Br. J. Pharmac. (1973), **48**, 144–147.

Some observations on the β-adrenoceptor agonist properties of the isomers of salbutamol

R. T. BRITTAIN, J. B. FARMER* AND R. J. MARSHALL†

Department of Pharmacology, Allen & Hanburys Limited, Ware, Herts.

Summary

1. The pharmacological activities of the optical isomers of salbutamol have been examined. (−)-Salbutamol was much more potent than (+)-salbutamol on β-adrenoceptors.

2. Both (−)- and (+)-salbutamol showed high selectivity for β-adrenoceptors in bronchial muscle compared to cardiac muscle, in this way resembling racemic salbutamol.

3. The use of isomeric activity ratio to detect differences between receptors was examined in the light of the results obtained with the isomers of salbutamol.

Introduction

Lands and his colleagues (Lands, Arnold, McAuliff, Luduena & Brown, 1967; Lands, Luduena & Buzzo, 1967) proposed that β-adrenoceptors be classified into β_1 and β_2 types. Stimulation of mammalian cardiac muscle is mediated by β_1-receptors and relaxation of bronchial, arterial and uterine muscle by β_2-receptors. Skeletal muscle also contains β_2-receptors. This classification, which was based on the relative potencies of N- and α-substituted catecholamines in different tissues, has gained more general acceptance since the discovery of highly selective β-adrenoceptor agonists (see Brittain, Jack & Ritchie, 1970). Salbutamol is a β-adrenoceptor agonist which is more active on bronchial smooth muscle than on cardiac muscle (Cullum, Farmer, Jack & Levy, 1969; Daly, Farmer & Levy, 1971). This drug contains an asymmetric centre and so it was of interest to ascertain whether activity resided mainly in the laevo (−) isomer (R configuration), as in the case with other sympathomimetic amines acting on adrenoceptors, and whether the isomers showed the same selectivity of action as the racemate. Recently Hartley & Middlemiss (1971) prepared the (−) and (+) isomers of salbutamol and this paper describes some pharmacological properties of these compounds.

Methods

Guinea-pigs of either sex, weighing 250–400 g were anaesthetized with urethane, 1·25 g/kg i.p., and prepared for measurement of bronchial resistance (Konzett & Rössler, 1940). Temporary increases in bronchial resistance, measured with a low pressure transducer connected to a Devices recorder, were produced by sub-maximal doses of acetylcholine, histamine or 5-hydroxytryptamine injected intravenously at intervals of 5 minutes. β-Adrenoceptor agonists were injected intravenously 5 min before intravenous injection of the spasmogens.

* Present address: Department of Pharmacology, Fisons Ltd., Loughborough, Leicestershire.
† Present address: University Department of Surgery, Western Infirmary, Glasgow, and Department of Pharmacology, University of Strathclyde, Glasgow.

Beagle dogs of either sex, weighing 7–12 kg, were anaesthetized with pentobarbitone sodium, 30 mg/kg intravenously. The animals were intubated with a cuffed endotracheal tube and allowed to breathe spontaneously, except in those experiments involving pulmonary resistance tests. Arterial blood pressure was measured from a carotid artery or femoral artery by means of a Bell & Howell blood pressure transducer connected to a Devices recorder. Heart rate was measured with a Devices instantaneous rate meter triggered by the pulse pressure. Measurement of pulmonary air flow, pressure and volume were made as described by Diamond (1967). Pulmonary resistance was calculated by the method of Amdur & Mead (1958). Temporary changes in pulmonary resistance were induced by intravenous injection of acetylcholine at 5 min intervals. β-Adrenoceptor agonists were given 1 min before the injection of acetylcholine. In all experiments drugs were injected intravenously through a cannula in a femoral vein.

Cumulative concentration-effect curves for the β-adrenoceptor agonists were determined for the reduction of induced tone in the isolated intact trachea preparation of the guinea-pig (Farmer & Coleman, 1970) and for their positive inotropic and chronotropic effects on isolated left and right atrial strips of the guinea-pig (Blinks, 1966) by adding geometrically increasing doses of drug to the tissue bath without changing the bathing fluid. The relative activities of the β-adrenoceptor agonists were expressed as the doses equipotent with isoprenaline (=1) at 50% of the maximal effect.

The following drugs were used: (±)-isoprenaline sulphate, (±)-salbutamol hydrochloride, (−)- and (+)-salbutamol acetate monomethanolate, pronethalol hydrochloride and propranolol hydrochloride. (±)-Isoprenaline was dissolved in 0·9% w/v NaCl solution (saline) containing ascorbic acid, 1 μg/ml. All other drugs were dissolved in saline. In the text, concentrations refer to the free base; (±)-isoprenaline is referred to as isoprenaline.

Results

Effects on bronchospasm in the anaesthetized guinea-pig

Isoprenaline and (−)-, (+)- and (±)-salbutamol given intravenously inhibited acetylcholine-induced bronchospasm in anaesthetized guinea-pigs. The dose-effect curves for the isomers and (±)-salbutamol were similar in slope and maxima to those obtained with isoprenaline. Effective intravenous doses were found in the ranges of 1–10 μg/kg for isoprenaline, 2·5–100 μg/kg for (−)- and (±)-salbutamol and 0·05–5·0 mg/kg for (+)-salbutamol. The mean equipotent doses for (−)-, (+)- and (±)-salbutamol compared to isoprenaline (=1) were 2·93 (1·30–5·29), 112 (57–233) and 3·75 (1·88–7·75) respectively. Similar orders of potencies were obtained when the spasmogen used was histamine or 5-hydroxytryptamine. The effects of (−)- and (+)-salbutamol were mediated through β-adrenoceptors because they were prevented by a prior injection of pronethalol, a β-adrenoceptor blocking agent.

Effects on acetylcholine-enhanced pulmonary resistance and on blood pressure and heart rate in the anaesthetized dog

Isoprenaline, (−)-, (+)- and (±)-salbutamol caused dose-dependent inhibition of the rise in pulmonary resistance induced by intravenous acetylcholine. The

effective intravenous dose-ranges were isoprenaline 0·1–2·0 μg/kg, (−)-salbutamol 1–4 μg/kg, (+)-salbutamol 50–400 μg/kg and (±)-salbutamol 4–20 μg/kg. The mean equipotent doses of (−)-, (+)- and (±)-salbutamol compared to isoprenaline (=1) were 2·6 (0·5–13·9), 138 (59–322) and 6·0 (3·0–12·2) respectively. Isoprenaline, (±)-salbutamol and the isomers, at the dose-ranges quoted, also caused falls in diastolic blood pressure (5–50 mmHg) but only isoprenaline caused significant increases in heart rate (10–60 beats/minute). Indeed very large doses of (−)- and (±)-salbutamol caused only small increases in heart rate; for example, the tachycardia after 100 μg/kg of either drug was only 20–25 beats/minute. (+)-Salbutamol, 400 μg/kg, had no significant effect on heart rate.

Effects on isolated tissue preparations

The effects of isoprenaline and (−)-, (+)- and (±)-salbutamol on isolated tracheal and atrial preparations of the guinea-pig are summarized in Table 1.

TABLE 1. *β-Adrenoceptor agonist activities of isoprenaline, (−)-, (+)- and (±)-salbutamol on isolated tissue preparations of the guinea-pig*

Preparation	Receptor type (Lands *et al.*, 1967)	β-Adrenoceptor agonist potency (mean equipotent doses* relevant to isoprenaline=1)			
		Isoprenaline	(−)-Salbutamol	(+)-Salbutamol	(±)-Salbutamol
Intact trachea	β_2	1	6·2 (3·2–11·9)	425 (345–522)	5·6 (3·4–9·4)
Atria (left)	β_1	1	>10,000†	V. weak negative inotropic response	>10,000†
Atria (right)	β_1	1	>10,000†	>10,000†	≏1,000†

* Calculated on weight/volume basis. † Partial agonist.

On the intact guinea-pig trachea the β-stimulants caused dose-dependent decreases in the rise of intraluminal pressure induced by transmural stimulation. The effective concentrations were: isoprenaline 5–50 ng/ml (12·9–129 nM), (−)- and (±)-salbutamol 20–300 ng/ml (84–1,260 nM) and (+)-salbutamol 1–25 μg/ml (4·20–105 μM). The concentration-effect curves for the isomers and (±)-salbutamol were similar in slope and maxima to those obtained with isoprenaline. The drug effects were antagonized by propranolol (100 ng/ml). The actions of (−)-, (+)- and (±)-salbutamol on isolated atrial preparations of the guinea-pig were quantitatively and qualitatively different from those of isoprenaline. Isoprenaline, 0·1–5 ng/ml (0·24–12·0 nM) caused dose-dependent positive chronotropic effects on cardiac muscle whereas concentrations of 0·2–20 μg/ml (0·84–84 μM) of (−), (+)- and (±)-salbutamol were required to elicit a positive chronotropic effect; even then the maximum responses to salbutamol were not more than 50% of those obtained with isoprenaline. In their inotropic actions (−)- and (±)-salbutamol were weak partial agonists; surprisingly (+)-salbutamol, 10–40 μg/ml (42–168 μM), caused a very weak negative inotropic effect (5–20%).

Discussion

Detailed studies with the isomer of isoprenaline (Beccari, Beretha & Lavendal, 1953) showed that the pharmacological activity of the racemate resides mainly in the (−) isomer. It was not surprising therefore, to find that (−)-salbutamol was

much more active than (+)-salbutamol. More interesting was the fact that both isomers resembled the racemic compound in being very much more active on tracheobronchial muscle than on cardiac muscle. This result makes it unnecessary to postulate that the relative inactivity of racemic salbutamol on cardiac muscle results from an interaction between the isomers in the tissue.

Patil (1969) has argued that if β-adrenoceptors are dissimilar, then the ratios of activity for the optical isomers of a β-adrenoceptor agonist in different tissues containing β-receptors should be different. Buckner & Patil (1971) determined isomeric activity ratios of (−)- and (+)-isoprenaline in isolated atrial and tracheal preparations of the guinea-pig and concluded that the β-adrenoceptors in these tissues were not different. While the isomeric activity ratio of (−)- and (+)-salbutamol on isolated tracheal muscle is 1:68 it is impossible to calculate a ratio on cardiac muscle because the isomers are virtually inactive on this tissue. As discussed elsewhere (Brittain *et al.*, 1970) the selectivity of action of salbutamol probably stems from the nature of the N-substituent and the 3-substituent in the phenyl ring and not the configuration about the asymmetric carbon. The results presented in this paper are in accord with Lands' proposals that β-adrenoceptors in tracheal smooth muscle can be differentiated from those in cardiac muscle.

REFERENCES

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

BECCARI, E., BERETHA, A. & LAVENDAL, J. S. (1953). Resolution of isopropyl noradrenaline into optical isomers and their pharmacological potency ratio. *Science*, **118**, 249–250.

BLINKS, J. R. (1966). Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. *J. Pharmac. exp. Ther.*, **151**, 221–235.

BRITTAIN, R. T., JACK, D. & RITCHIE, A. C. (1970). Recent β-adrenoceptor stimulants. *Adv. Drug Res.*, **4**, 197–253.

BUCKNER, C. K. & PATIL, P. N. (1971). Steric aspects of adrenergic drugs. XVI Beta Adrenergic receptors of guinea-pig atria and trachea. *J. Pharmac. exp. Ther.*, **176**, 634–649.

CULLUM, V. A., FARMER, J. B., JACK, D. & LEVY, G. P. (1969). Salbutamol: a new selective β-adrenoceptive receptor stimulant. *Br. J. Pharmac.*, **35**, 141–151.

DALY, M. J., FARMER, J. B. & LEVY, G. P. (1971). Comparison of the bronchodilator and cardiovascular actions of salbutamol, isoprenaline and orciprenaline in guinea-pigs and dogs. *Br. J. Pharmac.*, **43**, 624–637.

DIAMOND, L. (1967). Utilization of changes in pulmonary resistance for the evaluation of bronchodilator drugs. *Archs int. Pharmacodyn.*, **168**, 239–250.

FARMER, J. B. & COLEMAN, R. A. (1970). A new preparation of the isolated intact trachea of the guinea-pig. *J. Pharm. Pharmac.*, **22**, 45–50.

HARTLEY, D. & MIDDLEMISS, D. (1971). Absolute configuration of the optical isomers of salbutamol. *J. med. Chem.*, **14**, 895–896.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsandordnung zu Untersuchunger an der Bronchialmuskulatur. *Arch. exp. Path. Pharmak.*, **195**, 71–74.

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., LUDUENA, F. P. & BUZZO, H. J. (1967). Differentiation of receptors responsive to isoproterenol. *Life Sci.*, **6**, 2241–2249.

PATIL, P. N. (1969). A use of the isomeric ratio as a criterion to differentiate adrenergic receptors. *J. Pharm. Pharmac.*, **21**, 628–629.

(*Received December* 18, 1972)