ANTITUSIVE ACTIVITY AND OTHER RELATED PHARMACOLOGICAL PROPERTIES OF 2-ALLYLOXY-4-CHLORO-N-(2-DIETHYLAMINOETHYL) BENZAMIDE

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The drug is a synthetic antitussive, which was developed by Laboratoires de Recherches Mauvernay in France, having the following chemical structure. No formal report has been out yet, but it is said that the antitussive activity of the drug is as potent as that of codeine. It is the purpose of this paper to investigate its antitussive action especially its site of action and other pharmacological properties associated with the main effect.

\[
\begin{align*}
\text{Cl} & \quad \text{CONHCH}_2\text{CH}_2\text{N} \quad \text{C}_2\text{H}_5 \quad \text{HCl} \\
\text{OCH}_3 & \quad \text{CH}_3 = \text{CH}_2
\end{align*}
\]

MATERIALS AND METHODS

The drug (abbreviated as 264CE) is a white or somewhat cream-colored and odorless powder (m.p. 125–130°C), and has a bitter taste. On use, it was dissolved in distilled water, physiological saline solution and other nutritive solutions.

Toxicity: The dd-strain mice of both sexes weighing 15–20 g were used, and one group consisted of 6 mice and more than 5 groups were used for a series of experiment. After the drug was injected subcutaneously at the back, its toxic symptoms were observed. LD₅₀ and its fiducial limit (p=0.05) were calculated from the lethality observed within 24 hours using the method of Litchfield-Wilcoxon (1). Mongrel dogs of both sexes weighing 7–12 kg were also used for the observation of toxic signs.

Antitussive activity: The “coughing dog and cat” methods were used for this test. Dogs were used without anesthesia, while cats were lightly anesthetized with pentobarbital (20 mg/kg, i.p.). Cough was induced by mechanical stimulation on the mucosa of tracheal bifurcation (2). Evaluation of the effect was done by the changes in amplitude and frequency of the tracing of cough in respiration. 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' rest, 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 5 animals. From the effectiveness of the drug given intravenously, 50% antitussive dose (abbreviated hereafter as AtD<sub>50</sub>) and its fiducial limit were calculated by the method of Litchfield-Wilcoxon (p = 0.05).

As one of the procedures for investigating the site of antitussive action, equi-active doses among various routes of administration were compared. In the dogs lightly anesthetized with pentobarbital, 264CE was given through polyethylene tubes inserted previously into the common carotid artery, the vertebral artery and the cerebello-medullar cistern, and the doses necessary to obtain the same degree of antitussive effects as that by intravenous injection were studied.

Effect on stretch receptor impulses: The chest of a guinea pig anesthetized with urethane was opened under an artificial ventilation. The impulses from the alveolar stretch receptors were led via a bipolar silver electrode from the left vagus nerve trunk sectioned at the cervical region, and observed on an oscilloscope and recorded with a long-recording camera. The drugs were given intraperitoneally.

Effects on the centrally evoked cough response and on the sustained inspiratory response: In cats lightly anesthetized with pentobarbital (20 mg/kg i.p.), a bipolar stainless steel electrode was inserted into the expiratory pacemaker area (3-5), and square-wave pulses were given (parameter of stimulus: frequency 10 cps, duration 1 msec, voltage 0.5-4.0 V), to obtain spasmodic respiration 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 antitussive as cough responses produced reflexly by peripheral stimulation.

The sustained inspiration was induced by electric stimulation (50 cps, 1 msec, 0.5-7.0 V) through a bipolar stainless steel electrode inserted into the inspiratory center of Pitts (6) (the inspiratory integrator of Brodie and Borison (5)). Changes in the excitability of respiratory center due to the drug can be evaluated by the changes in the amplitude of the sustained inspiratory response and/or by the changes in the threshold of stimulus to induce the response.

In both the experiments described above, the respiratory changes were recorded by tambours connected to the tracheal cannula for tracing of intra-tracheal pressure and to thoracic and/or abdominal pneumographs.

Effect on the centrifugal respiratory pathway: The head of a cat lightly anesthetized with pentobarbital was fixed with the aid of a stereotaxic instrument. The spinal processes of the I, II, and III cervical vertebrae were resected to expose the cervical cord. In order to prevent troublesome movement of the spinal cord, a steel wire penetrating through a vertebral body was fixed tightly at both sides of the instrument and, furthermore, a spinal process of the vertebra caudal to the exposed cervical cord was clamped tightly. A bipolar electrode was inserted into one side of the lateral fascicule of the II cervical cord for stimulation (50 cps, 1 msec, 0.25-2.0 V). Changes in the movements of chest and
abdominal walls indicating the contraction of respiratory muscles were recorded by thoracic and/or abdominal pneumographs (7). When the centrifugal pathway below the stimulated area was depressed by the drug, the amplitude of contraction decreased. In many cases, the experiments were done along with the experiments described in the preceding section.

**Effect on the bronchial muscle:** *In vivo.* In the rabbits anesthetized with urethane, the tonus of bronchial muscle were recorded with the method of Jackson (8). *In vitro.* The tracheal muscle preparation of a guinea pig was made with the method of Takagi et al. (10) which is a modified method of Castillo and de Beer (9).

**Potentiating and prolonging effect on anesthesia:** Using the dd-strain male mice weighing 14-18 g, a dose of 0.5 mg/10 g of methylhexabital-Na (MH) was administered intraperitoneally, and the duration of anesthesia was determined. Three days later, the test drug was given intraperitoneally to the same mice and, 20 minutes later, the same dose of MH was given in order to study how the duration of anesthesia was changed. Using the same mice, the similar experiment was done with codeine.

**Local anesthetic activity:** Surface anesthesia was examined by corneal reflex method in guinea pigs (11); infiltration anesthesia was examined by intracutaneous wheal method in guinea pigs (11).

**Effect on the intestines:** Effect on the transportation of intestinal contents was examined with the charcoal meal test (12). Effect on the isolated intestines was examined with the Magnus method using a strip of the ileum of a guinea pig.

**Effects on respiration and blood pressure:** The blood pressure in 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 the dogs anesthetized with barbital.

**RESULTS AND DISCUSSION**

**I. Toxicity**

**1. Acute toxic symptoms**

Toxic symptoms appeared with doses more than 0.96 mg/10 g. A dose of 0.96 mg/10 g of 264CE caused the following symptoms: An increase of spontaneous motility followed by depressed behavior, a slight respiratory stimulation, stiffness of the extremities, occasional elevation of the tail, and occasional jumping. With the doses more than 1.15 mg/10 g, the following symptoms were anew added to those described above: A sudden bending of the body with teeth chattering and vocalization, alternate appearance of clonic convulsion and a position like embracing with the anterior extremities standing on the posterior extremities, and postictal depression. With 1.39 mg/10 g, dyspnea and respiratory arrest occurred during convulsion in two out of 6 mice. With 2.41 mg/10 g, death occurred in all six mice.

As presented above, toxic signs in mice are chiefly those of central excitation and death occurred with respiratory paralysis.
2. LD\textsubscript{50}

The LD\textsubscript{50} (mg/10 g) with its fiducial limit of 264CE was 1.55 (1.20-2.01) as HCl-salt, 1.38 (1.07-1.79) calculated as base. Those of other antitussives such as codeine phosphate, dihydrocodeine phosphate, dextromethorphan hydrobromide, and noscapine hydrochloride were 1.91 (1.78-2.05), 2.14 (1.56-2.94), 1.53 (1.34-1.74), and 19.8 (17.0-23.1), respectively. Therefore, the toxicity of 264CE is 0.98 times that of codeine, 1.17 times that of dihydrocodeine, 0.81 times that of dextromethorphan, and 13.1 times that of noscapine, when compared in terms of those of each base.

3. Toxicity in dogs

A dose of 30 mg/kg of 264CE was administered intravenously to 2 dogs, while 50 mg/kg to other 2 dogs. In all cases, severe clonic convulsions began to appear during the injection and repeated. In one of the dogs receiving 50 mg/kg, respiratory arrest occurred during tonic convulsion after 5 minutes, but another case showed an almost complete recovery after 4 hours. In both the dogs receiving 30 mg/kg, clonic convulsion repeated, but a recovery was seen after 150 and 190 minutes, respectively. Although only 4 cases were studied, the intravenous lethal dose of 264CE seemed to be around 50 mg/kg (45 mg/kg calculated as its base). Since LD\textsubscript{50} on intravenous injection of codeine phosphate is 97.8 mg/kg (69.0 mg/kg as its base), the toxicity of 264CE (base) is about 1.5 times that of codeine (base).

II. Antitussive Activity

1. Determination of potency

1) Dogs: The intravenous administration of 6.2 mg/kg of 264CE decreased the amplitude of tracing of cough in some of the cases, but the effect did not last for more than 10 minutes. Therefore, it was not considered to be effective in all 5 cases. With 7.5 mg/kg, it was effective in one out of 5 cases. One of the other cases showed 10% decrease of the amplitude but it lasted only for 10 minutes, so it was concluded to be ineffective. In 3 cases including one effective case, excitation and clonic convulsion appeared for several minutes. With 9.0 mg/kg it was effective in 2 cases (30% decrease of the amplitude for 30 minutes). With 10.7 mg/kg, it was effective in 3 cases (about 50% decrease of the amplitude and about 10% decrease of the frequency for 20-45 minutes) (Fig. 1). At the same time, excitation and clonic convulsion were seen in all cases.

With 12.8 mg/kg, it was effective in all cases (60% decrease of the amplitude and 30% decrease of the frequency for 30-50 minutes), but clonic convulsion increased and lasted for 15-35 minutes, though it did not develop into tonic one.

2) Cats: In 2 out of 5 cases receiving 7.5 mg/kg of 264CE, 15% decrease was seen only in amplitude for about 10 minutes. In 3 other cases, no change occurred. With 9.0 mg/kg it was effective in 2 cases (35% decrease of the amplitude for about 20 minutes). In 3 other cases, a slight inhibition was seen, but it was not considered to be effective. With 10.7 mg/kg it was effective in 3 cases (50% decrease of the amplitude, 20% decrease
of the frequency for 30–40 minutes). With 12.8 mg/kg it was effective in all cases (60% decrease of the amplitude, 30% decrease of the frequency for 25–60 minutes). In cats, since anesthetized lightly, convulsion and other excitation symptoms were not seen at all. Note the inhibition of the cough response for 25 minutes.

Table 1. Comparison of antitussive effect of 264CE with other control drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dog (Unanesthetized)</th>
<th>Cat (Pentobarbitalized)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AtD_{50} mg/kg i.v.</td>
<td>No. of animals used</td>
</tr>
<tr>
<td>264CE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCl-salt</td>
<td>10.8* (8.5–13.8)</td>
<td>25</td>
</tr>
<tr>
<td>Base</td>
<td>9.6 (7.6–12.3)</td>
<td></td>
</tr>
<tr>
<td>H_{2}PO_{4}-salt</td>
<td>3.1 (2.5–3.8)</td>
<td>25</td>
</tr>
<tr>
<td>Base</td>
<td>1.8–2.7</td>
<td></td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>15.3* (12.9–18.2)</td>
<td>20</td>
</tr>
<tr>
<td>HBr-salt</td>
<td>11.2 (9.4–13.3)</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>17.0* (13.8–21.0)</td>
<td></td>
</tr>
<tr>
<td>Noscapine</td>
<td>HCl-salt</td>
<td>25</td>
</tr>
<tr>
<td>Base</td>
<td>15.5 (12.6–19.1)</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parenthesis show the fiducial limit (p=0.05).
* Excitation and clonic convulsion occurred.

From the AtD_{50} of 264CE, as shown in Table 1, its effect in dogs is 1/3–1/4 that of
codeine, and much the same in cats. In dogs, convulsion was seen with the effective doses, though it was weaker than that caused by dextromethorphan or noscapine. In the case of intravenous administration, the duration of effect is not exceedingly prolonged.

2. Safety margin

On intravenous injection to dogs, safety margin (LD/AtD_{50}) was calculated to be about 5 and it is much smaller than that of codeine being 26.0.

3. Site of action

1) Comparison of equi-active doses among various routes of administration: In lightly pentobarbitalized dogs, 264CE was given into the common carotid artery, the vertebral artery and the cerebello-medullar cistern, and the doses necessary to obtain the same degree of antitussive effects as that by intravenous injection were studied.

In 5 cases, the ratio of the doses (mg/kg) necessary to obtain antitussive effect to the same extent is as follows: [Routes] vein : common carotid artery : vertebral artery : cistern = [Doses] 15.0 : 3-7.5 : 3-5 : 0.2-0.5. When the dose of intravenous injection is taken as 1, the ratio above is 1.0 : 1/2-1/5 : 1/3-1/5 : 1/30-1/75.

Thus, when the routes directing the drug to the brain stem were used, much smaller dose was enough to obtain the same effect as that by intravenous injection. Therefore, the site of action is suggested to be central.

2) Effect on the stretch receptor impulses: The impulses from the alveolar stretch receptors act on the respiratory center, not only regulating the depth of normal respiration (Hering-Breuer reflex), but also relating to an intensity of cough. The intensity of expiration on cough is influenced by the depth of the preceding inspiration. The deeper and faster the inspiration may be, the stronger the following expiration, that is, resulting in violent attack of cough. The antitussive action of benzonatate* is said to be due to selective local anesthesia of the receptors, resulting in a decrease of impulses (13). The drug has a local anesthetic action a little less potent than procaine, so this action was studied if there was any relation to its antitussive activity.

In the control group, 2-3 mg/kg of benzonatate decreased the number of the impulses, and 4 mg/kg resulted in a complete disappearance for about 25 minutes. In contrast, 264CE showed no effect in a dose range from 1 to 5 mg/kg. With an increased dose of 10 mg/kg, 3 out of 4 cases died and the rest showed a slight decrease in the number of the impulses.

3) Influence of decerebration: In order to study if the higher centers than the brain stem contribute to the antitussive activity of the drug, a mid-collicular transection at the corpora quadrigemina was done in cats, and the brain tissue rostral to the upper corpora was all scraped out. The experiments were done after the influence of operation went completely.

In 3 cats, 10 mg/kg of 264CE was injected intravenously, and its antitussive effects

* Tessalon®
before and after the decerebration were compared, but no discernible change in antitussive effect and duration of the effect was observed. This result suggests that the site of action of 264CE is at the brain stem below the transected area.

4) Effects on the centrally evoked cough response and on the sustained inspiratory response: With an intravenous dose of 8.2 mg/kg of the drug (the lower limit of ED₉₀), the frequency of tracing of cough induced by mechanical stimulation was not affected but the amplitude was reduced to about a half at 5 minutes and returned to normal at 60 minutes after administration. No change occurred both in the threshold of stimulus for cough induced by central stimulation and in the amplitude. On the other hand, threshold of sustained inspiration slightly increased without accompanying any change in amplitude.

As shown in Fig. 2, 9.8 mg/kg (ED₉₀) of the drug slightly decreased the frequency of the tracing of mechanical cough and it also showed 80% decrease of the amplitude at 5 minutes and 50% decrease at 15 minutes, and returned to normal at 60 minutes after the administration. On the other hand, the threshold for centrally evoked cough increased from 2.5 to 3.0V and the amplitude of each cough response decreased to some extent, and these changes returned to normal at 40 minutes. No change was observed in the amplitude of sustained inspiration but the threshold elevated from 6.0 to 7.0V.

In one out of 2 cases receiving 11.7 mg/kg (the upper limit of ED₉₀), 50–90% decrease of both the amplitude and frequency of mechanical cough were noted; the threshold for central cough elevated from 0.5 to 1.5V accompanying with a decrease in amplitude to
some extent; the threshold of sustained inspiration elevated from 1.0 to 1.5V accompanying with the reduction of amplitude to greater extent.

From the results presented above, 264CE decreases the sensitivity of the cough center with the AtD50, but it seems not to have a remarkable effect on the excitability of the respiratory center with the AtD50. However, it depresses the respiratory center when dose was increased more than the AtD50.

5) Effect on the centrifugal respiratory pathway: In order to confirm that a drug acts directly on the respiratory center and the cough center, it is necessary to exclude the actions on the centrifugal respiratory pathway which extends from the spinal cord to respiratory muscles.

In 3 cases receiving 12.8 mg/kg of 264CE intravenously, coughs evoked centrally and peripherally were completely inhibited. Although the intraspinal pathway was stimulated during the time when the amplitude of sustained inspiration was slightly inhibited, no change in the magnitude of muscle contraction was seen. Thus, with the AtD100 of the drug, the centrifugal pathway of respiration seemed not to be affected.

6) Effect 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 relaxation of tonus of the bronchial muscles results in a decrease in the intensity of cough. Therefore, the effect of the drug on the bronchial muscles was studied in vivo and in vitro.

With intravenous injection of 1.0-5.0 mg/kg of the drug alone, no effect was seen on the muscular tone recorded with the method of Jackson (8). The similar effect was seen with an increased dose of 10 mg/kg, but some cases died of hypotension.

With 10 μg/kg of histamine given intravenously, the amplitude of the tracing of respiration decreased markedly, becoming 10-20% of that seen before the administration. With the pretreatment of 1.0-5.0 mg/kg of 264CE, no effect was seen, but the contraction was rather strengthened in some cases given 5.0 mg/kg.

In vitro experiment, a weak relaxation was observed with concentrations more than 2 x 10^-4 g/ml of 264CE alone. Antiacetylcholine, anti-BaCl2 and antihistamine actions were 1/100,000 of atropine, 1/150 of papaverine, and 1/800 of diphenhydramine, respectively.

From the data above, it is concluded that 264CE has neither a direct action on the bronchial muscles nor the action preventing the contraction induced by histamine in rabbits, and that it does not have any significant action on the isolated bronchial muscles either.

Therefore, the site of antitussive action of the drug is concluded to be in the cough center.

7) Differences from narcotic antitussives: Differences in mechanism of action of the drug from narcotic antitussives were investigated on the following three points: Antagonism of N-allylnormorphine to the drug in antitussive activity, development of tolerance by repeated administrations of the drug, and formation of crossed tolerance between the
drug and morphine.

However, the results definitely showed that the mechanism of antitussive action of 264CE is quite different from those of narcotics.

III. Actions on the CNS

1. Potentiating and prolonging effect on anesthesia

In mice, a significant prolongation of the duration of anesthesia was observed with the doses more than 0.1 mg/10 g of 264CE and with 1.0 mg/10 g of codeine. Comparing the results obtained with such the same doses of both 264CE and codeine as 0.5 mg/10 g or 1.0 mg/10 g, a significant difference was seen at the levels of \( p = 0.01 \) or 0.001, respectively. Thus, the action of 264CE on the prolongation of anesthesia is much stronger than that of codeine.

2. Analgesic action

Analgesic activity was examined with the method of d’Amour-Smith (14) in mice. Doses up to 1/2 LD50 were administered, but no significant effect was seen.

IV. Local Anesthetic Action

The drug was 4/5 as potent as procaine in superficial anesthesia and 7/10 as potent as procaine in infiltration anesthesia both in guinea pigs.

V. Actions on the Intestines

1. Inhibitory effect on transportation of intestinal contents

On clinical application of narcotic antitussives such as codeine, constipation is one of the important side-effects. So, an inhibitory effect of the drug on the transportation of intestinal contents in mice was studied.

Using 6 groups for 264CE and 5 groups for codeine, the ED50 with fiducial limit were 0.33 (0.20-0.54) mg/10 g and 0.11 (0.08-0.14) mg/10 g, respectively. There was a significant difference between the two at a level of \( p = 0.05 \). Thus, the inhibitory effect of 264CE is only 1/3 that of codeine.

2. Actions on the isolated intestines

Anti-ACh, anti-BaCl2, and antihistamine effects were studied using the ileum of guinea pigs. On comparing with effects of atropine, papaverine and diphenhydramine, its anti-BaCl2 action was 1/4-1/5 that of papaverine, while the anti-ACh and antihistamine effects were 1/5,000 that of atropine and 1/50-1/60 that of diphenhydramine, respectively.

VI. Actions on the Respiration and Blood Pressure

Intravenous doses more than 0.5 mg/kg of 264CE caused a transitory blood pressure fall in the dogs anesthetized with barbital and the degree of fall depended upon the doses given. Respiration showed an increase concomitantly with the fall of blood pressure. With 10 mg/kg (equals to the AtD50 in unanesthetized dogs), the blood pressure fell by
50 mmHg during the injection. Increase in amplitude and frequency of respiration were associated with the decrease in blood pressure and it persisted for 3 to 4 minutes, and then respiratory depression occurred. Respiratory depression, especially decrease in frequency was most remarkable at 30 minutes after administration. Blood pressure began to rise at 30 seconds and returned to the pretreatment level within 30 minutes, though fluctuation of pressure associated with respiratory depression was observed. The respiration returned to the pretreatment level within 90 minutes, as shown in Fig. 3.

With 20 mg/kg, the blood pressure precipitously fell already during the injection and it was followed by apnea for several seconds. Then, respiratory stimulation developed. The blood pressure, however, continued to fall, and the respiration was also inhibited gradually, followed by respiratory arrest in 2 minutes. All the dogs used died with this dose.

On the other hand, intravenous doses less than 2.0 mg/kg of codeine showed no change in respiration and blood pressure. With the doses more than 5.0 mg/kg, the blood pressure fell temporarily, and the respiration increased concomitantly with the fall of blood pressure, followed by long-lasting depression. With 20 mg/kg, however, no death occurred, though the changes in respiration and blood pressure were remarkable.

Thus, 264CE seems to have more remarkable influences on respiration and blood pressure, especially on the latter, than codeine.

**SUMMARY**

The antitussive action and its site of action of 2-allyloxy-4-chloro-N-(2-diethylaminoethyl) benzamide HCl (264CE) were studied. The antitussive effect was 1/3-1/4 as potent as codeine in dogs and cats. The site of antitussive action was confirmed to be the cough center itself, but its mechanism of antitussive effect is different from those of narcotic
ones. The drug is as toxic as codeine in mice, but it is more toxic than codeine in dogs. Other pharmacological properties related to its main effect such as actions on the CNS, intestines, respiration and blood pressure, and local anesthetic action were also described.

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