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Effect of adrenal steroids on insulin release from cultured rat islets of Langerhans

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Summary. The effect of additions to the culture medium of some natural or synthetic corticosteroid hormones was studied in cultured rat islets of Langerhans. The steroids decreased glucose-induced insulin release. The extent of inhibition by dexamethasone was 18–55%, prednisolone 23%, hydrocortisone 21% and aldosterone 18%. None of them affected the basal secretion of insulin or had any effect on diameter or insulin content of the islet. The inhibitory action of dexamethasone on insulin release was observed in the range 63 nmol/l to 6.3 μmol/l. At 6.3 μmol/l during two h, dexamethasone (a) inhibited insulin response to glucose concentrations above 5 mmol/l (b) caused a delay in the first phase and markedly reduced the second phase of insulin release of perifused islets, and (c) decreased the incorporation of [H^3]-leucine into total islet proteins without affecting [H^3]-leucine-incorporation into insulin plus proinsulin. It is suggested that steroids, by directly acting on the islets of Langerhans, may modulate the insulin-release response to secretagogues.

Key words: Insulin release, insulin biosynthesis, dexamethasone, prednisolone, hydrocortisone, aldosterone, cultured islets.

Direct inhibitory effects of corticosterone on insulin secretion from the perfused rat pancreas and isolated islets have been shown [1, 2]. However, direct effects of other adrenal steroid hormones have not been explored. We now report effects of dexamethasone, prednisolone, hydrocortisone and aldosterone on insulin release and biosynthesis in islets directly exposed to these steroids in culture.

Materials and methods

Islets were obtained by collagenase digestion [3] from pancreas of male, fed Sprague-Dawley rats (200–300 g). Batches of 50 islets were cultured in 5 ml medium for 2 days with change to fresh medium at 24 h. The culture medium was RPMI 1640 (Gibco, Grand Island, NY, USA) containing glucose (11 mmol/l), penicillin (0.1 mg/ml), streptomycin (0.1 mg/ml) and 10% (v/v) inactivated calf serum (Gibco). The pH was buffered at 7.4 with N-2-hydroxyethyl piperazine-N′-2-ethane sulphonate (Hepes) 25 mmol/l. The islets were carefully matched for size, and the cultures were maintained in a metabolic incubator at 37 °C in an atmosphere of humidified air: CO_2 (95:5). After 48 h in culture, the medium was supplemented with the steroid or solvent solution as described. Sodium phosphate salts of dexamethasone (Merck, Sharpe & Dohme, Rayway, NJ, USA), prednisolone (Merck, Sharpe & Dohme) and hydrocortisone (Sigma, St. Louis, MO, USA) were dissolved in an aliquot of the culture medium. The sodium hemisuccinate salt of aldesterone (Sigma) was solubilized in the culture medium with 10% (v/v) ethanol; ethanol was also added to the matched control medium. After exposure, treated and control islets were used for the measurement of the various parameters. For insulin release, batches of five islets were preincubated for 30 min at 37 °C in bicarbonate medium pH 7.4 [4] containing fatty acid-free albumin (Sigma) 2 mg/ml and glucose 2 mmol/l followed by removal of the medium. The islets were then incubated for 2 h in the same medium containing glucose at the stated concentrations. Insulin released into the medium was measured by radioimmunossay [5] using rat insulin standard supplied by Novo Research Laboratories, Copenhagen, Denmark. Islets were also perifused as described previously [6] in two chambers run in parallel, each containing 100 treated or control islets. The perifusate was assayed for insulin release [5]. For insulin content, treated or control islets in batches of 5 were transferred from the culture medium and extracted in 0.5 ml 0.154 mol/l NaCl pH 2, containing bovine serum albumin (2 mg/ml), by sonication (15 s at position 2 on a Fisons Soniprep 150). The sonicate was diluted and assayed for insulin [5]. Islet diameter of treated and control islets was measured in the same culture dish using an eye-piece micrometer in a Nikon stereoscopic dissecting microscope. For measurement of insulin plus proinsulin and total islet protein synthesis, treated and control islets in batches of 7 were incubated in bicarbonate medium containing glucose (20 mmol/l), albumin (2 mg/ml) and [4,5-^{3}H]-leucine (4 μCi, 50 Ci/mmol) at 37 °C for 90 min. The incorporation of [^{3}H]-leucine into insulin plus proinsulin was measured using insulin-binding affinity columns and into total islet protein by trichoroacetic acid precipitation as previously described [7]. The results are expressed as mean ± SEM for the number of separate batches of islets given in parenthesis. The statistical significance was assessed by the two-tailed Student's t-test.

Results

The time course of the effect of dexamethasone (6.3 μmol/l) on insulin release is shown in Table 1-A. No effect was found on insulin release at glucose

Table 1. Effect of dexamethasone on insulin release by cultured rat islets

A	Dexamethasone	IRI, μU/h per islet[a] at glucose 2 or 20 mmol/l after culture with dexamethasone[b] for 1-3 h					
		1 h		2 h		3 h	
		2	20	2	20	2	20
	-	3±0.3 (30)	113±6 (67)	5±1 (31)	129±7 (38)	5±0.7 (41)	100±6 (53)
	+	4±0.5 (29)	93±4 (66)**	4±1 (31)	81±5 (40)**	4±0.4 (40)	63±5 (54)**
B	Dexamethasone, mol/l[c]	0	6.3×10^{-8}	6.3×10^{-7}	6.3×10^{-6}		
	IRI, μU/h per islet	85±6 (29)	66 ± 4 (29)*	51 ± 5 (29)**	38 ± 3 (29)**		

C	IRI, uU/h per islet (a) at glucose mmol/l								
	Dexamethasone,[d]	0	2	5	7.5	10	12.5	15	20	30
	−	7±1 (6)	8±1 (14)	11±2 (18)	23±3 (19)	33±4 (21)	62±5 (18)	72±4 (21)	86±6 (21)	132±8 (14)
	+	5±1 (6)	7±1 (13)	11±2 (18)	12±2 (19)**	17±2 (21)**	38±4 (18)**	58±4 (21)*	69±5 (21)*	98±7 (14)**

[a] Rates of insulin release (IRI) are given as mean ± SEM for the number of observations shown in parenthesis.
[b] 6.3 μmol/l in culture medium
[c] At glucose 20 mmol/l for 2 h
[d] 6.3 μmol/l for 2 h
Statistical significance from control: $+p<0.05$, $*p<0.01$, $**p<0.001$

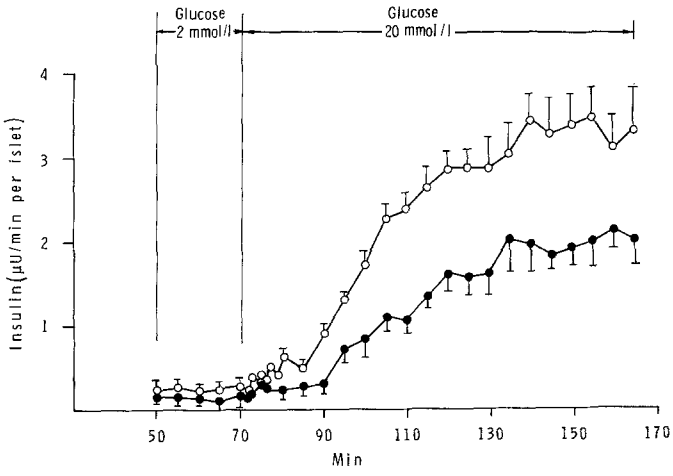

Fig. 1. Effects of dexamethasone on insulin response to glucose of perifused rat islets. After culture for 2 days, the islets were cultured for 2 h in the presence (●) or in the absence (○) of dexamethasone (6.3 μmol/l). Results are given as mean ± SEM of 5 experiments. Difference of rates of insulin release at 2 mmol/l glucose were not statistically significant ($p>0.05$). The differences of rates of insulin release at 20 mmol/l glucose were statistically significant between 80 min and 170 min at $p<0.05$ to $p<0.001$.

2 mmol/l, while the response to glucose 20 mmol/l was inhibited by 18% in islets previously cultured with dexamethasone for 1 h and by 37% after 2 and 3 h. Treatment of cultured islets for 2 h with 3 different concentrations of dexamethasone (Table 1-B) showed a dose-related reduction of insulin secretion in response to glucose 20 mmol/l to a maximum of 55% inhibition at dexamethasone 6.3 μmol/l. Study of the effects of dexamethasone treatment (6.3 μmol/l for 2 h) on the response of islets to varying concentrations of glucose (Table 1-C) indicated that exposure to the steroid caused significant inhibition of the insulin release evoked by glucose at concentrations from 7.5-30 mmol/l. The treatment had no effect on the estimated Km for glucose (14 mmol/l) but decreased the V_{max} from 132 to 98 μU/l per islet ($p<0.001$). In the perifusion system, dexamethasone pretreatment of the islets in culture (6.3 μmol/l, 2 h) did not change the basal rate of insulin secretion. The response to glucose 20 mmol/l was delayed and markedly reduced in both the early and late phases (Fig. 1). The integrated stimulated area was decreased from 211±18 to 117±15 μU per islet ($df=8$, $p<0.005$). Dexamethasone (6.3 μmol/l, 2 h) reduced the incorporation of [^{3}H]-leucine into total proteins of the islets by 7%, from the control value of 16449±174 ($n=56$) to 15454±174 ($n=56$) dpm/90 min per islet ($p<0.001$), but had no effect on [^{3}H]-leucine incorporation into insulin plus proinsulin. The duration of the effect of dexamethasone on insulin response to glucose was investigated after transferring the islets to fresh culture medium in the absence of added substances at the end of the 2 h treatment. The response to glucose 20 mmol/l was studied at 0,2 and 4 h of reculture. The secretory response to glucose was lowered by 27% at 0 h, by only 10% at 2 h and was fully re-established at 4 h.

The effect of dexamethasone (6.3 μmol/l, 2 h) on insulin release was compared in parallel experiments to those of equimolar concentrations of prednisolone and hydrocortisone and also to the effect of aldosterone (8 nmol/l); these concentrations of hydrocortisone and aldosterone were ten times the upper physiological human plasma levels. There was no effect of these steroids on basal insulin secretion. The response to glucose 20 mmol/l, as percent of control, was decreased 28.6±1.4 ($n=56$) by dexamethasone, 22.5±1.4 ($n=31$) by prednisolone, 21.0±1.3 ($n=40$) by hydrocortisone and 17.8±1.3 ($n=36$) by aldosterone. There was no effect of the tested steroids on insulin content or diameter of the islet.

Discussion

The results show a consistent inhibition of glucose-stimulated insulin release from islets treated directly with dexamethasone, prednisolone, hydrocortisone and aldosterone without affecting the basal secretion of insulin. The effect was observed at a concentration of dexamethasone (6.3 μmol/l) equivalent to 'pathophysiological' concentration of cortisol and also at a much lower concentrations within the 'physiological' range (6.3 nmol/l). We also found similar inhibitory actions of hydrocortisone and prednisolone at physiological levels (results not shown). These findings are of interest since glucose intolerance is commonly associated with excessive secretion of cortisol and aldosterone and also with the therapeutic administration of dexamethasone or prednisolone. This reduced tolerance and the reduction of insulin secretion by steroids may be causally related. We have shown here that the acute effects of dexamethasone on islets are reversible. The effects of a more prolonged treatment are unknown.

Dexamethasone had no effect on insulin plus proinsulin biosynthesis and on insulin content of the islets. It apparently exerts its effect by impairing the capacity for release of insulin from a previously synthesized store. It is possible that the decreased insulin response to glucose may be mediated by impairment of islet glucose metabolism since glucocorticoids have been shown to diminish glucose uptake and oxidation in other tissues [8]. However, this may not be the sole cause of impaired insulin release; corticosterone treatment of perifused islets has been shown to decrease calcium inflow associated with insulin release mediated by leucine and non-nutrients carbamylcholine and gliclazide [9], suggesting altered calcium-handling as a site for steroid action. The present study shows a small but significant decrease in islet total protein synthesis by dexamethasone that may involve some key protein(s) implicated in the release of insulin. Therefore, islet calmodulin and protein kinases and their substrates deserve further exploration. In this context, the actions of dexamethasone and other gluco- and mineralocorticoids in decreasing stimulated insulin release may be multifactorial. The present acute results produced by glucocorticoid treatment of islets in culture are in agreement with recent observations of reduced insulin response to glucose in mice treated with hydrocortisone [10], and indicate a possible role for adrenal steroids in the modulation of insulin release by secretagogues.

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