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EVALUATION OF A NEW BETA-ADRENERGIC BLOCKING AGENT, CARTEOLOL, BASED ON METABOLIC RESPONSES IN RATS—I.

BLOCKADE *IN VIVO* OF EPINEPHRINE- AND ISOPROTERENOL-INDUCED ALTERATIONS OF BLOOD CONCENTRATIONS OF CARBOHYDRATE AND LIPID INTERMEDIARY METABOLITES

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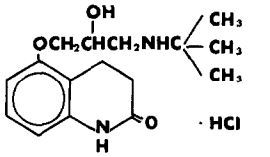
Abstract—Increases in blood concentrations of glucose induced by epinephrine, of lactate induced by epinephrine and isoproterenol, and of glycerol and insulin induced by isoproterenol in rats showed graded responses dependent on the dose of the catecholamines and were blocked also progressively by increasing doses of 5-(3-tert-butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril hydrochloride (carteolol), a new beta-adrenergic blocking agent. The ED_{50} estimated for these metabolic parameters, other than blood insulin, was 100–200 μg/kg of body wt for epinephrine and 10–20 μg/kg for isoproterenol, while the "corrected ID_{50}" for carteolol, reflecting the dissociation constant of the antagonist–receptor complex, was 1–3 μg/kg. It is concluded that the beta-adrenergic receptors of the same character concerning the affinity to agonists and antagonists mediate the increase of blood levels of these carbohydrate and lipid intermediary metabolites, and that the affinity of carteolol is one order higher than that of isoproterenol, which in turn is ten times higher than that of epinephrine. In contrast, much higher ED_{50} for isoproterenol and "corrected ID_{50}" for carteolol were obtained when hyperinsulinemia was used as a measure of beta action, suggesting that the beta-adrenergic receptor mediating pancreatic secretion of insulin is distinct in nature from the receptors involved in the control of carbohydrate and lipid metabolism. Comparison of the potency of carteolol with those of propranolol and pindolol showed that carteolol is the most potent beta-adrenergic blocking agent.

It is well known that most of the effects of epinephrine and other catecholamines on carbohydrate and lipid metabolism are mediated by beta-adrenergic receptors [1]. Some of these metabolic alterations have been the subject of extensive biochemical studies, which have revealed the chain of events from activation of adenylate cyclase to the change in metabolic parameters via phosphoprotein generated by cyclic AMP-dependent protein kinase. The beta-receptor-mediated activation of adenylate cyclase is known to occur in a wide variety of tissues and organs including the liver, skeletal and cardiac muscle, adipose tissues and secretory organs, resulting in modifications of enzymic activities and rates of metabolic flows in these tissues and eventually leading to changes in blood concentrations of metabolites. These metabolic changes are readily determined *in vivo* and *in vitro*. Thus, such biochemical parameters can serve as a good index for assessing activity of beta-adrenergic agonists as well as their antagonists.

The purpose of this series of studies is to evaluate a new beta-adrenergic blocking agent, 5-(3-tert-butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril hydrochloride (carteolol, OPC-1085) [2], by means of assessing its antagonistic effects on beta-stimulant-induced metabolic alterations. Yabuuchi and Kinoshita [3] have recently reported the blockade of cardiovascular actions of isoproterenol by this agent. In the present paper, epinephrine or isoproterenol was injected into rats to cause a rise of blood concentrations of some metabolites, the inhibition of which was then induced by carteolol in comparison with the inhibition induced by propranolol or pindolol. The results show that carteolol is a specific and potent blocking agent of the beta-adrenergic receptor-mediated metabolic functions. The chemical structure of carteolol is shown in Fig. 1.

MATERIALS AND METHODS

Male albino rats of the Wistar strain, weighing 120–250 g, were used before or after 20 hr starvation. Rats were anesthetized by the intraperitoneal injection of 50 mg pentobarbital/kg of body weight. Blood



5-(3-tert-butylamino-2-hydroxy) propoxy -3,4- dihydrocarbostyril hydrochloride

Fig. 1. Chemical structure of carteolol (OPC-1085).

samples were withdrawn from the inferior vena cava of the anesthetized rats through a heparinized syringe at the time of sacrifice. After centrifugation at 4°, the plasma was analyzed for glucose [4], lactate [5], pyruvate [6], glycerol [7], free fatty acids [8] and immunoreactive insulin [9]. The route, time and dose of injection of beta-stimulants, beta-blockers and other chemicals are described in the legends to Figs. 2–5.

The sources of reagents are: epinephrine, Merck, Sharp & Dohme; isoproterenol, Sigma; 5-methoxy-indole-2-carboxylic acid (MICA), Aldrich Chemical Co.; "assay kit" for radioimmunoassay of blood insulin, Dainabot Radioisotope Laboratories Ltd., Tokyo. Other reagents are of analytical grade from commercial sources.

RESULTS

Blockade by carteolol of the epinephrine- or isoproterenol-induced increases in blood levels of glucose, lactate, free fatty acids, glycerol and insulin. Intraperitoneal injection of epinephrine (200 μg/kg of body wt) or isoproterenol (500 μg/kg) into fasted rats caused roughly 2-fold and 3- to 4-fold increases in blood levels of glucose and lactate respectively (Fig. 2 panels A and B). It is also seen in Fig. 2 that the increments of the metabolite concentrations became smaller with increasing doses of carteolol, which was injected 60 min prior to the agonist. There was a complete abolition of the catecholamine actions at the dose of 1 mg/kg. Figure 3 shows that the changes in blood lactate concentration induced by the catecholamines with or without carteolol were associated

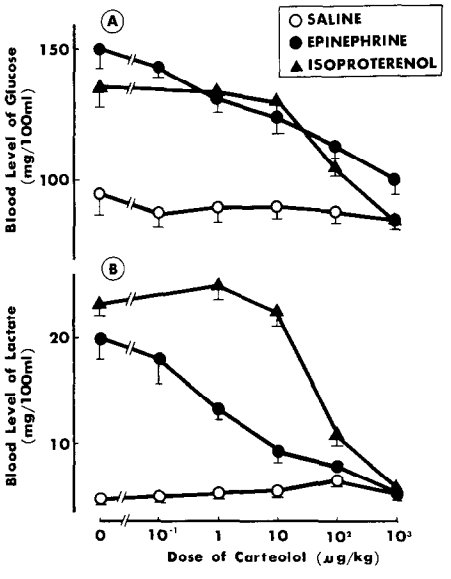


Fig. 2. Dose-dependent blockade of epinephrine- or isoproterenol-induced hyperglycemia (A) and hyperlactacidemia (B) by carteolol. Carteolol was injected subcutaneously 1 hr before the intraperitoneal injection of saline (○), epinephrine (●) or isoproterenol (▲) into fasted rats, which were sacrificed for blood sampling 30 min later. The doses of epinephrine and isoproterenol were 200 and 500 μg/kg of body wt respectively. Each point represents the mean value from five observations with S.E.M. shown as a vertical line.

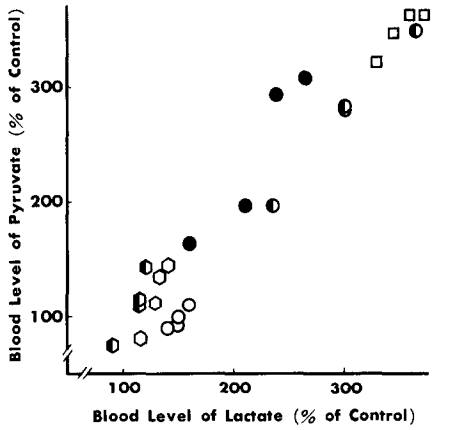


Fig. 3. Correlation between blood levels of pyruvate and lactate in the rats treated with epinephrine (□) or epinephrine plus carteolol at various doses: (◐) 0.1 μg, (●) 1 μg, (○) 10 μg, (◑) 100 μg, and (◐) 1000 μg/kg. Experimental conditions are the same as in Fig. 2.

with the same directional changes in blood pyruvate (correlation coefficient, r, is 0.965, $P < 0.001$).

Figure 4 shows the effects of the increasing doses of carteolol on isoproterenol-induced increases in blood concentrations of free fatty acids (FFA), glycerol and immunoreactive insulin. Epinephrine was

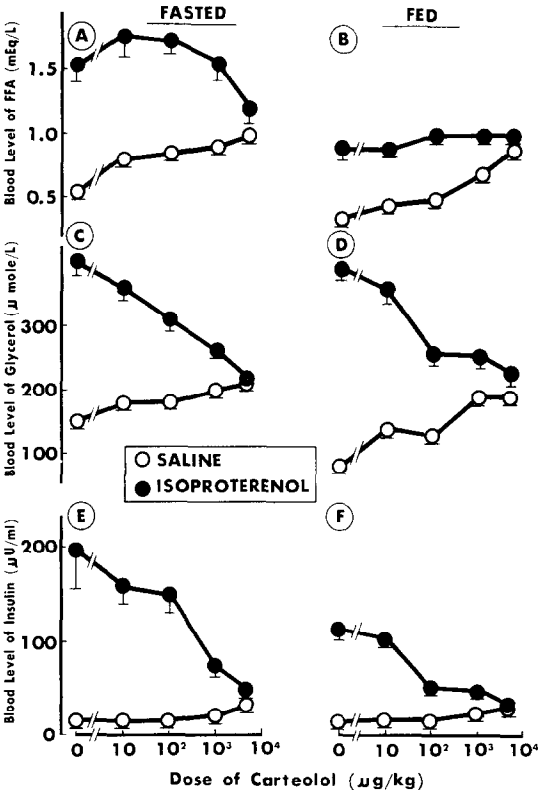


Fig. 4. Dose-dependent inhibition of isoproterenol-induced increases in blood concentrations of FFA (top), glycerol (middle) and immunoreactive insulin (bottom) in fasted (left) and fed (right) rats. Carteolol was injected subcutaneously into rats 15 min before subcutaneous injection of isoproterenol. Other experimental conditions are the same as in Fig. 2. The number of observations is four.

Table 1. ED_{50} for epinephrine and isoproterenol and ID_{50} for carteolol

	Metabolic parameters			
	Glucose	Lactate	Glycerol	Insulin
		(μg/kg body wt)		
ID_{50} for carteolol*				
With epinephrine (200 μg/kg)	10	1		
With isoproterenol (500 μg/kg)	20	30	50	500
ED_{50} for epinephrine	100	200		
	(0.5)†	(1)		
ED_{50} for isoproterenol		10	20	100
		(0.05)	(0.1)	(0.5)
"Corrected ID_{50}" calculated for carteolol‡				
For epinephrine	3	1		
	(0.01)	(0.003)		
For isoproterenol		1	2	100
		(0.003)	(0.005)	(0.3)

* Obtained from the plots in Figs. 2 and 4.
† Figures in parentheses show approximate μmoles/kg.
‡ Calculated from the values in Lines 1–4 according to the equation presented in text.

not employed in this experiment because it, even at the largest dose, caused an only slight elevation of FFA and insulin in rats in accordance with previous findings [7, 10, 11]. Stimulatory actions of isoproterenol on blood concentrations of glycerol and insulin were significantly reduced by the prior treatment of rats with 0.1 to 1 mg/kg of carteolol, regardless of whether the rats had been fasted (left panel) or fed (right panel). These stimulatory actions were no longer observable when the dose of carteolol was increased to 5 mg/kg. In the case of FFA, however, there was no significant difference in the blood concentration after isoproterenol between the rats receiving no beta-blocker and those treated with carteolol at 1 mg/kg or smaller doses. This apparent failure of carteolol to lower the blood FFA levels in isoproterenol-treated rats could be accounted for, at least in part, by an elevation of blood FFA level induced by carteolol alone. Thus, FFA accumulated in blood gradually as the dose of carteolol injected alone was raised until no significant increase was induced by isoproterenol in the rat pretreated with 5 mg/kg of carteolol.

Activity of carteolol as a blocking agent of metabolic actions of epinephrine and isoproterenol. The dose–response relationships in Figs. 2 and 4 enable us to estimate the dose of carteolol required for the half-maximal inhibition of the action of epinephrine or isoproterenol (ID_{50}). Since there was no graded response in blood FFA (Fig. 4, panels A and B), estimation of ID_{50} was made for the metabolic parameters other than FFA, the results being presented in Lines 1 and 2 in Table 1. In general, the dose of an agonist required for half-maximal stimulation (ED_{50}) can be considered to reflect the dissociation constant of the agonist–receptor complex, the formation of which eventually leads to the metabolic alteration observed. "Corrected ID_{50}," which likewise reflects the dissociation constant of the antagonist–receptor complex, can be obtained from ID_{50}, ED_{50} and the dose of agonist used for the measurement of ID_{50} according to the following equation.*

$$\text{"Corrected } ID_{50}\text{"} = \frac{ID_{50}}{1 + \dfrac{\text{dose of the agonist used}}{ED_{50}}}$$

For the purpose of obtaining ED_{50}, the effects of epinephrine and isoproterenol on blood levels of glucose, lactate, glycerol and insulin were studied at their various doses. The resultant dose–response relationships (not shown but see Ref. 12) were used for rough estimation of ED_{50}, which are recorded in Lines 3 and 4 in Table 1. It was impossible to obtain ED_{50} for epinephrine-induced hyperinsulinemia and ED_{50} for isoproterenol-induced hyperglycemia, because there were no graded responses for these parameters. It is seen that the beta-receptors which are involved in the increase of blood levels of lactate and glycerol show a one-order higher affinity to isoproterenol than to epinephrine, in good agreement with the fact that iso-

* This equation is derived as follows. Since the response to an agonist is proportional to the amount of the agonist–receptor complex, the response (R) at any dose of the agonist (A) is expressed as follows,

$$\frac{MR}{R} = 1 + \frac{ED_{50}}{A} \quad (1)$$

where MR represents the maximal response obtained with saturating dose of the agonist and ED_{50} reflects the dissociation constant of the agonist–receptor complex in terms of the dose of agonist. When an antagonist at a certain dose (I) is administered with this dose of the agonist, the response (R') is written as

$$\frac{MR}{R'} = 1 + \frac{ED_{50}}{A}\left(1 + \frac{I}{ID'_{50}}\right) \quad (2)$$

where ID'_{50} reflects the dissociation constant of the agonist–receptor complex in terms of the dose of antagonist, which equals the "corrected ID_{50}" in the present paper.

When $R/R' = 2$, then $I = ID_{50}$. Thus, the following equation is obtained by a combination of Equations 1 and 2.

$$2\left(1 + \frac{ED_{50}}{A}\right) = 1 + \frac{ED_{50}}{A}\left(1 + \frac{ID_{50}}{\text{"corrected } ID_{50}\text{"}}\right) \quad (3)$$

Arrangement of Equation 3 affords the equation used in this and the subsequent papers. The present authors are indebted to Professor M. Ui for derivation of these equations.

Table 2. Failure of carteolol to inhibit metabolic responses to MICA, ACTH and heparin*

Dose of stimulant (per kg)	Dose of carteolol (μg/kg) 0	100	1000
(Increment due to stimulant)			
MICA-induced hyperlactacidemia (mg/100 ml)			
25 mg	7.0	5.9	9.0
50 mg	16.9	17.7	13.5
MICA-induced increase in blood FFA (mEq/liter)			
25 mg	1.17	1.09	0.99
50 mg	1.30	1.30	1.26
ACTH-induced increase in blood FFA (mEq/liter)			
5 units	0.50	0.33	0.44
10 units	0.57	0.70	0.39
ACTH-induced hyperglycerolemia (μmoles/liter)			
5 units	44	46	71
10 units	59	71	53
Heparin-induced increase in blood FFA (mEq/liter)			
0.1 mg	0.34	0.36	0.27
1 mg	0.28	0.24	0.42
Heparin-induced hyperglycerolemia (μmoles/liter)			
0.1 mg	64	61	32
1 mg	45	34	24

* Carteolol or saline was injected subcutaneously 15 min before ACTH or heparin or 30 min before MICA. Blood levels of metabolites were determined at their peak levels, i.e. 60 min after MICA (i.p.), 30 min after ACTH (s.c.) and 5 min after heparin (i.v.) The values in this table show the effects of these stimulants (mean from four observations) expressed as the differences from the control which was obtained without the stimulant at each dose of carteolol under the same conditions.

proterenol is ten times more potent than epinephrine as a stimulator of adipose tissue lipolysis [13].

"Corrected ID_{50}" was then calculated by the use of the above equation and presented in Lines 5 and 6 in Table 1. It was 1–3 μg/kg for all the metabolic parameters other than isoproterenol-induced hyperinsulinemia. This value is one-order smaller than ED_{50} for the isoproterenol stimulation of these metabolic activities even in terms of a molar dose, showing that carteolol has an extremely high affinity to beta-receptors. An additional important finding in Table 1 is that ED_{50} and "corrected ID_{50}" calculated for "insulin" were much higher than those for other metabolic parameters. This will be discussed later (see Discussion).

Selectivity of carteolol-induced inhibition of metabolic activities. Blood concentrations of lactate, glycerol and FFA can be elevated without the mediation of the beta-receptor stimulation. Several examples are shown in Table 2. MICA caused increases in blood lactate due to an inhibition of hepatic gluconeogenesis [14] and in blood FFA as a result of hypoglycemia [15]. Both blood FFA and glycerol were raised by adrenocorticotropic hormone (ACTH) and heparin by stimulations of hormone-sensitive lipase and lipoprotein lipase respectively. Table 2 shows that these changes in blood metabolites were not affected by the high doses of carteolol. It is strongly suggested, therefore, that the action of carteolol is due to a selective blockade of the beta-receptor-mediated functions.

Comparison of the activity of carteolol with other beta-adrenergic blocking agents. In order to study the relative potencies of beta-adrenergic blocking agents, the inhibitory action of carteolol on the epinephrine- and isoproterenol-induced metabolic alterations was compared with those of propranolol and pindolol (LB-46). Percent inhibitions induced by these blockers were plotted against their doses in Fig. 5. Table 3 shows ID_{50} estimated from these plots. In accordance with the results of previous studies [3] using cardiovascular responses of dogs as a measure of beta-receptor-mediated actions, pindolol was roughly ten times as effective as propranolol in blocking metabolic and insulin-releasing actions of epinephrine and isoproterenol. The dose of carteolol required for the half-maximal inhibition of the epinephrine- or isoproterenol-induced activations was several-fold smaller than the dose of pindolol in all cases recorded in Table 3. Thus, it is concluded that carteolol is the most potent beta-adrenergic antagonist among these three drugs studied.

DISCUSSION

In the present study, blood concentrations of glucose, lactate, pyruvate, FFA, glycerol and insulin were employed as the measure of beta-receptor-mediated functions, based on the view that metabolic processes involved in the liberation of these metabolites from tissues into the circulation are dependent on the beta-receptor stimulation. It should be emphasized here,

Table 3. ID_{50} for the beta-blocking actions of carteolol, pindolol and propranolol calculated from plots in Fig. 5

Metabolites	Agonist	Fed or fasted	ID_{50} (μg/kg body wt) Carteolol	Pindolol	Propranolol
Glucose	Epinephrine	Fasted	10–20 (7)*	50 (2)	100 (1)
	Isoproterenol	Fasted	10–20 (7)	100 (1)	100 (1)
Lactate	Epinephrine	Fasted	1 (50)	10 (5)	50 (1)
Pyruvate	Epinephrine	Fasted	2 (50)	20 (5)	100 (1)
Glycerol	Isoproterenol	Fasted	50 (20)	100 (8)	1000 (1)
	Isoproterenol	Fed	50 (70)	100 (30)	3000–5000 (1)
Insulin	Isoproterenol	Fed	50 (40)	100 (20)	2000 (1)

* Figures in parentheses are relative potencies.

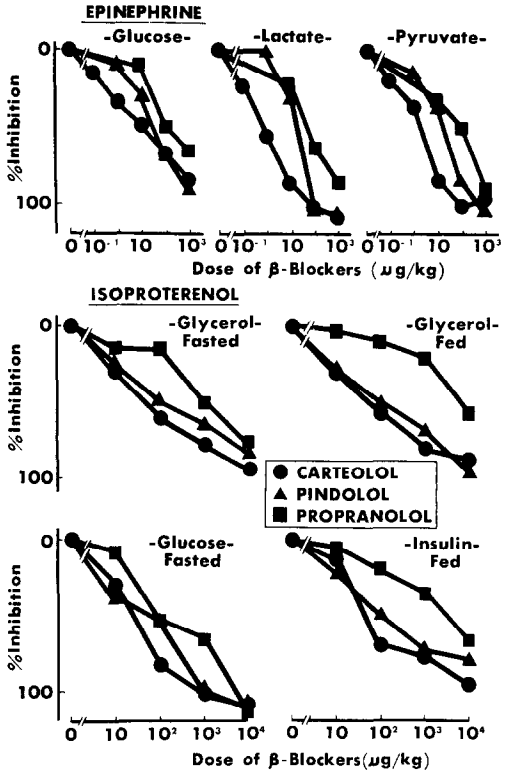


Fig. 5. Comparison of blocking activity of carteolol with propranolol and pindolol. Experimental conditions are the same as in Fig. 2. The number of observations is four to five.

however, that the beta-receptor-mediated function is not the only determinant of blood levels of these metabolites. Accordingly, some of them are, but others are not, suitable for this purpose, as will be discussed below.

The blood level of glucose is determined by both hepatic production and peripheral utilization. Gluconeogenesis and glycogenolysis in rat liver, major contributions to hepatic glucose liberation in the fasted state, are independent of alpha- and beta-receptor-mediated adrenergic control *in vivo* [16–18] and *in vitro* [18–20]. In contrast, Shikama and Ui have recently reported that the epinephrine-induced inhibition of peripheral glucose utilization, which plays an essential role in epinephrine-induced hyperglycemia [21], resulted from beta-stimulation [17]. It is reasonable, therefore, to assume that hyperglycemia induced by beta-stimulants as well as its inhibition by beta-blocking agents depends on the alteration of the rate of peripheral glucose utilization of blood glucose. The situation appears further complicated when isoproterenol is employed as a beta-stimulant, because it also gives rise to the secretion of insulin, which, in turn, interferes with hyperglycemia by stimulating peripheral glucose utilization [22, 23]. This would likely be the reason why there was no graded glycemic response to isoproterenol.

There was no change in the lactate/pyruvate ratio in blood after the beta-stimulants (Fig. 3), suggesting that observed changes in blood lactate resulted from altered metabolic activities rather than a shift of cellular oxidation–reduction state. Since tissue utilization of lactate is largely proportional to its blood level [24], it is very likely that the enormous increase elicited by catecholamines selectively reflects enhanced glycolysis in peripheral tissues which is known to be mediated by beta-stimulation.

Lipolysis activated via the beta-stimulation in the adipose tissue liberates FFA and glycerol into the blood stream [25]. FFA in the blood is then oxidized by various tissues including the muscle, or alternatively is re-esterified by the adipose tissue, while glycerol serves as a substrate of hepatic gluconeogenesis. The re-esterification of FFA is known to proceed as glucose becomes available to the tissue, as exemplified by the lowering of its blood level after feeding (Fig. 4). Probably, the increase of FFA liberation from the adipose tissue via beta-stimulation was counterbalanced by its re-esterification, which was potentiated by hyperglycemia or hyperinsulinemia. In this regard, blood glycerol served as a much better parameter than FFA of the beta-receptor-mediated lipolysis in adipose tissues. Finally, the beta-receptor-mediated insulin secretion has been well documented in rats [7, 26] as well as in man [27]. Since epinephrine can reduce blood insulin level due to alpha-receptor stimulation [28], it is reasonable that isoproterenol was much more potent than epinephrine as a stimulator of insulin secretion.

Based on these considerations, we were led to the conclusion that epinephrine-induced hyperglycemia, epinephrine- and isoproterenol-induced hyperlactacidemia, isoproterenol-induced hyperglycerolemia and hyperinsulinemia are utilizable as the measure of the beta-receptor-mediated responses in the rats. Although a possibility would still exist that the degree of these metabolic changes is, in a strict sense, not solely dependent on stimulation of beta-receptors, it should be noted that ED_{50} for epinephrine or isoproterenol as well as ID_{50} for the inhibition by carteolol were of essentially the same order, whether any of these metabolic changes were employed for these estimations (Table 1). This is a strong indication that these metabolic changes reflect, even in a quantitative sense, functions of the receptors of a similar type. Furthermore, ED_{50} and "corrected ID_{50}" calculated with respect to metabolic responses in Table 1 are in just the same order as those calculated with respect to blood cyclic AMP response in our companion paper [12], making it very likely that these metabolic responses resulted from beta-receptor stimulation via adenylate cyclase activation.

Though ED_{50} or "corrected ID_{50}" obtained here gives only a rough approximation related to the dissociation constant of the agonist– or antagonist–receptor complex, it is of utmost interest that these values estimated for isoproterenol-induced hyperinsulinemia were one or two orders higher than the corresponding values obtained for other metabolic responses. This fact strongly suggests that the beta-adrenergic receptors mediating the regulation of glycolysis and lipolysis are distinct from the receptors involved in the secretion of insulin. This idea is in accord with the finding that insulin is secreted from the pancreatic islet via the receptor of a beta-2 type [29], while lipolysis is enhanced by the beta-1 receptor stimulation in the adipose tissue [30, 31].

The inhibitory action of carteolol on these metabolic changes was due to beta-blockade so selectively that it failed to interfere with similar changes if these changes were induced by the agents other than beta-stimulants (Table 2). In conclusion, carteolol is a very potent beta-adrenolytic agent; it is more potent than pindolol and propranolol, and its affinity to the beta-adrenergic receptor is ten times higher than that of isoproterenol and 100 times higher than that of epinephrine. The findings (Fig. 4) that blood levels of FFA and glycerol tended to rise upon the injection of this blocking agent alone might be explained by assuming that it has a weak sympathomimetic action in addition to its potent sympatholytic action just as has been reported for pindolol [32]. This problem will be dealt with in detail elsewhere.*

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