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INFLUENCE OF SUBSTITUTED TETRAHYDROISOQUINOLINES AND CATECHOLAMINES ON LIPOLYSIS, *IN VITRO*—II

STEREOSELECTIVITY*†

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Abstract—Utilizing glycerol release as an index of lipolysis in rat epididymal fat tissue, intrinsic activity and affinity (pD_2 values) constants were calculated for various tetrahydroisoquinoline (THI) and catecholamine derivatives. Differences were noted in the pD_2 values and to a lesser degree in the intrinsic activity constants of the *d*-*l*-isomers and deoxy derivatives of the agonists studied. In all cases, the *l*-isomers proved to be more active than the *d*-isomers or deoxy derivatives in the release of glycerol from adipose tissue, *in vitro*. Significant differences in the pD_2 values for the more potent *l*-isomers were calculated and the rank order was observed to be 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI > isoproterenol > norepinephrine ≥ 1-benzyl-6,7-dihydroxy-1,2,3,4-THI. The deoxy derivatives of isoproterenol and norepinephrine possessed nearly identical pD_2 values to their respective *d*-isomers. *N*-isopropyldopamine and dopamine, however, were unable to elicit a maximum lipolytic response and did not possess equivalent intrinsic activity constants to *d*-isoproterenol and *d*-norepinephrine. Propranolol inhibited the lipolysis induced by 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI and norepinephrine in a competitive manner, whereas a noncompetitive inhibition was observed in the presence of the *alpha*-antagonist, phentolamine. The activity-differences for the isomers of 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI (795-fold) and isoproterenol (316-fold) were clearly greater than the isomeric-activity differences observed for norepinephrine (100-fold) and 1-benzyl-6,7-dihydroxy-1,2,3,4-THI (25-fold). These results indicate: (a) that the THI derivatives which do not possess an alcoholic (β-hydroxyl) group on the ethylamino-side chain are potent lipolytic agonists, and (b) that the tetrahydroisoquinoline derivatives act at a receptor system in adipose tissue similar, if not identical, to the interaction observed with norepinephrine. It may be suggested, therefore, that the β-hydroxyl group is not a necessary requirement for potent lipolytic activity as previously believed.

CONSIDERABLE interest has been placed upon studies designed to elucidate the steric aspects of adrenergic drugs.[1,2] For example, a great deal of knowledge has been gained as to the stereochemical requirements of sympathomimetic amines. Although several studies have been carried out with a variety of tetrahydroisoquinoline (THI) derivatives in adrenergic systems,[3–7] little information is available regarding the activity of optical isomers of isoquinoline derivatives.[8,9]

In this report, we will present data concerning the activity of *d*- and *l*-isomers of 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI and 1-benzyl-6,7-dihydroxy-1,2,3,4-THI on the release of glycerol from rat adipose tissue, *in vitro*. Differences in

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lipolytic activity between the isomers of these THI's are compared to the isomeric differences of the potent sympathomimetic amines norepinephrine and isoproterenol. Moreover, only a few reports have attempted to quantitate the stereoselective action of norepinephrine and isoproterenol on lipolysis, *in vitro*.[10,11]

EXPERIMENTAL

Methods. Non-fasted, white, male Harlan Wister rats, weighing 220 ± 30 g, were stunned and decapitated. The anterior one-third of the epididymal fat pads from four to six rats per experiment was transferred to Krebs–Ringer bicarbonate buffer (pH 7·4) and minced with scissors to yield adipose tissue fragments of 5–15 mg. For each test, 300 mg adipose tissue minces were added to 2·5 ml of the buffer solution containing 4% bovine albumin. The incubation of fat pads and the assay of glycerol release were conducted by procedures described previously.[7] In experiments using propranolol or phentolamine, the inhibitors were preincubated with adipose tissue fragments and buffer solution for 15 min prior to the addition of the agonist.

The rates of glycerol release, expressed as micromoles of glycerol released per gram of tissue per hour, were calculated from the data obtained after the addition of the agonist. Glycerol released in the absence of agonist was subtracted from the amount in the presence of the agonist to give the net rate of glycerol release. In each experiment, a maximal rate of glycerol release was observed in the presence of *l*-norepinephrine, which was employed as an internal standard in these studies. This maximal figure, found to be 5·4 μmoles/g/hr, was employed to calculate the per cent response of adipose tissue to varying concentrations of agonist to obtain the dose–response relationships. Experiments were repeated at least four times at various concentrations of agonist (ranging from 10^{-3} to 10^{-7} M) to establish the dose–response relationships. Values plotted in the figures refer to the mean ± S.E.

For each of the compounds tested, the affinity and intrinsic activity constants were determined according to the terminology described by Ariens and Simonis.[12,13] Intrinsic activity constants are expressed as the ratio of the maximum response obtained with each agonist to the maximal response obtainable in this system and the affinity constants are expressed as pD_2 values,[14] defined as the negative logarithm of the agonist concentration required to produce a response equal to 50 per cent of its maximal response.

RESULTS

Dose–response relationship of the d- and l-isomers of the tetrahydroisoquinolines (THI), isoproterenol and norepinephrine on the release of glycerol, in vitro. From the dose–response relationships presented in Figs. 1–4, the *l*-isomers of 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI, 1-benzyl-6,7-dihydroxy-1,2,3,4-THI, isoproterenol and norepinephrine were observed to be considerably more active in the release of glycerol from adipose tissue than the corresponding *d*-isomers. Moreover, the *d*-isomers of isoproterenol and norepinephrine elicited a maximal rate of glycerol release identical to that found with the *l*-isomers whereas the deoxy derivatives, which were also found to be weakly active, were unable to produce a maximum lipolytic effect (see Figs. 3 and 4).

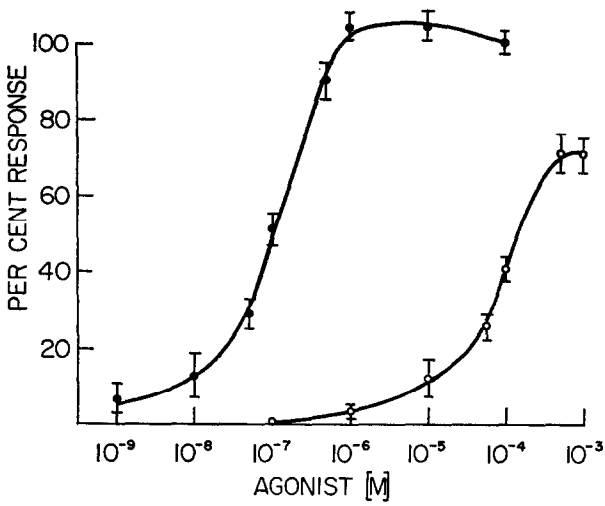

FIG. 1. Dose–response curves for the isomers of 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline on the release of glycerol from rat epididymal fat tissue *in vitro*. Key: (●——●), *l*-isomer; (○——○), *d*-isomer.

Effect of the adrenergic blocking agents, propranolol and phentolamine, on the release of glycerol induced by 1-(3′,4′,5′-*trimethoxybenzyl*)-6,7-*dihydroxy*-1,2,3,4-*THI and l-norepinephrine.* In an attempt to determine the mode of action for the tetrahydroisoquinoline derivatives on lipolysis it was of interest to examine the influence of propranolol and phentolamine as antagonists in this lipolytic system. As shown in Figs. 5–8, the inhibition of 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI and norepinephrine-induced lipolysis by these adrenergic blocking agents were quite

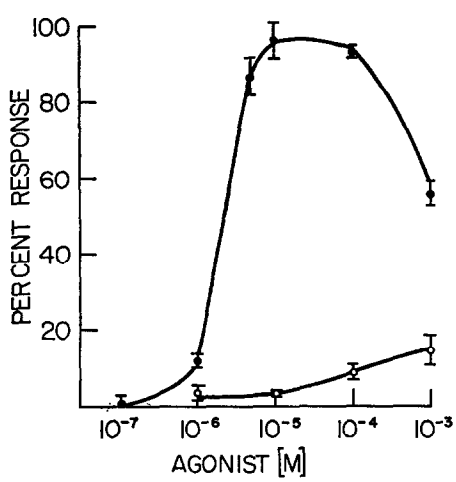

FIG. 2. Dose–response curves for the isomers of 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline on the release of glycerol from rat epididymal fat tissue *in vitro*. Key: (●——●), *l*-isomer; (○——○), *d*-isomer.

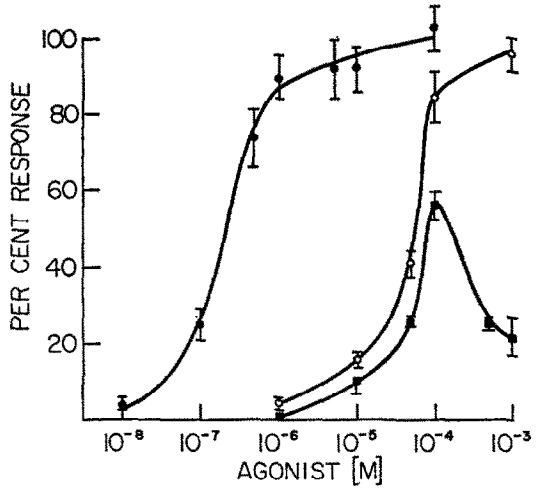

FIG. 3. Dose-response curves for *l*-isoproterenol (●——●), *d*-isoproterenol (○——○) and *N*-isopropyldopamine (■——■) on the release of glycerol from rat epididymal fat tissue *in vitro*.

similar. Propranolol (see Figs. 5 and 6) exhibited a competitive inhibition in the presence of either agonist whereas non-competitive inhibition was observed with phentolamine (see Figs. 7 and 8). In the presence of 10^{-5} M propranolol, a nearly identical parallel shift of 1·5 log units was noted with both agonists. Further, the maximal response of these agonists was reduced 20–30 per cent in the presence of 10^{-4} M phentolamine.

Intrinsic activity and affinity constants for the tetrahydroisoquinoline (THI) and catecholamine derivatives. The intrinsic activity and affinity (pD_2 values) constants for all agonists employed in this study are summarized in Table 1. As evidenced by the

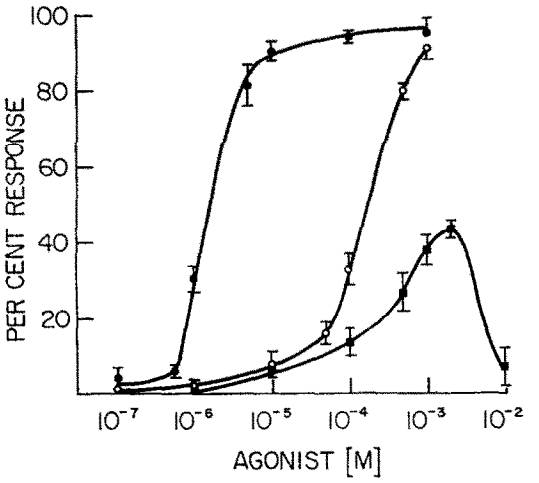

FIG. 4. Dose-response curves for *l*-norepinephrine (●——●), *d*-norepinephrine (○——○) dopamine (■——■) on the release of glycerol from rat epididymal fat tissue *in vitro*.

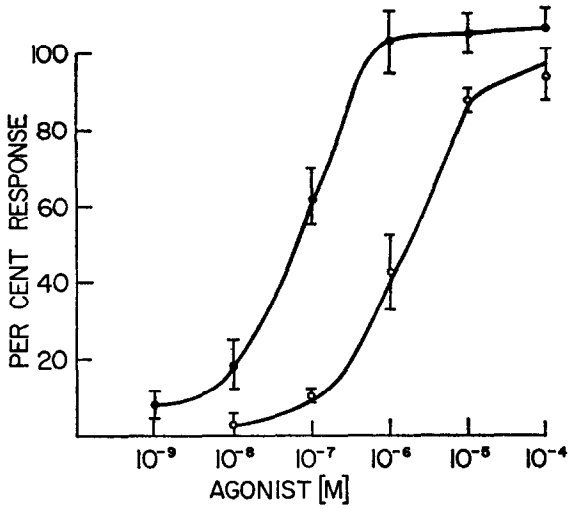

FIG. 5. Dose–response curves for *d*, *l*-1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline on the release of glycerol from rat epididymal fat tissue *in vitro*, in the presence (○——○) or absence (●——●) of 10^{-5} M propranolol.

pD_2 values for the agonists, the *l*-isomers were clearly more active than the *d*-isomers in the adrenergic-adipose tissue system. Of the THI's and catecholamines tested, the rank order of lipolytic activity for the more active *l*-isomers was 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI > isoproterenol > norepinephrine ≥ 1-benzyl-6,7-dihydroxy-1,2,3,4-THI. The *d*-isomers of the THI's and catecholamines possessed lower pD_2 values, thus indicating a reduction in the affinity of the agonist molecule. Moreover, it was found that *N*-isopropyldopamine and dopamine, the deoxy derivatives of isoproterenol and norepinephrine, respectively, were unable to induce a

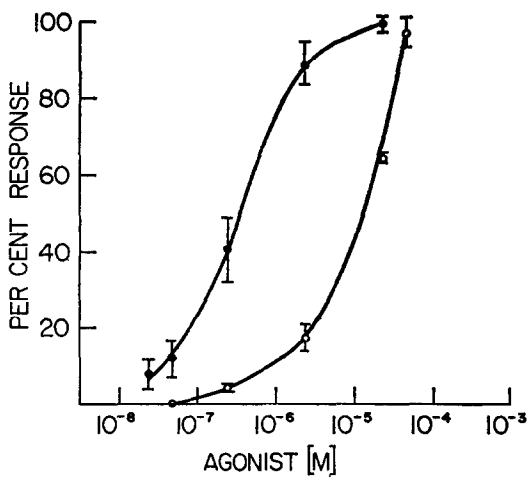

FIG. 6. Dose–response curves for *l*-norepinephrine on the release of glycerol from rat epididymal fat tissue *in vitro*, in the presence (○——○) or absence (●——●) of 10^{-5} M propranolol.

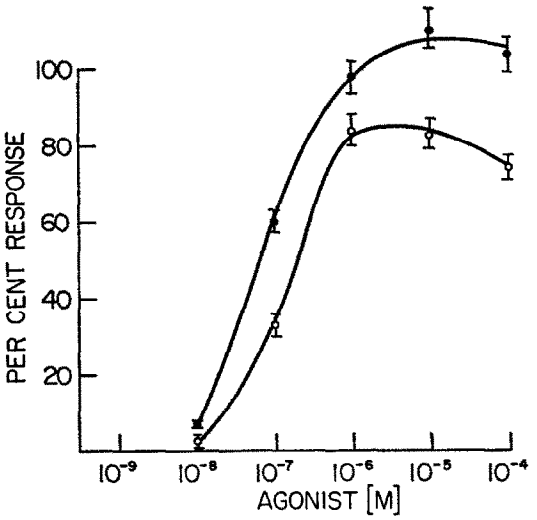

FIG. 7. Dose–response curves for *l*-1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline on the release of glycerol from rat epididymal fat tissue *in vitro*, in the presence (○——○) or absence (●——●) of 10^{-4} M phentolamine.

maximal release of glycerol from adipose tissue. The pD_2 values for these deoxy derivatives, however, were comparable to those obtained for the corresponding *d*-isomers of the catecholamines, isoproterenol and norepinephrine. In addition, it should be noted that the intrinsic activity constants for the *d*-isomers of the substituted THI's were also decreased.

In order to correlate the relative stereoselectivity of the THI's, isoproterenol and norepinephrine on the release of glycerol, the isomeric-activity differences were calculated and included in Table 1. As can be seen, the isomeric-activity difference of 2·9

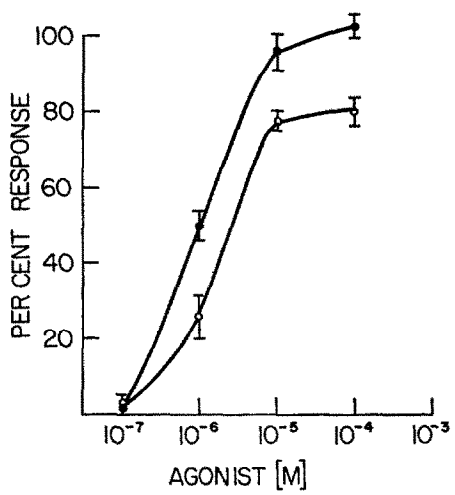

FIG. 8. Dose–response curves for *l*-norepinephrine on the release of glycerol from rat epididymal fat tissue *in vitro*, in the presence (○——○) or absence (●——●) of 10^{-4} M phentolamine.

TABLE 1. EFFECTS OF OPTICAL ISOMERS OF TETRAHYDROISOQUINOLINE (THI) AND CATECHOLAMINE DERIVATIVES ON THE RELEASE OF GLYCEROL FROM RAT ADIPOSE TISSUE, *in vitro*

Compound	Isomer	pD_2 Value ± S.D.	Isomeric-activity difference*	I.A.†
1-(3′,4′,5′-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI	*l*	7·0 ± 0·2	2·9	1·0
	d	4·1 ± 0·2		0·71
1-Benzyl-6,7-dihydroxy-1,2,3,4-THI	*l*	5·7 ± 0·1	1·4	1·0
	d	4·3‡		0·15
Isoproterenol	*l*	6·7 ± 0·1	2·5	1·0
	d	4·2 ± 0·2		1·0
N-isopropyldopamine		4·2 ± 0·2		0·57
Norepinephrine	*l*	5·8 ± 0·2	2·0	1·0
	d	3·8 ± 0·1		1·0
Dopamine		3·5 ± 0·2		0·43

* Isomeric-activity difference = [(negative molar log ED_{50} of *l*-isomer)−(negative log molar ED_{50} of *d*-isomer)].

† Intrinsic activity.

‡ Slopes were divergent and did not permit an accurate estimation of pD_2 values in these experiments.

log units for 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI was larger than the value of 2·5 log units observed for isoproterenol, a potent β-agonist. In addition, the isomeric activity differences for 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI and isoproterenol were greater than the ratio values of 2·0 and 1·4 log units as calculated for norepinephrine and 1-benzyl-6,7-dihydroxy-1,2,3,4-THI respectively.

DISCUSSION

Several investigators[15–17] have characterized adrenergic receptor systems as *alpha*- and *beta*-type. In response to stimulation by catecholamines, adipose tissue has been classified as an adrenergic receptor system of the *beta*-type, e.g. isoproterenol > epinephrine ≤ norepinephrine. In the present paper, the results clearly show that *l*-isoproterenol is more potent than *l*-norepinephrine on lipolysis, thus substantiating earlier reports.[10,17–19] According to Easson and Stedman,[2] the stereospecific interaction of sympathomimetic amines with adrenergic receptor systems has been proposed to involve a three-point attachment. They suggested that the *d*-isomers and deoxy derivatives of catecholamines should possess similar pharmacological activities. In this study, the deoxy derivatives, *N*-isopropyldopamine and dopamine, exhibited an affinity for the adrenergic–adipose tissue receptor system nearly identical to the corresponding *d*-isomers. It should be noted, however, that the intrinsic activities for the deoxy derivatives were less than those observed for the *d*-isomers. The equivalent potencies of deoxynorepinephrine (dopamine) and *d*-norepinephrine have been

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