Antilipolytic Activity of 4-(2-Hydroxy-3-isopropylaminopropoxy) Acetanilide (Practolol)[1] (35348)

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In the past few years a number of compounds have been synthesized which have *beta*-adrenergic blocking activity (1). Most of these compounds were equally effective at blocking all responses mediated by the *beta*-adrenergic receptor. Recently, a preliminary report has been published describing a compound which selectively blocked some *beta*-adrenergic responses while having little effect on others (2). This compound, practolol, was reported to have a relative specificity for the effects of catecholamines on heart rate, myocardial force of contraction and free fatty acid mobilization. The compound had a rather low specificity for the effects of the catecholamines on tracheal relaxation and vasodilation. The purpose of the present work was to more fully examine the effects of this compound on hormone-stimulated lipolysis. For this purpose, the activity of the compound was compared to that of propranolol, using the isolated fat cell preparation.

Materials and Methods. Fed, male Holtzman rats, weighing 160–200 g, were stunned by a blow to the head and killed by exsanguination. The fat pads were removed; and isolated fat cells were prepared by the method of Lech and Calvert (3). Aliquots of the fat cells were placed in polyethylene flasks containing Krebs–Ringer bicarbonate buffer (pH 7.4) with 4% bovine serum albumin and the appropriate drugs. Incubations were carried out at 37°, with gentle shaking, in an atmosphere of 95% O_2–5% CO_2 for 60 min. The final volume was 3.0 ml.

The reaction was terminated by adding an aliquot of the cells and medium to 5% trichloroacetic acid, and the rate of lipolysis was determined by measuring the production of glycerol by the method of Korn (4). Appropriate blank values were obtained for all drugs used. Glucose-1-^{14}C oxidation by fat cells was determined by measuring the production of CO_2-^{14}C as previously described (5). The protein content of the fat cells was determined as described by Lech and Calvert (3).

All results are expressed as the mean ± standard error of the mean. Unless otherwise stated, values for p were calculated by using Student's t test for paired comparisons. The method of Blinks (6) was used to determine pA_2 values, using at least three concentrations of the antagonist.

Bovine serum albumin (Fraction V) and the dibutyryl analog of cyclic 3′,5′-adenosine monophosphate (dibutyryl cyclic AMP) were purchased from Sigma Chemical Co. (St. Louis, Mo.). The *dl*-propranolol (*l*-isopropylamine-3-naphthyloxy-2-propanol) and the practolol (4-[2-hydroxy-3-isopropylaminopropoxy] acetanilide; ICI 50, 172; AY 21,011) were generously supplied by Ayerst Laboratories (New York, N.Y.). The *dl*-isoproterenol (1-[3,4-dihydroxy-phenyl]-2-isopropylaminoethanol) was kindly supplied by Winthrop Laboratories (New York, N.Y.).

Results. The antilipolytic activity of practolol was examined using the isolated fat cell preparation. At a concentration of 10^{-5} *M* this compound competitively inhibited isoproterenol-stimulated lipolysis (Fig. 1). For comparison purposes the antilipolytic activity of propranolol was examined. At a concentration of 10^{-7} *M* this *beta*-adrenergic blocking agent also competitively inhibited the lipolytic response to isoproterenol (Fig. 1). Neither practolol nor propranolol significantly ($p>0.50$) altered basal lipolysis.

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TABLE I. Intrinsic Lipolytic Activity of Practolol in Isolated Fat Cells.

Practolol conc (moles/liter)	Glycerol release[a] (μmole/mg of protein/hr)	p
0	0.08 ± 0.01	—
10^{-5}	0.09 ± 0.01	>0.50
10^{-4}	0.08 ± 0.01	>0.50
10^{-3}	0.25 ± 0.05	<0.01

[a] Results are the mean ± SEM for 8 expts.

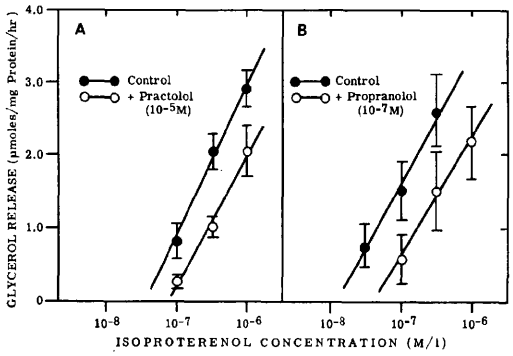

FIG. 1. Inhibition of isoproterenol-stimulated lipolysis by practolol (A) and propranolol (B): (abscissa) concentration of isoproterenol; (ordinate) glycerol release (μmoles/mg of protein/hr); practolol concentration, 10^{-5} M; propranolol concentration, 10^{-7} M; all values are the mean ± SE of seven observations.

The ability of practolol to increase lipolysis (intrinsic activity) was examined in eight experiments. At concentrations of 10^{-5} and 10^{-4} M this compound produced no alteration in lipolytic activity. At 10^{-3} M, however, there was a significant increase in lipolytic rate (Table I).

Table II shows the results of six experiments which measured the ability of practolol and propranolol to inhibit isoproterenol and dibutyryl cyclic-AMP-stimulated lipolysis. Isoproterenol-stimulated lipolysis was reduced by 50% by practolol and propranolol at concentrations of 10^{-5} and 10^{-7} M, respectively. Both of these compounds at a concentration of 10^{-3} M nearly abolished isoproterenol-stimulated lipolysis.

Propranolol (10^{-7} M) and practolol (10^{-5} M) did not significantly alter the lipolytic response to dibutyryl cyclic AMP. Propranolol at a concentration of 10^{-3} M, however, reduced the lipolytic response to this agent by more than 85%. At the same concentration, practolol had no significant effect on dibutyryl cyclic AMP stimulated lipolysis. Even when the concentration of practolol was increased to 10^{-2} M, there was no significant reduction in the response to this agent.

The relative abilities of propranolol and practolol to inhibit isoproterenol-stimulated lipolysis were compared by determining pA_2 values for the compounds. The pA_2 value for

TABLE II. Effect of Propranolol and Practolol on Isoproterenol and Dibutyryl Cyclic AMP-Stimulated Lipolysis.

Drug (moles/liter)	Inhibitor (moles/liter)	Glycerol release[a]	p
Isoproterenol (3×10^{-7})	None	1.99 ± 0.25	
	+ Propranolol (10^{-7})	0.95 ± 0.14	<0.005
	+ Practolol (10^{-5})	0.97 ± 0.18	<0.005
	+ Propranolol (10^{-3})	0.41 ± 0.04	<0.005
	+ Practolol (10^{-3})	0.24 ± 0.01	<0.005
DBC AMP[b] (2×10^{-3})	None	3.95 ± 0.51	
	+ Propranolol (10^{-7})	3.60 ± 0.31	>0.30
	+ Practolol (10^{-5})	3.67 ± 0.46	>0.10
	+ Propranolol (10^{-3})	0.46 ± 0.06	<0.005
	+ Practolol (10^{-3})	3.62 ± 0.69	>0.50
DBCAMP[b] (2×10^{-3})	None	3.77 ± 0.10	
	+ Practolol (10^{-2})	3.41 ± 0.10	>0.05

[a] Mean ± SEM of 4 to 6 expts. (μmole/mg of protein/hr).
[b] DBCAMP = dibutyryl cyclic AMP.

TABLE III. Effect of Propranolol and Practolol on ACTH-Stimulated Lipolysis.

Drug	Inhibitor	Glycerol release[a]	p[b]
ACTH (0.1 U/ml)	None	1.29 ± 0.27	—
	+ Propranolol (10^{-7} M)	1.53 ± 0.21	<0.01
	+ Practolol (10^{-5} M)	1.50 ± 0.30	<0.01
	+ Propranolol (10^{-3} M)	0.40 ± 0.08	<0.005
	+ Practolol (10^{-3} M)	1.94 ± 0.30	<0.001

[a] Mean ± SEM of 6 expts. (μmoles/mg of protein/hr).
[b] As compared to ACTH alone.

propranolol calculated from a series of six experiments was 7.92 ± 0.19 (mean ± SE). From a series of five experiments the pA_2 value for practolol was found to be 6.00 ± 0.11 (mean ± SE). Analysis of these data by Student's t test for group comparison revealed that the difference between these two pA_2 values was highly significant ($p<0.001$). These data indicate that propranolol was more than 80 times as potent than practolol as an inhibitor of isoproterenol-stimulated lipolysis.

Low concentrations of both propranolol (10^{-7} M) and practolol (10^{-5} M) failed to inhibit the lipolytic response to ACTH (Table III). Propranolol at a concentration of 10^{-3} M significantly inhibited the action of ACTH while the same concentration of practolol actually increased the lipolytic response.

TABLE IV. Effect of Propranolol and Practolol on Oxidation of Glucose-1-^{14}C by Isolated Fat Cells.

	Drugs	Glucose-1-^{14}C oxidation[a] (% control)
I	Control	100
	+ Propranolol (10^{-7} M)	113
	+ Propranolol (10^{-3} M)	30
	+ Practolol (10^{-5} M)	95
	+ Practolol (10^{-3} M)	126
	Insulin (1 mU/ml)	211
	+ Propranolol (10^{-7} M)	190
	+ Propranolol (10^{-3} M)	36
	+ Practolol (10^{-5} M)	190
	+ Practolol (10^{-3} M)	215
II	Control	100
	+ Practolol (10^{-2} M)	91
	Insulin (1 mU/ml)	328
	+ Practolol (10^{-2} M)	319

[a] Results of means of 3 to 5 expts.

As reported previously (5), propranolol at high concentrations inhibits glucose oxidation in fat cells. Concentrations of practolol as high as 10^{-2} M failed to alter glucose-1-^{14}C oxidation either in the presence or absence of insulin (1 mU/ml) (Table IV).

Discussion. Practolol is a new *beta*-adrenergic blocking agent which is reported to be selective *in vivo* for the effects of isoproterenol on the heart and mobilization of the free fatty acids (2). This agent is reported to have low specificity for tracheal relaxation and peripheral vasodilation. Barrett *et al.* (2) reported that practolol was ⅓ to ½ as potent as propranolol as an inhibitor of the *in vivo* effects of isoproterenol on free fatty acid mobilization, heart rate, and myocardial force of contraction. In the same experiments practolol had only 1/150 and 1/370 the potency of propranolol on isoproterenol-induced tracheal relaxation and vasodilation, respectively.

Results of *in vitro* experiments reported here show that practolol is a competitive inhibitor of isoproterenol-stimulated lipolysis, with a low level of intrinsic activity. On the basis of the pA_2 values for propranolol and practolol, propranolol appears to be more than 80 times as potent as practolol. This conclusion is substantiated by the results reported in Table II. Fifty percent inhibition of the lipolytic response to isoproterenol was produced by propranolol at a concentration of 10^{-7} M. One hundred times as much practolol (10^{-5} M) was needed to produce the same degree of inhibition.

These results do not agree with the *in vivo* results reported by Barrett *et al.* (2). Although no explanation is readily available for this discrepancy, it is well known that the

catecholamines and *beta*-adrenergic blocking agents have many effects which could modify the *in vivo* mobilization of free fatty acids. Such effects include changes in plasma levels of glucose (7), insulin secretion (8), and glucose utilization (5). Any or all of these reactions could complicate the estimation of the antilipolytic potency of practolol.

In addition to their effects on the *beta*-receptor, (presumably adenylcyclase) (9) propranolol and other *beta*-adrenergic blocking agents have been shown to have an inhibitory effect on the lipolytic response to the dibutyryl analog of cyclic AMP (10, 11). These results are substantiated by results reported here.

At a concentration of 10^{-3} *M*, propranolol inhibited dibutyryl cyclic AMP-stimulated lipolysis by greater than 85%. Practolol, however, at the same concentration, had no significant effect on dibutyryl cyclic AMP-stimulated lipolysis. In fact, increasing the concentration of practolol to 10^{-2} *M*, still did not significantly inhibit the response to dibutyryl cyclic-AMP. It should be noted here that practolol has been reported to have no local anesthetic activity (2) while propranolol has been reported to be quite potent in this regard (12–14). It is possible, therefore, that the ability of propranolol to inhibit dibutyryl cyclic AMP-stimulated lipolysis is a reflection of its local anesthetic activity. On the basis of this hypothesis practolol, not having any local anesthetic activity, would not inhibit the lipolytic response to the cyclic-nucleotide analog.

This local anesthetic action of propranolol also appears to provide an explanation of the inhibitory action of the compound in ACTH-stimulated lipolysis and glucose oxidation. Because practolol does not alter either of these metabolic processes it is unlikely that this action of propranolol is dependent on blockade of the *beta*-receptor. The ability of practolol to augment the lipolytic activity of ACTH remain unexplained.

Summary. The antilipolytic activity of the *beta*-adrenergic blocking drug, 4-(2-hydroxy-3-isopropylaminopropoxy) acetanilide (practolol), was examined using the isolated fat cell preparation. The compound was found to be a competitive inhibitor of hormone-stimulated lipolysis with low intrinsic activity. As determined by pA_2 values the compound was 80–100 times less potent than propranolol. In contrast to propranolol, practolol did not inhibit the lipolytic response to dibutyryl cyclic AMP nor ACTH. Practolol also failed to inhibit glucose oxidation.

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