EFFECTS OF ANALGESICS AND CNS-ACTING DRUGS ON STRUGGLING FOLLOWING REPETITIVE STIMULATION OF THE TAIL, AND FLEXOR REFLEX TO A SINGLE STIMULATION OF THE SCIATIC NERVE IN RATS

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Abstract—Utilizing struggling to a repetitive stimulation of the tail, as a pain response, flexor reflex to a single stimulation of the sciatic nerve, as a spinal reflex activity, the analgesic activity and the muscle relaxant activity of certain analgesics were assessed alternately. Stable and reproducible response to a repetitive stimulation of the tail or a single stimulation of the sciatic nerve was obtained over a period of 5 hr, and quantitative measurement of struggling could be made. The 50% inhibitory doses of various drugs to struggling and flexor reflex were as follows (mg/kg, i.p.): morphine, 3.30 & 6.60, respectively; codeine, 12.80 & 24.00; pentazocine, 7.80 & 12.20; indomethacin, 4.60 & no effect; aspirin, 94.00 & no effect; baclofen, 1.04 & 2.20; chlorpromazine, 2.45 & 0.75; diazepam, 0.45 & 0.86; mephenesin, 36.00 & 32.00. Based on these results, it is suggested that the method is useful for quantitative measurement of analgesia and muscle relaxation.

An intra-arterial administration of bradykinin induces vocalization of animals and being considered one of the indicators of pain response in animals has frequently been employed for the assay of analgesic drugs (1–7). It was recently reported that the sensitivity of the assay was increased when the struggling and biting in addition to the vocalization were measured as the indicator of pain response to bradykinin (8). The struggling may be a reflection of complicated reflex activities, and it is therefore conceivable that the response may be susceptible to either analgesics or muscle relaxants. In our preliminary experiments using a Haffner method, however, the vocalization and biting were suppressed by treatment of animals with muscle relaxants, whereas struggling was not affected.

In the present study, the measurement of not only struggling but also flexor reflex was made in an attempt to evaluate the analgesic action of drugs, separately from the muscle relaxing action. The feasibility of this method in assessment of the weak as well as strong analgesics was also demonstrated.

MATERIALS AND METHODS

All experiments were carried out on male rats of Wistar-strain weighing approximately 150 g. The animals were anesthetized with α-chloralose (40 mg/kg, s.c.) and urethane (400 mg/kg, s.c.) and were placed under artificial respiration. An experimental arrangement is schematically illustrated in Fig. 1.
FIG. 1. A schematic representation of the tail stimulation and the sciatic nerve stimulation, and recordings of responses. A. Typical tracing of struggling response to a repetitive stimulation of the tail. B. All movements were integrated. C. Typical tracing of flexor reflex to a single stimulation of the contralateral sciatic nerve.

The effects of analgesics were determined on the basis of an inhibitory action on the struggling induced by the electrical stimulation of the tail. When the tail was repetitively stimulated, the rat struggled violently. The movement of the head was significantly increased in parallel with struggling. Movement of the head was then recorded as the indicator of struggling, by means of a force displacement transducer (Nihon-Kohden, SB-1T). Repetitive stimulation was given at 30 min intervals following drug administration until the extent of struggling returned to the pre-medication level. The repetitive stimulation of the tail was performed for 15 sec each time. For stimulation, rectangular wave pulses generated from a Nihon-Kohden SEN 1101 stimulator were applied through a pair of stainless steel electrodes placed on the root of the tail, at a frequency of 50 Hz. The intensity and duration of the pulse was 10–15 V and 2.5 msec, respectively.

Flexor reflex induced by a single stimulation of the sciatic nerve was also recorded from the contralateral hindlimb which was also connected to a force displacement transducer, as shown in Fig. 1. The frequency of stimulation was 0.1 Hz, and pulse duration was 1 msec. The exposed sciatic nerve was bathed in warm paraffin. Stimulation of the sciatic nerve was given immediately following the stimulation of the tail, and measurements of flexor reflex and struggling were alternately performed. Recordings were begun 1 hr after anesthesia (control response) and the drugs were administered 2 hr later. All drugs were administered i.p. through an implanted cannula.

To examine the depth of anesthesia, electroencephalogram (EEG) was recorded. The
rats were prepared under anesthesia with ether, immobilized with gallamine triethiodide, artificially ventilated and placed on a stereotaxic apparatus. The locus for insertion of an electrode was determined according to the stereotaxic atlas of Pellegrino and Cushman (9). A stainless steel concentric bipolar electrode was implanted in the dorsal hippocampus (A: +2.4, L: 3, H: +2). EEG was recorded by a polygraph (Nihon-Kohden, RM-150). The drugs used were morphine hydrochloride, mephenesin, chlorpromazine (Shionogi), codeine phosphate (Shionogi), pentazocine (Sankyo), aspirin, aminopyrine, indomethacin (Milan), baclofen (CIBA-Geigy) and diazepam (Hoffman-La Roche).

RESULTS

Examination of frequency of stimulation and depth of anesthesia: Since the animal should be as lightly anesthetized as possible to prevent synergistic effects of the drug with anesthetics, the influence of various doses of α-chloralose+urethane was examined. There was no appreciable influence on the response to electrical stimulation up to 50 mg/kg, s.c. of α-chloralose+500 mg/kg, s.c. of urethane. As shown in Fig. 2, for example, when 40 mg/kg of α-chloralose and 400 mg/kg of urethane were given to rats, reproducible responses to electrical stimulation of the tail or the sciatic nerve were obtained over a considerable period, at least up to 5 hr. Therefore, in this experiment, the anesthesia given was a dose of 40 mg/kg of α-chloralose and 400 mg/kg of urethane.

The depth of anesthesia was also examined in EEG patterns of rats. Subcutaneous administration of 40 mg/kg of α-chloralose and 400 mg/kg of urethane produced a decrease in the frequency of waves in the cortex and desynchronization of rhythmical theta waves in the hippocampus (Fig. 3). However, these inhibitory responses in EEG turned into arousal responses by tail stimulation at 5 V for 10 sec, indicating that the pain response persisted even during the state of anesthesia.

When the frequency of stimulation was changed from 1 to 100 Hz, the most intensive struggling was obtained when 50 Hz stimulation was given (Fig. 4). Five successive repetitive

![Fig. 2. Changes in struggling and flexor reflex after anesthesia with α-chloralose and urethane. Left index shows % of control for struggling, and right index the tension development of flexor reflex. Each symbol represents means±S.E.](image-url)
stimulations were given at intervals of 30 sec in each experiment, but no sign of adaptation was observed.

**Effects of drugs on struggling and flexor reflex:** Both weak and potent analgesics as well as other CNS-acting drugs were used. Morphine was markedly effective in inhibiting struggling but less effective in suppressing flexor reflex (Fig. 5). Aspirin also had an inhibitory effect on struggling but no effect on the flexor reflex (Fig. 6). In contrast to mor-

**Fig. 3.** Effects of the tail stimulation on EEG patterns after anesthesia with 40 mg/kg, s.c. of α-chloralose and 400 mg/kg, s.c. of urethane in an immobilized rat. Inhibited EEG patterns by anesthesia changed to arousal patterns by the tail stimulation (5 V, 1 msec, 50 Hz) as indicated by the horizontal line. FC: Frontal cortex; PC: Parietal cortex; HPC: hippocampus.

**Fig. 4.** Frequency of stimulation of the tail was varied from 1 to 100 Hz. Measurement of struggling was determined by the area of struggling circumscribed by a baseline. Each symbol represents means±S.E.

**Fig. 5.** Effects of morphine (7.5 mg/kg, i.p.) on struggling and flexor reflex.
phine and aspirin, chlorpromazine and mephenesin exerted an inhibitory action on flexor reflex rather than on struggling as shown in Figs. 7 and 8, respectively.

The effect of drugs was changed with time and was dose-dependent (Figs. 9, 10). The time to peak effect of morphine, aspirin, chlorpromazine and mephenesin was 30, 60, 30 and 15 min, respectively. Even 2 hr after drug administration, struggling and flexor reflex had not returned to the control level when high doses were used.

As shown in Fig. 11, the inhibitory effect of morphine on both struggling and flexor reflex appeared in a dose-dependent manner, but a higher dose of the drug were required

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**Fig. 6.** Effects of aspirin (100 mg/kg, i.p.) on struggling and flexor reflex.

**Fig. 7.** Effects of chlorpromazine (1 mg/kg, i.p.) on struggling and flexor reflex.

**Fig. 8.** Effects of mephenesin (60 mg/kg, i.p.) on struggling and flexor reflex.
for inhibition of the flexor reflex. Non-narcotic analgesics such as aspirin and indomethacin inhibited struggling, whereas they had substantially no effect on the flexor reflex, as shown in Fig. 11. Chlorpromazine was unique in that it exerted a more potent action on flexor

Fig. 9. Duration and onset of peak activity of anti-struggling and anti-flexor reflex of morphine and aspirin. Each symbol represents means ± S.E.

Fig. 10. Duration and onset of peak activity of anti-struggling and anti-flexor reflex of mephenesin and chlorpromazine. Each symbol represents means ± S.E.
FIG. 11. Dose-response curves for inhibitory effects of various drugs to struggling and flexor reflex.

TABLE 1. The 50% inhibitory doses of various drugs to struggling and flexor reflex

| Drugs       | N  | Struggling Peak time (hr) | ID50 (95% C.L.) mg/kg, i.p. | Flexor reflex Peak time (hr) | ID50 (95% C.L.) mg/kg, i.p. |
|-------------|----|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| Morphine    | 20 | 0.25–0.50                 | 3.30 (1.98–5.51)            | 0.50–0.75                   | 6.60 (4.39–9.92)            |
| Codeine     | 12 | 0.25–0.50                 | 12.80 (5.67–28.93)          | 0.50–0.75                   | 24.00 (8.62–66.85)          |
| Pentazocine | 12 | 0.25–0.50                 | 7.80 (3.83–15.90)           | 0.50                        | 12.20 (7.31–20.36)          |
| Aspirin     | 16 | 0.75–1.00                 | 94.00 (62.53–141.31)        | no effect                   |                             |
| Aminopyrine | 12 | 0.50                      | 47.00 (25.49–86.66)         | no effect                   |                             |
| Indomethacin| 12 | 0.50–1.00                 | 4.60 (2.49–8.48)            | no effect                   |                             |
| Baclofen    | 16 | 0.50–0.75                 | 1.04 (0.85–1.28)            | 0.75–1.00                   | 2.20 (1.32–3.67)            |
| Mephenesin  | 16 | 0.25                      | 36.00 (16.96–76.42)         | 0.25–0.50                   | 32.00 (15.47–66.17)         |
| Diazepam    | 16 | 0.25–0.50                 | 0.45 (0.24–0.83)            | 0.25–0.50                   | 0.86 (0.08–8.97)            |
| Chlorpromazine| 16 | 0.50                      | 2.45 (1.33–4.52)            | 0.50–0.75                   | 0.75 (0.27–2.06)            |
reflex than on struggling. However, mephenesin had a nearly equipotent action on flexor reflex and struggling. In the present experiment, baclofen had a more potent inhibitory action on struggling than on flexor reflex, indicating that this analgesic seems independent of muscle relaxation.

Table 1 summarizes ID50 (95% confidence limits) and peak time of efficacy of all drugs used in the present experiment.

DISCUSSION

Guzman et al. (10), Lim et al. (1) and Taira et al. (11–12) have reported that the screening method of analgesics which utilizes vocalization responses in conscious animals as the indicator of pain is more advantageous in comparison with conventional screening methods which use escape response to thermal, mechanical or electrical stimulation in that it brings about quantitative and reproducible results. Guzman et al. (2) reported, however, that spontaneous diminution of vocalization response to i.a. administration of bradykinin was observed in some dogs. Hirata et al. (13) have reported that marked tachyphylaxis in vocalization response to i.a. administration of bradykinin took place in adult dogs, and a recent report suggested that the sensitivity of the assay was increased when the struggling and biting were added to the vocalization as an indicator of pain response to bradykinin (8). However, there is still controversy as to whether such a response as struggling is a good indicator of pain as this response seems to be due to complicated polysynaptic reflex activities and not exclusively to the response to pain.

In the present study, it was demonstrated that adaptation of struggling response to electrical stimulation did not develop, even when the stimulus was successively given at an interval of 30 sec and that, furthermore, spontaneous diminution of struggling did not occur. If struggling indeed reflects the pain response, such may be a promising approach in the assay of analgesic drugs.

The non-narcotic analgesic drugs, aspirin and indomethacin, exerted a favourable inhibitory action on the struggling but not on the flexor reflex. Chlorpromazine which possess an inhibitory action on a spinal reflex activity suppressed the action on the flexor reflex but to a much lesser extent on the struggling. These results indicate that the struggling is scarcely dependent on the spinal reflex activities but is unequivocally attributed to the pain induced by electrical stimulation of the tail.

The narcotic analgesics, morphine and codeine, produced a suppression of not only struggling but also flexor reflex. This can be explained by assuming that the narcotics possess muscle relaxant action in addition to the well known analgesic action. This assumption is supported by the evidence that morphine does depress the polysynaptic reflex activities in spinal animals (14–16). It is noteworthy, however, that the analgesic and muscle relaxant actions of these drugs could be quantitatively separated by using the present method (Fig. 11). O'Dell (17) also distinguished the dose of analgesics for the analgesic action from that for 'central dulling' action, i.e. a decreasing action on motor activity in mice.

Baclofen is a clinically effective muscle relaxant (18). In the present study, however,
it produced more potent inhibitory action on the struggling than on the flexor reflex, indicating that it may possess a strong analgesic action. This gains support from studies, where it was found that baclofen shows a potent analgesic action in a variety of analgesic tests (19-20).

It is of particular interest that chlorpromazine exerted such a potent inhibitory action on the flexor reflex. The mode of action of this drug is complicated (21) and it appears likely that it may act on various areas of the central nervous system, thereby resulting in intensive suppression of the polysynaptic reflex. The analgesic action of chlorpromazine is in agreement with the evidence so far provided (22).

All these findings taken together suggest that the struggling response induced by electrical stimulation of the rat tail is an excellent indicator of pain and can be utilized for the analgesic test. The present method appears to be advantageous in the following points: First, stable and reproducible struggling and flexor responses to a repetitive stimulation of the tail and to a single stimulation of the sciatic nerve, respectively, can be obtained over a period of 5 hr. Secondly, by measuring struggling and flexor reflex simultaneously, an analgesic-like action of the drug resulting from muscle relaxation can be favorably separated from an analgesic action itself. With respect to the mechanisms of action of various drugs as related to struggling, further studies are under way.

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