Phenethylamine Inhibitors of Partially Purified Rat and Human Pancreatic Lipase

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Abstract □ Methodology for the preparation of rat and human pancreatic lipase (EC 3.1.1.3) is described, which resulted in good yield of partially purified, stable enzyme useful for kinetic studies. Apparent K_m values for the rat (6.5 m*M*) and human (3.5 m*M*) enzyme were determined with triolein as the substrate. Several compounds of the phenethylamine class were found to be inhibitors of both rat and human pancreatic lipase. The structural feature in the phenethylamine series tested, which appeared to be necessary for lipase inhibition, was a halogenated substituent on the 3 or 4 position of the aromatic ring as in flutiorex, fenfluramine, *N*-benzyl-β-methoxy-3-(trifluoromethyl)phenethylamine (I), chlorphentermine and *p*-chloroamphetamine. A chloro group at the 2 position was ineffective (chlortermine). Alterations in the ethylamine portion of the molecule did not cause significant changes in the inhibitory properties of the active phenethylamines.

Keyphrases □ Phenethylamines—inhibition of pancreatic lipase, rats and humans □ Kinetics—competitive inhibition of pancreatic lipase, rats and humans □ Pancreatic lipase—inhibition by phenethylamines, rats and humans

The anorectic effect of many compounds of the phenethylamine class has been recognized for almost 40 years (1). In addition to anorexia, some phenethylamines show marked effects on lipid metabolism. Amphetamine and fenfluramine have been shown to decrease the postprandial rise in plasma triglycerides in corn oil loaded rats (2–4) and in humans (5), and to elevate plasma free fatty acid concentration (5, 6). Fenfluramine also was reported to inhibit hepatic lipogenesis (2), reduce intestinal motility (4), and interfere with postabsorptive reesterification by inhibiting rat intestinal palmitolyl CoA monolein transferase activity (7). Furthermore, fenfluramine and an analog, benfluorex, were reported to inhibit pancreatic lipase (2, 8, 9). Although these activities were postulated previously (4, 10) to explain (in part) weight reduction by fenfluramine through decreased dietary triglyceride absorption, a study in healthy humans revealed that fenfluramine did not alter fat absorption (11).

In addition to phenethylamines, several chemically unrelated compounds which inhibit the release of free fatty acids from adipose tissue reportedly interfere with postprandial lipemia (12, 13). Barboriak, *et al.* (12) demonstrated that 3,5-dimethylpyrazole, salicylic acid, and nicotinic acid inhibit plasma triglyceride rise in rats given corn oil. In these studies, reduced fat absorption was observed in animals receiving salicylic acid. Another report (13) demonstrated the antilipolytic activity of two α-blockers, phentolamine and phenoxybenzamine. These studies were furthered by examination of inhibitory effect of phentolamine and phenoxybenzamine on rat epididymal fat and pancreatic lipases (14). The epididymal fat lipase appeared to be inhibited by both α-blockers; pancreatic lipase was inhibited by phenoxybenzamine but stimulated by phentolamine.

The present study reports the effectiveness of various phenethylamines and other antilipolytic agents as inhibitors of rat and human pancreatic lipase. A systematic *in vitro* evaluation of representative compounds was initiated using freshly prepared, partially purified preparations of rat and human pancreatic lipase. The results were compared with those obtained with diethyl-*p*-nitrophenyl phosphate, a known active site inhibitor of hog pancreatic lipase (15).

MATERIALS AND METHODS

Pancreatic Lipase Preparations—Rat pancreatic proteins were prepared by adaptation of a method described for the guinea pig pancreas (16). In a representative experiment, six female Sprague-Dawley rats[1], weighing 180–220 g were fed a 20% corn oil diet *ad libitum* for 8 to 10 weeks. This diet was shown to increase pancreatic lipase levels ~50% (17, 18). Prior to sacrifice the rats were fasted for 24 hr to accumulate zymogen granules in the acinar cells of the pancrease (19). The pancrease was removed quickly after sacrifice and immersed in oxygenated Krebs-Ringer bicarbonate. The pancreatic lobules were prepared as described previously (16). Eight lobules corresponding to ~50 mg wet weight per flask were incubated for 4 hr in 5 ml of the oxygenated Krebs-Ringer bicarbonate containing carbachol (10^{-5} *M*), soybean trypsin inhibitor (10 μg/ml), benzamidine (10 m*M*), and phenylmethylsulfonyl fluoride (5 m*M*). After incubation the flask contents were centrifuged at 100,000×*g* for 30 min. The resulting juice was frozen immediately in liquid nitrogen and stored at −70°.

Human pancreatic juice was obtained from a patient with acute pancreatitis whose pancreatic duct had been cannulated. Benzamidine (8.2 m*M*) and soybean trypsin inhibitor (0.5 mg/ml) were added to the collection vessels to deter enzymatic degradation of the lipase. The juice was stored at −70°.

Rat and human pancreatic juices were purified by gel filtration chromatography[2] (2.5 × 100 cm) using 0.05 *M* tromethamine[3] buffer, pH 8 at 25°. Protein profiles were monitored at 280 nm[4]. Active fractions were combined, concentrated[5], and stored in 1-ml aliquots at −70°. These preparations remained fully active for over 6 months and were the source of enzymes for all experiments.

Pancreatic Lipase Assays—Two methods were utilized for the pancreatic lipase assay. The first was developed for use in screening large numbers of compounds and was based on the method of Krauss *et al.* (20). Glycerol tri[1-^{14}C]oleate (32 m*M*) was emulsified in 0.2 *M* tromethamine, pH 8 at 36° containing 0.15 *M* NaCl, bovine serum albumin (15 mg/ml, fatty acid free), and sodium taurocholate (1.4 m*M*) by sonication for three 1-min intervals at 80 watts[6]. The final assay volume was 1.0 ml. The reaction was initiated by the addition of enzyme and was allowed to proceed for 10 min. Under these conditions the reaction was linear for up to 20 min. The liberated free fatty acids were extracted and quantitated in a liquid scintillation counter[7]. The second method used was the titrimetric assay described by Maylie *et al.* (15). An olive oil–sodium taurocholate (1.4 m*M*) emulsion stabilized in 2% gum arabic was the substrate. The molecular weight of triolein (885 g) was used to calculate the substrate concentration. The final assay volume was 3.0 ml. The reaction was initiated by the addition of enzyme. The long-chain fatty acids released at 25° (pH 8) were continuously titrated with the aid of a recording pH-stat[8]. The lipase activity was determined directly from the slope of the linear portion of the curve.

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] Sephadex G-200, Pharmacia, Piscataway, N.J.
[3] Trizma, Sigma Chemical Co., St. Louis, Mo.
[4] Uvicord II, LKB, Sweden.
[5] PM10 filter, Amicon Corp., Lexington, Mass.
[6] Labsonic 9100, Labline Instruments, Melrose Park, Ill.
[7] Packard model 3380, Downers Grove, Ill.
[8] Radiometer, Copenhagen, Denmark.

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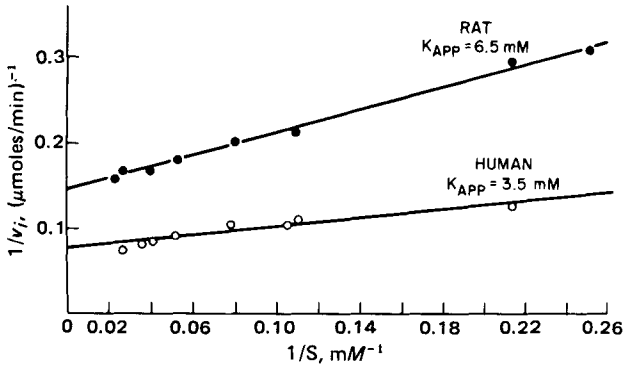


Figure 1—*Lineweaver–Burke plot of rat and human G-200 lipase activity. Assays were run using the described pH-stat method. Key: ●, rat G-200 lipase (protein concentration, 8.6 μg/ml); and ○, human G-200 lipase (protein concentration, 9.2 μg/ml).*

Inhibitors were added to the assays as solutions in either 90% ethanol or water, pH 8; 90% ethanol had no effect on pancreatic lipase activity assayed by either method.

Protein determinations were performed using the Lowry method (21).

Materials—Glycerol tri[1-^{14}C]oleate[9] was used without further purification. Sodium taurocholate, norepinephrine, epinephrine, phenylephrine, bovine serum albumin (fatty acid free), dopamine, phenethylamine, phenylpropanolamine, *l*-ephedrine, *d*-ephedrine, tyramine, and diisopropylfluorophosphate were purchased commercially[10]. Hydroxyamphetamine, amphetamine, phenoxybenzamine and *N*-benzyl-β-methoxy-3-(trifluoromethyl)phenethylamine (I) were used as received[11]. Diethylpropion[12], protokylol[12], metaraminol[13], nordefrin[13], tuaminoheptane[14], cyclopentamine[14], methoxyphenamine[15], clortermine[16], phentermine[17], nylidrin[18], metaproterenol[19], benzphetamine[20], phenmetrazine[21], phentolamine[21], chlorphentermine[22], fenfluramine[23], flutiorex[24], *p*-chloroamphetamine[25], and diethyl-*p*-nitrophenylphosphate[26] were used without further purification.

RESULTS

Characterization of Pancreatic Lipase Activities—Gel filtration of rat and human pancreatic juices by chromatography resulted in partial purification of both enzymes. The enzyme activities emerged 2.2 void volumes for rat lipase and 2.0 void volumes for human lipase resulting in a twofold purification of rat lipase and a sevenfold purification of human lipase.

Molecular weights were determined on the chromatographic column by comparing the elution of the lipases to standard proteins. The rat lipase appeared to have a molecular weight near 37,000; the human lipase molecular weight was ~48,000 (22).

The kinetics of the lipolytic reactions of the two lipase preparations were analyzed using the Michaelis–Menten formula as has been done for hog lipase (23) and phospholipases (24–26). The effects of varying substrate concentration on the initial velocity are shown in Fig. 1. Apparent K_m values obtained for rat and human G-200 lipase were of the order of 6.5 m*M* and 3.5 m*M*, respectively. It is not known whether the similar apparent K_m values are due to enzyme similarities or the physical characteristics of the emulsion assay system.

To determine whether the G-200 preparations contained colipase, purified hog colipase was incubated for 10 min at 37° with both the rat and human G-200 preparations at a ratio of 2 moles pure colipase to 1 mole of enzyme. The enzymic activities were assessed from initial velocity measurements using the pH-stat assay. As seen in Figs. 2A and 2B, there was no enhancement of either rat or human lipase activity after addition of exogenous colipase, indicating the G-200 lipase preparations were saturated with colipase. However, the additional colipase provided some protection of enzymic activity at high taurocholate concentrations (5–14 m*M*).

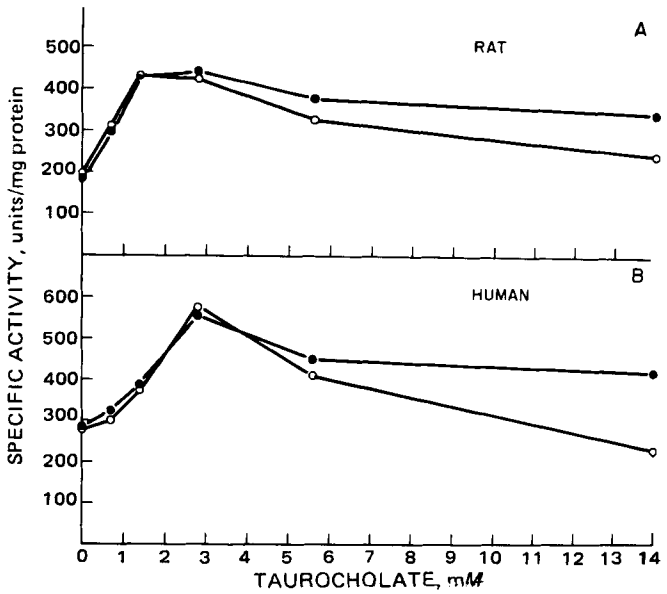


Figure 2—*Effect of added colipase on rat and human G-200 lipase. Two moles of pure colipase per mole of lipase were incubated with lipase for 10 min at 37°. An aliquot of the incubated enzyme–colipase mixture was added to the assay medium and the initial velocity recorded using the pH-stat assay. Key: ○, minus colipase; and ●, added colipase. (A), rat G-200 lipase; and (B), human G-200 lipase.*

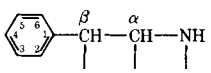
Inhibition of Rat G-200 Pancreatic Lipase—Many phenethylamines with central nervous system effects were inactive as inhibitors of pancreatic lipase. Table I shows the results of a structure–activity profile of the phenethylamine series evaluated in the carbon 14 and pH-stat titrimetric assays. All compounds were tested either to 20 m*M* or 100% inhibition. The catecholamines were either ineffective or slightly stimulatory toward rat pancreatic lipase. Interestingly, the five compounds in this series, which exhibited good inhibition, were the compounds related to amphetamine but with halogenated substituents on the 3 or 4 position of the aromatic ring. Inhibition constants summarized in Table I, as determined by a graphical method (27), were calculated for the five active phenethylamines from initial velocity data recorded during the titrimetric assay at 6 and 28 m*M* substrate concentrations. The lines were determined from linear regression analyses of the initial velocity data points which appeared to lie along a straight line. Cooperative and competitive inhibitory phenomena were observed for flutiorex, fenfluramine, and chlorphentermine. Using this method chlorphentermine, flutiorex, fenfluramine, *p*-chloroamphetamine, and I gave K_i values of 1.9, 3.0, 3.3, 6.2, and 11 m*M*, respectively. Analysis of the carbon 14 assay data resulted in K_i values of 9.6 m*M* for chlorphentermine, 6 m*M* for *p*-chloroamphetamine, 3.5 m*M* for flutiorex, 9.0 m*M* for fenfluramine, and 11 m*M* for I (Table I). Variation in the initial velocity was observed at the low substrate concentrations (6 m*M* triolein). However, this was <10% variation in the resulting K_i values. Interestingly, chlortermine, a phenylamine having a chlorine in the 2 position of the benzene ring, was not an inhibitor of rat pancreatic lipase, whereas the 4-chloro derivatives were inhibitors.

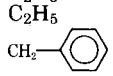
Two α-blockers were examined as inhibitors of rat G-200 pancreatic lipase and compared with diethyl-*p*-nitrophenylphosphate. The data were obtained and analyzed in the same manner as was done for the phenethylamines. The results are given in Table II. Phenoxybenzamine, which appeared to be a very strong inhibitor in the carbon 14 screen (0.9 m*M*), was a much weaker inhibitor when evaluated in the titrimetric assay (10 m*M*). This discrepancy was due to the fact that phenoxybenzamine delayed the initial velocity reaction for up to 6 min. However, after the initial lag time the enzymatic reaction proceeded and required relatively high phenoxybenzamine concentrations for inhibition.

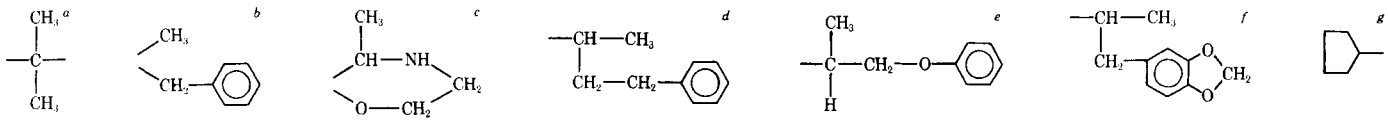
Phentolamine was inhibitory in both the carbon 14 and titrimetric

[9] Amersham, Arlington Heights, Ill.
[10] Sigma Chemical Co., St. Louis, Mo.
[11] Smith, Kline & French, Philadelphia, Pa.
[12] Merrell-National Laboratories, Cincinnati, Ohio.
[13] Winthrop Laboratories, New York, N.Y.
[14] Eli Lilly, Indianapolis, Ind.
[15] Ganes Chemical Works, Carlstadt, N.J.
[16] USV Pharmaceuticals, Tuckahoe, N.Y.
[17] Beecham-Messengil Pharmaceuticals, Bristol, Tenn.
[18] Millmaster Chemicals, New York, N.Y.
[19] Boehringer-Ingelheim, Ltd., Elmsford, N.Y.
[20] Upjohn, Kalamazoo, Mich.
[21] Geigy Pharmaceuticals, Ardsley, N.Y.
[22] Warner-Lambert, Morris Plains, N.J.
[23] A. H. Robins, Richmond, Va.
[24] Synthelabo, France.
[25] Leo Pharmaceuticals, Denmark.
[26] Aldrich, Milwaukee, Wis.

Table I—Chemical Structures of Phenethylamines and Inhibitory Properties on Rat Pancreatic Lipase



					Pancreatic Lipase Activity	
					^{14}C Assay	Titrimetric Assay
					K_i (mM)	
Flutiorex	3-SCF_3	H	CH_3	C_2H_5	3.5	3.0
Fenfluramine	3-CF_3	H	CH_3	C_2H_5	9.0	3.3
N-Benzyl-β-methoxy-3-(trifluoromethyl)phenethylamine	3-CF_3	OCH_3	H		11.0	11.0
Chlorphentermine	4-Cl	H	*a*	H	9.6	1.9
p-Chloroamphetamine	4-Cl	H	CH_3	H	6.0	6.2
					Carbon 14 Assay (10 mM)	
					% of Control	
Phenethylamine		H	H	H	100	
Amphetamine		H	CH_3	H	96	
Methamphetamine		H	CH_3	CH_3	85	
Benzphetamine		H	CH_3	*b*	100	
Phentermine		H	*a*	H	100	
Phenylpropanolamine		OH	CH_3	H	100	
d-Ephedrine		OH	CH_3	CH_3	119	
l-Ephedrine		OH	CH_3	CH_3	112	
Diethylpropion		O	CH_3	$(C_2H_5)_2$	109	
Phenmetrazine		*c*			100	
Methoxyphenamine	2-OCH_3	H	CH_3	CH_3	90	
Clortermine	2-Cl	H	*a*	H	91	
Phenylephrine	3-OH	OH	H	CH_3	90	
Metaraminol	3-OH	OH	CH_3	H	111	
Tyramine	4-OH	H	H	H	117	
Hydroxyamphetamine	4-OH	H	CH_3	H	85	
Nylidrin	4-OH	OH	CH_3	*d*	126	
Isoxsuprine	4-OH	OH	CH_3	*e*	88	
Dopamine	3-OH, 4-OH	H	H	H	80	
Norepinephrine	3-OH,4-OH	OH	H	H	111	
Epinephrine	3-OH,4-OH	OH	H	CH_3	113	
Nordefrin	3-OH,4-OH	OH	CH_3	H	106	
Isoproterenol	3-OH,4-OH	OH	H	$CH(CH_3)_2$	106	
Protokylol	3-OH,4-OH	OH	H	*f*	100	
Metaproterenol	3-OH,5-OH	OH	H	$CH(CH_3)_2$	100	
Cyclopentamine	*g*	H	CH_3	CH_3	84	
Tuaminoheptane	$CH_3(CH_2)_3$	H	CH_3	H	104	



assay. Inhibition constants of 5.8 mM for the former assay and 1.6 mM for the titrimetric assay were determined from Dixon plots (27). Diethyl-p-nitrophenylphosphate, an active site inhibitor of hog lipase (15), gave comparable K_i values for rat G-200 lipase in both the carbon 14 assay (0.1 mM) and the titrimetric assay (0.1 mM). Diisopropylfluorophosphate, an inhibitor of enzymes having an essential serine residue in the active site, produced no inhibition of rat G-200 lipase at 20 mM. This agrees with data obtained with hog lipase (15).

Inhibition of Human G-200 Pancreatic Lipase—The active inhibitors of rat G-200 pancreatic lipase were tested as inhibitors of human G-200 pancreatic lipase using the pH-stat titrimetric assay. Except for minor variations, the inhibitors of rat G-200 lipase were also effective as inhibitors of human G-200 lipase (Table III).

Diethyl-p-nitrophenylphosphate was the most effective inhibitor of both rat and human G-200 lipase giving identical inhibition constants of 0.1 mM. p-Chloroamphetamine was a stronger inhibitor of human G-200 lipase with an inhibition constant of 3.5 mM compared with rat G-200 lipase, 6.2 mM. The other active compounds gave inhibition constants for human G-200 lipase that were comparable or identical to the inhibition constants obtained for rat G-200 lipase.

DISCUSSION

Based on the reported specific activities for purified rat lipase and purified human lipase (28–30), the present preparations of rat and human G-200 lipases were 47 and 65% pure, respectively. A molecular weight of 37,000 was calculated for the rat lipase and agreed with the reported value of 36,000 (28). The human lipase had a calculated molecular weight of 48,000, slightly higher than the reported values of 45,000 (30) and 46,000 (29).

The fact that gel filtration did not appear to separate lipase from colipase was a major advantage of the present preparations. The presence of sufficient colipase for maximum activity with rat or human lipase was demonstrated in the experiments where high taurocholate concentrations (14 mM) did not inhibit significantly and added colipase did not stimulate significantly (Fig. 2).

The reversal of bile salt inhibition of lipase by colipase is most notable using the nonphysiological bile salt, taurodeoxycholate (28, 31, 32) and the nonphysiological substrate, tributyrin (23, 28). With a more physiological substrate (33), taurocholate at concentrations of 1–3 mM stimulated lipase hydrolysis of olive oil whether or not exogeneous colipase was added (Fig. 2). In the absence of exogeneous colipase, taurocholate at concentrations >6 mM produced similar decreases in rat and human lipase activity in agreement with previous results (34). Exogenous colipase protected the activity from this mild inhibition.

The results from conventional kinetic analysis were valuable despite difficulties due to the complex nature of the enzyme systems. The apparent K_m values calculated for rat (6.5 mM) and human (3.5 mM) lipases (Fig. 1) were on the same order as reported by Maylie, *et al.* for hog lipase using olive oil as substrate (23). In that communication, the reported K_m values were 2 mM in the presence and 3.5 mM in the absence of colipase, with 1.6 and 0.4 mM taurodeoxycholate, respectively. These K_m values do not represent the affinity between lipase and triglyceride as would be interpreted in aqueous systems. However, as suggested in another report (35) the K_m values do reflect the affinity of the enzyme molecule for the emulsion interface and could be considered the enzyme–interface dissociation constant, thereby lending a physical concept to the value.

The evaluation of compounds as inhibitors of lipase was validated by the agreement between the two lipase assays. The single-time point carbon 14 assay was valuable since a large number of compounds could be evaluated quickly. However, it did not have the ability to distinguish compounds which delayed the initial velocity from the true enzyme inhibitors. The pH-stat assay, which recorded initial velocities, could distinguish easily between the two types of compounds. Phenoxybenzamine was an inhibitor that caused a long delay in the initial velocity of rat G-200 lipase. Previous studies which indicated that phenoxybenzamine was a potent inhibitor of pancreatic lipase (1 mM resulting in 80% inhibition),

Table II—Inhibition Constants of Non-Phenethylamine Inhibitors of Rat Pancreatic Lipase

Compound	Pancreatic Lipase: Assay Carbon 14 K_i (mM)	Pancreatic Lipase: Titrimetric Assay K_i (mM)
Diethyl-*p*-nitrophenylphosphate	0.1	0.2
Phenoxybenzamine	0.9	10.2
Phentolamine	5.8	1.6
Diisopropylfluorophosphate	No inhibition at 20 mM	

Table III—Comparison of Inhibition Constants of Inhibitors of Rat and Human Pancreatic Lipase

Compound	Pancreatic Lipase: Rat K_i (mM)	Pancreatic Lipase: Human K_i (mM)
Diethyl-*p*-nitrophenylphosphate	0.1	0.1
Phentolamine	1.6	1.1
Chlorphentermine	1.9	2.1
Flutiorex	3.1	2.6
Fenfluramine	3.3	3.8
p-Chloroamphetamine	6.2	3.5
Phenoxybenzamine	10.0	8.9
N-Benzyl-β-methoxy-3-(trifluoromethyl)phenethylamine	11.0	12.0

utilized a single-time point assay and eliminated bile salt from the assay medium (36). Either condition might be responsible for the discrepancy between the present results and those published previously. Because of the complexity of the lipase system, phenoxybenzamine and the other inhibitors may interfere with an organization step in the enzyme–colipase–substrate–bile salt complex, thereby causing the lag time and/or inhibition of the initial velocity. A disruption of the lipase–colipase interaction by fenfluramine has been reported recently (37).

The utility of classical kinetics in studying the competitive inhibitors of lipase was evident in the use of the Dixon plot and resulting K_i values for ranking the efficacy of lipase inhibitors. Cooperative and competitive inhibitory properties were observed for fenfluramine, flutiorex, chlorophentermine, and phentolamine at concentrations >10 mM. A halogenated substituent on the 3 or 4 position of the aromatic ring (flutiorex, fenfluramine, I, chlorphentermine, *p*-chloroamphetamine) appeared to be necessary for lipase inhibition in the phenethylamine series tested (Table I). A chloro group at the 2 position was ineffective (chlortermine). Alterations in the ethylamine portion of the molecule did not cause significant changes in the inhibitory properties of the active phenethylamines. These data confirm the inhibitory effect of fenfluramine on pancreatic lipase (2, 8) and extended the observation to include a structure–activity profile of a number of phenethylamines.

REFERENCES

(1) M. F. Lesses and A. Myerson, *N. Engl. J. Med.*, **218**, 119 (1938).

(2) K. Comai and A. C. Sullivan, *Biochem. Pharmacol.*, **27**, 1987 (1978).

(3) S. Garattini, M. E. Hess, M. T. Tocconi, E. Veneroni, and A. Bizzi, in Advances in Experimental Medicine and Biology," W. L. Holmes, R. Paoletti, and D. Kritchevsky, Eds., Plenum, New York, N.Y., 1972, p. 103.

(4) A. Bizzi, E. Veneroni, and S. Garattini, *Eur. J. Pharmacol.*, **23**, 131 (1973).

(5) G. L. D. Pawn, in "Amphetamines and Related Compounds," E. Costa and S. Garattini, Eds., Raven, New York, N.Y., 1970, p. 641.

(6) J. Duhault and M. Boulanger, *Rev. Fr. Etudes Clin. Biol.*, **X**, 215 (1965).

(7) W. N. Dannenburg, B. C. Kardian, and L. Y. Norrell, *Arch. Int. Pharmacodyn. Ther.*, **201**, 115 (1973).

(8) W. N. Dannenburg and J. W. Ward, *ibid.*, **191**, 58 (1971).

(9) J.-J. Bernier, *Nouv. Presse Med.* **29**, 971 (1975).

(10) S. Garattini, *S. Afr. Med. J., Suppl.*, **45**, 21 (1971).

(11) E. Evans, P. D. Samuel, D. S. Miller, and W. L. Burland, Postgrad. *Med. J., Suppl. 1*, **51**, 115 (1975).

(12) J. J. Barboriak, R. C. Meade, J. Owenby, and R. A. Stiglitz, *Arch. Int. Pharmacodyn. Ther.*, **176**, 249 (1968).

(13) E. Wertheimer, M. Hamosh, and E. Shafrir, *Am. J. Clin. Nutr.*, **8**, 705 (1960).

(14) S. Ramachandran, Y. K. Yip, and S. R. Wagle, *Eur. J. Biochem.*, **12**, 201 (1970).

(15) M. F. Maylie, M. Charles, and P. Desnuelle, *Biochim. Biophys. Acta*, **276**, 162 (1972).

(16) G. A. Scheele and G. E. Palade, *J. Biol. Chem.*, **250**, 2660 (1975).

(17) L. I. Gidez, *J. Lipid Res.*, **14**, 169 (1973).

(18) A. Bucko and Z. Kopec, *Nutr. Dieta*, **10**, 276 (1968).

(19) J. P. Reboud, A. Benabdeljilil, and P. Desnuelle, *Biochim. Biophys. Acta*, **58**, 326 (1962).

(20) R. M. Krauss, H. G. Windmueller, R. I. Levy, and D. S. Fredrikson, *J. Lipid Res.*, **14**, 286 (1973).

(21) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(22) K. Weber and M. Osborn, *J. Biol. Chem.*, **244**, 4406 (1969).

(23) M. F. Maylie, M. Charles, M. Astier, and P. Desnuelle, *Biochem. Biophys. Res. Commun.*, **52**, 291 (1973).

(24) J. A. Blain, J. D. E. Patterson, C. D. Shaw, and M. Waheed Akhtar, *Lipids*, **11**, 553 (1976).

(25) J. Scandella and A. Kornberg, *Biochemistry*, **10**, 4447 (1971).

(26) D. A. White, D. J. Pounder, and J. N. Hawthorne, *Biochim. Biophys. Acta*, **242**, 99 (1971).

(27) M. Dixon and E. C. Webb, in "Enzymes," Academic, New York, N.Y., 1964, p. 329.

(28) B. Borgstrom and C. Erlanson, *Eur. J. Biochem.*, **37**, 60 (1973).

(29) A. Vandermeers, M. C. Vandermeers-Piret, J. Rathe, and J. Cristophe, *Biochim. Biophys. Acta*, **370**, 257 (1974).

(30) E. Forssell, *Ann. Acad. Sci. Fenn. [Medica]*, **166**, 1 (1974).

(31) "Hawks' Physiological Chemistry," B. L. Oser, Ed., McGraw-Hill, New York, N.Y. 1965, p. 491.

(32) B. Borgstrom and C. Erlanson, *Biochim. Biophys. Acta*, **242**, 509 (1971).

(33) P. Desnuelle and P. Savary, *J. Lipid Res.*, **4**, 369 (1963).

(34) R. G. H. Morgan and N. E. Hoffman, *Biochim. Biophys. Acta*, **248**, 143 (1971).

(35) H. Brockerhoff and R. G. Jensen, in "Lipolytic Enzymes," Academic, New York, N.Y., 1974, p. 15.

(36) K. Santhanam, Y. K. Yip, S. Ramachandran, D. O. Allen, and S. R. Wagle, *Life Sci.*, **10**, 437 (1971).

(37) B. Borgstrom and C. Wollesen, *FEBS Lett.*, **126**, 25 (1981).

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