A METHOD OF INDUCING AND RECORDING COUGH AND EXAMINATION OF THE ACTION OF SOME DRUGS WITH THIS METHOD

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INTRODUCTION

During the past 10 years various methods for measuring antitussive effect have been devised, and it is through these methods that various pharmacological basic observations have been added one by one to the numerous antitussives. An outline of the studies on the pathological physiology and pharmacology of cough can be obtained by reading Bucher's article (1). The methods hitherto used for inducing cough are chemical stimulation (2-5), electrical stimulation (6-8) or mechanical stimulation (9, 10).

The present authors have devised a new method for recording artificial cough induced by electrical stimulation of a dog's tracheal mucosa, and the effects of several drugs have been examined with this method. Through this study a new substance has been discovered from sympathomimetic amine.

METHOD OF EXPERIMENT AND RESULTS

1. The Selection of Experimental Animals

The animals used were monkeys, cats, dogs, rabbits, guinea pigs and rats, and artificial cough could be induced in all animals excepting rabbits and rats. Since monkeys are expensive and dogs are convenient for continued experimental study, dogs weighing between 7-10 kg were chiefly used in the present study. The dogs were bred for a certain period before they were used for the experiment so that they could become tamed to the person handling the experiment.

II. Careful Selection of Experimental Method

The authors have reviewed several of hitherto reported methods of inducing cough and have studied the following items which are the basic problems in examining antitussive agents.

1) The sensitivity of tracheal mucosa

Cough is said to be readily induced in the interarytenoid, posterior wall of the larynx, bifurcation tracheae and paries membranaceus. The above two portions in the trachea,
being a sensitive area, are richly distributed with multipolar nerve cells. In order to find out which portion of the trachea is most adequate in stimulating and inducing cough, a tracheal fistula was made about 3 cm below the cricoid cartilage in a dog, and the tracheal mucosa was directly stimulated from this portion. When the mucosa was stimulated gradually towards the bifurcatio tracheae from the opening of the fistula, a mind cleaning the throat was induced at a certain area (Fig. 1, stimulation point A) and coughing became remarkable as the stimulation moved towards the deeper part of the trachea (stimulation point B) and reached its maximum as it reached the bifurcatio tracheae (stimulation point C).

The relation between the stimulation points and the degree of cough is indicated in Fig. 2.

2) The induction of cough by electric stimulation of tracheal mucosa

It goes without saying that the selection of an adequate stimulation for inducing cough is of special importance. The following method which can give a constant stimulation and can express it quantitatively and cause only a minimal organic change at the point of stimulation was device for inducing cough.
A spinal wave stimulation apparatus and stimulation electrode such as shown in Fig. 3 were used for electric stimulation. The rubber tube was bent so that it would adhere closely to the tracheal mucosa by its own elasticity. The voltage and frequency were combined in many ways, and point A, B and C (Fig. 1) were stimulated to reveal three reaction curves. Each stimulation was given 5 times—for 2 sec and at intervals of 1 min. The anode was closely adhered to the shaved gluteal region with a binding agent.

3) Examination of the degree of adequate excitation
Since the determination of the effect of the drug is greatly influenced by the degree of cough induced artificially, the degree of adequate excitation at points A, B and C were examined, and it was found that the adequate excitation for inducing cough could be obtained with 3 V 20 c/sec (Fig. 4).

4) The sensitivity of tracheal mucosa at a horizontal plane
The afore-mentioned electrodes were closely adhered to the anterior, posterior and lateral walls of each stimulation point of trachea, and a spinal wave of 3 V 20 c/sec was given and observed. Although the posterior wall seemed to be slightly more sensitive than the other walls, no specific difference could be confirmed with the apparatus.

5) Method of determining antitussive effect
Before administration of drug, stimulation was given and three reaction curves were obtained. The drug under examination was then administered, and they were comparatively studied periodically 5, 10, 15 minutes and thereafter every 15 minutes for 60–90 minutes. When A and B reactions were remarkably inhibited, the drug was
considered to have an antitussive effect, and C reaction was referred to as an aid for the determination (Fig. 5). In the present method when the drug possesses an antitussive action, an effective curve of three types (three forms of inverse L, V and H) is obtained by the recovery of inhibition of the reaction curves.

III. Antitussive Action of Various Agents in the Present Experiment

1) Codeine phosphate (central depressant)

Thirty to fifty percent inhibition was observed in all 3 cases after administration of 1 mg/kg. In 3 out of 4 cases the three stimulations were remarkably inhibited 5 minutes after injection of 2-2.5 mg/kg, and they return to the standard cough curves after 60 minutes. A marked inhibition was found in all 3 cases given 3.5 mg/kg.

2) 2-Methoxy-5-methyl-β-phenyl-isopropyl-dimethyllamine hydrochloride (M-6) (sympathomimetic agent)

A remarkable inhibitory action was confirmed 5 minutes after injection of 10 mg/kg in 4 out of 6 cases, and continued to remain effective for about 60 minutes. The inhibitory effect was about 20-50% in the remaining 2 cases.

3) β-Dimethylaminoethyl benzyl amide hydrochloride (DBH) (parasympatholytic agent)

Although the inhibitory action was weak when given 12 mg/kg, a conspicuous inhibition was confirmed 5 minutes after injection of 15 mg/kg in all 4 cases, and continued to remain effective for 60 minutes. A nearly complete recovery was found 75-90 minutes later.

4) 3-(2'-Methoxyphenoxy) propane-1,2-diol (MPD) (tracheal smooth muscle relaxant)

In 3 out of 6 cases the stimulations were remarkably inhibited from 30 minutes to about 120 minutes after injection of 15 mg/kg. A 30-50% inhibition was observed in the other 2 cases.
5) Polyethylenglycol-p-n-butyaminobenzoate methyl ether (PBM) (anesthetics of stretch receptor)

Although its action was mild when given less than 2 mg/kg, the three stimulation points were remarkably inhibited in all three cases when administered 3 mg/kg, and the reactions all recovered after 30 minutes. However, with this dose, respiration of the dogs was disturbed and agony continued for about 10 to 15 minutes.

6) N-(2-dimethylamino-9-propyl) phenothiazine hydrochloride (NPH) (tranquilizer)

Stimulation was remarkably inhibited about 15 minutes after injection of 2 mg/kg in all 5 cases, and returned to the standard curve in about 60-75 minutes.

The experimental results of other central depressants and sympathomimetic agents are shown in Table 1.

7) When 0.4 mg/kg of morphine hydrochloride and 5 mg/kg of codeine phosphate are
TABLE 1. A comparison of cough inhibiting effect of various drugs.

<table>
<thead>
<tr>
<th>Name of agent</th>
<th>Chemical structure</th>
<th>$ED_{50}$ (mg/kg)</th>
<th>$LD_{50}$ (mg/kg)</th>
<th>$LD_{50}/ED_{50}$</th>
<th>M/C</th>
<th>Effective dose (mg/kg)</th>
<th>Degree of inhibition</th>
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<tr>
<td>Morphine hydrochloride</td>
<td></td>
<td>0.1</td>
<td>360 (s.c.)</td>
<td>3600</td>
<td>18.0</td>
<td>0.1—0.2</td>
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<td>Hydrocodeine phosphate</td>
<td></td>
<td>1.5</td>
<td>150 (i.v.)</td>
<td>100</td>
<td>1.3</td>
<td>2.5—3.0</td>
<td>++</td>
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<tr>
<td>Codeine phosphate</td>
<td></td>
<td>1.5</td>
<td>120 (i.v.)</td>
<td>80</td>
<td>1.0</td>
<td>2.5—3.5</td>
<td>++</td>
</tr>
<tr>
<td>Hydrocodeine N-oxide</td>
<td></td>
<td>15.0</td>
<td>1050 (i.v.)</td>
<td>70</td>
<td>0.8</td>
<td>15.0—20.0</td>
<td>+</td>
</tr>
<tr>
<td>Codeine N-oxide</td>
<td></td>
<td>12.0</td>
<td>*767 (i.v.)</td>
<td>64</td>
<td>0.8</td>
<td>15.0—20.0</td>
<td>+</td>
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<tr>
<td>Dextromethorphan hydrobromide</td>
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<td>2.0</td>
<td>*157 (s.c.)</td>
<td>78</td>
<td>0.4</td>
<td>2.0—3.0</td>
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<td>75 (i.v.)</td>
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<td>0.1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>15.0—25.0</td>
<td>±</td>
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<tr>
<td>dl-N-methyl ephedrine hydrochloride</td>
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<td></td>
<td></td>
<td></td>
<td>10.0—20.0</td>
<td>+</td>
</tr>
<tr>
<td>o-Methoxy-p-phenyl-isopropylmethyl-</td>
<td></td>
<td>15.0</td>
<td>*241.4 (s.c.)</td>
<td>20</td>
<td>0.08</td>
<td>20.0—25.0</td>
<td>++</td>
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<tr>
<td>amine hydrochloride</td>
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</table>

* Cited from other studies.
injected intravenously once a day at the same time, a tolerance is acquired in about 7-10
days. If a repeated application of the same dosis of codeine is given at 10 day intervals
from right after complete disappearance of its antitussive effect, its efficiency rate after the
second administration drops further as compared to the initial administration (Fig, 12).

8) Evaluation of antitussive effect

In evaluating antitussive effect the LD₅₀/ED₅₀ of the antitussive agent is first calculated
and applied as the active coefficient of the agent, and then compared with that of codeine.
ED₅₀ was determined by referring to the continuity of the antitussive effect, frequency of
cough and amplitude of the curves. LD₅₀ was calculated after subcutaneous injection when
the figures were below a definite value after intravenous injection.

Since the ED₅₀ of codeine phosphate is 1.5-1.6 mg/kg, the following coefficients are
obtained:

subcutaneous injection \[ \frac{L_{D_{50}}}{E_{D_{50}}} = \frac{325 \text{ mg}}{1.5 \text{ mg}} = 200 \]
intravenous injection \[ \frac{L_{D_{50}}}{E_{D_{50}}} = \frac{120 \text{ mg}}{1.5 \text{ mg}} = 80 \]

The antitussive effect of various agents can therefore be indicated as \( P = \frac{kM}{C} \). This
has been applied in the present experiment (\( M \) stands for coefficient of the agent under
study; \( C \) stands for the coefficient of codeine phosphate; \( k \) stands for the specificity of the
agent and is usually excluded, since it changes relatively according to the symptoms.)

DISCUSSION

1. Rat, guinea pigs, rabbits, dogs, cats and monkeys were used in the present study,
and it was found that cough could readily induced artificially in the animals excepting
rats and rabbits. Dogs were most convenient for a long term observation.

2. The mucous membrane of the bifurcatio tracheae is the most sensitive to stimula-
tion in dogs, and this sensitivity becomes gradually weaker towards the upper portion of
the trachea. This portion was named stimulation point C and two other points, each located
1-2 cm towards the upper portion of the trachea, were called B and A. The three reactions
obtained by stimulating the above three points were indicated as the curves of the cough.

3. The most adequate stimulation for inducing cough artificially is 3 V 20 c/sec.

4. No significant difference could be confirmed between the sensitivity of the anterior,
posterior and lateral walls of trachea in each of the three stimulating points at a horizontal
plane.

5. Since it is desirable for the stimulation inducing cough to be similar to that of
a physiologic one, the selection of the sort of stimulation, portion of stimulation, degree
of stimulation and other adequate conditions is most important in determining the effect
of antitussive agents.

6. The antitussive effect of several drugs has been studied by the author's method.
It is said that 2-3.5 mg/kg of codeine remarkably inhibit artificial cough and that hydro-
7. An inhibitory action can be confirmed by administration of 20-25 mg/kg of \textit{a}-methoxyphenamine (sympathomimetic drug), while a conspicuous action can be found after administration of 10 mg/kg of M-6, a derivative of the former. Reports (11) have already been made on 27 of these derivatives, and those having remarkable antitussive effects are shown in Table 1. A mild antitussive action can be confirmed with administration of 10-20 mg/kg of N-methylephedrine.

8. Both DBH and MPD demonstrate conspicuous antitussive action with a dose of 15 mg/kg. Although 3 mg/kg of PMD remarkably inhibits coughing for about 15 min, the dogs fall into an agonizing condition. An inhibitory action can be confirmed with administration of 2 mg/kg of NPH.

9. Repeated administration of morphine and codeine leads to nearly complete disappearance of inhibitory action in 7-10 days, and when repeated injections of codeine are given at intervals of 10 days, it becomes less effective after the second injection as compared to the initial one. In other words, no marked difference in tolerance can be confirmed between morphine and codeine.

**SUMMARY**

1. Among the various animals used the dogs seem to be most fit in inducing cough artificially. The experiment can be carried out without anesthetics.

2. The sensitivity of the tracheal mucosa in dogs to stimulation differs according to the site of stimulation and the bifurcatio tracheae is most sensible. There is no marked difference in sensibility of the anterior, posterior and lateral walls of trachea at a horizontal plane.

3. The authors have devised a new method in inducing cough by electric stimulation of the tracheal mucosa in dogs. A 3 V 20 c/sec stimulation is most adequate. With this method the stimulation can be defined quantitatively and the reaction of the three portions having a different sensitivity to the stimulation may be recorded and observed, and effect of the antitussive agents can thus be examined.

4. Among the derivatives of morphine, morphine hydrochloride possesses the highest antitussive action, and the antitussive effect of codeine and hydrocodeine is below 1/10 of morphine. The N-oxide of codeine and hydrocodeine has a weaker toxicity and antitussive effect. The antitussive effect of dextromethorphan is weaker than that of codeine, and although an inhibitory action can be confirmed with intravenous injection of narcotine,
a conspicuous antitussive effect cannot be expected with a safe dose. Papaverine demonstrates a definite antitussive action.

5. 0-Methoxyphenamine, its four derivatives and dl-N-methylephedrine indicate a definite antitussive action.

6. A definite antitussive effect can be confirmed with DBH, MPD and NPH. Although the action of PBM appears rapidly, its duration is short.

7. The tolerance of codeine phosphate is about the same degree as that of morphine hydrochloride.

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