ON THE SITE OF ANTITUSSIVE ACTION OF 1-(2-BENZYLPHENOXY)-2-PIPERIDINOPROPANE PHOSPHATE (PIREXYL)

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1-(2-Benzylphenoxy)-2-piperidinopropane phosphate (abbreviated as ASA 158/5) which was introduced by the Pharmacia Research Laboratory (Sweden) is a compound with the following chemical structure (1). According to an unofficial and unpublished report from the Laboratory (2), it has the antitussive potency 2 to 4 times as potent as that of codeine phosphate and its antitussive action may be mainly due to the prevention of impulses from the pulmonary stretch receptors. The present purpose of this study is to confirm the site of antitussive action of the drug.

\[
\text{O-CH}_2\text{-CH-}\text{N}\underline{\text{CH}_3}\cdot\text{H}_3\text{PO}_4
\]

MATERIALS AND METHODS

The drug is a white crystalline powder (m.p. 149-151°C) and was supplied by the Pharmacia in Sweden as Pirexyl® powder. On use, the powder was dissolved in distilled water or physiological saline solution.

Toxicity

Male mice of dd-strain weighing 20±2 g were used, and one group consisted of 10 mice and more than 5 groups were used. After the drug was administered intravenously, intraperitoneally, subcutaneously and orally, acute toxic symptoms were observed. LD₅₀ and its fiducial limit (p=0.05) were calculated from the lethality within 24 hours using the method of Litchfield-Wilcoxon (3). Mongrel dogs of both sexes weighing 7-12 kg were also used for the observation of toxic symptoms.

Antitussive activity

a) Mechanical stimulation

The “coughing dog and cat” methods (4) were used for this test. Dogs were used
without anesthesia, while cats were lightly anesthetized with pentobarbital sodium (20 mg/kg, i.p.). Cough was induced by mechanical stimulation on the mucosa at the tracheal bifurcation. Evaluation of the effect was made by the changes in amplitude and frequency of cough recorded on a smoked paper. When the amplitude and/or the frequency were decreased by more than 20% as compared with the control, and also when such a decrease lasted for more than 20 minutes, the effect was considered to be significant. As the same animals can be used in this method repeatedly for the experiment after 2 or 3 days' interval, the errors arising from individual differences in animals were able to be kept minimal. More than 4 groups of animals were used and each group consisted of more than 6 animals. From the effect of the drug given intravenously, 50% antitussive dose (abbreviated as AtD50) and its fiducial limit were calculated by the method of Litchfield-Wilcoxon (p=0.05).

\[ \text{b) Electrical stimulation} \]

Electrical stimulation was induced by electrical stimulation of the superior laryngeal nerve (parameter of stimulus; frequency 20 cps, duration 1 msec, voltage 0.5–2 V) in a cat lightly anesthetized with 20 mg/kg of pentobarbital sodium given intraperitoneally (5). The antitussive effect was determined from the elevation of threshold voltage and the decrease in amplitude and frequency of tracing of cough.

\[ \text{Effects on stretch receptor impulses} \]

The chests of urethanized guinea pigs and pentobarbitalized cats were opened under an artificial ventilation. The impulses from the alveolar stretch receptors were led through a bipolar silver electrode from the peripheral stump of the left vagus nerve sectioned at the cervical region, and observed on an oscilloscope and recorded with a long-recording camera. The drugs were given intravenously.

\[ \text{Effects on the centrally-evoked cough response and on the sustained inspiratory response} \]

In cats lightly anesthetized with pentobarbital sodium (20 mg/kg, i.p.), a bipolar stainless steel electrode was inserted into the expiratory pacemaker area in the brainstem (6–9), and square-wave pulses were given (parameter of stimulus; 20 cps, 1 msec, 1–8 V) to obtain spasmodic respiration just like cough. It is still remained to be ascertained if this respiration is entirely identical to cough, but it is sure to be affected similarly by known antitussives as the cough produced reflexly by peripheral stimulation (7). The sustained inspiration was induced by electrical stimulation (50 cps, 1 msec, 1–8 V) through a bipolar stainless steel electrode inserted into the inspiratory center of Pitts (10) [the inspiratory integrator of Brodie and Borison (8)] in the medulla.

Changes in the excitability of respiratory center due to the drug can be evaluated by the changes in the amplitude of the sustained inspiratory responses and/or by those in the threshold necessary to induce the inspiratory response. In both the experiments above, the respiratory changes were recorded by tambours connected to the tracheal cannula for tracing of intratracheal pressure and to thoracic and abdominal pneumographs.
Effect on the descending respiratory pathway (9)

The head of a cat lightly anesthetized with pentobarbital was fixed with the aid of a stereotaxic instrument. The spinous processes of the I, II, and III cervical vertebrae were resected to expose the cervical cord. In order to prevent troublesome movement of the vertebral column, a steel wire penetrating through a vertebral body was fixed tightly at both sides of the instrument and, furthermore, a spinous process of the vertebra caudal to the exposed cervical cord was clamped tightly. A bipolar stainless steel electrode (the tip was 100 μ in diameter) was inserted into the anterior part of lateral funicle of either side in the II cervical cord for stimulation (50 cps, 1 msec, 0.3-2 V).

Changes in the movements of chest and abdominal walls indicating the contraction of respiratory muscles were recorded by thoracic and/or abdominal pneumographs. When the descending pathway caudal to the stimulated area was depressed by the drug, the amplitude of contraction decreased. In many cases, the experiments were done along with those described in the preceding section.

Effects on the bronchial muscles

In vitro: The tracheal muscle preparation of a guinea pig was made with the method of Castillo and De Beer (12) modified by Takagi et al. (11).

In vivo: In rabbits anesthetized with urethane, the tone of the bronchial muscles was recorded with the method of Jackson (13).

Other experimental methods will be described in each section concerned.

RESULTS AND DISCUSSION

I. Toxicity

The LD₅₀ of ASA 158/5 and codeine in mice determined by administration through various routes are shown in Table 1.

The toxicity of ASA 158/5 was twice as much as that of codeine when the drugs were administered intravenously, while it was approximately equal to that of codeine when administered intraperitoneally. By oral administration, however, it was 1/4 to 1/3 of that of codeine.

In mice, toxic symptoms caused by ASA 158/5 were salivation, ataxia and convulsion, and death occurred with respiratory paralysis during tonic convulsion. In dogs, toxic symptoms caused by intravenous doses ranging from 30 to 40 mg/kg of the drug

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Routes</th>
<th>i.v.</th>
<th>i.p.</th>
<th>s.c.</th>
<th>p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA 158/5</td>
<td></td>
<td>32</td>
<td>139</td>
<td>710</td>
<td>1,100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(29-36)</td>
<td>(154-144)</td>
<td>(612-144)</td>
<td>(1,027-1,177)</td>
</tr>
<tr>
<td>Codeine</td>
<td></td>
<td>62</td>
<td>140</td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>phosphate</td>
<td></td>
<td>(55-70)</td>
<td>(117-168)</td>
<td></td>
<td>(232-357)</td>
</tr>
</tbody>
</table>

Figures in parentheses show the fiducial limits (p=0.05).
were transitory apnea followed by respiratory stimulation, licking, salivation, vomiting, ataxia and clonic convulsions. Death occurred with doses more than 50 mg/kg.

II. Effects on Respiration and Blood Pressure

The blood pressure of the femoral artery was recorded with the routine Hg manometer method and the respiration was recorded with a Marey’s tambour via tracheal cannula in rabbits anesthetized with urethane.

With intravenous doses more than 2 mg/kg of ASA 158/5, a remarkable but transitory blood pressure fall occurred. Respiration showed an increase in respiratory rate for 15 to 20 minutes following the apnea immediately after injection. After the vagus nerves were cut bilaterally at the superior cervical region, the changes in blood pressure and respiration described above did not occur any longer. Carotid occlusion pressor response was not affected by any doses of the drug. The results indicate the reflex mechanism of these effects.

III. Evaluation of Antitussive Activity

As shown in Fig. 1, both the amplitude and frequency of tracing of cough in an unanesthetized dog induced by mechanical stimulation were decreased to 50% of those of the control at 5 minutes after intravenous injection of 4.3 mg/kg of ASA 158/5, and its effect lasted for as long as 45 to 50 minutes (see Fig. 1).

On the other hand, a definite antitussive activity was also observed with an intravenous dose of 3.0 mg/kg of the drug in a cat lightly anesthetized with pentobarbital (see Fig. 1).

The AtD₅₀ of ASA 158/5 in unanesthetized dogs and in lightly pentobarbitalized cats

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**Fig. 1.** Antitussive effects of ASA 158/5 in an unanesthetized dog and a pentobarbitalized cat.

The drug was injected intravenously.

For explanation, see text.

RESP.: Intratracheal pressure (expiration is recorded upwards), STIM.: mechanical stimulation of the tracheal mucosa, TIME: in minute.
and those of codeine phosphate in both of animals were shown in Table 2. Thus, the antitussive activity was approximately equal to that of codeine or slightly stronger.

The coughs induced by electrical stimulation of the superior laryngeal nerve in cats were depressed with intravenous doses of 1.5 to 2.0 mg/kg of the drug, that is, elevation of threshold voltage and decrease in both amplitude and frequency of the tracing of cough were observed in 3 out of 4 cases. The efficacy of the drug was approximately equal to that of codeine.

Thus, the coughs induced by mechanical stimulation or electrical stimulation of the superior laryngeal nerve were depressed by approximately the same dose of the drug.

Safety margin: On intravenous injection to dogs, safety margin (LD/AtD₅₀) was calculated to be about 14 and it is about a half of that of codeine 26.0.

IV. Site of Antitussive Action

The following steps of experiments were used for elucidation of the site of action.

1) Comparison of equi-active doses for various routes of administration

As a measure for investigation of the site of antitussive action, equi-active doses for various routes of administration were compared. In dogs, lightly anesthetized with pentobarbital, the drug was given through polyethylene tubes inserted previously into the common carotid artery, the vertebral artery and the cerebello-medullary cistern, and the doses necessary to indicate the same degree of antitussive effects that obtained by administration into the femoral vein were studied.

Following the intravenous administration of 2–3 mg/kg of the drug, both the amplitude and frequency of the tracing of cough elicited by mechanical stimulation in dogs were decreased to 50–60% of those of the control, and such inhibition lasted for about 30 to 50 minutes after injection. In 4 cases, the doses (mg/kg) necessary to obtain antitussive effect to the same extent as above were as follows: (Routes) femoral vein: common carotid artery: vertebral artery: cistern = (Doses) 2–3: 1–2: 0.4: 0.2. When the intravenous dose was taken as 1.0, the ratio above was 1.0: 1–1/2: 1/3–1/4: 1/8–1/10, respectively. On the other hand, the ratio of effective doses of codeine phosphate was
1.0: 1/2–1/3: 1/4–1/5: 1/40–1/50, respectively. Therefore, the finding above indicates that the site of action of the drug is somewhat different from that of codeine, which is believed to act exclusively on the cough center per se.

2) Effect on the stretch receptor

The impulses form the alveolar stretch receptors action respiration, not only regulating the depth of normal respiration (Hering-Breuer reflex), but also affecting on the intensity of cough. The expiratory intensity of cough is influenced by the depth of the preceding inspiration. The deeper and faster the inspiration, the stronger is the subsequent expiration, that is, it may result in violent attack of cough. The antitussive action of benzonatate (Tessalon®) is said to be due to selective stretch receptor anesthesia, resulting in a decrease of impulses from the receptors (14).

The drug is said to have a local anesthetic action more potent than procaine (2) and it was emphasized that antitussive action may be due primarily to the inhibition of impulses from the pulmonary stretch receptor (2). So, the potency of local anesthetic activity was first determined and then the action on stretch receptors was also studied if there was any relation to its antitussive activity.

a) Local anesthetic action

(i) Surface anesthesia: In rabbits, the corneal reflex was obviously disappeared in 10 minutes after the application of 0.1% solution of ASA 158/5 and complete recovery was observed at 60 to 120 minutes after application. This effect was approximately equal to that of 1% procaine employed as a control drug. One per cent solution of ASA 158/5 showed much more potent anesthetic action. On the other hand, such a highly concentrated solution caused local irritation, e.g., remarkable lacrimation and corneal opacity or even corneal ablation.

(ii) Conduction anesthesia: The action potentials of the sciatic nerve of frogs observed on an oscilloscope, were reduced by about 20% in amplitude with 0.05 to 0.1% solution of ASA 158/5, and about 50% with 0.2 to 0.3% solution of the drug. On the other hand, 0.05 to 0.1% solution of procaine reduced the amplitude to about 50%. Therefore, the conduction anesthesia of ASA 158/5 is 1/4 to 1/3 of that of procaine. The action potentials inhibited by procaine easily returned to normal by washing with Ringer solution several times, but in the case of ASA 158/5, recovery was quite slower and no recovery occurred when solution of more than 0.2% in concentration was employed for the test.

b) Depressing action on stretch receptor impulses

In guinea pigs, 2 to 3 mg/kg of benzonatate decreased the voltage of the impulses and 4 mg/kg resulted in a complete disappearance of the impulses for 20 to 25 minutes. On the other hand, ASA 158/5 decreased the voltage of impulses with a dose of 7.5 mg/kg and showed complete disappearance for 20 to 30 minutes with a dose of 10 mg/kg (see Fig. 2).

In a cat, ASA 158/5 showed no effect with a dose of 2.5 mg/kg which had been enough to produce antitussive effect in the same animal. With an increased dose up to 7.5 mg/
kg, the voltage of impulses was decreased and 10 mg/kg resulted in complete disappearance (see Fig. 2).

The results suggest that anesthesia of the stretch receptors does not play a decisive role in the production of antitussive action, that is, mechanism of action of the drug is different from that of benzonatate.

3) Influence of decerebration

In order to study whether the higher centers than the brain stem contribute to the antitussive activity of ASA 158/5, a mid-collicular transection was done in cats under ether anesthesia, and the cranial contents rostral to the superior colliculi were all removed. The experiments were carried out after the influences of anesthesia and operation had completely disappeared.

Before the decerebration, a dose of 2.5 mg/kg of ASA 158/5 showed definite antitussive effect and it lasted for 40 to 50 minutes. After the decerebration, no discernible change in antitussive effect was observed with the same dose, except that the duration of effect was prolonged in one out of three cases.

From the results presented above, the site of action of ASA 158/5 seems to be present at the brain stem below the transected area.

4) Effects on the centrally-evoked cough response and on the sustained inspiratory response

Whereas cough responses induced by mechanical stimulation of the tracheal mucosa and electrical stimulation of the superior laryngeal nerve were definitely depressed by 2 mg/kg of the drug, neither centrally-evoked cough nor sustained inspiratory response were affected with this dose. As shown in Fig. 3, 4 mg/kg of the drug slightly decreased the frequency of the tracing of mechanical cough, it also showed 60% decrease in the am-

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**Fig. 2.** Effects of ASA 158/5 (10 mg/kg, i.v.) on the stretch receptor impulses recorded from the left vagus of a urethanized guinea pig and a pentobarbitalized cat.

For explanation, see text.

Left : Guinea pig.
Right : Cat.

IMP. : Impulses, RESP. : intratracheal pressure (inspiration is recorded upwards).
Amplitude at 5 minutes, 50% decrease at 40 minutes, and returned to normal at 80 minutes, after administration. The cough induced by the electrical stimulation of the superior laryngeal nerve was also markedly depressed and it returned to normal at 80 minutes after administration. On the other hand, the amplitude of centrally-evoked cough was reduced to about a half at 5 minutes after administration and threshold of stimulus was elevated from 3.0 to 5.0 V and returned to normal at 40 minutes after administration. The amplitude of the sustained inspiratory response slightly decreased with this dose but no change occurred in the threshold of stimulus.

Thus, it was found that somewhat larger dose was necessary to depress the centrally-evoked cough when compared with the doses necessary to depress the cough responses peripherally induced by mechanical or electrical stimulation. The result shows that the antitussive action of ASA 158/5 may be due mainly to a decrease of excitability of cough center and, in addition, partly to the peripheral effect such as a decrease of pulmonary stretch receptor impulses and the relaxation of the bronchial muscles (see the following).

5) Effect on the descending respiratory pathway

In the preceding section, it was found that ASA 158/5 depressed the cough center accompanying with a slight depression of centrally-evoked inspiratory response. How-
ever, in order to confirm the fact that a drug should act directly on the respiratory center and the cough center, it is necessary to exclude the actions on the descending respiratory pathway extending from the spinal cord to the respiratory muscles.

The centrally- or/and peripherally-evoked coughs were definitely depressed by the intravenous dose of 4 mg/kg of the drug. The intraspinal pathway was stimulated during the time when the amplitude of centrally-evoked inspiratory response was slightly inhibited but no change occurred in the magnitude of muscle contraction.

This result suggests that ASA 158/5 had no effect on the descending respiratory pathway with a dose producing the depressions of cough center and inspiratory center.

6) Effects on the bronchial muscles

On cough attacks, the bronchial muscles are contracted, and the lumen of respiratory tract is narrowed to such an extent that the air stream is greatly accelerated. A reduction in tone of the bronchial muscles results in a decrease in the intensity of cough. Therefore, the effects of the drug on the bronchial muscles were studied in vitro and in vivo.

FIG. 4. Effects of ASA 158/5 on the tracheal and bronchial muscles.

Upper: Isolated tracheal muscle preparation of a guinea pig.
Effect of ASA 158/5 on the contraction induced by $10^{-6}$ g/ml of histamine (Hist.) was examined. The drugs were added to the bath.

Lower: The tone of the bronchial muscles of a urethanized rabbit.
Effects of ASA 158/5 (1 mg/kg, i.v.) and papaverine (1 mg/kg, i.v.) on the bronchial muscle contraction induced by an intravenous dose of 10 $\mu$g/kg of histamine (Hist.) were examined.

RESP.: Respiration (intratracheal pressure) given by artificial ventilation is maintained at a constant state and reductions in amplitude of tracing show increase in tone of the bronchial muscles.

B.P.: Blood pressure in mmHg. TIME: in minute.

For explanation, see text.
TABLE 3. Effects of ASA 158/5 on the various responses related to cough.

<table>
<thead>
<tr>
<th>Doses (mg/kg i.v.)</th>
<th>Animals used</th>
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<tbody>
<tr>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
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</table>

In vitro: The contraction of the tracheal muscle preparation of a guinea pig caused by $10^{-6}$ g/ml of histamine HCl was slightly reduced by an addition of $10^{-6}$ g/ml of ASA 158/5, and $10^{-5}$ g/ml of the drug caused remarkable reduction (Fig. 4). This action of ASA 158/5, however, was somewhat weaker than that of papaverine, as shown in Fig. 4.

In vivo: With intravenous injection of 5 mg/kg of the drug alone, no effect was seen on the muscular tone recorded by the method of Jackson (14). The contraction of the bronchial muscles by intravenous doses of 10 to 15 µg/kg of histamine or 15 to 20 µg/kg of acetylcholine was completely inhibited for 20 to 30 minutes by the pretreatment with 1 to 2 mg/kg of ASA 158/5. The similar effect was seen with 1 mg/kg of papaverine but the duration of the effect (for 5 to 10 minutes) was much shorter than that of ASA 158/5.

Thus, ASA 158/5 definitely decreased the tone of the bronchial muscles like papaverine and this action may contribute partly to the antitussive activity of the drug.

The results obtained are shown in a summarized form in Table 3. This will help for elucidation of the site of antitussive action of ASA 158/5.

7) Differentiation from narcotic antitussives

Antitussive actions of narcotic drugs such as morphine, codeine, and so forth were rapidly and completely abolished by the administration of N-allylnormorphine. So, this antagonism was applied to differentiate the mechanism of antitussive action between the drug and narcotic ones.

In unanesthetized dogs, intravenous doses of 0.4 to 0.8 mg/kg of N-allylnormorphine did not affect the antitussive effect of intravenous dose of 4 mg/kg of ASA 158/5 at all. The results show that the mechanism of antitussive action of ASA 158/5 is quite different from those of narcotics.
SUMMARY

The site of antitussive action of 1-(2-benzylphenoxy)-2-piperidinopropane phosphate (Pirexyl) was studied.

1. It has been found that antitussive activity of the drug is approximately equal to that of codeine in dogs and cats.

2. When the drug was administered through three routes such as the common carotid artery, the vertebral artery and the cerebello-medullary cistern, much smaller doses were sufficient to produce the same degree of the effect that shown by intravenous administration, however, the ratio in equi-active doses shown by the administration of the drug to four routes was somewhat different from that of codeine, which acts exclusively on the cough center.

3. Somewhat larger dose was necessary to depress the centrally-evoked cough than those necessary to depress the cough responses peripherally induced by mechanical or electrical stimulation.

4. In the cat, the stretch receptor impulses were not affected with an antitussive dose (2.5 mg/kg) of the drug, however, they were decreased or abolished when the dose was increased up to 7.5 or 10 mg/kg. In the guinea pig, the similar effects were also observed.

5. The drug showed spasmolytic actions not only on the bronchial muscles in rabbits (in vivo) but also on the tracheal muscles in guinea pigs (in vitro).

6. The effect of the drug was not influenced by decerebration after mid-collicular transection.

7. The drug showed no effect on the efferent respiratory pathways extending from the spinal cord to the respiratory muscles.

From the results described above, it is concluded that the antitussive activity is displayed mainly through the decrease in excitability of the cough center per se and partly through the peripheral effects such as the decrease of pulmonary stretch receptor impulses and the relaxation of the bronchial muscles.

The mode of depressing action of the drug on the cough center was different from those of narcotic antitussive agents such as codeine, because the antitussive activity of the drug is not antagonized by N-allylnormorphine at all.

REFERENCES

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4) Kase, Y.: This Journal 2, 7 (1952); Pharm. Bull. 2, 298 (1954); This Journal 4, 130 (1955)
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