Vol. 102, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1981 Pages 458-465

EARLY EFFECT OF STEROIDS ON ^{45}CALCIUM UPTAKE BY MOUSE THYMOCYTES

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Received May 19,1981

SUMMARY : The effects of various steroids on ^{45}calcium uptake were investigated in parallel with the viability of mouse thymocytes. Dexamethasone, a synthetic compound with glucocorticoid activity, induced a rapid increase in membrane permeability to calcium. This effect was still measurable using 10^{-7} M dexamethasone and appeared specific for compounds with glucocorticoid potency. In addition, calcium efflux from prelabeled cells was not altered in the presence of dexamethasone, indicating an increased total cell concentration. It is therefore suggested that calcium ions play a role in steroid-induced cell lysis.

Although alterations in calcium metabolism and other divalent cations have been reported following long term in vivo treatment with steroids in several hormone-responsive tissues, little information is available on the cellular exchange of calcium shortly after administration of steroids to isolated cells. It has been shown that estradiol-17 ß, even at physiological concentrations, influences rates of cellular exchange in uterine cells as early as 2.5 min after in vitro addition of hormone (1). Progesterone has been observed to produce very rapid increase in intracellular free calcium levels in fully grown oocytes (2). Glucocorticoids similarly affect calcium transport across the intestinal mucosa within 1 hr (3) Although direct data are scanty, the possibility that glucocorticoids can influence the action of cyclic AMP through intracellular calcium ion metabolism has been suggested by Rasmussen and Tenenhouse (4). In lymphoid tissue, the effect of glucocorticoids are well documented (5) and the calcium ion has been suggested to play a role in steroid-induced as well as in ionophore A_{23187}-induced cell lysis (6,7). In this paper, evidence is presented for a rapid in vitro effect of glucocorticoids on ^{45}calcium uptake by isolated thymocytes.

0006-291X/81/170458-08$01.00/0

MATERIALS AND METHODS

Animals and reagents

The animals used in all investigations were intact female $C_{57}Bl_6$ mice, 6-8 weeks old. Unlabeled steroids were obtained from Sigma Chemical Corp. (St. Louis, Mo). Each steroid was dissolved in absolute ethanol and diluted to the appropriate concentrations before each experiment. The final ethanol concentration was always lower of equal to 0.01 % and did not alter ^{45}calcium uptake or cell viability. Calcium concentrations were adjusted using a calcium standard solution (Titrisol, Merck). EGTA (ethylene glycol-bis (ß-aminoethyl- ether) NN'-tetra acetic acid) was purchased from Sigma. Culture medium (minimum essential medium, MEM, calcium free, ref 138) and the various supplements (sodium pyruvate, L-glutamine, penicillin-streptomycin and non essential amino acid solution) were obtained from GIBCO. Sterile ^{45}calcium chloride solution (20-25 Ci/g) was purchased from CEA (Saclay, France).

Isolation of thymocytes

The procedure for thymocyte isolation has been previously described in detail (7,8). Cell suspensions were adjusted to contain 10^7 cells/ml in MEM supplemented with sodium pyruvate (1mM), L-glutamine (2mM), 1 % (v/v) non essential amino acid solution, 100 U/mL penicillin and 100 µg/ml streptomycin and usually 1 mM Ca^{++} (medium A). Cell preparation was carried out at room temperature and the cell suspensions were preincubated at 37°C for 30 min in an atmosphere of 5 % CO_2 in air before each experiment (9.).

Measurement of calcium uptake

At the beginning of the experiments, aliquots of the cell suspension were added to glass tubes containing ^{45}calcium (1 µCi/10^7 cells) and either steroid or vehicle only (control) in a final volume of 4 ml. After various periods of incubation at 37°C, 100 µl aliquots of the cell suspensions were rapidly filtered on Whatman GF/A filters. The filters were washed with 2 x 10 ml of ice cold buffer (135 mM NaCl, 5 mM KH_2PO_4, 5 mM Tris-HCl, 1 mM $MgCl_2$, 5 mM glucose, pH 7.4). The radioactivity collected on the filters was counted by liquid scintillation spectrometry.

In the experiments designed to follow calcium efflux, cell suspensions were incubated first with ^{45}calcium (1 µCi/10^7 cells) for 2 h at 37°C, in order to allow tracer equilibration. Cells were then rapidly washed twice by centrifugation (Eppendorf 3200 centrifuge, 11,000 g, 15 sec) and resuspended in medium A supplemented or not with steroid or EGTA (time zero of the efflux). At various intervals following resuspension, samples were filtered as described above. At each time the amount of ^{45}Ca associated with the cells was expressed as a percentage of the total bound calcium at the moment of steroid addition (time zero).

Determination of cell viability

Viability of the cells was estimated by the trypan blue exclusion technique (10) and was always over 95 % at the end of the isolation procedure.

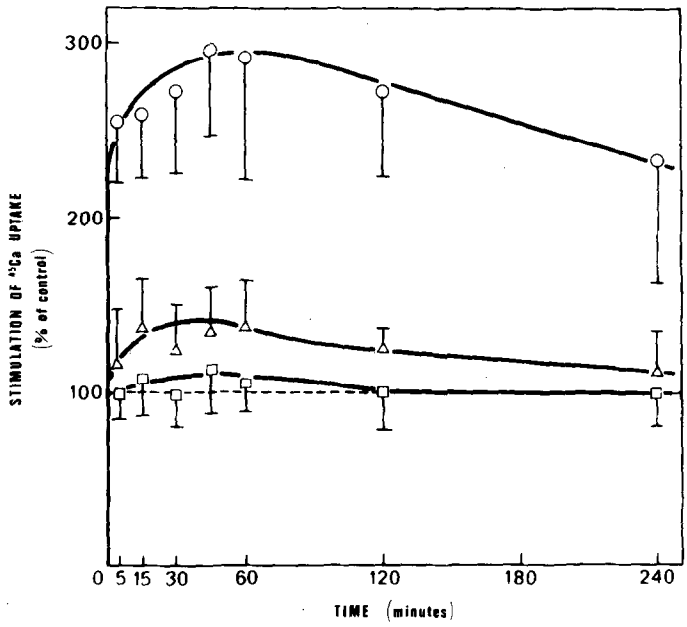

Figure 1 : Dose-response curves of the action of dexamethasone on ^{45}calcium uptake.

The concentration of dexamethasone tested were 10^{-8}M (□), 10^{-7} (△), 10^{-6}M (○). Each value is expressed as a percentage of the control sample (100 % : control) and represents the mean (± S.D) of triplicate determination in 6 experiments.

RESULTS

As previously described, in the absence of drugs the uptake of ^{45}calcium increased progressively with time to reach a plateau after 60 min incubation (7,9).

Following the addition of 10^{-6}M dexamethasone (a synthetic glucocorticoid, 9 fluoro-11ß, 17,21 trihydroxy-16α-methylpregna-1-4 diene-3,20-dione) to the cell suspension a rapid and transient increase in calcium uptake was demonstrated (Figure 1). This peak which occured as early as 5 min after steroid addition was followed by a gradual decline in the rate of ^{45}calcium accumulation. It should be emphasized, as reported by Jensen and Rasmussen (11) and Kaiser and Edelman (12), that a large variability was observed in the kinetics of calcium uptake and response to drugs among different animal groups on different days. Although the curves from several experiments were qualitatively similar, both the magnitude of the respon-

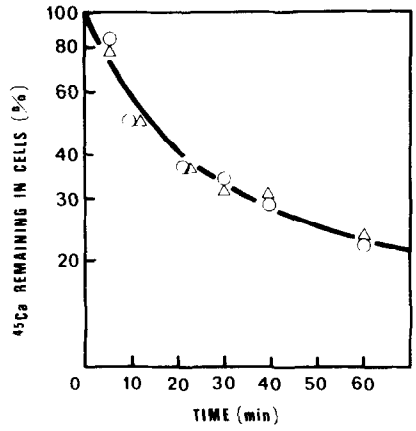

Figure 2 : Effect of dexamethasone on ^{45}calcium efflux.

The cells were first incubated with ^{45}Ca for 2 h at 37°C and then rapidly washed twice. At various intervals following resuspension in medium A containing or not 10^{-6}M dexamethasone, aliquots were filtered on whatman GF/A filters. At each time the amount of ^{45}Ca associated with the cells was expressed as a percentage of the total bound calcium at the moment of resuspension. Each value is the mean of triplicate determination in a typical experiment. (○) control, (△) 10^{-6}M dexamethasone. This experiment was repeated 3 times and gave qualitatively similar results.

se to dexamethasone and the length of time required to obtain the peak were variable. Therefore, kinetic experiments were always used to study the dose-response and the specificity of this effect. As also shown in Figure 1, this stimulation of ^{45}calcium uptake was dependent upon the concentration of dexamethasone and was still detectable at 10^{-7}M.

As shown in Figure 2, addition of 10^{-6}M dexamethasone did not modify ^{45}calcium efflux from prelabeled cells, whereas addition of the calcium chelator EGTA enhanced the efflux (results not shown).

In order to explore the specificity of this phenomenon, we have studied the effects of various steroids on calcium uptake (Table I). At 10^{-6}M, other glucocorticoids (corticosterone and cortisol) also enhanced the uptake of ^{45}calcium, whereas testosterone, progesterone and diethylstilbestrol, a non steroidal compound with estrogenic activity did not stimulate calcium uptake.

Table I : Effects of various steroids (10^{-6}M) on ^{45}Ca uptake by isolated thymocytes. Each value is the mean(± SD) of triplicate determinations from 3-6 experiments and is expressed as a percentage of control incorporation (100 % : control).

	TIME					
	5'	15'	30'	60'	90'	180'
DEXAMETHASONE	226.9 ± 55	239.9 ± 71	217.8 ± 55.3	244.9 ± 81.5	227.4 ± 89.9	197.1 ± 40.2
CORTICOSTERONE	215.8 ± 37.7	233.9 ± 107.6	254.8 ± 116.8	243.6 ± 68.9	200.7 ± 67.8	213.7 ± 66.4
CORTISOL	293.3 ± 101.9	315.7 ± 184	323.1 ± 167.2	351.6 ± 137.2	261.6 ± 101.6	259.6 ± 93.7
TESTOSTERONE	115.2 ± 37.2	116 ± 27.7	117.6 ± 10.4	79.5 ± 10.9	76.4 ± 17.3	96.8 ± 39.3
DIETHYLSTILBESTROL	129.6 ± 31.4	111.4 ± 38.3	118.4 ± 13	92.1 ± 19.7	99.9 ± 25.3	98.2 ± 37
PROGESTERONE	111.8 ± 21.6	113.3 ± 52.5	103.5 ± 13	86.6 ± 13.8	108.8 ± 15.6	122.4 ± 15.6

Table II : Determination of cell viability after 4 hr of incubation in the absence or presence of 10^{-6} steroids. The number of viable cells was determined using trypan blue exclusion procedure.

	Cell viability	
Control	90.2 % ± 3.6	n = 13
Dexamethasone	70.7 % ± 19.6	n = 5
Corticosterone	81.6 % ± 10.2	n = 5
Cortisol	81.4 % ± 4.2	n = 5
Testosterone	91.6 % ± 1.5	n = 3
Diethylstilbestrol	91.2 % ± 2.1	n = 3
Progesterone	88.5 % ± 4.8	n = 3

In parallel experiments, we showed that the viability of the cells after 4h of incubation with the various drugs only began to decline when glucocorticoid compounds were present (Table II).

DISCUSSION

Calcium ions play a major role in the modulation of many biochemical and biological processes of mammalian cells (13) and appear to be intimately involved in the control of cell proliferation (14) and of cell lysis (15,16). Despite all the available information concerning the interaction of glucocorticoids with their receptors and the early metabolic effects triggered by the hormone in lymphoid cells, little is known on those events linked to cell death. It has been suggested by Kaiser and Edelman (6) that one of the mechanisms responsible of thymocytolysis may be a glucocorticoid-induced change in cytoplasmic Ca^{++} activity. However, this explanation was discar-

ded because of the lack of any early changes in calcium ion uptake by lymphoid cells in the presence of steroids (17). We have recently described a relationship between the extent of cell lysis which increases with the stimulation of calcium uptake promoted by ionophore A_{23187} (7). We thus reinvestigated the effect of glucocorticoids on ^{45}calcium uptake. Our results demonstrate that glucocorticoids rapidly stimulate the uptake of ^{45}calcium in a manner similar to that described in lymphoid cells after ionophore treatment (7,11). In the case of ionophore this biphasic pattern has been attributed to a rapid increase in the permeability of the plasma membrane followed by a redistribution of the intracellular calcium (11).

This effect of steroids on calcium uptake in thymocytes appears specific for glucocorticoids as sex steroids at 10^{-6}M did not significantly alter calcium permeability. In contrast with calcium uptake, tracer efflux from preloaded cells was not modified by dexamethasone, thus suggesting that glucocorticoid action may be associated with an elevation of intracellular calcium concentration. Preliminary experiments using atomic absorption spectrometry have shown that the cell calcium content was significantly elevated 2 h after steroid addition, that is long before the first signs of cell death.

This observation is of extreme importance because it has already been demonstrated that the increase of nuclear fragility, which represents the first biochemical event unequivocally linked to glucocorticoid-induced cell death can also be observed in the presence of increasing concentrations of calcium (17). Further experiments are now in progress to study the link between cellular changes in calcium and steroid action in corticosensitive and corticoresistant lymphoid cell populations.

ACKNOWLEDGEMENT

The authors are grateful to Dr Dominique Duval for his continued support and advice and to Dr Marie-Aude Devynck for helpful discussions.

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