CONCANAVALIN A INDUCED GLUCOCORTICOID RESISTANCE IN RAT THYMOCYTES IN RELATION TO GLUCOSE METABOLISM AND GLUCOCORTICOID RECEPTORS

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Summary

Concanavalin A stimulates glucose uptake in isolated rat thymocytes, at concentrations as low as 1.0 μg/ml. The magnitude of the response is dose-dependent with maximal glucose uptake (greater than 100% over control) observed over the 50-500 μg/ml range. The response to concanavalin A occurs within 5 minutes and is maximal by 1 hour. Exposure of thymocytes to physiological concentrations of cortisol has been shown previously to lead to a 15-30% reduction in glucose uptake within 20 minutes (1). Cortisol added simultaneously to or after concanavalin A has no effect on glucose uptake, but when it is added before concanavalin A, a glucocorticoid response is observed even in the face of concanavalin A-induced glucose uptake. Unlike the cortisol effect, the concanavalin A-induced increase in thymocyte glucose uptake is not inhibited by either cycloheximide or cordycepin. The antagonism between cortisol and concanavalin A may in part exist at the specific glucocorticoid receptor level, since exposure of cells to concanavalin A rapidly and significantly reduces the number of specific, saturable glucocorticoid binding sites in isolated thymocytes.

Introduction

Plant lectins, such as Concanavalin A,* can bind to peripheral blood lymphocytes in culture (2), induce blast transformation and ultimately initiate mitosis. These changes are preceded by alterations in RNA synthesis, phosphorylation and distribution (3,4,5). Glycolysis appears to be the major source for energy required to sustain these alterations in cellular activities (6,7). Guanosine 3'-5' cyclic monophosphate has been suggested as a possible intracellular mediator of some actions of these mitogenic compounds on lymphocytes (8).

Conversely, when rat thymocytes are exposed to glucocorticoids, cell death occurs (9). Both actinomycin D and cycloheximide sensitive steps (10,11) appear to mediate a decrease in glucose metabolism which precedes cell death

* Concanavalin A = Con A in text.

in response to cortisol (12).

The present investigation explores the actions of Con A and cortisol alone and in combination on glucose metabolism in isolated thymocytes.

Materials and Methods

Thymus cell suspensions from male Sprague-Dawley rats adrenalectomized 4-6 days prior to use were prepared in Krebs-Ringer bicarbonate buffer* (pH 7.4) maintained in equilibrium with 95% O_2:5% CO_2 for all glucose uptake studies. The same buffer containing 5 mM glucose was used for studies where steroid hormone binding was assessed.

Reagents utilized in glucose uptake studies were prepared in KRB with 10 mM glucose so the final reaction media contained 5 mM glucose. Cortisol (Calbiochem) was used at a final concentration of 1 μM. Con A (Pharmacia) was prepared at the final concentrations shown in the figures or legends. Cycloheximide (Sigma) was used at a final concentration of 2.5×10^{-4}M and cordycepin (Sigma) was used at 1 mg/ml.

For measurement of glucose uptake, 40 μl aliquots of thymocyte cell suspension at a cytocrit (V) of 0.1-0.12 milliliters of packed cells per ml cell suspension containing approximately 2.5×10^7 cells were placed in Eppendorf plastic tubes and incubated as previously described (2). Except where noted the incubation was for 1 hour at 37°C. Subsequently the cells were cooled, sedimented in an Eppendorf centrifuge (Model 3200) for 2 minutes at maximum force. Aliquots of the supernatant were sampled for analysis of remaining medium glucose via enzymatic determination (glucostat, Worthington Biochem.). The results are expressed in terms of micromoles glucose uptake per ml of packed cells as calculated from determination of standards and estimation of the cytocrit (V) via the microhematocrit method.

For assay of steroid hormone receptors 0.5 ml aliquots of thymus cell suspensions prepared in KRB with 5 mM glucose at a V of 0.3-0.35 were incubated in glass vials with or without Con A for 45 min at 37°C. The samples were then placed on ice and transferred to a cold room at 3°C. Each sample was divided into two 0.25 ml aliquots to which [^{3}H]dexamethasone (New England Nuclear, S.A. 27 curies per mm) or unlabeled dexamethasone plus [^{3}H]dexamethasone were added. The final concentration of the [^{3}H]dexamethasone was 10 nM, that of the unlabeled dexamethasone was 1 μM. The vials were gassed with 95% O_2:5% CO_2, capped and allowed to shake gently for 90 minutes. Specific cytoplasmic and nuclear binding capacities were measured by the $MgCl_2$-dextran coated charcoal and the $MgCl_2$ dilution procedures described by Munck and Wira (13). Receptor binding was determined as the saturable fraction, i.e., the difference between incubations containing only [^{3}H]dexamethasone and [^{3}H] dexamethasone plus unlabeled dexamethasone. The results are shown as this difference per ml of packed cells based upon the initial cytocrit.

Results

As shown in Fig. 1, Con A at doses of 1 μg/ml cell suspension significantly ($P < 0.05$) increased glucose uptake by isolated thymocytes. Maximal uptake (greater than 100% over control values) is achieved at doses of 100 μg/ml.

* Krebs-Ringer bicarbonate buffer = KRB in text.

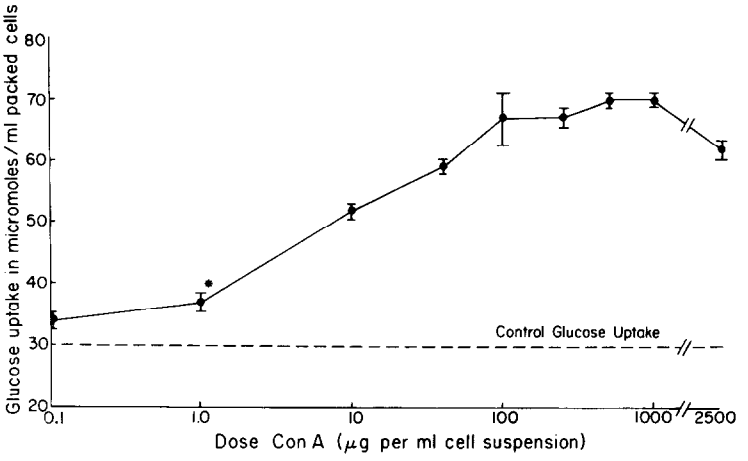

Fig. 1. Influence of Con A concentration on glucose uptake in isolated rat thymocytes. Forty μℓ aliquots of cell suspension V 0.1-0.12 were incubated with Con A for 1 hour at 3°. The final glucose concentration in the incubation media was 5 mM. The results are expressed as micromoles glucose uptake/ml of packed cells and represent the means ± s.e. of 5-6 individual determinations from a representative experiment. Values are significantly greater than control at the $P < 0.5$ level.

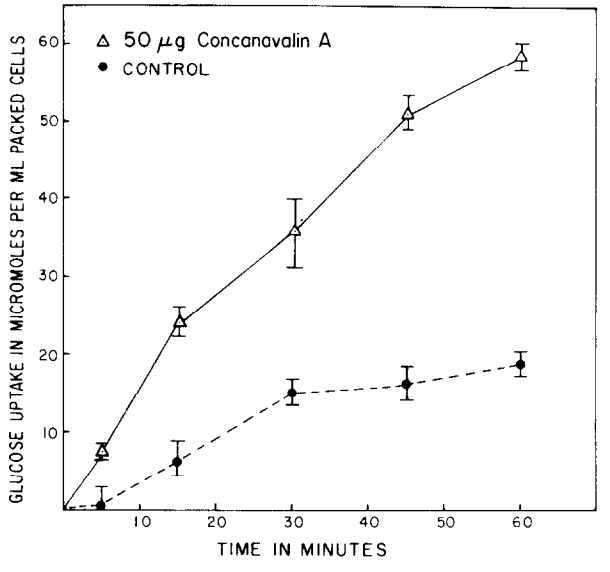

Fig. 2. Influence of the time of incubation at 37° on Con A altered glucose uptake by isolated rat thymocytes. Forty μℓ aliquots of cell suspension (V 0.1-0.12) were incubated as described in Fig. 1, in the presence of Con A (50 mg/ml) or KRB (5 mM glucose) alone for the time periods indicated. Cells were pelleted by centrifugation and 20 ml aliquots of supernatant sampled for glucose determination as described. Values shown are the mean ± s.e. of at least 4-6 individual determinations from a representative experiment. All Con A treated groups differ significantly from appropriate controls at the $P < 0.5$ level or smaller.

Concentrations greater than 1 mg/ml result in a reduction of total uptake but values remain significantly greater than controls. Similar biphasic dose response curves have been observed for Con A in a variety of lymphocyte systems (14).

The results in Fig. 2 demonstrate that Con A at 50 μg/ml significantly stimulates glucose uptake by thymocytes within 5 minutes. Similar 5-minute effects were also observed at either 2 or 100 μg concentrations. Glucose uptake is significantly greater than controls at all time intervals.

In Fig. 3(A) evidence is presented that Con A abolishes the cortisol effect when cells are exposed to Con A and steroid simultaneously. As shown by the first three bars, cortisol by itself significantly reduces glucose uptake by 17%, whereas Con A stimulates glucose uptake by more than 100%. Comparison of the last two bars shows that cortisol does not have a measurable effect when it is added together with Con A. This observation has been obtained repeatedly and consistently. The results in Fig. 3(B), however, demonstrate that when cortisol is added before Con A in the absence of glucose, a cortisol effect similar in magnitude to that produced by cortisol alone is observed superimposed on the Con A effect.

Evidence that Con A and cortisol affect glucose uptake by different mechanisms is given in Fig. 4. Neither cycloheximide nor cordycepin at concentrations previously demonstrated capable of abolishing the cortisol effect on glucose uptake (11,15) alter the Con A-induced stimulation of glucose uptake. The inhibitors themselves did not significantly alter glucose uptake.

In an attempt to understand the mechanism by which Con A blocks the effect of cortisol on glucose uptake, I have investigated the influence of Con A on the interaction of glucocorticoids with their cytoplasmic and nuclear receptors (11,16). As shown in Fig. 5, exposure of cells to Con A prior to measurement of saturable cytoplasmic and nuclear glucocorticoid receptors, results in a significant 35% reduction in both cytoplasmic and nuclear fractions. As expected (9), at low temperatures only a small proportion of the receptor

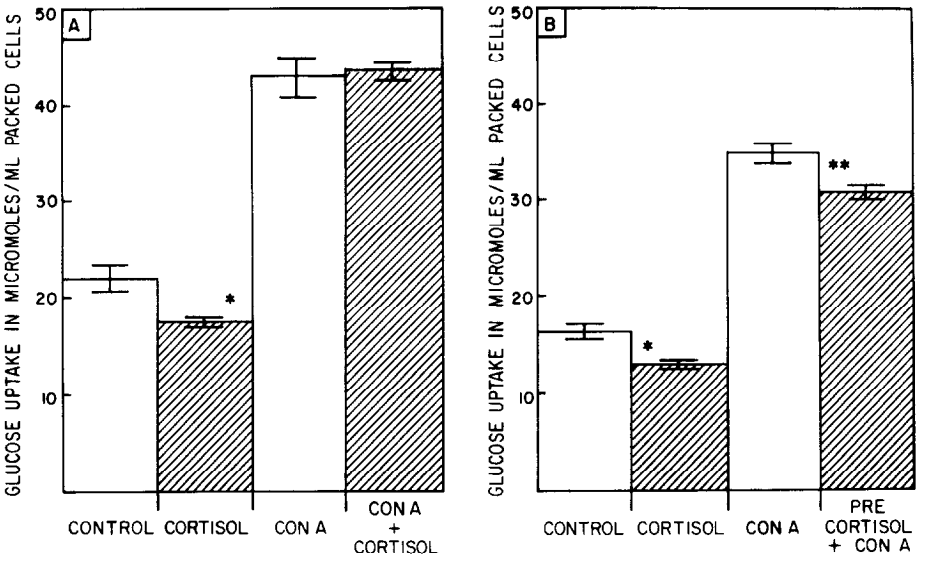

Fig. 3. (A) The influence of cortisol and Con A alone and in combination on glucose uptake in thymocyte suspensions. Forty µℓ aliquots of thymocytes (V 0.1-0.12) were incubated with either cortisol (1 µM) or Con A (250 µg/ml) alone or in combination for 1 hour at 37°, as described. Glucose uptake was assessed as in Fig. 1. Values are the means ± s.e. of 4-6 individual determinations from a representative experiment.

(B) Aliquots of cells were incubated with or without 1 µM cortisol for 20 minutes at 37° (in the absence of glucose) prior to exposure to Con A (250 µg/ml) as described in A. Values are the mean ± s.e. of 5-6 individual determinations from a typical experiment. * Differs significantly from control ($P < .05$). ** Differs significantly from Con A ($P < .05$).

bound radioactivity is found in the nucleus. Preliminary data suggest the results are due to decreased number of receptors rather than a decreased association constant.

Discussion

Cell suspensions of immature thymocytes were found to respond to Con A via rapid stimulation of glucose uptake, much as has been shown for the action of phytohemagglutinin* on bovine peripheral lymphocytes (17). PHA appears to activate functional membrane carriers in cultured lymphocytes (17). The

* Phytohemagglutinin = PHA in text.

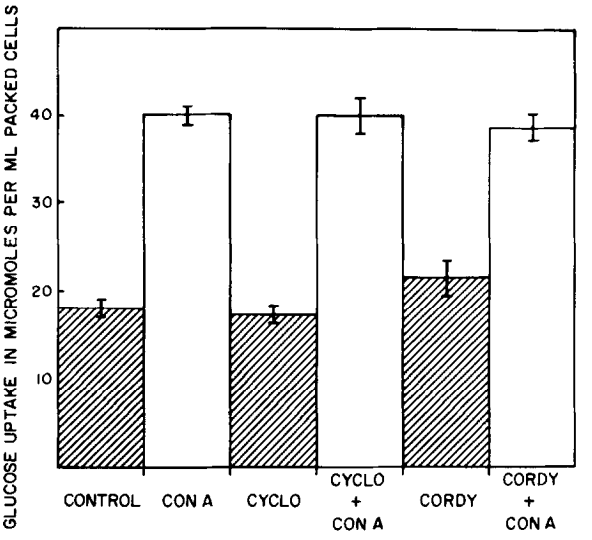

Fig. 4. Influence of inhibitors of RNA and protein synthesis on Con A induced glucose uptake. Forty µℓ of thymocyte suspension (V 0.1-0.12) were incubated with medium KRB (5 mM glucose), Con A (125 µg/ml), cycloheximide alone, or cordycepin alone for 1 hour at 37° as described. Glucose uptake was determined as designated. Typical results are expressed as the mean ± s.e. for 4-5 individual determinations.

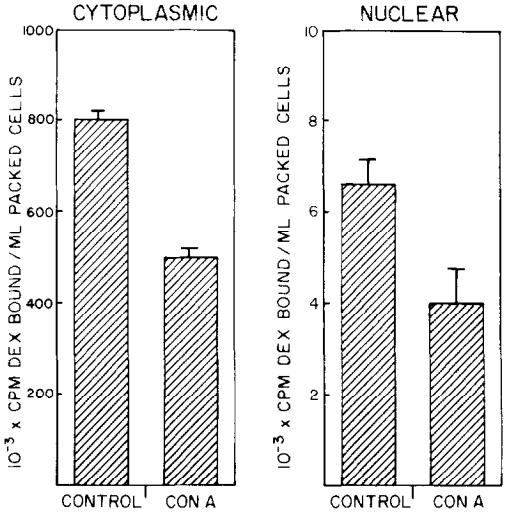

Fig. 5. Influence of Con A on thymocyte cytoplasmic and nuclear saturable glucocorticoid receptor concentrations. Five hundred µℓ aliquots of thymocytes (7.5 x 10^8 cells) were incubated with or without Con A (500 µg/ml) for 45 minutes at 37°C. Vials containing cell suspensions were then placed on ice and all subsequent procedures conducted at 3°C. Cell suspensions were incubated for an additional 90 minutes with 10 nM [^{3}H] Dexamethasone or [^{3}H] Dexamethasone plus 1 µM unlabeled dexamethasone. Following this period cytoplasmic and nuclear receptor concentrations were assayed as described in Materials and Methods. The values shown represent only the saturable portion of binding ([^{3}H]dexamethasone - [^{3}H]dexamethasone plus unlabeled dexamethasone). The figures represent the means ± s.e. of 4 individual determinations from a typical experiment.

rapid action of the Con A effect on glucose uptake in thymocytes and the lack of effect by cycloheximide or cordycepin suggest that Con A may exert its action in a similar manner.

Studying human peripheral lymphocytes in culture, Nowell (18) has shown that prednisolone at physiological concentrations is capable of antagonizing phytohemagglutinin-augmented mitosis. This effect was absent, however, when cultures received glucocorticoid following mitogen exposure. Similar time-relationships have been found for the cytolytic action of prednisolone on cultured lymphocytes (19). The findings reported here that Con A reduces receptor number suggest that the suppression of the cortisol effect on glucose uptake in thymocytes by simultaneous or prior exposure of cells to Con A, may at least in part result from a Con A-induced alteration of glucocorticoid receptor metabolism. Con A exposure rapidly reduces the receptor binding capacity, thus possibly altering glucocorticoid induced gene activation.

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