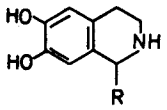
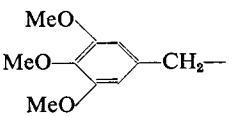
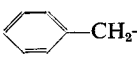
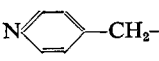
Influence of substituted tetrahydroisoquinolines on lipolysis, *in vitro*

The important structural requirements involved in the mobilization of free fatty acids (FFA) in adipose tissue induced by adrenergic agents, particularly noradrenaline, isoprenaline, and adrenaline include a catechol nucleus, an alcoholic hydroxyl group on the β-carbon atom, and bulkyl alkyl or arylalkyl functions on the amino-nitrogen of the ethylamino side-chain (Feller & Finger, 1966; 1970; Wenke, 1966; Cernohorsky, Cepelik & others, 1966). While it is well-known that the adrenergic activity of substituted phenethylamines is greatly enhanced by *N*-substitution, few studies have been reported on the influence of tetrahydroisoquinoline (THI) derivatives in adipose tissue receptor systems. Holtz, Stock & Westermann (1964) found the isoquinoline derivative, 3,4 dihydroxybenzyl-6,7 dihydroxy-THI, to be 6 times less active than noradrenaline in the mobilization of FFA, *in vivo* and *in vitro*. In other studies (Iwasawa & Kiyomoto, 1967; Yamato, Hirakura & Sugasawa, 1966) it was reported that several THI analogues were more active than adrenaline on tracheal relaxation. Of the THI's tested 1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI was found to be the most potent. Since tracheal relaxation and lipolysis are considered to be β-adrenergic receptor systems (Wenke & others, 1966), it was of interest to test additional THI derivatives for their ability to release glycerol from adipose tissue, *in vitro*.

Epididymal fat pads from 4–6 male Harlan-Wistar rats (200–250 g) per experiment were transferred to Krebs Ringer bicarbonate buffer, pH 7·4 and minced with scissors to yield adipose tissue fragments. Various concentrations (10^{-3} — 10^{-9}M) of each agonist and 300 mg of adipose tissue were incubated at 37° for 60 min in 2·5 ml of bicarbonate buffer which contained 4% bovine serum albumin (Finger, Page & Feller, 1966). After 60 min, the reaction was terminated by the addition of 1 ml of trichloroacetic acid (10%). The rate of lipolysis was determined from the glycerol formed, by oxidation according to Lambert & Neish (1950) and assay of the resulting formaldehyde by the method of Nash (1953). A maximal release of glycerol calculated to be 5·4 μmol glycerol/g tissue h^{-1} was observed for all agonists with the exception of dopamine. This value was then used to calculate the percent response of adipose tissue to varying concentrations of agonist to obtain dose-response relations. Experiments were repeated at least 4 times to establish dose-response curves to permit the calculation of the intrinsic activity and affinity (pD_2 values) constants as described by Miller, Becker & Tainter (1948) and Ariens & Simonis (1962). From a comparison of the pD_2 values shown in Table 1, the rank order of activity for the compounds was THI (I) > noradrenaline > THI (II) $\geqslant$ THI (III) >> dopamine. The trimethoxy-THI analogue (I) was the most active derivative tested and was found to be about 5 times more potent than noradrenaline. Although the 1-benzyl (II) and 1-(4′-pyridylmethyl) (III)- THI's were less active than noradrenaline, they were much more effective than dopamine. The difference in pD_2 values observed between noradrenaline and dopamine is attributed to the presence or absence of the β-hydroxyl group respectively. While this chemical moiety has been ascribed an important role for the catecholamine-induced mobilization of FFA; the THI derivatives which lack the β-hydroxyl group retain potent lipolytic activity in this adrenergic adipose tissue system. We have also observed that the β-blocker, propranolol, is a competitive inhibitor of the lipolysis induced by noradrenaline and the 3,4,5-trimethoxy compound (I). In the presence of 10^{-5}M propranolol, a parallel shift of 1·5 log dose units was obtained for both of these compounds (see Table 1). This evidence suggests that the THI derivatives may interact at the same adrenergic receptor system in adipose tissue as noradrenaline. Coupled with the previous study on the effects of

Table 1. *Intrinsic activity and affinity (pD_2 values) constants for substituted tetrahydroisoquinolines* (THI) *on the release of glycerol from adipose tissue*, in vitro.



No.	Compound	R	$pD_2 \pm$ s.d.	Intrinsic activity
I	1-(3′,4′,5′-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI		7·2 ± 0·4	1·0
	1-(3′,4′,5′-Trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-THI & 10^{-5}M (±)-propranolol		5·7 ± 0·3	1·0
	(—)-Noradrenaline		6·5 ± 0·3	1·0
	(—)-Noradrenaline & 10^{-5}M (±)-propranolol		4·9 ± 0·1	1·0
II	1-Benzyl-6,7-dihydroxy-1,2,3,4-THI		5·8	1·0
III	1-(4′-Pyridylmethyl)-6,7-dihydroxy-1,2,3,4-THI		5·6	1·0
	Dopamine		3·9	0·3

THI compounds on tracheal relaxation, our results clearly indicate that appropriately substituted THI's are potent β-adrenergic agonists.

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REFERENCES

ARIENS, E. J. & SIMONIS, A. M. (1962). *Acta Physiol. Pharmac. neerl.*, **11**, 151–172.

CERNOHORSKY, M., CEPELIK, J., LINCOVA, D. & WENKE, M. (1966). *J. Pharm. Pharmac.*, **18**, 188–189.

FELLER, D. R. & FINGER, K. F. (1970). *Biochem. Pharmac.*, **19**, 705–713.

FINGER, K. F. & FELLER, D. R. (1966). *J. pharm. Sci.*, **55**, 1051–1054.

FINGER, K. F., PAGE, J. G. & FELLER, D. R. (1966). *Biochem. Pharmac.*, **15**, 1023–1032.

HOLTZ, P., STOCK, K. & WESTERMANN, E. (1964). *Arch. exp. Path. Pharmak.*, **248**, 387–405.

IWASAWA, Y. & KIYOMOTO, A. (1967). *Jap. J. Pharmac.*, **17**, 143–152.

LAMBERT, M. & NEISH, A. C. (1950). *Canad. J. Res.*, **23**, 83–89.

MILLER, L. C., BECKER, T. J. & TAINTER, M. L. (1948). *J. Pharmac. exp. Ther.*, **92**, 260–268.

NASH, T. (1953). *Biochem. J.*, **55**, 416–421.

WENKE, M. (1966). *Adv. Lipid Res.*, **4**, 69–105.

WENKE, M., LINCOVA, D., CERNOHORSKY, M. & CEPELIK, J. (1966). *J. Pharm. Pharmac.*, **18**, 190–191.

YAMATO, E., HIRAKURA, M. & SUGASAWA, S. (1966). *Tetrahedron*, Suppl., **8**, Part I, 129–134.