For many years there has been an extensive search for agents as effective as morphine in the relief of pain yet free from the hazard of addiction. Until recently it had appeared that this search would be fruitless.

Authors have investigated acetylpiperazine derivatives, and found that 1,4-bis (2-methoxy-4-propylphenoxyacetyl) piperazine had an analgesic activity without narcotic action (1). This drug, however, does not have good solubility in water and cannot be absorbed from the intestine easily. Thus, this drug appeared to have less analgesic potency when administered orally.

In order to overcome this disadvantage, we have carried out further investigations on many other piperazines and found that 1-butyryl-4-cinnamylpiperazine hydrochloride (hereafter referred to as AP-237) had high analgesic potency (2) and good solubility in water. This paper describes the results of more detailed studies on the analgesic effects of AP-237.

METHODS

AP-237, white crystalline powder, is easily soluble in water. The structural formula of AP-237 is as follows.

\[
\begin{align*}
\text{CH}_2\text{CH} = \text{CH} - \text{CH}_3 - \text{N} - \text{CO} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 \cdot \text{HCl} \\
1\text{-Butyryl-4-cinnamylpiperazine hydrochloride (AP-237)}
\end{align*}
\]

In order to determine the analgesic efficacy, following methods were employed; pressure method (3) by Takagi and Kameyama et al., modified hot plate method (4) by Takagi and Kameyama, the D’Amour-Smith’s method (5) and the writhing method (6).

1) Pressure method

Experimental animals employed were ddk-strain male mice weighing 20–25 g, Wistar strain albino rats of both sexes weighing 100–150 g and guinea pigs weighing 200–250 g. Ten each of the above animals were made into one group. Pain stimuli was given by pressing the root of the tail of the mouse, the toe of the hind-limb of the albino rat (7) and...
the fore-limb of the guinea pig (8). The drugs were given per os, and thereafter, pressure stimuli was given 6 times at intervals of 20 minutes. The pain threshold was determined by the pressure at which the response to pain was first observed. For the mice and albino rats, the dose-response curve was delineated with the highest threshold among 6 times pressure stimulations. For the guinea pigs, the dose-response curve was delineated with the mean value of 6 pain thresholds.

2) Hot plate method

ICR-JCL strain female mice weighing 20–25 g were employed. After administering the drug, the mice were put on the heated plate 6 times at intervals of 20 minutes, and the time was measured from when they were put on the hot plate until they exhibited the second reaction (jumping response). When this time was longer than 10 seconds, the dose of the tested compounds was considered to be effective and the ED$_{50}$ was calculated.

3) D’Amour-Smith’s method

Animals employed were ICR-JCL strain female mice weighing 25–30 g and Wistar strain albino rats of both sexes weighing 100–150 g. The tips of the tails of animals were smeared with Indian ink, and after administering the drug, thermic rays were irradiated 4 times at intervals of 30 minutes, and the time until the animal exhibited an escape reaction was measured. When this time extended longer than 10 seconds, the dose of the drug was evaluated to have an analgesic effect and the ED$_{50}$ was calculated. The calculation of the ED$_{50}$ was performed by the method of Litchfield-Wilcoxon (9) in the mice and also by the “up and down” method in the rats.

4) Benzoquinone writhing method

Animals employed were ICR-JCL strain mice weighing 20–25 g and Wistar strain albino rats of both sexes weighing 100–150 g, each group consisted of ten animals. Twenty minutes after administration of drugs, 0.2 ml of 0.02% benzoquinone was intraperitoneally injected to the animal. Thereafter, the stretching counts during 20 minutes were determined and the rate of inhibitory effects on stretching were calculated as compared with placebo control.

In above pharmacological studies, AP-237 and control drug (aminopyrine) were all administered per os.

Acute toxicity was studied on both sexes of ICR-JCL strain mice and Wistar strain albino rats by means of oral, subcutaneous and intravenous administrations. Acute toxicity in guinea pigs was studied by oral administration. The observation period covered one week after the administration of the drug. The LD$_{50}$ values were calculated by the method of Litchfield-Wilcoxon (9).

RESULTS

1) Analgesic effect in the pressure method

Fig. 1 shows the dose-response curve of the analgesic effect of the drug determined by the pressure method employing mice, albino rats and guinea pigs. Fig. 2 shows the time-response curve of the analgesic action of the drug on mice. The administration of
FIG. 1. Dose-response curve of analgesic activity of AP-237 and aminopyrine in mice, rats and guinea pigs (pressure method).

![Graph showing dose-response curves for AP-237 and aminopyrine in mice (a), rats (b), and guinea pigs (c).](image)

Fig. 2. Effect of AP-237 and aminopyrine on the pressure pain in mice.

- a) AP-237 administered orally to male mice (●—● 25 mg/kg; ○.....○ 50 mg/kg; ×—× 100 mg/kg; ▲—▲ 200 mg/kg)
- b) Aminopyrine administered orally to male mice (●—● 167 mg/kg; △—△ 200 mg/kg; ×.....× 240 mg/kg)

TABLE 1. Effect of AP-237 and aminopyrine on the pressure pain in mice, rats and guinea pigs.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Sex</th>
<th>ED* (100 mmHg)</th>
<th>ED* (120 mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AP-237 (mg/kg)</td>
<td>Aminopyrine (mg/kg)</td>
</tr>
<tr>
<td>Mice</td>
<td>♦</td>
<td>38.5</td>
<td>165</td>
</tr>
<tr>
<td>Rats</td>
<td>♦</td>
<td>10.8</td>
<td>240</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>♦</td>
<td>10.6</td>
<td>224</td>
</tr>
</tbody>
</table>

Drugs were all administered orally.

* ED (100 mmHg) and ED (120 mmHg): Necessary dose of drugs for increasing pain threshold up to 100 or 120 mmHg, calculated from the dose-response curve in Fig. 1.
AP-237 resulted in a marked elevation of pain threshold even after 20 minutes. The onset of the analgesic effect of AP-237 was earlier than that of aminopyrine. As shown in Fig. 1, the dose-response curve approached a straight line. Table 1 shows the calculated dose necessary for elevating the pain threshold up to 100 mmHg or 120 mmHg from the above dose-response curve.

2) Analgesic effect in the D'Amour-Smith's method

Table 2 shows the analgesic effect determined by the D'Amour-Smith's method employing mice and albino rats. AP-237 showed considerably potent analgesic effect also in this method. A marked sex-difference was observed only in albino rats; its analgesic effect was about nine times more potent in female than in male rats.

3) Analgesic effect in the hot plate method

The $ED_{50}$ value of AP-237 for the analgesic action on mice determined by the hot plate method was 161 (97.5–265.7) mg/kg, while oral administration of 300 mg/kg aminopyrine did not show any significant analgesic effect in this method.

4) Analgesic effect in the writhing method

Fig. 3 shows the relation between the dose of the analgesic drugs and the inhibition of stretching induced by intraperitoneal injection of benzoquinone in mice and albino rats. Table 3 shows 50% inhibiting values calculated from the above dose-response curve.

![Fig. 3. Effect of AP-237 and aminopyrine on the writhing syndromes of mice and rats induced by intraperitoneal injection of benzoquinone.](image_url)
TABLE 3. Analgesic potency of AP-237 and aminopyrine determined by the benzoquinone writhing method.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Sex</th>
<th>AP-237 (mg/kg)</th>
<th>Aminopyrine (mg/kg)</th>
<th>Acetylsalicylic acid (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>♂</td>
<td>61.0</td>
<td>92</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>65.4</td>
<td>94</td>
<td>320</td>
</tr>
<tr>
<td>Rats</td>
<td>♂</td>
<td>2.7</td>
<td>5.2</td>
<td>83.2</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>2.4</td>
<td>4.5</td>
<td>83.3</td>
</tr>
</tbody>
</table>

All drugs were administered orally.

In this method, aminopyrine showed a considerably potent inhibitory action. However, AP-237 seemed to be more potent than aminopyrine in its inhibitory effect on benzoquinone writhing.

5) Acute toxicity

Table 4 shows the acute toxicity of AP-237. There were no marked differences among the species nor sexes of animals used.


<table>
<thead>
<tr>
<th>Animals</th>
<th>Sex</th>
<th>p.o. (mg/kg)</th>
<th>s.c. (mg/kg)</th>
<th>i.v. (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>♂</td>
<td>755 (634~980)</td>
<td>529 (503~566)</td>
<td>80.8 (75.5~86.5)</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>846 (776~922)</td>
<td>435 (416~457)</td>
<td>98.5 (92.3~104.9)</td>
</tr>
<tr>
<td>Rats</td>
<td>♂</td>
<td>748 (629~890)</td>
<td>527 (484~574)</td>
<td>75.5 (71.9~79.3)</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>653 (616~692)</td>
<td>339 (313~366)</td>
<td>70.3 (66.3~74.5)</td>
</tr>
<tr>
<td>Guinea</td>
<td>♦</td>
<td>700 (556~882)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pigs</td>
<td>♠</td>
<td>700 (667~735)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

All fiducial limits are significant at 0.05 probability level.

DISCUSSION

The potency of the analgesic effect of AP-237 varies according to the methods and species of animals employed. Comparison of the analgesic potency of AP-237 with aminopyrine could not be allowed only from this experiment, because AP-237 and aminopyrine seemed to be different in the mode of action. However, when it was supposed to be able to compare the potencies of the drugs, AP-237 was about 4~20 times as potent as aminopyrine in the D'Amour-Smith's method and about 2 times in the benzoquinone writhing method. In the hot plate method, aminopyrine did not show any analgesic effect, while AP-237 did exert an analgesic effect even in this method.

The analgesic effect of AP-237 in the pressure method had reached its maximum in 20 minutes after administration, thus the onset of the effect was proved to be prompt. This fact may be due to the good absorption of AP-237 from the intestine.

The pain threshold was markedly elevated by administration of AP-237. Especially
in mice, elevation up to 140 mmHg was observed at the maximum. In the pressure method employing mice, it was reported that the elevation of the pain threshold of antipyretic analgesics was far less than that of morphine-like analgesic drugs (3). As is clearly seen in Fig. 2, AP-237 showed a high elevation of the pain threshold as compared with that of aminopyrine; in this respect, AP-237 seemed to exert some modes of action different from that of antipyretic analgesics such as aminopyrine. When administered subcutaneously to mice, in a preliminary examination, the necessary doses of AP-237 and morphine for increasing the pain threshold up to 120 mmHg were 7.0 and 2.0 mg/kg, respectively.

The potency ratio of AP-237 as compared with aminopyrine was the lowest in the writhing test, and far higher in the pressure method and in the D'Amour-Smith's method. Stretching state, induced by peritoneal injection of irritants as in benzoquinone writhing test, is thought to be prevented by inhibiting the local reaction to irritants and also by blocking the central nervous transmission in the pathway for pain (10). Approved anti-inflammatory drugs such as aminopyrine, sodium salicylate, phenylbutazone, etc. were reported to have a favorable inhibitory action on the stretching state even in small doses (10). In our experiments, aminopyrine exerted a potent analgesic effect in the writhing method. This was thought to be due to its anti-inflammatory effects on peripheral sites. AP-237 exerted a potent effect in the pressure method and in the D'Amour-Smith’s method which are considered not to be greatly influenced in the evaluation of the analgesic effect by an anti-inflammatory action on the peripheral sites, thus, most of the analgesic effects of AP-237 seemed to occur in the central nervous system.

A marked sex difference was observed in the analgesic effect of AP-237 in the evaluation by the D'Amour-Smith’s method employing albino rats. That is, the analgesic effect on female rats was about 9 times more potent than that on male rats. This sex difference, however, was not observed in mice nor guinea pigs. If AP-237 is considered to be degraded by the drug metabolizing enzymes of liver microsomes, it is possible that the sex difference is observed only in rats. However, aminopyrine, which is considered to be inactivated by microsomal enzymes, exhibited only a little more marked analgesic action in the female rats than in the male rats from the viewpoint of the ED$_{50}$ values, and the sex difference arising from the drug metabolizing enzymes seemed to exert great influences upon the duration of action rather than on the ED$_{50}$ values of aminopyrine. Moreover, the sex difference in the analgesic effect of AP-237 was not observed in the pressure method nor in the writhing method even when albino rats were employed. From these results, it was thought to be difficult to explain the sex difference of the analgesic effect of AP-237 observed in the D'Amour-Smith’s method only with the relation to the drug metabolism in microsomes. Upon consideration of the specific character of the methodology of the D'Amour-Smith’s method, further studies should be made.

As shown in Table 4, the acute toxicity of AP-237 was at 700–800 mg/kg by oral administration to any of the species of animals employed. The authors also have evaluated the acute toxicity of aminopyrine by oral administration, the results being 930 (845–1023) mg/kg in male mice, 1320 mg/kg in male albino rats and 1440 mg/kg in female albino rats.
Therefore, the acute toxicity of AP-237 seemed to be not so elevated, though its potency of the analgesic effect was higher than that of aminopyrine. It can be said from these results that the safety-range of AP-237 seems to be wider than that of aminopyrine.

SUMMARY

The analgesic effect of a new compound, 1-butyryl-4-cinnamylpiperazine hydrochloride (AP-237), was evaluated by the use of the pressure method, the hot plate method, the D'Amour-Smith's method and the writhing method. AP-237 showed significant analgesic effect in all methods employed, and the onset of its effect was observed to be prompt. Aminopyrine did not show any significant analgesic effect in the hot plate method, and seemed to be less potent in other methods compared with AP-237. It was suggested that AP-237 might exert some modes of action different from that of antipyretic analgesic drugs.

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