

(黒田嘉代志)

The Fundamental Studies on the Cough-Reflex and Antitussive
Action (Agents)

[Abstract]

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It is a common knowledge that the coughing (cough paroxysm) is a pathognomonic symptom of respiratory disease. Use of antitussive agents for suppressing the cough paroxysm as the symptomatolytic treatment is profitable extremely in the domain of clinical veterinary science, animal husbandry (zootechny) and clinical medicine.

On the other hand, it is well known that codeine phosphate (codeine) can be expected to give a certain effect on the clinical application while many of non-narcotic antitussives are ineffective. Further, the use of narcotic antitussives is controlled by the "Narcotics Control Law" and induces the side-effects (adverse reactions) such as respiratory suppression, alimentary disturbance (constipation), circulatory disturbance (postural hypotension), development of tolerance and dependence (physical or psychic). In addition, it can possibly bring about social- or national-troubles such as drug-abuse and atrocious crime.

For these reasons, the development of not only a new analgesics but also antitussives that are non-narcotic and effective certainly, has been demanded socially for a long time in each domain.

From reasons of above mentioned, isoaminile ; α -(isopropyl)- α -(β -dimethylaminopropyl)phenylacetonitril was synthesized newly in order to overcome these disadvantages by Stühmer and Funke, and the antitussive activities and

other pharmacological and toxicological properties of isoaminile were investigated in detail by the author. The results satisfied generally the social demand of above-noted and also induced some important new findings in chemical and life science. The following is a summary.

1. First of all, evaluating method of antitussive activity in animals must be established for the development of a new antitussive agent. In comparative methodology, a) "coughing dog" method (Kase) was most excellent as primary screening and/or evaluating method, and b) "coughing cat" method (Kase and Yuizono) and c) the electric stimulation method of cat's superior laryngeal nerve (Domenjuz) followed in order of the practical use, and ultimately d) the inhalation method of ammonia-aerosol in guinea-pig (Winter and Flataker) was unsuitable under the conditions practised in this time.

2. Using the above noted procedures a) b) c) and further the electric stimulation method of cat's dorso-lateral part of the medulla (Borison), the antitussive activities of isoaminile were examined generally. The antitussive activities of isoaminile had no difference between "citrate" and "cyclamate", and was approximately equal to that of codeine in compared with the median antitussive effective dose (AtD50 value), and was more potent than those of dextromethorphan hydrobromide (d-methorphan) and noscapine hydrochloride (noscapine) which had been frequently for clinical treatments. (Fig. 1 and Table 1).

3. The safety margin (dog's LD50 value/dog's AtD50 value) of isoaminile, both "citrate" and "cyclamate" was larger than ten. This was small compared with that of codeine, but was larger than those of d-methorphan and noscapine. From the above results, it is suggested that isoaminile is excellent substance in antitussive activity and in selectivity. (Table 1).
4. The antitussive action of isoaminile had no influence by the treatment of narcotic antagonist; levallorphan bitartrate (levallorphan) (Fig. 2), and had no development of tolerance by repeated administration for a long time (for 40 days) (Fig. 3), and had no development of cross-tolerance between that of codeine (Fig. 4).
5. a) After repeated administration to dogs for a long time (for 40 days), the dogs had no withdrawal symptoms in both cases that isoaminile was abstinenced suddenly and that levallorphan was substituted instead of isoaminile. b) After repeated administration to rats for a long time (for 77 days), the rats had no abstinence syndrome (body weight loss) in both cases that isoaminile was stopped suddenly and that levallorphan was given in place of isoaminile. c) The abstinence syndroms of morphine hydrochloride (morphine) dependent-rats could not be prevented by the substitution of isoaminile.
6. The main common poisoning syndromes in mice, rat and

dogs induced by isoaminile were increase in spontaneous movement, tremor, ataxia of hind-limbs, clonic and tonic convulsions.

There were no difference of the median lethal dose. (LD50 value) of isoaminile between different "salts" and species of animal used. Comparing with LD50 values in mice, acute toxicity of isoaminile was nearly equal to codeine by subcutaneous injection, but was less than codeine by intravenous and oral administration.

7. Isoaminile had an analgesia-like effect (d'Amour-Smith and Haffner's methods) in high dosage with poisoning syndromes, but this phenomenon was different completely from those of codeine and morphine that is antagonized by levallorphan.

8. In high dosages with poisoning symptoms, isoaminile had a marked inhibitory effect on the flexer response of a hind-leg of spinal mice which were steeped in warm water and also on the spinal action potentials of spinal cats.

9. With high dosages more than approx. 5 to 10 times of anti-tussive effective dose, isoaminile produced a moderate inhibitory effect on the central nervous system such as depression of consciousness-level, prolongation of anesthesia, anti-convulsive effects and anti-tremor effect.

10. In vivo, isoaminile had no influence on the normal tension of bronchial muscle, but had a dilating effect on the bronchial-spasm induced by histamine dihydrochloride (histamine).

11. Local anesthetic actions of isoaminile were equal to that of procaine hydrochloride.
12. The effects of isoaminile on the respiratory system, circulatory system, alimentary tract system and autonomic nervous system were much less compared with that of codeine.
13. The safety maximal losage in the subacutely treated rats (successive oral administration of isoaminile for 28 to 33 days) was approx. 50 to 75 times as much as the presumptive clinical daily dose.
14. The safety maximal dosage in the chronically treated rats (successive oral administration of isoaminile for 136 days) was approx. 10 to 20 times as much as the presumptive clinical daily dose.
15. The safety maximal dosage in the chronically treated dogs (successive oral administration of isoaminile for 180 days) was approx. 7.5 to 11.3 times as much as the presumptive clinical daily dose.

In conclusion, judging from the results of pharmacological and toxicological studies, it is suggested that isoaminile is more excellent antitussive agent in the safety as much low acute and chronic toxicity than codeine.

Furthermore, an attempt was made in order to clearly demonstrate the mode (site) of antitussive action of isoaminile by examining the effects on the cough reflex arc

(Fig. 5) in order and in detail. The following is a summary.

1. When isoaminile was given by the routes directly getting to the brain stem such as the cerebello-medullar cistern, far smaller doses were sufficient to obtain the same effect as that by intravenous administration.
2. It exerted no effects on both of the sensory receptors for cough reflex in the tracheal mucosa and the pulmonary stretch receptors in spite of possessing a local anesthetic action as potent as that of procaine.
3. It had no effect on the evoked postsynaptic potentials in the solitary tract nucleus by electrical stimulation of the ipsilateral superior laryngeal nerve.
4. The antitussive effect of isoaminile was not influenced by decerebration after mid-collicular transection (Fig. 6).
5. It had no effect on the descending respiratory pathways extending from the cervical cord to the respiratory muscles.
6. It possessed a spasmolytic action on histamine-induced contraction of bronchial muscles in doses more than antitussive effective dose, though it showed no effect on the normal tone of bronchial muscles.
7. It depressed the centrally-induced coughs in antitussive effective dose which was surely able to depress peripherally-induced coughs. On the other hand, it had no pronounced effect on the respiratory center with the same dose. (Fig. 7).
8. It significantly diminished the burst discharges of the

recurrent nerve induced by frequent electric stimulation of the superior laryngeal nerve as active as that of codeine (Fig. 8). That is to say, isoaminile may suppress the integrating polysynaptic pathway, probably acting as special central substrates important for cough production and localizing between the solitary tract nucleus and the ambiguous nucleus.

9. Its central antitussive action was clearly differentiated from that of codeine in the mode of action, and it was confirmed by following results. a) Antitussive activity of isoaminile was not inhibited by a narcotic antagonist; levallorphan (Fig. 2). b) It had no influence on the respiratory center activity even in much larger doses than antitussive effective dose (Fig. 7).

In conclusion, it is suggested that the antitussive action of isoaminile is due to selective depression of cough center per se.

Putting the generalization finally. The achievements in this study were as follow. 1) Establishment of evaluating method of antitussive activity in animals, which was closely correlated to clinical efficacy. 2) Development of new non-narcotic antitussive agent; isoaminile (Perocan[®], "cyclamate" was permitted on 3th September 1969, "citrate" was permitted on 12th January 1974) which was excellent in the potency and safety, and had been demanded socially for

a long time. 3) Explanation of mode of antitussive action of isoaminile.

This clearly indicates that not only it contributes to clinical veterinary medicine, animal husbandry science (zootechny) and clinical medicine but also it furnishes new important knowlege in following viewpoints on the physiology, pharmacology, pharmacy, toxicology and veterinary medicine.

1) Synthesis of new non-narcotic antitussives (analgesics) from the narcotic parental compounds (basic structure). 2) Physiology of cough-reflex, in particular, natural relation of "cough-center" and respiratory center, 3) Explanation of "cough-center" in cellular level. 4) Interaction between antitussive action and analgesic action. 5) Interaction between antitussive action and narcotic property.

Table 1 Antitussive activity and safety margin of isoaminile in dogs.

Drug	salt	M.S.				M.S.		
		AtD50 (mg/kg, i.v.)	Confidence limits (p=0.05)	Ratio ^{a)}	Safety margin (LD50/AtD50)	AtD50 (mg/kg, p.o.)	Confidence limits (p=0.05)	Ratio ^{a)}
Isoaminile	citrate base	8.00	5.09-11.0	0.30	10.0	19.0	13.5-26.6	0.44
		4.32	2.74-5.94	0.39		10.3	7.29-16.36	0.58
Isoaminile	cycamate base	7.72	5.25-11.3	0.31	10.9	21.0	16.5-26.6	0.40
		4.40	2.99-6.44	0.39		12.0	9.41-15.2	0.50
Codeine	phosphate base	2.40*	2.07-2.89	1.00	31.9	8.4*	7.53-9.38	1.00
		1.70	1.53-2.12	1.00		6.0	5.57-6.96	1.00
Dextromethorphan	HBr-salt base	15.3**	12.9-18.2	0.16	2.0	Ineffective up to 62.0**		0.14 ^{b)}
		11.2	9.50-13.3	0.16				
Noscapipe	HCl-salt base	17.0**	13.8-21.0	0.14	4.7	80.0** (minimal effective dose)		0.11 ^{b)}
		13.6	12.6-19.2	0.11				

M.S. : Mechanical stimulation of tracheal mucosa
 * Depression of respiration and sedation occurred
 ** Clonic convulsion occurred
 a) Ratio of efficacy to that of codeine

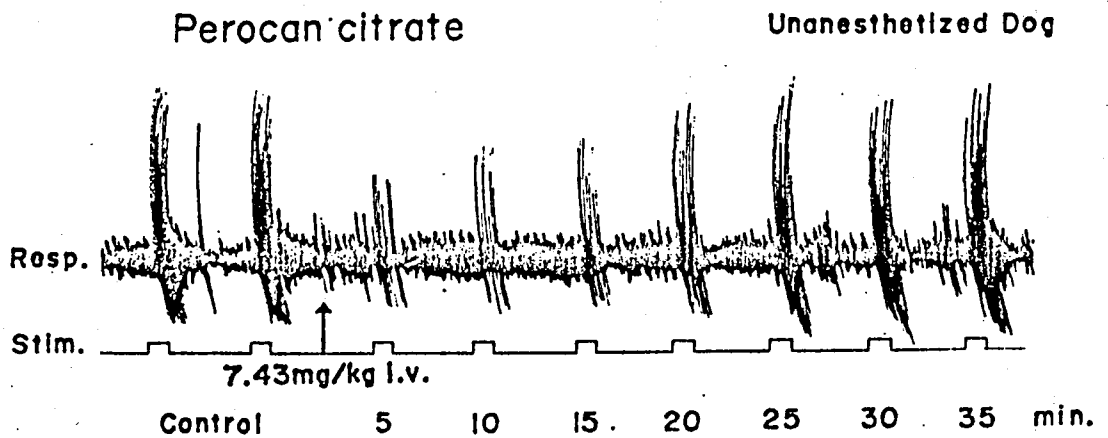


Fig. 1 Antitussive effect of isoaminile in an unanesthetized dog.

Tested by "Coughing dog" method. At the arrow, 7.43mg/kg of isoaminile citrate (Perocan citrate) was administered intravenously. Resp.: respiration (intratracheal pressure. expiration recorded upwards). Stim.: Stimulation. The amplitude and frequency in tracing of coughs were decreased to about 60% of those of pretreatment control 5 min after the drug, and the depression of coughs lasted for 30 min.

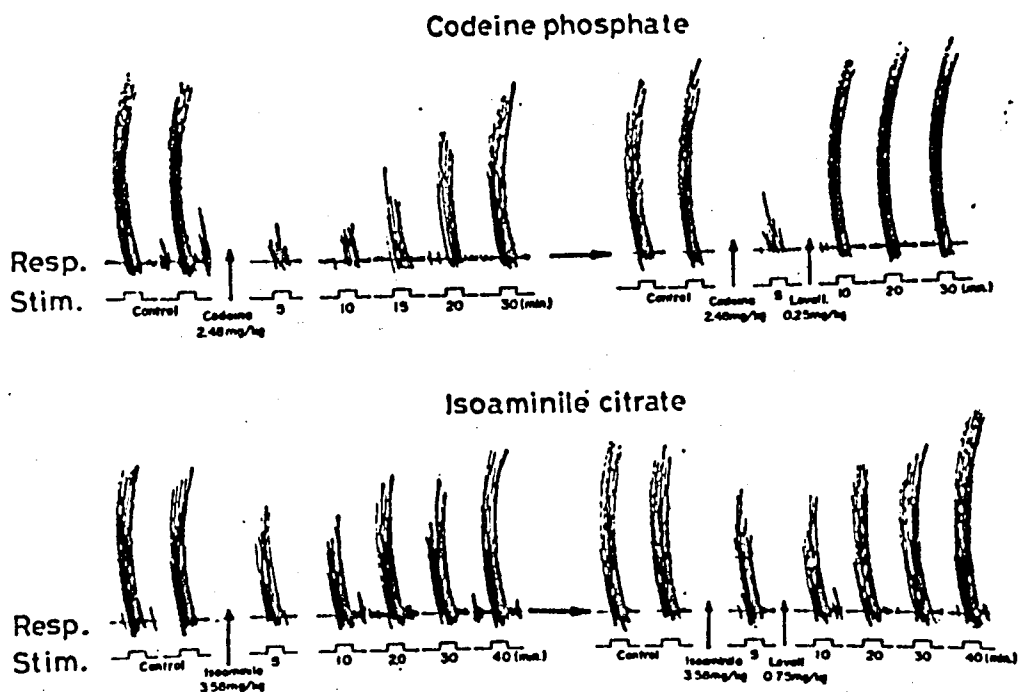


Fig. 2 Levallorphan antagonism

Antitussive effect of isoaminile citrate was determined by the "Coughing dog" method. In an i.v. dose of 3.58mg/kg of the drug, it showed 30-40% decrease in the amplitude and 40-50% decrease in the frequency of tracing of coughs and the effects lasted for 30-35min. Five days later, the same dose of the drug as before was given by the same route again. The antitussive effect of the drug was confirmed to be almost the same as before 5 min after the administration, thereafter, levallorphan 0.75mg/kg; corresponding to 1/5 of that of isoaminile, was administered. However, the effect of isoaminile was not influenced by levallorphan and it returned to the pretreatment state at 40 min. In contrast to this, antitussive effect of codeine phosphate 2.48mg/kg, i.v. was immediately abolished by levallorphan 0.25mg/kg, i.v.; corresponding to 1/10 of that of codeine.

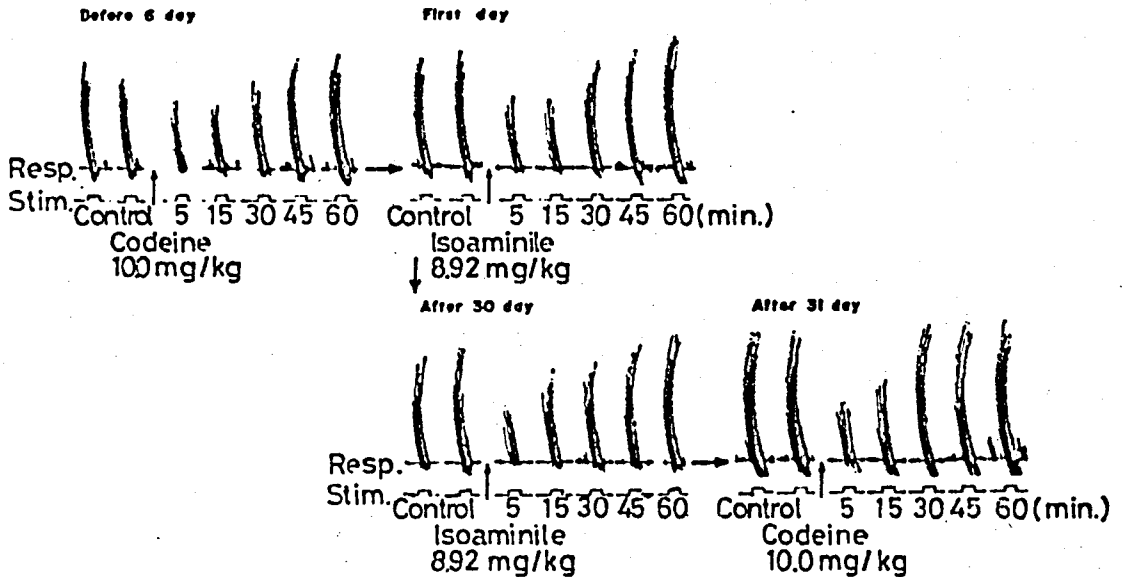


Fig. 3 Development of tolerance in antitussive activity.

Isoaminile cyclamate 8.92mg/kg were given intravenously twice a day for 40 consecutive days. Antitussive effect was examined by the "Coughing dog" method every two weeks and the effects of the drug on behaviour, condition of health, and body weight were also carefully checked every day. No significant difference was observed in antitussive effect and its duration between the first day and 40th day (30th day). Furthermore, the symptoms were observed on the first day neither decreased, nor disappeared, nor increased by repeated daily administration of isoaminile. In addition to this, no significant changes in body weight were observed.

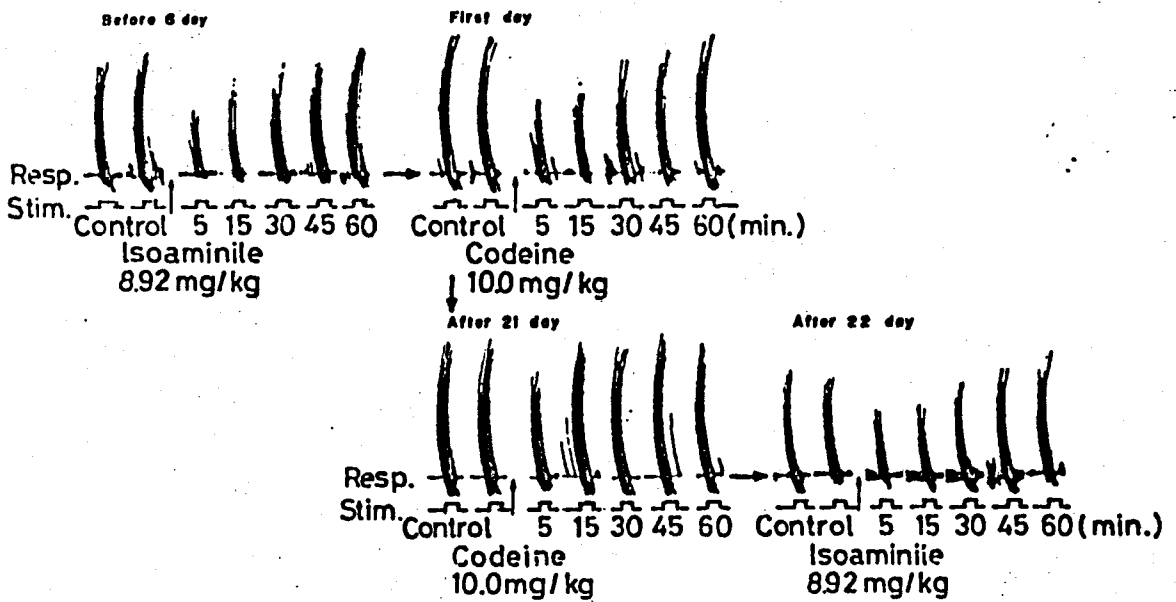


Fig. 4 Cross-tolerance between isoaminile and codeine in antitussive activity

The dogs, which had been used for the experiment in the preceding section (development of tolerance), were employed. Antitussive effects of isoaminile and codeine were determined prior to this experiment, that is, the isoaminile group was given an i.v. dose of 10mg/kg of codeine phosphate, and the codeine group was treated with 8.92mg/kg of isoaminile. The isoaminile group received the same dose of isoaminile twice a day for 40 consecutive day, on the other hand, the codeine group was given the same dose of codeine twice a day for 19-21 days. On the 31-35th day, isoaminile group was given the same doses of codeine as before, and antitussive effect of the drug was determined by the "Coughing dog" method (Fig. 3). The antitussive activity of codeine group was decreased by continued daily administration and disappeared ultimately on the 16th (3 cases), 17th (one case), and 19th (one case) days, respectively. On the 19-21th day, codeine group was given the same doses of isoaminile as before.

However, no significant differences were observed in antitussive effects of isoaminile between before and after the continued daily administration of the drugs. Thus, the result indicates that no cross-tolerance was established between the two drugs in antitussive effect.

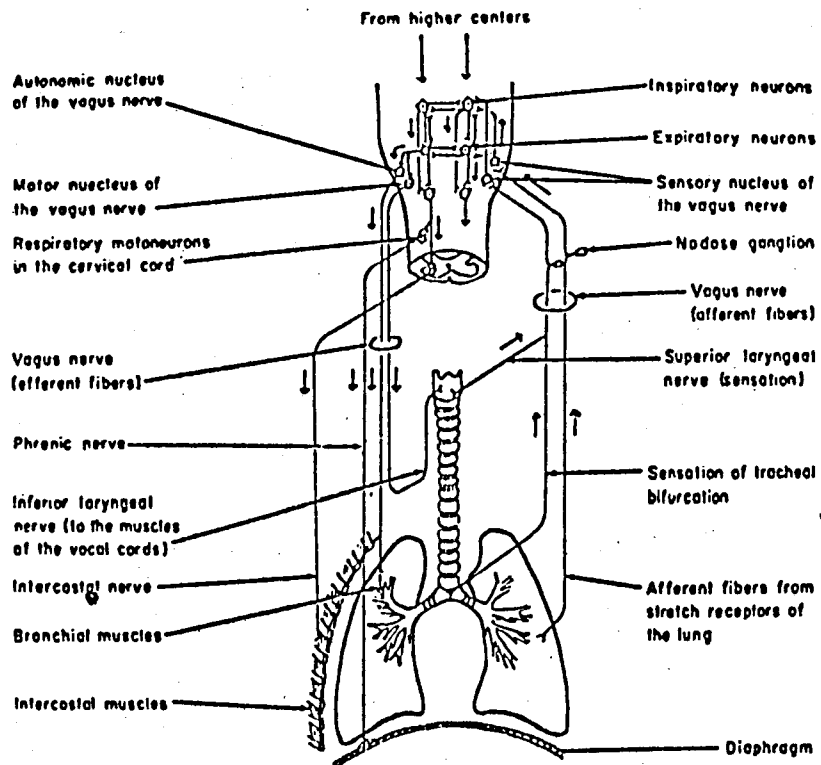


Fig. 5 Cough reflex arc

Afferent pathway at one side, efferent pathway at the other side.

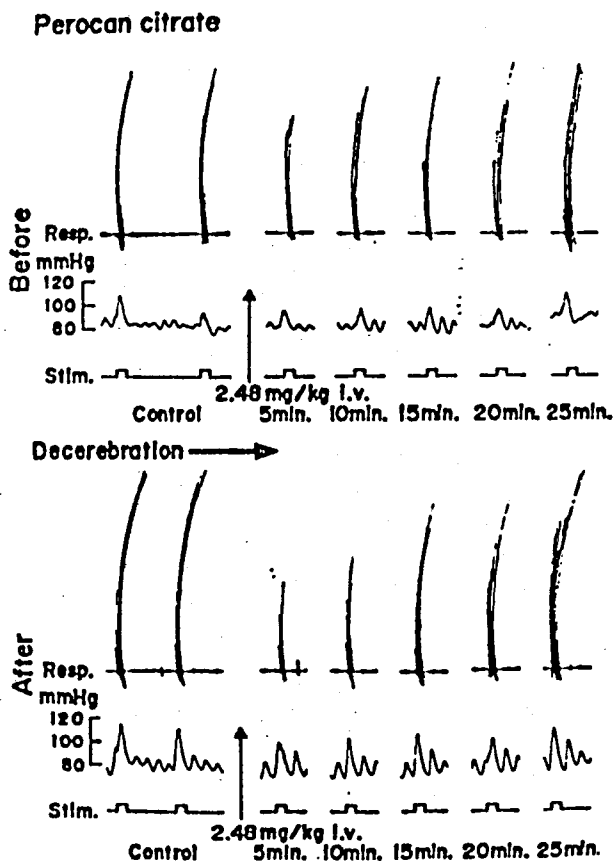


Fig. 6 Influence of decerebration

In order to know whether the centers higher than the brain stem contribute to the antitussive activity of the drug, decerebration was carried out in cats, and antitussive effects of isoaminile were compared before and after decerebration. Cough responses were evoked either by mechanical stimulation of tracheal mucosa or electrical stimulation of dorso-lateral part of the medulla. However, no discernible changes in the potency or in the duration of the effect of isoaminile citrate 2.07-2.48mg were observed. The above results demonstrated that the sites of action of isoaminile are in the lower brain stem.

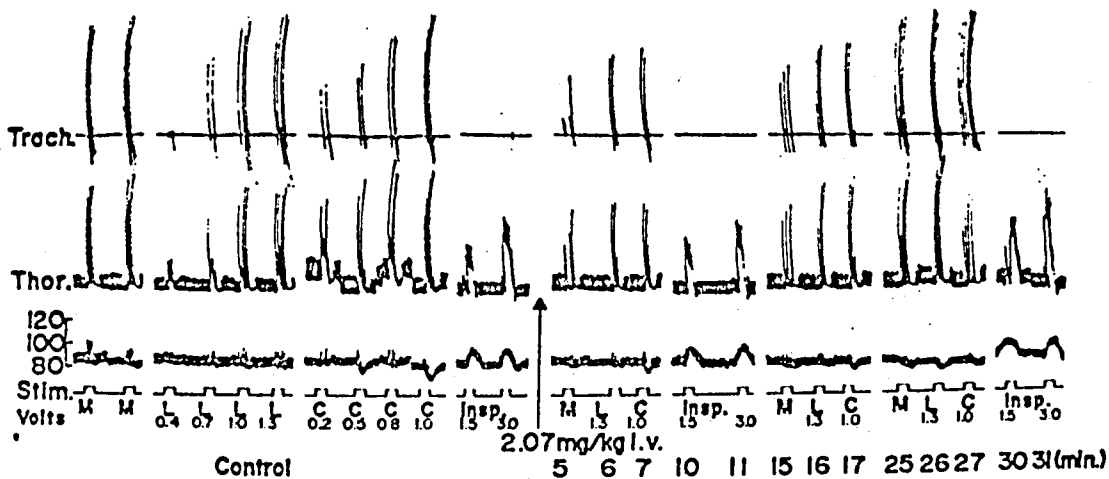
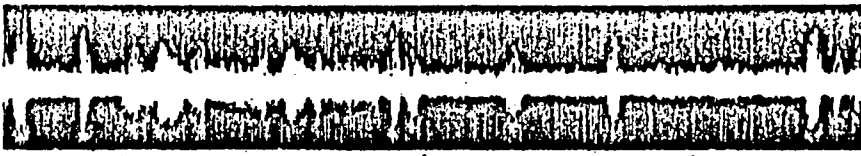


Fig. 7 Effects on the peripherally and, centrally induced cough responses, and on the centrally evoked sustained inspiratory response.

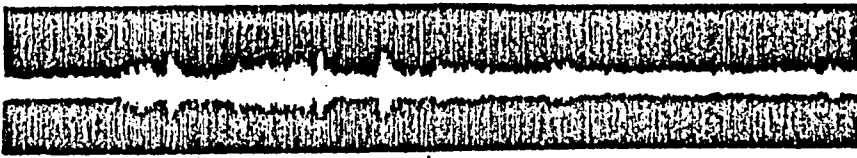
Cough responses were induced either by mechanical stimulation of tracheal mucose (M), electrical stimulation of the superior laryngeal nerve (L) or electrical stimulation of the expiratory pace-maker area at the dorso-lateral part of the medulla (C). The sustained inspiratory responses (Insp.) were induced by electric stimulation of the inspiratory center (Pitts). The caudal part of the cerebellum was removed. Trach.: intratracheal pressure (expiration recorded upwards), Thor.: respiration recorded by a thoracic pneumograph (inspiration recorded upwards), Stim.: stimulation, At the arrow; 2.07mg/kg of isoaminile citrate was given intravenously. Cough responses induced by peripherally and centrally were markedly depressed and returned to the pretreatment state 25-27 min after medication. In contrast to this, no change occurred in sustained inspiratory response. The results show that isoaminile was able to depress both types of peripherally and centrally induced cough responses with the same dose level, however, it seems not to exert marked effect on normal respiratory activity, in other wards, the antitussive action of isoaminile may be due mainly to a selective suppression of the cough center per se.

Before



→ Perocan 5.0 mg/kg, i.v.

5 min



[100 μ V

5sec

Fig. 8 Effects on action potentials of the recurrent nerve evoked by stimulation of the superior laryngeal nerve.

Evoked action potentials were elicited by repetitive stimulation (20cps) of the superior laryngeal nerve of cat with immobilized by gallamine triethiodide. The duration of stimulation is shown by a bar under each record. The evoked potentials were reduced 5 min after i.v. administrations of isoaminile citrate (Perocan). These burst discharges were possibly formed on the integrating circuit consisting of large number of synapses. 5mg/kg of codeine phosphate or dextromethorphan hydrobromide inhibited also the discharges. These results will suggest that the acting site of the antitussives may exist in this pathway, that is, the antitussive agents may affect the neurons in the pathway playing a major role in the formation of coughs.