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EFFECTS OF QUINTERENOL (1-(5-(8-HYDROXYQUINOLYL))-2-ISOPROPYLAMINOETHANOL) ON THE METABOLISM OF ADIPOSE TISSUE

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Abstract—1. The partial β-adrenergic agonist and catecholamine analogue, quinterenol (1-(5-(8-hydroxyquinolyl)-2-isopropylaminoethanol) was examined for its effects on adipose tissue metabolism *in vitro*. The drug promoted glycerol release from isolated fat cells in a dose-dependent hyperbolic manner with a maximum effect at 10^{-3} M. 2. Quinterenol promoted an enhancement of lipolysis stimulated by a serum fat-mobilizing factor (lipolysin) over a narrow dose range (10^{-5} M to 10^{-4} M) and produced a similar narrow-band enhancement (at the single dose of 10^{-5} M) of noradrenaline-stimulated lipolysis. This synergism has been interpreted as phosphodiesterase inhibitory activity. 3. Propranolol at 10^{-3} M totally inhibited both noradrenaline (10^{-4} M) and quinterenol (10^{-3} M)-induced lipolysis; and had reduced, though still significant effects, when at 10^{-6} M. It is suggested that quinterenol promotes lipolysis via the cyclic AMP system in similar fashion to noradrenaline. 4. Quinterenol alone was not able to enhance the incorporation of [U-^{14}C]glucose into total fat cell lipid. However, quinterenol did further stimulate insulin-induced lipid synthesis in fat cells obtained from both fasted and fed rats.

Many β-adrenergic blocking agents bear a structural resemblance to the beta agonist isoprenaline (isoproterenol). It has been suggested [1] that the side chain isopropyl-substituted secondary amine probably determines interaction with β receptors whereas the substituents on the aromatic ring(s) determine agonism or antagonism. Because of this close structural similarity, some β-blocking compounds show partial agonist activity (e.g., dichloroisoproterenol, pronethalol). A 5-(8-hydroxyquinoline) analogue of the catecholamines, quinterenol (1-(5-(8-hydroxyquinolyl))-2-isopropylaminoethanol) initially shown to be a β-adrenergic stimulant [2, 3] but subsequently shown to have β-stimulatory and β-blocking effects [4], would appear to be one of these partial agonist types of compound. Iorio and Moore (1971) [4] showed that quinterenol-induced free fatty acid (FFA) release from adipose tissue incubated *in vitro* was positively dose-related from $5{\cdot}4 \times 10^{-9}$ M to $5{\cdot}4 \times 10^{-5}$ M, but at $5{\cdot}4 \times 10^{-3}$ M, auto-inhibition occurred. Because of these interesting effects on adipose tissue, an investigation was made of quinterenol on basal and hormone-induced fat cell lipolysis, *in vitro*, in the presence and absence of the β-blocking compound propranol; and on incorporation of [U^{14}C]glucose into total fat cell lipid.

MATERIALS AND METHODS

DL-Propranolol was kindly provided by Imperial Chemical Industries Limited. Quinterenol hydrochloride, a gift from Pfizer Incorporated, is reputedly unstable in solution (Jones, E. R. M., personal communication, 1972) therefore, various physico-chemical tests were performed to investigate this. [U-^{14}C]glucose was obtained from the Radiochemical Centre, Amersham, Bucks.

Isolated fat cells were prepared in Krebs Ringer bicarbonate buffer (containing albumin 3·5 g 100 ml and glucose 45 mg/100 ml) at pH 7·4 by collagenase digestion of epididymal adipose tissue obtained from male Sprague–Dawley rats weighing 190–210 g [5]. Aliquots of fat cell suspension (equivalent to approximately 4 μmoles of triglyceride per flask) derived from fasted rats, were incubated in buffer, with or without noradrenaline (10^{-4} M), serum (1·1 ml per vial), propanolol, in the presence and absence of quinterenol, for 90 min in a shaking water bath at 37°C.

Glycerol release was employed as an index of lipolysis and was measured by an enzymatic method [6] as described previously [7]. Lipolysis was expressed as nmole of glycerol released/mg lipid during 90 min incubation. Total lipid was determined by a technique [8] modified from Folch, Lees and Sloan-Stanley [9]. In other experiments fat cells from fasted or fed rats were incubated with tracer doses of [U^{14}C]glucose and quinterenol (at 10^{-3} M or 10^{-6} M) in the presence and absence of insulin (100 micro units ml^{-1}). Incorporation of the tracer amounts of [U^{14}C]glucose into total fat was employed as a measure of cell lipid synthesis. Lipid was extracted from the fat cells as described previously [8] using chloroform/methanol, an aliquot evaporated, the lipid residue taken up in toluene phosphor and radioactivity was

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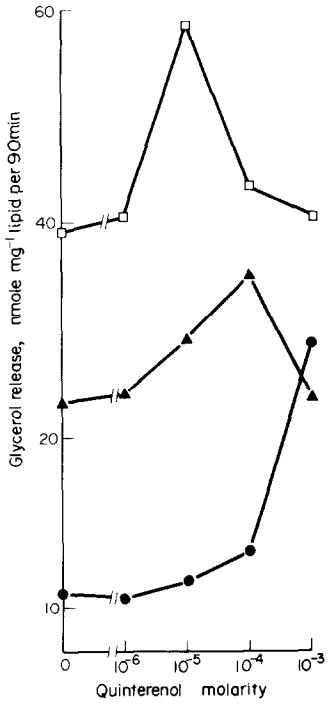

Fig. 1. Dose-response curves for glycerol release from rat isolated fat cells, following stimulation by quinterenol (●——●), quinterenol plus lipolysin (▲——▲), quinterenol plus noradrenaline (□——□).

measured in a Packard Tricarb model 2450 spectrometer.

RESULTS

There was no change in the u.v. spectrum of quinterenol solutions at pH 7, after exposure to light for 70 hr, 0·1 N HCl for 40 hr, or carbonate/bicarbonate buffer (pH 10) for 12 hr.

Fig. 1 indicates that there is a dose-related increase of basal glycerol release with maximum effect at the highest drug used, i.e. 10^{-3} M. Both noradrenaline and the serum fat-mobilizing factor (lypolysin) produced a significant ($P < 0{\cdot}01$) elevation of glycerol release above the no-drug control value. When quinterenol was also present there was an unexpected synergism in both cases. With noradrenaline this occurred at 10^{-5} M, and with lipolysin between 10^{-5} M and 10^{-4} M.

The data in Table 1 show that propanolol at 10^{-3} M strongly inhibited the lipolytic effect of noradrenaline (10^{-4} M) and quinterenol (10^{-3} M), and at 10^{-6} M there was a smaller but still significant reduction in the glycerol release provoked by these lipolytic agonists.

From Table 2 it can be seen that, overall, total lipid synthesis was greater in fat cells from fed rats than in cells from fasted rats. In all cases there was a significant enhancement of glucose incorporation in the presence of insulin; whereas the presence of quinterenol alone, at 10^{-6} M or 10^{-3}, manifested no significant effects on total lipid synthesis. Only in fat cells from fed rats (and in the presence of insulin) did the higher concentration of quinterenol have conspicuously greater effects than when at 10^{-6} M.

DISCUSSION

Since the earliest publications of the beta adrenoceptor activity of quinterenol on muscle [2, 3], only one published investigation had dealt with the activity of this drug in connection with adipose tissue [4], as mentioned above. The results reported here appear to be somewhat at variance with those of Iorio and Moore [4] who found auto-inhibition by quinterenol at 5×10^{-3} M on rat epididymal adipose tissue pieces, whereas at 1×10^{-3} M, the results of this study using isolated fat cells, indicated maximum stimulation. Since pieces of adipose tissue were used,

Table 1. Effects of propranolol on noradrenaline- and quinterenol-induced lipolysis in fat cells obtained from fasted rats

Lipolytic agent	Propranolol Molarity NIL	10^{-6} M	10^{-3} M
Control	5·2 ± 0·8	4·5 ± 0·2	3·9 ± 0·2
Noradrenaline (10^{-4} M)	39·4 ± 0·6	15·7 ± 1·3**	5·2 ± 0·1
Quinterenol (10^{-3} M)	28·9 ± 0·6***	20·8 ± 0·1**	5·0 ± 0·5

Data are nanomoles of glycerol released/mg lipid ± S.E.M. of 3 observations during 90 min incubation. Significance of difference from respective control values: **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

Table 2. Effects of quinterenol and insulin on the incorporation of [U-^{14}C]glucose into fat cell lipid

Source of fat cells	Quinterenol molarity: Control Insulin absent	Control Insulin present	10^{-6} M Insulin absent	10^{-6} M Insulin present	10^{-3} M Insulin absent	10^{-3} M Insulin present
Fasted rats	747 ± 37	1747 ± 14***	995 ± 80*	1468 ± 135**	703 ± 25	2310 ± 108***
Fed rats	1812 ± 303	2563 ± 76	1402 ± 49	2528 ± 64	1962 ± 145	4005 ± 221**

Data are cpm per 50 mg of fat cell lipid, mean of 3 observations ± S.E.M. Significance of difference from respective control values: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

which manifest *in vitro* a low surface area to volume ratio, it is possible that the observed auto-inhibition of lipolysis was due to intracellular accretion of FFA and this produced the inhibition. With isolated fat cells where each cell presents a large surface to the medium, the total surface area to volume ratio is considerably increased, allowing maximum passage of chemical substances.

The non-additive effects in glycerol release provoked by quinterenol at 10^{-6} M, 10^{-4} M and 10^{-3} M in the presence of noradrenaline suggest that quinterenol and noradrenaline are working through the common mechanism of β-adrenergic stimulation, adenyl cyclase activity and cyclic AMP formation. The large increment in physiological response (synergism) at the single drug dose level of 10^{-5} M is compatible with phosphodiesterase inhibition as the cause, since many phosphodiesterase inhibitors are only active in this context over very narrow dose range. The narrow dose range of activity of quinterenol in enhancing lypolysin activity may also be due to phosphodiesterase inhibition. The effects of propranolol at 10^{-3} M in reducing both noradrenaline and quinterenol-induced lipolysis to control values but having a similarly lesser effect when at 10^{-6} M are also compatible with a common mechanism of noradrenaline- and quinterenol-induced fat cell lipolysis.

The fact that quinterenol could not, of itself, stimulate total lipid synthesis whereas the compound did further enhance insulin-stimulated lipid synthesis, epitomizes its partial agonist nature. It has been suggested [7], that when cyclic AMP (cAMP) is generated by a noradrenaline stimulus different functions of the cyclic AMP structure are responsible, respectively, in lipolysis and lipogenesis. However, Goldman and coworkers [10] maintain the theory of Dualism or the Yin-Yang hypothesis of cAMP and cyclic GMP (cGMP) having equal and opposite effects. It is conceivable therefore that the partial agonist character is due to stimulation of both cAMP and cGMP simultaneously, resulting in a physiologic antagonism.

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