by atropine (5 mg kg⁻¹ i.p.), by the choline uptake blocker HC-3 (1 μg/mouse i.c.v.) and by the 5-HT₁A antagonist NAN-190 (0.5 mg kg⁻¹ i.p.) (Table I). Conversely, no modification in sumatriptan antinociception was obtained by pretreating the mice with the opioid antagonist naloxone (1 mg kg⁻¹ i.p.) and the GABA₉ antagonist CGP-35348 (100 mg kg⁻¹ i.p.).

The dose-response curve of sumatriptan administered i.p. and i.c.v. on the abdominal constriction test is shown in Fig. 1. Sumatriptan, injected i.p. at the doses of 10, 20 and 30 mg kg⁻¹, statistically decreased the number of abdominal constrictions, with a maximum effect 15 and 30 min after treatment. Likewise, sumatriptan injected i.c.v. at 50–100 μg per mouse induced antinociception with the maximum effect 15 min after administration (Fig. 1). The antinociceptive effect of sumatriptan (20 mg kg⁻¹ i.p.) was prevented, as in the hot-plate test, by pretreatment with HC-3 but not by reserpine (2 mg kg⁻¹ i.p.), a monoamine store depletor, administered twice, 48 and 24 h before the test (Fig. 1). Sumatriptan, in the antinociceptive dose range, did not elicit any change of motor coordination as revealed by the rota-rod test (data not shown).

Discussion

Sumatriptan, like 8-OH-DPAT, is able to induce antinociception in mice in the presence of thermal (hot-plate test) or chemical (abdominal constriction test) stimuli. Sumatriptan exerts its antinociceptive effect by acting centrally for the following reasons: i) It is possible to reach the same intensity of analgesia by injecting doses (50–100 μg per mouse) of sumatriptan directly into the cerebral ventricles which are considerably lower than those needed parenterally; ii) i.c.v. administration of HC-3 is able to antagonize the increase in the sumatriptan pain threshold. Sumatriptan antinociception is dependent on central cholinergic activation mediated via 5-HT₁A receptor stimulation since it is prevented not only by the 5-HT₁A antagonist NAN-190, but also by the muscarinic antagonist atropine and the ACh depletor.
HC-3. The effectiveness of the central cholinergic system is, therefore, fundamental for sumatriptan antinociception. Other neurotransmitter systems are not involved in sumatriptan antinociception since the opioid antagonist naloxone, the GABA<sub>3</sub> antagonist CGP-35348 and the polyamine depletor reserpine are all unable to prevent the effect of sumatriptan. The doses and administration schedules of the above-mentioned drugs are suitable for preventing antinociception induced respectively by morphine (10), GABA<sub>3</sub> agonists (11) and by the antidepressant drug clomipramine (12). Skingle et al. (13) have reported that sumatriptan has little or no antinociceptive activity against a range of noxious stimuli in rodents. Since the doses of sumatriptan injected by these authors were in the same range as ours, it is probable that the striking discrepancy between our results and their depends on the excessive delay with which these authors detected the pain threshold. Since Skingle et al. (13) compared sumatriptan with morphine, we suppose that they have evaluated both drugs 30 min after administration, at which time morphine reaches its maximum analgesic activity while the antinociceptive effect of sumatriptan almost disappears.

In summary, our results show that sumatriptan is able to potentiate endogenous cholinergic activity. It remains to be clarified whether the sumatriptan antinociception observed in rodents contributes to the antimigraine activity elicited by the drug in humans.
Table 1. Dose-response curve of sumatriptan and effects of atropine, HC-3, naloxone, CGP-35348 and NAP-190 on sumatriptan anti-nociception in the mouse hot-plate test

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Treatment</th>
<th>mg kg⁻¹ i.p.</th>
<th>Before pretreatment</th>
<th>15min</th>
<th>30min</th>
<th>45min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Saline</td>
<td>13.7 ± 0.6</td>
<td>14.2 ± 0.8</td>
<td>16.6 ± 1.4</td>
<td>13.9 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sumatriptan</td>
<td>14.5 ± 1.1</td>
<td>15.7 ± 1.5</td>
<td>16.3 ± 1.4</td>
<td>14.3 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>Saline</td>
<td>13.6 ± 0.9</td>
<td>19.2 ± 1.7</td>
<td>17.3 ± 1.3</td>
<td>14.8 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>5mg kg⁻¹ i.v.</td>
<td>Sumatriptan</td>
<td>12.4 ± 1.0</td>
<td>25.7 ± 2.1</td>
<td>22.2 ± 1.5</td>
<td>17.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>HC-3</td>
<td>Saline</td>
<td>13.7 ± 0.6</td>
<td>13.9 ± 0.9</td>
<td>14.1 ± 0.5</td>
<td>14.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>1mg mouse i.c.v.</td>
<td>Sumatriptan</td>
<td>15.6 ± 1.2</td>
<td>15.7 ± 1.9</td>
<td>15.2 ± 2.2</td>
<td>16.3 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Naloxone</td>
<td>Saline</td>
<td>13.6 ± 0.4</td>
<td>13.5 ± 0.8</td>
<td>13.2 ± 0.6</td>
<td>13.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>1mg kg⁻¹ i.v.</td>
<td>Sumatriptan</td>
<td>13.6 ± 0.5</td>
<td>14.0 ± 0.9</td>
<td>13.6 ± 0.9</td>
<td>15.0 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>CGP-35348</td>
<td>Saline</td>
<td>13.6 ± 0.7</td>
<td>11.6 ± 0.9</td>
<td>12.7 ± 1.1</td>
<td>12.3 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>100mg kg⁻¹ i.p.</td>
<td>Sumatriptan</td>
<td>13.6 ± 0.7</td>
<td>12.4 ± 1.5</td>
<td>18.7 ± 1.5</td>
<td>16.6 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>NAP-190</td>
<td>Saline</td>
<td>13.6 ± 0.6</td>
<td>15.6 ± 1.4</td>
<td>16.3 ± 2.1</td>
<td>13.7 ± 1.8</td>
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</tr>
<tr>
<td>0.5mg kg⁻¹ i.p.</td>
<td>Sumatriptan</td>
<td>14.6 ± 0.9</td>
<td>16.7 ± 1.6</td>
<td>14.9 ± 1.7</td>
<td>13.6 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean of at least 8 mice.
Atropine, naloxone and NAP-190 were injected 15 min before sumatriptan; HC-3 and CGP-35348 respectively 5h and 5min before sumatriptan.
* p < 0.01; ^ p < 0.05 in comparison with saline-saline; † p < 0.01 versus saline-sumatriptan.

References


Galietti N., Ghelardini C., Bartolini A. Involvement of the serotonergic system in the analgesic effect of tricyclic antidepressants. Behav Pharmacol. 6 (Suppl. 1), 20. 1995.
