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THE EFFECTS OF PENTOBARBITONE AND CHLORALOSE ANAESTHESIA ON THE VAGAL COMPONENT OF BRONCHOCONSTRICTION PRODUCED BY HISTAMINE AEROSOL IN THE ANAESTHETIZED DOG

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1 Total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were measured in dogs anaesthetized with pentobarbitone or chloralose and subjected to aerosols of histamine during 4 successive inspirations.

2 Histamine caused concentration-dependent increases in R_L and decreases in C_{dyn}. A significant vagal component was involved, but only when chloralose was employed and then only in the R_L response.

3 The resting values of R_L and C_{dyn} were similar regardless of which anaesthetic was used and remained essentially the same if the vagi were cooled.

4 Electrical stimulation of the efferent vagi caused large increases in R_L of dogs given chloralose and these effects were attenuated by the administration of pentobarbitone. Such stimulation was relatively ineffective in dogs given pentobarbitone alone.

5 *In vitro*, electrical field stimulation caused contractions of dog trachealis muscle. The responses were reduced by pentobarbitone in concentrations approximating to plasma levels in the anaesthetized dogs (1 to 5×10^{-4} M), but the effects of exogenous acetylcholine were unaltered. The inhibition was dose-dependent, reversed by washing and unaltered by hexamethonium.

6 The results suggest that pentobarbitone inhibits the vagal component of histamine-induced bronchoconstriction in the dog by an action on the efferent pathway. Furthermore, pentobarbitone acts either by blocking transmission along postganglionic parasympathetic nerves or by preventing the release of acetylcholine from the nerve endings in the lung.

Introduction

Studies both in animals and man have shown that many different bronchoactive agents can cause a vagally mediated bronchoconstriction (Gold, 1973). Although the effects of local anaesthetic aerosols on reflex bronchoconstriction have been investigated in the dog (Dain, Boushey & Gold, 1974; 1975; Cross, Guz, Jain, Archer, Stevens & Reynolds, 1976) little is known about the effects of intravenous general anaesthetics on this mechanism of bronchoconstriction. For this reason we have investigated the effects of two intravenous general anaesthetics commonly used in animal experimentation, namely chloralose and pentobarbitone, on the reflex component of histamine-induced bronchoconstriction in dogs. We have also conducted *in vitro* experiments, with canine isolated trachealis muscle, to help explain our *in vivo* findings. Parts of this work have been communicated to the British Pharmacological Society (Richards & Jackson, 1976).

Methods

In vivo *experiments*

Light surgical anaesthesia was induced in Beagle dogs of either sex (9–12 kg) by the intravenous injection of pentobarbitone 30 mg/kg or chloralose 80 mg/kg. The level of anaesthesia was maintained by a continuous infusion of 0.1 mg kg^{-1} min^{-1} pentobarbitone or by a bolus injection of 10–15 mg/kg chloralose every 15 minutes. Immediately after induction of anaesthesia a cuffed Magill endotracheal tube was inserted, to be replaced after tracheotomy by a plastic cannula with its end just below the cricoid cartilage. The animals were ventilated with air at constant pressure (0.98 kPa) by means of a Bird Mark VII respirator. The lungs were inflated to 1.96 kPa transpulmonary pressure (TPP) after severe histamine-induced falls in lung compliance and also periodically to reverse the gradual decrease in lung volume, which occurs during positive pressure

ventilation. A mid-line thoracotomy was carried out routinely.

A catheter was inserted into the muscularis branch of the right femoral artery for recording blood pressure, using a Statham P23Db pressure transducer and heart rate was derived from the blood pressure signal with a Devices instantaneous rate meter. Catheters were placed in the right medial saphenous vein and the muscularis branch of the left femoral artery for the injection of drugs and the removal of arterial blood for blood gas analysis respectively.

Both cervical vagosympathetic nerves were carefully exposed and placed on silver thermodes, 2.5 cm long, through which water at 0.5°C could be circulated. When the nerves were cooled, chilled saline (0.9% w/v NaCl solution) swabs at approximately 0.5°C were placed on the nerves which passed over the thermodes. In some dogs both nerves were placed on shielded bipolar platinum electrodes and stimulated by two Devices square wave stimulators with pulses of 1 ms width, supramaximal voltage, at a frequency of 20 Hz for 20 s periods.

The body temperature of the dogs was maintained at 38.5°C with a thermostatically controlled heating blanket in conjunction with a rectal thermocouple.

The partial pressures of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) in the arterial blood and the blood pH were monitored regularly by means of a Radiometer ABL 1 acid-base analyser. By adjusting the ventilation of the animal at the beginning of the experiment, or during control periods, it was possible to maintain $P_{O_2} > 13.3$ kPa, P_{CO_2} between 3.32 kPa and 5.32 kPa and pH between 7.36 and 7.46.

Air flow rate was measured with a Fleisch pneumotachograph (Type 0; 9.8 Pa ≡ 43.16 ml/s) connected to a Furness Controls micromanometer (100–0–100 Pa). Tidal volume was obtained by electrical integration of the flow signal with a Devices integrator. TPP was measured by a Furness Controls micromanometer (1–0–1 kPa), one side of which was connected to the tracheal cannula and the other side of which was left open to the atmosphere. All recordings were displayed on a Devices M19 recorder.

Total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were measured by a manual graphic method using the displayed signals of flow, volume and TPP (Amdur & Mead, 1958). The respiratory computer described by Carney, Pugh & Sheard (1972) was also used for the calculations of R_L and C_{dyn}, its displayed output being calibrated and checked for accuracy by comparison with simultaneous manual determinations of R_L and C_{dyn}. The computer was accurate to ± 0.02 kPa l^{-1} s for R_L and to ± 6.2 ml kPa^{-1} for C_{dyn}.

Aerosols were generated on inspiration only using a Vaponefrin inhalajet nebuliser modified to deliver a significant proportion of large (12.8 μm) as well as small (0.5 μm) particles. The nebuliser was modified by removing the impingement ball.

The effects of histamine aerosols on resting R_L and C_{dyn} were investigated in 5 dogs anaesthetized with pentobarbitone (by the use of histamine solutions of 0.125, 0.5, 1.0 and 2.0%), or with chloralose (by the use of histamine solutions of 0.0625, 0.125, 0.25 or 0.5%). In addition, the effects of similarly administered aerosols of saline were investigated in two dogs anaesthetized with chloralose. The following experimental procedure was used: the vagus nerves were maintained at 38.5°C and 4 breaths were administered of histamine aerosol in a concentration selected at random from the previous list. Identical histamine challenges were repeated at 30 min intervals until two successive responses were consistent. Next the histamine was given after cooling both cervical vagi to approximately 0.5°C. The nerves were then rewarmed to 38.5°C and the procedure repeated with a different concentration of histamine. A total of 3 observations was made on the effects of vagal cooling on each concentration of histamine.

Measurement of plasma levels of pentobarbitone

Venous blood samples were taken from dogs approximately 4 h after the induction of pentobarbitone anaesthesia. After centrifugation, the plasma concentration of pentobarbitone was measured as described by Udenfriend, Duggan, Vasta & Brodie (1957).

In vitro *experiments*

Beagle dogs of either sex (9–12 kg) were anaesthetized with intravenous chloralose 80 mg/kg and the cervical trachea removed. Trachealis muscle preparations were then prepared from single tracheal rings (Yamaguchi, Hitzig & Coburn, 1976). The trachealis muscle was attached by thread to a tissue holder made from a 5 ml syringe case in the wall of which were fixed two platinum wires for electrical field stimulation of the muscle. The holder was placed in a 25 ml organ bath containing Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2 and maintained at 38.5°C. Under a resting tension of 1 g, contractions of the muscle were measured isometrically with a Grass FT.03 transducer. At 10 min intervals, the tissue was stimulated for 15 s with square wave pulses (width 2 ms, frequency 20 Hz and supramaximal voltage) delivered from a Grass S4 stimulator and using a Devices Digitimer. When at least 4 consistent responses to field stimulation had been obtained pentobarbitone (1×10^{-4} M, 2.5×10^{-4} M or 5×10^{-4}M) was added to the organ bath and left until there was no further reduction in the response. The drug was then washed from the bath and the response recovered to control values. Atropine 1.8 μM was then added. Similar experiments were conducted in which hexamethonium 3.6 μM was added to the bath 30 min before the addition of pentobarbitone. In other

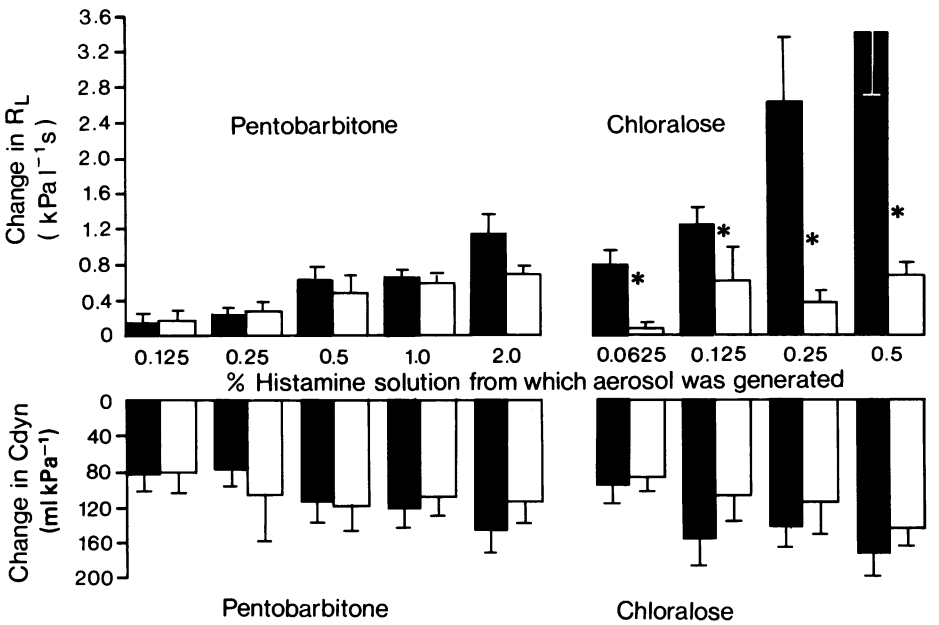

Figure 1 Changes in total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) produced by 4 breaths of histamine aerosol generated from different concentrations of histamine solution in dogs anaesthetized with pentobarbitone or chloralose. The solid columns are the responses with the vagi at body temperature and the open columns are the responses with the vagi cooled to 0.5°C. Bars indicate s.e. mean, $n=6$ for solid columns, $n=3$ for open columns. * $0.05>P>0.01$.

experiments, cumulative dose-response curves to acetylcholine were obtained in the absence and presence of 5×10^{-4} M pentobarbitone. The pentobarbitone was added to the bathing solution and 30 min allowed before the first dose of acetylcholine was given.

Drugs and solutions

The drugs used were: histamine acid phosphate (BDH), chloralose (Koch-Light Laboratories), pentobarbitone sodium (Sagatal, May and Baker), acetylcholine chloride (Koch-Light Laboratories), atropine sulphate (Sigma) and hexamethonium bromide (May and Baker).

Drug solutions were freshly prepared in isotonic saline and concentrations are given in terms of bases.

The Krebs-Henseleit solution contained (g/l): NaCl 6.87, $MgSO_4 7H_2O$ 0.29, $NaH_2PO_4 2H_2O$ 0.18, dextrose 1.0, $NaHCO_3$ 2.1, KCl 0.43 and $CaCl_2$ 0.28.

Results

In vivo *experiments*

Four breaths of histamine aerosol administered to 5 dogs anaesthetized with pentobarbitone produced dose-dependent increases in R_L and falls in C_{dyn} (Figure 1). Cooling the cervical vagi did not significantly affect the increases in R_L or the decreases in C_{dyn} ($P>0.05$).

In 5 dogs anaesthetized with chloralose, 4 breaths of histamine aerosol produced marked increases in R_L even when the aerosol was generated from comparatively low concentrations of histamine solution (Figure 1). The increases in R_L were significantly inhibited by vagal cooling, whereas the falls in C_{dyn} were unaffected ($P>0.05$). Four breaths of an aerosol generated from normal saline did not affect R_L or C_{dyn}.

The initial resting values of R_L and C_{dyn} were similar regardless of the anaesthetic employed and cooling the cervical vagi did not significantly affect any of these values. The initial resting value of R_L in dogs anaesthetized with chloralose was 0.26 ± 0.05 kPa l^{-1} s compared with 0.23 ± 0.003 kPa l^{-1} s with pentobarbitone, whilst the respective values of C_{dyn} were 213 ± 25 ml kPa^{-1} and 245 ± 16 ml kPa^{-1}; all values are mean ± s.e. mean, $n=5$.

The results showed that histamine aerosol was unable to produce a large reflex bronchoconstriction in dogs anaesthetized with pentobarbitone. To establish whether this block was occurring on the afferent or efferent side of the reflex the vagus nerves were electrically stimulated. To prevent cephalad

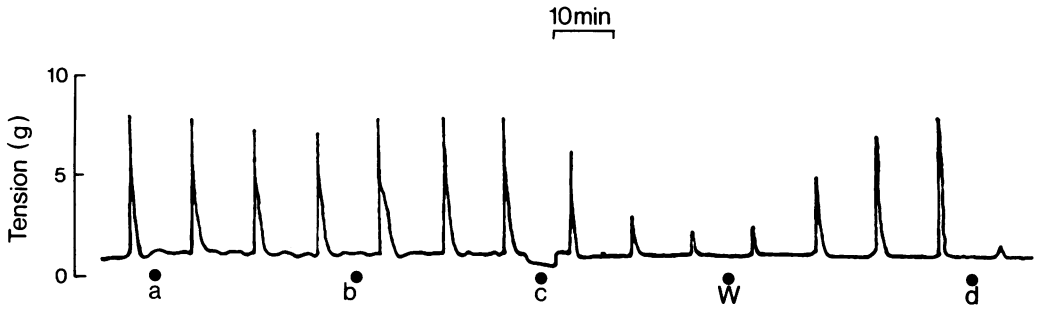

Figure 2 The effect of hexamethonium 1.8 μM (given at a and b); pentobarbitone 5×10^{-4} M (given at c) and atropine 1.8 μM (given at d) on the response of canine trachealis muscle to field stimulation. The bath was washed at W.

stimulation the electrodes were placed on the vagus nerves caudal to the point of cooling.

Stimulation of the vagi in dogs anaesthetized with pentobarbitone produced an increase in R_L of 0.12 ± 0.4 kPa l^{-1} s while in dogs anaesthetized with chloralose a much greater increase occurred in R_L of 1.2 ± 0.18 kPa l^{-1} s (mean ± s.e. mean, $n=6$). In both groups of animals vagal stimulation stopped the heart with subsequent 'vagal escape'.

When intravenous pentobarbitone was given to dogs anaesthetized with chloralose the response to electrical stimulation of the vagus nerves 10 min later was significantly reduced although resting values of R_L were unaffected. Addition of 5 mg/kg reduced the response by 49% ± 14.5% and 10 mg/kg by 69.5% ± 11.3% (mean ± s.e. mean, $n=7$).

Plasma levels of pentobarbitone

The plasma concentrations of the samples of venous blood taken from 4 dogs anaesthetized with pentobarbitone were 1.42×10^{-4} M; 1.73×10^{-4} M; 1.55×10^{-4} M and 1.73×10^{-4} M pentobarbitone. These values agree with those quoted by Altura & Altura (1975).

Table 1 The effect of acetylcholine on canine isolated trachealis muscle in the absence and presence of pentobarbitone 5×10^{-4} M

Acetylcholine (M)	*Response (g)*	*Response in presence of pentobarbitone (g)*
2.2×10^{-8}	0.4 ± 0.1	0.5 ± 0.2
8.8×10^{-8}	1.0 ± 0.3	1.1 ± 0.4
3.5×10^{-7}	2.1 ± 0.5	2.2 ± 0.6
1.4×10^{-6}	4.0 ± 0.7	4.2 ± 1.0
5.5×10^{-6}	5.4 ± 0.9	5.7 ± 1.2
2.2×10^{-5}	7.3 ± 1.2	7.6 ± 1.4
8.8×10^{-5}	9.5 ± 1.6	9.4 ± 1.6

In vitro *experiments*

Pentobarbitone 5×10^{-4} M did not affect the dose-response curve to acetylcholine (Table 1). However, 1×10^{-4}, 2.5×10^{-4} and 5×10^{-4} M pentobarbitone significantly inhibited the response of trachealis muscle to field stimulation by 29.4 ± 8.3% (mean ± s.e. mean, $n=9$), 62.7 ± 7.2% ($n=8$) and 64.4 ± 9.4% ($n=7$) respectively. The maximum inhibition occurred 30 min after administration of pentobarbitone and the tissue had recovered completely 30 min after washing the drug from the bath. Hexamethonium 3.6 μM did not affect the response to field stimulation and in its presence pentobarbitone 5×10^{-4} M still inhibited the response (Figure 2). Atropine 1.8 μM blocked completely the effects of field stimulation in all the preparations used in this study.

Discussion

Dennis & Douglas (1970) showed that pentobarbitone protected guinea-pigs from the respiratory effects of histamine aerosol. Douglas, Dennis, Ridgway & Bouhuys (1972) suggested that this inhibition may have been caused by the action of pentobarbitone on the central nervous system resulting in a disturbance of the sympathetic and parasympathetic control of the airways. Our results extend these earlier observations.

In dogs anaesthetized with pentobarbitone we found it impossible to produce large reflex increases in R_L with histamine aerosol. In contrast, in dogs anaesthetized with chloralose, histamine aerosol produced a severe bronchoconstriction which was almost entirely reflex. Electrical stimulation of both cervical vagosympathetic nerves produced small increases in R_L (0.12 kPa l^{-1} s) with pentobarbitone anaesthesia and large increases (1.2 kPa l^{-1} s) with chloralose. Nerve stimulation stopped the heart in both groups of animals. Moreover, the addition of small quantities of pentobarbitone to dogs anaesthetiz-

ed with chloralose significantly reduced the response of the airways to electrical stimulation of the cervical vagosympathetic nerves. We concluded, therefore, that pentobarbitone inhibited reflex bronchoconstriction by an action on the efferent arm of the reflex.

In dogs anaesthetized with chloralose the increase in R_L produced by electrical stimulation of the cervical vagosympathetic nerves was approximately 30% of the increase in R_L produced by 4 breaths of histamine aerosol generated from a 2.0% solution. The inability of electrical nerve stimulation to match the bronchoconstriction produced by reflex action can probably be attributed to the way in which the nerves were electrically stimulated in these experiments. Unlike the cat, the cervical vagus nerve of the dog cannot be separated from sympathetic fibres (Miller, 1964) unless the nerve is cut and a microdissection performed. In our experiments, where this was not done, sympathetic fibres were electrically stimulated with the cervical vagi. Olsen, Colebatch, Mebel, Nadel & Staub (1965) have shown, using the cat, that electrical stimulation of the cervical vagi is considerably more effective in increasing R_L than vagosympathetic stimulation.

In vitro experiments showed that pentobarbitone, at concentrations likely to be encountered in the plasma of dogs anaesthetized with this drug, did not affect the response of canine trachealis muscle to acetylcholine. Thus anaesthetic concentrations of pentobarbitone did not depress tracheal muscle directly or have an atropine-like action; however, they did significantly inhibit the response of trachealis muscle to field stimulation. The inhibition was concentration-dependent, reversible and occurred in the presence of a ganglion blocking agent. Since the response of the trachealis muscle to field stimulation was completely blocked by atropine we conclude that in this preparation pentobarbitone either prevents the release of acetylcholine from parasympathetic nerve endings, or inhibits transmission along postganglionic parasympathetic nerves. Further experiments are needed to establish which of these two proposed modes of action is operating. The barbiturates have been reported to have both local anaesthetic actions and an effect on transmission at autonomic neuroeffector junctions in a variety of tissues (Goodman & Gilman, 1975). The proposed modes of action for pentobarbitone in our experiments are therefore not novel for this class of drug.

It is interesting to note that the effects of vagal stimulation on the heart were not inhibited by pentobarbitone, indicating a selectivity of action of the drug on the parasympathetic post-ganglionic nerves in the lung.

Reflex bronchoconstriction produced by histamine aerosol is probably the result of an increase in the activity of lung irritant receptors which, via central connections, produces higher vagal tone to the airways (Mills, Sellick & Widdicombe, 1969; Gold, Kessler & Yu, 1972; Sampson & Vidruk, 1975). Sellick (1970) investigated the effect on lung irritant receptor activity in the rabbit of Nembutal (Abbott), a commercial preparation of pentobarbitone sodium dissolved in ethyl alcohol and propylene glycol. She found that Nembutal stimulated the irritant receptors and that this stimulation was caused by the solvent and not pentobarbitone, which did not affect the discharge pattern of the receptors. Sagatal, the preparation used in this study, is similar in constitution to Nembutal and therefore it is unlikely that it suppresses the activity of lung irritant receptors: more probably it stimulates them.

The role of the vagus nerves in histamine-induced bronchoconstriction in anaesthetized dogs is not clear. Gold *et al.* (1972) claim that histamine bronchoconstriction is entirely reflex, while others attribute the bronchoconstriction solely to the direct action of histamine on bronchial smooth muscle (Krell, Chakrin & Wardell, 1976). Our results may explain this particular discrepancy. Gold *et al.* (1972) used chloralose and urethane as the anaesthetic agents whereas Krell *et al.* (1976) used pentobarbitone. However, DeKock, Nadel, Zwi, Colebatch & Olsen (1966) using dogs anaesthetized with pentobarbitone, injected histamine directly into the bronchial arteries and obtained a reflex bronchoconstriction. The reason for the discrepancy between our and their observations may be related to the quantity of pentobarbitone administered because they used paralysed animals and did not indicate in their paper the maintenance dose of anaesthetic.

The changes in C_{dyn} produced by histamine aerosol were not significantly affected by cooling the vagus nerves in dogs anaesthetized with either pentobarbitone or chloralose. This finding is consistent with the view that the peripheral airways are not well innervated by the parasympathetic fibres of the autonomic nervous system (Nadel, 1974).

Although Fleisch & Calkins (1976) have pointed out some differences between the responses of rabbit tracheal and bronchial smooth muscle to certain pharmacological agents, the *in vitro* responses obtained in this study agree in most part with our *in vivo* observations. However, it should be noted that the highest dose of pentobarbitone used *in vitro* (5×10^{-4}M) inhibited the response to field stimulation of canine trachealis muscle by 64% whereas, *in vivo,* in dogs anaesthetized with this anaesthetic, histamine aerosol failed to produce a reflex bronchoconstriction. Further the increase in R_L produced by electrical stimulation of the cervical vagosympathetic nerves in these animals was only one tenth of that produced in dogs anaesthetized with chloralose.

We offer the explanation, therefore, that pentobarbitone inhibits reflex bronchoconstriction

principally, but not solely, by an action on the parasympathetic postganglionic nerve fibres, or endings in the lung. We cannot say whether this conclusion extends to other species.

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References

ALTURA, B.T. & ALTURA, B.M. (1975). Pentobarbital and contraction of vascular smooth muscle. *Am. J. Physiol.*, **229**, 1635–1640.

AMDUR, M.O. & MEAD, J. (1958). Mechanics of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.*, **192**, 364–368.

CARNEY, I., PUGH, I. & SHEARD, P. (1972). Application of an inexpensive analogue computer for the continuous determination of airways dynamic compliance and resistance. *Br. J. Pharmac.*, **46**, 578–579P.

CROSS, B.A., GUZ, S.K., JAIN, S.A., ARCHER, S., STEVENS, J. & REYNOLDS, F. (1976). The effect of anaesthesia of the airway in dog and man: a study of respiratory reflexes, sensations and lung mechanics. *Clin. Sci. mol. Med.*, **50**, 439–454.

DAIN, D., BOUSHEY, H.A. & GOLD, W.M. (1974). Apparent vagal blockade produced by an aerosol of local anaesthetic in dogs Am. Rev. resp. Dis., **109**, 693.

DAIN, D.S., BOUSHEY, H.A. & GOLD, W.M. (1975). Inhibition of respiratory reflexes by local anaesthetic aerosols in dogs and rabbits. *J. appl. Physiol.*, **38**, 1045–1050.

DEKOCK, M.A., NADEL, J.A., ZWI, S., COLEBATCH, H.J.H. & OLSEN, C.R. (1966). New method for perfusing bronchial arteries: histamine bronchoconstriction and apnea. *J. appl. Physiol.*, **21**, 185–194.

DENNIS, M.W. & DOUGLAS, J.S. (1970). Control of bronchomotor tone in spontaneously breathing unanaesthetized guinea-pigs. In *Airway Dynamics, Physiology and Pharmacology*, ed. Bouhuys, A., pp. 253–262. Springfield, Illinois: Charles C. Thomas.

DOUGLAS, J.S., DENNIS, M.W., RIDGWAY, P. & BOUHUYS, A. (1972). Airway dilation and constriction in spontaneously breathing guinea-pigs. *J. Pharmac. exp. Ther.*, **180**, 98–109.

FLEISCH, J.H. & CALKINS, P.J. (1976). Comparison of drug-induced responses of rabbit trachea and bronchus. *J. appl. Physiol.*, **41**, 62–66.

GOLD, W.M. (1973). Cholinergic pharmacology in asthma. In *Asthma: Physiology, Immunopharmacology and Treatment*. Proceedings of an International Conference on Asthma, Airle, Virgina, 1972, ed. Austen, K.F. & Lichtenstein, L.M., pp. 169–184. New York and London: Academic press.

GOLD, W.M., KESSLER, G.F. & YU, D.Y.C. (1972). Role of vagus nerves in experimental asthma in allergic dogs. *J. appl. Physiol.*, **33**, 719–725.

GOODMAN, L.S. & GILMAN, A. (1975). Hypnotics and sedatives—barbiturates. In *The Pharmacological Basis of Therapeutics*. 5th edition, pp. 102–123. New York: Macmillan.

KRELL, R.D., CHAKRIN, L.W. & WARDELL, J.R. (1976). The effect of cholinergic agents on a canine model of allergic asthma. *J. Allergy clin. Immun.*, **58**, 19–50.

MILLER, M.E. (1964). The autonomic nervous system. In *The Anatomy of the Dog*, p. 630. Philadelphia and London: Saunders.

MILLS, J.E., SELLICK, H. & WIDDICOMBE, J.G. (1969). Activity of lung irritant receptors in pulmonary microembolism, anaphylaxis and drug-induced bronchoconstrictions. *J. Physiol., London.*, **203**, 337–357.

NADEL, J.A. (1974). Factors influencing airway smooth muscle. In *Evaluation of Bronchodilator Drugs*. An Asthma Research Council Symposium, Royal College of Physicians. ed. Burley, D.M., Clarke, S.W., Cuthbert, M.E., Paterson, J.W. & Shelley, J.H., pp. 11–14. London: The Trust for Education and Research in Therapeutics.

OLSEN, C.R., COLEBATCH, H.J.H., MEBEL, P.E., NADEL, J.A. & STAUB, N.C. (1965). Motor control of pulmonary airways studied by nerve stimulation. *J. appl. Physiol.*, **20**, 202–208.

RICHARDS, I.M. & JACKSON, D.M. (1976). An investigation of histamine aerosol induced reflex bronchoconstriction in the anaesthetized dog. *Br. J. Pharmac.*, **57**, 439P.

SAMPSON, S.R. & VIDRUK, E.H. (1975). Properties of irritant receptors in canine lung. *Resp. Physiol.*, **25**, 9–22.

SELLICK, H.J. (1970). *Nervous Control of Breathing in Mammals*. Thesis, University of Oxford.

UDENFRIEND, S., DUGGAN, D.E., VASTA, B.M. & BRODIE, B.B. (1957). A spectrophotofluorometric study of organic compounds of pharmacological interest. *J. Pharmac. exp. Ther.*, **120**, 26–32.

YAMAGUCHI, B., HITZIG, B. & COBURN, R.F. (1976). Endogenous prostaglandins and mechanical tension in canine trachealis muscle. *Am. J. Physiol.*, **230**, 1737–1743.

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