ANALGESIC POTENCIES OF NON-NARCOTIC, NARCOTIC AND ANESTHETIC DRUGS AS DETERMINED BY THE BRADYKININ-INDUCED BITING-LIKE RESPONSES IN RATS

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Abstract—Aversive (nociceptive) biting-like responses induced by micro-application of bradykinin solution onto rat tooth pulp were dose-dependently suppressed by non-narcotic drugs such as baclofen and lidocaine as well as carbamazepine and phenytoin, which are employed for clinical treatment of trigeminal neuralgia. The potency order of these drugs on a molar basis is baclofen (4.20) >carbamazepine (1.00) >lidocaine (0.94) >phenytoin (0.19). Such responses were also inhibited by morphine, pentazocine and cyclazocine (potency ratio of the three general analgesics, 1.00:0.46:8.11), indomethacin (a non-narcotic and anti-inflammatory analgesic) and α-chloralose (an anesthetic). The latter drug produced an analgesic effect at doses much lower than those used for anesthesia. These findings suggest that our method is feasible for evaluating the activities of general and particular analgesic drugs in the trigeminal regions.

Hitherto, usual experimental analgesic tests such as the tail-pinch, tail-flick and hot-plate methods have been extensively employed in the investigations of analgesics. Though these methods have contributed much to the advancement of pharmacological and clinical studies, they are not without discrepancies. Narcotic agonist-antagonist analgesic drugs such as pentazocine and cyclazocine are ineffective in the tail-flick and hot-plate methods without marked motor impairment (1), though these drugs have been shown to be potent analgesics in humans. The primary agent for the treatment of trigeminal neuralgia, carbamazepine, produces a slight analgesic effect on tail-flick and tail-pinch methods at doses exceeding 200 mg/kg p.o. (2). Responses evoked by high-frequency and single-shock electrical stimulation of the tooth pulp in rabbits show no apparent effects with carbamazepine at doses of more than 50 mg/kg i.p. (Foong et al., unpublished data). Recently, we have introduced a simple and reliable method in evaluating the effects of drugs on trigeminal pain (3). In the present experiments the spectrum of analgesic potencies of various drugs including baclofen, lidocaine, indomethacin, morphine, pentazocine, cyclazocine and α-chloralose as well as carbamazepine and phenytoin was examined with this method.

Materials and Methods

1. Animals and preparations: Male Sprague-Dawley rats (n=207) weighing between 200–300 g were used in all experiments. Implantation of the bradykinin-filled cannula onto the tooth pulp and the fixation of it on the lower incisor surfaces were carried out under ethyl ether anesthesia. At least 2 hr were allowed to elapse between
the discontinuance of the anesthetic and the
beginning of the experiment. Details of the
procedures have already been described in
our previous report (3).

2. Analgesic assay: A micro-application of
bradykinin (Protein Research Foundation,
Mino: 0.63–1.25 ng in 0.5–1.0 μl of distilled
water) onto the tooth pulp of either one of
the lower incisors produced biting-like
responses and some other aversive behaviors
such as jumping, struggling, rubbing,
scratching, escape, head-jerk and body-
jerks, with a latency of 1 min or less. The
biting-like response was taken as the measure
for assaying analgesic potencies of drugs
because this response had the best re-
producibility. As 440 out of 481 rats (91%)
tested so far showed a duration of 20 min or
more for the biting-like responses before
drug administration, only rats with such
responses were used for further experimen-
tation. Bradykinin was repetitively applied at
intervals of 60 min through the implanted
cannula in each animal before and after the
administration of a drug. When the duration
of the biting-like response induced by
bradykinin was shortened to 5 min or less
after drug administration (observed time:
10 min), the effect of drug was considered
analgesic. This criterion seems equivalent to
that employed in the previous experiments
(3) because almost all those animals in which
the durations of the biting-like responses were
shortened to 10% or less than the control
mean duration (i.e. ≥90% inhibition) by the
drugs tested possessed response durations
of 5 min or less after the administration of
drugs. Recovery was regarded as positive
when rats showed a response duration exceed-
ing 5 min following bradykinin ap-
lication. This is a simplified version of our
previously described method (3).

3. Materials: The drugs used were baclofen (a gift from Ciba-Geigy (Japan), Ltd.,
Takarazuka), lidocaine HCl (Fujisawa Phar-
mac., Co., Ltd., Osaka), indomethacin (a
gift from Sumitomo Chem. Ind., Co., Ltd.,
Osaka), morphine HCl (Takeda Chem. Ind.,
Ltd., Osaka), pentazocine HCl and cyc-
lozocine (gifts from Torii-Winthrop Japan,
Tokyo), naloxone HCl (a gift from Endo
Labs., Garden City, U.S.A.) and α-chloralose
(E. Merck AG, Darmstadt, F.R.G.). Indo-
methacin was suspended in 0.03% carboxy- 
methyl cellulose sodium (C.M.C.) solution,
and α-chloralose was dissolved in propylene
glycol. These drugs were administered intra-
peritoneally. The other drugs were dissolved
in either physiological saline or distilled
water and administered subcutaneously. Each
animal was given only one dose of a drug.

4. Statistical analysis: At least 19 rats
were used to determine the dose-response
curve and ED50 value of a drug. The ED50
values with 95% confidence limits were
determined by the method of Litchfield and
Wilcoxon (4).

Results

Baclofen suppressed the biting-like
responses induced by micro-application of
bradykinin onto the tooth pulp in a dose-
dependent manner. As shown in Fig. 1,
baclofen at doses of 1.5, 3.0, 5.0 and 7.5
mg/kg s.c. produced an analgesic effect in
1 out of 6 rats tested (16.7%), 4 out of 9
(44.4%), 5 out of 7 (71.4%) and all of 7
(100%), respectively, 30 min after s.c.
treatment with the drug. The ED50 value
was calculated as 3.30 mg/kg s.c. After the
administration of baclofen, the effects were
long-lasting and disappeared at 90 min for
the lowest dose, but at 150 min for the other
doses. The drug at the highest dose used
(7.5 mg/kg) did not elicit head-drop and
ptosis, but slight motor impairment was
observed.

Lidocaine at doses of 8.0, 20, 30 and 50
mg/kg s.c. produced analgesic effects in 2
out of 9 rats examined (22.2%), 3 out of 6
Fig. 1. Dose-dependent suppressive effects of baclofen (s.c.) on bradykinin-induced biting-like responses. ▲: 1.5 mg/kg (6 rats), ◆: 3.0 mg/kg (9 rats), ◇: 5.0 mg/kg (7 rats) and ●: 7.5 mg/kg (7 rats). The ordinate shows the percentage equivalent to the number of rats suppressed over the total number of rats used for a particular dose, and the abscissa indicates the time after drug administration in min.

(50.0%), 4 out of 7 (57.1%) and 6 out of 7 (85.7%), respectively, 15 min after s.c. treatment with the drug. The ED50 value was 16.0 mg/kg s.c. Recovery from the effect was obtained, at the latest, at 75 min after drug treatment.

Indomethacin suppressed the bradykinin-induced biting-like responses in a dose-dependent manner within a dose-range of 60–150 mg/kg i.p. The ED50 value calculated from the data at 30 min after the administration was 98.0 mg/kg i.p. The analgesic effect of indomethacin was no longer observed 90 min after the treatment with the drug, except at the highest dose (150 mg/kg). Lidocaine and indomethacin did not induce any behavioral abnormalities in the doses used.

The suppressive effects of morphine on the biting-like responses were examined 30, 90 and 150 min after drug administration in the present experiments. Such an effect was observed in 4 out of 10 rats tested (40.0%), 5 out of 8 (62.5%) and 7 out of 9 (77.8%) at doses of 2.0, 3.0 and 4.0 mg/kg s.c., respectively, 30 min after the treatment. The ED50 value determined was 2.40 mg/kg s.c.

Pentazocine and cyclazocine produced dose-related analgesic effects in dose ranges of 2.5–10 and 0.125–0.5 mg/kg s.c., respectively. The time courses of the effects of both drugs were similar, and almost all rats recovered by 75 min after administration. The ED50 values of pentazocine and cyclazocine were 5.29 and 0.24 mg/kg s.c., respectively. Pentazocine and cyclazocine as well as morphine did not elicit any behavioral abnormalities at the doses used. The suppressive effects of the three drugs on the bradykinin-induced biting-like responses were antagonized by the pretreatment with naloxone (0.5 mg/kg s.c.) 5 min before drug administration.

α-Chloralose suppressed the bradykinin-induced biting-like responses in a dose-dependent manner within a rather small dose range of 5.0–20 mg/kg i.p. The suppressive effects were long-lasting and disappeared at 90 min for the lowest dose, but at 150 min for the other doses after the administration of α-chloralose. The ED50 value calculated from the data, 30 min after the injection of the drug, was 7.35 mg/kg i.p. In these doses used, α-chloralose did not produce any
The dose-response curves of the drugs used in the present and previous (3) experiments as determined by the suppression of bradykinin-induced biting-like responses are represented in Fig. 2, and the ED50 values with 95% confidence limits of the drugs used are summarized in Table 1.

### Discussion

The present experiments demonstrated that an analgesic test, using inhibition of the biting-like response induced by micro-application of bradykinin onto rat tooth pulp as an index, was feasible in determining analgesic potencies of various drugs in addition to carbamazepine, phenytoin and morphine which have been previously reported (3).

Cutting and Jordan (5) and Levy and Proudfit (6) reported that baclofen, only at doses of 5.0–10 mg/kg (i.p. or s.c.) or more, produced significant analgesic effects in usual analgesic tests such as the hot-plate, benzoquinone-writhing and tail-flick tests with the mouse. In the present experiments, however, baclofen was effective at smaller doses, and the ED50 value was 3.30 mg/kg s.c. Such a dose is compatible to that employed by Fromm et al. (7) who showed an inhibitory action of baclofen on single anesthesia and other behavioral abnormalities.

Table 1. Analgesic activity of various drugs evaluated by suppression of the bradykinin-induced biting-like response in rats

| Drug            | ED50 mg/kg (95% confidence limits) | Route |
|-----------------|------------------------------------|-------|
| Baclofen        | 3.30 (2.39–4.55)                   | s.c.  |
| Lidocaine       | 16.0 (8.10–31.6)                   | s.c.  |
| Carbamazepine*  | 13.1 (7.81–22.0)                   | i.p.  |
| Phenytoin*      | 75.0 (27.2–267)                    | i.p.  |
| Indomethacin    | 98.0 (78.7–121)                    | i.p.  |
| Morphine        | 2.40 (1.66–3.49)                   | s.c.  |
| Pentazocine     | 5.29 (2.96–9.47)                   | s.c.  |
| Cyclazocine     | 0.24 (0.14–0.40)                   | s.c.  |
| α-Chloralose    | 7.35 (4.97–10.9)                   | i.p.  |
| Pentobarbital*  | N.D. up to 15.0 mg/kg             | i.p.  |

*: the data from ref. (3). N.D.: non-detectable.
neuronal responses in the spinal trigeminal nucleus oralis of the cat to maxillary nerve stimulation. They also reported clinical effectiveness of the drug on trigeminal neuralgia.

Systemic administration of lidocaine reportedly inhibits epileptiform cortical after-discharges elicited by cortical stimulation in the cat and monkey (8) and is employed for treatment of trigeminal neuralgia by some clinicians (9). Such a profile of the effects of lidocaine is similar to that of carbamazepine and phenytoin. The present study showed that the potency of lidocaine in suppressing the trigeminal pain induced by micro-application of bradykinin onto the tooth pulp is of the same order in magnitude as that of carbamazepine (Table 1). For drugs which are clinically employed in treating paroxysmal pain such as trigeminal neuralgia, the potency rank expressed on a molar basis in a decreasing order is baclofen (4.20) > carbamazepine (1.00) > lidocaine (0.94) > phenytoin (0.19). This rank is roughly parallel to the clinical potencies of these drugs.

In the present experiments, indomethacin, a non-narcotic and anti-inflammatory analgesic, produced a weak but dose-dependent analgesic effect. Its ED50 value is 2.4 times larger than that obtained in an analgesic test in which the inhibition of the flexor reflex of rat hind-limb induced by intra-arterial injection of bradykinin was used as an index (10). Pentazocine and cyclazocine have respective ED50 values of 5.29 and 0.24 mg/kg s.c. that are compatible to the corresponding values obtained in the bradykinin-induced hind-limb flexor reflex test in the rat (11). The potency rank for general analgesic drugs in this study is cyclazocine (8.11) > morphine (1.00) > pentazocine (0.46) > indomethacin (0.03). when expressed on a molar basis.

Foong et al. (12) have reported that morphine (2.0–4.0 mg/kg i.v.), pentazocine (5.0–10 mg/kg i.v.) and cyclazocine (0.5–1.0 mg/kg i.v.) suppressed the evoked potential recorded at the dorsal hippocampus following electrical stimulation of the rabbit tooth pulp, but not that at the trigeminal subnucleus caudalis. This indicates that the suppressive effects of these drugs on the biting-like responses induced by chemical stimulation of the rat tooth pulp in the present experiments may be due to the inhibitory action of the drugs on painful impulses at the hippocampus and/or the afferent pathways to the hippocampus from the trigeminal subnucleus caudalis. However, a blockade of the impulses from the tooth pulp to the trigeminal subnucleus caudalis and the surrounding reticular nuclei probably contributes to the suppressive effects of these drugs in the present experiments as the drugs inhibited single neuronal response recorded at the nuclei to the same chemical stimulation (13).

A potent analgesic activity of α-chloralose was demonstrated in this study. The ED50 value of the drug was 7.35 mg/kg i.p., which is much less than the doses recommended for anesthetic purposes (80–90 mg/kg). On the other hand, pentobarbital produced insignificant suppression of the bradykinin-induced biting-like response at 10–15 mg/kg i.p., dose at which the drug elicits ataxia and sedation (3).

In conclusion, the present experiments establish the spectrum of application in the studies of general and particular analgesic activities in the trigeminal regions of certain drugs.

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