EFFECTS OF ANISOTROPINE METHYLBROMIDE (VALPIN®) AND ITS MIXTURE WITH SULPYRINE ON VOCALIZATION RESPONSE AND SPASM OF INTESTINE INDUCED BY ACETYLCHOLINE IN DOGS

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Anisotropine methylbromide (A.M.; Valpin®) is a tropine ester related structurally to atropine and homatropine methylbromide, and has been synthesized by Gordon and Weiner (1). The chemical name is 2-propylpentanoyl tropinium methylbromide and structural formula is as shown in Fig. 1.

\[
\text{CH}_3 \quad \text{CH} \quad \text{CH}_3 \\
\text{(CH}_3\text{)}_2 \text{N}^+ \quad \text{CH} = \text{O} \text{-CO} \text{-CH} \quad \text{CH}_3 \text{CH}_2 \text{CH}_3 \quad \text{Br}^- \\
\text{CH}_2 \quad \text{CH} \quad \text{CH}_3
\]

\[\text{C}_{17}\text{H}_{32}\text{O}_2\text{NBr} : 362.36\]

FIG. 1. Chemical structure of 2-propylpentanoyl tropinium methylbromide.

In previous experiments, Taira et al. (2) found that the vocalization response of dogs to acetylcholine (Ach) administered into a branch of the mesenteric artery was selectively abolished by atropine. On the other hand, sodium salicylate was also found to be effective for prevention of the vocalization response to Ach, although a larger dose was needed in the mesenteric artery than in the femoral. They concluded that the vigorous contraction of the intestine through a muscarinic mechanism probably plays an important role in the induction of the nociceptive response, although possible involvement of other mechanisms cannot be ruled out. In the present experiments the analgetic effect of A.M. and of its mixture with sulpyrine was assessed, the procedure being essentially the same as described by Taira et al. (2) and Nakayama et al. (3).

METHODS

1) Experiments on vocalization of conscious dogs (chronic experiments)

Fifteen dogs of both sexes, weighing 2.5 to 5.0 kg were used. Procedures employed
in this study were essentially the same as those described in a previous paper (2). Two or three days prior to the experiment the animals were anesthetized with sodium pentobarbital, 30 mg/kg, i.v., and laparotomized under an aseptic condition. Polyvinyl tubing (ID 0.5 mm; OD 0.8 mm) which had been filled with \( \omega \)-heparin, 1000 U/ml, was inserted retrogradely into a branch of the mesenteric artery. The distal end of the catheter was plugged with a needle and fixed to the skin at the nape. The catheter was flushed with \( \omega \)-heparin every day. Mycillin (Kaken), antibiotic preparation, was administered to prevent infection (1 vial daily). Experiments were performed after the animals recovered completely, which was several days. The animal was restrained loosely on a hanging girth. Compounds were administered into the mesenteric artery through the catheter. Vocalization was picked up by means of a non-directional microphone (Matsushita Electric, MC-2010) and recorded on magnetic tapes by using a tape recorder (Matsushita Electric, RQ-703). Afterwards the tapes were played back, and output was rectified and integrated by means of a passive circuit with a time constant of 2 sec. The integrated responses were finally displayed on charts by using an ink-writing oscillograph (Nihon Kohden, WI-260). The threshold dose of Ach for inducing vocalization response was determined by increasing its dosage by two-fold steps, and the four or eight times threshold dose was selected as a test dose. The test dose of Ach was given two or three times to obtain control responses before an analgetic effect of a given test compound was examined. The analgetic effect was determined in the following way: 3 min after an intra-arterial injection of the compound, the test dose of Ach was given, and then it was repeated at 10 min intervals until the response returned to control. Fig. 3 shows actual tracings of vocalization in response to 2 \( \mu \)moles of Ach obtained as above. To obtain the inhibition-time curve, areas of the integrated vocalization responses were measured by means of a planimeter and their per cent decreases from control were plotted against time after injection of the test compound. In the inhibition curves thus constructed, the area circumscribed by the abscissa, ordinate and the curve was measured and termed as total inhibition (% min).

2) Measurement of intraluminal pressure of the small intestine in laparotomized dogs

All dogs used previously in the chronic experiments were anesthetized with sodium pentobarbital, 30 mg/kg, i.v., and laparotomized. A portion of the intestine which drugs injected through the tubing would reach was confirmed by 0.01 % solution of pontamine sky blue 6B (Tokyo Kasei). In order to measure intraluminal pressure in this portion, a water-filled rubber balloon was placed and connected to an electromanometer (Nihon Kohden, MP-4). The respiratory movement and the systemic blood pressure were also monitored. All recordings were made on an ink-writing oscillograph (Nihon Kohden, WI-380).

Compounds used in the present experiments were acetylcholine (Ach), sulpyrine and anisotropine methylbromide (A.M.). Each compound was dissolved at a desired concentration with 0.9 % physiological saline. Ach and A.M. were injected into the catheter in a volume of 0.1 ml and sulpyrine was in a volume of 1 ml. All materials injected into the catheter were flushed in with 0.4 to 0.7 ml of 0.9 % saline at a rate of 0.05 ml/sec.
RESULTS

1) Results obtained in conscious dogs (chronic experiments)

The effects of A.M., sulpyrine and a mixture of both were assessed against the vocalization response to Ach. In the present experiments, animals vocalized consistently in response to 2 \( \mu \)moles of Ach (0.36 mg as salts) which were selected as a test dose. Sulpyrine in doses from 13.6 to 67.8 \( \mu \)moles (4.5 to 22.6 mg as salts) failed to inhibit the Ach-induced vocalization response. Fig. 2 illustrates such a typical experiment where 67.8 \( \mu \)moles of sulpyrine were ineffective. However, addition of sulpyrine to A.M., i.e., simultaneous administration of both, prolonged the duration of blocking effect of A.M. on the Ach-induced vocalization. As indicated in Table 1, total inhibition attained with combined administration of A.M. and sulpyrine was significantly greater (P<0.01) than that with injection of A.M. alone. Although maximum inhibition caused by a mixture of A.M. and sulpyrine appeared slightly greater than that by A.M. alone, there was no statistical difference between the two. Fig. 3 depicts an example in which the duration of inhibitory effect of A.M. was

![Fig. 2. Failure of prevention of the Ach-induced vocalization by sulpyrine injected into the mesenteric artery of a conscious dog.](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of animals</th>
<th>Inhibitory action on the Ach-induced vocalization</th>
<th>Inhibitory action on the Ach-induced intestinal spasm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maximum inhibition (%)</td>
<td>Total inhibition (% min)</td>
</tr>
<tr>
<td>Anisotropine methylbromide (0.5 ( \mu )mole)</td>
<td>7</td>
<td>85.1 \pm 14.2</td>
<td>1489 \pm 545</td>
</tr>
<tr>
<td>Mixture of anisotropine methylbromide (0.5 ( \mu )mole) and sulpyrine (13.6 ( \mu )moles)</td>
<td>7</td>
<td>97.0 \pm 3.0</td>
<td>3722 \pm 651</td>
</tr>
</tbody>
</table>

*Statistical analysis, paired 't' test*
obviously prolonged by combination with sulpyrine.

2) Effects of A.M. and its mixture with sulpyrine on Ach-induced intestinal spasm in anesthetized and laparotomized dogs (acute experiments)

When administered into a branch of the mesenteric artery, Ach in small doses augmented only the intestinal motility. When doses of Ach were increased up to those which had caused vocalization consistently in the chronic experiments, Ach produced a spastic contraction of the intestine. The intestinal blood flow appeared to be interrupted by the spastic contraction and the intestine became pale. At the same time respiratory excitation preceded by momentaneous arrest and fluctuation of the systemic blood pressure were observed as pseudosomatic reflex (4). Height and duration of the intestinal contraction increased with the increment of the dose of Ach administered (Fig. 4).

In each preparation, the effects of single and combined injections of sulpyrine and A.M. were studied on the spastic contractions produced by 2 μmoles of Ach. In all the experiments injection of sulpyrine alone failed to inhibit the spastic contractions produced by
Fig. 5. Failure of prevention by sulpyrine of Ach-induced contractions of the small intestine in an anesthetized dog.

Fig. 6. Effects of A.M. and a mixture of A.M. and sulpyrine on the Ach-induced contractions of the small intestine in an anesthetized dog.

Ach. Fig. 5 illustrates such an example where sulpyrine even at a large dose of 150 μmoles (50.0 mg as salts) was ineffective in inhibiting the spastic contraction caused by 2 μmoles of Ach. A.M. at a dose of 0.5 μmole abolished the intestinal contraction produced by 2 μmoles of Ach (Fig. 6, upper row). Although administration of sulpyrine alone was ineffective, its administration simultaneous with A.M. prolonged the duration of the inhibitory effect of A.M. on the contractile response to Ach. (Fig. 6, lower row). Total inhibition and maximum inhibition were summarized in Table 1. The duration of spasmolytic action of the mixture of A.M. and sulpyrine was significantly longer (p<0.01) than that of A.M.

DISCUSSION

In experiments on conscious animals the vocalization response to injection of Ach into a branch of the mesenteric artery was effectively inhibited by intra-arterial injection of A.M. in doses about 1/4 those of Ach. In acute experiments on anesthetized and laparotomized
animals the Ach-induced spastic contraction was also abolished by A.M. in doses about 1/4 those of Ach. These facts support the suggestion in the previous paper (2) that intestinal spasm rather than neural chemoreception plays an important role in inducing the noxious response from the intestine.

As clearly demonstrated in the present experiments sulpyrine per se lacks an antimuscarinic or spasmolytic action. However, the combined use of sulpyrine with A.M. definitely prolonged not only the blocking action of A.M. on the Ach-induced vocalization response but also its spasmolytic effect. According to Randall (5) and Guzman et al. (6) aminopyrine has a peripheral analgetic action. Furthermore, as to mechanism of action the latter authors suggested that aminopyrine may block neural chemoreceptors in both somatic and visceral areas. Since sulpyrine is a congener of aminopyrine, it may share such activity. However, the present results were negative. At present, there is no reasonable explanation of the mechanism for sulpyrine to potentiate the effect of A.M. It is, however, noteworthy that sulpyrine definitely potentiates analgetic and spasmolytic actions of A.M. by its peripheral action although sulpyrine per se has no analgetic or spasmolytic action in the intestine.

SUMMARY

In conscious dogs the analgetic effect of anisotropine methylbromide (A.M.; Valpin®) and that of its mixture with sulpyrine were compared on the vocalization response to acetylcholine injected into a branch of the mesenteric artery. The effect of these drugs on the intestinal spasm produced by intra-arterial administration of acetylcholine was also investigated in anesthetized and laparotomized dogs. Sulpyrine prolonged not only the action of A.M. in blocking the vocalization response to acetylcholine but also its antimuscarinic and spasmolytic action, although sulpyrine alone was entirely ineffective on both responses.

REFERENCES