A NEW ANALGESIC TESTING METHOD USING ULTRASONIC STIMULATION

I. EFFECTS OF NARCOTIC AND NONNARCOTIC ANALGESICS

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Abstract—A quantitative method for measuring pain threshold by the use of ultrasonic stimulation in mice has been designed. The method has the advantage of precision, simplicity of technique, rapidity of measurement, and the fact that the stimulus is innocuous upon repeated application. The nature of the sensations induced by ultrasonic stimulus is somewhat like that felt with a prick type of pain. Pentazocine (30, 100, 150 mg/kg i.p.), aminopyrine (15, 50, 100, 150 mg/kg i.p.), phenacetin (100, 150, 200, 250 mg/kg i.p.), sodium salicylate (150, 200, 250 mg/kg i.p.) and other antipyretic analgesics were active in a wide range of doses indicating that this technique is sensitive to the narcotic antagonist and to the weak analgesics as well as to the narcotic analgesics such as morphine (2.5, 5, 10, 15 mg/kg i.p.), codeine (10, 20, 25, 30, 50 mg/kg i.p.), and pethidine (5, 10, 15, 20, 25 mg/kg i.p.). The ultrasonic method is, therefore, applicable in screening procedures when attempting to evaluate the analgesic potency of a wide variety of chemical agents.

A variety of methods for analgesic testing in laboratory animals have been reported. They can be classified according to the method of noxious stimulation as follows: thermal (1, 2, 3, 4, 5), mechanical (6, 7, 8, 9, 10), chemical (11, 12, 13), and electrical (14, 15, 16). Most of these methods have drawbacks, however, in that they are capable of detecting only narcotic analgesics or reactions to many agents which are not analgesic in man, and cannot, in general, be used for testing analgesics in man.

We have already reported that ultrasonic stimulation can cause a prick type of pain in toads and a cough in dogs (17).

In the present work, we used ultrasonic stimulation in mice in an attempt to design a better analgesic screening method. Narcotic and nonnarcotic analgesics for analgesic activity were thus tested.

MATERIALS AND METHODS

Male dd-strain mice weighing 14–18 g were used for most all experiments. The apparatus for measuring pain threshold is presented schematically in Fig. 1. The ultrasonic stimulus was provided by an ultrasonic vibrator (UR-150P, Tomy Seiko, Co., Ltd.). The stimulating parameters were as follows: frequency of ultrasonic wave, 20 KHz; voltage 70 V (obtained with a voltage regulator); stimulus intensity, 52.0 W/cm² (0.74 A), and in the experiment of varying stimulus intensity, in addition, 17.5 W/cm² (0.25 A), 24.5 W/cm² (0.35 A) and...
98.0 W/cm² (1.40 A). The tip, that is, the peripheral end from which the ultrasonic wave irradiates, measured 1 cm².

Each mouse was placed in a perspex box. The tail was allowed to protrude, and was placed in the shallow groove of a wet sponge (1.5 cm in length, 1.5 cm in width, and 0.5 cm in thickness) fixed on a wooden plate. The tip of the generator was attached to the base of the tail in all experiments, except for those specifically mentioned. By ultrasonic stimulation on the tail, mice pushed themselves forward to avoid stimulation or turned their heads to bite the stimulating tip. They sometimes made crying sounds. The biting behavior was similar to that seen by pressure stimulus in Haffner’s method. A forelimb or hindlimb muscle twitching response which was commonly observed in both avoiding and biting behavior was selected as the index of pseudoaffective responses. As a pain threshold, the reaction time which means the time required between the beginning of ultrasonic stimulation and the onset of pseudoaffective responses seen when the animal felt a pain to the stimulus was recorded with a digital stop-watch having 0.01 sec scaling (Takei Kiki Kogyo, Co., Ltd.). The stop-watch is operated by the same foot switch which provides the current. In evaluating analgesic potencies of drugs, mice possessing a reaction time of less than 2 sec before drug administration were used. After obtaining normal thresholds, an analgesic drug was administered to animals and the change in threshold to pain was followed for 2 hr. The measurements of reaction time were made on each animal 15, 30, 45, 60, 90 and 120 min after the i.p. administration of the tested dose. The time required for a single measurement was usually less than 1 min. By a preliminary stimulation of the fingers of the investigator’s hands, the nature of the sensations induced by ultrasonic stimulus was confirmed to be merely a prick type of pain and not a thermal one. Each group consisted of ten mice. All experiments were carried out at a room temperature of 23 ± 1°C.

For the estimation of ED50 values of drugs, mice which showed a reaction time of more than 4 sec after drug administration were determined positive for analgesic effect. Using the method of Litchfield and Wilcoxon (18), dose response curves were plotted, and ED50 values and relative potencies were determined. Statistical significance was determined by the use of Student’s t test, the level of significance being P = 0.05.

The drugs administered in the present study were as follows: in the narcotic class, morphine hydrochloride, pethidine hydrochloride and codeine phosphate; in the class of nonnarcotic analgesics, pentazocine lactate, aminopyrine, sulpyrine, antipyrine, acetaminophen, phenacetin, acetylsalicylic acid, sodium salicylate, phenylsalicylate and salicylamide. These drugs were dissolved in 0.9% saline or suspended in saline solution of 0.2%, carboxymethylcellulose and given i.p. in a volume of 0.1 ml/10 g body weight. The solvents had no
effect on the reaction time to stimulation.

RESULTS

Stimulus intensity and pain threshold

Each group of 10 male mice weighing 14–18 g was tested over a range of ultrasonic stimulation to the basal area of the tail. It was found that the reaction time shortened with increased stimulus intensity (Fig. 2). A ten sec period of stimulations at 15 min intervals for 75 min at these intensities caused no injury to the tail, and no adaptation, that is, shortening of the reaction time to the repeated ultrasonic stimuli occurred.

Site difference in the tail and sex difference of pain threshold

Ten mice of each sex weighing 14–18 g were subjected to ultrasonic stimuli at the intensity of 52.0 W/cm². As shown in Fig. 3A, sensitivity of the site of the tail to stimulation was in the following order: base > middle > tip. Thus the base of the tail was chosen as a suitable site for stimulation in the following experiments due to its high sensitivity and little variation. Female mice tended to be more sensitive than males at every site.

Age difference of pain threshold

As shown in Fig. 3B, there was no significant difference in either sex between the mice at the age of 35 days (body weight 14–18 g) and those at the age of 70 days (body weight 20–23 g).

Frequency distribution of normal threshold

The measurements of normal threshold on the 350 male mice subjected to ultrasonic stimulus at the intensity of 52.0 W/cm² are shown in Fig. 4. The mean, mode and median values of normal pain threshold were almost identical, being 1.5 sec.

Effects of narcotic analgesics

The time-action curves for morphine in doses from 2.5 to 15 mg/kg i.p. are shown as an example (Fig. 5). The rise in threshold was significant in every dose. This rise was observed 15 min following injection and lasted for more than 120 min. The height of the threshold-raising effect increased with the dose administered. In Fig. 6, dose-response curves for morphine, pethidine and codeine are shown. Their relative potencies were: morphine, 1; pethidine, 0.56; codeine, 0.37, on the basis of the potency of morphine.

Effects of pentazocine

The time-action curves for pentazocine in doses of 30, 100 and 150 mg/kg i.p. are shown in Fig. 7. Each dose of pentazocine raised pain threshold for more than 2 hr, although a
Fig. 3. Site (A), sex (A, B) and age (B) differences in the threshold to ultrasonic pain in mice.
In both A and B, stimulus intensity at 52.0 W/cm² was chosen. To determine site difference in the tail, the base, middle and tip were stimulated. To determine age difference, mice at the age of 35 days and those at the age of 70 days were used, and stimulation was given to the base of the tail.

Fig. 4. Frequency distribution curves of normal pain thresholds with ultrasonic stimulation in 350 male mice. The base of the tail of each mouse was stimulated at the intensity of 52.0 W/cm².

Fig. 5. Analgesic action of morphine in mice. Time-action curves were shown. Ordinate: reaction time in sec. Abscissa: time after i.p. injection of the drug. Each point is the mean for ten male mice with standard error.

Fig. 6. Analgesic activity of narcotic analgesics in mice tested by the ultrasonic method. Analgesic response was represented as % of mice which showed more than 4 sec of reaction time after drug administration.
The time-action curves for aminopyrine in doses from 15 mg/kg to 150 mg/kg i.p. are shown (Fig. 8). The analgesic activity of doses of aminopyrine as low as 50 mg/kg can be detected by our method. Dose-response relationships for various antipyretic analgesics are presented in Fig. 9. Effects of salicylates are presented in Fig. 10. All salicylates used showed no significant effect in doses of 50 and 100 mg/kg i.p. A relatively high dose (150 mg/kg) of sodium salicylate or salicylamide significantly raised the threshold, while 150 mg/kg of phenylsalicylate and acetylsalicylic acid did not.

**DISCUSSION**

The ultrasonic wave method has been only rarely applied in the pharmacological field and has been limited mostly to measurements of blood flow rate. We have found that ultrasonic irradiation can cause a prick type of pain in toads and a cough in dogs (17). Those techniques have, however, not been widely used as an adequate ultrasonic apparatus for the
purpose of concentrating beam of waves on local areas of the animals had not been designed.

Ultrasonic stimulation causes a prick type of pain not accompanied by a thermal or electrical sensation. The mechanism of inducing such a pain is still unknown. The stimulus intensity used in the study causes no injury to the tissue irradiated, thus this method has advantages over other methods, i.e. mechanical stimulus such as Haffner's method (6) influencing the sense of pressure, thermal stimulus such as hot plate or hot water methods influencing heat related factors as well as those of pain, and chemical stimulus such as acetic acid or phenylquinone-induced writhing methods leading to tissue injury like inflammation, and electrical stimulus producing other sensations such as electrical reflex different from that of pain.

The pseudoaffective response produced by ultrasonic stimulation is similar to that produced by the tail pressure method in mice, although with the former method, a forward movement to avoid stimulation was occasionally observed as well as attempts to bite the stimulating apparatus. The sensitivity to pain in the different sites of the tail in mice was in the following order: base > middle > tip, the results paralleling those observed in the pressure method by Takagi, et al. (19). Normal pain thresholds between 35 and 70 day old mice were not significantly different. Young mice would, however, be preferable for the analgesic screening test, since sensitivity to analgesics has been reported to decrease with age in the experiment using the hot plate and pressure procedures (20, 21, 22).

The ED50 values of some analgesic drugs tested by the ultrasonic method are compared with those tested by other methods in Table 1. Codeine is reportedly 1/4 as potent as morphine while pethidine lies between these compounds being more potent than codeine, but less potent than morphine (23, 24). This is approximately the ratio of effectiveness found in our present work. Our data on the intensities of analgesic action of narcotic and non-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Analgesic activity of drugs tested by various methods in mice (i.p. administrations)</th>
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<tbody>
<tr>
<td>Method</td>
<td>Morphine</td>
</tr>
<tr>
<td></td>
<td>ED 50</td>
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<tr>
<td>Pressure</td>
<td>7.8 (6.4–9.4)</td>
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<tr>
<td>Thermal (Receptacle)</td>
<td>4.6 (2.5–7.8)</td>
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<tr>
<td>Ultrasonic</td>
<td>7.3 (6.6–8.1)</td>
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<tr>
<td>Method</td>
<td>Aminopyrine</td>
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<tr>
<td></td>
<td>ED 50</td>
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<tr>
<td>Writting</td>
<td>24 (17–34)</td>
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<tr>
<td>Thermal (Hot Plate)</td>
<td>133 (101–169)</td>
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<tr>
<td>Ultrasonic</td>
<td>90 (55–131)</td>
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ED50 values (given in mg/kg) with corresponding 95% confidence intervals. Note relative potencies of narcotic analgesics to morphine.
narcotic compounds are in general agreement with those obtained by other methods (5, 9, 25, 26) where mice were used as the experimental animals and drugs were administered i.p. Our method is considered sensitive enough for antipyretic analgesics, and can detect the analgesic activity of a narcotic antagonist analgesic such as pentazocine to the same extent as the chemical induced writhing method (27, 28). The failure to obtain a clear dose-dependent relationship for pentazocine may be ascribed to a self-antagonism due to its analgesic antagonistic action (29).

The tail-flick (6, 7, 8) and hot plate procedures (3, 4, 5) are, in general, relatively specific mainly for narcotic analgesics. On the other hand, the chemically induced writhing procedure (11, 12, 13), as seen in each paper, lacks specificity and reacts to many agents that are clinically ineffective, such as alkaline agents, tranquilizers, antihistamines, amphetamine, and some vitamins as well as to narcotic antagonist analgesics, antipyretic and narcotic analgesics. Our method is capable of detecting not only strong analgesics but also mild analgesics such as antipyretic agents, although salicylates are less sensitive. The spatial preference procedure recently introduced by Houser and Paré (16) makes use of operant techniques with electrical stimulus reportedly sensitive to the weak analgesics. This method includes a complex operating procedure and a critical limitation of unavailability for the analgesic test in man.

The ultrasonic method designed by the present authors has the following advantages: the nature of the sensations produced by the stimulus is merely a prick type of pain; the response to stimulus can be quantitatively represented; the stimulus can be repeated in rapid succession without adaptation to stimuli and with no injury to the tissue; the necessary apparatus is simple and the operating procedure is uncomplicated; many animals can be tested within a given time; the relation between the stimulus intensity and the strength of pain sensation is clear; the prick type of pain is felt quite sharply and the pain threshold can be clearly obtained; both weak and strong analgesics can be screened; the method can be applied to humans as well as laboratory animals, and potencies of analgesics can be compared in different species. A follow-up paper will report the analgesic test as applied directly in humans.

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