AN EFFECT OF DEXAMETHASONE ON GLYCOGEN METABOLISM IN HEPATOCYTE CULTURES

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1. Introduction

The role of glucocorticoids in the regulation of hepatic glucose production by metabolic processes such as glycogenolysis and gluconeogenesis has been termed 'permissive' since they are essential for the maintenance of normal responsiveness of cells to hormones which act through cyclic AMP [1]. In isolated perfused livers from adrenalectomised rats maximal concentrations of glucagon activate phosphorylase normally but physiological concentrations do not [2,3]. Further, a lack of adrenal corticosteroids caused a significant reduction in total phosphorylase activity with no change in the level of active phosphorylase [4]. Glucagon-stimulated cyclic AMP accumulation by livers perfused in vitro has been reported as normal or increased [2]. Several laboratories have reported no change in cyclic AMP-dependent protein kinase activity in liver homogenates obtained from normal or adrenalectomised rats [5,6], but others have found only 75% of the normal activity of type I cyclic AMP-dependent protein kinase in liver homogenates obtained from adrenalectomised rats [7].

Thus, despite extensive investigation, the mechanisms by which glucocorticoids participate in the regulation of hepatic glycogen metabolism have yet to be clearly defined. This is due in part to the use of adrenalectomised animals for such studies and the attendant problem of hormone interaction inherent in such an in vivo system. Hepatocytes in primary monolayer culture are viable for several days in a totally defined environment [8–10] and as such should provide a useful system in which to study glucocorticoid effects on glycogen metabolism. Therefore, a study of the enzymes of hepatic glycogen metabolism was undertaken in hepatocytes maintained in primary monolayer culture in the presence and absence of a biologically active synthetic glucocorticoid, dexamethasone.

2. Materials and methods

Isolated hepatocytes were made by in vitro perfusion of liver [11] from male rats of a random-bred Wistar strain (180–200 g). The isolated hepatocytes were suspended at 2×10^6 cells/ml in Leibovitz-15 tissue culture medium (L-15) supplemented with 25 mM Hepes, 20 mM glucose, 100 units penicillin/ml and 10% foetal calf serum. Aliquots of the cell suspension (2.5 ml) were innoculated onto 60 mm diam. tissue culture dishes (Falcon plastics, Calif.) and incubated at 37°C in humidified air. After 6 h the medium was removed and exchanged for serum-free L-15 with or without 1 μM dexamethasone. After 24 h in vitro the culture medium was aspirated, the cells washed and suspended in an appropriate buffer, and homogenised by 20 strokes of an A pestle in a Dounce homogeniser.

The activities of the following enzymes were measured directly on the homogenate by standard methods: glycogen synthase [12], phosphorylase *a* [13], total phosphorylase after in vitro activation with muscle phosphorylase kinase [14], total phosphorylase *b* kinase [15], protein kinase [16] and cyclic AMP phosphodiesterase [17]. Phosphorylase phosphatase activity was measured by the rate of inactivation of phosphorylase *a*. Hepatocyte cultures were preincubated with glucagon for 5 min to elevate phosphorylase *a*. The cultures were homogenised and incubated at 30°C in 25 mM MES (pH 6.8),

5 mM EDTA, 45 mM mercaptoethanol, which prevents the conversion of phosphorylase *a* to *b*, but allows expression of phosphatase activity. At 2 min intervals, aliquots were diluted in 50 mM MES (pH 6.1), 200 mM NaF, 10 mM EDTA, 45 mM mercaptoethanol and 50 μl of the mixture assayed for phosphorylase *a* [13]. The phosphorylase *a* activity plotted against time approximated first-order kinetics. Since endogenous phosphorylase *a* was the substrate and since it was not possible to measure the initial reaction velocity, results are expressed as a first-order rate constant for the reaction rather than in conventional enzyme units. Protein [18], cyclic AMP [19] and glycogen [20] were measured by standard techniques.

3. Results and discussion

The hepatocyte cultures synthesised glycogen from glucose present in the culture medium (table 1) as reported [8,9,10]. However, in contrast to reports that the level of glycogen declined over a 3-day culture period [10,21] the concentration of glycogen doubled between 24 and 48 h in vitro. Hepatocytes cultured in the presence of dexamethasone had less glycogen at both 24 and 48 h than cells cultured in the absence of this glucocorticoid. This is in contrast

Table 1
Glycogen content of hepatocytes cultured in the presence or absence of 1 μM dexamethasone for 24 and 48 h

	24 h		48 h	
	–	+	–	+
Glycogen (μg glycogen/mg protein)	10.43[a] ±2.30 (8)	4.43 ±0.95 (8)	26.59[a] ±3.90 (11)	10.63 ±1.31 (11)

[a] $P < 0.01$ for dexamethasone-treated cells compared to control cells; *P*-values were calculated using Student's *t*-test for paired variates

Table 2
Enzyme activities and cyclic AMP concentration of hepatocytes cultured in the presence or absence of 1 μM dexamethasone for 24 h

	Dexamethasone	
	–	+
Total glycogen synthase (nmol . min^{-1} . mg protein^{-1})	4.18 ± 0.58 (6)	3.95 ± 0.48 (6)
Active glycogen synthase (nmol . min^{-1} . mg protein^{-1})	0.47 ± 0.06 (6)	0.42 ± 0.05 (6)
Total phosphorylase (nmol . min^{-1} . mg protein^{-1})	93.03 ± 17.78 (9)	93.89 ± 17.39 (9)
Phosphorylase *a* (nmol . min^{-1} . mg protein^{-1})	12.75 ± 1.31 (8)	31.7 ± 4.4 (8)
Phosphorylase phosphatase (s^{-1})	0.05 ± 0.01 (5)	0.05 ± 0.01 (5)
Total phosphorylase kinase (mU phosphorylase *a* . min^{-1} . mg protein^{-1})	28.25 ± 7.80 (8)	27.63 ± 8.43 (8)
Total protein kinase (nmol . min^{-1} . mg protein^{-1})	57.6 ± 7.9 (7)	58.6 ± 7.7 (7)
Cyclic AMP (pmol . mg protein^{-1})	1.00 ± 0.11 (5)[a]	1.64 ± 0.11 (5)
Low K_m cyclic AMP phosphodiesterase (pmol . min^{-1} . mg protein^{-1})	8.84 ± 0.66 (5)[a]	8.04 ± 0.84 (5)
High K_m cAMP phosphodiesterase (pmol . min^{-1} . mg protein^{-1})	1421 ± 142 (5)	1370 ± 161 (5)

[a] $P < 0.01$; *P*-values were calculated using Student's *t*-test for paired variates

A milliunit of enzyme activity is that amount of enzyme that incorporated 1 nmol [^{14}C]G-1-P into glycogen/min under the conditions of the assay

to in vivo studies where liver glycogen was decreased by adrenalectomy then slowly increased by glucocorticoid administration [22,23]. However, using a different synthetic glucocorticoid (betamethasone dipropionate) glucocorticoid suppression of glycogen accumulation was observed in foetal rat liver [24].

The presence of dexamethasone in the culture medium had no effect on total glycogen synthase activity and the amount of enzyme in the active form (table 2). If it is assumed that glycogen synthase is the rate-limiting enzyme for glycogen synthesis, then the differences in glycogen content must be due to an increased mobilisation and therefore be reflected in one or more of the enzymes responsible for glycogen degradation. It was found that dexamethasone produced a 2-fold increase in phosphorylase *a* activity with no increase in total phosphorylase. This is similar to the observation that phosphorylase *a* in foetal rat liver was increased after treatment with glucocorticoid for 24 h [24]. Hepatocytes isolated from adrenalectomised rats are reported to have decreased activities of both total and active phosphorylase compared to hepatocytes from normal rats [25].

The increase in active phosphorylase could be due to a decrease in activity of phosphorylase phosphatase or an increase in activity of phosphorylase *b* kinase. However, the activities of phosphorylase phosphatase and phosphorylase *b* kinase were unaffected by culture of the hepatocytes in the presence of dexamethasone (table 2). Dexamethasone also had no effect on total cyclic AMP-dependent protein kinase activity. Due to the low total protein kinase activity of the hepatocyte cultures, the assay used was not sensitive enough to measure protein kinase in the absence of added cyclic AMP (active protein kinase).

The level of cyclic AMP found in the hepatocyte cultures (table 2) is higher than that in freshly isolated hepatocytes presumably due to lowered phosphodiesterase activity of hepatocytes in culture [26]. Dexamethasone treatment of the cultures resulted in an increase in the level of cyclic AMP and a decrease in activity of the low K_m form of cyclic AMP phosphodiesterase. We have shown [26] that dexamethasone treatment of hepatocyte cultures has no effect on adenylate cyclase activity and therefore the observed increase in cyclic AMP concentrations in the dexamethasone-treated cultures is probably due to the effect of this glucocorticoid on the low K_m phosphodiesterase. Dexamethasone treatment of the hepatocyte cultures had no effect on the high K_m phosphodiesterase (table 2). There is conflict concerning the effect of glucocorticoids on hepatic phosphodiesterase activity. Phosphodiesterase activity was unchanged following adrenalectomy [27] or on incubation of hepatoma tissue culture cells with dexamethasone [28]. Conversely, glucocorticoid administration decreased the activity of phosphodiesterase in several tissues [29] and in hepatoma tissue culture cells [30].

Incubation of hepatocyte cultures with dexamethasone causes a lowering of the glycogen content of the cells. It appears to be due to an increase in mobilisation of glycogen associated with an activation of glycogen phosphorylase. The glucocorticoid also produces an elevation in tissue cyclic AMP which would indicate that the hormone is probably exerting its effects by activation of protein kinase and phosphorylase *b* kinase. However, so far it has not been possible to demonstrate direct effects on these enzymes and an increase in phosphorylase *a* independent of changes in cyclic AMP concentration cannot be ruled out.

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