CHARACTERIZATION OF β -ADRENOCEPTOR SUBTYPE MEDIATING THE METABOLIC ACTIONS OF SALBUTAMOL

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1 The following four intravenous treatments were administered in a balanced, randomized, Latin square design to four healthy volunteers: (1) saline injection (10 min); salbutamol infusion ($0.15 \mu\text{g kg}^{-1} \text{ min}^{-1}$ for 60 min) (sS), (2) propranolol injection (0.3 mg kg^{-1} for 10 min); salbutamol infusion (pS), (3) practolol injection (2 mg kg^{-1} for 10 min); salbutamol infusion (PS) and (4) saline injection; saline infusion (ss).

2 Heart rate was recorded and venous blood taken for estimation of insulin, glucose and potassium before and after each injection (0 and 12–15 min) and at various times during the infusion (30, 45, 60 and 75 min).

3 The mean, peak % heart rate changes from baseline, control were +65.8%, +23.0%, –10.9% and –12.1%, the mean, peak % glucose changes, +61.0%, +7.1%, +3.6% and +6.9%, and the mean, peak % insulin changes, +298%, +28%, –28% and –43% during the infusions for sS, PS, ss and pS respectively.

4 The mean serum potassium levels before the injections and at the completion of the infusions were 3.98 and 3.08, 4.03 and 3.63, 4.07 and 4.15, and 4.03 and 4.13 meq/l for sS, PS, ss and pS respectively.

5 Propranolol completely abolished the cardiac and metabolic responses of salbutamol.

6 Practolol produced only partial cardiac β -adrenoceptor blockade, but almost completely inhibited the metabolic actions of salbutamol.

Introduction

β_2 -adrenoceptor stimulating drugs, e.g. salbutamol, by intravenous administration are currently used in the treatment of severe asthma (Fitchett, McNicol & Riordan, 1975; Marlin & Turner, 1975a & b; May, Paterson, Spiro & Johnson, 1975; Spiro, Johnson, May & Paterson, 1975; Williams, Parrish & Seaton, 1975), cardiovascular shock (Wyse, Gibson & Branthwaite, 1974) and premature labour (Liggins & Vaughan, 1973; Korda, Lineham & Jones, 1974). Numerous metabolic effects including insulin release, hypokalaemia, lipolysis and glycogenolysis, which have important clinical implications, have been reported with salbutamol in animals (Loubatieres, Mariani, Sorel & Savi, 1971) and man (Goldberg, van As, Joffe, Krut, Bersohn & Seftel, 1975; Massara, Fassio, Camanni & Molinatti, 1975; Leitch, Clancy, Costello & Flenley, 1976; Taylor, Gaddie, Murchison & Palmer, 1976; Wang & Clansen, 1976). Although insulin release is mediated by stimulation of pancreatic β -adrenoceptors (Porte, 1967; Imura, Kato, Ikeda, Morimoto & Yawata, 1971; Loubatieres *et al.*, 1971; Goldberg *et al.*, 1975), the subtype of β -adrenoceptor, i.e. β_1 or β_2 , involved has not been clearly defined.

The purpose of this study was to compare the blocking effects of propranolol and practolol on the

metabolic actions of intravenous salbutamol in man in order to determine the nature of the β -adrenoceptors mediating these responses.

Methods

Four healthy males, aged from 30–33 years, and weighing from 68–82 kg, volunteered for this study after informed consent was obtained. The study was a single-blind, balanced, randomized, Latin square design. The four treatments were as follows: (1) saline injection, salbutamol infusion (sS), (2) propranolol injection, salbutamol infusion (pS), (3) practolol injection, salbutamol infusion (PS) and (4) saline injection, saline infusion (ss). The doses of the intravenous injections over ten minutes were: (1) propranolol, 0.3 mg kg^{-1} , (2) practolol, 2 mg kg^{-1} and (3) 0.9% sodium chloride injection B.P. and for the intravenous infusions over 60 min, (1) salbutamol, $0.15 \mu\text{g kg}^{-1} \text{ min}^{-1}$ and (2) 0.9% sodium chloride injection B.P. All injections and infusions were made up to equal volumes respectively with 0.9% sodium chloride injection B.P. and the infusions were delivered by a constant infusion pump (Infusomat, B. Braun Melsungen).

Following an overnight fast the subjects rested for 15 min after which baseline, control heart rate was recorded with an electrocardiogram and a venous blood sample collected. The 10 min intravenous injection was then given (0–10 min) and further heart rate recording and venous blood sample taken (12–15 min). The 60 min intravenous infusion was administered (15–75 min) during which heart rate was recorded and venous blood collected at 30, 45, 60 and 75 min. All venous blood samples were assayed for insulin (immunoassay), glucose (glucose oxidase method) and potassium (autoanalyser method).

The results were submitted to statistical analysis using the paired Student's *t*-test.

Results

The mean $\pm$ s.e.mean control, baseline values before the treatments for the four subjects for heart rate were 75 ± 8 , 68 ± 5 , 73 ± 6 and 74 ± 9 beats/min, for plasma insulin, 13 ± 4 , 15 ± 3 , 22 ± 4 and 13 ± 8 mU/ml, for blood glucose, 74 ± 3 , 73 ± 4 , 74 ± 4 and 77 ± 7 mg% and for serum potassium, 4.0 ± 0.2 , 3.8 ± 0.1 , 4.0 ± 0.3 and 4.1 ± 0.2 meq/l with treatments sS, pS, PS and ss respectively. There were no significant differences between these mean, control values before the treatments for the four parameters. The results for heart rate, blood glucose and plasma insulin were expressed as % changes from the control, baseline values before the treatments. The mean $\pm$ s.e.mean % heart rate, blood glucose and plasma insulin changes from control and the mean $\pm$ s.e.mean serum potassium levels for all four treatments with the four subjects are shown in Table 1.

During the intravenous infusions, the mean % heart rate increase from control for sS was significantly greater than for ss, pS and PS at all times from 30–75 min ($P < 0.05$) and that for PS significantly greater than for ss (45–75 min) and pS (60 and 75 min) ($P < 0.05$). There was a substantial mean % insulin increase from control during the salbutamol infusion in sS, significantly greater than in ss and pS at 45 min ($P < 0.05$). During the infusions, the individual, peak % insulin increases from control with sS ranged from 121% to 650% and for any subject, the peak % insulin increases from control with the other treatments were 13%, 11% and 54% for ss, pS and PS respectively. There was a significant difference between the mean % glucose increase from control for sS from 45–75 min and those for ss, pS and PS ($P < 0.05$). Serum potassium levels during the infusion in sS were significantly less than in ss at 60 and 75 min ($P < 0.05$) and in pS from 45–75 min ($P < 0.05$). There were no other statistically significant differences found with any of the four parameters at any of the times as shown in Table 1.

All subjects reported palpitations and muscle tremor during the infusion in treatment sS.

Discussion

This study demonstrates that salbutamol by the intravenous route in man produces a substantial increase in insulin secretion from the pancreas. This insulin release appears to be mediated by direct stimulation of pancreatic β -adrenoceptors rather than being secondary to blood glucose elevation which was relatively less in magnitude and slower in onset. These results with salbutamol are similar to those with isoprenaline, a non-selective β -adrenoceptor agonist, and are in agreement with those of other workers (Goldberg *et al.*, 1975; Massara *et al.*, 1975; Leitch *et al.*, 1976; Taylor *et al.*, 1976). The pronounced fall in serum potassium during the salbutamol intravenous infusion results from a movement of potassium from extracellular to intracellular compartments secondary to stimulation of insulin release and glycolysis. This hypokalaemic effect of salbutamol has important clinical implications because falls in serum potassium may provoke cardiac dysrhythmias particularly in patients receiving digoxin. Thus, whenever intravenous infusions of salbutamol are administered, serum potassium should be monitored.

The term 'selectivity' when used with respect to β -adrenoceptor agonists and antagonists refers to their relative predilection in having preferential actions on β -adrenoceptor-mediated effects in different tissues. With agonists, 'selectivity' relates to their ability to stimulate bronchial β -adrenoceptors and to spare those in the heart, whereas with antagonists the opposite phenomenon is implied. Salbutamol is known to preferentially stimulate the β_2 -subtype of adrenoceptors, e.g. bronchial, skeletal muscular and peripheral vascular, and to spare the β_1 -subtype in the heart compared with isoprenaline (Brittain, Farmer, Jack, Martin & Simpson, 1968). Because of the selective action of salbutamol some authors have suggested that insulin release stimulated by this drug is mediated by β_2 -adrenoceptors (Massara *et al.*, 1975; Taylor *et al.*, 1976). However, salbutamol by intravenous infusion is capable of producing considerable dose-related tachycardia. The selectivity of salbutamol appears to be lost when administered intravenously and when the dosage is increased (Marlin & Turner, 1975b). Dunlop & Shanks (1968) showed that the heart rate increase produced by β -adrenoceptor agonists by the parenteral route as well as being due to direct cardiac β_1 -adrenoceptor stimulation might also be related to reflex tachycardia initiated by a fall in mean blood pressure due to stimulation of β_2 -adrenoceptors in vascular smooth muscle. Moreover, the tachycardia produced by intravenous isoprenaline even after pre-treatment with atropine was only incompletely blocked by practolol, a widely recognized selective β_1 -adrenoceptor antagonist (Brick, Hutchison, McDevitt, Roddie & Shanks, 1968). In this study there was a significant positive correlation between the heart rate increase and both insulin release ($r = 0.82$, $P < 0.001$)

and glucose increase ($r = 0.65$, $P < 0.005$) produced by salbutamol in sS. If cardiac β_1 -adrenoceptor stimulation is the predominant mechanism for the production of this tachycardia, these positive correlations would suggest that the metabolic responses are mediated by β_1 -adrenoceptors. On the other hand, if it could be shown that significant insulin release without an accompanying tachycardia could be produced by smaller doses of salbutamol than those used in this study, this would favour involvement of β_2 -adrenoceptors. However, Goldberg *et al.* (1975) showed that 50 μg salbutamol by intravenous bolus injection produced both a small insulin release and tachycardia. Furthermore, studies in asthmatic patients have demonstrated that with smaller in-

travenous doses of salbutamol than in the present study, tachycardia of some degree accompanies significant bronchial β_2 -adrenoceptor stimulation, i.e. bronchodilation (Marlin & Turner, 1975a & b; May *et al.*, 1975; Spiro *et al.*, 1975). Thus, salbutamol does not possess sufficient β_2 -adrenoceptor selectivity by the intravenous route to test the above hypothesis and even in small doses, stimulation of peripheral vascular β_2 -adrenoceptors would still occur.

In this study, blockade of the heart rate response with practolol was incomplete, particularly during the latter half of the salbutamol infusion. This might be related to uninhibited peripheral vascular β_2 -adrenoceptor responses. Alternatively, compared with propranolol, the dose of practolol might have been less

Table 1 The mean $\pm$ s.e.mean % heart rate, blood glucose and plasma insulin changes from control, baseline values before each treatment and serum potassium levels before and after each treatment for the four subjects for ss, sS, PS and pS.

Time (min)	Treatments			
	ss	sS	PS	pS
% heart rate change from control				
15	-3.7 ± 4.7	-4.0 ± 2.3	-1.4 ± 1.3	-8.5 ± 3.3
30	-4.1 ± 3.6	32.5 ± 7.2	1.3 ± 5.4	-12.1 ± 4.4
45	-5.9 ± 3.0	47.2 ± 9.2	8.4 ± 2.5	-10.6 ± 4.8
60	-10.9 ± 5.9	61.2 ± 7.9	9.9 ± 4.0	-10.6 ± 4.0
75	-6.5 ± 4.5	65.8 ± 12.5	23.0 ± 6.0	-9.0 ± 4.4
% blood glucose change from control				
15	1.0 ± 5.7	-1.2 ± 1.7	-3.8 ± 3.8	1.6 ± 2.7
30	3.6 ± 4.7	4.6 ± 4.2	-3.9 ± 4.2	2.6 ± 3.0
45	1.9 ± 3.1	28.3 ± 5.2	-0.8 ± 3.8	2.2 ± 3.0
60	-1.0 ± 4.7	48.3 ± 11.1	4.8 ± 5.8	6.9 ± 3.0
75	1.5 ± 3.8	61.0 ± 12.8	7.1 ± 8.3	4.8 ± 3.0
% plasma insulin change from control				
15	4 ± 11	19 ± 18	-34 ± 14	-21 ± 10
30	-28 ± 20	95 ± 40	-10 ± 25	-28 ± 5
45	-15 ± 10	172 ± 77	-14 ± 20	-40 ± 8
60	-15 ± 13	298 ± 108	5 ± 19	-43 ± 18
75	-7 ± 7	295 ± 123	28 ± 24	-19 ± 14
Plasma potassium levels (meq/l)				
0	4.07 ± 0.23	3.98 ± 0.23	4.03 ± 0.28	4.03 ± 0.26
15	3.88 ± 0.13	4.05 ± 0.20	3.85 ± 0.21	3.88 ± 0.14
30	3.90 ± 0.11	3.80 ± 0.15	3.95 ± 0.23	4.03 ± 0.22
45	3.93 ± 0.14	3.45 ± 0.14	3.85 ± 0.21	4.03 ± 0.13
60	3.98 ± 0.17	3.43 ± 0.27	3.83 ± 0.23	4.10 ± 0.09
75	4.15 ± 0.23	3.08 ± 0.19	3.63 ± 0.19	4.13 ± 0.06

Treatments ss: saline injection, saline infusion

sS: saline injection, salbutamol infusion ($0.15 \mu\text{g kg}^{-1} \text{ min}^{-1}$ for 60 min)

PS: practolol injection (2 mg kg^{-1}), salbutamol infusion

pS: propranolol injection (0.3 mg kg^{-1}), salbutamol infusion

The intravenous injections were administered for 10 min (0–10 min) and the constant intravenous infusions for 60 min (15–75 min)

potent in producing cardiac β_1 -adrenoceptor blockade. Kumana, Marlin, Kaye & Smith (1974) showed that whereas propranolol (0.3 mg kg^{-1}) and practolol (2 mg kg^{-1}) produced similar reductions in exercise heart rate, practolol had a definite sparing effect on bronchial β_2 -adrenoceptors as judged by changes in peak expiratory flow rate at rest and during vigorous exercise. In the present study, propranolol, a non-selective β -adrenoceptor antagonist, abolished the salbutamol-induced insulin release, glucose elevation and potassium fall. With practolol, these metabolic effects were almost completely inhibited. This finding again suggests that the metabolic effects are mediated by β_1 -adrenoceptors. However, it is not possible to distinguish with certainty as to whether the minimal metabolic changes following practolol are due to incomplete β_1 -adrenoceptor blockade or to a sparing effect on β_2 -adrenoceptors. Loubatieres *et al.* (1971) in dogs showed that propranolol but not practolol inhibited insulin release induced by both isoprenaline and salbutamol suggesting involvement of β_2 -

adrenoceptors, although no indication was given as to the cardiac responses with these drugs. Goldberg *et al.* (1975) demonstrated in man that insulin release following salbutamol was blunted by practolol and suggested that both β_1 and β_2 -adrenoceptors might be involved. These authors also concluded that the mild glucose rise induced by salbutamol which was suppressed by practolol also might be mediated by β_1 -adrenoceptors by stimulation of liver glycogenolysis.

In conclusion, the results of this study suggest that the insulin release produced by salbutamol is mediated by β_1 -adrenoceptors. However, until drugs are discovered which demonstrate *in vivo* in man more selectivity towards the possible subtypes (β_1 and β_2) of β -adrenoceptors, the assessment of the particular receptor which mediates a given response of the adrenergic nervous system will remain unclear.

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