Br. J. Pharmac. (1979), **66**, 229–233

EFFECTS OF β_2-ADRENOCEPTOR STIMULATION ON CARDIAC METABOLISM IN THE CONSCIOUS DOG

E. ANNE SHANAHAN, MARK L. WAHLQVIST & ERROL G. WILMSHURST

Department of Surgery & Department of Medicine, Monash University, Prince Henry's Hospital, Melbourne and Department of Endocrinology, Royal North Shore Hospital, Sydney, Australia

1 The effects of the β_2-adrenoceptor stimulant, salbutamol, on cardiac metabolism have been studied in conscious mongrel dogs. The potential effects of anaesthesia on the study of cardiac metabolism have been avoided by prior implantation of arterial (A) and coronary sinus (CS) catheters for blood sampling and a central venous catheter for infusion. Extraction of substrates for myocardial energy metabolism (C_{A-CS}) was assessed 3 to 24 days post-operatively. A 100 μg bolus of salbutamol was given followed by an infusion of 3 μg/min for 1 h.

2 Although heart rate increased significantly from 106 to 165 beats/min, fractional extraction of oxygen tended to fall from 84% to 77%. Thus an increase in coronary blood flow rather than in oxygen extraction must have maintained an oxygen supply commensurate with the salbutamol-induced tachycardia.

3 Neither C_{A-CS} glucose nor fractional glucose extraction altered significantly during salbutamol infusion despite increases in arterial concentration (C_A) of glucose and arterial insulin immunoreactivity and a decrease in C_A of free fatty acids (FFA). This suggests that an insulin-antagonistic action accompanies the infusion of salbutamol.

4 The fractional extraction of lactate increased during salbutamol infusion. In part, this may have been a reflection of a decreased myocardial extraction of FFA with salbutamol in this model.

Introduction

Afterload reduction consequent on peripheral vasodilatation has recently been under investigation as a method for decreasing the extent of myocardial infarction (Forrester, Diamond, Chatterjee & Swan, 1976; Forrester, Diamond & Swan, 1977). The β_2-adrenoceptor stimulator, salbutamol, in addition to its bronchodilator properties (Spiro, Johnson, May & Paterson, 1975), is also vasodilator (Gibson & Coltart, 1971; Turnheim & Kraupp, 1971). There is some evidence that salbutamol is of therapeutic value in low output states due to cardiogenic shock (Gibson & Coltart, 1971; Lal, Savidge, Davies, Ali & Soni, 1972; Wyse, Gibson & Branthwaite, 1974; Sharma, Goodwin & Steiner, 1976).

Unlike other adrenoceptor stimulants, salbutamol produces an increase in cardiac output without depression of cardiac efficiency (Nayler & McInnes, 1972). There may be metabolic as well as haemodynamic reasons why this may be so. It now appears that β_2-receptors are agonist for insulin secretion (Loubatières, Mariani, Sorel & Savi, 1971; Goldberg, Van As, Joffe, Krut, Bersohn & Sefrel, 1975; Massara, Fassio, Camanni & Molinatti, 1975). Salbutamol also elevates blood glucose concentrations (Goldberg *et al.*, 1975; Taylor, Gaddie, Murchison & Palmer, 1976). Combined insulin and glucose infusions have been used for the reduction of myocardial ischaemia (Chiong, West & Parker, 1976; Rogers, Stanley, Breinig, Prather, McDaniel, Moraski, Mantle, Russell & Rackley, 1976; Russell, Rogers, Mantle, McDaniel & Rackley, 1976). Thus it became of interest to examine the effects of salbutamol on cardiac metabolism *in vivo*. Any such effects could be of interest not only in cardiac disease, but also in other situations such as status asthmaticus (Spiro *et al.*, 1975) and premature labour (Korda, Lyneham & Jones, 1974), where salbutamol is given intravenously.

Methods

Adult male mongrel dogs with body weights from 18 to 25 kg were studied. Between 3 and 24 days before study, arterial (aortic via the common carotid or femoral artery), central venous (via the internal jugular or femoral vein) and coronary venous catheters, made of vinyl, were implanted. The coronary venous catheter was introduced into the coronary sinus on

0007-1188/79/060229-05 $01.00

the dorsal aspect of the heart about 2 cm from its ostium via a right thoracotomy and secured in place with a purse string suture. A suture from between two catheter collars about 2 mm apart to the epicardium prevented the catheter from slipping out of the coronary sinus. Catheter patency was maintained by daily flushes of sterile 0.17 mol/l sodium chloride solution and each line was filled with sodium heparin (100 u/ml). The dogs were studied the morning after an overnight fast. At the start of the study, the heparin solution was aspirated from the catheters; the catheters were then flushed with 17 mmol/l sodium citrate as anticoagulant in 0.17 mol/l sodium chloride. The animals stood in a loose harness for the duration of the study. Blood pressure was detected with a P23 Db Statham transducer connected to the arterial line and recorded with a Grass polygraph (Model 7).

Blood was sampled simultaneously from the arterial and coronary sinus catheters, before and during salbutamol infusion into the central venous line. Haemoglobin, haematocrit and blood gases, insulin immunoreactivity and substrate concentrations were determined. Salbutamol (Ventolin, Allen and Hanburys) was given as a 100 μg bolus followed by a constant infusion of 3 μg/min for 1 h. The bolus was given to allow a steady state to be reached earlier than it would have been with infusion alone. A Harvard infusion pump was used to deliver the salbutamol at 0.19 ml/min.

Whole blood glucose was determined with a glucose oxidase electrode system (Clark, 1971) and whole blood lactate enzymatically (Calbiochem, 1976). Plasma free fatty acids were extracted according to the method of Trout, Estes & Friedberg (1960) and determined by the colorimetric method of Duncombe (1964). Plasma insulin was measured by a double antibody radioimmunoassay based on that of Hales & Randle (1963). Blood gases were determined with an Eel-Corning model 165 Mark I blood-gas analyser. Total oxygen in blood was calculated as the sum of physically-dissolved oxygen, on the basis of the Po_2, and haemoglobin associated oxygen from a knowledge of haemoglobin concentration and oxygen saturation (Holmgren & Pernow, 1959). Oxygen dissociation curves for dog haemoglobin were those constructed by Rossing & Cain (1966). The oxygen extraction ratios (OER) for substrates examined were calculated thus:

$$OER\% = \frac{C_{A-CS}S \cdot O_2EqS}{C_{A-CS}O_2} \times 100$$

where

C_{A-CS} = arterial-coronary sinus difference in concentration, S = substrate. O_2Eq = oxygen equivalent. (Oxygen equivalent for glucose = 6; lactate = 3; FFA = 24.5).

The concentration of plasma FFA was adjusted to a concentration in whole blood by use of the haematocrit values. Negative arterio-venous differences were regarded as zero for the purpose of calculation of the OER since OER implies utilization of substrate.

Tests of statistical significance were carried out as indicated, according to Snedecor (1961).

Results

After salbutamol infusion for 1 h, heart rate had increased significantly from 106 to 165 beats/min for the 5 dogs studied (Table 1). Mean blood pressure did not change significantly (Table 1). Arterial oxygen content and the myocardial extraction of oxygen (C_{A-CS} O_2) also did not change significantly after 1 h of salbutamol infusion (Table 1).

Although glucose concentration rose significantly by 36% as a result of salbutamol infusion, there was no evidence of an increase in myocardial glucose extraction (Table 2). In this context, it is also noteworthy that plasma insulin rose from a control value of 87 ± 12 pmol/l to 1583 ± 378, 766 ± 219 and

Table 1 Effect of salbutamol on cardiac performance

	Mean blood pressure (mmHg)	*Heart rate (beats/min)*	*Oxygen (μmol/l)* C_A	C_{A-CS}
Control	133 ± 8	106 ± 5	6810 ± 570	5750 ± 570
Salbutamol	124 ± 12^{NS}	165 ± 9**	6840^{NS} ± 560	5140^{NS} ± 280

Mean ± s.e.mean of 5 studies are shown.
C_A refers to arterial concentration, C_{A-CS} to the arterial-coronary sinus difference in concentration.
Significance of difference from control is indicated by: NS i.e. $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

517 ± 192 pmol/l at 20, 40 and 60 min respectively after the start of salbutamol infusion.

Plasma FFA concentrations and myocardial FFA extractions fell significantly during salbutamol infusion (Table 2). Blood lactate concentrations tended to rise with salbutamol infusion. The fractional extraction of lactate, which was not significant under control conditions, rose significantly with salbutamol infusion (Table 2).

Overall, myocardial glucose extraction was not significantly related to arterial glucose concentration as assessed by linear regression analysis (Table 3). However, the myocardial extractions of FFA and lactate were significantly related to their respective arterial concentrations (Table 3).

Discussion

The fractional extraction of oxygen did not increase, but rather tended to fall from 84% to 77%, with salbutamol infusion, at a time when heart rate had increased by 56%. Since arterial oxygen content was unchanged, salbutamol must have allowed an increase in coronary blood flow, sufficient to supply the increased oxygen requirements which would have accompanied the tachycardia, without an increased myocardial extraction of oxygen. This is of particular interest since the physiological tachycardia of exercise does lead to an increased myocardial extraction of oxygen, as well as an increased coronary flow (Kaijser, Lassers, Wahlqvist & Carlson, 1972). These findings are consistent with those of Nayler (1971) in respect of a direct coronary vasodilator effect of salbutamol and of Nayler & McInnes (1972) in regard to the maintenance of myocardial efficiency with salbutamol. The findings also lend encouragement to the further evaluation of salbutamol as a therapeutic aid in states of low cardiac output (Gibson & Colthart, 1971; Lal *et al.*, 1972; Wyse, Gibson & Branthwaite, 1974; Sharma *et al.*, 1976).

Salbutamol induced at least three changes in the dog which might have led to increased myocardial extraction of glucose. These were increases in blood glucose concentration and plasma immunoreactive insulin and decreases in plasma FFA (Wahlqvist, Kaijser, Lassers, Löw & Carlson, 1973b). Of course, the utilization of glucose by the myocardium (C_{A-CS} × coronary flow) will have increased with salbutamol as coronary flow increased, but the relative contribution of glucose to myocardial energy utilization, as reflected in the OER for glucose, was unchanged. This suggests either that glucose, insulin and FFA are not important determinants of myocardial glucose extraction under the circumstances of these experiments or that salbutamol induces insulin-antagonism. One possibility to be considered is that salbutamol promoted the utilization of endogenous myocardial substrates, namely glycogen and triglyceride, in preference to blood glucose. This would seem unlikely as a direct effect of a β_2-agonist on an organ with a dominant β_1-receptor population. It would be of interest to know what changes salbutamol induced in blood concentrations of hormones such as glucagon, glucocorticoids, growth hormone and catecholamines which

Table 3 Dependence of a substrate's myocardial extraction on its arterial concentration

	r
Glucose	0.00^{NS} (10)
FFA	0.78** (10)
Lactate	0.97*** (8)

'*r*' is the correlation coefficent. Number of observations is shown in parentheses. Significance is indicated by: NS i.e. $P > 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2 Effect of salbutamol on myocardial substrate metabolism in the conscious dog

	Blood glucose		*Blood lactate*		*Plasma FFA*	
	Control	*Salbutamol*	*Control*	*Salbutamol*	*Control*	*Salbutamol*
C_A (μmol/l)	3530 ± 180	4810 ± 460*	440 ± 40	3840 ± 1270^{NS}	870 ± 120	500 ± 90**
C_{A-CS} (μmol/l)	210 ± 120	120 ± 40^{NS}	−30 ± 20	1270 ± 580^{NS}	260 ± 50	70 ± 30*
% extraction	5.6 ± 3.1	2.8 ± 1.1^{NS}	−18.9 ± 16	30.4 ± 4.2*	31.2 ± 6.7	12.4 ± 4.0^{NS}
OER (%)	17.8 ± 11.8	17.7 ± 6.4^{NS}	0.4 ± 0.4	53.4 ± 23.8^{NS}	57.6 ± 13.4	25.6 ± 10.8

Mean ± s.e.mean of 5 studies are shown: in the case of lactate $n = 4$. % extraction is ($C_{A/CA-CS}$) × 100; OER is the oxygen extraction ratio. For further details see footnote to Table 1.

would have been antagonistic to insulin (Imura, Karo, Ikeda, Morimoto & Yawata, 1971).

The fall in plasma FFA concentrations with salbutamol in the dog contrasts with the rise in FFA seen in man (Goldberg *et al.*, 1975). This presumably means the antilipolytic effect of stimulated insulin secretion is greater in the dog than the direct lipolytic action of salbutamol.

The increased fractional extraction of lactate with salbutamol means that, given a linear relationship between C_{A-CS} lactate and C_A lactate, a relatively greater proportion of lactate has been extracted in the presence of salbutamol. This could be due to the decreased extraction of FFA and an inverse relationship between the extractions of the two substrates (Wahlqvist, Kaijser, Lassers & Carlson, 1973a). Nevertheless, the movement of glycolytic products into the tricarboxylic acid cycle is presumably enhanced in this case. This may have therapeutic implications for the management of the ischaemic myocardium where lactate production rather than uptake is characteristic.

The technical assistance of Miss Diane Newnan and Mr John Standing is gratefully acknowledged. This study was supported by a grant-in-aid from the National Heart Foundation.

References

CALBIOCHEM (1976). *Rapid Lactate Reagents*. Doc. No. L03053. La Jolla, California: Calbiochem.

CHIONG M.A., WEST R. & PARKER J.O. (1976). The protective effect of glucose-insulin-potassium on the response to atrial pacing. *Circulation*, **54**, 37–46.

CLARK L.C. JR. (1971). A new family of polarographic electrodes for the measurement of glucose, ethanol, amino acids and other oxidase substrates. *Proc. Int. Union Physiol. Sciences*, **9**, 115.

DUNCOMBE W.G. (1964). The colorimetric micro-determination of non-esterified fatty acids in plasma. *Clin. Chim. Acta*, **9**, 122–125.

FORRESTER J.S., DIAMOND G., CHATTERJEE K. & SWAN H.J.C. (1976). Medical therapy of acute myocardial infarction by application of hemodynamic subsets. *N. Engl. J. Med.*, **295**, 1356–1362; 1404–1413.

FORRESTER J.S., DIAMOND G.A. & SWAN H.J.C. (1977). Correlative classification of clinical and hemodynamic function after acute myocardial infarction. *Am. J. Cardiol*, **39**, 137–145.

GIBSON D.G. & COLTART D.J. (1971). Haemodynamic effects of intravenous salbutamol in patients with mitral valve disease: comparison with isoprenaline and atropine. *Post-grad. med. J.*, Suppl. **47**, 40–44.

GOLDBERG R., VAN AS M., JOFFE B.I., KRUT L., BERSOHN I. & SEFTEL H.C. (1975). Metabolic responses to selective β-adrenergic stimulation in man. *Post-grad. med. J.*, **51**, 53–58.

HALES C.N. & RANDLE P.J. (1963). Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.*, **88**, 137–146.

HOLMGREN A. & PERNOW B. (1959). Spectrophotometric measurements of oxygen saturation of blood in the determination of cardiac output. A comparison with the Van Slyke method. *Scand. J. clin. Lab. Invest.*, **11**, 143–149.

IMURA H., KATO Y., IKEDA M., MORIMOTO M. & YAWATA M. (1971). Effect of adrenergic-blocking or-stimulating agents on plasma growth hormone, immunoreactive insulin, and blood free fatty acid levels in man. *J. clin. Invest.*, **50**, 1069–1079.

KAIJSER L., LASSERS B.W., WAHLQVIST M.L. & CARLSON L.A. (1972). Myocardial lipid and carbohydrate metabolism in fasting men during prolonged exercise. *J. appl. Physiol.*, **32**, 847–858.

KORDA A.R., LYNEHAM R.C. & JONES W.P. (1974). The treatment of premature labour with intravenously administered salbutamol. *Med. J. Aust.*, **1**, 744–746.

LAL S., SAVIDGE R.S., DAVIES D.M., ALI M.M. & SONI V. (1972). Intravenous salbutamol and cardiogenic shock. *Br. med. J.*, **1**, 853–854.

LOUBATIÈRES A., MARIANI M.M., SOREL G. & SAVI L. (1971). The action of β-adrenergic blocking and stimulating agents on insulin secretion. Characterisation of the type of β-receptor. *Diabetologia*, **7**, 127–132.

MASSARA F., FASSIO V., CAMANNI F. & MOLINATTI G.M. (1975). Salbutamol-induced increase in plasma insulin in man. *Horm. Metab. Res.*, **7**, 94.

NAYLER, W.G. (1971). Some observations on the pharmacological effects of salbutamol with particular reference to the cardiovascular system. *Post-grad. med. J.*, Suppl. **47**, 16–21.

NAYLER W.G. & MCINNES I. (1972). Salbutamol and orciprenaline-induced changes in myocardial function. *Cardiovasc. Res.*, **6**, 725–733.

ROGERS W.J., STANLEY A.W., BREINIG J.B., PRATHER J.W., MCDANIEL H.G., MORASKI R.E., MANTLE J.A., RUSSELL R.O. & RACKLEY C.E. (1976). Reduction of hospital mortality rate of acute myocardial infarction with glucose-insulin-potassium infusion. *Am. Heart J.*, **92**, 441–454.

ROSSING R.G. & CAIN S.M. (1966). A nomogram relating PO_2, pH, temperature and hemoglobin saturation in the dog. *J. appl. Physiol.*, **21**, 195–201.

RUSSELL R.O., ROGERS W.J., MANTLE J.A., MCDANIEL M.G. & RACKLEY C.E. (1976). Glucose-insulin-potassium, free fatty acids and acute myocardial infarction in man. *Circulation*, **52**, Suppl. I, 207–209.

SHARMA B., GOODWIN J.F. & STEINER R.E. (1976). An improvement in cardiac function by salbutamol in severe heart muscle disease. *Circulation*, **54**, Suppl. II, 11–31.

SNEDECOR G.V. (1961). *Statistical Methods*. Ames, Iowa: Iowa State College Press.

SPIRO S.G., JOHNSON A.J., MAY C.S. & PATERSON J.W. (1975). Effects of intravenous injection of salbutamol in asthma. *Br. J. clin. Pharmac.* **2**, 495–501.

TAYLOR M.W., GADDIE J., MURCHISON L.E. & PALMER K.N.V. (1976). Metabolic effects of oral salbutamol. *Brit. med. J.* **1**, 22.

TROUT D.L., ESTES E.H. & FRIEDBERG S.J. (1960). Titration of free fatty acids of plasma: a study of current methods and a new modification. *J. Lip. Res.*, **1**, 199–202.

TURNHEIM K. & KRAUPP O. (1971). Pulmonary and systemic circulatory effects and β-adrenergic selectivity of hexoprenaline, salbutamol, oxyfedrine and isoproterenol. *Eur. J. Pharmac.*, **15**, 231–239.

WAHLQVIST M.L., KAIJSER L., LASSERS B.W. & CARLSON L.A. (1973a). Fatty acid as a determinant of myocardial substrate and oxygen metabolism in man at rest and during prolonged exercise. *Acta. med. scand.*, **193**, 89–96.

WAHLQVIST M.L., KAIJSER L., LASSERS B.W., LÖW H. & CARLSON L.A. (1973b). The role of fatty acid and of hormones in the determination of myocardial carbohydrate metabolism in healthy fasting men. *Eur. J. clin. Invest.*, **3**, 57–65.

WYSE S.D., GIBSON D.G. & BRANTHWAITE M.A. (1974). Haemodynamic effects of salbutamol in patients needing circulatory support and open-heart surgery. *Br. med. J.*, **II**, 502–503.

(Received August 1, 1978.
Revised November 1, 1978.)