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Effect of Adrenalectomy on Acceleration of Gluconeogenesis by Calcium Ions, Adenosine 3′:5′-Cyclic Monophosphate and Adrenaline in Rat Kidney Tubules

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1. Gluconeogenesis from pyruvate was measured in renal-cortical-tubule fragments prepared from fed male rats 6–8 days after adrenalectomy or sham adrenalectomy. The response of this process to 3′:5′-cyclic AMP and adrenaline was compared in these two states at two Ca^{2+} concentrations. 2. Adrenalectomy decreased the percentage stimulation of gluconeogenesis by 3′:5′-cyclic AMP, but increased percentage stimulation by adrenaline. Cortisol treatment of adrenalectomized rats (50mg/kg, twice daily for 2 days) did not reverse the change in responsiveness to 3′:5′-cyclic AMP and adrenaline. 3. Stimulation of gluconeogenesis by 1 μM-adrenaline was unaffected by 10 μM-propranolol (β-blocker) in either state. Phentolamine (α-blocker; 10 μM) totally blocked stimulation of gluconeogenesis by 1 μM-adrenaline in the sham-operated condition, but was only partially effective in this respect after adrenalectomy.

Adrenal corticosteroids have long been implicated in the control of carbohydrate metabolism. In recent years several studies have revealed a 'permissive' role of glucocorticoids in several tissues, namely that a normal response to other hormones is lost after adrenalectomy and quickly regained after administration of glucocorticoid. *In vivo* there is a marked decrease after adrenalectomy in the hyperglycaemic response after administration to rats of adrenaline or 3′:5′-cyclic AMP (Schaeffer *et al.*, 1969), and *in vitro* the perfused rat liver has a decreased glycogenolytic response to exogenous 3′:5′-cyclic AMP (Exton *et al.*, 1972). The liver phosphorylase *b*-to-*a* interconversion caused by exogenous 3′:5′-cyclic AMP, glucagon or adrenaline is muted after adrenalectomy (Schaeffer *et al.*, 1969; Exton *et al.*, 1972; Saitoh & Ui, 1975). In the perfused rat heart, a similar failure of glycogenolysis to respond to adrenaline after adrenalectomy can be corrected by raising the perfusate Ca^{2+} concentration, implying, in this system at least, that a Ca^{2+}-mediated or -stimulated process was affected by adrenalectomy (Miller *et al.*, 1971). The regulation of hepatic gluconeogenesis has also been found to be profoundly altered after adrenalectomy. In perfused livers from starved adrenalectomized rats stimulation of glucose formation from lactate by glucagon, adrenaline or exogenous 3′:5′-cyclic AMP is greatly decreased (Exton *et al.*, 1972). Stimulation by exogenous 3′:5′-cyclic AMP of [^{14}C]lactate conversion into glucose or glycogen is also muted in livers from fed adrenalectomized rats (Exton *et al.*, 1972). Gluconeogenesis in rat renal cortex, which may not primarily be concerned with provision of glucose for body tissues in general (McCann, 1962), is extremely sensitive to Ca^{2+} (Krebs *et al.*, 1963; Rutman *et al.*, 1965; Nagata & Rasmussen, 1970; Alleyne *et al.*, 1973). In addition, this process can be stimulated by exogenous 3′:5′-cyclic AMP (Guder *et al.*, 1971; Roobol & Alleyne, 1973) and by adrenaline through an α-receptor (Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977). Here we have investigated the effect of adrenalectomy on the response of renal-tubule gluconeogenesis to these agents.

Materials and Methods

Chemicals

Sodium pyruvate, enzymes, $NADP^+$ and ATP were obtained from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K., or from International Enzymes Ltd., Windsor, Berks., U.K. 3′:5′-Cyclic AMP (sodium salt), L-adrenaline, cortisol (hydrocortisone) 21-sodium succinate, DL-propranolol hydrochloride DNA (type V, sodium salt, 'highly polymerized', from calf thymus) and bovine plasma albumin powder (fraction V) were from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. The albumin was defatted by the method of Chen (1967), with minor modifications (Saggerson, 1972). Phentolamine mesylate was purchased from CIBA Laboratories, Horsham, Sussex, U.K.

Animals

Two sources of animals were used. Charles River, Margate, Kent, U.K., supplied adrenalectomized and sham-adrenalectomized male Sprague–Dawley rats 1 day after operation at 120–130g body weight. Male Sprague–Dawley rats from the University College London Biochemistry Department animal colony were adrenalectomized or sham-adrenalectomized in this laboratory under chloral hydrate anaesthesia at 130–140g body wt. Animals were killed 6–8 days after operations, during which time they were maintained on cube diet GR3 EK (E. Dixon and Sons, Ware, Herts., U.K.). Adrenalectomized rats were given 0.9% (w/v) NaCl in tap water to drink. The effectiveness of adrenalectomy was assessed by checking for complete absence of the glands after death. Where indicated, cortisol dissolved in 0.9% (w/v) NaCl was administered subcutaneously (50mg/kg body wt.) twice daily at approx. 10:00 and 17:30h for 2 days. Controls for these experiments received injections of 0.9% (w/v) NaCl at the same times.

Preparation of renal-cortical-tubule fragments

This was performed as described by Macdonald & Saggerson (1977), but in Ca^{2+}-free Krebs-Ringer bicarbonate medium containing collagenase (2mg/ml) and fatty acid-poor albumin (20mg/ml), followed by washing in Ca^{2+}-free Krebs–Ringer bicarbonate medium containing fatty acid-poor albumin (10mg/ml). Finally each tubule preparation was made up in the latter medium to a stock suspension in which the tissue from each original kidney cortex was dispersed in 5ml. Each preparation of tubules from adrenalectomized rats was accompanied by a parallel preparation from sham-operated animals.

Incubation techniques

Incubations were always commenced immediately after the preparation of tubule fragments. Portions (1.0ml) of the stock suspension were dispensed into 25ml silicone-treated Erlenmeyer flasks and incubated with shaking at 37°C in a final volume of 4ml Krebs–Ringer bicarbonate medium containing fatty acid-poor albumin (10mg/ml), 5mM-sodium pyruvate and other indicated additions. The flask contents were continuously gassed with O_2/CO_2 (19:1).

Analytical methods

Glucose was measured enzymically (Slein, 1965)

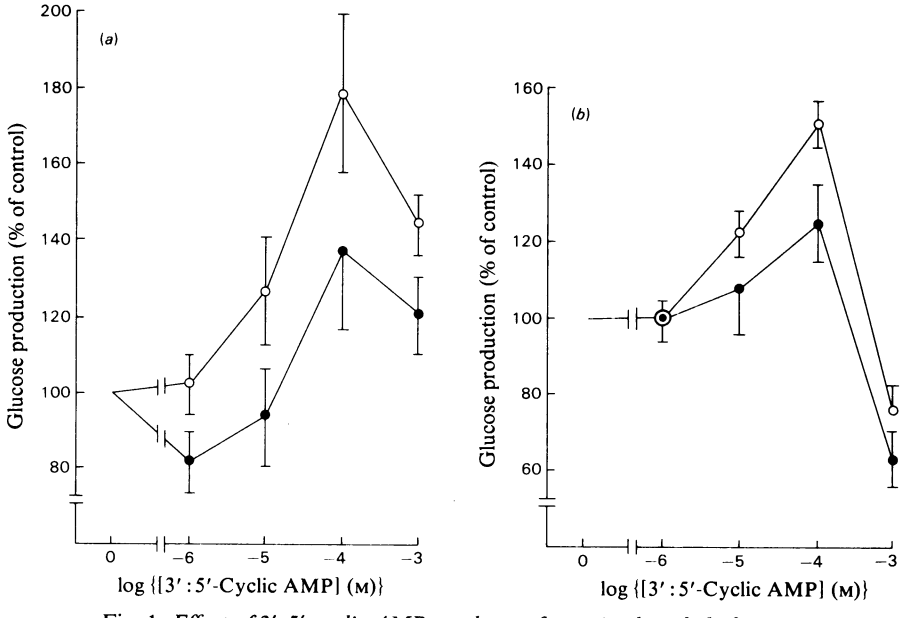

Fig. 1. *Effect of* 3′:5′*-cyclic AMP on glucose formation by tubule fragments*

Tubule fragments were prepared in Ca^{2+}-free media and then incubated for 1h in Krebs–Ringer bicarbonate medium containing fatty acid-poor albumin (10mg/ml), 100μM-disodium EGTA, 5mM-sodium pyruvate and with addition of either 0.2mM- or 2mM-$CaCl_2$. The values are means for seven independent experiments and the bars indicate S.E.M. The mean amounts of tubule DNA per ml of flask contents were: sham-operated (○), 49μg; adrenalectomized (●), 46μg. (*a*) With 0.2mM-Ca^{2+}; basal rates of glucose formation were: sham-operated, 0.67 ± 0.03μmol/h per mg of DNA: adrenalectomized, 0.51 ± 0.28μmol/h per mg of DNA. (*b*) With 2mM-Ca^{2+}; basal rates of glucose formation were: sham-operated, 3.70 ± 0.15μmol/h per mg of DNA; adrenalectomized, 2.29 ± 0.28μmol/h per mg of DNA.

in extracts prepared by deproteinization of incubation-flask contents with 0.5ml of ice-cold 45% (w/v) $HClO_4$. These extracts were neutralized by addition of 1M-triethanolamine hydrochloride and concentrated K_2CO_3 (approx. 5M). In all experiments the small amount of glucose initially present in non-incubated preparations was also determined and subtracted from experimental values.

DNA was measured by the method of Burton (1956) in duplicate 1.0ml portions of stock tubule suspensions.

Statistical methods

Analysis of data was performed on a paired basis and statistical significance determined by Student's *t* test.

Results and Discussion

General considerations

Tubule fragments were prepared in Ca^{2+}-free media because this investigation was associated with other studies in which changes in the Ca^{2+}-dependence of gluconeogenesis after adrenalectomy was investigated. In these parallel studies (results not shown) inclusion of 100μM-EGTA in all incubations was necessary, since, in some instances, gluconeogenesis was studied in the absence of Ca^{2+}. EGTA (100μM) was therefore added throughout to maintain comparability between all studies.

In view of the amelioration of the adrenalectomy lesion in cardiac glycogen metabolism that can be achieved by raising perfusate [Ca^{2+}] (Miller *et al.*, 1971) it was decided to perform the present studies at a low and a high Ca^{2+} concentration. These were obtained by additions of 0.2mM- or 2mM-Ca^{2+} respectively.

The alterations attributable to adrenalectomy in the effects of adrenaline or 3′:5′-cyclic AMP shown below were much more apparent in tubule preparations made in the absence of Ca^{2+} than in those made without Ca^{2+} depletion of the tissue. The reason for this is unclear. Qualitatively, though, similar effects of adrenalectomy were seen in non-Ca^{2+}-depleted preparations (results not shown).

Effect of adrenalectomy on the response to exogenous 3′:5′-cyclic AMP

On incubation of tubules with 0.2mM-Ca^{2+} (+0.1mM-EGTA), maximum stimulation of gluconeogenesis by 0.1mM-3′:5′-cyclic AMP in tubules from adrenalectomized rats was only 37%, whereas in the sham-operated condition this change was 78% (Fig. 1*a*). In 2mM-Ca^{2+} (+0.1mM-EGTA) stimulation

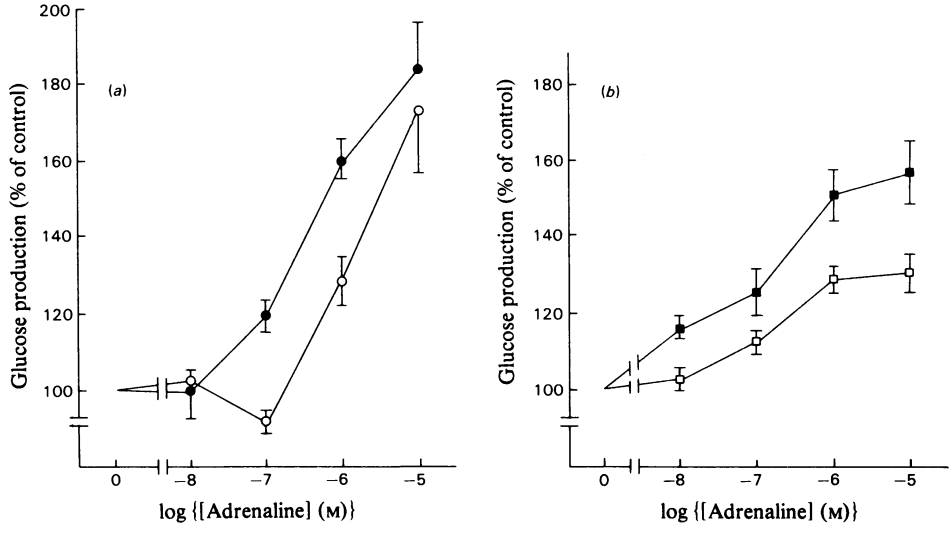

Fig. 2. *Effect of adrenaline on glucose formation by tubule fragments*

Tubule fragments were prepared in Ca^{2+}-free media and then incubated for 1h in Krebs–Ringer bicarbonate medium containing fatty acid-poor albumin (10mg/ml), 100μM-disodium EGTA, 5mM-sodium pyruvate and with addition of either 0.2mM- or 2mM-disodium EGTA, 5mM-sodium pyruvate and with addition of either 0.2mM- or 2mM-$CaCl_2$. The values are means for six independent experiments and the bars indicate S.E.M. The mean amounts of tubule DNA per ml of flask contents were: sham-operated (○), 49μg; adrenalectomized (●), 45μg. (*a*) With 0.2mM-Ca^{2+}; basal rates of glucose formation were: sham-operated, 0.68 ± 0.14μmol/h per mg of DNA; adrenalectomized, 0.52 ± 0.07μmol/h per mg of DNA. (*b*) With 2mM-Ca^{2+}; basal rates of glucose formation were: sham-operated, 4.08 ± 0.35μmol/h per mg of DNA; adrenalectomized, 2.75 ± 0.33μmol/h per mg of DNA.

of gluconeogenesis by 3′:5′-cyclic AMP again was less after adrenalectomy (Fig. 1*b*). With 0.1mM-3′:5′-cyclic AMP the percentage stimulation seen in the sham-operated condition was 51 ± 6%, which is significantly greater ($P < 0.05$) than the 25 ± 10% increase seen after adrenalectomy.

Effect of adrenalectomy on the response to adrenaline

In these experiments it was found that dose responses to adrenaline were decidedly shifted after adrenalectomy (Figs. 2*a* and 2*b*) and that with 2mM-Ca^{2+} the maximum response to the hormone was considerably increased (Fig. 2*b*). Adrenalectomy significantly increased the percentage stimulation of gluconeogenesis by adrenaline under the following conditions: with 0.2mM-Ca^{2+} (+0.1mM-EGTA), at 0.1μM- ($P<0.001$) and 1μM-adrenaline ($P<0.01$); with 2mM-Ca^{2+} (+0.1mM-EGTA), at 10nM- ($P<0.01$), 1μM- ($P<0.02$) and 10μM-adrenaline ($P<0.01$). It is conceivable that these changes could arise from retention of circulatory catecholamine by the tissue preparation. This should be lower after adrenalectomy. However, appreciable retention of catecholamines through the preparation procedure seems most unlikely, since the collagenase digestion was performed with about 2g wet wt. of tissue per 10ml followed by thorough washing twice with 10ml of medium and then dilution of no more than 100mg wet wt. of tissue into a 4ml incubation volume. Exton *et al.* (1972) found that adrenaline stimulation of gluconeogenesis from lactate was impaired in livers from starved adrenalectomized rats, but did not observe any change in adrenaline activation of [^{14}C]glucose synthesis from [^{14}C]lactate in livers from fed adrenalectomized rats. In rat liver, gluconogenesis can be enhanced through stimulation of both α- and β-adrenergic receptors (Exton & Harper, 1975). The present results show that, in renal cortex from fed rats, where adrenergic activation of this process appears to be solely through an α-receptor (Guder & Rupprecht, 1975; Macdonald & Saggerson, 1977), there is an actual enhancement of the adrenaline stimulation after adrenalectomy.

Table 1 shows that stimulation of gluconeogenesis by 1μM-adrenaline was still unaffected by a 10-fold excess of the β-blocker propranolol after adrenalectomy. However, a 10μM concentration of the α-blocker phentolamine, which completely inhibited the action of 1μM-adrenaline in the sham-operated condition, was only partially effective in this respect after adrenalectomy. It is possible therefore that adrenalectomy changed some characteristic of the α-receptor. It is noteworthy that, with tubules prepared in Ca^{2+}-free media, 10μM-phentolamine and 10μM-propranolol both appreciably decreased glucose formation. This was not previously found to be the case with tubule fragments prepared in 1.27mM-Ca^{2+} (Macdonald & Saggerson, 1977). This would appear to be another example of an effect that is amplified by prior Ca^{2+} depletion of the tissue.

Effect of cortisol therapy on adrenalectomized rats

Cortisol treatment of starved adrenalectomized rats restores the responsiveness to glucagon and adrenaline of gluconeogenesis in perfused livers from these animals (Exton *et al.*, 1972). Cortisol therapy

Table 1. *Effects of adrenergic blocking agents on adrenaline stimulation of gluconeogenesis in tubule fragments from adrenalectomized and sham-operated rats*

Tubule fragments were prepared in Ca^{2+}-free medium and then incubated for 1h in Krebs–Ringer bicarbonate with fatty acid-poor albumin (10mg/ml), 2mM-$CaCl_2$, 100μM-disodium EGTA and 5mM-sodium pyruvate. The values are means ± S.E.M. for six independent experiments in every case and are expressed as μmol of glucose/h per mg of DNA. The values in parentheses are 'adrenaline-stimulated' values expressed as percentages of the appropriate controls. Mean values for tubule DNA per ml of flask contents were: Expt. 1: sham-operated, 63μg; adrenalectomized, 60μg. Expt. 2: sham-operated, 65μg; adrenalectomized, 57μg. * $P<0.02$, ** $P<0.01$ and *** $P<0.001$ for effects of adrenaline.

Expt. no.	Additions	Sham-operated	Adrenalectomized
1	None	3.96 ± 0.54	2.57 ± 0.39
	Adrenaline (1μM)	5.16 ± 0.69***	3.83 ± 0.60***
		(131 ± 4%)	(150 ± 7%)
	Phentolamine (10μM)	3.29 ± 0.48	2.11 ± 0.39
	Adrenaline (1μM) + phentolamine (10μM)	3.24 ± 0.49	2.53 ± 0.41*
		(99 ± 4%)	(122 ± 5%)
2	None	4.51 ± 0.44	2.83 ± 0.25
	Adrenaline (1μM)	5.85 ± 0.59***	4.10 ± 0.48**
		(129 ± 4%)	(144 ± 7%)
	Propranolol (10μM)	3.67 ± 0.46	2.43 ± 0.26
	Adrenaline (1μM) + propranolol (10μM)	4.86 ± 0.72**	3.31 ± 0.30***
		(131 ± 6%)	(138 ± 5%)

Table 2. *Effects of 3′:5′-cyclic AMP, adrenaline and α-adrenergic blockade on gluconeogenesis in tubule fragments from adrenalectomized rats treated with cortisol*

Tubule fragments were prepared from cortisol or saline-injected adrenalectomized rats in Ca^{2+}-free medium and then incubated for 1 h in Krebs–Ringer bicarbonate medium containing fatty acid-poor albumin (10 mg/ml), 100 μM-disodium EGTA, 5 mM-sodium pyruvate and the indicated addition of $CaCl_2$. The values are means ± S.E.M. for six independent experiments in every case and are expressed as μmol of glucose/h per mg of DNA. The values in parentheses are 'cyclic AMP' or 'adrenaline-treated' values expressed as percentages of the appropriate controls. Mean amounts of tubule DNA per ml of flask contents were: saline-injected, 55 μg; cortisol-treated, 59 μg. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ for effects of cyclic AMP or adrenaline.

Ca^{2+} addition	Other additions	Adrenalectomized, saline-injected	Adrenalectomized cortisol-treated
0.2 mM	None	0.59 ± 0.05	0.74 ± 0.10
	3′:5′-cyclic AMP (0.1 mM)	0.96 ± 0.07***	1.20 ± 0.07***
		(166 ± 8%)	(171 ± 11%)
	Adrenaline (1 μM)	0.78 ± 0.07**	0.89 ± 0.06
		(135 ± 7%)	(127 ± 8%)
2 mM	None	2.63 ± 0.29	3.37 ± 0.34
	3′:5′-Cyclic AMP (0.1 mM)	3.69 ± 0.42***	4.56 ± 0.52**
		(140 ± 2%)	(135 ± 7%)
	Adrenaline (1 μM)	3.76 ± 0.49**	4.90 ± 0.51**
		(140 ± 5%)	(146 ± 7%)
	Phentolamine (10 μM)	2.30 ± 0.35	3.20 ± 0.35
	Adrenaline (1 μM) + phentolamine (10 μM)	2.52 ± 0.32*	3.11 ± 0.37
		(116 ± 8%)	(96 ± 2%)

after adrenalectomy also restores the glycogenolytic response to adrenaline of rat heart (Miller *et al.* 1971), the activation of hepatic phosphorylase by glucagon or adrenaline (Saitoh & Ui, 1975), the hyperglycaemic effects of adrenaline or 3′:5′-cyclic AMP in the intact rat (Schaeffer *et al.*, 1969) and the lipolytic response to catecholamines of rat adipose tissue (Allen & Beck, 1972). Table 2 shows that cortisol therapy in fed adrenalectomized rats increased glucose formation in renal tubules prepared from these animals, although this change was not statistically significant. In addition, cortisol therapy reversed the change in sensitivity of the adrenergic receptor to phentolamine (α-blocker). Cortisol treatment did not alter the percentage effects of 0.1 mM-3′:5′-cyclic AMP or 1 μM-adrenaline on gluconeogenesis in the presence of 0.2 mM- or 2 mM-Ca^{2+} additions. These findings imply that glucocorticoid depletion after adrenalectomy cannot be the only factor responsible for the altered response of renal gluconeogenesis to 3′:5′-cyclic AMP or adrenaline.

General conclusions

The present study shows that adrenalectomy alters the response of renal gluconeogenesis to adrenaline and exogenous 3′:5′-cyclic AMP. It is particularly noteworthy that the changes in percentage responsiveness to 3′:5′-cyclic AMP and α-adrenergic stimulation are in opposite directions and are unaffected by glucocorticoid therapy.

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