

## DIFFERENTIATION OF METABOLIC ADRENOCEPTORS

T. LOAKPRADIT & R. LOCKWOOD

Department of Pharmacology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok, Thailand

- 1 Cardiovascular and metabolic responses to intravenous infusion of isoprenaline were measured in fasted, anaesthetized cats.
- 2 Isoprenaline ( $0.2 \mu\text{g kg}^{-1} \text{min}^{-1}$  for 15 min) decreased diastolic blood pressure and increased heart rate, blood glucose, blood lactate and plasma free fatty acids.
- 3 Oxprenolol (0.5 mg/kg) antagonized all cardiovascular and metabolic effects of isoprenaline non-selectively.
- 4 Para-oxprenolol (0.25 mg/kg) and practolol (4 mg/kg) antagonized the effects of isoprenaline on heart rate and free fatty acids selectively.
- 5 H 35/25 ((1-(4-methylphenyl)-2-isopropyl aminopropanol) hydrochloride, 3 mg/kg) antagonized the effects of isoprenaline on blood pressure, glucose and lactate selectively.
- 6 It is concluded that metabolic adrenoceptors are differentiated into subtypes similar to those mediating cardiostimulation and vasodilatation.

### Introduction

It has been proposed that  $\beta$ -adrenoceptors mediating pharmacological responses to sympathomimetic agonists are differentiated into two subtypes designated ' $\beta_1$ ' and ' $\beta_2$ ' (Lands, Arnold, McAuliff, Luduena & Brown, 1967). This classification is based on the relatively selective activity of certain agonists in producing these responses. Thus, adrenoceptors on which noradrenaline has strong activity, such as those mediating cardiostimulation, have been assigned to the  $\beta_1$  subtype. Conversely, adrenoceptors on which noradrenaline has weak activity, including those mediating vasodilatation and bronchodilatation, have been assigned to the  $\beta_2$  subtype. For each response, the activity of noradrenaline has been assessed in comparison with that of other agonists, primarily adrenaline and isoprenaline, which appear to have strong activity on both the  $\beta_1$  and  $\beta_2$  subtypes of adrenoceptor.

The selective agonist activity of noradrenaline has also been used to classify  $\beta$ -adrenoceptors mediating metabolic responses. Thus, due to the strong activity of noradrenaline on lipolytic adrenoceptors mediating release of free fatty acids (FFA), these adrenoceptors have been assigned to the  $\beta_1$  subtype (Lands *et al.*, 1967). Conversely, liver and muscle glycogenolytic adrenoceptors mediating release of glucose and lactate, respectively, have been assigned to the  $\beta_2$  category due to the relatively weak activity of noradrenaline in producing these responses (Arnold, McAuliff, O'Connor & Brown, 1968).

Metabolic responses to adrenaline and isoprenaline have also been compared with those produced by

selective non-catecholamine agonists. Tazolol, or ITP, selectively produces cardiostimulation (Strosberg & Roszkowski, 1972); this agonist increases plasma FFA but does not increase glucose or lactate (Lockwood & Lum, 1974). Tazolol is therefore a presumably  $\beta_1$  selective agonist which supports the Lands' classification of  $\beta$ -adrenoceptors. However, the actions of presumably  $\beta_2$  selective agonists are less consistent with this classification. Thus, salbutamol selectively produces bronchodilatation (Cullum, Farmer, Jack & Levy, 1969) yet this agonist is more active in producing increases in plasma FFA and less active in producing increases in blood lactate (Kelly & Shanks, 1975) than would be expected in a selective  $\beta_2$  agonist.

In the present study, an attempt has been made to provide evidence regarding differentiation of metabolic adrenoceptors by the use of  $\beta$ -adrenoceptor antagonists which are presumed to be non-selective or selective for either cardiac or vascular  $\beta$ -adrenoceptors. Cardiovascular and metabolic responses to intravenous infusion of isoprenaline have been measured in control animals. These responses have been compared to those observed in animals pretreated with practolol, an antagonist which selectively blocks isoprenaline-induced cardiostimulation (Dunlop & Shanks, 1968) and with H 35/25, an antagonist which selectively blocks isoprenaline-induced vasodilatation (Levy & Wilkenfeld, 1969). Responses to isoprenaline have also been examined in animals pretreated with oxprenolol or para-oxprenolol, antagonists which have

been reported to have dissimilar selectivity for cardiovascular  $\beta$ -adrenoceptors (Vaughan Williams, Bagwell & Singh, 1973).

## Methods

Fasted adult cats (2.0–4.1 kg) of either sex were anaesthetized with intravenous pentobarbitone sodium 30 mg/kg. Supplemental intravenous doses of pentobarbitone were administered as needed to maintain anaesthesia. Both vagosympathetic nerve trunks were ligated and severed in the neck. Systolic and diastolic blood pressures (BP) were monitored via a Statham PT 06 transducer from a catheter inserted into a carotid artery. Heart rate was determined by counting the number of systolic peaks on the blood pressure recording. Recordings were made on a Hewlett Packard Model 7700 polygraph. A femoral artery and vein were cannulated to obtain arterial blood samples and to administer drugs, respectively. The trachea was cannulated and animals were allowed to respire spontaneously. Following completion of surgical procedures, heparin sodium (250 units/kg) was administered intravenously to prevent clotting of blood samples.

Arterial blood glucose levels were determined by the *o*-toluidine method of Dubowski (1962). Arterial blood lactate levels were determined by the lactate dehydrogenase method of Marbach & Weil (1967). Arterial plasma FFA were measured by the copper complexation method of Duncombe (1964).

The following drugs were used: ( $\pm$ )-isoprenaline hydrochloride (Winthrop Laboratories, Inc.), oxprenolol hydrochloride (Ciba-Geigy, Ltd.), para-oxprenolol hydrochloride (Ciba-Geigy, Ltd.), practolol (Imperial Chemical Industries, Ltd.) and H 35/25 (1-(4-methylphenyl)-2-isopropyl aminopropanol hydrochloride, Axel Kistner AB). The dosages of isoprenaline and practolol are in terms of the bases; the dosages of oxprenolol, para-oxprenolol, H 35/25 are in terms of the salts. Isoprenaline was administered by continuous intravenous infusion; infusions were performed with a Harvard Model 975 infusion pump calibrated to deliver a volume of 0.5 ml of isoprenaline solution in 0.9% w/v NaCl solution (saline) per minute. Oxprenolol, para-oxprenolol, practolol and H 35/25 were administered over a 5 min period which was completed 15 min before beginning infusion of isoprenaline. Arterial blood samples of 2.5 ml each were removed for analysis immediately before administration of isoprenaline and  $\beta$ -adrenoceptor antagonists and at 5, 10 and 15 min after beginning isoprenaline infusion.

Statistical analyses were performed using Student's *t* test for group comparison or the *t* test for paired data as appropriate. A *P* value of less than 0.05 was considered to be significant.

## Results

### *Cardiovascular responses to isoprenaline*

Heart rate and diastolic BP were recorded just before (time 0) and at 5, 10 and 15 min after beginning isoprenaline infusion. Isoprenaline significantly increased heart rate and decreased diastolic blood pressure; these effects persisted throughout the period of infusion but were maximal at 5 min after beginning isoprenaline administration (Table 1).

### *Metabolic responses to isoprenaline*

Blood glucose, blood lactate and plasma FFA levels were determined in samples taken just before (time 0) and at 5, 10 and 15 min after beginning isoprenaline infusion. Isoprenaline significantly increased glucose, lactate and FFA levels; these effects were progressive throughout the period of infusion and therefore were maximal at 15 min after beginning isoprenaline administration (Table 1).

### *Cardiovascular and metabolic responses to $\beta$ -adrenoceptor antagonists*

Cardiovascular and metabolic parameters were measured just before and at 15 min after administration of oxprenolol, para-oxprenolol, practolol or H 35/25. The doses of antagonists used were those found to produce significant antagonism of at least one of the cardiovascular responses to isoprenaline infusion. Oxprenolol, para-oxprenolol and practolol produced small but significant increases in blood glucose (Table 2). As shown in Table 2, para-oxprenolol was the only antagonist which produced significant changes in diastolic blood pressure, blood lactate and plasma FFA while oxprenolol was the only antagonist which produced a significant change in heart rate.

### *Effect of antagonists on cardiovascular responses to isoprenaline*

Heart rate and diastolic BP were recorded at time 0, which was 15 min after administration of antagonists and just before beginning isoprenaline, and at 5, 10 and 15 min after beginning isoprenaline infusion. Maximal changes in heart rate and diastolic BP produced by isoprenaline in animals pretreated with antagonists were compared with maximal changes produced in non-pretreated controls (Table 3). Oxprenolol non-selectively antagonized both the increase in heart rate and the decrease in blood pressure produced by isoprenaline in controls. In contrast to this, para-oxprenolol and practolol selectively antagonized the isoprenaline-induced increase in heart rate while H 35/25 selectively

**Table 1** Cardiovascular and metabolic responses to isoprenaline ( $0.2 \mu\text{g kg}^{-1} \text{min}^{-1}$ )

Time	Heart rate (beats/min)	Diastolic BP (mmHg)	Blood glucose (mg %)	Blood lactate (mg %)	Plasma FFA (mEq/l)
0	$112 \pm 10$	$88 \pm 8$	$85 \pm 5$	$18.6 \pm 1.3$	$0.44 \pm 0.03$
5	$162 \pm 9^*$	$51 \pm 7^*$	$126 \pm 7^*$	$23.8 \pm 0.9^*$	$0.57 \pm 0.05^*$
10	$161 \pm 9^*$	$62 \pm 10^*$	$156 \pm 7^*$	$31.1 \pm 0.7^*$	$0.71 \pm 0.10^*$
15	$159 \pm 8^*$	$66 \pm 9^*$	$167 \pm 3^*$	$33.1 \pm 0.6^*$	$0.77 \pm 0.09^*$

Number of animals = 7. Time represents time in minutes after beginning isoprenaline infusion.

\* Significantly different from initial (time 0) values ( $P < 0.05$ ).

**Table 2** Cardiovascular and metabolic responses to  $\beta$ -adrenoceptor antagonists

Antagonist	n	$\Delta$ Heart rate (beats/min)	$\Delta$ Diastolic BP (mmHg)	$\Delta$ Blood glucose (mg%)	$\Delta$ Blood lactate (mg%)	$\Delta$ Plasma FFA (mEq/l)
Oxprenolol (0.5 mg/kg)	3	$-28 \pm 10^*$	$-5 \pm 3$	$6 \pm 1^*$	$1.6 \pm 0.9$	$-0.01 \pm 0.01$
Para-oxprenolol (0.25 mg/kg)	5	$3 \pm 5$	$-8 \pm 3^*$	$28 \pm 3^*$	$1.5 \pm 0.5^*$	$0.03 \pm 0.01^*$
Practolol (4 mg/kg)	6	$-13 \pm 9$	$-8 \pm 4$	$15 \pm 4^*$	$1.0 \pm 0.78$	$0.003 \pm 0.008$
H 35/25 (3 mg/kg)	6	$-5 \pm 3$	$-3 \pm 3$	$1 \pm 1$	$0.1 \pm 0.1$	$0.01 \pm 0.01$

n = number of animals.  $\Delta$  represents change from initial values.

\* Significant change from initial values ( $P < 0.05$ ).

**Table 3** Effect of antagonist pretreatment on cardiovascular and metabolic responses to isoprenaline ( $0.2 \mu\text{g kg}^{-1} \text{min}^{-1}$ )

Pretreatment	n	$\Delta$ Heart rate (beats/min)	$\Delta$ Diastolic BP (mmHg)	$\Delta$ Blood glucose (mg%)	$\Delta$ Blood lactate (mg%)	$\Delta$ Plasma FFA (mEq/l)
None (controls)	7	$50 \pm 6$	$-37 \pm 4$	$82 \pm 5$	$14.5 \pm 4.0$	$0.33 \pm 0.08$
Oxprenolol (0.5 mg/kg)	3	$11 \pm 1^*$	$-3 \pm 2^*$	$28 \pm 5^*$	$2.7 \pm 1.4^*$	$0.01 \pm 0.01^*$
Para-oxprenolol (0.25 mg/kg)	5	$14 \pm 2^*$	$-42 \pm 8$	$77 \pm 5$	$13.2 \pm 0.6$	$0.04 \pm 0.02^*$
Practolol (4 mg/kg)	6	$20 \pm 1^*$	$-40 \pm 4$	$79 \pm 5$	$11.9 \pm 1.9$	$0.02 \pm 0.01^*$
H 35/25 (3 mg/kg)	6	$43 \pm 3$	$-2 \pm 4^*$	$16 \pm 6^*$	$0.9 \pm 0.8^*$	$0.28 \pm 0.07$

n = number of animals.  $\Delta$  represents maximal change from initial values.

\* Significantly different from response to isoprenaline in Controls ( $P < 0.05$ ).

antagonized the isoprenaline-induced decrease in diastolic blood pressure.

*Effect of antagonists on metabolic responses to isoprenaline*

Blood glucose, blood lactate and plasma FFA levels were determined in samples taken at time 0, which was 15 min after administration of antagonists and just before beginning isoprenaline infusion, and 5, 10 and 15 min after beginning isoprenaline infusion. Maximal changes in blood glucose, blood lactate and plasma FFA produced by isoprenaline in animals pretreated with antagonists were compared with maximal changes produced in non-pretreated controls (Table 3). Oxprenolol non-selectively antagonized all metabolic responses to isoprenaline. In contrast to this, para-oxprenolol and practolol selectively antagonized the isoprenaline-induced increase in plasma FFA while H 35/25 selectively antagonized the isoprenaline-induced increases in blood glucose and blood lactate.

### Discussion

The metabolic adrenoceptors mediating isoprenaline-induced increases in blood glucose, blood lactate and plasma FFA appear to consist exclusively of  $\beta$ -adrenoceptors. Accordingly, it has been established that these metabolic responses to isoprenaline can be completely blocked by the  $\beta$ -adrenoceptor antagonist, propranolol (Barrett & Cullum, 1968). Unlike isoprenaline, other sympathomimetic agonists such as adrenaline are associated with an  $\alpha$ -adrenoceptor-mediated inhibition of pancreatic insulin release which presumably contributes to the increase in blood glucose produced by these agonists (Altszuler, Gottlieb, Steel & Bjerknes, 1974). Although the increase in blood glucose produced by isoprenaline appears to be due primarily to stimulation of liver glycogenolysis, it is possible that this response is enhanced by simultaneous stimulation of muscle glycogenolysis. This is consistent with the observation that elevation of blood lactate (by i.v. infusion of sodium lactate) significantly increases blood glucose (Miller, Issekutz, Paul & Rodahl, 1964). This is probably due to the increased availability of lactate for liver gluconeogenesis. On the other hand, elevation of blood lactate or glucose may diminish increases in plasma FFA due to stimulation of lipolytic adrenoceptors by facilitating re-esterification to triglycerides (Miller *et al.*, 1964). This interaction may partly account for the relatively large increases in plasma FFA characteristic of noradrenaline, an agonist which produces relatively small increases in blood lactate or glucose.

Previous attempts to differentiate metabolic

adrenoceptors have been concerned primarily with differences between activities of noradrenaline and other sympathomimetic agonists in producing responses attributable to glycogenolysis or lipolysis. For example, noradrenaline has been demonstrated to have very weak activity compared to isoprenaline in producing stimulation of muscle glycogenolysis (Fleming & Kenny, 1964). Since many responses to noradrenaline can be potentiated by pretreatment of animals with neuronal uptake blockers such as cocaine, it may be argued that weak activity of noradrenaline relative to other catecholamines is due to rapid inactivation of noradrenaline by neuronal uptake. However, except for increases in blood glucose, metabolic responses to noradrenaline are only slightly potentiated by cocaine pretreatment and such potentiation requires doses of cocaine greater than those necessary to potentiate other noradrenaline responses such as vasoconstriction (Hardman & Mayer, 1965). Furthermore, neuronal uptake inactivation does not explain the relatively strong activity of noradrenaline compared to other catecholamines in producing increases in plasma FFA (Barrett, 1965). In general, studies with noradrenaline and other selective agonists appear to support the concept that metabolic adrenoceptors are heterogeneous and are differentiated into subtypes.

Relatively few studies have used selective antagonism of metabolic responses to sympathomimetic agonists to differentiate metabolic adrenoceptors. Practolol, a presumably  $\beta_1$  selective antagonist, selectively inhibited adrenaline-induced increases in plasma FFA in one study but the dose of practolol which was used did not alter adrenaline-induced increases in heart rate (Cash, Woodfield & Allan, 1970) and therefore blockade of the 'cardiac'  $\beta$ -adrenoceptor subtype is problematical. Butoxamine, an antagonist which selectively antagonizes isoprenaline-induced vasodilatation (Parratt & Wadsworth, 1970), also antagonizes isoprenaline-induced increases in glucose and lactate (Salvadore, April & Lemberger, 1966). However, butoxamine has also been reported to block increases in plasma FFA produced by sympathomimetic agonists (Colville, Lindsay & Burns, 1965) and this action would not be expected from a presumably  $\beta_2$  selective antagonist. Blockade of metabolic responses to sympathomimetic agonists by other selective  $\beta$ -antagonists such as para-oxprenolol or H 35/25 does not appear to have been investigated.

In the present investigation, non-selective antagonism of cardiostimulant and vasodilator responses to isoprenaline by oxprenolol was accompanied by antagonism of all metabolic responses measured. In contrast to this, selective antagonism of the cardiostimulant responses to isoprenaline by para-oxprenolol or practolol was accompanied by selective antagonism of the effect of

isoprenaline on plasma FFA. The apparent failure of practolol pretreatment to antagonize glycogenolytic adrenoceptors is consistent with the results of Pogatsa, Kaldor & Vizi (1970) who found that practolol, unlike the non-selective  $\beta$ -antagonist propranolol, did not inhibit stimulation of glycogenolysis or glucose release by isoprenaline in perfused rat liver. The selective antagonism of the vasodilator response to isoprenaline by H 35/25 was accompanied by selective antagonism of the effects of isoprenaline on blood glucose and lactate. The apparent failure of H 35/25 to antagonize lipolytic adrenoceptors is in contrast to the blockade of FFA release reported for butoxamine and may reflect a greater degree of selectivity of H 35/25 for  $\beta_2$ -adrenoceptors compared to butoxamine.

The results of the present investigation support the concept that metabolic adrenoceptors generally classified as ' $\beta$ ' are heterogeneous and can be further differentiated into subtypes comparable to those proposed by Lands *et al.* (1967). Thus, lipolytic adrenoceptors appear to resemble adrenoceptors mediating cardiostimulant responses to sympathomimetic agonists whereas glycogenolytic adreno-

ceptors appear to resemble adrenoceptors mediating vasodilator responses. The results of this study do not necessarily confirm the  $\beta_1$ ,  $\beta_2$  classification of Lands *et al.* since other tissues may contain  $\beta$ -adrenoceptors which are not equivalent to those mediating either cardiostimulation or vasodilatation. However, the ability of selective  $\beta$ -adrenoceptor antagonism to dissociate lipolysis from other adrenergic responses has potential clinical value in view of evidence that increases in FFA may facilitate production of cardiac arrhythmias by adrenoceptor agonists (Kurien, Yates & Oliver, 1971). Due to the apparent differentiation of adrenoceptors, it is possible that selective lipolytic blockade can be achieved without interfering with desired responses (such as bronchodilatation) to therapeutic doses of sympathomimetic drugs.

The authors would like to express their appreciation to Prof. H. Brunner and Dr K. Scheibli of Ciba-Geigy, Ltd. (Basle) for the generous gift of oxprenolol and paroxprenolol and to M. Wharmby of ICI Ltd. for the generous gift of practolol.

## References

- ALTSZULER, N., GOTTLIEB, B., STEELE, R. & BJERKNES, C. (1974). Metabolic effects of stimulation of alpha and beta adrenergic receptors in the dog. *Fedn Proc.*, **33**, 486.
- ARNOLD, A., McAULIFF, J., O'CONNOR, W. & BROWN, T. (1968). Beta-2 receptor mediated glycogenolytic responses to catecholamines. *Arch. int. Pharmacodyn.*, **176**, 451-457.
- BARRETT, A. (1965). The mobilization of free fatty acids in response to isoprenaline in the rat. *Br. J. Pharmac. Chemother.*, **25**, 545-566.
- BARRETT, A. & CULLUM, V. (1968). The biological properties of the optical isomers of propranolol and their effects on cardiac arrhythmias. *Br. J. Pharmac.*, **34**, 43-55.
- CASH, J., WOODFIELD, D. & ALLAN, A. (1970). Adrenergic mechanisms in the systemic plasminogen activator response to adrenaline in man. *Br. J. Haemat.*, **18**, 487-494.
- COLVILLE, K., LINDSAY, L. & BURNS, J. (1965). Metabolic and other blocking properties of butoxamine. *Pharmacologist*, **7**, 750.
- CULLUM, V., FARMER, J., JACK, D. & LEVY, G. (1969). Salbutamol: a new selective beta adrenoceptive receptor stimulant. *Br. J. Pharmac.*, **35**, 141-151.
- DUBOWSKI, K. (1962). An *o*-toluidine method for body fluid glucose determination. *Clin. Chem.*, **8**, 215-235.
- DUNCOMBE, W. (1964). Colorimetric determination of non-esterified fatty acids in plasma. *Clin. Chim. Acta*, **9**, 122-125.
- DUNLOP, D. & SHANKS, R. (1968). Selective blockade of adrenoceptive beta receptors in the heart. *Br. J. Pharmac.*, **32**, 201-218.
- FLEMING, W. & KENNY, A. (1964). The effect of fasting on the hyperglycaemic responses to catecholamines in rats. *Br. J. Pharmac.*, **22**, 267-274.
- HARDMAN, J. & MAYER, S. (1965). The influence of cocaine on some metabolic effects and the distribution of catecholamines. *J. Pharmac. exp. Ther.*, **148**, 29-39.
- KELLY, J. & SHANKS, R. (1975). Metabolic and cardiovascular effects of isoprenaline and salbutamol in the dog. *Br. J. Pharmac.*, **53**, 157-162.
- KURIEN, V., YATES, P. & OLIVER, M. (1971). The role of free fatty acids in the production of ventricular arrhythmias after coronary artery occlusion. *Eur. J. Clin. Invest.*, **1**, 225-231.
- LANDS, A., ARNOLD, A., McAULIFF, J., LUDUENA, F. & BROWN, T. (1967). Differentiation of receptor systems activated by sympathomimetic amines. *Nature, Lond.*, **214**, 597-598.
- LEVY, B. & WILKENFELD, B. (1969). An analysis of selective beta receptor blockade. *Eur. J. Pharmac.*, **5**, 227-234.
- LOCKWOOD, R. & LUM, B. (1974). Selective adrenergic stimulant actions of 1-isopropylamino-3-(2-thiazoloxo)-2-propranolol (ITP) in anaesthetized cats. *Life Sci.*, **14**, 73-81.
- MARBACH, E. & WEIL, M. (1967). Rapid enzymatic measurement of blood lactate and pyruvate. *Clin. Chem.*, **13**, 314-324.
- MILLER, H., ISSEKUTZ, B., PAUL, P. & RODAHL, K. (1964). Effect of lactic acid on plasma free fatty acids in pancreatized dogs. *Amer. J. Physiol.*, **207**, 1226-1230.
- PARRATT, J. & WADSWORTH, R. (1970). Effect of selective beta adrenoceptor blocking drugs on myocardial circulation. *Br. J. Pharmac.*, **39**, 296-308.

- POGATSA, G., KALDOR, A. & VIZI, E. (1970). The inhibitory effect of sulphonylurea derivatives on liver glycogenolysis increased by catecholamines. *J. Pharm. Pharmac.*, **22**, 447-451.
- SALVADORE, R., APRIL, S. & LEMBERGER, L. (1967). Inhibition by butoxamine, propranolol and MJ 1999 of the glycogenolytic action of the catecholamines in the rat. *Biochem. Pharmac.*, **16**, 2037-2041.
- STROSBERG, A. & ROSZKOWSKI, A. (1972). A selective myocardial beta stimulant. *Fedn Proc.*, **31**, 567.
- VAUGHAN WILLIAMS, E., BAGWELL, E. & SINGH, B. (1973). Cardiospecificity of beta receptor blockade. *Cardiovasc. Res.*, **7**, 226-240.

(Received June 7, 1976.  
Revised August 1, 1976.)