SITE OF ANALGESIC ACTION OF ZOMEPIRAC SODIUM, A POTENT NON-NARCOTIC ANALGESIC IN EXPERIMENTAL ANIMALS

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Abstract—Zomepirac sodium inhibited the reflex hypertension caused by an injection of bradykinin into the splenic artery of anesthetized dogs, but not that by injection of bradykinin plus PGE₁. In the rat acetic acid writhing test, the potency ratio of intraperitoneal (ED₅₀=0.41 μg/kg) to intravenous (ED₅₀=33.5 μg/kg) anti-writhing activity of zomepirac sodium was 79.2 (37.1–173), though the ratio of codeine phosphate (373 μg/kg, i.p., 352 μg/kg, i.v.) was 0.934. When equipotent doses of zomepirac sodium were administered to rats receiving intraperitoneal acetate acid, the plasma zomepirac level after i.v. administration was more than 200 times that after i.p. administration, while the peritoneal exudate zomepirac contents were nearly equal after administration by both routes. Zomepirac sodium (5 μg/kg) did not produce significant anti-writhing activity after intracerebroventricular administration. From these results, it was suggested that zomepirac sodium produced analgesic action through a strong blockade of the hyperalgesia in the peripheral system.

Materials and Methods

Drug: Sodium 5-(4-chlorobenzoyl)-1,4-dimethyl-1H-pyrrole-2-acetate dihydrate (zomepirac sodium), codeine phosphate and morphine hydrochloride were used. Drugs...
were dissolved in saline for intravenous and intraperitoneal administration and dissolved in 0.5% gum tragacanth aqueous solution for oral administration. Dosages were expressed as the salt form of the drug. In all experiments, zomepirac sodium was administered as the sodium salt dihydrate; 100 mg zomepirac sodium is equivalent to 83.33 mg zomepirac.

**Bradykinin evoked visceral pain:** Mongrel dogs weighing 8 to 12 kg were anaesthetized with hexobarbital sodium (30 mg/kg, i.v.) and α-chloralose (30–50 mg/kg, i.v.). Under anaesthesia, a branch of the splenic artery and cephalic vein were cannulated with polyethylene tubes for bradykinin and drug injection, respectively. The femoral artery was also cannulated for measuring blood pressure. After waiting 2 hr or more, a starting dose of 1 µg of bradykinin (Protein Research Foundation, Osaka) was injected intraarterially into the spleen through the cannula assembly under light anaesthesia, and only animals showing an increase in blood pressure (hypertension) by bradykinin was measured before and 5, 15 and 30 min after intravenous administration of the test drug (8–10).

**Anti-writhing test:** Male Wistar rats weighing 100 to 110 g were used in the acetic acid writhing test. Each rat was given intraperitoneally 1 ml of a 1% acetic acid aqueous solution and was settled into a cylindrical cage (24.5 cm in diameter) made of transparent acrylic resin. Only the rats showing a writhing syndrome within 15 min after acetic acid injection were used for the experiment. Drugs were administered to the rats 60 min after acetic acid injection, and then the number of writhes was counted for 20 min (11, 12).

Female ddN mice weighing 18 to 22 g were used in the phenylquinone writhing test. Each mouse was given intraperitoneally 0.1 ml/10 g body weight of a 0.03% phenylquinone solution in 5% aqueous ethanol, and the number of writhes was counted for 15 min beginning from 5 min after phenylquinone injection. Drugs were administered orally 30 min before phenylquinone injection (13, 14).

A reduction in writhing counts greater than 50% of the vehicle control value was considered to be significant (effective). The anti-writhing ED50-value was calculated from the effective rates (percentages of number of animals effective/tested) according to the method of Litchfield and Wilcoxon (15).

**Intracerebroventricular injection:** Male Wistar rats weighing 100 to 110 g were used. A guide cannula was stereotactically implanted into the lateral ventricle of the brain under hexobarbital sodium anaesthesia, and 5 days later, the rats were used for the acetic acid-induced writhing test. After intracerebroventricular (i.c.v.) administration of test drugs, the injected site was confirmed by injecting Evan's blue dye in the same manner.

**Determination of zomepirac sodium in plasma and peritoneal exudate:** The blood was obtained by heart puncture from the rat receiving intraperitoneally acetic acid under ether anaesthesia, and then after death, the exudate in the peritoneal cavity was washed out with 10 ml of distilled water. Zomepirac in plasma and the exudate was extracted and determined according to the method of Kojima and Yoshida in our laboratories as follows: A half ml of plasma was put in a centrifuge tube (15 ml vol.), acidified with 3 ml of 6 N H₂SO₄, shaken with 5 ml of n-heptane containing 1.5% isoamylalcohol for 15 min, and then centrifuged at 2,200 rpm for 10 min. Four ml of the n-heptane layer was evaporated by bubbling with air in a water bath at 50°C. The residue was dissolved in 0.1 ml of methanol, and 10 to 20 µl of the methanol solution was injected into the column of a high-performance liquid chromatograph (HPLC). The peritoneal exudate with washings was pooled, and 5 ml of this was ex-
traced by the same procedure as used for
the plasma.

A Waters Model 204-chromatograph and
μ-Bondapack NH₂ column were used with a
Model 6000A Solvent Delivery System,
Model U6K Universal Injector and Model
UV440 Absorbance Detector. The eluent
constituent consisted of a mixture of 1%
acetic acid, methanol and acetonitrile (1/1/5
by vol.). The flow rate was 2.5 ml/min and
the effluent was monitored at 254 nm.
Retention time of zomepirac is 3.8 min. A
known amount of zomepirac sodium was
added to the pooled plasma and the pooled
exudate from the rat receiving an intra-
peritoneal injection of acetic acid, and the
calibration curve was estimated. Zomepirac
contents in the sample were determined from
the calibration curve.

Statistics: The potency ratio was deter-
mained according to the method of Finney
(16), and statistical significance was deter-
mained by Student's t-test.

Results
Tolerance and naloxone antagonism of
anti-writhing action: The anti-writhing ED50-
value of zomepirac sodium has been reported
to be 0.91 mg/kg given orally in the mouse
phenylquinone writhing test (17). Thus, 5
mg/kg of zomepirac sodium was given
orally to the mice once a day for 7 days; and
on the 8th day, the anti-writhing activity of
zomepirac sodium was compared with that
in the mice repeatedly given the vehicle in
the phenylquinone writhing test. The anti-
writhing ED50-values (95% confidence
limits) of both groups were the same: 0.982
(0.191-5.06) mg/kg. p.o. Thus, analgesic
tolerance to zomepirac sodium was not
produced. Under the same experimental
conditions, tolerance to codeine phosphate
has been found to be produced (18).

Anti-writhing activity (inhibitory rate=70.0%, n=7) of zomepirac sodium (3 mg/kg,
p.o.) was not reversed by 0.04 (65.8%, n=7)
and 0.4 (67.5%, n=7) mg/kg, s.c. of naloxone
hydrochloride in the phenylquinone writhing
test in mice.

Effect of zomepirac sodium on bradykinin-
and bradykinin plus PGE₁-induced hyper-
tension: An increase in blood pressure of
about 25 mmHg was produced by injecting
bradykinin at a dose of 1 μg/dog into the
splenic artery. This hypertension was
enhanced by the simultaneous administration
with 5 μg/dog of prostaglandin E₁ (PGE₁),
while PGE₁ (5 μg/dog) alone did not
produce any pressure change. In the pressure
activity, the simultaneous administration of
0.5 μg/dog of bradykinin with 5 μg/dog of

![Graph](image_url)

**Fig. 1.** Effect of zomepirac sodium and codeine
phosphate on the hypertension caused by intra-
arterial injection of bradykinin or bradykinin plus
PGE₁ in lightly anaesthetized dogs. The reflex
hypertension caused by intraarterial injection of
bradykinin (1 μg/dog, open column) or bradykinin
(0.5 μg/kg) plus PGE₁ (5 μg/kg, stippled column)
was determined just before and 5, 15 and 30 min
after a single intravenous administration of each
drug. Ordinate scale represents the maximal in-
hibitory effect as compared with each pre-drug
value among the 3 determinations. The vertical bar
represents S.E. •0.01<P<0.05, significantly different
from each vehicle control. •0.01<P<0.05,
significantly different from the bradykinin group.
PGE₁ was approx. equivalent to 1 μg/dog of bradykinin. When bradykinin or bradykinin plus PGE₁ was given intraarterially to the dog 3 to 5 times at 10 min intervals, constant pressure responses were found to be evoked.

When zomepirac sodium was given intravenously to the dog at doses of 4 to 16 mg/kg, the hypertension by bradykinin was inhibited in a dose-dependent manner. Eight mg/kg, i.v., of zomepirac sodium produced the maximum reduction of 60.4±15.5% (n=4) (among three determinations as compared with the pre-drug value) of the hypertension by bradykinin, but only 18.6±6.5% (n=4) of that by bradykinin plus PGE₁ (Fig. 1). In contrast, 8 mg/kg, i.v., of codeine phosphate reduced both the hypertensions by almost the same degree.

Comparison in anti-writhing potencies of intraperitoneally and intravenously administered zomepirac sodium: Zomepirac sodium was administered into the peritoneal cavity or into the tail vein of rats 60 min after injection of acetic acid, and then the number of writhes was counted for 20 min. Zomepirac sodium produced a dose-dependent decrease in the writhing counts at dosage ranges of 0.25 to 1 μg/kg, i.p., and 20 to 80 μg/kg, i.v.; and their effects were significant in comparison with the vehicle control (Table 1). The effective rates, percentages of number of rats effective/ tested are shown in Fig. 2. Anti-writhing ED50 calculated from the effective rates was 0.41 μg/kg, i.p., and 33.5 μg/kg, i.v. (Table 1); and the potency ratio (95% confidence limits) of intraperitoneally to intravenously administered zomepirac sodium was 79.2 (37.1–173). n=60. Namely, the anti-writhing activity of zomepirac sodium after intraperitoneal administration was about

<table>
<thead>
<tr>
<th>Route</th>
<th>Drug</th>
<th>Dose μg/kg</th>
<th>n</th>
<th>No. of writhing/20 min mean±S.E. (%)</th>
<th>ED50 in mg/kg (95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>0.25</td>
<td>10</td>
<td>8.4±1.3*</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td>Zomepirac sodium</td>
<td>0.5</td>
<td>10</td>
<td>5.6±0.9**</td>
<td>53.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>10</td>
<td>4.3±0.9**</td>
<td>64.5</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td></td>
<td>14</td>
<td>13.4±0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Codeine phosphate</td>
<td>200</td>
<td>7</td>
<td>9.3±1.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>7</td>
<td>8.3±1.3**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>800</td>
<td>7</td>
<td>2.9±0.9**</td>
<td></td>
</tr>
<tr>
<td>i.v.</td>
<td>Saline</td>
<td>20</td>
<td>10</td>
<td>9.3±1.1**</td>
<td>37.6</td>
</tr>
<tr>
<td></td>
<td>Zomepirac sodium</td>
<td>40</td>
<td>10</td>
<td>7.2±1.6**</td>
<td>51.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>10</td>
<td>4.4±0.9**</td>
<td>70.5</td>
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<tr>
<td></td>
<td>Saline</td>
<td>13</td>
<td>14</td>
<td>14.9±0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Codeine phosphate</td>
<td>200</td>
<td>7</td>
<td>10.1±1.8*</td>
<td>34.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>7</td>
<td>8.1±1.6**</td>
<td>47.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>800</td>
<td>7</td>
<td>1.9±0.8**</td>
<td>87.7</td>
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</table>

Drugs were administered intraperitoneally or intravenously to the rat 60 min after acetic acid injection, and then the number of writhes was counted for 20 min. The ED50-value was calculated from the effective rates (See Fig. 2 explanation). *0.01<P<0.05 and **P<0.01, significantly different from each saline control. n: number of rats used.
Fig. 2. Comparison of anti-writhing activities of zomepirac sodium and codeine phosphate by intraperitoneal and intravenous administration in rats. Each drug was administered intraperitoneally (open symbols, broken lines) and intravenously (closed symbols, solid lines) to the rat. When the rat showed half or less of the number of writhes counted in the vehicle control group, the dose was considered to be effective. The ordinate scale represents the percentages of number of rats in which the dose was effective/number of rats tested.

Table 2. Exudate and plasma zomepirac levels after intraperitoneal or intravenous administration of zomepirac sodium in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose µg/kg</th>
<th>Route</th>
<th>n</th>
<th>Zomepirac level (mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>exudate (ng/rat)</td>
</tr>
<tr>
<td>Zomepirac sodium</td>
<td>4</td>
<td>i.p.</td>
<td>7</td>
<td>111±14.6</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>i.v.</td>
<td>7</td>
<td>58.6±4.5</td>
</tr>
</tbody>
</table>

Zomepirac sodium was administered intraperitoneally or intravenously to the rat 60 min after acetic acid injection; and 5 min later, zomepirac contents in the peritoneal exudate and plasma were determined.

80 times more potent than that after intravenous administration. In contrast, ED50-values of codeine phosphate were 373 µg/kg, i.p., and 352 µg/kg, i.v. (Table 1); and the potency ratio of codeine phosphate was 0.934 (0.398–1.95), n=42.

Zomepirac levels in plasma and peritoneal exudate: Four and 160 µg/kg of zomepirac sodium were administered intraperitoneally and intravenously, respectively, to the rat 60 min after acetic acid injection; and 5 min later, zomepirac levels in the plasma and peritoneal exudate were determined. As shown in Table 2, zomepirac level in the exudate after intraperitoneal administration was about 2 times greater than that after intravenous administration, though the plasma level was less than 1/200. This finding suggests that the average zomepirac contents in the exudate during the counting period of 20 min after intraperitoneal administration may be nearly equal to or greater than that after intravenous administration.

Anti-writhing activity of intracerebroventricularly administered zomepirac sodium: Zomepirac sodium was dissolved in sterile saline and 2 µl of it was infused into the lateral cerebroventricle of the rat brain.
Table 3. Anti-writhing activity of zomepirac sodium and morphine hydrochloride after intracerebroventricular administration in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose µg/kg i.c.v.</th>
<th>n</th>
<th>No. of writhing/20 min mean±S.E.</th>
<th>inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2±1</td>
<td>7</td>
<td>14.1±1.4</td>
<td></td>
</tr>
<tr>
<td>Zomepirac sodium</td>
<td>5</td>
<td>8</td>
<td>10.1±1.8</td>
<td>28.4</td>
</tr>
<tr>
<td>Morphine HCl</td>
<td>5</td>
<td>7</td>
<td>2.7±0.6**</td>
<td>80.9</td>
</tr>
</tbody>
</table>

Drugs were administered intracerebroventricularly (i.c.v.) to the rat 60 min after acetic acid injection, and then the number of writhes was counted for 20 min. **P<0.01, significantly different from the saline control. °: µl/kg, i.c.v.

through the guide cannulae implanted previously. Then, the number of writhes was counted for 20 min after zomepirac sodium administration. Zomepirac sodium did not produce significant anti-writhing activity after i.c.v. administration of 5 µg/kg. In contrast, morphine hydrochloride showed a potent anti-writhing activity after a single administration of 5 µg/kg, i.c.v., and its inhibitory rate was 80.9% (P<0.01) (Table 3).

Discussion

It has been reported that zomepirac sodium produces a potent analgesic activity in man (2-6) despite its chemical structure which is related to the acidic non-steroidal anti-inflammatory drugs. Thus, it may be possible that zomepirac sodium might partly have a central mechanism of analgesic action or mode of analgesic action different from other non-steroidal anti-inflammatory analgesics. Thus, the site of analgesic action of zomepirac sodium was compared with that of codeine, which is a centrally acting analgesic.

Strong narcotic analgesics like morphine induce tolerance, and their analgesia is reversed with naloxone. Zomepirac sodium, unlike codeine, did not produce tolerance after repeated administration for 7 days, and its anti-writhing action was unaffected by naloxone. These results are in accord with those by Pruss et al. (1). These properties of zomepirac sodium were different from those of morphine-like analgesics.

It is widely accepted that intraarterially injected bradykinin induces reflex hypertension due to stimulation of pain receptors, but not due to the chemical constriction of the spleen in the spleen of lightly anaesthetized dogs (9, 19). Ferreira et al. (20) have reported that PGE$_1$ sensitizes pain nerve endings in the spleen of lightly anaesthetized dogs and potenciates the reflex hypertension of bradykinin and that the spleen generates and releases an E-like prostaglandin continuously into the venous outflow, and this release is readily increased by intraarterial bradykinin. The pain producing activity of bradykinin in the presence of exogenous PGE$_1$ or PGE$_2$ is unaffected by aspirin-like drugs (20, 21), but is blocked by morphine-like drugs. Zomepirac sodium inhibited the hypertension of bradykinin, but did not inhibit significantly the hypertension of bradykinin plus PGE$_1$. Codeine, however, blocked both the hypertensions. The mode of this action of zomepirac sodium supports the results that zomepirac sodium is a potent inhibitor of prostaglandin synthetase in vitro (1, 7).

Nakamura and Shimizu (22) have reported that the technique of estimating the potency difference between intraperitoneal and intravenous administration of the test drug is useful for investigation of the site of action of analgesic drugs. In this technique, the
anti-writhing activity of zomepirac sodium after intraperitoneal administration was about 80 times more potent than that after intravenous administration, and also there was a correlation between the anti-writhing activity and the zomepirac contents in the peritoneal exudate. Thus, it is suggested that site for the anti-writhing action of zomepirac sodium, unlike codeine and morphine, is mostly in the peritoneum (peripheral system).

A significant anti-writhing action was not found when zomepirac sodium was administered intracerebroventricularly at 5 μg/kg (about 10 times of the i.p. ED50). The slight inhibitory action of zomepirac sodium might be due to a direct action of zomepirac sodium on the central nervous system and/or an action of zomepirac sodium which had leaked out into blood from the cerebroventricle, or it might be due to a non-specific action of zomepirac sodium. This lack of analgesic action by zomepirac sodium is in accord with the data of Capetola et al. (23) who showed that zomepirac sodium (total doses of 25 and 50 μg), but not morphine sulfate (10 μg), failed to cause significant analgesic effects after direct injection into the lateral ventricle in the rat adjuvant arthritic flexion assay. It has been reported that the brain zomepirac level (0.48 μg/g) is 2.08% of the plasma level (23.1 μg/ml) 6 hr after a single oral administration of 6 mg/kg of zomepirac, and the peak time in the plasma level is about 4 hr after dosing (24). This brain level is lower than the other organs tested: one-third and one-ninth that in the muscle and skin, respectively. Thus, it is improbable that zomepirac accumulates highly in the brain after intravenous or intraperitoneal administration of zomepirac sodium. In contrast, morphine hydrochloride produced marked anti-writhing activity after i.c.v. administration of 5 μg/kg, lower than its equipotent dose (about 40 μg/kg) (22) after intravenous or intraperitoneal dosing. Prostaglandins have been postulated to be involved in the transmission of pain impulses within the brain (25–27). Thus, this postulation suggests the possibility that inhibitors of prostaglandin synthetase may produce their analgesic action through both the central and peripheral mechanisms. The present results, however, ruled out the possibility that an inhibitor of prostaglandin synthetase, zomepirac sodium, may modify the response to pain in the brain caused by peripheral stimulation, at least after systemic administration of its analgesic doses.

These findings demonstrate that zomepirac sodium (1) produces an analgesic action dissimilar to morphine-like analgesics; (2) blocks the hyperalgesia brought about by bradykinin, prostaglandins etc.; and (3) acts in the peripheral system. In general, noxious stimuli produce pain by direct action on the nociceptors and/or through the release of algesic chemicals such as bradykinin, prostaglandins and substance P, which cause the hyperalgesia by several mechanisms. Zomepirac sodium inhibits strongly the pain-like responses caused by algesic chemicals (17), and its anti-writhing ED50-value (0.41 μg/kg) after intraperitoneal (action site) administration was extremely low as compared with that of oral zomepirac sodium (330 μg/kg) (17), codeine phosphate (352 μg/kg, i.v.) and morphine hydrochloride (12.5 μg/kg, i.v. and i.p.) (22). Consequently, the present results may demonstrate that the strong blockade of the hyperalgesia in the peripheral system brings about comparatively potent analgesia in man.

References
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