CONTINUOUS DETERMINATION OF TRACHEOBRONCHIAL SECRETORY ACTIVITY IN DOGS

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Abstract—We have designed a new method for continuous recording of the output of respiratory tract fluid, the approach representing a modification and combination of the intratracheal electrode and stopper methods which we had employed originally. Electrical resistance change indicated alteration in the output of respiratory tract fluid, without alteration in composition and temperature of fluid, tracheal muscular tone or respiratory movement, at least, within a physiological range. Among the expectorant drugs thus tested, pilocarpine, 100 and 200 µg/kg given intravenously, significantly and in a dose dependent manner augmented the output of the fluid about 5 minutes after the administration. Given orally in a dose of 2 mg/kg, the output of fluid increased in about 20 minutes. Senega syrup, 0.3 ml/kg had no effect when given intravenously, yet, 2 ml/kg given orally increased the output of fluid within 5 minutes, thereby suggesting that the related secretagogic activity is due to a reflex action following stimulation of the gastric mucosa. Emetine, 2 mg/kg given orally increased the output about 20 minutes after administration while glyceryl guaiacolate, 50 mg/kg had no effect. We propose that our method be used to evaluate expectorants for clinical use as the continuous monitoring of the output of respiratory tract fluid apparently provides a more accurate assessment.

In pulmonary disorders such as bronchial asthma and chronic bronchitis, a considerable amount of airway mucus is synthesized and excreted from glandular and goblet cells of the trachea and bronchi. Basic research on respiratory tract secretion and the evaluation and application of expectorants have not progressed as there was no adequate laboratory model for determining mucus production.

To evaluate airway secretory activity, Henderson and Taylor in 1910 (1) attempted to measure the increase in weight of a calcium chloride tube attached to a tracheal cannula. In 1940, Sakuno (2) indirectly estimated the activity by measuring the amount of dye leaked from the lungs following systemic dye injection. A quantitative method for collecting respiratory tract fluid (RTF) was first described by Perry and Boyd in 1941 (3) and with this method, the volume output of RTF draining from a tracheal cannula during a 2 to 4 hr period can be measured with a graduated test tube connected to the cannula. These methods are however, inadequate.

We developed the stopper method in which airway secretory activity can be evaluated by measuring the output of RTF accumulating in the trachea, under conditions of normal ventilation (4, 5). This method, however, cannot detect slight changes in the output or allow for an accurate determination of the time course of secretory conditions. In continuing studies, we found that airway secretory activity could be estimated by determining
the electrical resistance between intratracheal and indifferent electrodes (6). By combining the two aforementioned methods and making appropriate modifications we found that we could monitor continuously the output of RTF, and using this method we studied the secretagogic effects of expectorants. Our data are reported herein.

MATERIALS AND METHODS

When there is a direct current between the electrodes, polarization is unavoidable, therefore, we designed an apparatus which utilizes an alternating current (0.3 mA) between platinum electrodes (o.d. 0.2 mm) and found that we could continuously record electrical resistance (ER). To do this, we used a modified bridge box for alternating current (Yokogawa Electric Works, Kohlrausch bridge Type 2758) as shown in Fig. 1B. ER changes were thus recorded on a polygraph (Nihon Kohden, RM-85) via an integrator (Nihon Kohden, RFJ-5; mean 0.3 sec) and DC amplifier (Nihon Kohden, RDH-5). An oscilloscope (Nihon Kohden, VC-8) which was inserted into the above apparatus allowed for monitoring and calibration of ER change (Fig. 1A).

To determine the influence of composition and temperature of fluid on ER changes, the electrodes were set perpendicularly at a distance of 5 mm, and were in contact with an electrolyte solution in a small petri dish. ER change between the electrodes was evoked by infusing the solution into the dish in a constant flow using a microtube pump (Tokyo Rika Kikai, MP-201).

In in vivo experiments, male mongrel dogs weighing about 10 kg were used. Food was withheld for 16 hr before intragastric administration of expectorants. The animals were anesthetized with pentobarbital Na (30 mg/kg i.v.) and placed on a board, head downward, with the back of the animal at an angle of 15°. A polyethylene stopper (4, 5) (Fig. 1A)

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**Fig. 1.** A method for continuously evaluating airway secretory activity using intratracheal electrodes. A. diagram of the method. B. circuit of bridge box.
which prevents secretion moving toward the larynx was inserted into the trachea. Normal ventilation through the nasopharynx could thus be maintained. We modified the stopper in the following manner; the electrodes were attached to the stopper at a distance of 5 mm near a dam equipped with a stopper. The output of RTF can be evaluated continuously by measuring cumulative ER change caused by immersion of the electrodes into the RTF. This fluid which then accumulates at the dam of the stopper can be collected and measured. For the calibration of RTF volume in in vivo experiments, saline in a volume of 0.025 ml was sequentially infused into the trachea near the stopper via a small cannula, at the beginning of each experiment. The ER changes produced by saline infusions were recorded on a polygraph. The calibration curve for an in vivo experiment was thus obtained from each animal. Before administration of the expectorants, the infused saline was removed from the dam of the stopper. In some experiments, tracheal muscular tonus was measured with a special strain-gauge (Nihon Kohden, 5 mm x 8 mm) sutured at paries membranaceus. An intratracheal temperature measured with a needle-type thermister (Nihon Kohden, MGAIII-219) was inserted into the trachea and respiratory movement was recorded with a respiratory pick-up (Nihon Kohden, MCR-2TA) to determine the influence of in situ factors other than the output of RTF. Arterial blood pressure and heart rate were monitored from the right femoral artery.

Compounds used were chondroitin sulfate sodium (Tokyo Kasei), egg albumin (Tokyo Kasei), pilocarpine hydrochloride (Torii), emetine hydrochloride (Tokyo Kasei), senega syrup (Yoshida Seiyaku) and glyceryl guaiacolate (Kyoto Yakuhin). Doses of all drugs used were expressed in terms of the salts. All drugs were dissolved or diluted in saline for i.v. administration and in distilled water when given orally. Drug solutions were given i.v. in a dose of 0.1 ml/kg and be gavage in a dose of 0.5 ml/kg, except in the case of senega syrup.

RESULTS

Influence of composition and temperature of fluid

In the in vitro experiments, 0.9% NaCl aqueous solution (saline) at 36°C was placed in a petri dish and here the ER decreased from 7.5 kΩ to 1.9 kΩ (Fig. 2). The ordinate in Fig. 2 represents a decrease in ER, that is, a rise in the depth of fluid. The ER change was particularly sensitive up to about 2 mm in the depth of fluid. When 0.6 or 1.2% NaCl aqueous solution was used instead of saline, the same curve as in Fig. 2 was obtained. Substitution of 131 mM NaCl for KCl (up to 50 mM) did not significantly alter the results. The findings were the same with the addition of 3% chondroitin sulfate and 0.5% egg albumin. Increases in temperature to 32°, 36° or 40°C had no effect on the results.

Determination of effects of some expectorants

At the beginning of each experiment, a calibration curve was obtained by recording the ER change provoked when saline was infused into the trachea in a dose of 0.025 ml. By using this calibration curve, ER change produced by drugs was referred to in terms of volume (ml). Ten min prior to drug administration, tracheal mucosa adjacent to the entotracheal stopper was gently cleaned with saline-moistened cotton. Intravenous administration of
saline (0.1 ml/kg) caused a slight and gradual increase in RTF volume, this probably indicating the normal secretory activity (Figs. 3 and 6). Total RTF volume for two hr after p.o. administration of distilled water (0.5 ml/kg) was 0.038 ± 0.004 ml (N=5). Respiratory movement had little influence on the RTF recording.

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i) Pilocarpine: With administration of pilocarpine (200 μg/kg, i.v.), RTF volume began to increase 2–5 min later, and thereafter rapidly increased over 60 min after administration (Figs. 4 and 6). Total RTF volumes during one hr were 0.340 ± 0.050 ml (N=5) with a dose of 200 μg/kg i.v., and 0.081 ± 0.007 ml (N=5) with 100 μg/kg i.v. of pilocarpine. Both increases are highly significant (p<0.001) as compared to the control. RTF recording was not directly altered by a strong tracheal constriction provoked by an i.v. administration of pilocarpine (Fig. 4). Intratracheal temperature was little change by ventilation and
pilocarpine-induced respiratory excitation. Oral administration of pilocarpine in a dose of 2 mg/kg produced an increase in RTF volume about 20 min later (Figs. 5 and 6). Total RTF volume during two hr was 0.380±0.096 ml (N=5, significant at p<0.001).

ii) Senega syrup: Intravenous administration of senega syrup, 0.3 ml/kg (equivalent to about 13.5 mg/kg), had no more effect than did saline. Total RTF volume during one hr was 0.010±0.006 ml (N=4). On the contrary, oral administration of senega syrup, 3 ml/kg (about 90 mg/kg), produced an increase in RTF volume within 5 min of administration, and 30 min later there was a rapid increase in volume (Fig. 7). Total RTF volume in two hr was 0.114±0.029 ml (N=5, significant at p<0.001).

iii) Emetine: Emetine, 2 mg/kg p.o., produced an increase in RTF volume about 20 min after administration (Fig. 8). Total RTF volume during two hr was 0.129±0.030 ml
FIG. 6. Effect of pilocarpine on the output of respiratory tract fluid in anesthetized dogs. Each point represents the mean ± S.E. for five experiments. R.T.F.: respiratory tract fluid.

FIG. 7. Time course of the output of respiratory tract fluid when senega syrup, 2 ml/kg, was given p.o. to an anesthetized dog.

FIG. 8. Time course of the output of respiratory tract fluid when emetine, 2 mg/kg, was given p.o. to an anesthetized dog.
iv) Glyceryl guaiacolate: Glyceryl guaiacolate, 50 mg/kg p.o., had no effect, the total RTF volume during two hr being 0.040±0.011 ml (N=5).

DISCUSSION

The present methods used to determine airway secretory activity have drawbacks. In the method of Henderson and Taylor (1), increase in the weight of the tube containing the calcium chloride probably indicates the water content of the expired gas. In Sakuno’s method (2), the amount of dye leaked from the lungs, following injection of the material, more or less indicates the permeability of the capillaries in the lungs. We found no apparent relationship between the volume of RTF and the amount of dye in the lavage fluid (7). Using the method of Perry and Boyd (3), the volume of RTF collected in the tube varies markedly depending on the air which passes through the tracheal cannula. Actually, with our newly devised method, the normal RTF volume was 0.016±0.005 ml during one hr under conditions when ventilation through the nasopharynx was maintained. On the other hand, the RTF volume measured by the method of Perry and Boyd was 0.17±0.03 ml or 0.19±0.04 ml, under conditions where the insufflated air in the tracheal cannula was 38–39°C and the humidity 100% (8, 9). In addition, overhydration leads to a greater yield of RTF and an inaccurate assessment of the action of the expectorants administered. The method has another disadvantage in that drainage of RTF into the test tube is hindered at the site of insertion of the tracheal cannula. In 1970, Wardell et al. (10) reported the canine tracheal pouch method, however, with this approach, the secretory activity throughout the airways, including bronchi and bronchioles, cannot be measured. All these methods are unsatisfactory since slight changes in RTF volume and the time course of secretory conditions cannot be accurately determined.

In our newly designed method, we applied an alternating current in the circuit and attached electrodes to the endotracheal stopper. By so doing, we were able to obtain a more accurate determination of the output of RTF since secretions were all stored at the dam of the stopper, the RTF volume could thus be represented in terms of ml determined by calibration at the beginning of each experiment and could be confirmed at the end of the experiment by collecting the fluid. We found no changes in the ER between the electrodes altered by composition of fluid, such as electrolytes, mucus and protein, or by change in the temperature of the fluid. Addition of mucopolysaccharide and protein in amounts reportedly contained in RTF (11) and tracheal muscular constriction and respiratory movement also had no substantial effect on ER. It was therefore shown that changes in ER are exclusively capable of detecting change in the volume of RTF.

Secretagogic effects of some expectorants were determined by the present method. A parasympathetic effect was reproduced by pilocarpine, the same drug which evoked the secretagogic effect in the human and rat bronchial glands maintained in culture (12, 13). The stimulatory effect of pilocarpine on the airway secretory tissues was also confirmed in vivo by administration of the agent into the bronchial artery of dogs (7). We found in
In the present work it was found that pilocarpine in an oral dose which has no significant effect on systemic blood pressure, can induce an increase in the output of RTF. The finding that ER change occurred later than tracheal contraction when pilocarpine was given i.v. is probably due to the delay in the accumulation or arrival of RTF at the stopper. The delay in onset of action when pilocarpine was given p.o. can probably be attributed to the time required for absorption of the drug.

Senega is an extract of dried root of Polygala senega L. or Polygala senega L. var. latifolia Torrey et Gray (Polygalaceae), which contains saponins, and since the 18th century has been used as an expectorant. The mechanism of its secretagogic action on the airway is still, however, poorly understood. Boyd (14) ascribed the action partly to a reflexogenic origin due to its stimulating effect on the gastric mucosa. Our finding of a rapid increase in RTF volume with oral administration of senega, despite the fact that i.v. administration produced no effect, supports the reflexogenic action proposed by Boyd. Boyd and Knight (15) reported that emetine, 1-5 mg/kg p.o., increased RTF volume by 20-30% in rabbits and cats. In the present study, emetine, 2 mg/kg p.o., increased the volume by 240%, the onset of action being about 20 min after administration. Glyceryl guaiacolate has been used in the treatment of cough, upper respiratory infections and the common cold (16). Evidence of clinical efficacy of the agent, however, remains to be determined. The secretagogic effect of glyceryl guaiacolate (5 g/kg p.o. or 10 mg/kg i.p.) in rabbits has been reported in experiments using the method of Perry and Boyd (3, 17). We found that this agent had no expectorant effect whatever, when given orally to dogs.

Our newly designed method should be most applicable to laboratory assessment of clinically prescribed expectorants.

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