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EVALUATION OF A NEW BETA-ADRENOCEPTOR AGONIST, PROCATEROL, BASED ON METABOLIC RESPONSES IN RATS

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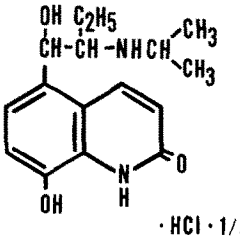
Abstract—Intravenous injection of 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate (procaterol) or isoproterenol into fasted rats caused increases in blood levels of glucose, lactate, free fatty acids (FFA), glycerol, immunoreactive insulin and cyclic AMP. Procaterol-induced alterations of these metabolic parameters, other than FFA, were durable; the increases were observable over a period longer than 2 hr, in contrast to a much shorter duration of isoproterenol-induced metabolic changes. These actions of procaterol were antagonized by propranolol, were observed in adrenodemedullated rats, and were enhanced by theophylline. It is thought therefore, that metabolic changes induced by procaterol are actually mediated via β-adrenoceptors, as are its pharmacological actions. Procaterol caused hyperlactacidemia at molar doses ten to one hundred times lower than those required for isoproterenol, trimetoquinol or salbutamol. A much higher dose of procaterol was required to increase blood levels of FFA, glycerol and insulin. In view of our findings that butoxamine, a selective β_2-adrenoceptor antagonist, antagonized the isoproterenol-induced blood lactate, while practolol, a selective β_1-antagonist, effectively inhibited the stimulatory actions of isoproterenol on FFA, glycerol and insulin, it is concluded that procaterol is a more selective β_2-adrenoceptor agonist than isoproterenol or trimetoquinol, and is more potent than salbutamol.

It is well known that most of the effects of isoproterenol and other catecholamines on carbohydrate and lipid metabolism are mediated by β-adrenoceptors [1]. Accordingly, some metabolic parameters in the blood of rats could serve as a good index for β-adrenergic responses *in vivo* [2, 3].

The purpose of the present paper is to describe the metabolic responses of rats to a new β-adrenoceptor agonist, 5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate (procaterol, OPC-2009) synthesized by Yoshizaki *et al.* [4, 5], in comparison with those to isoproterenol, salbutamol or trimetoquinol. Procaterol has been reported to stimulate β_2-adrenoceptors rather selectively based on its cardiovascular and bronchodilator actions [6–8]. The present results suggest that some metabolic responses to β-adrenoceptor agonists are mediated via β_2-adrenoceptors whereas other responses are mediated via β_1-adrenoceptors, and are consistent with the view that procaterol is a selective β_2-adrenoceptor agonist. The chemical structure of procaterol is shown in Fig. 1.



5-(1-hydroxy-2-isopropylaminobutyl)
8-hydroxycarbostyril hydrochloride
hemihydrate

Fig. 1. Chemical structure of procaterol (OPC-2009).

MATERIALS AND METHODS

Male albino rats of the Wistar strain, weighing 160–200 g, were used after 18 hr of starvation. The rats were anesthetized by intraperitoneal injection of 45 mg pentobarbital/kg of body weight. Blood samples were withdrawn from the inferior vena cava of the anesthetized rats through a heparinized syringe when they were killed. The blood was analyzed for glucose [9], lactate [10], glycerol [11], free fatty acids (FFA) [12] and immunoreactive insulin [13]. In the experiments for the determination of blood cyclic AMP level, 50 μl blood was quickly mixed with 500 μl of an isotonic solution of 10 mM EDTA. The supernatant fluid was separated by centrifugation and assayed for cyclic AMP [14] using a cyclic AMP assay kit obtained from Yamasa Shoyu Co., Chiba, Japan. The route, time and dose of injection of β-adrenoceptor agonists, β-adrenoceptor antagonists and other drugs are described in the legends to the figures. The drugs used in this study were *dl*-procaterol (Otsuka), *l*-isoproterenol (Sigma Chemical Co., St. Louis, MO), *dl*-salbutamol (Leiras), *l*-trimetoquinol (Tanabe), *dl*-propranolol (ICI), *dl*-practolol (Leiras) and *dl*-butoxamine (Burroughs Wellcome). Other chemicals were of analytical grade from commercial sources.

RESULTS

Changes of metabolic parameters due to procaterol-induced β-adrenoceptor stimulation. Metabolic changes induced by the intravenous injection of procaterol (1 μmole/kg of body wt) are illustrated in Fig. 2, in comparison with those induced by the same dose of isoproterenol. The dose of procaterol was chosen to cause submaximal increase in blood lactate. Procaterol, as well as isoproterenol, caused increases in blood levels of glucose, lactate, FFA, glycerol, cyclic AMP and insulin. The most remarkable feature was that procaterol-induced alterations in all metabolic parameters other than FFA continued longer than 2 hr after injection, whereas changes induced by isoproterenol terminated within 1 hr.

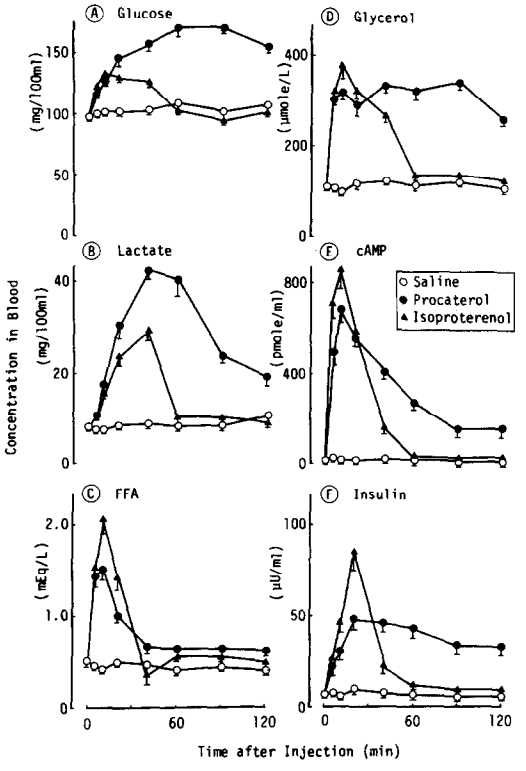


Fig. 2. Increases in metabolic parameters after injection with procaterol or isoproterenol. Saline (○), procaterol (●, 1 μmole/kg) or isoproterenol (▲, 1 μmole/kg) was injected intravenously into the tail vein of fasted rats at zero time and the rats were killed for blood sampling at times shown on abscissa. Each point represents the mean value from five observations with S. E. M. shown as a vertical line.

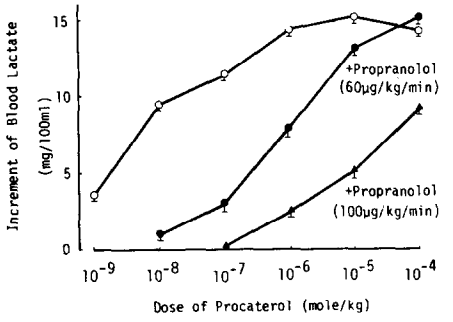


Fig. 3. Blockade of procaterol-induced hyperlactacidemia by propranolol. Propranolol was given by intravenous infusion at the constant rates of 60 (●) or 100 (▲) μg/kg/min after a priming dose of 10 mg/kg. Saline was injected and infused (○). Procaterol (1 nmole–0.1 m-mole/kg) was injected intravenously 20 min after the start of constant infusion of propranolol; 10 min later, the rats were killed for blood sampling. $N = 5$.

Figure 3 shows the inhibitory effect of a β-adrenoceptor antagonist, propranolol, on the procaterol-induced increase in blood concentration of lactate. In this experiment, propranolol was administrated by a primed-infusion technique; a single intravenous injection of 10 mg/kg of propranolol was followed by a constant infusion into the femoral vein at the rate of 60 or 100 μg per kg per min. The dose–response curve of procaterol causing hyperlactacidemia was shifted to the right by propranolol, indicating that the

Table 1. Failure of adrenodemedullation and ganglionic blocking to suppress metabolic changes to procaterol*

Parameters in blood	Sham-operated procaterol		Adrenodemedullated procaterol	
	−	+	−	+
Glucose (mg/100 ml)	102.8 ± 1.7	123.2 ± 1.9	95.5 ± 3.6	111.2 ± 7.1
Lactate (mg/100 ml)	5.4 ± 0.5	21.0 ± 0.4	6.6 ± 0.4	17.2 ± 2.4
FFA (m-equiv./liter)	0.67 ± 0.06	1.34 ± 0.11	0.49 ± 0.05	1.17 ± 0.05
Glycerol (μmoles/liter)	86.9 ± 19.5	264.3 ± 12.6	69.7 ± 8.7	284.2 ± 8.9
cAMP (pmoles/ml)	26.5 ± 8.5	579.4 ± 61.3	19.4 ± 2.7	462.5 ± 186.4

*The rats, 1 wk after operation, were injected intravenously with saline (−) or procaterol (+, 1 μmole/kg). Adrenodemedullated rats were injected intraperitoneally with hexamethonium (20 mg/kg) 10 min before injection with saline or procaterol. Ten minutes after injection of procaterol, the rats were killed for blood sampling. $N = 5$.

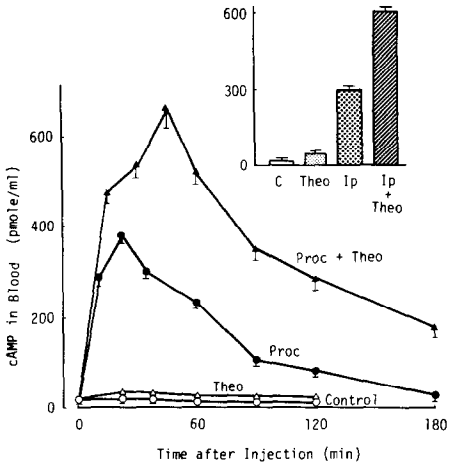


Fig. 4. Effect of theophylline and procaterol on the blood level of cyclic AMP. Theophylline (Theo), 100 mg/kg as aminophylline, was injected subcutaneously 20 min before the intravenous injection of procaterol (Proc) at 1 μmole/kg. In the inset, isoproterenol (Ip), 30 nmoles/kg, was injected intravenously 20 min after the injection of theophylline into rats, which were killed for blood sampling 10 min later. $N = 4$.

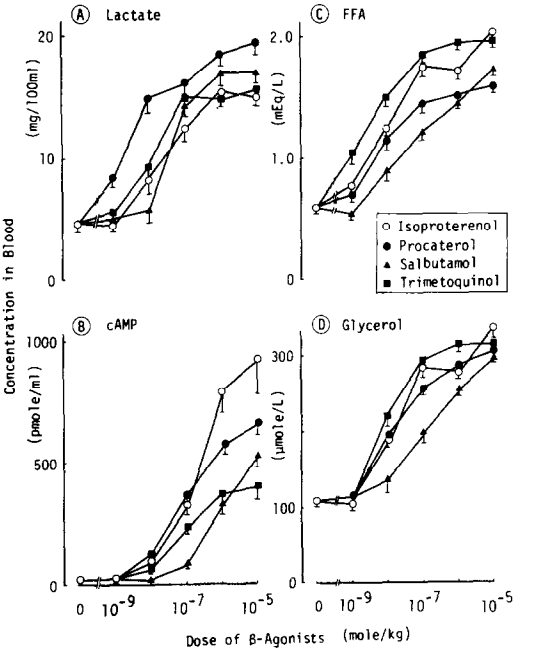


Fig. 5. Dose-dependent increases in blood levels of metabolic parameters by isoproterenol (○), procaterol (●), salbutamol (▲) and trimetoquinol (■). Rats were killed for blood sampling 10 min after the intravenous injection of the β-adrenoceptor agonists. $N = 6$.

hyperlactacidemic action of procaterol was effectively antagonized by propranolol.

Procaterol (1 μmole/kg, i.v.)-induced increases in blood concentrations of the various metabolic parameters in adrenodemedullated and hexamethonium-treated rats were almost the same as in sham-operated rats (Table 1). Thus, these metabolic alterations induced by the injection of procaterol appear to reflect the stimulation of β-adrenoceptors rather directly without mediation of autonomic ganglia or catecholamines secreted from the adrenal medulla.

It is seen in Fig. 4 that the prior injection of theophylline (100 mg/kg s.c. as aminophylline), despite its failure to affect the cyclic AMP level by itself, was very effective in enhancing the isoproterenol-(inset) or procaterol-induced increases in the blood cyclic AMP level.

Comparison of the activity of procaterol with other β-adrenoceptor agonists. In order to study the relative potencies of β-adrenoceptor agonists, the action of procaterol was compared with the actions of isoproterenol and trimetoquinol [15], and salbutamol [16], a selective β_2-agonist. Figures 5 and 6 show increases in blood levels of lactate, cyclic AMP, FFA, glycerol and insulin, which are dependent on the doses of these agonists. Determinations were made with blood samples taken at 10 min in Fig. 5 and at 20 min in Fig. 6 after the injection of the drug.

Procaterol produced a significant increase in blood lactate level at a dose as low as 1 nmole/kg (Fig. 5A). In this regard, procaterol was 10–100 times more potent than isoproterenol, trimetoquinol or salbutamol (Figs. 5A and 6A). In contrast, trimetoquinol and isoproterenol produced greater increases in blood concentrations of FFA (Fig. 5C), glycerol (Fig. 6C) and insulin (Fig. 6D) than procaterol and salbutamol did at the same molar doses. At a dose of 10 μmoles/kg, the highest dose employed here, procaterol or salbuta-

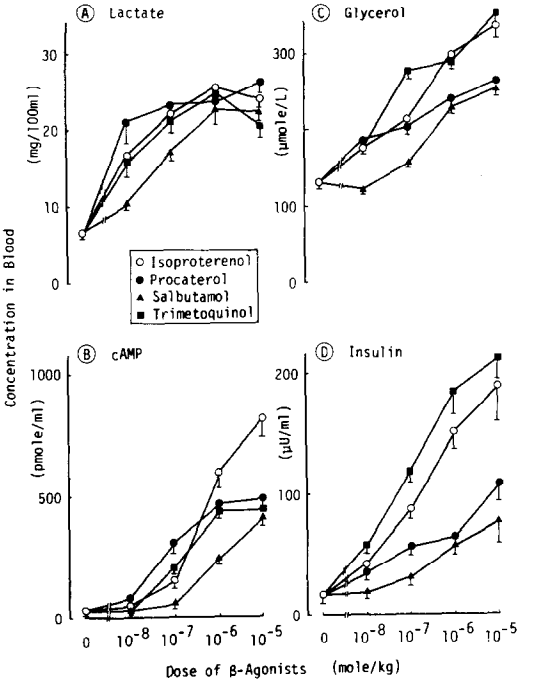


Fig. 6. Dose-dependent increases in blood levels of metabolic parameters by isoproterenol (○), procaterol (●), salbutamol (▲) and trimetoquinol (■). Rats were killed for blood sampling 20 min after the subcutaneous injection of the β-adrenoceptor agonists. $N = 6$.

mol caused hyperinsulinemia to a much smaller degree than isoproterenol and trimetoquinol. Thus, procaterol and salbutamol appeared to act as partial agonists when the blood insulin level was employed as an index of β-adrenoceptor response.

In the case of blood cyclic AMP, procaterol was as potent as (Fig. 5B, at 10 min after intravenous injection), or more potent (Fig. 6B, at 20 min after subcutaneous injection) than, isoproterenol at their lower doses (10–100 nmoles/kg), whereas isoproterenol was the most potent among the four β-agonists at their high doses (1–10 μmoles/kg). Thus, the relative potency of these β-agonists greatly depends on the metabolic indices employed for evaluation of potency. It is conceivable, therefore, that more than one type of β-adrenoceptor mediates these metabolic changes induced by different drugs.

Blockade by practolol or butoxamine of isoproterenol-induced increases in blood levels of metabolic parameters. Figure 7 shows the difference in the inhibitory effect of propranolol (a non-selective β-adrenoceptor antagonist), practolol (a selective β_1-antagonist) [17] and butoxamine (a selective β_2-antagonist [18] on isoproterenol (0.1 μmole/kg s.c.)-induced increases in blood levels of metabolic parameters. The increase in the blood lactate level induced by isoproterenol at 10 min after (Fig. 7A) or 30 min after (Fig. 7D) was inhibited by pretreatment with propranolol in a dose-dependent manner. There was a complete abolition when the dose of propranolol was increased to 10 μmoles/kg. The isoproterenol-induced hyperlactacidemia was reduced significantly by butoxamine at the dose of 0.1 m-mole/kg, whereas it was not affected at all by any dose of practolol employed.

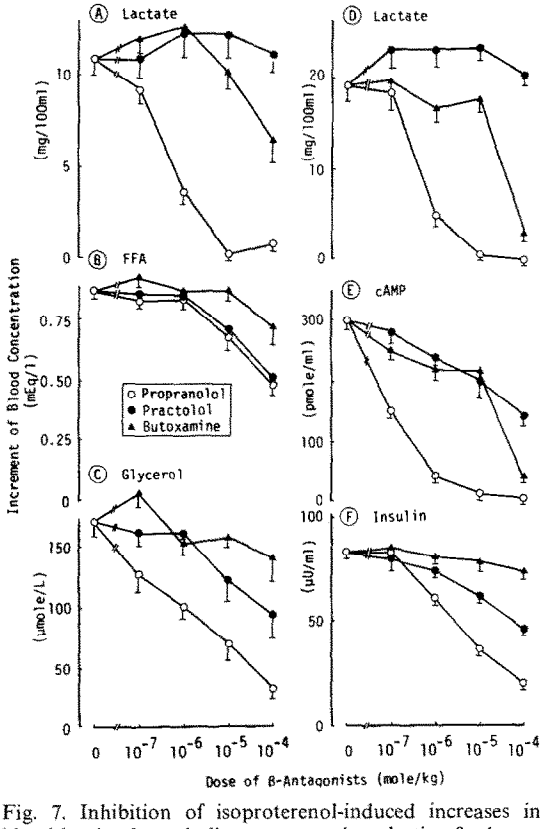


Fig. 7. Inhibition of isoproterenol-induced increases in blood levels of metabolic parameters by selective β-adrenoceptor antagonists. Propranolol (○), practolol (●) or butoxamine (▲) was injected subcutaneously 30 min before isoproterenol. Rats were killed for blood sampling 10 min (A, B and C) or 30 min (D, E and F) after the subcutaneous injection of isoproterenol at 0.1 μmole/kg. $N = 6$.

Stimulatory actions of isoproterenol on the blood concentrations of glycerol and insulin were inhibited dose dependently by practolol although the inhibition produced was 10–100 times less potent than that produced by propranolol. But butoxamine was without effect in this regard, even at the dose of 0.1 m-mole/kg. The increase in FFA induced by isoproterenol was rather resistant to antagonists; it was antagonized by propranolol or practolol only partly. The isoproterenol-induced increase in the blood cyclic AMP was abolished completely by 1–10 μmoles/kg of propranolol. There was only a partial inhibition of the cyclic AMP increase when practolol or butoxamine was injected at doses lower than 10 μmoles/kg. At 100 μmoles/kg, butoxamine was more potent than practolol (Fig. 7E).

DISCUSSSION

The injection of procaterol into fasted rats gave rise to increases in blood concentrations of various metabolites such as glucose, lactate, glycerol, FFA, insulin and cyclic AMP. Most of these metabolic changes induced by procaterol were sustained over 2 hr or longer, in contrast to only a 1-hr duration for these changes induced by isoproterenol at the same molar dose. The duration of the procaterol action may be accounted for partly by the long (55 min) half-life of the drug in plasma as compared to isoproterenol [19].

The following findings clearly indicate that the interaction of procaterol with β-adrenoceptors is principally responsible for the metabolic alterations observed. First, these metabolic changes were effectively antagonized by a β-adrenoceptor antagonist, propranolol (Fig. 7). Second, procaterol was effective in adrenodemedullated and hexamethonium-treated rats as well, making it unlikely that endogenous catecholamines secreted from the adrenal medulla are involved in the procaterol action. Third, metabolic changes induced by procaterol were associated with increases in blood cyclic AMP, which should reflect changes in its amounts within cells of various organs [3, 14]. Tissue levels of cyclic AMP also increased in rats upon injection with procaterol (to be published). The procaterol-induced increase in blood cyclic AMP was potentiated by theophylline, an inhibitor of cyclic nucleotide phosphodiesterase, and was antagonized by propranolol, suggesting that adenylate cyclase activation via β-adrenoceptors occurred after procaterol administration.

Either β_1-or β_2-adrenoceptors appear to be involved in each of the metabolic responses of rats to β-adrenoceptor agonists. Isoproterenol-induced hyperlactacidemia was antagonized by butoxamine, a β_2-antagonist, but not by practolol, a β_1-antagonist, even at the highest dose employed (Fig. 7A and D), whereas increases in blood FFA, glycerol and insulin were prevented by practolol more efficiently than by

butoxamine (Fig. 7B, C and F). Thus, it is likely that adrenoceptors of the β_1-type and β_2-type are predominantly responsible for lipolysis and glycolysis, respectively, in the rat, in accord with previous findings on the dog and cat [20–23]. The present results that practolol was more effective than butoxamine in antagonizing isoproterenol-induced hyperinsulinemia are at variance with previous reports [24, 25], in which insulin was reported to be released from dog pancreas *in vivo* via the stimulation of β_2-receptors. This discrepancy might be explained in terms of species differences between rats and dogs, because Furman and Tayo [26] reported that practolol effectively inhibited insulin secretion in rats. The increase in blood cyclic AMP induced by isoproterenol was antagonized by either practolol or butoxamine, though butoxamine was somewhat more potent than practolol in this regard. It is very likely that blood cyclic AMP originated from various tissues, in some of which β_1-receptors, and in others β_2-receptors, are involved in adenylate cyclase activation.

Based on the foregoing discussion, metabolic responses of rats to a β-adrenoceptor agonist would be employed as a means of classifying the agonist as the β_1-type, the β_2-type or the non-specific β-type. Hyperlactacidemia may reflect stimulation of β_2-receptors rather than β_1-receptors, whereas stimulation of β_1-receptors may cause increases in blood FFA, glycerol and insulin preferentially to the increase in blood lactate in the rat. The metabolic responses to a β-agonist may depend on its potency as well as on its relative affinity to the β_1- and β_2-receptors. The injection of procaterol into rats caused marked hyperlactacidemia at much lower doses than required of other β-agonists, including isoproterenol. Blood levels of FFA and glycerol were also elevated by procaterol to essentially the same extent as by isoproterenol when their doses were less than 0.01 μmole/kg for intravenous injection (Fig. 5) and less than 0.1 μmole/kg for subcutaneous injection (Fig. 6). When the doses of the β-agonists were further increased, however, procaterol-induced increases in blood FFA and glycerol were much less than their increases induced by isoproterenol. Thus, procaterol is a very potent β-agonist, i.e. it exhibits high affinities in binding to β-receptors. In addition, procaterol stimulates β_2-receptors rather selectively, as revealed by marked hyperlactacidemia. Salbutamol was less potent than other agonists in raising blood levels of most metabolites so far studied. But, salbutamol would be also a β_2-agonist in a sense that hyperlactacidemia was induced by doses of salbutamol lower than caused increases in blood FFA and glycerol. Procaterol and salbutamol were less effective than other agonists in inducing insulin secretion (Fig. 6D), in accord with the foregoing proposal that insulin is secreted via stimulation of β_1-receptors rather than β_2-receptors in rats.

In summary, the determination of increases in blood concentrations of various metabolites in response to a β-agonist would be a useful means for studying whether the agonist is of the β_1-type or of the β_2-type. By this means, procaterol was found to be a very potent β_2-agonist, in accord with its cardiovascular and bronchodilator actions [6–8].

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