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Action of propranolol on lipolysis induced by catecholamine, dibutyryl cyclic AMP and serum

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LIPOLYSIS may be induced in isolated adipose tissue, *in vitro*, by a variety of agonists, e.g. catecholamines,[1,2] the dibutyryl derivative of cyclic AMP (DBCAMP),[3,4] serum.[5–10] Catecholamines stimulate lipolysis in adipose tissue via the beta-adrenergic receptors of the fat cell leading to adenyl cyclase action, cyclic AMP formation and activation of hormone sensitive lipase[3,11] resulting in the formation of free fatty acids (FFA) and glycerol. DBCAMP promotes lipolysis in adipose tissue, *in vitro*, by virtue of its lipid solubility with consequent penetration of the fat cell membranes coupled with the inability of lipocytes to differentiate, as regards lipolysis, between it and the native product.[12]

Fain[11] has proposed that the beta-adrenergic blocking drug propranolol may act in two ways to inhibit catecholamine-stimulated lipolysis: low concentrations of propranolol specifically antagonize the activation of adenyl cyclase (i.e. beta-blockade, *per se*), whilst higher concentrations prevent the action of cyclic AMP. It has been shown previously that propranolol may also antagonize serum-induced lipolysis in isolated fat cells.[13]

The aim of this study was to compare the action of propranolol blockade of catecholamine- and DBCAMP-provoked lipolysis with that induced by serum.

L-Adrenaline, L-noradrenaline, DBCAMP(N^6-2′-*O*-dibutyryl cyclic adenosine-3′-5′ monophosphate), aminophylline (theophylline) and caffeine, were obtained from the Sigma London Chemical Co. Ltd, Kingston-upon-Thames, Surrey. 1-isopropylamino-3-(1-naphthyloxy)propan-2-*ol*(*dl*-propranolol hydrochloride) was kindly provided by Imperial Chemical Industries Ltd (Pharmaceuticals Division) Alderley Park, Macclesfield, Cheshire.

Male albino Wistar rats weighing 190–210 g (obtained from A. Tuck & Son, Hullbridge, Essex and fed on Thompson's Rat Cube Diet) were fasted overnight but allowed water *ad lib.* They were killed by carbon dioxide anaesthesia and exsanguination. Epididymal adipose tissue was used to prepare suspensions of isolated fat cells in Krebs Ringer bicarbonate buffer (pH 7·4 containing glucose 45 mg/100 ml and bovine albumen (Armour) 3·5 g/100 ml) using a technique[8] modified from Rodbell.[14] Blood for serum was obtained from the same animals, and no special precautions were taken to prevent access of air. Aliquots (500 μl, containing approximately 30 mg of lipid) of free fat cell suspension were dispensed into polypropylene incubation vials containing pharmacological/biochemical reagent solution, and either 1·10 ml of fasted rat serum, or buffer. The contents of each vial were made up to a total volume of 2·0 ml before gassing with a mixture of 75% nitrogen, 20% oxygen and 5% carbon dioxide, capping and incubating for 90 min in a shaking water bath at 37°. The lipolytic action was "fixed" by freezing to −20°, and the samples were stored frozen. Glycerol release was employed as an index of lipolysis. This metabolite was measured before and after incubation, by an enzymatic method[15] using glycerokinase and glycerol-1-phosphate dehydrogenase (Boehringer Corporation, London). Glycerol release was related to the weight of intracellular lipid present in an aliquot of isolated fat cell suspension determined by a modified Folch[16] technique described elsewhere.[17]

The results are summarized in Table 1.

When isolated fat cells were incubated with the maximally effective dose of serum,[8,9,13] there was a conspicuous rise in glycerol release above the control value. The presence of catecholamine (total concentration 1×10^{-4} M-consisting of $0{\cdot}5 \times 10^{-4}$ adrenaline and $0{\cdot}5 \times 10^{-4}$ M noradrenaline) or DBCAMP 5×10^{-3} M, provoked a highly significantly ($P < 0{\cdot}001$) elevated lipolytic response. DBCAMP at concentrations of 5×10^{-4} M, 5×10^{-5} M produced glycerol eggresses of respectively $5{\cdot}4 \pm 0{\cdot}2$ and $4{\cdot}2 \pm 0{\cdot}6$ nmoles/mg lipid during the 90 min incubation, i.e. not statistically significantly ($P > 0{\cdot}05$) different from the control value.

Fat cells incubated with lipolytic agonist plus propranolol showed a reduction in glycerol release when compared with the values obtained following incubation in the absence of the beta-blocking compound in all cases except DBCAMP plus the low concentration (1×10^{-6} M) of beta-blocking drug. Propranolol at a concentration of 1×10^{-3} M was more effective an antagonizing lipolysis stimulated both by serum and by catecholamine than when at 1×10^{-6} M.

Serum plus DBCAMP did not produce a lipolytic response that was conspicuously different from catecholamine plus DBCAMP. When lipocytes were incubated in the presence of 5×10^{-3} M caffeine, the glycerol release was not significantly different ($P > 0{\cdot}05$) from that obtained in response to serum plus caffeine. No significant rise above the control value was obtained with caffeine or theophylline when used at concentrations of 5×10^{-4} M or 5×10^{-5} M, the respective values were: caffeine $5{\cdot}7 \pm 1{\cdot}0$ and $2{\cdot}8 \pm 1{\cdot}1$; theophylline $4{\cdot}4 \pm 1{\cdot}2$ and $2{\cdot}7 \pm 0{\cdot}2$.

TABLE 1. EFFECT OF SERUM AND CATECHOLAMINE WITH AND WITHOUT DIBUTYRYL CYCLIC AMP PROPRANOLOL OR CAFFEINE ON LIPOLYSIS IN THE ISOLATED FAT CELL

Treatment	Control (buffer only)	Serum (1·10 ml/vial)	Catecholamine (1×10^{-4} M)	DBCAMP (5×10^{-3} M)	Caffeine (5×10^{-3} M)
Agonist alone	3·2 ± 0·1	16·4 ± 0·7	34·5 ± 2·2	58·6 ± 3·1	47·4 ± 6·0
Plus DBCAMP (5×10^{-3} M)	—	63·6 ± 1·1	65·2 ± 1·9	—	—
Plus propranolol (1×10^{-6} M)	—	9·5 ± 0·8*	15·7 ± 1·3†	58·6 ± 3·7‡	—
Plus propranolol (1×10^{-3} M)	—	1·7 ± 0·7*	2·2 ± 0·4*	5·1 ± 0·7*	—
Plus caffeine (5×10^{-3} M)	—	55·5 ± 3·6	—	—	—

Values are the mean ± S.E.M. of three to five observations (nmoles glycerol released/mg lipid/90 min). Significance of difference from values obtained in the absence of propranolol. * $P < 0.001$, † $P < 0.01$, ‡ Not significant.

The results obtained with high and low doses of propranolol and catecholamine or DBCAMP, are compatible with the views of Fain.[11] The similar response of serum- and catecholamine-induced lipolysis to low and high doses of propranolol, and the results obtained of lipolysis provoked by serum or catecholamine in the presence of DBCAMP, are compatible with the view that serum induces lipolysis via a mechanism which is comparable to that prevailing in fat cells under the conditions of catecholamine stimulation. Support of this thesis is also indicated by the non-additive response of glycerol release obtained with 5×10^{-3} M caffeine without and with serum.

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