Central versus peripheral site of action of the tachykinin NK₁-antagonist RP 67580 in inhibiting chemonociception

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1 Many studies indicate an involvement of substance P in the transmission of nociceptive stimuli, without, however, presenting any conclusive evidence as to its exact site and mode of action. The present experiments tested the involvement of substance P in the mediation of chemical nociception using the non-peptidic specific tachykinin NK₁-receptor antagonist, RP 67580 (2-[1-imino-2-(2-methoxyphenyl)-ethyl]-7,7-diphenyl-4-perhydroisoindolone (3aR, 7aR)).
2 Mean arterial pressure (MAP) and intragastric pressure (IGP) were measured in anaesthetized rats. The reflex changes of these parameters in response to i.p. or s.c. injections of hydrochloric acid or capsaicin were taken to indicate nociception.
3 Intravenous administration of RP 67580 up to 5 mg kg⁻¹ had little influence on the reflex changes in MAP or IGP in response to hydrochloric acid or capsaicin. In contrast, the sensitization of rats to i.p. capsaicin by preinjection of prostaglandin E₂ was significantly reduced by 1 mg kg⁻¹ RP 67580.
4 Intrathecal injection of 5 µg RP 67580 inhibited the reflex changes of MAP and IGP in response to i.p. or s.c. capsaicin whereas the inactive enantiomer RP 68651 was ineffective.
5 The results indicate that spinal NK₁-receptors are involved in the acute transmission of chemically induced pain, while such receptors in the periphery take part in the sensitization by prostaglandin E₂. The rather minor ability of i.v. RP 67580 to inhibit the acute nociceptive reflex is attributed to an insufficient penetration of the blood-brain-barrier.

Keywords: Pain; chemonociception; substance P; NK₁-antagonist; sensitization

Introduction

Both anatomical and functional studies indicate an involvement of substance P in the spinal transmission of noxious stimuli (see Otsuka & Yoshioka, 1993). Up to now, however, no conclusive evidence exists as to whether substance P acts as a transmitter per se at the first spinal synapse or whether it only facilitates the action of glutamate (Dougherty & Willis, 1991; Mjellem-Joly et al., 1992; Rusin et al., 1992; 1993). Part of the evidence stems from the fact that non-peptidic antagonists of the NK₁-receptor, like CP-96,345 or RP 67580, display antinoceptive action, mainly in models of chemonociception (Garret et al., 1991; 1992; Yamamoto & Yakh, 1991; Berge & Stährberg, 1993; Chapman & Dickenson, 1993; Yashpal et al., 1993). Regarding models of thermal nociception, however, reports on the effectiveness of these antagonists are conflicting (Lecci et al., 1991; Radhakrishnan & Henry, 1991; Berge & Stährberg, 1993; Szolcsányi et al., 1993). Contrary to the early antagonist substance P analogues, the new compounds are devoid of neurotoxic effects and are therefore suitable for administration to the spinal cord (Yamamoto & Yakh, 1991).

The present study investigated the action of the selective NK₁-receptor antagonist, RP 67580, in a model of visceral and somatic chemonociception in anaesthetized rats. RP 67580 was chosen because of its higher affinity for the rat NK₁-receptor than CP-96,345 (Fong et al., 1992; Fardin et al., 1993b).

Methods

Experimental setup

Sprague-Dawley rats of either sex (200–300 g body weight; Forschungsanstalt für Versuchstierkunde, Himberg, Austria) were fasted overnight with free access to tap water. The experimental setup has been described in detail earlier (Holzer-Petsche, 1992). In brief, under phenobarbital anaesthesia (250 mg kg⁻¹, i.p.) the trachea was cannulated and the oesophagus ligated in the neck. Blood pressure was monitored from a carotid artery, a jugular vein was cannulated for continuous i.v. infusion of a glucose-bicarbonate buffer (1.5 ml h⁻¹) and drug injections. After laparotomy, a cannula was introduced into the stomach via the pylorus for measurement of isometric gastric contractions at a constant volume of 5 ml isotonic saline. A fall in mean arterial blood pressure (MAP) and intragastric pressure (IGP) was taken to indicate nociception (Cerervo & McRitchie, 1982; Holzer-Petsche, 1992).

For injection of algesic chemicals, up to five PE-10 cannulae were positioned in the peritoneal cavity between folds of the mesentery, taking care to avoid direct contact of the tips of the i.p. catheters with the stomach. In some experiments similar cannulae were also introduced into the abdominal subcutis. Body temperature was kept constant at 37–38°C by means of a heating pad.

HCl and capsaicin were used as algesic compounds and always injected in a volume of 100 µl. At the beginning of each experiment the threshold dose of each test substance at i.p. or s.c. administration was established for each rat. Then the antagonist or the control solution was administered i.v. (up to 2.5 ml kg⁻¹) and 10 and 20 min later the i.p. and s.c. injections of the previously established threshold doses of algesics were repeated.

In the experiments testing the effect of RP 67580 on sensitization, the threshold dose of i.p. capsaicin was arbitrarily set as '100'. After i.v. injection of RP 67580 or vehicle, 3 pmol PGE₂ was given i.p. immediately before, and through the same cannula as, each further administration of capsaicin at doses 3, 10, 30, and 100. This series of injections was always finished within 40 min after the i.v. administration of RP 67580, i.e. within the tested duration of action of the
antagonist. Because the magnitude of the responses did not correlate well with the doses of capsaicin, the results were evaluated as all-or-none effects.

For intrathecal injections, a PE-10 cannula was introduced at the beginning of the experiment through the cisterna magna so that its tip lay at the dorsal surface of the spinal cord at the lower thoracic/upper lumbar level. Injection volumes were 10 μl. The correct position of the cannula was verified by injecting 10 μl Evans blue at the end of the experiment and inspecting the distribution of the dye.

Statistics

Values are presented as means ± s.e.mean. Statistical comparisons were made using the Wilcoxon-Pratt test or Fisher’s exact test for 2 × 2 tables, both one-sided, rejecting the null hypothesis if the treatment reduced the reflex response. In the case of testing RP 67580 against the hypotensive effect of substance P the Quade test was used (Conover, 1980). P < 0.05 was regarded as statistically significant.

Substances

RP 67580 (2-[1-imino-2-(2-methoxyphenyl) ethyl]-7,7 diphenyl-4-phenyldioindolone (3aR,7aR)) and the inactive 3aS,7aS enantiomer RP 68651 were generously supplied by Dr C. Garret, Rhône-Poulenc Rorer, Vitry, France. RP 67580 was dissolved in 23 mM HCl at a concentration of 5 mg ml⁻¹, RP 68651 in 9 mM HCl at 4 mg ml⁻¹. Capsaicin was obtained from Serva (Heidelberg, Germany) and dissolved in 10% ethanol, 10% Tween80 and 80% isotonic NaCl at 10 mg ml⁻¹ (33 μmol l⁻¹). A stock solution of 1 mM 1⁻¹ substance P (Cambridge Research Biochemicals, Northwich, United Kingdom) was made up with distilled water. All dilutions were made with isotonic saline.

Results

Effect of i.v. RP 67580

Both i.p. and s.c. injection of capsaicin or HCl caused a prompt and transient fall in MAP and IGP (Figure 1). The threshold doses varied widely between rats: for HCl i.p. they ranged from 0.03–10 mmol l⁻¹, for HCl s.c. from 0.03–100 mmol l⁻¹, for capsaicin i.p. from 0.01–10 pmol, and for capsaicin s.c. from 0.003–100 pmol.

In control experiments, vehicle (HCl) was administered i.v. at a concentration of 4.6 mmol l⁻¹ (1 ml kg⁻¹) corresponding to 1 mg kg⁻¹ RP 67580 or at a concentration of 11.5 mmol l⁻¹ (2 ml kg⁻¹), which corresponded to 5 mg kg⁻¹ of the antagonist. These injections did not significantly influence the reflex changes of MAP and IGP in response to i.p. or s.c. injection of threshold doses of HCl or capsaicin (Table 1). As to RP 67580, 5 mg kg⁻¹ (11.4 μmol kg⁻¹) significantly inhibited only the changes in IGP after i.p. injection of capsaicin while having no statistically significant effect on the other parameters measured (Table 1). For reasons of solubility, higher doses of RP 67580 could not be tested.

In most control rats (vehicle i.v.), 3 pmol PGE₂ injected prior to, and through the same cannula as, capsaicin caused sensitization. This means that changes in MAP and IGP occurred in response to previously subthreshold doses of capsaicin. After RP 67580 (1 mg kg⁻¹, i.v.), the proportion of

<table>
<thead>
<tr>
<th>A</th>
<th>Stimulus</th>
<th>Dose of RP 67580</th>
<th>Fall in MAP (mmHg) before</th>
<th>Fall in MAP (mmHg) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RP67580</td>
<td>71.50 ± 1.0</td>
<td>85.0 ± 1.0</td>
</tr>
<tr>
<td>HCl i.p.</td>
<td>1 mg kg⁻¹</td>
<td>12 ± 2</td>
<td>15 ± 2 (6)</td>
<td>11 ± 0.04 (6)</td>
</tr>
<tr>
<td>HCl s.c.</td>
<td>1 mg kg⁻¹</td>
<td>14 ± 3</td>
<td>18 ± 2 (9)</td>
<td>10 ± 0.02 (9)</td>
</tr>
<tr>
<td>Cap i.p.</td>
<td>1 mg kg⁻¹</td>
<td>15 ± 2</td>
<td>15 ± 4 (13)</td>
<td>13 ± 0.03 (13)</td>
</tr>
<tr>
<td>Cap s.c.</td>
<td>5 mg kg⁻¹</td>
<td>14 ± 3</td>
<td>7 ± 1 (6)</td>
<td>14 ± 0.02 (6)</td>
</tr>
<tr>
<td>Cap s.c.</td>
<td>5 mg kg⁻¹</td>
<td>19 ± 3</td>
<td>16 ± 3 (8)</td>
<td>14 ± 0.02 (8)</td>
</tr>
<tr>
<td></td>
<td>5 mg kg⁻¹</td>
<td>13 ± 3</td>
<td>3 ± 2 (5)</td>
<td>14 ± 0.02 (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Stimulus</th>
<th>Concentration of vehicle</th>
<th>Fall in IGP (kPa) before</th>
<th>Fall in IGP (kPa) after</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl i.p.</td>
<td>4.6 mmol l⁻¹</td>
<td>1 ml kg⁻¹</td>
<td>12 ± 2</td>
<td>12 ± 2 (7)</td>
</tr>
<tr>
<td>HCl s.c.</td>
<td>4.6 mmol l⁻¹</td>
<td>1 ml kg⁻¹</td>
<td>13 ± 2</td>
<td>14 ± 2 (7)</td>
</tr>
<tr>
<td>Cap i.p.</td>
<td>4.6 mmol l⁻¹</td>
<td>1 ml kg⁻¹</td>
<td>18 ± 3</td>
<td>17 ± 3 (11)</td>
</tr>
<tr>
<td>Cap s.c.</td>
<td>4.6 mmol l⁻¹</td>
<td>2 ml kg⁻¹</td>
<td>17 ± 5</td>
<td>5 ± 3 (5)</td>
</tr>
</tbody>
</table>

Table 1 Effect of RP 67580 (A) or vehicle (HCl; B) injected i.v. on falls in mean arterial blood pressure (MAP) and intragastric pressure (IGP) induced by threshold doses of i.p. or s.c. HCl or capsaicin (Cap)
Table 2 Effect of RP 67580 (1 mg kg⁻¹, i.v.) on sensitization to i.p. capsaicin (Cap) by i.p. prostaglandin E₂ (PGE₂) (3 pmol)

<table>
<thead>
<tr>
<th>Dose of Cap after PGE₂</th>
<th>Fall in MAP (vehicle)</th>
<th>Fall in MAP (RP 67580) (vehicle)</th>
<th>Fall in IGP (RP 67580)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>11/14 (79%)</td>
<td>7/16 (44%)</td>
<td>11/15 (73%)</td>
</tr>
<tr>
<td>10</td>
<td>10/14 (71%)</td>
<td>4/16 (25%)*</td>
<td>9/14 (64%)</td>
</tr>
<tr>
<td>30</td>
<td>13/14 (93%)</td>
<td>8/16 (50%)*</td>
<td>13/15 (87%)</td>
</tr>
<tr>
<td>100</td>
<td>11/15 (73%)</td>
<td>13/18 (81%)</td>
<td>12/15 (80%)</td>
</tr>
</tbody>
</table>

Data are the number of rats responding to capsaicin vs. total n, the corresponding percentage is given in parentheses. The response is defined as fall in mean arterial pressure (MAP) and fall in intragastric pressure (IGP) caused by capsaicin. The threshold dose of capsaicin was set as 100.

*P<0.05 vs. vehicle (Fisher’s exact test for 2 × 2 tables, one-sided)

Figure 2 Effect of intrathecal injections of RP 67580 (5 μg), its inactive enantiomer RP 68651 (5 μg) or of vehicle (10 μl) on the reflex fall in mean arterial blood pressure (MAP) and intragastric pressure (IGP) in response to threshold doses of i.p. and s.c. capsaicin (Cap). (1) Before / (2) after vehicle (n = 4); (3) before / (4) after RP 67580 (n = 11–13); (5) before / (6) after RP 68651 (n = 5–8). Columns denote means, with s.e.mean.

![Figure 2](image)

**Discussion**

The present study shows that i.v. administration of the NK₁-antagonist RP 67580 up to 5 mg kg⁻¹ does not substantially antagonize reflex responses to acute chemical stimuli. This contrasts with the marked antinociceptive effect of only 1 mg kg⁻¹ of this antagonist in the phenylbenzoquinone writhing test (Garret et al., 1992), where pain-related behaviour is also induced by i.p. injection of an irritant. This lack of antagonism cannot, however, be attributed to an insufficient blockade of NK₁-receptors, because in the present study 1 mg kg⁻¹ RP 67580 inhibited substance P-induced hypotension.

Contrary to the lack of effect of i.v. administered RP 67580, i.t. injection reduced the reflex responses to i.p. or s.c. administered painful chemicals. Thus one has to conclude that spinal NK₁-receptors do take part in acute chemonociception, either somatic or visceral. The observed discrepancy can be explained by the limited ability of RP 67580 to cross the blood-brain barrier (Fardin et al., 1993a). When tested i.t., the enantiomer RP 68651 proved ineffective, indicating that the action of RP 67580 was indeed due to the blockade of NK₁-receptors and not an unspecified effect as might be concluded from the findings of Rupniak et al. (1993).

However, 1 mg kg⁻¹ RP 67580 administered peripherally, i.e. intravenously, was active against the sensitization of peritoneal nociceptors: the proportion of rats becoming sensitized to i.p. capsaicin by preinjected PGE₂ was lower in the RP 67580 group than in the vehicle group. Such an attenuation of sensitization phenomena by RP 67580 has also been observed by Amann & Donnerer (1993).

In assessing the above findings, one has to examine those models of nociception where NK₁-antagonists have proven active: non-peptide NK₁-antagonists display antinociceptive actions preferably in models of nociception involving inflammatory or sensitizing events. These include thermal hyperalgesia induced by heat (Yashpal et al., 1993) as well as the second phase of pain-related behaviour in the formalin test (Yamamoto & Yaksh, 1991; Bergé & Stähler, 1993; Yashpal et al., 1993; Chapman & Dickinson, 1993). This suggests that substance P plays a role in the development of the inflammation or of the hyperalgesia accompanying this inflammation rather than in the transmission of the acute noxious stimulus. Reports on acute antinociceptive actions of NK₁-antagonists are equivocal, but the tendency for the substances to block nociceptive reflexes seems to be greater when they are administered directly to the spinal cord rather than in the periphery (Radhakrishnan & Henry, 1991; Chapman & Dickinson, 1993).
While CP-96,345 has been shown to penetrate the blood-brain well enough (Lecci et al., 1991), the present study indicates the opposite for RP 67580, as is also emphasized by Fantini et al. (1991). This differs from the conclusion drawn by Laird et al. (1993) from their observation that i.v. administered RP 67580 inhibits the facilitation of a nociceptive spinal flexion reflex in anaesthetized rats. However, their results might also be interpreted as RP 67580 inhibiting facilitation at a peripheral level: the conditioning stimulus to the afferent C-fibres might release enough substance P from the peripheral nerve endings in order to lead to a short-lived sensitization of the afferent fibres.

Several studies indicate a peripheral role of substance P via NK1-receptors in sensitizing afferent nerve endings. Kessler et al. (1992) have demonstrated on a rat skin-nerve preparation in vitro that substance P sensitizes nociceptors to a mixture of inflammatory mediators. Yamamoto et al. (1993) have shown that carrageenin-induced thermal hyperalgesia of the rat paw can be prevented by i.v. but not i.t. administration of the NK1 antagonist CP-96,345 or the combined NK1/NK2 antagonist FK224. From differences in efficacies between i.p. and i.t. administered CP-96,345 Berge & Stålberg (1993) deduce a peripheral site of action of the antagonist in inhibiting the second phase of the formalin test. Analogous, though unrelated to nociception, is the ability of CP-96,345 to block hypoxia-induced afferent activity in the carotid sinus nerve of cats (Prabhakar et al., 1993). According to these authors this might imply that substance P from glomus cells is involved in exciting afferent nerve endings via NK1-receptors. Thus there is quite some evidence in favour of substance P sensitizing or even exciting peripheral afferent endings.

Even the effectiveness of RP 67580 in the writhing test (Garret et al., 1992) might possibly be attributed to a peripheral action: the behaviour in the writhing test usually takes a few minutes to develop. It is conceivable that during this time lag sensitization phenomena are initiated, which might be blunted by peripherally acting NK1-antagonists.

The mechanism of such a sensitization is, however, still far from being elucidated. It is known that substance P releases prostaglandins from a variety of cells, for example, synoviocytes (Lottz et al., 1987), or spinal cord astrocytes (Marriott et al., 1991). These prostaglandins, in turn, can sensitize nociceptive nerve endings to subsequent stimuli. However, there are data indicating also the opposite sequence of events, i.e. prostaglandins apparently releasing substance P: Nakamura-Craig & Smith (1989) showed that repeated subplantar injections of substance P or PGE2 caused mechanical hyperalgesia in rats pretreated with indomethacin. A substance P antagonist blocked the sensitization by substance P as well as that by PGE2. In fact, in cultures of chick embryo dorsal root ganglion cells PGE2 was directly observed to release substance P (Nicol et al., 1992). Such a mechanism might also apply in the present model of sensitization by PGE2.

In conclusion, the results of the present study indicate that central NK1-receptors contribute to the direct transmission of painful chemical stimuli, while peripheral receptors play a role in sensitization phenomena.

The authors thank Mrs B. Brodacz for excellent technical assistance and Dr C Garret, of Rhône-Poulenc Rorer, for the generous supply of RP 67580 and RP 68651. T.R.N. held an ARGE Alpen-Adria Fellowship from the Government of Styria. The work was supported by the Austrian Research Foundation, grant no. P7858-MED.

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(Received July 18, 1994
Accepted October 25, 1994)