Interaction between Glucocorticoids and Glucagon in the Hormonal Modification of Calcium Retention by Isolated Rat Liver Mitochondria

By BERNARD P. HUGHES and GREGORY J. BARRITT
*Clinical Biochemistry Unit, Flinders University School of Medicine,
Flinders Medical Centre, Bedford Park, South Australia 5042, Australia*

(Received 2 October 1978)

1. The administration of dexamethasone to intact fed rats by intraperitoneal injection for 3 h was associated with a 6-fold increase in the time for which mitochondria subsequently isolated from the liver retain a given load of exogenous Ca^{2+} . This effect was blocked by the co-administration of cycloheximide with dexamethasone, and partially blocked by the co-administration of puromycin. Daily administration of dexamethasone for periods of 4–7 days resulted in liver mitochondria that exhibited a decreased ability to retain exogenous Ca^{2+} . 2. When glucagon was administered to fed adrenalectomized rats, the increase in mitochondrial Ca^{2+} -retention time that results from the action of this hormone was reduced by 50 % when compared with its effect on intact animals. The administration of dexamethasone to adrenalectomized rats partially restored the full effect of glucagon. 3. Dexamethasone did not enhance the effect of glucagon on mitochondrial Ca^{2+} -retention time when administered to intact fed rats. 4. It is concluded that these data support the hypothesis that the hormone-induced modification of liver mitochondria, which results in an increase in the time for which exogenous Ca^{2+} is retained, involves a step in which new protein is synthesized.

Previous studies in this laboratory have shown that the administration of glucagon or N^6O^2 -dibutyryl cyclic AMP to rats, or the perfusion of rat livers with these agents, leads to modification of the properties of mitochondria subsequently isolated from the liver (Hughes & Barritt, 1978; Barritt *et al.*, 1978). This modification includes increases in (a) the initial rates of Ca^{2+} and P_i transport, (b) the concentration of endogenous P_i and (c) the time for which exogenous Ca^{2+} is retained by the mitochondria (Hughes & Barritt, 1978; Barritt *et al.*, 1978). Two properties, namely sensitivity to cycloheximide and a relatively long time course of development (about 60 min), appear to distinguish these changes from some of the effects of glucagon on liver mitochondria reported by others (Yamazaki, 1975; Yamazaki *et al.*, 1977).

Glucocorticoids have also been shown to alter some properties of liver mitochondria (Adam & Haynes, 1969; Wakat & Haynes, 1977; Kimura & Rasmussen, 1977). Therefore it was considered important to investigate the effect of changes in glucocorticoid concentration *in vivo* on the ability of isolated liver mitochondria to retain exogenous Ca^{2+} , and to determine whether there is any interaction between glucocorticoids and glucagon in their effects on this parameter. Ca^{2+} retention was measured because previous studies (Hughes & Barritt, 1978) have shown that this exhibits the most sensitive

response to the changes induced in liver mitochondria by glucagon. The results show that administration of the synthetic glucocorticoid dexamethasone to rats for periods of 3 h increases the time for which liver mitochondria retain Ca^{2+} . In the adrenalectomized rat, the effect of glucagon on hepatic mitochondrial Ca^{2+} -retention time is significantly impaired.

Experimental

Animals

Male hooded Wistar rats (Institute of Medical and Veterinary Science, Adelaide), weighing 200–250 g and fed *ad libitum*, were used in all experiments. Dexamethasone (2 mg/100 g body wt.) and glucagon (25 μg /100 g body wt.) suspended in 0.9 % (w/v) NaCl, containing 50 mg of bovine serum albumin/l (NaCl/albumin), were administered by intraperitoneal injection as described previously (Dorman *et al.*, 1975). When used, cycloheximide (250 μg /100 g body wt.) and puromycin (5 mg/100 g body wt.) were administered in a similar manner. Injection of the hormone(s) under test was begun between 09:30 and 10:00 h. Control rats received injections of equal volumes of NaCl/albumin. After the times indicated, the rats were killed by decapitation and mitochondria sedimenting between 3000 and 107 000 g-min isolated from the liver as described previously (Hughes &

Barritt, 1978). In some experiments, mitochondria that sedimented at between 3000 and 22500g-min ('heavy' mitochondria as defined by Prpic *et al.*, 1978) were isolated.

Bilateral adrenalectomy was performed through incisions made in the dorsal area. Adrenalectomized rats (kept one to a cage) were maintained on a normal diet. The drinking water was supplemented with 5mM-glucose. Experiments with adrenalectomized rats were performed 5–7 days after removal of the adrenal glands.

Mitochondria and Ca^{2+} retention

Mitochondrial respiration, the protein content of mitochondrial suspensions, initial rates of Ca^{2+} transport at 0°C and the time for which mitochondria retain a load of exogenous $^{45}\text{Ca}^{2+}$ (100nmol/mg of mitochondrial protein) were measured as described previously (Hughes & Barritt, 1978). The medium used for the measurement of $^{45}\text{Ca}^{2+}$ retention contained, in a final volume of 2.0ml, 250mM-sucrose, 2.5mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], 1mM-succinate, 1mM-potassium phosphate (except where indicated otherwise), 150 μM - $^{45}\text{CaCl}_2$ (0.13 μCi) and 1.5mg of mitochondrial protein/ml. The pH was adjusted to 7.4 with KOH. Ca^{2+} -retention time is defined as the time that elapses between the addition of $^{45}\text{Ca}^{2+}$ and the point at which 50% of the accumulated $^{45}\text{Ca}^{2+}$ has been released (Hughes & Barritt, 1978).

Chemicals

Dexamethasone, glucagon and puromycin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. The sources from which other reagents were obtained have been described previously (Hughes & Barritt, 1978; Barritt *et al.*, 1978).

Results

Effects of dexamethasone on fed intact rats

Administration of the synthetic glucocorticoid dexamethasone to fed rats by intraperitoneal injection increased the time for which liver mitochondria retain a given load of $^{45}\text{Ca}^{2+}$ by about 6-fold (Fig. 1). Maximal increases in Ca^{2+} -retention time were observed when dexamethasone was administered 3h before the rats were killed. The effect on mitochondrial Ca^{2+} -retention time of dexamethasone treatment for 3h was not as great as that observed 1h after the administration of glucagon

Fig. 1. Comparison of the effects of dexamethasone and glucagon on mitochondrial Ca^{2+} -retention time

Dexamethasone (2mg/100g body wt.) and glucagon (25 μg /100g body wt.) were administered to intact fed rats, as described in the Experimental section, at 3 and 1h respectively before the rats were killed. The uptake and retention of $^{45}\text{Ca}^{2+}$ in mitochondria isolated from control rats ($\circ$), and rats treated with dexamethasone ($\bullet$) or glucagon (Δ) were measured as described in the Experimental section. The data are the means $\pm$ S.E.M. for the results of seven experiments.

Table 1. Effects of glucagon and dexamethasone administered to intact or adrenalectomized fed rats on the retention of Ca^{2+} in mitochondria subsequently isolated from the liver

Dexamethasone (2mg/100g body wt.) and glucagon (25 μg /100g body wt.) were administered 3 and 1h respectively before the rats were killed. Adrenalectomy, isolation of mitochondria and the measurement of Ca^{2+} -retention time were performed as described in the Experimental section. The values are the means $\pm$ S.E.M. with the numbers of experiments shown in parentheses. The degrees of significance, *P*, between the test condition and the control were determined by Student's *t* test.

Condition of rat	Agent administered	$^{45}\text{Ca}^{2+}$ -retention time (min)	<i>P</i>
Intact	Control	1.0 $\pm$ 0.6 (7)	
	Dexamethasone	5.9 $\pm$ 1.6 (3)	<0.025
	Glucagon	17.1 $\pm$ 2.0 (4)	<0.0001
Adrenalectomized	Control	1.5 $\pm$ 0.7 (6)	
	Glucagon	8.8 $\pm$ 2.6 (6)	<0.025
	Dexamethasone	5.6 $\pm$ 2.2 (5)	<0.05
	Glucagon + dexamethasone	13.3 $\pm$ 2.8 (5)	<0.001

Table 2. Comparison of the effects of a short exposure to glucagon in the presence of dexamethasone with exposure to glucagon alone on mitochondrial Ca^{2+} -retention time

Glucagon (25 µg/100g body wt.) and dexamethasone (2mg/100g body wt.) were administered to intact fed rats 15min and 3h respectively before the rats were killed. Control rats received injections of NaCl/albumin at both 3h and 15min before the rats were killed. Administration of the hormones, isolation of mitochondria and measurement of Ca^{2+} -retention time were performed as described in the Experimental section. The values given are means±S.E.M. with the numbers of experiments in parentheses. The degrees of significance, *P*, between the test condition and the control were determined by Student's *t* test.

Hormone administered	$^{45}\text{Ca}^{2+}$ -retention time (min)	<i>P</i>
Control	2.2 ± 1.1 (4)	
Dexamethasone	5.4 ± 2.1 (3)	
Glucagon	8.9 ± 1.9 (4)	<0.025
Dexamethasone plus glucagon	8.2 ± 1.7 (4)	<0.025

Table 3. Effect of the co-administration of puromycin or cycloheximide with dexamethasone on the retention of Ca^{2+} in mitochondria subsequently isolated from liver

Dexamethasone (2mg/100g body wt.) was administered to intact fed rats 3h before the rats were killed. Control rats received NaCl/albumin. When used, cycloheximide (250 µg/100g body wt.) or puromycin (5mg/100g body wt.) were injected at the same time as dexamethasone, or separately, 3h before the rats were killed. Administration of the agents, isolation of mitochondria which sedimented at between 3000 and 22500g·min ('heavy' mitochondria) and measurement of Ca^{2+} -retention time were performed as described in the Experimental section. The values are the means±S.E.M. or mean±range (where *n* = 2) with the numbers of experiments in parentheses. The degrees of significance, *P*, were determined by Student's *t* test; *dexamethasone versus control, *P* < 0.005; **dexamethasone versus dexamethasone plus cycloheximide, *P* < 0.025.

Agent(s) administered	$^{45}\text{Ca}^{2+}$ -retention time (min)
Control (6)	6.5 ± 1.2
Dexamethasone (4)	13.5 ± 0.7*
Dexamethasone plus puromycin (4)	9.2 ± 2.1
Dexamethasone plus cycloheximide (3)	6.7 ± 2.3**
Cycloheximide (2)	5.2 ± 1.7

(Fig. 1 and Table 1). Previous studies (Hughes & Barritt, 1978) have shown that these conditions yield maximal stimulation of mitochondrial Ca^{2+} retention by glucagon. The increase in Ca^{2+} -retention time

that followed the exposure of rats to elevated concentrations of glucagon for a suboptimal time of 15min (cf. Hughes & Barritt, 1978) was not further increased if the rats had previously been treated with dexamethasone for 3h (Table 2).

The increase in mitochondrial Ca^{2+} -retention time induced by dexamethasone was completely blocked by the administration of cycloheximide with this agent (Table 3). The administration of cycloheximide alone had no effect on mitochondrial Ca^{2+} -retention time (Table 3). The effect of dexamethasone was partially blocked by the co-administration of puromycin (Table 3). The experiments described in Table 3 were conducted with mitochondria which

Fig. 2. Effects of the administration of dexamethasone for 7 days on mitochondrial Ca^{2+} retention measured in the presence of 1.0 mM-(a) and 0.25 mM-(b) potassium phosphate

Dexamethasone (2mg/100g body wt.) was administered between 09:30 and 10:00h on each day as described in the Experimental section. Control rats received daily injections of similar volumes of NaCl/albumin. The rats were killed on the seventh day after the first injection of dexamethasone, liver mitochondria isolated from control rats (○) and rats treated with dexamethasone (●), and their Ca^{2+} -retention time was measured as described in the Experimental section. The data are the means±S.E.M. for the results of three experiments.

sedimented between 3000 and 22500g-min ('heavy' mitochondria; Prpic *et al.*, 1978). 'Heavy' mitochondria isolated from control rats have been shown to retain Ca^{2+} for longer periods of time than mitochondria isolated at higher centrifugal forces (Table 3 and Prpic *et al.*, 1978).

The exposure of intact rats to dexamethasone (administered daily) for periods of 4–7 days resulted in liver mitochondria that exhibited a decreased ability to retain Ca^{2+} when compared with mitochondria isolated from the livers of control rats (Fig. 2a). This difference was more clearly demonstrated when Ca^{2+} transport was measured in the presence of 0.25 mM-potassium phosphate (Fig. 2b). The change from conditions under which dexamethasone increased mitochondrial Ca^{2+} -retention time (Fig. 1) to those in which a decrease in retention time was observed (Fig. 2) was found to occur after about 3 days exposure to daily injections of the steroid.

Effects of dexamethasone and glucagon on fed adrenalectomized rats

Mitochondria isolated from the livers of fed adrenalectomized rats exhibited a small increase in Ca^{2+} -retention time when compared with mitochondria isolated from the livers of intact fed rats. However, under the conditions used for the measurement of Ca^{2+} -retention time, this effect was not statistically significant (Table 1). Administration of glucagon to adrenalectomized rats resulted in an increase in mitochondrial Ca^{2+} -retention time (Table 1). However, the effect of the hormone was only about one-half of that observed in intact rats. The ability of glucagon to increase mitochondrial Ca^{2+} -retention time was somewhat restored by the prior administration of dexamethasone to the adrenalectomized animals (Table 1). Dexamethasone alone produced an increase in Ca^{2+} -retention time similar in magnitude to that observed after its administration to intact rats (Table 1).

The administration of dexamethasone to intact or adrenalectomized rats for 3 h was found to have no significant effect on the ADP-stimulated (State 3) and ADP-depleted (State 4) rates of oxygen consumption, the ratio of these rates (the respiratory control ratio), and the initial rate of Ca^{2+} transport under the conditions employed for the measurement of these parameters.

Discussion

The results of the experiments reported here, in which dexamethasone was administered to rats for periods of 4 or more days, confirm the earlier observations of Kimura & Rasmussen (1977), who showed that the administration of dexamethasone to

rats for 3 days results in a decrease in the ability of liver mitochondria to retain Ca^{2+} . However, the present results also show that exposure to dexamethasone for shorter periods of time (between 3 h and 3 days) is associated with the opposite effect, that is, an increased ability of the mitochondria to retain Ca^{2+} . Since the action of corticosteroids on cells has been shown to involve changes in the rate of synthesis of intracellular proteins (Gorski & Gannon, 1976), these data are consistent with the idea (Hughes & Barritt, 1978) that the modification of mitochondria, which is manifested as an increase in the retention of exogenous Ca^{2+} , involves a step or steps in which new protein is synthesized. The observations that the effects of dexamethasone are blocked by cycloheximide or puromycin are consistent with this proposal.

The significant reduction in the effect of glucagon on mitochondrial Ca^{2+} -retention time observed in adrenalectomized rats suggests that, as in the case of the enzymes phosphoenolpyruvate carboxykinase (EC 4.1.1.32) and tyrosine aminotransferase (EC 2.6.1.5), which are induced by glucagon (Wicks *et al.*, 1974; Krone *et al.*, 1974), full expression of the effect of glucagon is dependent on normal concentrations of glucocorticoids. Further evidence for an interaction between these two hormones is provided by the observation that, although dexamethasone alone increases mitochondrial Ca^{2+} -retention time when administered to intact rats, the increase in retention time that follows the administration of both glucagon and dexamethasone is no greater than the increase produced by glucagon alone. This observation, as well as those on the changes induced by dexamethasone in mitochondrial Ca^{2+} retention in intact and adrenalectomized rats, is analogous to results reported for the effects of N^6O^2' -dibutyryl cyclic AMP and cortisol on phosphoenolpyruvate carboxykinase and tyrosine aminotransferase activities in the livers of intact and adrenalectomized rats (Krone *et al.*, 1974).

We are grateful to Kay Dalton and Peter Pascoe for skilled technical assistance. This work was supported by the National Health and Medical Research Council of Australia.

References

- Adam, P. A. J. & Haynes, R. C., Jr. (1969) *J. Biol. Chem.* **244**, 6444–6450
- Barritt, G. J., Thorne, R. F. W. & Hughes, B. P. (1978) *Biochem. J.* **172**, 577–585
- Dorman, D. M., Barritt, G. J. & Bygrave, F. L. (1975) *Biochem. J.* **150**, 389–395
- Gorski, J. & Gannon, F. (1976) *Annu. Rev. Physiol.* **38**, 425–450

- Hughes, B. P. & Barritt, G. J. (1978) *Biochem. J.* **176**, 295–304
- Kimura, S. & Rasmussen, H. (1977) *J. Biol. Chem.* **252**, 1217–1225
- Krone, W., Huttner, W. B., Seitz, H. J. & Tarnowski, W. (1974) *FEBS Lett.* **46**, 158–161
- Prpic, V., Spencer, T. L. & Bygrave, F. L. (1978) *Biochem. J.* **176**, 705–714
- Wakat, D. K. & Haynes, R. C., Jr. (1977) *Arch. Biochem. Biophys.* **184**, 561–571
- Wicks, W. D., Barnett, C. A. & McKibbin, J. B. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1105–1111
- Yamazaki, R. K. (1975) *J. Biol. Chem.* **250**, 7924–7930
- Yamazaki, R. K., Sax, R. D. & Hauser, M. A. (1977) *FEBS Lett.* **75**, 295–299