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SHORT COMMUNICATION

ABSENCE OF AN EARLY EFFECT OF GLUCOCORTICOIDS ON NONESTERIFIED FATTY ACID ACCUMULATION IN ISOLATED RAT THYMUS CELLS

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SUMMARY

Nonesterified fatty acid levels have been measured in isolated rat thymocytes incubated with or without 1.3 μM cortisol for 2 h. No significant cortisol effect on nonesterified fatty acid levels was observed, though the well-established early effect of cortisol on glucose uptake was present. Since other work has shown that by two hours thymus cells exposed to similar concentrations of cortisol already manifest a number of changes that appear closely related to subsequent cytolysis, it seems unlikely that stimulation of nonesterified fatty acid accumulation can be a primary cause of cytolysis or that it is involved in the early effect of glucocorticoids on thymus cells.

It is well established that physiological concentrations of glucocorticoids induce lysis of lymphocytes *in vivo* and *in vitro*. Disruption of nuclear structure can be seen as early as two hours after exposure of incubated mouse thymus cells to hormone [1].

Burton, Storr, and Dunn[1] suggested the hypothesis that glucocorticoids cause lysis by promoting the accumulation of nonesterified fatty acids which in turn alter nuclear membrane integrity. Turnell, Clarke, and Burton[2, 3] showed that after glucocorticoid treatment there is accumulation of fatty acids *in vivo* in mouse thymus and possibly lymphosarcoma cells. They also found that exogenous fatty acids *in vitro* can cause lysis of glucocorticoid-sensitive cells, but do not appear to affect glucocorticoid-insensitive cells.

So far as we are aware, however, there is no evidence that exposure of thymus or lymphosarcoma cells to glucocorticoids *in vitro* results in accumulation of nonesterified fatty acids. Since cytolysis can be produced by glucocorticoids added to isolated rat thymus cells [1, 4], it follows that if fatty acids cause this effect, an increase in fatty acids should be observable in the thymus cells prior to the earliest manifestations of cytolysis. The results we describe show that under conditions in which several other effects of glucocorticoids on thymus cells can be demonstrated, no increase in nonesterified fatty acids occurs.

By procedures described elsewhere [5], suspensions of thymus cells from adrenalectomized rats were prepared in Krebs–Ringer bicarbonate buffer containing 11.0 mM glucose and equilibrated with 95% O_2, 5% CO_2. Quadruplicate incubations were begun by adding 1.5 ml of cell suspension (cytocrit 0.14 or approximately 7×10^8 cells/ml) to flasks containing cortisol (California Corp.) in about 15 μl of buffer to achieve a final concentration of 1.3 μM, or the same volume of buffer alone. At 10, 30, 60 and 120 min after addition of cells 0.3-ml aliquots of suspension were removed and placed in 2.7 ml of an organic extraction solution [6], and extracted overnight as described by Cushman *et al.*[7]. 1.8 ml of heptane and 0.8 ml of water were added to each tube, the phases allowed to separate and aliquots of the organic phase removed for triplicate assay of nonesterified fatty acids (NEFA) using the ^{63}Ni binding method described by Ho[8] and modified by Cushman *et al.*[7]. Glucose uptake was assayed by measuring glucose disappearance from the medium at the end of the 2-h incubation [9].

Our results are presented in Table 1. No cortisol effect

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Table 1. Nonesterified fatty acid levels in rat thymus cells *in vitro* incubated with 11.0 mM glucose and with or without cortisol (1.3 μM)*

	NEFA (μmol/g wet weight)				Glucose uptake after 120 min (μmol/ml Cells)
	Incubation time (min)				
	10	30	60	120	
Control	1.23 ± 0.042	1.35 ± 0.028	1.51 ± 0.054	1.52 ± 0.031	26.9 ± 0.45
Cortisol	1.15 ± 0.044	1.27 ± 0.029	1.42 ± 0.044	1.47 ± 0.020	21.2 ± 0.35†

* Results are given as means ± 1 standard error, with 4 replicates in each group.
† $P < 0.005$.

on nonesterified fatty acid levels could be demonstrated. There was, however, a highly significant inhibitory effect of cortisol on glucose uptake, evidence for a normal cortisol action on the cells during the 2-h incubation. We have repeated these experiments under slightly different conditions with the same result.

We conclude that cortisol does not stimulate the accumulation of NEFA in isolated rat thymus cells for at least 2 h. By 2 h cortisol not only inhibits glucose uptake —an effect that is already present by 15-20 min [5]—but also protein synthesis [10, 11], and other metabolic activities [12]. In addition, cortisol has been shown to cause an increase in what has been termed "nuclear fragility" [13]. Both this latter phenomenon and the disruption of nuclear morphology that can be detected by electron microscopy after 2 h of exposure of mouse thymus cells to glucocorticoids [1], appear to be early direct manifestations of cytolysis. Since no effects of cortisol on levels of nonesterified fatty acids are observed by this time, it seems unlikely that stimulation of nonesterified fatty acid accumulation plays an important role in the early phases of the cytolytic actions of glucocorticoids.

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