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PRELIMINARY COMMUNICATIONS

FIBROBLAST SUSCEPTIBILITY TO β-ADRENERGIC STIMULANTS

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Fibroblasts were for a long time considered cells of limited function and responsiveness whose main purpose was to provide intracellular substratum and support for more highly specialized tissues. It has become increasingly clear that fibroblasts are, in fact, highly specialized cells and that their "support" role is tailored to the particular organ and stage of development [1,2]. What is more, their dependence in culture upon insulin [3] and a variety of growth factors [4,5,6,7,8] and their production of nerve growth factor [9] combine to cast the fibroblast in a new light with respect to its sensitivity to so-called "hormonal influences."

Chick embryo and normal human fibroblasts in monolayer cultures have recently been shown to preserve their ATP content for at least 24 hr when deprived of a carbon source.* Several non-metabolizable sugar analogues,*+ such as 3-O-methylglucose, 6-deoxyglucose and α-methyl-D-glucoside, were shown to have no influence upon that property of the cells. A somewhat unexpected result was obtained when β-adrenergic stimulants such as epinephrine or DL-isoproterenol were added to the starved cultures. Gradual loss of ATP results from the addition of as little as 5-10 μg isoproterenol/ml with a lag of 4-6 hr. The extent of ATP loss and its rapidity are functions of the

* G. Ev. Demetrakopoulos, B. Linn and H. Amos, manuscript in preparation.

+ G. Ev. Demetrakopoulos, B. Linn and H. Amos, unpublished data.

concentration of the β-adrenergic stimulants. L-epinephrine (adrenalin) has effects similar to those observed with isoproterenol. Such an effect is not observed with α-adrenergic stimulants, such as L-phenylepinephrine, while norepinephrine (primarily an α-adrenergic stimulant) has an intermediate effect (Table 1).

Table 1. Effect of adrenergic stimulants on the ATP content of human fibroblasts in culture cultivated in glucose-free media [Eagle's minimal medium (MEM glc^-) supplemented with 2% dialyzed fetal calf serum (DFCS)]*

Stimulant	Concentration (mM)	ATP content (nmoles/mg cell protein)
Control		30.1 ± 3
L-epinephrine bitartrate	0.13	10.3 ± 0.3
L-epinephrine bitartrate	0.27	2.0 ± 0.5
DL-isoproterenol HCl	0.23	10.5 ± 2
L-phenylepinephrine HCl	0.25	28 ± 3
Norepinephrine	0.29	22 ± 4

*Skin fibroblasts isolated from normal human individuals were grown in multiwell tissue culture plates in Eagle's minimal medium (MEM) supplemented with glucose (1 mg/ml) and 10% dialyzed fetal calf serum (DFCS). When the monolayers were near confluency the medium was changed to glucose-free MEM supplemented with 2% DFCS. The adrenergic stimulants were added to the media at concentrations as indicated, and the cells re-incubated at 37°. Six hr later, the ATP was extracted as described earlier (G. Ev. Demetrakopoulos, B. Linn and H. Amos, manuscript in preparation). The values presented are the arithmetic mean values of triplicate samples for each point with the average deviation for each set. This experiment is representative of the results obtained in each of 5 separate experiments.

Removal of the medium containing the β-adrenergic stimulant and re-incubation in fresh medium free of carbon source and stimulant delay the further loss in ATP, as shown in Fig. 1.

Since the β-adrenergic stimulants are known to increase adenyl cyclase activity of mammalian cells [10], it is conceivable that this is the mechanism responsible for the accelerated loss of cellular ATP. If that is the case, inhibitors of adenyl cyclase should prevent such an effect. Indeed, the presence of glucose, a known adenyl cyclase inhibitor [11,12], blocks the effect of the β-adrenergic stimulants on the ATP loss (catabolite repression). [This inhibition can be overcome by increasing the concentration of the

β-adrenergic stimulants (data not shown).] When carbon sources other than glucose are provided, the effect of the β-adrenergic stimulants on the ATP levels is still demonstrable although somewhat less pronounced when compared to that of fibroblasts maintained in sugar-free medium. We have evidence (data not shown) that the presence of glucose analogues, such as 3-O-methyl-glucose and 6-deoxyglucose, inhibits the β-adrenergic stimulant effect on the ATP. Such analogues are not phosphorylated and per se do not cause any change in the ATP. A similar protective effect has been effected by the presence of pyruvate in the media.

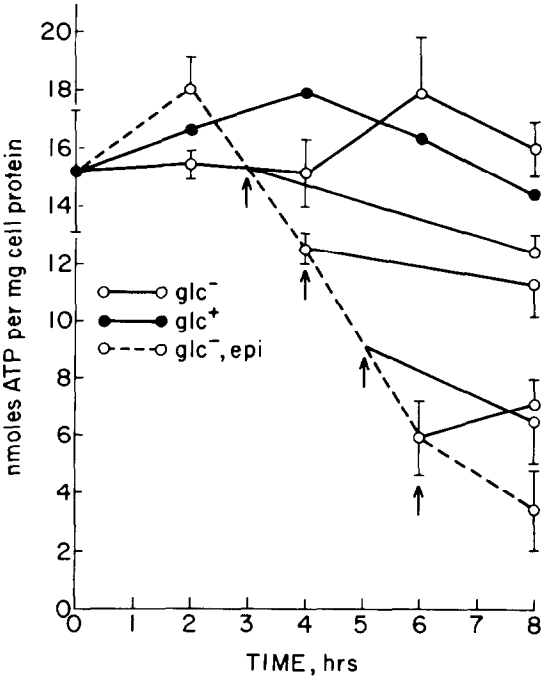

Fig. 1. ATP content of primary CEF cells, as a function of time of exposure to epinephrine. At "0" time, the monolayers were provided with fresh medium (BME + 2% DFCS) supplemented with glucose, 1 mg/ml (●——●); glucose-free medium (o——o); and glucose-free medium + L-epinephrine, 25 μg/ml (o---o). The arrows indicate the time of replacement of epinephrine containing medium with fresh carbon source and stimulant-free BME + 2% DFCS. The monolayers were re-incubated for the rest of the experiment. Each point represents the arithmetic mean value of six samples. The vertical lines indicate the average deviation of the samples. (BME = Eagle's Basal Medium.)

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