A NEW SCREENING METHOD FOR EVALUATING ANTITUSSIVES IN CONSCIOUS GUINEA PIGS

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In order to evaluate the effects of antitussive agents, a new method for inducing the cough reflex in guinea pigs was devised. Male guinea pigs weighing 300—450 g were fixed in a supine position under non-anesthesia. A puncture electrode made by a stainless steel wire (0.2 mm diameter, 10 cm length) was inserted into the trachea through a guiding cannula (needle size 23G, Terumo). The optimal parameters of electrical stimulation given on the tracheal mucous membrane were a square-wave pulse with a 40 Hz frequency, duration of application 5 s. Fifty percent of antitussive doses (AtD50) of the drugs used in this study were as follows: codeine, 3.35 mg/kg (i.p.), 7.5 mg/kg (p.o.); morphine, 1.2 mg/kg (i.p.), 2.5 mg/kg (p.o.); picoperidamine, 10.9 mg/kg (i.p.), 22.6 mg/kg (p.o.); benproperine, 14.5 mg/kg (i.p.), 22.6 mg/kg (p.o.); fominoben, 13.9 mg/kg (i.p.), 477 mg/kg (p.o.). These AtD50s are much less as compared with those obtained using other methods.

These results suggest that the present method for inducing the cough reflex in guinea pigs is useful for the first screening of antitussives, especially when only a small quantity of the materials is available.

Keywords — antitussive; cough reflex; codeine; guinea pig; electrical stimulation

INTRODUCTION

The evaluation of antitussive agents in experimental animals has been carried out in a variety of methods. Experimental methods of inducing cough reflex can be classified as due to mechanically1—10 chemically1—8,10—15 and electri-
cally16—22 induced stimulation.

The animal species for evaluating antitussive agents are also variable. Rodents are used more for pharmacological purpose, as they are more readily available in large numbers. Particularly, a small animal is suitable for screening test of a small sample. The guinea pig is described in the literature as a readily coughing animal23 and does not have many disadvantages compared to rat and mouse.24

The electrical stimuli in experimental methods of inducing cough reflex do the least damage to the tissue. We reported previously the method for inducing cough reflex by electrical stimulation in the unrestrained and un-
anesthetized dog.25 There are, however, no detailed reports in the guinea pig.

In the present study, we devised a simple and reliable method as the first screening to evaluate the antitussive effectiveness.

MATERIALS AND METHODS

Male guinea pigs weighing 300—450 g were used.

A puncture electrode was made of stainless steel wire (0.2 mm diameter, 10 cm length) and coated with cashew for insulation. Before use, the tip of puncture electrode was exposed (3 mm length).

Animals were fixed in a dorsal position under nonanesthesia. The electrode was inserted into the trachea through a guiding cannula (needle size 23G, Terumo). The guiding cannula was pulled out as soon as the electrode had been inserted into the trachea. The stainless steel needle placed arbitrarily into the muscle behind the ear was
used as an indifferent electrode against the electrode inserted into the trachea of animals.

The parameters of electrical stimulation used in inducing the cough reflex were a square-wave pulse with a 40 Hz frequency, with the duration of pulse of 1 ms, the voltage of 2–4 V and the duration of application of 5 s. The specific voltage necessary for cough stimulation and the site of electrode placement were carefully determined for each individual animal. Setting of stimulus intensity in each animal was made by increasing voltage strength from 2 V until the voltage produced 1–2 coughs/stimulation reproducibly and stably. The tip of electrode was placed near the bifurcation tracheae, because the bifurcation tracheae is the most sensitive site for coughing.23) 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 a vinyl tape.

Cough responses were measured using a respiratory pick up applied to the thorax of the guinea pigs. Recordings were made on a polygraph (Nihon Kohden, RM-150). The stimuli were given at intervals of 5, 10, 15, 30, 45, and 60 min after drug administration. When no cough response occurred even to one stimulus, the drug was regarded as effective. ED50 was calculated by the up and down method.24)

One animal was, in a part of experiments, used twice repeatedly with an interval of 10 or more days.

Drugs tested for antitussive activity were administered intraperitoneally or orally. When drugs were given orally, animals were fasted for 24 h before experiments.

Drugs used in this study were morphine hydrochloride (Takeda), codeine phosphate (Sankyo), picoperidamine hydrochloride (Takeda), benperone phosphate (Pfizer Taito), and fominoben hydrochloride (Boehringer). Doses of all drugs used refer to their bases. Solutions of fominoben were prepared by dissolving the base in a small volume of 0.1 N HCl.

**FIG. 1.** Relation between the Frequency and Voltage of Electrical Stimulation on the Number of Coughs Coughs were induced by electrical stimulation of the tracheal mucosa. Each column is the mean value with S.E. for five experiments.

- ■ 2.0 V
- □ 3.0 V
- □ 4.0 V
making up to required volume (about pH 4) with saline solution and distilled water. Mor- phine, codeine, picoperidamine and benproperine were dissolved in saline solution and dis- tilled water.

RESULTS
The most suitable parameters of electrical stimulation necessary to induce the cough reflex were examined. As the voltage was increased beyond 5 V, a diminished cough response and tremor of cervical muscle were noted. Figure 1 shows the effects of altering stimulus frequency on evoked cough reflex for a given number of pulses at 2, 3, and 4 V. In the range of 10 Hz, no cough reflex was elicited. On the other hand, in the range of higher frequencies (50, 60, 70, and 80 Hz), the number of cough reflex was decreased. Therefore, there was evidence for a high level of cough reflex occurring at 40 Hz for each stimulating voltage.

An intraperitoneal administration of saline used as a control had no effect on the cough reflex caused by electrical stimulation of the tracheal mucosa over a period of 60 min after administration, as shown in Fig. 2. An oral administration of saline also had no effect on the cough reflex over a 60 min period.

The dose ratios of drugs of intraperitoneal and oral administration were 1.5 and 2.0, respectively. All five drugs were administered either intraperitoneally or orally. The range of doses of codeine selected for intraperitoneal administration was 2.25 to 7.59 mg/kg. Figure 3 shows the remarkable antitussive effect of 3.38 mg/kg (i.p.) of codeine. In an oral administration of codeine, the range of doses was 4 to 16 mg/kg. Antitussive effects of oral administration of 8

![Graph showing the effects of different stimuli on cough reflex over time.](image-url)

**FIG. 2. Reproducibility of the Cough Reflex in the Guinea Pig**
The cough reflex was induced by electrical stimulation (ES) of the tracheal mucosa.
mg/kg of codeine are shown in Fig. 4. The 50% antitussive doses (AtD50) of codeine were 3.5 mg/kg and 7.5 mg/kg, respectively when the drug was administered intraperitoneally and orally.

In the other drugs used, the following ranges of doses were tested: morphine, 0.67–2.25 mg/kg (i.p.), 1–8 mg/kg (p.o.); picoperidamine, 7.59–17.09 mg/kg (i.p.), 8–64 mg/kg (p.o.); benproperine, 11.39–17.09 mg/kg (i.p.), 16–32 mg/kg (p.o.); and fominoben, 7.59–25.64 mg/kg (i.p.), 128–1024 mg/kg (p.o.).

Table I shows the AtD50 of each drug. Morphine had the most potent antitussive activity. The antitussive activity of morphine was about 3 times stronger than that of codeine. Antitussive effects of picoperidamine, benproperine and fominoben were about 0.3 times, 0.3–0.5 times and 0.25 times stronger than that of codeine when those drugs were administered intraperitoneally. The antitussive activity of fominoben was, however, much less (0.02 times of codeine) when administered orally.

**DISCUSSION**

The requirements for the models of antitussive test are that 1) the intensity of stimulus for coughing is quantitative, 2) the cough response induced is reproducible during an experimental period, 3) unanesthetized animals are suitable and 4) the surgical damage is minimal, especially on the trachea.

Three methods for inducing the cough reflex

![Diagram showing the effects of codeine on the cough reflex induced by electrical stimulation (ES) of the tracheal mucosa.](image)

**FIG. 3. Effects of Codeine on the Cough Reflex induced by Electrical Stimulation (ES) of the Tracheal Mucosa**

At the arrow, 3.38 mg/kg of the drug was administered i.p.
(mechanical, chemical, electrical) are known. The mechanical methods often require light anesthesia even when stimulus is given to induce coughs, because the surgical damage is so heavy. Moreover, in the mechanical method, the intensity of stimulus is difficult to be made quan-
titative. It is also questionable whether an irre-
ducibly minimal quantity of stimulus is given on the intratracheal mucous membrane.

In the present study, we used the electrical method. The tip of electrode was placed near the bifurcatio tracheae, because the bifurcatio tra-

\[\text{FIG. 4. Effects of Codeine on the Cough Reflex induced by Electrical Stimulation (ES) of the Tracheal Mucosa}\
\text{At the arrow, 8 mg/kg of the drug was administered p.o.}\]

\begin{table}
\caption{Comparison of Antitussive Effects of Various Drugs in the Guinea Pigs}
\begin{tabular}{lcc}
\hline
Drugs & AtD50 (mg/kg) & \\
 & i.p. & p.o. \\
\hline
Morphine \cdot HCl & 1.2 & 2.5 \\
Codeine \cdot H_3PO_4 & 3.5 & 7.5 \\
Picoperidamine \cdot HCl & 10.9 & 22.6 \\
Benproperine \cdot H_3PO_4 & 14.5 & 22.6 \\
Fominoben \cdot HCl & 13.9 & 477.8 \\
\hline
\end{tabular}
\end{table}

\text{AtD50: 50\% antitussive dose.}
cheae is the most sensitive site for coughing. 23)

The electrode was insulated with cashew except the tip of electrode. The impalement of the electrode into the trachea was easy and gave a minimal injury. Coughing occurred only when an electric current was applied on electrode.

The intensity of stimulus was adjustable in the present method; the irreducible minimum of intensity was selected for each animal in the beginning of experiment, and was used throughout the experiment. In this point, the chemical method is not so adjustable for selecting a minimal supramaximal threshold for each animal.

The five antitussive drugs, morphine, codeine, picoperidamine, benperoproine and fominoben, were used in this study for evaluating the cough-depressant effect. Similar to the results of Takagi et al. 10) (mechanical method) and Friebel et al. 13) (chemical method), the antitussive effect of morphine was about three times stronger than that of codeine in our experiment. The ATD50s of morphine and codeine were, however, much less (about 1/4—1/5) when using the present method, as were the cases of other antitussives used presently. To know the reproducibility of the results, three different tests for obtaining ATD50s of morphine (i.p.) were made. The ATD50s were 1.2, 1.2, and 1.6 mg/kg, respectively, indicating little variability in the results on antitussive activity.

The relatively low ATD50s obtained in the present study would be attributed at least in part, to the minimal suprathreshold intensity of stimulus for inducing coughs which was chosen for each animal.

In summary, the puncture electrode-induced cough (PEC) method in guinea pigs has the following advantages: 1) Conscious animals are used for the test, 2) the screening test of newly synthesized materials in small quantities is feasible, 3) reproducible and quantitative intensity of stimuli can be applied, 4) surgical damage to animals is minimal, and 5) antitussive effects can be evaluated sensitively.

The present method would be, thus, useful for the first screening method for antitussives.

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