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EFFECTS OF THE ADRENERGIC AGONISTS ISOPRENALINE AND NORADRENALINE AND THE α-BLOCKING AGENTS DIHYDROERGOTAMINE AND PHENTOLAMINE ON THE LIPOLYSIS IN ISOLATED FAT CELLS OF THE RAT, GUINEA PIG, DOG AND MAN

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Abstract—The actions of noradrenaline and isoprenaline and of the α-adrenoceptor antagonists dihydroergotamine (DHE) and phentolamine, on lipolysis in isolated fat cells from the rat, guinea pig, dog and man have been investigated. In isolated rat fat cells the efficacy of noradrenaline in stimulating lipolysis was equal to that of isoprenaline. In guinea pig, dog and, particularly in human fat cells the maximal effects elicited by noradrenaline were lower than those elicited by isoprenaline. In these tissues the efficacy of noradrenaline was elevated to isoprenaline levels in the presence of low concentrations of DHE or phentolamine. DHE alone produced a weak stimulation of lipolysis in rat fat cells and an intense stimulation in canine fat cells; phentolamine was without effect. In concentrations of 10^{-5} M and above both DHE and phentolamine inhibited catecholamine stimulated lipolysis in fat cells from all species tested, probably as a result of unspecific effects.

It is well established that catecholamine-induced lipolysis is the result of β-adrenoceptor stimulation. Although there are several reports in the literature concerning the influence of α-adrenoceptor antagonists on lipolysis, a certain amount of controversy exists as to the role of α-adrenoceptors in the control of this metabolic process.

In *in vivo* studies in man, dogs and rats, parenteral administration of α-adrenoceptor antagonists such as DHE, phentolamine, phenoxybenzamine or dibenamine is known to lead to increases in plasma levels of both free fatty acids and glycerol [1–8]. In isolated adipose tissue from the rat [9, 10] and particularly from the dog [11], DHE stimulates lipolysis. In isolated human fat cells, phentolamine, although itself devoid of lipolytic activity, has been reported to potentiate the lipolysis induced by adrenaline without influencing the responses to isoprenaline [12, 13]. In rat fat cells DHE, phentolamine and phenoxybenzamine have been variously reported to either inhibit [14–18], or to be without effect [19] on the lipolysis induced by catecholamines or histamine.

In an attempt to gain more insight into this problem we have investigated the effects of the α-adrenoceptor antagonists DHE and phentolamine on lipolysis in fat cells from the rat, guinea pig, dog and man.

MATERIALS AND METHODS

All chemicals used to prepare Krebs–Ringer buffer were of analytical grade from Merck Ltd. Bovine serum albumin (fraction V) was purchased from Armour Pharmaceutical Co., collagenase (type 1, 140 units/mg) from Worthington Biochemical Corp., L-noradrenaline was obtained from Fluka Ag, DL-isoprenaline sulfate and DL-dithiothreitol from Sigma, phentolamine from Ciba-Geigy. Dihydroergotamine methanesulfonate came from Sandoz Ltd.

Male Wistar rats weighing 180–200 g and guinea pigs (Sandoz strain) weighing 400–500 g were fasted for about 18 hr prior to sacrifice by decapitation or by a blow on the head and the epididymal fat pads removed.

Mongrel dogs were fasted overnight, anaesthetized with Numal^{R} 40 mg/kg i.v. and a piece of subcutaneous (s.c.) fat removed from the abdominal region.

Subcutaneous adipose tissue from the abdomen, was obtained from female patients aged 25–50 yr who had been fasted overnight before surgery. The patients underwent surgery for different reasons; however, none of them was grossly obese or had diabetes. The human tissue samples were placed in a buffer medium at 37° and were transported to the laboratory within 10 min of removal.

All tissue samples were washed with 0.9% NaCl solution and cut into small pieces, the adipocytes were isolated using the method described by Rodbell [20] in a glucose-free digestion medium.

Insulin, present in the commercially available albumin, was destroyed by stirring the 4% albumin phosphate buffer with DL-dithiothreitol (10 mg/100 ml) for a period of 2 hr. Aliquots of 500 μl of cell suspension (approx. 20 mg fat cells) were pipetted into plastic incubation flasks. Using these quantities, rate of lipolysis was linear for up to 2 hr of incubation. Each agent or combination of agents to be studied was added to the flask at the appropriate dose.

The total volume was made up to 1 ml and the flasks incubated under air in a Braun Melsungen

metabolic shaker at 37°. The incubation period was 1 hr for the rat (once 2 hr) and guinea pig fat cells, and 2 hr for the canine and human tissues. All points in the figures are derived from a single representative experiment. There was practically no variation between triplicate incubations.

Glycerol release, determined by the method of Laurell and Tibbling [21] adapted for the Auto-Analyzer, was used as an index of lipolysis and the results expressed in μmoles/m-mole triglycerides/hr. Basal lipolysis obtained in control experiments was subtracted from the total glycerol value and the difference taken as a measure of drug-induced lipolysis.

Triglycerides were analysed using the Biochimica Test Combination (Boehringer, Mannheim G.m.b.H.).

RESULTS

Comparison of the lipolytic action of DHE and isoprenaline in isolated fat cells from the rat and dog. As shown in Fig. 1 and Table 1 isoprenaline was approximately equipotent in inducing lipolysis in both rat and dog fat cells (pD_2 values 7.45 and 7.50 respectively) but the maximum lipolytic effect was lower in the latter species.

In the rat DHE induced lipolysis but was much less potent than isoprenaline (pD_2 5.71) and also had a lower efficacy corresponding to less than 10 per cent of the maximal isoprenaline effect. In the dog, although DHE was less potent than isoprenaline (pD_2 6.35) its lipolytic efficacy was almost as great as that of the latter compound.

In both rat and dog fat cells the lipolytic effect of DHE in concentrations of 10^{-5}M and above was less intense than those seen at lower concentrations.

Influence of the α-adrenoceptor antagonists DHE and phentolamine on the lipolytic effects of isoprenaline and noradrenaline in rat, guinea pig and human fat cells. In rat fat cells, although isoprenaline was more potent than noradrenaline, both agonists showed similar efficacy in stimulating lipolysis (Table 1).

At a concentration of 10^{-4}M both DHE and phentolamine produced a non-competitive antagonism of catecholamine-induced lipolysis while at lower concentrations neither of the antagonists produced any effect (not shown).

As shown in Fig. 2a and b, in guinea pig fat cells isoprenaline was not only more potent than noradrenaline but also possessed a greater efficacy than the latter compound. In this preparation low concentrations of either DHE or phentolamine were without effect on the responses to isoprenaline but increased the efficacy of noradrenaline to a level which was comparable to that of isoprenaline.

High concentrations (10^{-4}M) of either DHE or phentolamine produced a non-competitive antagonism of catecholamine-induced lipolysis.

Isoprenaline was much less effective in stimulating lipolysis in human fat cells than in tissues from the other species tested. In guinea pig and canine fat cells the maximal lipolytic effect of noradrenaline was lower than that of isoprenaline and in human tissue this difference in efficacy was

Table 1. pD_2 values, relative potencies and efficacy of isoprenaline, noradrenaline and DHE in stimulating lipolysis of rat, guinea pig, dog and human fat cells

	Rat				Dog			Guinea pig			Man		
Substance	*n*	*Ef*	pD_2	*RP*	*n*	*Ef*	pD_2	*RP*	*n*	*Ef*	pD_2	*RP*	*n*	*Ef*	pD_2	*RP*
Isoprenaline (1)	7	50.4	7.45	100	6	32.4	7.50	100	6	19.6	6.99	100	6	4.3	7.20	100
SE		3.6	0.05			1.2	0.05			0.9	0.08			0.9	0.03	
Noradrenaline (2)	7	50.1	6.51	11	6	22.2	6.66	13	6	13.8	5.96	9	6	0.8	5.30	1
SE		4.5	0.05			1.6	0.10			0.7	0.08			0.3	0.09	
p(1)			<0.001			<0.001	<0.01			<0.001	<0.001			<0.01	<0.001	
DHE	4	3.0	5.71	1.8	6	26.0	6.35	7								
SE		0.8	0.10			1.9	0.07			*No effect*				*No effect*		
p(1)		<0.001	<0.001				<0.001									
p(2)		<0.001	<0.001				<0.05									

n = number of experiments.
Ef = efficacy (μ moles glycerol/m-mole triglycerides/hr.
RP = relative potency.
The isolated fat cells were incubated in Krebs–Ringer buffer containing 4% bovine albumine at pH 7.4 and 37° one hour for rat and guinea pig and two hours for dog and human tissue.
Mean values ± standard error (SE) of pD_2 and *Ef* were calculated from individual concentration–response curves. See text for other experimental details.

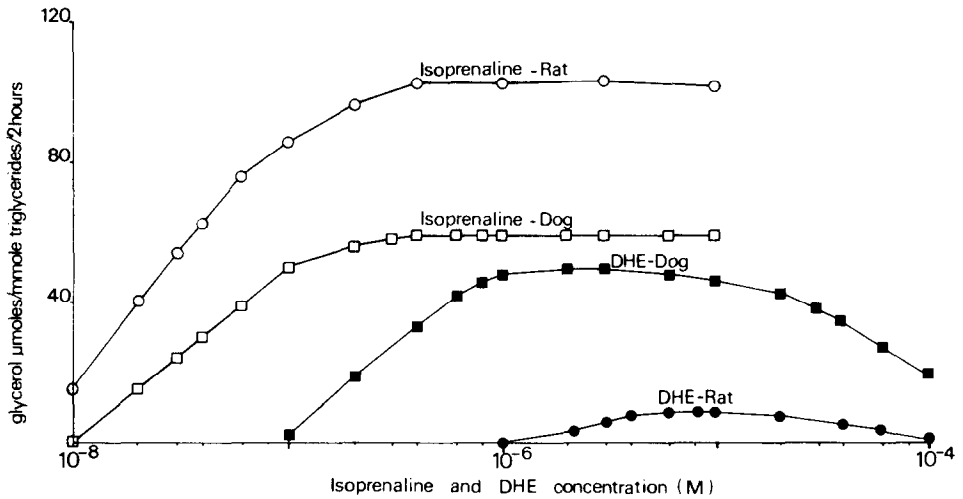

Fig. 1. Concentration-response curves for isoprenaline (open symbols) and DHE (closed symbols) for the release of glycerol in fat cells isolated from rat epididymal and dog subcutaneous adipose tissue. Experimental conditions as described in the legend to Table 1.

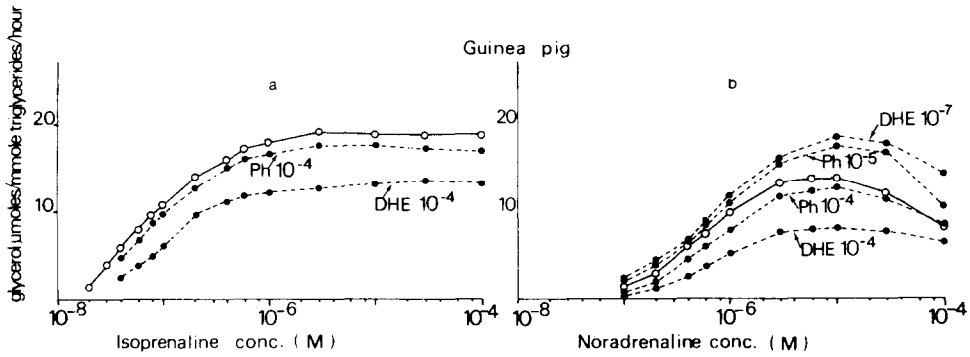

Fig. 2. Effect of DHE and phentolamine (Ph) on the concentration-response curves of glycerol release stimulated by isoprenaline (a) and noradrenaline (b) in guinea pig fat cells. Experimental conditions as described in the legend to Table 1.

even more pronounced. As seen in experiments in guinea pig fat cells, in human tissue addition of low concentrations of DHE was without influence on the response to isoprenaline but increased the lipolytic efficacy of noradrenaline to a level similar to that obtained with isoprenaline (Fig. 3a). In the presence of low concentrations of DHE the dose-response curves to noradrenaline were bell-shaped. Similar results were obtained in experiments with phentolamine (not shown). These results indicate that DHE and phentolamine are competitive α-adrenoceptor blockers and that the blockade can be overcome by high noradrenaline concentrations. Higher concentrations of either α-adrenoceptor antagonist produced a non-specific inhibition of the lipolytic effects of both isoprenaline and noradrenaline (Fig. 3b).

Influence of DHE on the lipolytic action of isoprenaline and noradrenaline in isolated canine fat cells. In canine adipose tissue, as in guinea pig and human fat cells the lipolytic efficacy and potency of isoprenaline was greater than that of noradrenaline (Fig. 4a and b, Table 1). The dose-response curves obtained in the presence of phentolamine (not shown) were similar to those in guinea pig fat cells. Subthreshold lipolytic concentrations of DHE increased the potency but not the efficacy of isoprenaline (Fig. 4a). In the case of noradrenaline, the increase in potency seen in the presence of DHE was even more marked and the maximum effect elicited was increased to a level which was comparable to that obtained with isoprenaline (Fig. 4b).

Again in these experiments high concentrations of DHE or phentolamine produced a non-competitive inhibition of the lipolytic actions of both catecholamines.

DISCUSSION

The results of these investigations show that marked species differences exist in the response of isolated fat cells to the different agonists and antagonists investigated.

In rat fat cells we confirmed the reports[12, 17], 22–25] that noradrenaline, like adrenaline, is a less potent lipolytic agent than isoprenaline, but has a comparable efficacy to isoprenaline. Furthermore, the finding that in this tissue, the lipolytic efficacy

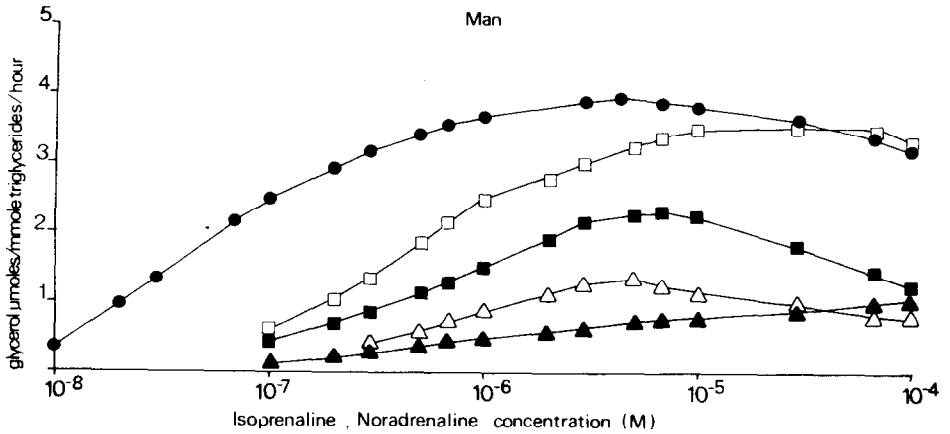

Fig. 3a. Typical concentration–response curves for catecholamine stimulated lipolysis of human fat cells. Influence of DHE on noradrenaline effect. Isoprenaline ●, noradrenaline ▲, noradrenaline + DHE 3×10^{-8}M ■, noradrenaline + DHE 10^{-7}M □, noradrenaline + DHE 10^{-4}M △. Experimental conditions as described in the legend to Table 1.

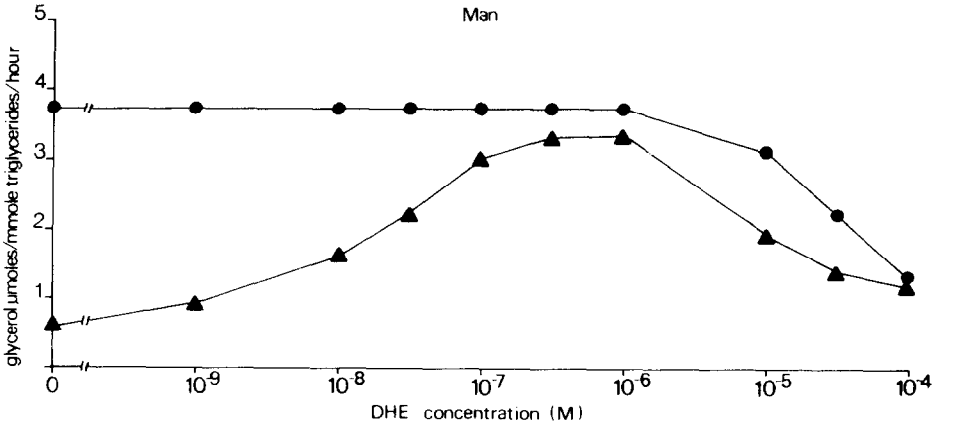

Fig. 3b. The effect of DHE on lipolysis induced by 3×10^{-6}M noradrenaline ▲ and 10^{-6}M isoprenaline ●. Experimental conditions as described in the legend to Table 1.

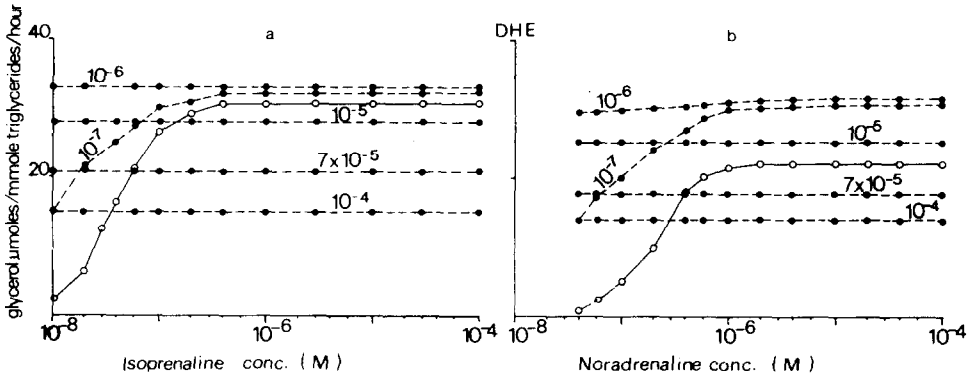

Fig. 4. Concentration–response curves in dog fat cells for isoprenaline (a) and noradrenaline (b) on glycerol release in the absence (open symbols) and presence (closed symbols) of DHE added in the molar concentrations indicated on the lines. Experimental conditions as described in the legend to Table 1.

of DHE was less than 1/10 of that of isoprenaline, while the potency was about 100 times less than that of the latter drug, is in good agreement with the results of Hotta *et al.* [10].

It seems questionable whether the lipolysis induced by DHE or phenoxybenzamine [14] results from β-adrenoceptor stimulation since Hotta *et al.* [10] have reported that the effect can be inhibited by either propranolol (5.6×10^{-6}M) or phentolamine (1.7×10^{-5}M). Furthermore, the type of antagonism involved is not clear since these authors used only one concentration of antagonist. In our experiments the low glycerol levels obtained after DHE administration (which are only marginally higher than the sensitivity of analytical method) did not permit us to investigate the effects of beta adrenoceptor blockade on the response.

As has been reported by Burns and Langley [12, 13] for human fat cells, in guinea pig and dog fat cells noradrenaline was not only less potent than isoprenaline but also its maximal lipolytic effect was markedly lower.

In contrast to phentolamine, DHE produced a marked lipolysis in isolated canine fat cells. Vogel *et al.* [11] have reported similar findings and suggested that the DHE-induced lipolysis does not result from β-adrenoceptor stimulation, but from a direct stimulation of the adenylate cyclase.

DHE, and to a lesser extent phentholamine, produced a non-competitive inhibition of catecholamine-induced lipolysis at concentrations in excess of 10^{-5}M. This finding would explain the inhibition of catecholamine-induced release of free fatty acids and glycerol seen after administration of DHE, phentolamine or phenoxybenzamine in the rat or in isolated fat cells from this species [10, 14–16, 26].

Ward and Fain [27] reported that DHE at a concentration of 10^{-4}M acts as a phosphodiesterase inhibitor and Fain [17] found that at concentrations of 2.3×10^{-4}M and 2.3×10^{-5}M the drug potentiated the lipolytic action of dibutyryl c-AMP.

Conversely, Nakano *et al.* [16] found that at a concentration of 10^{-4}M DHE inhibited the lipolytic action of the phosphodiesterase inhibitor theophylline and also the lipolysis induced by dibutyryl c-AMP. Furthermore, Kather *et al.*[28] have recently reported that DHE at a concentration of 10^{-4}M inhibits isoprenaline-induced stimulation of adenylate cyclase in human fat cell ghosts. These contradictory results are probably a reflection of the fact that at high concentrations (in excess of 10^{-5}M) DHE, and probably the other α-adrenoceptor antagonists tested, exert a variety of non-specific effects which are not relevant to the clinical situation.

In man, DHE is usually administered in doses of 0.5–5 mg/day. If one assumes an even distribution of the drug throughout the body, then this dose would give a maximum concentration of 10^{-8}–10^{-7}M although the actual concentration present at the receptor site would certainly be lower than this.

Although the effects of DHE on lipid metabolism in dog and man are qualitatively similar it would appear from our *in vitro* results that the mechanisms by which the drug produces its effects in these two species are different. In man, the increases in plasma levels of free fatty acids and glycerol seen after intravenous administration of therapeutic doses of DHE, phentolamine or phenoxybenzamine result from an unmasking of the β-adrenoceptor-mediated lipolytic effects of endogenous catecholamines after blockade of α-adrenoceptors in the adipose tissue.

In the dog, phentolamine appears to produce its effect as a result of an action similar to that seen in man. DHE on the other hand possesses a dual mode of action. In addition to its direct lipolytic effect the drug appears to facilitate noradrenaline-induced lipolysis as a result of its α-adrenoceptor antagonist activity since at a concentration of 10^{-7}M, which did not induce lipolysis, DHE increased both the potency and efficacy of this agonist.

If this hypothesis is correct then it is obvious that the results obtained in studies of the lipolytic activity of the ergot alkaloids in the dog cannot be extrapolated to man.

It may therefore be concluded that: (1) in the rat DHE is only weakly effective in stimulating lipolysis in comparison to the catecholamines, while in the dog the drug exerts a strong lipolytic action which is not mediated by β-adrenoceptor stimulation; (2) Guinea pig, dog and human fat cells all bear α-adrenoceptors, stimulation of which leads to an inhibition of lipolysis. In low concentrations both DHE and phentolamine potentiate noradrenaline-induced lipolysis in these tissues by blocking α-adrenoceptors thereby leaving the β-adrenoceptor-mediated lipolytic effects of the transmitter unopposed by α-adrenoceptor stimulation.

At high concentrations DHE and phentolamine inhibit lipolysis in fat cells from all species examined; however, this effect appears to be unspecific and is not considered to be relevant to the effect of the drug in man.

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