EFFECT ON FENFLURAMINE AND RELATED COMPOUNDS ON THE PANCREATIC COLIPASE/LIPASE SYSTEM

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1. Introduction

The anoretic drug fenfluramine (FF) is believed mainly to display its action in the central nervous system. However, in vitro and in vivo studies suggest that FF also causes an impairment of lipid digestion and absorption from the intestine in the rat by peripheral mechanisms [1–3]. As this effect is thought to be mediated in part by a FF-induced inhibition of pancreatic lipase activity [2,4], we have analysed in more detail the effects of this drug in vitro on the pancreatic colipase/lipase/substrate system. The results indicate that FF has only a limited effect on lipase alone but that in the presence of colipase and bile salt above its critical micellar concentration (CMC), FF can inhibit lipase action almost completely. The results also indicate that FF acts on the binding or penetration of colipase to the lipase substrate. We have also investigated the structure/function relationship through the effects of some related compounds on lipase/colipase.

2. Materials and methods

Fenfluramine ((trifluoromethyl-3-phenyl)-1-ethylamine-2-propane) was obtained from Boehringer, Ingelheim; phentermine (2-phenyl-1,1-dimethylethylamine) and chlorphentermine (1-(*p*-chlorophenyl)-2-methyl-2-aminopropane) were from Troponwerke, Köln; amfepramone (2-diethylamino-1-phenyl-1-propanone) was from Pharmacia, Uppsala; and phenylethylamine was a product of Fluka, Switzerland. They were used in 50 mM solutions in 150 mM NaCl. Taurodeoxycholate (TDC) was synthesized in this laboratory. Tributyrin was a product of Merck, FRG. Colipase-I-101 (the proform) and colipase-I-96 (the trypsin-activated form) were prepared from porcine pancreas as in [5]. Porcine pancreatic lipase low in colipase was prepared in this laboratory [6].

Lipase activity against tributyrin as substrate was determined using an automatic titration system (Mettler Instr., Zürich) in 10 ml total vol. as in [6]. The additions to the buffer or the bile salt-containing buffer were made in the order: FF; tributyrin; colipase and lipase; the latter two with 1 min interval. The reaction was run at pH 7.0 and 40°C.

3. Results

The effect of FF on the hydrolysis of tributyrin catalysed by pancreatic lipase in buffer (pH 7.0) was rather insignificant in the 1.0–10 mM range investigated (fig.1). This experiment was run at 25°C to minimize irreversible enzyme inactivation by the substrate.

In the presence of 4 mM taurodeoxycholate and colipase-I-101 (10^{-7} M), lipase (10^{-9} M) was inhibited by FF to 50% at ~3 mM (fig.2) and complete inhibition was seen at 10 mM FF. With colipase-I-96 higher concentrations of FF were needed for the same effects (~50% inhibition at 10 mM FF). Bile salts were necessary to obtain inhibition of lipolysis in the presence of FF (fig.1). This figure, besides showing the effect of FF alone, shows the effect of bile salt on the inhibition of lipase (10^{-9} M) with tributyrin as substrate in the presence of 10^{-7} M colipase-I-101 and 10 mM FF. Taurodeoxycholate results in an inhibition that is almost complete above the CMC of the bile salt. Taurocholate also inhibits lipase under these conditions but higher concentrations are

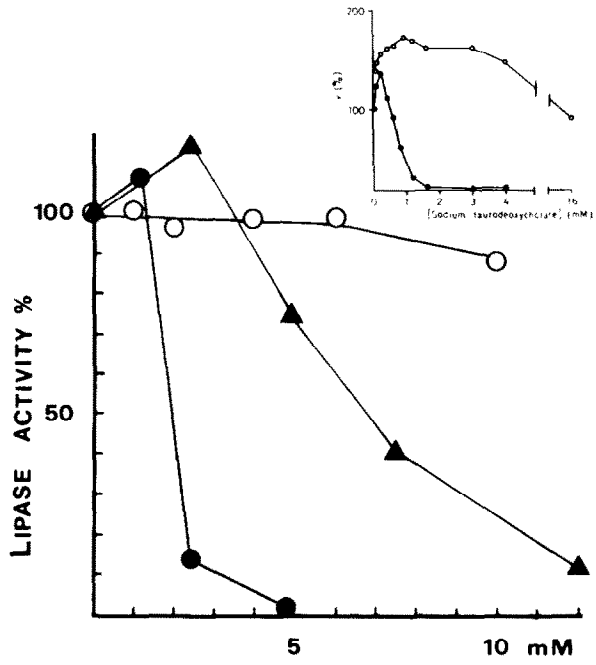


Fig.1. Effect of fenfluramine alone on the rate of hydrolysis of tributyrin dispersed in buffer (pH 7.0) at 25°C (○) lipase 2×10^{-9} M. Effect of varying concentration of TDC (●) and TC (▲) in the presence of 10 mM FF. Lipase 10^{-9} M, colipase-I-101 10^{-7} M (pH 7.0) 40°C. Insert taken from fig.1 in [8]: (●) in the absence and (○) in the presence of colipase.

needed what may be related to its higher CMC. The extent of inhibition caused by FF was also related to the concentration of colipase in the system. Fig.3 shows the effects of FF on the lipolysis in the pres-

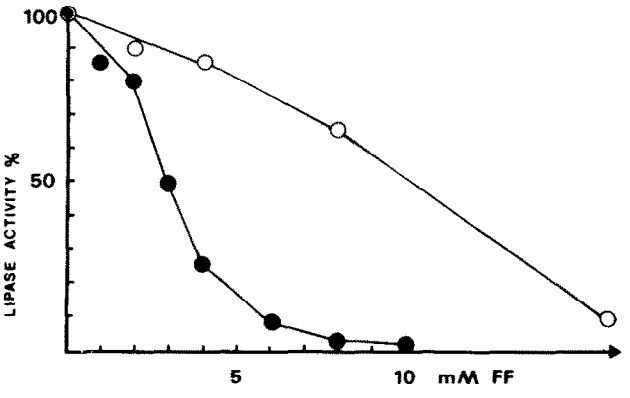


Fig.2. Effect of fenfluramine on lipase activity in the presence of 4 mM taurodeoxycholate: 10^{-7} M colipase-I-96 (○); colipase-I-101 (●) 10^{-7} M; lipase 10^{-9} M (pH 7.0).

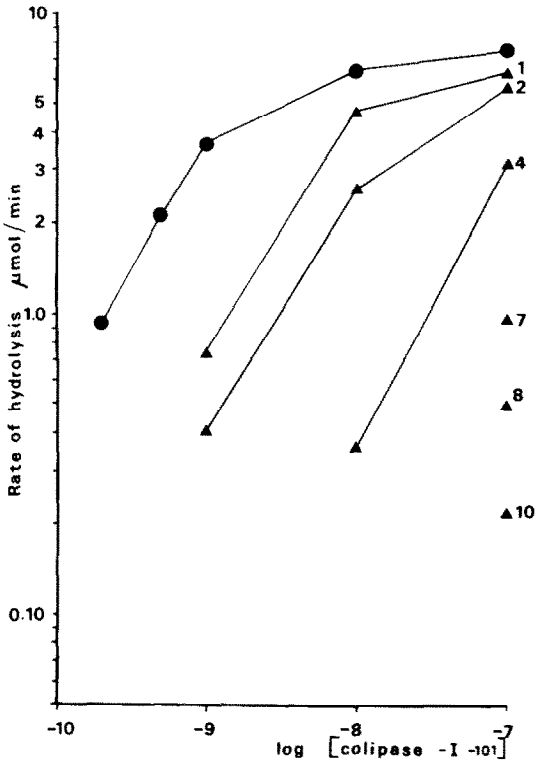


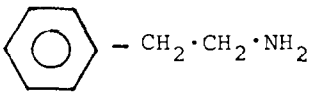
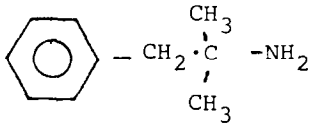
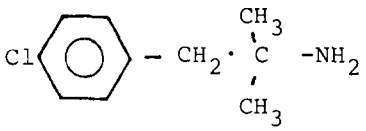
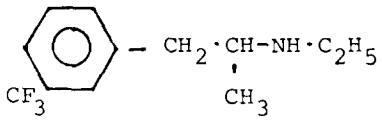
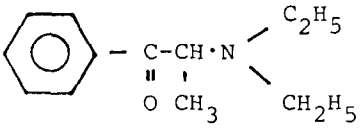
Fig.3. Effect porcine colipase-I-101 concentration on the rate of hydrolysis of tributyrin in the presence of 4 mM TDC and varying concentrations of FF (pH 7.0) 40°C: porcine pancreatic lipase 10^{-9} M; (●) no FF added; (▲) FF at the levels given (1–10 mM).

ence of different concentrations of colipase-I-101. In the absence of any inhibitor, the rate of lipolysis by pancreatic lipase (10^{-9} M) reaches a saturation level and above 10^{-8} M colipase increases lipolysis relatively slightly. FF inhibits the lipolysis but the extent of inhibition is related to the colipase concentration. The lower the colipase concentration the more marked is the effect of FF.

Separation of the substrate phase (oil phase) from the aqueous phase by centrifugation shows that inhibiting concentration of FF displaces colipase to the aqueous phase. FF at 10 mM was found not to affect the binding of lipase to colipase (C. Albertsson, personal communication).

The other compounds related to FF were tested at 4 mM with 10^{-8} M colipase-I-101 in 4 mM TDC. The effects are shown in table 1 and indicate that of the compounds available only FF and chlorphentermine had inhibitory effects, the latter to a lower degree.

Table 1
Effect of FF and related compounds at 4 mM on the rate of hydrolysis of tributyrin in the presence of 4 mM TDC and 10^{-8} M porcine colipase (pH 7.0) 40°C; porcine lipase was 10^{-9} M

Compound		Rate of lipolysis (%)
No addition		100
	Phenylethylamine	100
	Phentermine	95
	Chlorphentermine	14
	Fenfluramine	5
	Amfepramone	95

4. Discussion

Here we confirm that FF, an anoretic of the amphetamine type, is a potent inhibitor of the pancreatic lipase/colipase/bile salt system in vitro (the lipase preparation previously used most probably contained colipase) [4]. The use of highly purified lipase and colipase yielded some further information as to the mechanism of action of the drug within this system. The results show that lipase alone is only insignificantly inhibited by FF. While bile salts alone inhibit lipase, its activity is restored in the presence of colipase [7]. In the presence simultaneously of FF and bile salt, however, lipase can be completely inhibited in this in vitro system. FF in the presence of bile salts prevents the penetration or binding of colipase to the lipase substrate interface. FF may compete for the substrate interface and, in the presence of bile salt and FF, colipase may be extruded and lipolysis prevented. The curve for lipolysis in the presence of FF and colipase *vs* [TDC] in fig.1 is very similar to the curve obtained for TDC without colipase added (see fig.1 in [8]).

Colipase-I-96, the trypsin-activated form of colipase, is more resistent to the effect of FF; i.e., higher concentrations of FF are needed to displace it from the interface. Since it has been proposed that colipase-I-101 as the pro-colipase is activated by trypsin in the intestinal content [5], we should consider the activated form to be the physiological competitor of FF at the substrate interface. No experiments have been done yet to determine whether this FF effect can be shown under in vivo conditions. It could be expected, however, that intraluminal FF concentra-

tions, even at highest possible dosage, would be too low to have any considerable effect on the lipase/colipase-I-96 activity and therefore fail to have a significant influence on lipid digestion and absorption in vivo.

Of the compounds tested only FF and to some extent chlorphentermine, inhibited lipolysis. Thus, it appears that substitution in the phenyl ring is important in this respect and that changes in the sidechain are less effective. Therefore, no relation seems to occur between the anoretic effect and the inhibition of lipolysis of these drugs. The lipase/colipase system presented here might, however, be used in efforts to find compounds effective at therapeutical dosage levels.

The mechanism of action of FF may be elucidated in further experiments. However, the effect of FF is clearly potentiated by the simultaneous presence of bile salts.

References

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