A NEW METHOD FOR INDUCTION OF THE COUGH REFLEX

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Accepted December 3, 1973

Abstract—A new method for artificially inducing the cough reflex in the unrestrained and unanesthetized dog has been devised. The present method can be utilized for testing the relative effectiveness of antitussive drugs. A monopolar electrode is inserted into the dog tracheal mucosa in such a way as to minimize surgical stress. The most successful electrical stimulation has been obtained from square-wave pulses which were produced by a 1-3 volt, 20 Hz. The duration of the square-wave pulse was 1.0 msec and the stimulation was applied for 5 sec. This stimulation usually produced 3-5 cough reflexes. It has been found that electrical stimulation of the tracheal mucosa induces the cough reflex and this phenomenon can be successfully reproduced at intervals of at least 5 minutes duration. Our method allows for a lower threshold voltage for cough induction than those previously published, a greater utilization of each dog and a reduction of time required to standardize the cough reflex and the drug dosage for each animal. As the dogs are caged, there is more freedom of movement, thus the drug effect on posture and general behavior can be better observed. The method described may be applicable to induction of the cough reflex in a variety of experimental animals.

Following Craigie’s first experiments (1) on the artificially induced cough reflex in the dog, Larsell & Burget (2) and Ernst (3) further investigated mechanical and chemical stimulation of the tracheal mucosa to induce coughing in experimental animals. The known methods for cough induction were developed only after extensive research. These can be classified as due to electrically (4-14), mechanically (2, 3, 9, 15-21), and chemically (2, 16-18, 20, 22-25) induced stimulation. The receptors for cough induction can be divided into: a) the external mechanical stimulation receptor, which is situated in the upper tracheal mucosa; b) the chemical stimulation receptor, located in the trachea and throughout the bronchial trees (26). Since coughing is also induced by inhalation of hot or cold air, the cough reflex cannot be attributed solely to mechanical or chemical stimulation of the receptors. It is thus feasible to consider coughing as originating in the trachea regardless of the stimulation. It is necessary that any artificially induced coughing closely resemble the mechanism of that which occurs naturally. Previously reported experimental methods have not proven satisfactory and to induce a reproducible cough and minimize injury to the trachea, various methods of cough induction have been investigated in our laboratory (4, 5).

In the present study, the cough reflex was induced electrically, in the unrestrained and unanesthetized dog, by means of a flexible electrode. This method induces a cough which can be easily reproduced and facilitates study of the effectiveness of antitussive drugs.
1. Experimental animals

The animals used in these experiments were healthy mongrel dogs weighing 7–10 kg and guinea-pigs weighing 500–800 g. Both males and females were used. Food and water were provided ad libitum and the animals were maintained in a constant environment. The temperature and humidity were kept at 21°C and 60%, respectively.

2. Stimulating electrode

The electrodes were inserted in the dog trachea before each experiment. They were made of stainless steel wire (0.1 mm diameter) and coated with a thin polyvinyl film (Fig. 1). This coated electrode was sheathed by a stainless steel needle (size No. 1/2, Natsume). An adhesive Alon-alpha (Toagosei Chemical) was used for fixation of the polyvinyl coated electrode and the stainless steel needle sheath and also as insulation. Before use, the resistance of each electrode was tested. Each electrode was allowed to stand in water for a few minutes, after which it was wiped dry with gauze and the resistance between the needle sheath and the central electrode was measured by a galvanometer. Electrodes with a resistance greater than 25 MΩ between the central core and stainless steel sheath were used.

Electrodes used for guinea-pigs were unsheathed since the guinea-pig trachea is too narrow to accommodate the same type of electrode used for dogs.

A stainless steel needle (size No. 1/3, Natsume) placed arbitrarily into the muscle behind the ear was used as an indifferent electrode against the electrode inserted into the trachea of animals.

3. Induction of cough reflex

Dogs: The sheathed electrode was further threaded through a guiding cannula (needle of size No. 1/1, Natsume) until the tip touched the trachea (Fig. 2). The electrode was introduced through a shaved portion of the neck 4 cm below the bottom of the thyroid cartilage. The tip of the electrode was bent at an angle of 30–60° before insertion into the guiding cannula.

FIG. 1. Electrode for stimulation of the tracheal mucosa in the dog.

FIG. 2. Electrode introduction into the dog trachea.
The central core of the electrode was made of stainless steel wire with moderate flexibility to enable the tip of the electrode to pierce the tracheal mucosa without injuring the trachea. After insertion and placement of the electrode were completed, at least 5 minutes were allowed to pass as a short period of time is required to allow for the induction of coughing due to insertion. The guiding cannula was pulled out as soon as the electrode had been inserted into the trachea.

**Guinea-pigs:** Since the guinea-pig trachea is narrower than that of a dog, the electrode used was unsheathed and was covered only with a guiding cannula (needle size No. 1/2, Natsume). The non-anesthetized animals were fixed in a supine position and the electrode was introduced into the trachea.

General operation to induce cough reflex to the tracheal mucosa: The parameters of electrical stimulation necessary to induce 3-5 coughs were a square-wave pulse with a 20 Hz frequency, the duration of pulse 1.0 msec and the duration of application 5 seconds. The specific voltage necessary for cough stimulation and the site of electrode placement were carefully determined for each individual animal. When stimulation caused excessive coughing, the site of placement of the electrode was shifted upward (toward the throat). This prevented a too rapid exhaustion of the animal and prolonged the time during which coughs could be reproduced. When the site of optimal stimulation was obtained, the electrode was fixed to the neck with vinyl tape.

Macroscopical examination of the stimulation site of the tracheal mucosa of the dogs and guinea-pigs was performed on the same day as stimulation, and three, four and seven days after stimulation.

4. **Recording of the cough reflex**

A respiratory pick up (Nihon Kohden, MCR-2TA) was applied to the thorax of the dogs or guinea-pigs, and coughing was induced utilizing electric current. The respiratory pick up was connected to a pen recorder (Nihon Kohden, RM-150) through an amplifier (Nihon Kohden, RB-2). The amplitude of the pen movement was found to be in parallel with the severity of the cough. Studies were conducted in a shielded cage (Fig. 3).

5. **Standard criteria for evaluation of the antitussive effect of the drugs**

The amplitude and frequency of coughs were recorded continuously both before and after drug administration, on a pen recorder (Nihon Kohden, RM-150). Prior to drug administration, the amplitude of each cough was measured with a cm scale, and this average was multiplied by the number of coughs. At intervals of 5, 10, 15, 30, 45, 60, 90 and 120 min after drug administration, determination of the amplitude was made and the number of coughs

![Fig. 3. Unrestrained and non-anesthetized dog in a shielded cage.](image)
counted. The efficacy of the drug was defined as the percent decrease of amplitude multiplied by the number of coughs as a function of time after drug administration when a drug was found to be effective. The degree of antitussive activity of each drug was assessed by plotting the percentage of response inhibition as a function of time for the first 30 min after drug administration and determining the area under the curve. Maximal antitussive drug activity occurred during the 30 min period after drug administration and in order to determine the effectiveness of each drug, it was compared to the cough severity before the drug had been given. ED50 was calculated by the method of Litchfield and Wilcoxon (27).

6. Drugs used

In the present experiment, the following antitussive drugs were investigated, morphine hydrochloride (Takeda), codeine phosphate (Sankyo), N-2-Pyridylmethyl-N-phenyl-N-2-piperidinoethyl amine hydrochloride (picoperidamine hydrochloride, Takeda), 1-(2-hydroxyethoxyethyl)-4-p-chlorobenzylpiperidine hydrochloride (piclobetol, Sankyo) and 14-hydroxydihydro-6β-thebainol-4-methyl ether (oxymethetanol, Sankyo).

RESULTS

As a control, physiological saline solution was administered intravenously.

Saline administration i.v. does not affect cough severity or reproducibility caused by electrical stimulation of the dog tracheal mucosa over a period of 120 min after administration, or in the guinea-pig over a 60 min period as is shown in Fig. 4.

A slight redness of the tracheal mucosa of both these animals was observed following electrical stimulation applied during normal experiments, however, this disappeared within 3 days.

The effectiveness of codeine for cough suppression as a function of time for different dosages is shown in Fig. 5. Codeine is shown to act quickly and yields a maximum cough suppression from 5 to 15 min after i.v. administration, after which it decreases markedly with time.

As is shown in Fig. 6, the remarkable antitussive effect of a 10 mg/kg dose of piclobetol

![Fig. 4. Reproducibility of the cough reflex in the dog. The cough reflex was induced by electrical stimulation (3 V, 1.0 msec, 20 Hz, for 5 sec) of the tracheal mucosa in a male dog weighing 7.8 kg. Saline was administered i.v., at the arrow.](image-url)
was observed immediately after i.v. administration and coughing remained suppressed for 15 min. Thirty min after administration, a slight reflex appeared and after 45-60 min about 50% of the cough severity could be measured. This severity returned to pre-drug administration levels after 75-90 min.

Table 1 is a summary of the results obtained on antitussive effectiveness of the 5 drugs studied in this experiment. The narcotic drugs, morphine and oxymethabanol were found to be the most effective antitussive drugs. Codeine and picoperidamine possessed moderate suppressive ability against electrically induced coughing. Piclobetol was less than half as effective as picoperidamine.

<table>
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<tr>
<th>Drugs</th>
<th>ED50 (mg/kg, i.v.)</th>
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<tr>
<td>Morphine HCl</td>
<td>0.1 (0.08-0.13)</td>
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<tr>
<td>Codeine H3PO4</td>
<td>1.0 (0.72-1.39)</td>
</tr>
<tr>
<td>Picoperidamine HCl</td>
<td>2.0 (1.54-2.60)</td>
</tr>
<tr>
<td>Piclobetol</td>
<td>7.6 (5.71-10.11)</td>
</tr>
<tr>
<td>Oxymethabanol</td>
<td>0.1 (0.07-0.14)</td>
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Numbers in parentheses show the fiducial limits (P=0.05).
The comparative antitussive activity of morphine and codeine as determined by the present method and previous methods is presented in Table 2. It can be seen that of the 5 methods presented, our method has the greatest sensitivity for determining antitussive effectiveness as defined by ED50.

**DISCUSSION**

We have successfully induced a reproducible cough in the unrestrained and non-anesthetized dog. This cough is induced by a square-wave pulse, 1–3 volt, 20 Hz current with a pulse duration of 1.0 msec. Five seconds of electrical stimulation at the tracheal mucosa were required to produce 3–5 coughs which had similar electrical parameters when measured through an amplifier and chart recorder. The main advantages of the present method are that major surgery to permanently implant an electrode in the trachea can be done away with and fistulas can be prevented. Our method also allows the point of stimulation to be more easily varied. With the other methods it is difficult to maintain a constant level of stimulation because of the difficulty in locating precisely the site for optimum stimulation. Another problem with these methods is the frequency of bacterial infection during long term experiments. When the fistula method is used, there is a tendency for tracheal constriction to develop due to granuloma formation. This spontaneously produces a cough reflex, even without stimulation. Both surgical implantation and the fistula method are difficult to adapt to small animals. In contrast, our method causes minimal injury to the trachea as surgical stress is eliminated and the opening to the trachea is small and can be easily covered. The use of a guiding cannula reduces stress to the tissue surrounding the opening.

An autopsy of the tracheal mucosa at the site of stimulation was conducted 3 days after stimulation. Pathological indication of inflammation was not observed in either the dog or the guinea-pig. Thus this new method may be applicable to experimental animals of all sizes.

The antitussive effects of 5 drugs were studied using our method of cough induction. The values of ED50 obtained for morphine and codeine were lower than those previously

<table>
<thead>
<tr>
<th>Animals</th>
<th>Present Method</th>
<th>Yanaura</th>
<th>Kase</th>
<th>Domenjoz</th>
<th>Takagi et al.</th>
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<tr>
<td></td>
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<td>guinea-pig</td>
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<tr>
<td>Methods</td>
<td>E (a)</td>
<td>E (a)</td>
<td>M (b)</td>
<td>E (c)</td>
<td>C (d)</td>
</tr>
<tr>
<td>Morphine</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>0.25 (5/5)</td>
<td>5.9 (e)</td>
</tr>
<tr>
<td>Codeine</td>
<td>1.0</td>
<td>1.5</td>
<td>2.5</td>
<td>3.0 (2/2)</td>
<td>14.4 (e)</td>
</tr>
</tbody>
</table>

Morphine and codeine were administered i.v. except at places marked (e) which indicates s.c. administration. E(a) : Electrical stimulation of the tracheal mucosa. M(b) : Mechanical stimulation of the tracheal mucosa. E(c) : Electrical stimulation of the superior laryngeal nerve. C(d) : Chemical stimulation of the tracheal mucosa by SO2.
reported in the non-anesthetized dog (Table 2). The ED50 values obtained for picoperidamine (28) and piclobetol were also found to be lower than those previously reported.

These results indicate that our method may be useful for the investigation of the comparative potency of antitussive drugs and could provide a routine screening procedure to determine antitussive effectiveness. This method also allows for observation of spontaneous activity and posture of the animals during the course of each experiment. Thus the sedative activity and the pseudo-antitussive activity of many psychosedatives can be differentiated.

The resistance between the stainless steel central core of the experimental electrode and an indifferent electrode placed behind the ear of a dog and the resistance measured in the control experiment were compared. Measurement of each was done at 5 min intervals during a 150 min period and was arranged in such a way that the resistance was measured at the time of electrical stimulation. A marked reduction of resistance occurred approx. 90 min after insertion and stimulation of the dog trachea had begun. A cough of constant severity was observed during the first 120 min of observation. Further examination revealed that a reduction of the resistance between the experimental electrode and the indifferent electrode up to 20–50 kΩ (from the starting 1000 kΩ) did not affect the measurement of cough severity. However, pilocarpine (0.03 mg/kg, i.v.), which increased the tracheal secretion, greatly decreased the resistance of the electrode. These results indicate a significant relationship between the tracheal secretion and the reduction of effective voltage at the site of stimulation.

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