Br. J. Pharmac. (1969), **35**, 141–151.

Salbutamol: a new, selective β-adrenoceptive receptor stimulant

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1. Salbutamol is a β-adrenoceptive receptor stimulant. Its pharmacological actions are reduced or abolished by β-receptor antagonists.

2. In anaesthetized animals, salbutamol, given intravenously, was slightly less active than isoprenaline in preventing spasm of bronchial smooth muscle but was considerably less active as a cardiac stimulant and vasodepressor substance. Its duration of action was about 2 to 3 times that of isoprenaline.

3. Salbutamol given by mouth or aerosol to conscious guinea-pigs, greatly diminished bronchospasm caused by inhalation of acetylcholine. By mouth, salbutamol was more active and had a longer duration than isoprenaline or orciprenaline without affecting heart rate. By aerosol, salbutamol was approximately 10 times as active as isoprenaline and 100 times as active as orciprenaline. Its duration of action was much longer than that of isoprenaline or orciprenaline. Only isoprenaline produced an increase in heart rate by the aerosol route.

4. On isolated guinea-pig trachea salbutamol had about 1/10 the activity of isoprenaline, on isolated atria about 1/2000.

Sympathomimetic amines are widely used for the treatment of reversible airways obstruction. Isoprenaline has been extensively used, following demonstration of its bronchodilator activity in animals (Konzett, 1940a, b) and man (Stolzenberger-Seidel, 1940). Isoprenaline is, however, effective only when given by aerosol or sublingual routes because it is rapidly inactivated by catechol-O-methyl transferase (Ross, 1963). It can also cause marked side effects due to stimulation of β-adrenoceptive receptors (β-receptors) in the cardiovascular system. More recently, orciprenaline, the resorcinol analogue of isoprenaline, was shown to be an effective bronchodilator when given by aerosol or by mouth, with some selectivity for bronchial smooth muscle (Engelhardt, Hoefke & Wick, 1961). Orciprenaline is much less potent than isoprenaline but is longer acting. The action of orciprenaline showed that a catechol structure was not essential for β-receptor stimulant activity and that selective actions on β-receptors in different organs of the body were possible. This inference was supported by Lands & Brown (1964) who, working with variously N-substituted catecholamines, concluded that the optimal structural requirements for bronchodilator activity were different from those for cardiac stimulation.

It seemed possible that other chemical groupings might subserve for the catechol function in isoprenaline-like compounds to yield β-receptor stimulant drugs, which were not substrates for catechol-O-methyl transferase and had very high selectivity for bronchial smooth muscle. Indeed, very recently, Larsen, Gould, Roth, Comer, Uloth, Dungan & Lish (1967) have shown 2′-hydroxy-5′-(1-hydroxy-2-(isopropyl-amino)ethyl) methanesulphonanilide to be a β-receptor stimulant approaching isoprenaline in potency, but its degree of selectivity for different β-receptors *in vivo* is not yet clear. Of many compounds made and tested in these laboratories, 2-*t*-butylamino-1-(4-hydroxy-3-hydroxymethyl) phenylethanol (Salbutamol, AH 3365) was chosen for detailed study because it seems to have a very selective action on bronchial muscle. Some of the pharmacological results are given in this paper. The structure of salbutamol is given in Fig. 1 together with those of isoprenaline and orciprenaline.

Methods

Anaesthetized cats and dogs

Cats of either sex, weighing 2–3 kg, were anaesthetized with chloralose, 80 mg/kg intravenously, after induction with 3% halothane in nitrous oxide and oxygen (3 : 1 v/v). Beagles of either sex were anaesthetized with pentobarbitone sodium, 30 mg/kg intravenously. Arterial blood pressure was recorded using a Devices blood pressure transducer attached to a cannula placed in the right femoral artery. Heart rate was measured with a Nielson instantaneous ratemeter triggered by the pulse pressure of the *QRS* complex of the e.c.g. Respiration was recorded via a Magill cuffed endotracheal tube and a Statham low-pressure transducer. Bronchial resistance was measured by the method of Konzett & Rössler (1940). Increases in bronchial tone were effected by stimulating the intrathoracic portion of the right efferent vagus. Peripheral blood flow in the skinned pelvic limb of the dog was measured in a cannulated portion of the femoral vein using a photocell drop counter. Drugs were injected intravenously or intra-arterially.

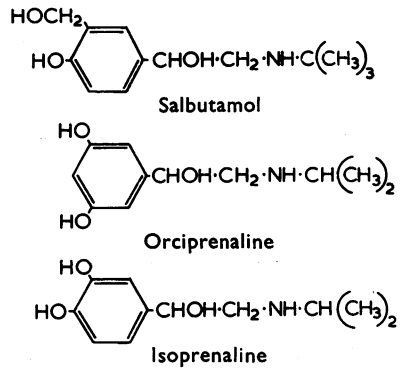

FIG. 1.

Anaesthetized guinea-pigs

Guinea-pigs were anaesthetized with urethane, 1.25 g/kg given intraperitoneally, and prepared for measurement of bronchial resistance (Farmer & Lehrer, 1966) by the method of Konzett & Rössler (1940). Temporary increases in bronchial resistance were produced by intravenous injections of acetylcholine, histamine, 5-hydroxytryptamine or bradykinin. Other drugs were given by intravenous injection or by aerosol.

Conscious guinea-pigs

Guinea-pigs were exposed to an aerosol of 1% acetylcholine in 0.9% w/v NaCl and the times for the animals to exhibit severe dyspnoea were noted. The maximum exposure time to acetylcholine was 7 min. The spray was produced by a Wright nebulizer operated at a pressure of 103.4×10^3 N/m^2 (15 lb. sq. in.). Salbutamol, orciprenaline, isoprenaline or saline were given orally or the animals were exposed for 1 min to aerosols of these substances. Groups of six animals were used for each dose of all compounds investigated and each group was exposed once only to the acetylcholine aerosol. The time interval between dosing and exposure to aerosol was varied so that the duration of drug action could be determined.

In separate experiments the effects of the above substances on heart rate were determined by the method of Farmer & Levy (1968). Guinea-pigs were trained to stand unrestrained on four plate electrodes and the e.c.g. obtained was used to trigger an instantaneous ratemeter. Drugs were administered orally or the animals were exposed for 1 min to aerosols of the compounds. Groups of four animals were used for each dose of any compound.

Isolated tissues

Tracheal chain of the guinea-pig. The tracheal chain was set up as described by Castillo & de Beer (1947) except that the tracheal rings were opened by severing the cartilage (Akcasu, 1959). The preparation was suspended in a salt solution of the following composition (g/l.): $NaHCO_3$, 1.0; NaH_2PO_4, 0.32; NaCl, 8.0; glucose, 1.0; $MgCl_2$, 0.42; KCl, 0.2; $CaCl_2$, 0.4. The solution was aerated and maintained at 37° C. Mechanical records were obtained by means of an isotonic lever.

Atria of the guinea-pig. The hearts of guinea-pigs were removed and placed in chilled McEwen solution (1956). The blood was gently squeezed from the heart; the atria were separated, cleared of fat and suspended in McEwen solution maintained at 32° C and gassed with 95% oxygen and 5% carbon dioxide. Contractions were recorded with an isometric strain gauge and Devices polygraph. Cumulative dose response curves for salbutamol, isoprenaline or orciprenaline were determined by adding geometrically increasing doses of drug without changing the bath fluid. Each concentration of drug was allowed to produce its maximum effect.

Drugs used. Acetylcholine chloride (Roche Products); isoprenaline sulphate (Burroughs Wellcome); orciprenaline (Boehringer); histamine acid phosphate (B.D.H.); bradykinin (Sandoz); 5-hydroxytryptamine creatinine sulphate (B.D.H.); pronethalol hydrochloride (I.C.I.); propranolol hydrochloride (I.C.I.).

Results

Comparison of the effects of salbutamol, isoprenaline and orciprenaline in anaesthetized animals

Antagonism of acetylcholine-induced bronchospasm in the guinea-pig

The activities of salbutamol, isoprenaline and orciprenaline against acetylcholine-induced bronchospasm in anaesthetized guinea-pigs are shown in Fig. 2 A and B. Salbutamol, 25–50 μg/kg intravenously, produced a similar intensity of response to isoprenaline, 20–40 μg/kg intravenously, but its duration of action was longer. The higher doses of salbutamol and isoprenaline had biological half-lives of about 23 and 8 min respectively. Salbutamol was about 40 times more active than orciprenaline and equiactive doses of both had similar durations of action (Fig. 2B). The results of continuous aerosol administration of salbutamol and isoprenaline are shown in Fig. 3. By this method, the maximal bronchodilator effect obtained with any concentration of the drugs used, occurred within 10–15 min. In these conditions salbutamol was about half as active as isoprenaline. Salbutamol, 50 μg/kg given intravenously, also inhibited bronchoconstriction due to intravenous injections of histamine, 5-hydroxytryptamine or bradykinin (Fig. 4). Intramuscular injection of pronethalol, 10 mg/kg, a β-receptor blocking agent, blocked these actions of salbutamol.

Actions on blood pressure, heart rate and respiration of dogs

Isoprenaline, 0.1 and 0.5 μg/kg intravenously, caused dose dependent falls in diastolic blood pressure of 40 and 55 mm Hg and increases in heart rate of 28 and 37 beats/min. The larger dose also caused cardiac arrhythmias. Respiration was

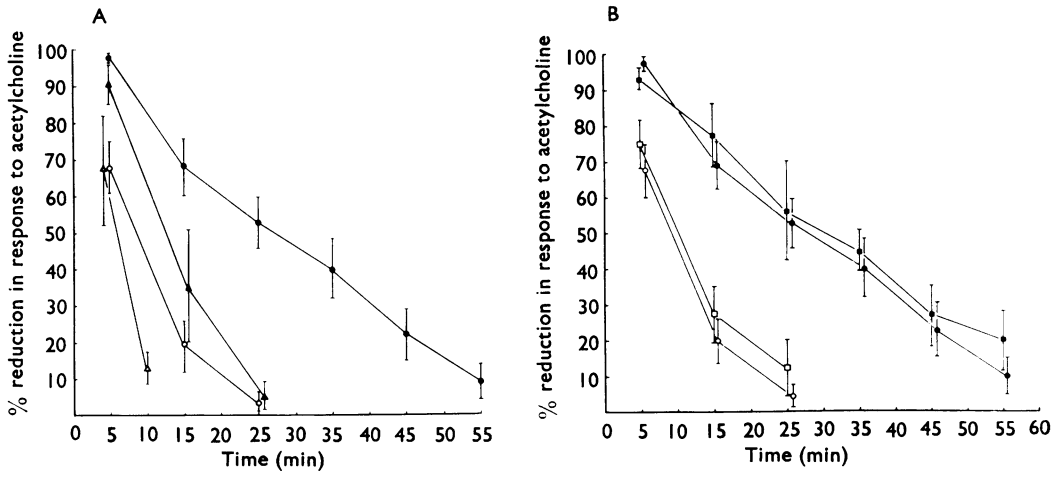

FIG. 2. Effects of salbutamol and isoprenaline (A), and salbutamol and orciprenaline (B), given intravenously, on the bronchoconstrictor action of acetylcholine (given intravenously at 5 or 10 min intervals) in anaesthetized guinea-pigs. Each point is the mean response obtained in at least four guinea-pigs ± S.E. Salbutamol, 25 μg/kg (○——○), 50 μg/kg (●——●); isoprenaline, 20 μg/kg (△——△), 40 μg/kg (▲——▲); orciprenaline, 1 mg/kg (□——□), 2 mg/kg (■——■).

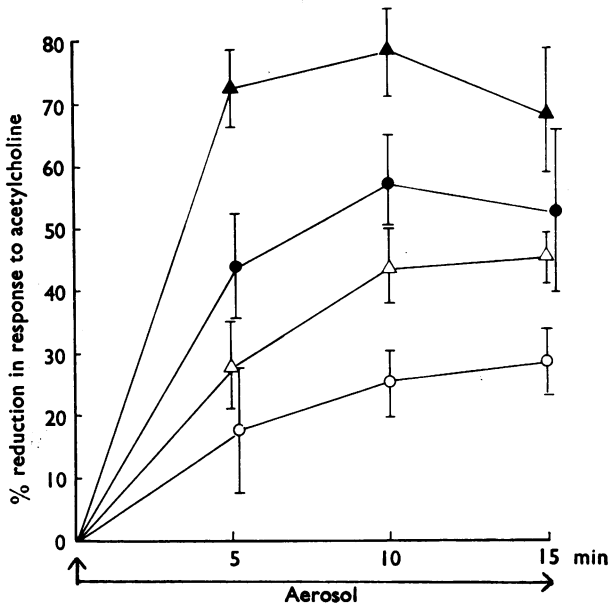

FIG. 3. Comparison of the effects of salbutamol and isoprenaline given by aerosol on the bronchoconstrictor action of acetylcholine (given intravenously at 5 min intervals) in anaesthetized guinea-pigs. Each point is the mean response obtained in at least four guinea-pigs ± S.E. The concentration of drug aerosol was isoprenaline 80 μg/ml. (△——△) and 250 μg/ml. (▲——▲); salbutamol 100 μg/kg (○——○) and 250 μg/ml. (●——●).

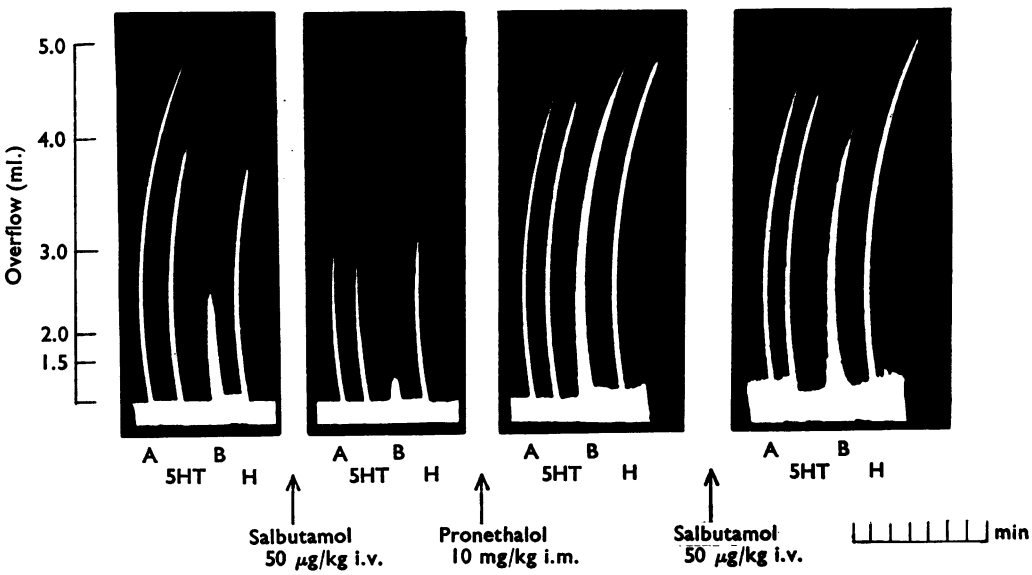

FIG. 4. Effects of salbutamol and pronethalol on the bronchoconstrictor action of acetylcholine (A, 10 μg) 5-hydroxytryptamine (5HT, 10 μg), bradykinin (B, 3 μg) and histamine (H, 5 μg) in the anaesthetized guinea-pig. The bronchoconstrictor agents were given intravenously.

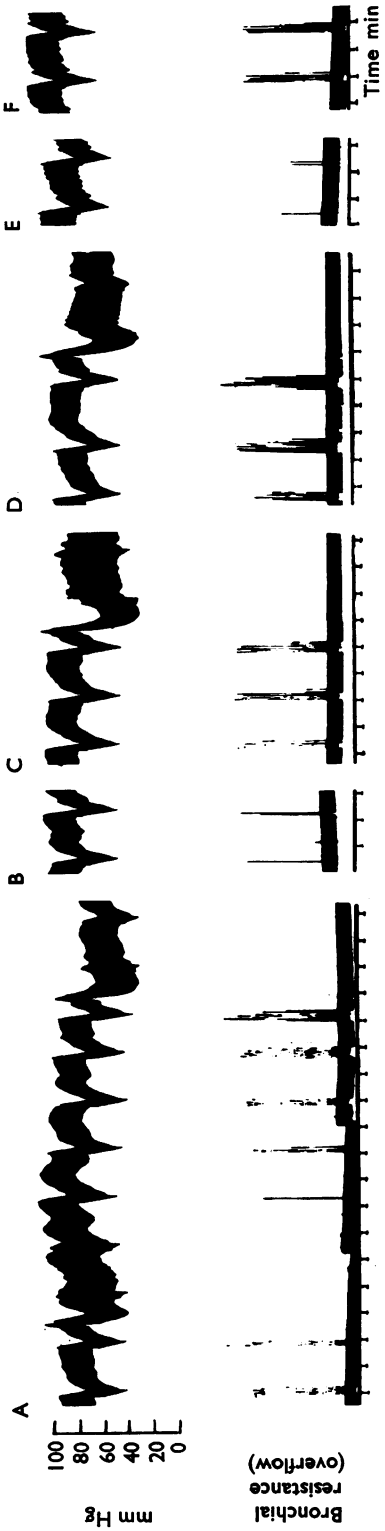

FIG. 5. Effect of salbutamol and isoprenaline given intravenously on the response of the bronchi to efferent vagal stimulation and on blood pressure and heart rate of an anaesthetized cat (male, 1.9 kg) 10 min elapsed between panels A and B, 5 min between B and C, 6 min between C and D, 45 min between D and E, and 20 min between E and F. ●, Efferent vagal stimulation 10 c/s, 1 msec, 10V, for 15 sec.

stimulated by both doses of isoprenaline. Salbutamol, 1 and 5 μg/kg, caused dose dependent falls in diastolic blood pressure of 20 and 45 mm Hg, and increases in heart rate of 7 and 24 beats/min. The duration of action of salbutamol on blood pressure and heart rate was longer than that observed with isoprenaline but no arrhythmias occurred. The larger dose of salbutamol caused respiratory stimulation. Intravenous infusion of propranolol 2 μg/kg per min, markedly reduced the effects of both drugs on blood pressure, heart rate and respiration.

Antagonism of bronchoconstriction caused by vagal stimulation in open chest cats and dogs

In anaesthetized cats both salbutamol and isoprenaline, 1–2.5 μg/kg given intravenously, abolished or reduced the response of bronchial muscle to efferent vagal stimulation but salbutamol had a longer duration of action. A typical experiment is illustrated in Fig. 5.

In anaesthetized dogs both compounds, 10–20 μg/kg intravenously, reduced or abolished bronchoconstriction caused by efferent vagal stimulation. The bronchodilatation caused by isoprenaline, unlike that due to salbutamol, was associated with a marked fall in blood pressure and increase in heart rate. In general, the cardiovascular responses of open-chested dogs to the β-receptor stimulants were less than those of animals with intact chests.

In open-chested dogs isoprenaline, 40 μg/kg intravenously, caused cardiac arrhythmias, and stimulation of the efferent vagus at this time proved fatal. Large doses of salbutamol did not affect the heart in this way.

Effects on peripheral blood flow in the skinned hind limb of the dog

Isoprenaline, 0.02 and 0.04 μg/kg injected into the femoral artery, increased femoral venous blood flow by 31 and 66% respectively. Salbutamol, 0.2 and 0.4 μg/kg, similarly injected caused increases of 48.5 and 97%. Salbutamol is therefore

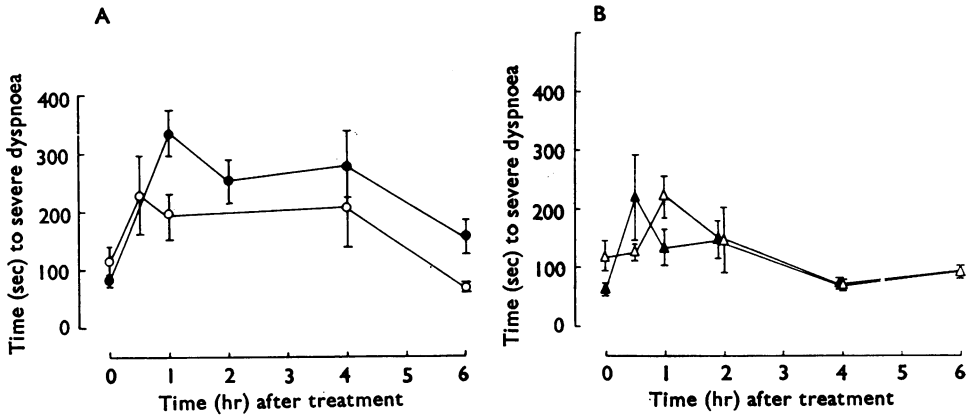

FIG. 6. Effects of (A) salbutamol 1 mg/kg (○——○) and 5 mg/kg (●——●) and (B) isoprenaline 1 mg/kg (△——△) and 5 mg/kg (▲——▲) given orally on the time elapsing before development of severe dyspnoea in conscious guinea-pigs exposed to a continuous aerosol of acetylcholine. Each point is the mean result ± S.E. for an individual group of at least six guinea-pigs.

approximately 1/10 as potent as isoprenaline as a vasodilator in skeletal muscle. Intravenous infusion of propranolol, 59 μg/kg per min for 20 min, greatly reduced the vasodilator responses to both drugs. In these conditions the increases in flow obtained with salbutamol were 13.0 and 19.4% and with isoprenaline 9.9 and 17%.

Comparison of the effects of salbutamol, isoprenaline and orciprenaline on acetylcholine-induced bronchospasm, and heart rate in conscious guinea-pigs

Oral administration. Salbutamol, 1 mg/kg, significantly increased the mean times to dyspnoea in animals exposed to acetylcholine aerosol at 0.5, 1 and 4 hr. The effects were more marked after salbutamol 5 mg/kg and persisted for at least 6 hr. The effects of isoprenaline, 1 and 5 mg/kg, were less marked and much shorter in duration. The results of these experiments are illustrated in Fig 6. Orciprenaline, 20 mg/kg, delayed the onset of dyspnoea from 42 sec to 185 sec at 30 min, and 134 sec at 1 hr but at 2 hr no drug effect was discernible. At 50 mg/kg, the time to dyspnoea increased from 42 sec to 185 sec at 30 min and to 201 sec at 1 hr but there was no increase at 2 hr. At 100 mg/kg, the only increase was from 42 sec to 92 sec at 30 min.

Salbutamol, 1 mg/kg, did not increase significantly the heart rate; 5 mg/kg caused an increase of about 30 beats/min after 30 min, which gradually declined to pre-dose level over a 6 hr period. Isoprenaline, 1 and 5 mg/kg, caused marked increases in heart rate. After the 5 mg/kg dose, heart rates exceeded 500 beats/min after 30 min and there was marked respiratory stimulation; heart rates returned to normal after 5 hr. Orciprenaline, 20 mg/kg, increased the heart rate by 34 beats/min 30 min after dosing. The rate returned to control levels within 2–3 hr. At 50 mg/kg, there was a similar increase in heart rate with a duration of 4–5 hr but none at 100 mg/kg.

Aerosol administration. The effects of salbutamol, isoprenaline and orciprenaline, given by aerosol, on the mean times to dyspnoea in animals exposed to acetylcholine aerosol are illustrated in Fig. 7. It was difficult to assess relative potencies in this test because the intensities and durations of action of the β-receptor stimulants tested varied considerably. It is obvious, however, that the protection afforded by salbutamol greatly exceeded that afforded by isoprenaline or orciprenaline. At peak intensity, salbutamol was about 10 times more potent than isoprenaline and about 100 times more potent than orciprenaline. The heart rates of animals exposed to salbutamol 0.1 and 1 mg/ml. were not significantly increased. Heart rates of animals exposed to isoprenaline aerosol 0.1 mg/ml. were unaffected but did increase after the 1 mg/ml. aerosol. Orciprenaline given by aerosol had no effect on heart rate.

Effects of salbutamol and isoprenaline on isolated tissues

Tracheal chain of the guinea-pig. Salbutamol, isoprenaline and orciprenaline were found to relax tracheal chains. Different chains varied greatly in their sensitivity to the drugs used but effective concentrations were usually found in the following ranges; salbutamol, 10–100 ng/ml.; isoprenaline, 1–10 ng/ml.; orciprenaline, 2–20 ng/ml.

Atria of the guinea-pig. Cumulative dose response curves for increases in rate and tension were obtained with salbutamol 0.4–20 μg/ml., isoprenaline 0.2–10 ng/ml. and orciprenaline 0.1–10 μg/ml. The curves for salbutamol were less steep and reached a lower maximum than those for isoprenaline or orciprenaline. The curves for salbutamol were not parallel with those of isoprenaline and orciprenaline so no simple ratio of potencies could be calculated. For a 50% increase in tension salbutamol was about 2000 times less active than isoprenaline. Propranolol, 1 and 5 ng/ml., caused concentration-dependent shifts of the dose response curves of the agonists.

Discussion

The pharmacological results show salbutamol to be a β-adrenoceptive receptor stimulant which is qualitatively and quantitatively different from isoprenaline. It is not easy to give potency ratios for salbutamol, isoprenaline and orciprenaline because different ratios are obtained from different *in vitro* and *in vivo* tests. The

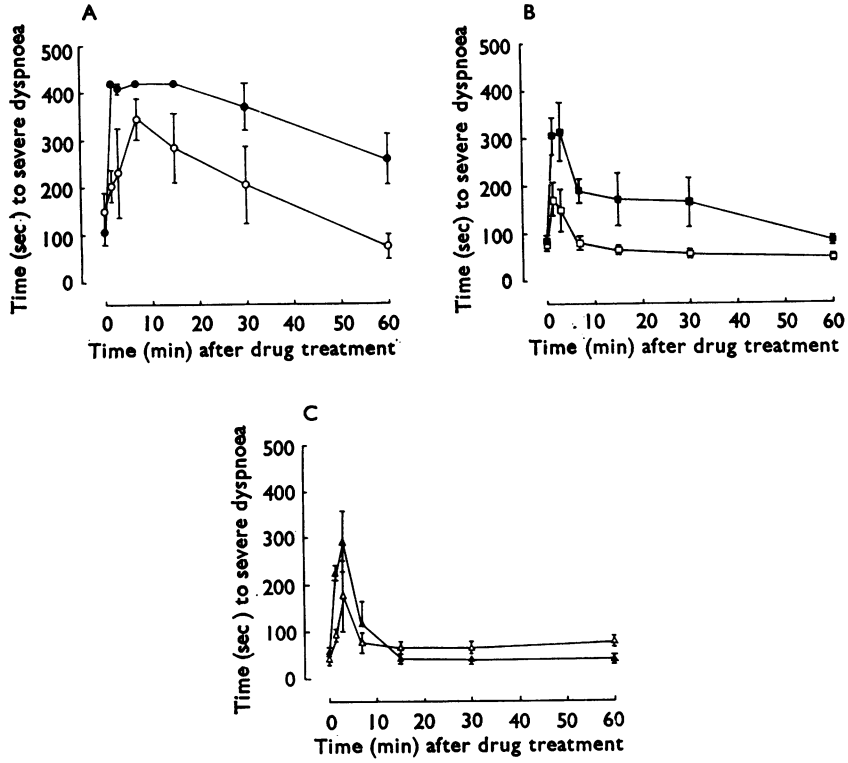

FIG. 7. Effect of (A) salbutamol 0.1 mg/ml. (○——○) and 1 mg/ml. (●——●), (B) orciprenaline 1 mg/ml. (□——□) and 10 mg/ml. (■——■) and (C) isoprenaline 0.1 mg/ml. (△——△) and 1 mg/ml. (▲——▲) given by aerosol (animals exposed for 1 min), on the time elapsing before development of severe dyspnoea in conscious guinea-pigs exposed to a continuous aerosol of acetylcholine. Each point is the mean response ± S.E. for an individual group at least six guinea-pigs.

route of administration greatly affects the results in *in vivo* tests. It is obvious, however, that salbutamol is much more active on bronchial smooth muscle than on cardiac muscle. This result is compatible with the proposal of Lands, Arnold, McAuliff, Luduena & Brown (1967) for subdividing β-receptors into β_1 receptors in cardiac muscle and β_2 receptors in bronchial smooth muscle. The degree of selectivity shown by salbutamol for β-receptors in bronchi is greater than that reported for any other β-receptor stimulant.

That inhibitory β-receptors in smooth muscle should be different from excitatory receptors in the heart is perhaps not surprising. A more surprising finding is that salbutamol acts with different intensities on vascular smooth muscle and bronchial smooth muscle both of which were supposed by Lands and his colleagues to contain β_2 receptors. For example, in dogs the vasodilator action of salbutamol is only about 1/10 that of isoprenaline but the drugs are nearly equiactive on bronchial muscle. Similar pharmacological effects in man could be important because effective bronchodilatation would be possible without unwanted cardiovascular side effects even with orally administered drug. Preliminary studies with human volunteers indicate this to be a real possibility (W. T. Simpson, personal communication). From a theoretical viewpoint, these results indicate that β_2 adrenoceptive receptors in different organs may vary sufficiently for drug-receptor complexes of differing affinities to be formed.

The duration of action of salbutamol is about twice that of isoprenaline when the drugs are given intravenously to dogs. This result is expected because salbutamol is not a substrate for catechol-O-methyl transferase the enzyme mainly responsible for limiting the action of isoprenaline. Salbutamol has been shown to be inactivated by metabolism to its phenolic glucuronide and by excretion of unchanged drug and its metabolite. The ratio of unchanged drug to metabolite varies in different species. For example, in the dog most of the drug is excreted unchanged but in the rat excretion of metabolite predominated (Brittain, Farmer, Jack, Martin & Simpson, 1968). The high oral activity of salbutamol is also possible because of its resistance to liver catechol-O-methyl transferase.

The greater potency and duration of action of salbutamol compared with isoprenaline or orciprenaline were most obvious when the drugs were given orally or by aerosol to protect guinea-pigs from bronchospasm induced by an acetylcholine aerosol. The greater selectivity of salbutamol for bronchial muscle than for cardiac muscle was also seen when the drugs were given by mouth. Only salbutamol prevented bronchospasm without an obvious increase in heart rate. Both isoprenaline and orciprenaline caused tachycardia, the severity and duration of which closely followed the protective action against acetylcholine. It is not easy to explain the relatively poorer effect of the higher doses of orciprenaline in this test. It is just possible that it is a partial agonist which in high doses acts as a blocker but there was no evidence that orciprenaline was a partial agonist on guinea-pig atria or tracheal muscle *in vitro*.

The long duration of action of small doses of salbutamol given by aerosol suggests that the drug is relatively slowly mobilized from the lungs. This conclusion was confirmed in human volunteer studies which showed that 100–200 μg of drug given by aerosol acted for longer that 4–10 mg doses given by mouth. Orally administered, the drug is short acting because it is quickly absorbed and rapidly excreted in the urine (Martin and Simpson, personal communication).

REFERENCES

AKCASU, A. (1959). The physiologic and pharmacologic characteristics of the tracheal muscle. *Archs int. Pharmacodyn. Thér.*, **122**, 201–207.

BRITTAIN, R. T., FARMER, J. B., JACK, D., MARTIN, L. E. & SIMPSON, W. T. (1968). α-[(*t*-Butylamino)methyl]-4-hydroxy-m-xylene-α^1, α^3-diol (AH 3365): a selective β-adrenergic stimulant. *Nature, Lond.*, **219**, 862–863.

CASTILLO, J. C. & DE BEER, E. J. (1947). The tracheal chain. *J. Pharmac. exp. Ther.*, **90**, 104–109.

ENGELHARDT, A., HOEFKE, W. & WICK, H. (1961). Pharmacology of the sympathomimetic amine drug 1-(3,5-dihydroxy phenyl)-1-hydroxy-2-isopropylamino ethane. *Drugs Germ.*, **4**, 121–131.

FARMER, J. B. & LEHRER, D. N. (1966). The effect of isoprenaline on the contraction of smooth muscle produced by histamine, acetylcholine or other agents. *J. Pharm. Pharmac.*, **18**, 649–656.

FARMER, J. B. & LEVY, G. P. (1968). A simple method for recording the electrocardiogram and heart rate from conscious animals. *Br. J. Pharmac. Chemother.*, **32**, 193–200.

KONZETT, H. (1940a). Neues zur Asthmatherapie. *Klin. Wschr.*, **19**, 1303–1306.

KONZETT, H. (1940b). Neue broncholytisch hochwirksame Körper der Adrenalinereihe. *Arch. exp. Path. Pharmak.*, **197**, 27–40.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch. exp. Path., Pharmak.*, **195**, 71–74, 27–40.

LANDS, A. M., ARNOLD, A., MCAULIFF, J. P., LUDUENA, F. P. & BROWN, T. G. (1967). Differentiation of receptor systems activated by sympathomimetic amines. *Nature, Lond.*, **214**, 597–598.

LANDS, A. M. & BROWN, T. G. (1964). A comparison of the cardiac stimulating and bronchodilator actions of selected sympathomimetic amines. *Proc. Soc. exp. Biol., Med.*, **116**, 331–333.

LARSEN, A. A., GOULD, W. A., ROTH, H. E., COMER, W. T., ULOTH, R. G., DUNGAN, K. W. & LISCH, P. M. (1967). Sulphonanilides. II Analogues of catecholamines. *J. med. Chem.*, **10**, 462–472.

MCEWEN, L. M. (1956). The effect on the isolated rabbit heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol., Lond.*, **131**, 678–689.

ROSS, S. B. (1963). In vivo inactivation of catecholamines in mice. *Acta Pharmac. Tox.*, **20**, 267–273.

STOLZENBERGER-SEIDEL, M. (1940). Klinische Untersuchungen zur Behandlung des Asthma bronchiale. *Klin. Wschr.*, **19**, 1306–1310.

(*Received August* 19, 1968)