Pharmacological Characterization of Capsaicin-Induced Body Movement of Neonatal Rat

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ABSTRACT—In neonatal rats, nociceptive responses induced by capsaicin were characterized pharmacologically. Capsaicin, injected subcutaneously (s.c.), induced body movement including scratching and struggling responses, and the responses were quantified by using a device composed of an audio speaker as a detector. The capsaicin-induced body movement was inhibited by a tachykinin NK₁-receptor antagonist RP-67580 with an ID₅₀ value of 3.5 mg/kg, s.c. Opioid analgesics, morphine, buprenorphine and pentazocine, also inhibited the body movement with ID₅₀ values of 0.085, 0.0079 and 0.92 (mg/kg, s.c.), respectively. Non-steroidal anti-inflammatory drugs, indomethacin, ibuprofen and acetaminophen, did not exert any effect on the capsaicin-induced body movement. Neither the sedative diazepam nor the sedative chlorpromazine inhibited the body movement. It is concluded that the capsaicin-induced body movement in neonatal rats, which is considered to be nociceptive responses mediated by substance P, is sensitive to centrally acting analgesics with μ-opioid receptor agonist activity.

Keywords: Capsaicin, Analgesia, Opioid, Quantification of nociceptive response, Neonatal rat

It has been demonstrated that in the neonatal stage in rats, the tail, forepaw and hindpaw react to nociceptive thermal stimulus (1–5) and that at this stage, subcutaneously (s.c.) injection of formalin into a hindpaw induces nociceptive behavioral responses (6, 7). Thus, in neonatal rats, the processing pathway of pain information from peripheral tissue to the central nervous system is already developed.

Previously, we reported that s.c. injection of capsaicin in the neonatal rat’s dorsa induced body movement including scratching and struggling responses and that the responses could be quantified by a device composed of an audio speaker as a detector (8). The characteristic and advantageous features of this method are that an analgesic effect is automatically quantified by merely putting animals on the detector, the techniques employed are less laborious for experimenters, small quantity of drug is required, and animals can be tested in series at intervals of 90 sec (8).

In adult rats, the behavioral response to s.c. injection of noxious chemicals is inhibited by tachykinin NK₁-receptor antagonists (9), opioids (10, 11), non-steroidal anti-inflammatory drugs (NSAIDs) (12–14) or sedatives (15). In neonatal rats, however, although it is known that opioids inhibit the nociceptive response induced by chemical stimulus (6, 11), the effects of tachykinin NK₁-receptor antagonists, NSAIDs and sedatives on the nociceptive response remain to be examined. In the present experiments, the capsaicin-induced body movement in the neonatal rat was characterized pharmacologically by using these drugs. Preliminary results of this study have been presented elsewhere (16).

MATERIALS AND METHODS

All experiments were carried out according to the guidelines provided by the Institutional Animal Care and Use Committee of Sankyo Co., Ltd. (Tokyo).

Animals

Pregnant and maternal Wistar-Imamichi rats (the Imamichi Institute of Animal Reproduction, Ibaraki) were housed with a 12-hr light-dark cycle and with food and water available ad libitum. Room temperature was maintained at 23±1°C. Both male and female 2- to 8-day-old neonatal rats were used. The day of birth was considered 0-day-old.

Quantification of capsaicin-induced body movement

Details of the method have been described elsewhere
(8). The body movements including scratching and struggling responses of the neonatal rat induced by s.c. injections of capsaicin were recorded by a device composed of an audio speaker as a detector. The animal was put in a plastic chamber that was attached to the diaphragm of the audio speaker. The body movement of the animal in the chamber generated a voltage change in the speaker coil. The signal of the voltage change was transferred to a matching transformer, an audio amplifier, a full-wave rectifier, a relay, a monostable multivibrator and a pulse-counter in turn. When the output signal from the speaker overshot a specified threshold level, digital pulses were generated by the monostable multivibrator, which were counted by the pulse counter.

Drugs

The following chemical substances were used: capsaicin, indomethacin, chlorpromazine hydrochloride, U-50488 methanesulfonate (Sigma Chemical Co., St. Louis, MO, USA); p-acetamidophenol (acetaminophen), diazepam (Wako Pure Chemical Industries, Ltd., Osaka); morphine hydrochloride, pentazocine hydrochloride, naloxone hydrochloride (Sankyo Co., Ltd., Tokyo); buprenorphine hydrochloride (Leptan; Otsuka Pharmaceutical Co., Ltd., Tokyo); RP-67580 (3aR,7aR)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)ethyl]perhydroisoindol-4-one: synthesized in the Medicinal Chemistry Research Laboratories, Sankyo Co., Ltd.,); S(+)-ibuprofen (Research Biochemicals International, Natick, MA, USA); TAN-67 dihydrobromide (Toeris Cookson Ltd., Bristol, UK).

A concentrated stock solution of capsaicin was prepared by dissolving it in 100% ethanol and working solutions were made by diluting the stock solution with saline. The solution was injected s.c. at 100 μl volume in the dorsa of animals. RP-67580, morphine, pentazocine, buprenorphine, naloxone, TAN-67 and chlorpromazine were each dissolved in saline, and diazepam was suspended in saline containing 0.5% Tween 80. Each drug solution was administered s.c. at 10 ml/kg of body weight 25 min prior to the capsaicin injection, except for naloxone, which was administered 20 min prior to the capsaicin injection. U-50488 was dissolved in saline and administered s.c. at 10 ml/kg of body weight, immediately before measuring body movement. Indomethacin, ibuprofen and acetaminophen were dissolved in 5% N,N-dimethylacetamide and 95% polyethylene glycol 400, and each drug solution was administered intraperitoneally (i.p.) 15 min prior to the capsaicin injection at 1 ml/kg of body weight.

Data analyses

Data were expressed as the mean±S.E.M. Data were evaluated by ANOVA followed by Dunnett’s multiple comparison test. In cases where a control group was to be compared with a single group treated with a drug, Student’s t-test was used. Potency of analgesics was expressed as ID50, the dose at which the nociceptive response was reduced to 50% of the mean control value. In calculating the ID50 value, the count of the response induced by the saline injection was subtracted from that induced by the capsaicin injection. ID50 values and 95% confidence limits were determined by linear regression analysis from the percent value.

RESULTS

Body movement induced by capsaicin

When capsaicin (1 ng–10 μg/100 μl/body) was injected s.c. in the dorsa of neonatal rats aged 2, 5 and 8 days, the rats scratched the administered area, struggled (i.e., thrashing about and rolling laterally), and sometimes squeaked. At all the ages tested, capsaicin induced the response immediately after injection, and the response peaked at 0–1 min and then decayed (Fig. 1A: a–c); the response increased dose-dependently (Fig. 1A: d). The level of response to capsaicin in 5-day-old rats was somewhat lower at low doses and higher at high doses than in 8-day-old rats, but they were not significantly different. The level of response to capsaicin at some doses for 2-day-old rats were significantly lower than that in 5- or 8-day-old rats. The response to vehicle in 2-day-old rats was also significantly smaller than that in 8-day-old rats. In the following experiments, the same aged rats, 4- to 8-day-old, were used in one type of experiments and sub-maximum effective doses of capsaicin (150 or 300 ng/100 μl/body) were used; the resultant body movement was measured for 1 min immediately after the injection of capsaicin.

Capsaicin is known to release substance P-like immunoreactivity from the central terminals of primary afferents (17, 18). It was investigated whether substance P was involved in the capsaicin-induced body movement, using a non-peptide tachykinin NK1-receptor antagonist, RP-67580. The drug (3–30 mg/kg), administered s.c. 25 min prior to the capsaicin injection, inhibited the body movement dose-dependently (Fig. 1B, Table 1).

Effect of opioids on capsaicin-induced body movement

A μ-opioid receptor agonist, morphine (0.03–0.3 mg/kg), administered s.c. 25 min prior to the capsaicin injection, inhibited the capsaicin-induced body movement dose-dependently (Fig. 2A: a, Table 1). Buprenorphine (0.003–0.03 mg/kg) and pentazocine (0.3–3 mg/kg) also inhibited the capsaicin-induced body movement dose-dependently (Fig. 2A: b and c, Table 1). These
effects of morphine and buprenorphine disappeared in animals treated with naloxone, but the effect of pentazocine was not fully reversed by naloxone (Fig. 2B).

In contrast to the effects of these opioids, a κ-opioid receptor agonist, U-50488 (0.3 and 1 mg/kg, s.c.) alone induced a marked increase of body movement (data not shown), being consistent with previous reports (19, 20). This made us unable to examine its analgesic effect.

A δ-opioid receptor agonist, TAN-67 (30 mg/kg, s.c.), did not inhibit the capsaicin-induced body movement.

Effect of NSAIDs and sedatives on capsaicin-induced body movement

The effects of NSAIDs and sedatives were investigated on the capsaicin-induced body movement. NSAIDs such as indomethacin (30 mg/kg, Fig. 3a), ibuprofen (30

![Graphs showing body movement responses](image)

**Fig. 1.** Capsaicin-induced body movement (A) and its inhibition by tachykinin NK₁-receptor antagonist (B). Graphs Aa–Ac show the time course of the response following subcutaneous injection of capsaicin in 2-, 5- and 8-day-old rats. Capsaicin at the doses (ng or μg/body) indicated was injected as a 100-μl saline solution (○ saline, △ 1 ng, □ 10 ng, ● 100 ng, ▲ 1 μg, ■ 10 μg). The response was counted during 3 sequential 1-min periods. Graph Ad shows dose-response relationships of the response during 1 min immediately after capsaicin injection (○ 2-day-old, △ 5-day-old, □ 8-day-old). RP-67580 was pre-treated and the response was counted during 1 min immediately after capsaicin injection (150 ng/body, B). In A, each point with vertical bar represents the mean ± S.E.M. of 5–6 animals. In B, each point with vertical bar represents the mean ± S.E.M. of 10 animals in the group not treated with the antagonists and of 6 animals in the other groups; 4-day-old rats were used. In A, *P<0.05, compared with 8-day-old animals treated with the corresponding doses; **P<0.05, ***P<0.001, compared with 5-day-old animals treated with the corresponding doses (Student’s t-test). In B, *P<0.05, **P<0.01, compared with animals not treated with the antagonist (Dunnett’s test subsequent to ANOVA).
Table 1. Inhibitory effect of opioid and NK1-receptor antagonist on capsaicin-induced body movement

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ID50 (mg/kg)</th>
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<tbody>
<tr>
<td>Morphine</td>
<td>0.085 (0.066–0.11)</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.0079 (0.0050–0.013)</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>0.92 (0.55–1.5)</td>
</tr>
<tr>
<td>RP-67580</td>
<td>3.5 (1.7–7.2)</td>
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</tbody>
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Each value represents ID50 with 95% confidence limits in parentheses. Values were obtained from the data for 3 doses. Five or six animals were used for each dose.

mg/kg, data not shown) and acetaminophen (300 mg/kg, data not shown), administered i.p. 15 min prior to the capsaicin injection, did not affect the capsaicin-induced body movement. Neither the sedative diazepam (30 mg/kg, Fig. 3b) nor the sedative chlorpromazine (5 mg/kg, Fig. 3c), administered s.c. 25 min prior to the capsaicin injection, inhibited the body movement.

Fig. 3. Effect of non-steroidal anti-inflammatory drug and sedative on capsaicin-induced body movement. Body movement was counted during 1 min immediately after capsaicin injection (150 ng/body). Each column with vertical bar represents the mean and S.E.M. of 8–11 animals in the groups not treated with the drugs and of 6 animals in the other groups. Five-day-old rats were used in graph a and 4-day-old rats in graphs b and c. N.S., not significantly different (Student’s t-test).

Fig. 2. Inhibitory effect of morphine (MOR), buprenorphine (BUP) and pentazocine (PEN) on capsaicin-induced body movement (A) and antagonist effect of naloxone (NAL) on the effect of opioid (B). Body movement was counted during 1 min immediately after capsaicin injection. Dose of capsaicin used was 150 ng/body except for 300 ng/body in graph Ba. Each point with vertical bar represents the mean ± S.E.M. of 5–10 animals, and 5-day-old rats were used in graph Aa and 4-day-old rats in graphs Ab and Ac. Each column with vertical bar represents the mean and S.E.M. of 8–11 animals in the group treated with neither opioids nor naloxone and 4–5 animals of the other groups, and 5-day-old rats were used in B. *P<0.05, **P<0.01, compared with animals not treated with agonist in A and compared with animals treated with agonist alone in B (Dunnett’s test subsequent to ANOVA).
DISCUSSION

Tachykinin receptor antagonists administered intrathecally are reported to inhibit the capsaicin-induced nociceptive responses of adult mice (21). The capsaicin-induced body movement of neonatal rats was also inhibited by the NK1-receptor antagonist RP-67580 in the present experiments. Although the stereoselectivity of RP-67580 was not examined in our experiments, it is reported that the formalin-induced nociceptive response of adult rats are inhibited by RP-67580 but not by the enantiomer RP-68651 (9). The doses used in the present experiments (3−10 mg/kg, s.c.) were in a range similar to those in the above report (3−10 mg/kg, s.c.) (9). Therefore, it is considered that the inhibitory effect of RP-67580 on the capsaicin-induced body movement may be the result of blockade of the NK1 receptors. According to the recent report using NK1-receptor knock-out mice, the receptor does not mediate the signaling of acute pain (22). However, there is accumulated pharmacological evidence indicating that NK1-receptor antagonists inhibit acute and/or tonic nociceptive responses (23). The present experiments first show in neonatal rats in vivo that the NK1-receptor antagonist is effective in inhibiting the nociceptive response.

In the present experiments, the amount of the capsaicin-induced nociceptive body movement was larger in 5- to 8-day-old rats than 2-day-old rats. Capsaicin is reported to release substance P-like immunoreactivity from the central terminals of primary afferents in the rat spinal cord (17, 18). Substance P-like immunoreactivity can be already detected in the rat dorsal root ganglion at birth and increases until 10 days of age (24, 25). The increase in response to capsaicin may be in part due to the increase in substance P content in the dorsal root ganglion.

It is well known that NSAIDs inhibit nociceptive responses to chemical stimuli in adult animals. Although acetaminophen suppresses the early and the late phase of the formalin-induced nociceptive response (12, 26), indomethacin and ibuprofen inhibit the late phase but the early phase only scarcely in adult rats and mice (13, 14, 26−28). The late phase, but not the early phase, seems to be due to an inflammatory response partly mediated by endogenous prostaglandins (26, 27). The present capsaicin-induced response was within the time range of the early phase and insensitive to the above NSAIDs at higher or the same doses than those in the previous reports (12−14, 26−28). It is, therefore, suggested that endogenous prostaglandins do not play a role in the capsaicin-induced body movement of neonatal rats.

Sedatives including diazepam and chlorpromazine are shown to inhibit the chemical stimulus-induced nociceptive responses in adult animals. Diazepam is effective in reducing the early and the late phase of the formalin-induced nociceptive responses and at the same time inhibits motor activity in adult rats (15). Chlorpromazine shows an analgesic effect in the acetate-induced writhing test in adult mice (29). In the present experiments, however, diazepam and chlorpromazine did not inhibit the capsaicin-induced nociceptive response at higher doses than those in the previous reports (15, 29). Thus, the capsaicin-induced nociceptive response is insensitive to sedatives as well as to NSAIDs.

Morphine and buprenorphine are more effective in neonatal rats than in adult rats in suppressing nociceptive responses (15). In neonatal rats, morphine and buprenorphine alleviate the monophasic formalin-induced nociceptive response with ED50 values of 0.42 and 0.012 mg/kg, i.p., respectively (11). In the present experiments, the capsaicin-induced body movement was inhibited by morphine and buprenorphine with ID50 values of 0.085 and 0.0079 mg/kg, s.c., respectively. Thus, it seems that in neonatal rats, the capsaicin response is more susceptible than the formalin response to μ-opioid receptor agonists. Based on these results, it is suggested that the capsaicin-induced body movement of neonatal rats, which is considered to be nociceptive responses mediated by substance P, is sensitive to centrally acting analgesics with μ-opioid receptor agonist activity but sensitive to neither NSAIDs nor sedatives.

REFERENCES


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