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Effect of β-adrenoceptive blocking agents on the response to bronchoconstrictor drugs in the guinea-pig air overflow preparation

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Appendix describing a new modification of the air overflow method

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Summary

1. Propranolol augmented the bronchoconstrictor response to methacholine or histamine, recorded by air overflow in the anaesthetized, vagotomized guinea-pig.

2. After adrenalectomy, propranolol was still active, though less so than before.

3. In the pithed guinea-pig, there was no augmentation of the effect of bronchoconstrictors on air overflow. The action of propranolol could thus be due to the β-adrenoceptor blockade of compensatory sympathetic bronchodilator activity, as concluded by McCulloch, Proctor & Rand (1967).

4. Electrical stimulation of the thoracic region of the spinal cord of the pithed guinea-pig reduced the effect of bronchoconstrictors on air overflow. This reduction could be blocked by propranolol; practolol was much less effective.

Introduction

Propranolol has been reported to increase airway resistance in asthmatics, an effect that makes its use undesirable in such patients (McNeill, 1964; Macdonald, Ingram & McNeil, 1967; Langer, 1967). Since a bronchoconstrictor effect is not readily demonstrable with propranolol in animal preparations, an investigation was undertaken using the reported action of β-adrenoceptive blocking agents in enhancing the response of the guinea-pig air overflow preparation to bronchoconstrictors (Collier, James & Piper, 1965; Farmer & Lehrer, 1966).

Methods

Guinea-pigs of body wt 250–400 g were anaesthetized with urethane, 2·5–3 g/kg intraperitoneally, to eliminate spontaneous respiration and inflated by a Palmer respiration pump at 72 strokes/minute. The effects of drugs were studied by an air overflow method, derived from that described by Konzett & Rössler (1940). The details of the modifications that result in simplification and improved sensitivity

are described in the Appendix to this paper. Increases in air overflow were produced by intravenous injections of methacholine or histamine, and rapid recovery was effected by occluding the overflow tube for a time, as suggested by Collier, Holgate, Schachter & Shorley (1960). The animals were always bilaterally vagotomized as this improved the regularity of response.

Anaesthetized guinea-pigs were sometimes further bilaterally adrenalectomized while others were pithed under urethane anaesthesia. A No. 14 plastic covered metal knitting needle was used as the pithing rod and in some experiments this was previously stripped over the length that would lie in the thoracic region ; the nerve roots were stimulated electrically through the rod left in position after pithing, in a similar manner to that previously described for lumbar stimulation in rats (Gillespie & Muir, 1967 ; Parkes & Gerrard, 1970). A needle thrust through the skin of a hindlimb acted as an indifferent electrode. Square pulses of 2 ms duration and 80 V were used at 10 Hz for periods of 30 seconds. D-Tubocurarine, 1 mg/kg, was given intravenously to prevent skeletal muscle twitches and the animals were also given phenoxybenzamine, 2 mg/kg intravenously, to prevent the α-adrenoceptive consequences of sympathetic stimulation, particularly pulmonary vasoconstriction. Because of the antagonism of phenoxybenzamine toward histamine, methacholine was used to increase air overflow in these experiments.

Drugs used were : propranolol hydrochloride, I.C.I.; practolol (I.C.I. 50172), I.C.I.; lignocaine hydrochloride, Williams Francis, Ltd. ; histamine acid phosphate, B.D.H. ; methacholine, Koch-Light ; D-tubocurarine chloride, Burroughs Wellcome ; phenoxybenzamine hydrochloride, Smith, Kline & French Ltd.; urethane, Koch-Light.

Results

Anaesthetized guinea-pigs

Figure 1 shows the effects of repeated doses of propranolol in augmenting the effects of a small dose of histamine on air overflow. The potentiation was dose dependent.

In determining changes in sensitivity to bronchoconstrictors, three concentrations of histamine or methacholine were each given twice before, and again twice after, the dose of blocking agent. The heights of record were plotted against dose and regression lines fitted by eye. From these could be calculated the percentage change in dose required for a given effect on air overflow before and after the blocking agent. The dose-response relationship for propranolol in reducing the dose of histamine required for this effect is seen in Fig. 2.

In large doses, lignocaine was also found to augment the effects of methacholine and histamine, which the β-adrenoceptive blocking agent practolol (I.C.I. 50172) was only weakly effective.

Adrenalectomized guinea-pigs

After bilateral adrenalectomy, sensitivity to the effects of bronchoconstrictors on air overflow was somewhat enhanced. In the anaesthetized animal with intact adrenals, an increase of air overflow by 40% of the maximum obtainable required a significantly greater dose of histamine intravenously than after adrenalectomy (Table 1). Similarly, as Fig. 2 shows, propranolol was somewhat less effective in

potentiating the response to bronchoconstrictors after adrenalectomy than in animals with intact adrenals, the difference in activity being significant at the 5% level (Table 2). Adrenalectomy did not affect the activity of practolol (Fig. 2), whereas lignocaine was inactive after this procedure.

Pithed guinea-pigs

Neither propranolol nor practolol caused any enhancement of the response to methacholine or histamine in doses up to 2 mg/kg (Fig. 3). Moreover, the dose of

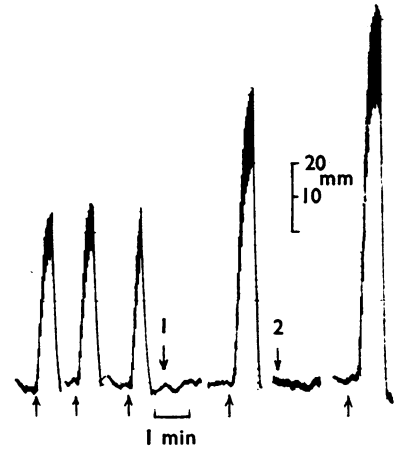

FIG. 1. Record of air overflow in an anaesthetized, vagotomized guinea-pig. At ↑, histamine 5 μg/kg intravenously; at 1, propranolol 100 μg/kg intravenously; at 2, propranolol 100 μg/kg intravenously: total dose, 200 μg/kg. Histamine was injected 2 min after propranolol and 5 min was allowed for recovery from bronchoconstriction; the chart was stopped between injections.

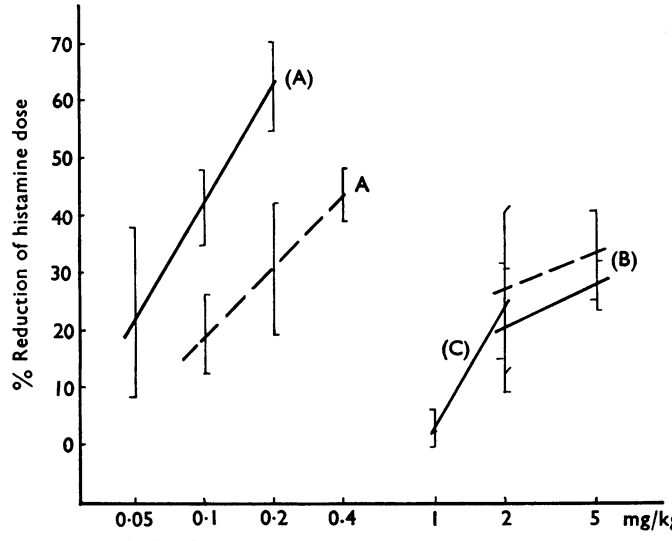

FIG. 2. Dose-response relationships for potentiation of the effect of histamine on air overflow in the anaesthetized, vagotomized guinea-pig (——) and after bilateral adrenalectomy (– – –). (A) Propranolol; (B) practolol; (C) lignocaine. Regression lines for propranolol are computed. Vertical bars extend to mean ±S.E. of values for doses used.

histamine required for a 40% increase was significantly less, at the 0·1% level, than that required in animals with an intact nervous system (Table 1).

TABLE 1. *Intravenous dose of histamine required to increase air overflow by 40% of the maximum obtainable in anaesthetized, vagotomized guinea-pigs*

	Dose (μg/kg)±S.E.	*P*
Otherwise intact	12·3±1·5	
After adrenalectomy	8·2±1·0	<0·05
After pithing	5·6±0·7	<0·001

TABLE 2. *Effect of bilateral adrenalectomy on the activity of propranolol in potentiating the increase of air overflow by histamine in the anaesthetized, vagotomized guinea-pig*

	From dose-response regression (Fig. 2)	
	$b \pm s_b$	ED50 (95% fiducial limits)
Intact adrenals	66·0±23·3	0·13 (0·095–0·175) mg/kg
After adrenalectomy	40·7±17·3	0·58 (0·32–1·03) mg/kg

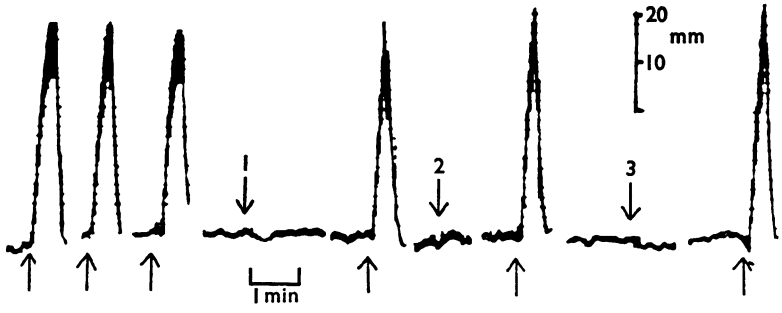

FIG. 3. Record of air overflow from a pithed guinea-pig. At ↑, methacholine, 8 μg/kg intravenously; at 1, propranolol, 0·1 mg/kg intravenously; at 2, propranolol, 0·5 mg/kg intravenously; at 3, propranolol, 2·0 mg/kg intravenously. Methacholine was injected 2 min after propranolol and 5 min was allowed for recovery from bronchoconstriction; the chart was stopped between injections.

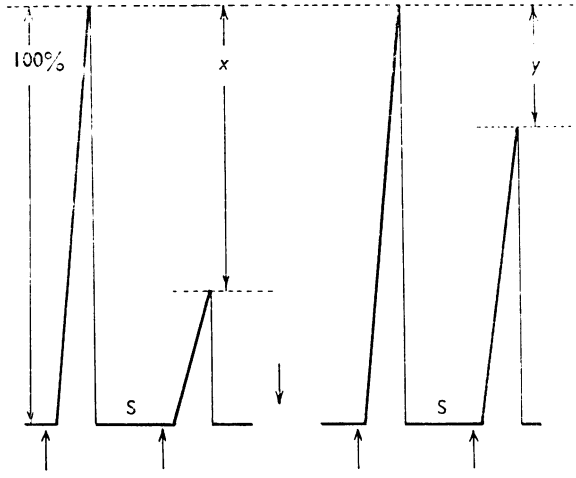

FIG. 4. Illustration of the expression of activity in reducing the effect of thoracic cord stimulation on air overflow under the influence of bronchoconstrictors. At S, electrical stimulation; at ↑, standard dose of bronchoconstrictor; at ↓, β-adrenoceptive blocking agent.

Electrical stimulation in pithed guinea-pigs

The expression of the action of β-adrenoceptor blocking agents on the effects of thoracic cord stimulation on the response to bronchoconstrictors in the pithed guinea-pig was derived as shown in Fig. 4. Three doses of methacholine were given prior to stimulation and the reduction of the effect on air overflow (x) was expressed as the percentage of the mean control response. After recovery of the response, this was again tested after giving the dose of β-adrenoceptor blocking agent and yet again after stimulation. The reduction now occurring (y) was also expressed as the percentage of control, and the difference (x-y) was then expressed as a percentage of x, the control effect of stimulation, for comparison with the dose of antagonist. Figure 5, for instance, shows that electrical stimulation for 30 s of the thoracic cord prior to a dose of histamine or methacholine that was previously effective in increasing air overflow, reduced the response and that pretreatment with propranolol

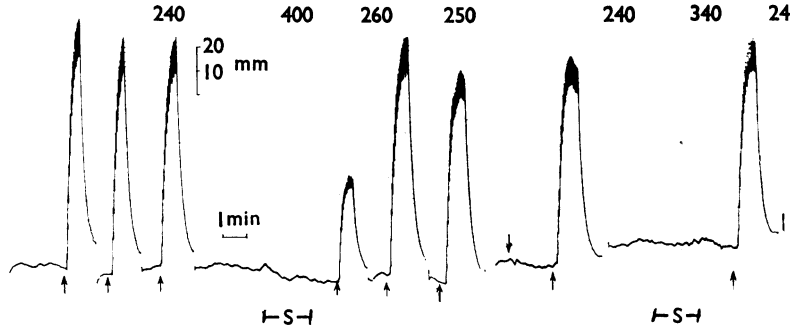

FIG. 5. Record of air overflow from a pithed guinea-pig. At S, electrical stimulation of the thoracic region of the spinal cord for 30 s; at ↑, methacoline, 6 μg/kg intravenously; at ↓, propranolol, 10 μg/kg intravenously. The figures above the record give the heart rate at that time, in beats/minute. Methacholine was injected 30 s after stimulation and 2 min after propranolol. Five minutes were allowed for recovery from bronchoconstriction; the chart was stopped between injections.

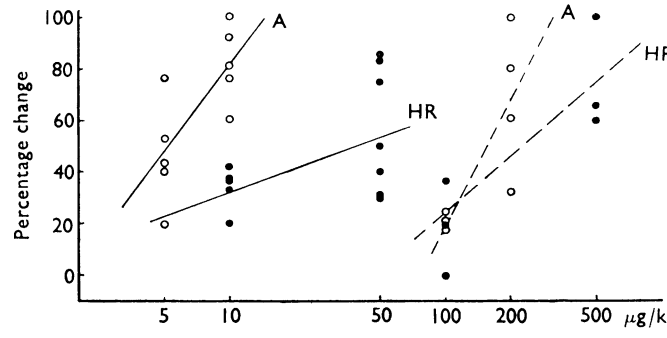

FIG. 6. Dose-response relationships for the actions of propranolol (——) and practolol (– – –) in the pithed guinea-pig on two effects of thoracic cord stimulation. (A) Decreasing the air overflow response to a constricting dose of methacholine (○); percentage change derived as shown in Fig. 4. (HR), Tachycardia (●); percentage reduction of increase in heart rate.

prevented this reduction. The effect of propranolol was dose dependent, as seen in Fig. 6; practolol was far less effective (see also Table 3). Both drugs were active in approximately one-tenth of the doses effective in potentiating bronchoconstrictors (Fig. 2). Simultaneous observation of heart rate showed that tachycardia accompanied the effect of electrical stimulation. Figure 6 shows that this was reduced by the β-adrenoceptive blocking agent, though, whereas propranolol was more effective against bronchoconstriction than against tachycardia, practolol reduced both similarly (Table 2).

Discussion

These results agree with the similar findings for the action of propranolol on bronchospasm in the guinea-pig reported by McCulloch *et al.* (1967). We agree with their conclusion that a compensating reflex, involving the sympathetic nervous system, comes into play and limits the bronchoconstrictor effect of agents like histamine. The finding that the activity of propranolol is reduced, though not abolished, by adrenalectomy suggests that adrenaline liberated from the adrenal glands may play some part in this limitation of bronchoconstrictor response. This is supported by the increase in sensitivity to bronchoconstrictors after adrenalectomy. The slight activity of lignocaine, abolished by adrenalectomy, suggests that local anaesthetic activity may be effective in blocking this contribution to compensation. Propranolol has a measurable local anaesthetic activity (Morales-Aguilerá & Vaughan Williams, 1965) that could be responsible for this component of its action, whereas the lack of effect of adrenalectomy on the action of practolol in potentiating bronchoconstrictors gives further confirmation, since this drug is devoid of local anaesthetic activity (Dunlop & Shanks, 1968).

Guinea-pigs are most sensitive to the bronchoconstrictor action of histamine or methacholine when pithed and the absence of any potentiating action of either propranolol or practolol in pithed animals confirms that the response to bronchoconstrictors is normally limited by an action of the intact nervous system that is susceptible to β-adrenoceptive blockade.

The reduction of bronchoconstrictor responses by stimulation of the thoracic outflow in the pithed animal could also be lessened by the β-adrenoceptive blocking agents, providing a demonstration of the mechanism adduced to explain potentiation in the normal animal. The evidence does not suggest a reason for the 10-fold greater effectiveness of the β-adrenoceptive blocking agents in reducing the effect of cord stimulation than in potentiating bronchoconstrictors. The weaker activity of practolol compared with that of propranolol, both in potentiating bronchocon-

TABLE 3. *Effect of propranolol and practolol on two consequences of thoracic cord stimulation in the pithed guinea-pig*

	From dose-response regressions (Fig. 6) (A)		(HR)	
	$b \pm sb$	ED50 (95% fiducial limits) μg/kg	$b \pm sb$	ED50 (95% fiducial limits) μg/kg
Propranolol	117·5±38·3	5·3 (4·3–6·65)	30·1±13·3	39·5 (26–60)
Practolol	163·9±54	155 (124–193)	72·4±19·3	221 (130–300)

(A) Reduction of the effect of methacholine on air overflow; (HR) tachycardia.

strictor responses and in blocking the effects of cord stimulation, are in general agreement with its reported activity as a β-adrenoceptive blocking agent on tracheal smooth muscle (Dunlop & Shanks, 1968). This method, therefore, appears suitable for determining the activity of β-adrenoceptive blocking agents on the bronchial tree.

The relative activities of propranolol and practolol quoted by Dunlop & Shanks for β-adrenoceptor blockade on the heart and tracheal chain do not allow a derivation of the relative activities of either drug for these two forms of muscle. The results reported here show that, in the guinea-pig, whereas propranolol shows some selectivity in that it is relatively more effective in blocking bronchodilatation than in reducing tachycardia due to cord stimulation, practolol appears to be non-selective, since it blocks both with similar effectiveness. It should be recalled, however, that selectivity has been demonstrated for practolol in man in experiments in which isoprenaline-induced bronchodilatation was unaffected, although the accompanying tachycardia was blocked (Powles, Shinebourne & Hamer, 1969).

Appendix

Describing a new modification of the air overflow method

Introduction

The method of Konzett & Rössler (1940) detects changes in resistance to inflation of the lungs *in vivo* by using the resistance of a water column to ensure their inflation to constant pressure at each delivery of a constant volume of air by the pump. Increased resistance of the lungs causes some of this air to escape through the column and, in the original method, this causes excursion of a volume recorder. A mercury valve was used to direct this excess air into the recorder and to permit its escape between inflations (Fig. 7a).

When used with guinea-pigs (e.g. Collier *et al.*, 1960) the maximum changes in air overflow obtainable are of the same order as the fluctuations occurring at each pump stroke, due to displacement of the water filling the bore of the tube immersed in the water column. Greater difference between this basal excursion and that representing a response to a bronchoconstrictor agent can be made by adjusting the mercury valve so that only a fraction of the air displaced during basal ventilation reaches the recorder (e.g. as used by Farmer & Lehrer, 1966). The volume recorder can be replaced by a pneumotachograph (Fleisch tube) recording via a differential pressure transducer (e.g. as used by Lessin & Kramer, 1968; Fig. 7b); the mercury value now serves only the function of limiting the basal excursion. Moreover, satisfactory adjustment is a critical and difficult matter.

With the method described here, this function of the mercury valve has been replaced by an integrating circuit operating on the output from the pressure transducer. This almost entirely balances out the positive- and negative-going fluctuations of signal caused by the movements of air in the Fleisch tube during basal ventilation, permitting maximal representation of the out-of-balance signals that arise from the overflow of air during bronchoconstriction.

Methods

The arrangement of pump, water column and Fleisch tube are shown in Fig. 7c. The output of the photoelectric micromanometer ('Mercury', Model M3) is fed into the circuit shown in Fig. 8, consisting of an Ancom general purpose amplifier, Model 15A-1, with a gain determined by the ratio of feedback resistance to input resistance. The addition of a capacitor in parallel with the feedback resistor pro-

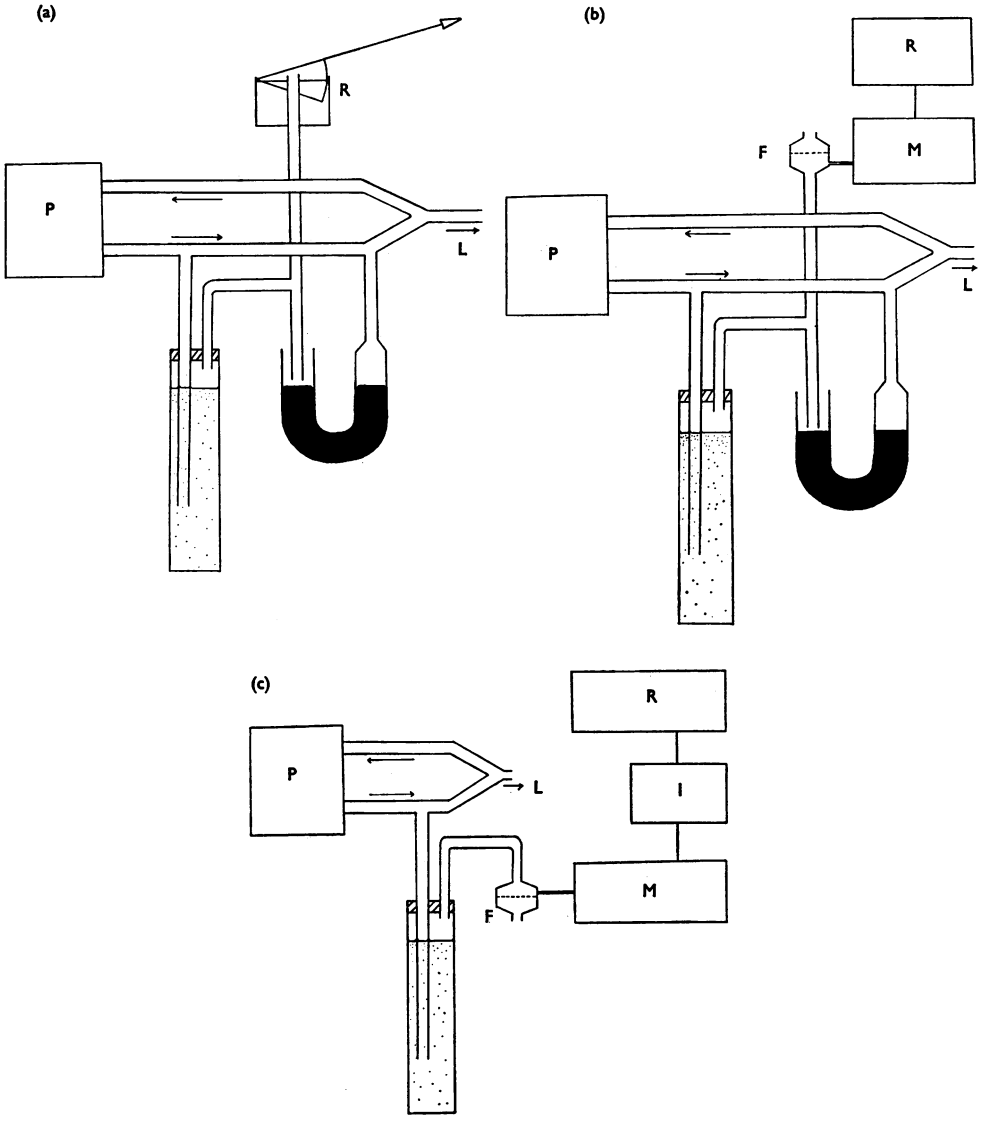

FIG. 7. Diagrams of the arrangement used to record changes in air overflow; (a) by the original method of Konzett & Rössler (1940); (b) as modified for electrical recording via a pneumotachograph and differential manometer as used by Lessin & Kramer (1968); (c) as described in the text, with integration of the differential manometer output. (P) Respiration pump; (L) tracheal cannula; (F) pneumotachograph (Fleisch tube); (M) differential manometer; (I) integrating circuit; (R) recording device.

duces integration of the input signal over a time interval dependant on the product of the parallel resistance and capacitance values (in this case about 10 s). This must be chosen in relation to the rate of ventilation, to balance damping of the basal excursions against the time to charge the integrating capacitor to a level proportional to the constrictor response.

The operation of the system in the absence of this circuit, and without the mercury valve device of earlier forms of the method, is seen in Fig. 9a, which shows the changes in resistance to inflation on intravenous injection of various doses of methacholine into a heavily anaesthetized, vagotomized guinea-pig. Figure 9b shows the record of a similar series where the integrating circuit has been used and it will be noted that the effect of integration is a greater differentiation of response from the baseline, a greater sensitivity and a greater differentiation between responses to different doses.

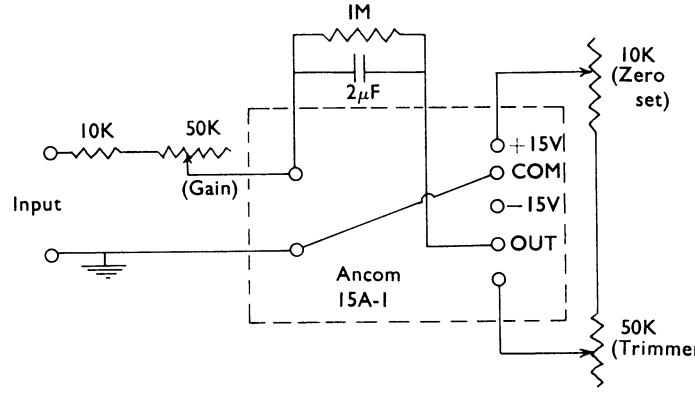

FIG. 8. Circuit diagram of the electronic integrator.

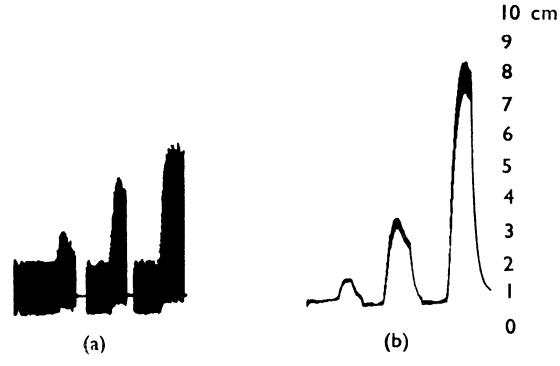

FIG. 9. Record of air overflow in an anaesthetized, vagotomized guinea-pig, injected intravenously with methacholine in successive doses of 4, 6 and 10 μg/kg using the method described in the text. (a) Without integration of the differential manometer output, recorder sensitivity, 0·1V FSD ; (b) with integration of the output, recorder sensitivity, 1·0V FSD.

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