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Adrenergic receptors in human adipocytes—Divergent effects on adenosine 3′,5′-monophosphate and lipolysis*

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THE ROLE of adenosine 3′,5′-monophosphate (cyclic AMP) in mediating many of the effects of a number of hormones, including the catecholamines, is now widely accepted. The catecholamines are capable of interacting with at least two types of adrenergic receptors, known as alpha and beta receptors, and it has been postulated that cyclic AMP may be involved in both types of interaction.[1] According to this hypothesis, beta receptors mediate an increase in the intracellular level of cyclic AMP, secondary to the stimulation of adenyl cyclase, whereas an interaction with alpha receptors leads to a fall in the level of cyclic AMP, and consequently an opposite effect on cell function. In a previous study,[2] the effects of phentolamine (an alpha adrenergic blocking agent) and propranolol (a beta adrenergic blocking agent) on the basal and epinephrine-stimulated rates of lipolysis of human and rat adipocytes were observed. The results of these observations indicated that human fat cells possessed both alpha and beta adrenergic receptors, mediating inhibition and stimulation of lipolysis, respectively, whereas rat cells possessed only beta receptors. Comparable results have since been reported by others.[3] To obtain more direct information about the possible role of cyclic AMP in these responses, we have now carried out experiments with human fat cells in which both cyclic AMP levels and glycerol release have been measured. As predicted on the basis of the above hypothesis, the results indicate that cyclic AMP levels and lipolysis are indeed influenced by alpha and beta receptors in a closely parallel fashion.

Isolated human fat cells were prepared from subcutaneous adipose tissue obtained from surgical patients using a method previously described.[4] Krebs albumin buffer, cells, and test substances (epinephrine, phentolamine, and propranolol, each at a final concentration of 10^{-5} M) were placed in plastic flasks and incubated with gentle shaking at 37° under an atmosphere of 95% O_2 and 5% CO_2. At the end of 30 min, the contents of one set of flasks were rapidly frozen in liquid nitrogen and stored at −70°. They were later homogenized in the presence of 0·1 M HCl, and cyclic AMP was measured in purified extracts by a method based on the activation of liver phosphorylase.[5] The remaining flasks were incubated for 4 hr, following which filtrates were prepared for glycerol analysis by the method of Garland and Randle.[6]

Prior to the basic study just described, a series of preliminary experiments were done in which both parameters were measured at various intervals up to 4 hr. The effect of epinephrine in the presence of phentolamine is illustrated in Fig. 1. The level of cyclic AMP under these conditions reaches a peak at around 30 min, whereas glycerol continues to be released at an increased rate for at least 4 hr. This type of relationship is compatible with a mechanism involving the phosphorylation and hence activation of a lipase under the catalytic influence of a cyclic AMP-sensitive protein kinase.[7,8] Cyclic AMP was detected in increasing concentrations in the buffer during the first hour of incubation, but thereafter its concentration remained relatively stable.

The results of an experiment involving both blocking agents are shown in Fig. 2. The addition of epinephrine by itself increased the level of cyclic AMP, and this effect was greatly enhanced in the presence of phentolamine. In the presence of propranolol, by contrast, epinephrine reduced the level below that seen in control flasks. Neither phentolamine nor propranolol at these concentrations had

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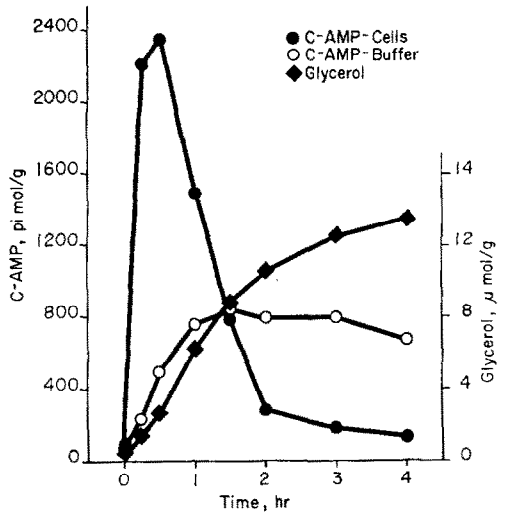

FIG. 1. Effect of epinephrine and phentolamine on cyclic AMP levels and glycerol release. Drugs were added at zero time to a suspension of human fat cells, and incubation was stopped at various times between 15 min and 4 hr. Cyclic AMP (C–AMP) and glycerol are expressed in terms of picomoles and micromoles, respectively, per gram dry weight of lipid initially present in the cell suspension.

any effect when added in the absence of epinephrine. Thus, changes in cyclic AMP paralleled the changes in glycerol release previously reported.[2] In some experiments, an almost perfectly linear relationship between the two parameters was observed. In other experiments, the increase in cyclic AMP produced by the epinephrine–phentolamine combination was disproportionately larger than the effect on glycerol release. However, in all experiments in which this design was followed (Table 1), phentolamine enhanced the positive effect of epinephrine and propranolol reduced it.

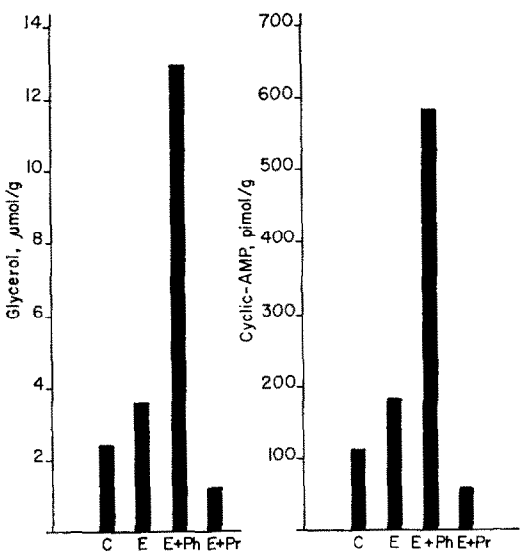

FIG. 2. Effect of epinephrine alone (E) and in the presence of phentolamine (E + Ph) and propranolol (E + Pr) on lipolysis, as measured by glycerol release, and on the level of cyclic AMP in human fat cells. Glycerol was measured 4 hr and cyclic AMP 30 min after the indicated combination of drugs. Controls (C) received no drugs.

TABLE 1. EFFECT OF EPINEPHRINE ALONE AND IN THE PRESENCE OF ADRENERGIC BLOCKING AGENTS ON THE INTRACELLULAR LEVEL OF CYCLIC AMP AND GLYCEROL RELEASE*

	Control		Epinephrine		Epinephrine + phentolamine		Epinephrine + propranolol	
Expt No.	cAMP	Glyc.	cAMP	Glyc.	cAMP	Glyc.	cAMP	Glyc.
1	123	2·40	184	3·58	858	12·92	59	1·22
2	65	1·57	107	4·53	1743	9·17	49	0·95
3	104	2·02	175	4·28	525	14·29	72	1·65
4	148	4·36	244	8·87	2838	12·69	140	0·99
5	134	1·94	194	3·65	1309	14·94	101	1·01

* cAMP = cyclic AMP, expressed as picomoles per gram at 30 min; Glyc. = glycerol, expressed as micromoles per gram per 4 hr. Each experiment was done using cells from a different patient.

It is difficult to interpret these results other than by assuming the existence in human fat cells of two types of adrenergic receptors, influencing the level of cyclic AMP in opposite directions. It would appear that beta receptors normally predominate, since epinephrine by itself causes an increase in cyclic AMP and a corresponding increase in the rate of lipolysis. The nature of this effect as the resultant of two opposing actions is revealed by the adrenergic blocking agents. When the antagonistic alpha receptors are blocked by phentolamine, epinephrine interacts with the unopposed beta receptors to produce very large increases in the level of cyclic AMP and the rate of lipolysis. Conversely, when the beta receptors are blocked by propranolol, then the inhibitory influence of the alpha receptors becomes unmasked. A similar explanation has been offered to account for the effects of epinephrine on insulin release by the pancreas,[9] although it should be noted that in that case the level of cyclic AMP was measured in pancreatic islets rather than in a single type of cell. On the assumption that the observed changes occurred primarily in beta cells, however, it is of interest that alpha receptors rather than beta receptors appeared to predominate in the absence of blocking agents.

As mentioned previously, the increase in cyclic AMP in response to the epinephrine–phentolamine combination was much greater in some experiments than in others. It should be noted that this difference is not simply an artifact related to the time chosen for measurement, because, even in samples showing a linear relation between cyclic AMP and lipolysis, the cyclic AMP was found to peak at about 30 min. Whether this represents anything more than a quantitative difference in sensitivity among the samples remains to be seen. All of the samples were from subcutaneous tissue, and there was nothing distinctive about the clinical status of the donors. The disproportionately large increase in the level of cyclic AMP in response to epinephrine plus phentolamine in some experiments is consistent with the results of many other studies, indicating that the level of cyclic AMP can increase far beyond that required for the maximal stimulation of cell function (see, for example, ref. 10–13).

These results can be added to many previous observations suggesting that beta adrenergic effects in general are mediated by increased levels of cyclic AMP, secondary to the stimulation of adenyl cyclase. They also support the hypothesis[1,9] that at least some alpha adrenergic effects are the result of a fall in the level of cyclic AMP. The mechanism by which alpha receptors mediate this effect is still obscure. The physiological significance of this arrangement (two types of receptors in the same cells, mediating divergent effects on the level of cyclic AMP) also requires further study.

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Action of dopamine and noradrenaline on synaptic transmission in sympathetic ganglia of brown fat

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BROWN adipose tissue has a dual sympathetic nerve supply.[1,2] There are postganglionic fibres of neurones in the paravertebral ganglia, supplying blood vessels and mediating vasodilatation. Another set arise from ganglia embedded within the adipose tissue. They supply the adipocytes, mediating lipolysis, stimulation of respiration and thermogenesis. This latter system has an easily measurable action on target tissue (stimulation of respiration) and offers a useful model for the study of synaptic transmission. In this communication it will be shown that dopamine produces sustained facilitation of synaptic transmission across the cholinergic synapse. This may be due to increased prostaglandin E_2 synthesis in the postsynaptic neurone. It is also suggested that noradrenaline may inhibit synaptic transmission by enhancement of prostaglandin E_3 formation in ganglion cells.

Methods

Interscapular brown fat was taken from 21-day-old rats maintained at animal house temperature (22°). Respiration rate was measured on individual lobules suspended in Ringer–Locke solution in a well-type oxygen electrode, maintained at 25°. Various drugs were added to the well after the establishment of a basal oxygen consumption rate as shown in Fig. 1.

The rate of prostaglandin synthesis in paravertebral ganglia was determined by the method of Van Dorp *et al.*[3] Rat paravertebral ganglia (0·1 g) were homogenized with a glass hand homogenizer in 1 ml of ice-cold solution containing 130 mM KCl, 20 mM tris buffer pH 7·4 and 5 mM $MgCl_2$. Homogenate (0·1 ml) was added to 1 ml of solution containing 1 mM (50,000 counts/min) of either 1-C 14 labelled arachidonic acid (PGE_2 precursor) or 1-C 14 tagged eicosapentaenoic acid (PGE_3 precursor) together with 0·3 mM reduced glutathione, 0·3 mM hydroquinone, 100 mM tris buffer pH 7·4 and 5 mM $MgCl_2$. After incubation for 1 hr at 37° the reaction was stopped by adding 1 ml of 0·2 M citric acid. Prostaglandins were extracted with two aliquots of ether. The ether extract was resolved on silica thin layer chromatoplates developed in benzene–dioxan–acetic acid (20:20:1, by vol.). Areas corresponding to a carrier prostaglandin E_1 were scraped for radioactivity measurement by liquid scintillation counting. 1-C 14 labelled arachidonic acid and eicosapentaenoic acid