A MODIFICATION OF HAFFNER'S METHOD FOR TESTING ANALGESICS

HIROSHI TAKAGI, TOSHIYA INUKAI
AND MOTOTAKA NAKAMA

Department of Pharmacology, Faculty of Pharmaceutical Sciences,
Kyoto University, Sakyo-ku, Kyoto

Received for publication April 21, 1966

Haffner's tail-pinch method (1) for testing analgesic drugs has been adopted in many laboratories either in the original method (2-4, 17) or in the varieties of modifications (5-7). Certain objections can, however, be raised against the use of the Haffner's method; the intensity of the painful stimulus produced by this method is not shown in figures, neither is it varied quantitatively.

The improvement of these disadvantages has been carried out by Eddy (8), Friend and Harris (9), Fleish and Dolivo (10), Brodie et al. (11), Green and Young (12), Yanai (13), Takagi and Kameyama (14), and so on, but there seems still no ideal method exists.

The Authors describe here a simple tail-pinch method, which the intensity of pressure stimulus is varied quantitatively and which satisfies the sensitivity and reproducibility for testing non-narcotic analgesics as well as narcotics.

METHODS

Measurement of the clip pressure: A 5 cm long artery clip with the branches of 2 cm long, 1.5 mm in diameter is used to press the base of mouse's tail. An arrangement of the apparatus for measuring clip pressure was illustrated in Fig. 1. The spring balance (A) (with the scale calibrated in 10 g increments and permits readings that ranges from 0 to 1,000 g) is hanged from an arm of stand and hook of spring balance is connected with a fine wire to one branch of the clip (B), of which another branch is connected to the fixed arm of stand. An iron wire (JIS No. 12; Diameter 2.6 mm, 4 cm long) was adopted as a model of mouse's tail and inserted into the branches of the clip. When the spring balance is raised by means of the screw of the stand, the branches of the clip (B) open. This enables the iron wire inserted into the branches of the clip to move at the critical point. The scale is read on the balance which shows the pressure of the clip. By this arrangement several artery clips were chosen of which intensities were 50, 100, 300, 500 and 800 g. The pressure of ready-made artery clip can be changed by treatment with pinchers.
Animals: Experiments were conducted with mice, ddK strain, 13 g body weight. In each experiment 10 mice were used. In a preliminary experiment, no significant difference between the sexes has been observed both in normal pain threshold and the sensitivity to the analgesics. Thereafter, both sexes of mice were used.

Drugs used: Morphine hydrochloride, codeine phosphate, aminopyrine were dissolved in distilled water and antipyrine, phenacetine and acetyl salicylic acid were dissolved in 50% propylene glycol. All the drugs were administered subcutaneously.

RESULTS

1. Relation between the intensity of the pressure stimuli and the rate of the appearance of reflex response in normal mice

Mice are selected for experiments which show reflex response to noxious stimulation within 2 seconds after the base of a tail is pressed by various pressures of clips.

The left graph of the Fig. 2 shows the relationship between the percent of mice which showed reflex response and the in-
tensity of the pressure stimulus.

The linear relationship of the right graph shows that the reflex response to noxious stimuli of normal mice is distributed normally to the logarithm of the pressure given (12-14).

2. Criteria for the measurement of analgesic potency

When a base of mouse's tail was pinched with an artery clip (500 g) before or after drug administration, the mouse's responses were classified into 3 classes:

"N" (Normal response): turns his head to the tail and bites an artery clip within 2 seconds.

"P" (Partial analgesia): shows the above mentioned behaviors in 2-6 seconds after the application of artery clip.

"C" (Complete analgesia): does not show the above mentioned behaviors over 6 seconds after the tail-pinch.

The pressure stimuli were not applied over 15 seconds to avoid the injury of tail. Fig. 3 shows some typical behaviors of "N", "P", and "C", after the application of artery clip.

3. Relation between the intensity of the pressure stimulus and the ED 50 of morphine

The dose-response curve of morphine analgesia using 50, 100, 300, 500 and 800 g pressure stimuli was shown in Fig. 4. All curves were parallel both by "C" and "C+P" responses. The ED 50 of morphine was calculated on each clip pressure by the Lichfield-Wilcoxon method (15) and shown in Table 1. On the morphine analgesia, there is no significant difference between group I (50 g and 100 g) and group II (300 g and 500 g) by the statistical calculation of the Lichfield-Wilcoxon method, while significant difference between group I and group III (800 g) or group II and group III were detected.

Within the pressure range of 50 g to 800 g the logarithm of the pressure (g) is linear to the logarithm of the ED 50 values of morphine. The pressure stimulus by forceps that we used before (16) was estimated between 1,000-2,000 g by this relation.
Fig. 4. The dose-response curve of morphine analgesia using 50, 100, 300, 500 and 800 g clips.
Left : C response was used as a criteria for measurement.
Right : C+P response was used as a criteria for measurement.

Thus the conditions adopted for the screening purpose as follows:

a) The pressure stimulus is fixed to 500 g, which is higher than the normal pain threshold (100-300 g) and not significantly different from 300 g on the ED 50 of morphine. By this pressure, 97% of the normal ddK-mice can be selected for the experiment.

b) As a criterion for measuring analgesia, "C" is adopted so as to minimize the error responsible for the investigator's side. "C+P" which seems to be useful for the evaluation of mild analgesics is also recorded for the reference purpose.

Table 1. ED50 of morphine measured by two kinds of criteria in mice.

<table>
<thead>
<tr>
<th>Pressure (g)</th>
<th>ED50 of morphine (mg/kg) s.c.</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.0 (1.5-2.6)*</td>
<td>1.0 (0.8-1.2)*</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2.5 (1.7-3.7)*</td>
<td>1.3 (1.0-1.6)*</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>4.3 (3.6-5.1)*</td>
<td>2.2 (2.0-2.4)*</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>4.7 (4.1-5.3)*</td>
<td>2.3 (1.9-2.8)*</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>7.0 (5.3-9.3)*</td>
<td>3.5 (2.8-4.4)*</td>
<td></td>
</tr>
</tbody>
</table>

C : complete analgesia
P : partial analgesia
* : fiducial limits of ED50 (P = 0.95)

Fig. 5. Analgesic time courses of morphine (5 mg/kg s.c.) and aminopyrine (100 mg/kg s.c.).
Hatched bar : C response
Open bar : C+P response
4. Time courses of analgesic actions of morphine and aminopyrine

Fig. 5 shows the time courses of morphine 5 mg/kg and aminopyrine 100 mg/kg measured with the 500 g pressure clip. The maximal analgesic potencies of both drugs were obtained in 30 minutes after subcutaneous injection and then their effects gradually decreased.

5. Estimation of the analgesic potency of several drugs

Analgesic potencies of drugs were estimated at 30 minutes after drug administration.

Fig. 6 shows the dose-response curves of several analgesics. Table 2 shows the analgesic potencies of several analgesics, measured by the above mentioned method. If the potency of morphine is 1, relative potencies of codeine, aminopyrine and antipyrine are 0.2, 0.03, 0.01 respectively.

The analgesic potencies of phenacetine and acetyl salicylic acid compared to that of antipyrine are shown in Table 3. Mice administered phenacetine 200 mg/kg or 500 mg/kg sc. showed a muscle relaxation which made the measurement of analgesic action rather difficult. Acetyl salicylic acid showed weak analgesia only when administered the doses near to the lethal dose.

6. Difference of the sensitivity between the strains of the mice to the morphine analgesia

The ED 50 of morphine obtained on the ddK, SM, ICR, CF₁ strains are shown in

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED₅₀ mg/kg</th>
<th>C Relative potency (morphine = 1)</th>
<th>C + P Relative potency (morphine = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>4.7</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>(4.1–5.4)*</td>
<td></td>
<td>(1.9–2.8)*</td>
</tr>
<tr>
<td>Codeine</td>
<td>23.9</td>
<td>0.2</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>(13–44)*</td>
<td></td>
<td>(9.0–13.5)*</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>120.0</td>
<td>0.03</td>
<td>83.0</td>
</tr>
<tr>
<td></td>
<td>(91–144)*</td>
<td></td>
<td>(68–98)*</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>360</td>
<td>0.01</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>(263–494)*</td>
<td></td>
<td>(168–315)*</td>
</tr>
</tbody>
</table>

C : complete analgesia
P : partial analgesia
*: fiducial limits of ED5₀ (P = 0.95)
Table 4. Of the strains tested, SM was the most sensitive to morphine while ddK, CF, and ICR are sensitive in descending order.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg s.c.</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C% C + P% C% C + P%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipyrine</td>
<td>60  80</td>
<td>20  40</td>
<td></td>
</tr>
<tr>
<td>Phenacetine</td>
<td>55  80</td>
<td>20  40</td>
<td></td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>10  20</td>
<td>0    0</td>
<td></td>
</tr>
</tbody>
</table>

C : complete analgesia
P : partial analgesia

Discussion

It is recognized by many investigators that the stimulant pressures needed to elicit "pain response" in untreated animals are distributed normally when transformed to the logarithm of the pressures (12, 13). The average values of the pain threshold which has
been reported by various investigators and the Authors were shown in Table 5.

With the same animal, the pain threshold of stimulant pressure varies to a fairly large extent according to the investigators. This may be due to the following causes;

1) The position of stimulant pressure is on the anal side or on the tip side of a tail.

2) The applied stimulus is sharp or not.

3) The differences in the strain, age, and the conditional situation of the test animal.

The supra-threshold stimulus (500 g) was usual analgesiometry in order to get good precision, though normal pain threshold lay between 100 and 300 g.

All through the present experiment no damage was noticed at the positions to which the stimulus was applied by clip-pressure of 500 g, nor any adaptation or hypersensitization was noticed at all when the repetitive stimuli were applied to a normal mouse for 3 hours at an interval of 15 minutes, but when this interval was reduced a tendency to hypersensitivity was noticed. Green and Young (12) recognized the similar tendency with rats in their tail-pressure method.

Two seconds and six seconds which are adopted as the reaction times for measuring analgesic potency are not the one based on the strict statistical procedure but they gave a very satisfactory result in our experiences.

The judgement on "P" response meets a great difficulty in mice with the muscle rigidity or muscle relaxation induced by drugs. In such a case, it is recommended that the potency of analgesic drugs be measured by the "C" response. However only when the effect of analgesics goes parallel with the judgement "C" and "C+P" in each dose, can the data of "C+P" be taken as a criterion.

Various results have been obtained by many investigators on the evaluation of potencies of analgesics. The present results which are shown on Table 2 are considered to be proper in comparing with the data of other investigators (1-15) when relative potencies of analgesics (morphine=1) is taken as a measure. The reproducibility of results were good when experiments were repeated by two investigators independently.

The sensitivity of a mouse to the stimulant pressure or analgesic drugs tends to decrease with the increase of its weight and considerable differences in sensitivity are observed in various strains.

In the present experiment, clip-pressure of 500 g was used taking into consideration the number of selectable mice, but by using less clip-pressure and young mice of 10 g or less which are more sensitive to the stimulant pressure, there is a possibility to be able to evaluate a potency of less active analgesic drug than aminopyrine or antipyrine.

SUMMARY

1. A simple method was devised for measuring the intensity of pressure of artery clip was used in Haffner's tail-pinch method for testing analgesics. By this device various pressures of clips ranged from 50 to 800 g were chosen. This arrangement made it possible to control and to duplicate the intensity of pressure stimulus.
2. The supra-threshold stimulus (500 g) was used for the usual analgesiometry in order to reduce observer’s error, though normal pain threshold in the present experiment lay between 100 and 300 g.

3. As a criterion for judging analgesia, loss of head turn response and biting behavior were adopted, and they are graded by their reaction time, complete (C) and partial (P) analgesia.

4. By the present modification, it was capable to measure potencies of weak analgesics. ED 50 of various analgesics estimated by the judgement “C” and “C+P” are described. The weak analgesic potencies of phenacetine and acetylsalicylic acid could be observed but ED 50 of these drugs were difficult to measure.

The essentials of this paper were presented at the 27th Kinki Area Regional Meeting of the Japanese Pharmacological Society (May 23, 1965).

Acknowledgement: The Authors wish to express their gratitude to Prof. J. Okada of the Pharmaceutical Engineering for his helpful suggestion in the measurement of the pressure of artery clip. We are indebted to Prof. G. Condouris, Department of Pharmacology, New Jersey College of Medicine and Dentistry and Dr. H. Fujimori, Department of Anesthesiology, Albert Einstein College of Medicine, Yeshiva University, for advise in the preparation of the manuscript.

REFERENCES

1) Haffner, F. : Dtsch. med. Wschr. 55, 731 (1929)
3) Hesse, E. : Ibid. 158, 233 (1930)
4) Schumann, O. : Ibid. 196, 109 (1940)
5) Straub, W. AND Friemel, E. : Ibid. 195, 481 (1940)
6) Fujimura, H. : Yakugaku Zasshi 73, 437 (1953)
9) Freind, F.J. AND Harris, S.C. : Ibid. 93, 161 (1948)