Adrenal and Plasma Corticosterone and Pituitary and Plasma ACTH in Alloxan Diabetic Rats[1,2] (37343)

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Many investigators have reported that the chronic metabolic stress of uncontrolled experimental diabetes produced a state of adrenal hyperactivity (1–11). Adrenal hyperplasia of alloxan diabetes (1–9) was accompanied by increased plasma corticosterone (10, 11), increased (2, 3), or normal (7) *in vitro* adrenal corticosteroidogenesis as well as lymphoid involution (9, 12). Pituitary ACTH concentration was found to be normal (8, 13, 14) and hypophysectomized rats responded to pituitary extracts of diabetic animals with normal adrenal hypertrophy (13) and normal adrenal ascorbic acid depletion (8). In addition, the adrenal hypertrophy response to unilateral adrenalectomy was found to be normal in alloxanized animals (14). However, there was a significant decrease in the ability to respond to acute stress (adrenal ascorbic acid depletion parameter) which could not be attributed to decreased adrenal responsiveness (8) nor decreased pituitary ACTH reserve (8, 13, 14) and could be reversed by insulin administration (8). The latter work suggested that there was a block at the hypothalamo–hypophyseal level.

It was felt that further elucidation on the state of the pituitary–adrenal axis in uncontrolled experimental diabetes was needed. This investigation was, therefore, undertaken to explore parameters of pituitary–adrenal activity which had not been previously considered such as plasma ACTH and *in vivo* adrenal corticosterone. In addition, it was felt that the validity of previous reports (8, 13, 14) on pituitary ACTH reserve should be tested by using a more precise ACTH assay method. This study was, therefore, designed to measure both pituitary and plasma ACTH in alloxan diabetes using the plasma corticosterone response of dexamethasone[3] suppressed rats. In addition, simultaneous determination of plasma corticosterone and adrenal corticosterone of alloxan diabetic rats were made.

Materials and Methods. Rats were maintained under constant lighting (14 hr artificial light) and temperature (26°) and were fed Purina laboratory chow and water *ad libitum* except when indicated. Male Wistar rats (100–150 g) were fasted for 48 hr before injection of alloxan or vehicle. Female Sprague–Dawley rats (200–250 g) used as assay animals were given dexamethasone (20 μg/ml) in the drinking water the night before use.

Experimental diabetes was produced by intraperitoneal injection of 2.0% alloxan monohydrate solution which was freshly prepared in a citrate–phosphate buffer (pH 4.00). A dose of 20 mg/100 g or an equal volume of buffer was administered and the animals were individually housed for the next 72 hr. Qualitative determination of glycosuria (Testape) was made 48 and 72 hr after injection. Only those animals with a 2.0% minimum of glycosuria were considered suitable for the study since in a preliminary screening this level was found to be associated with glucose levels of above 350 mg%. All sacrifices were made by decapitation 72 hr after injection (between 10:00 AM and 12.00 noon) and within 30 sec after removal from the cages.

Corticosterone determination of plasma and adrenals were made in 8 diabetic and 8 con-

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[3] Dexamethasone supplied by Schering Corporation.

trol rats using fluorometric measurements at 470 mU activation with an acid–ethanol fluorescent reagent. Trunk blood was collected in heparinized beakers and immediately centrifuged in heparinized tubes. Plasma was stored frozen in 0.5 ml quantities in teflon lined screw capped tubes for later corticosterone determination according to the method of Purves and Sirett (15). Both adrenal glands were removed, cleaned, and weighed. Homogenates of each gland were made in 5 ml of cold 20% v/v ethanol–saline solution using a Cenco-Potter glass homogenizer. Homogenates were stored frozen in teflon-lined screw-capped tubes for later corticosterone determination according to the method of Vernikos-Danellis *et al.* (16).

ACTH was assayed in both pituitary and plasma by a modification of the method of Purves and Sirett (15). Pituitary suspensions, plasma, or vehicle (2% gelatine–0.01 *N* HCL–saline) were injected into the exposed femoral vein of the anesthetized (6.0 mg pentobarbital/100 g ip) dexamethasone suppressed rat. Blood was collected from the abdominal aorta with a heparinized syringe 18 min after injection. Plasma of the assay animal was stored frozen in 0.5 ml quantities in teflon lined screw capped tubes for later corticosterone determination (15).

Pituitary ACTH was determined by using pooled pituitaries from 10 diabetic and 10 control rats. Pituitary pools of 5 pituitary/ml of vehicle were stored frozen and homogenized with a teflon pestle just before use. Pituitary suspensions of 0.1 pituitary or 0.2 pituitary/0.2 ml of vehicle were made by addition of vehicle at the time of homogenation. Pituitary suspensions were administered as 0.2 ml/100 g. There were 4 recipients for each dose of control pituitary suspensions, and 5 recipients for each dose of diabetic pituitary suspensions, and 3 recipients receiving vehicle only.

For plasma ACTH, trunk blood was collected from 34 diabetic and 35 control rats and the plasma of 16–18 animals were pooled and frozen using the precautions suggested by Purves and Sirett (17) to minimize loss of ACTH potency. Plasma was thawed just before use by holding the vial under cold tap

TABLE I. Adrenal Weights and Corticosterone in Adrenals and Plasma of Normal and Diabetic Male Rats.[a]

Group	*N*	Relative adrenal wt (mg/100 g)	Corticosterone		
			Adrenals[d]		Plasma (μg/100 ml)
			Total μg	μg/100 mg	
Control[b]	8	20.43 ± 2.94	1.43 ± 0.21	4.86 ± 0.48	16.00 ± 3.62
Diabetic[c]	8	30.48 ± 2.29	2.71 ± 0.09	8.15 ± 0.80	33.00 ± 3.65
Diabetic vs controls		+10.05 ± 3.73 (+50% $p < 0.02 > 0.01$)	+1.28 ± 0.37 (+90% $p < 0.005 > 0.001$)	+3.29 ± 1.13 (+67% $p < 0.01$)	+17.00 ± 5.14 (+106% $p < 0.01 > 0.005$)

[a] All animals were fasted 48 hr prior to injection. Sacrifices 72 hr after ip injection.
[b] Citrate–phosphate buffer (pH 4.00) 0.1 ml/100 g.
[c] Alloxan (2%) in citrate–phosphate buffer (pH 4.00) 20 mg/100 g. Glycosuria 2% minimum.
[d] Based on adrenal pairs.

water. There were 6 recipients receiving control plasma, 6 recipients receiving diabetic plasma, and 9 recipients receiving vehicle only.

Results and Discussion. In acutely diabetic rats, adrenal hyperplasia, increased adrenal and plasma corticosterone (Table I) were associated with increased plasma ACTH (Table II) and normal pituitary ACTH (Table III). The 90% increase in total adrenal corticosterone was accompanied by a 50% increase in adrenal weight as well as a 67% increase in adrenal corticosterone concentration (Table I). Adrenal size was not the only factor in the increased corticosterone output since there was an increase in the amount of steroid output per 100 mg of adrenal tissue (Table I). These findings agree with *in vitro* studies (3) in which 38% increase in steroid production per 100 mg of tissue was observed. It is in conflict however, with *in vitro* studies in which either normal corticosteroidogenesis (7) or increased corticosterone output based on adrenal hyperplasia only (2) had been observed.

The remarkable correlation between 106% increase in plasma corticosterone and a 90% increase in total adrenal corticosterone (Table I) and the significantly higher levels of plasma ACTH (Table II) support the concept that the increased plasma corticosterone was due to increased pituitary–adrenal activity. Decreased hepatic inactivation of corticosteroids (18) may have played a role in these observations.

Despite the obvious increased activity of the pituitary–adrenal axis, there has been no evidence that pituitary ACTH had been depleted (Table III) which could have been responsible for the previously observed impairment of the acute stress response (8). Large amounts of ACTH apparently can be synthesized and simultaneously released into the plasma in the basal state of acute diabetes, but the ability to synthesize and release ACTH in response to acute stress has been impaired. The acute stress response depends upon the ability to rapidly synthesize new ACTH (19) and not upon pituitary concentration (20). However, it is felt that this rapid ACTH synthesis depends upon the rapid synthesis of a factor or factors responsible for ACTH synthesis (20). Therefore it is possible to attribute the partially impaired acute stress response observed in diabetic animals (8) to a partial block at the hypothalamo–hypophyseal level which interfered with the factor or factors responsible for

TABLE II. Plasma ACTH of Normal and Diabetic Male Rats Assayed in Dexamethasone Suppressed Rats.

Recipient groups[a]	*N*	Plasma corticosterone (μg/100 ml)	Response[e]
Controls[b]	9	2.32 ± 0.34	
Normal plasma[c]	6	3.48 ± 0.28	$+1.16 \pm 0.44$ ($p < 0.02 > 0.001$)
Diabetic plasma[d]	6	8.71 ± 0.53	$+6.39 \pm 0.63$ ($p < 0.001$)
Diabetic plasma vs normal plasma		$+5.23 \pm 0.63$ ($p < 0.001$)	

[a] Sprague-Dawley female rats (200–250 g) receiving dexamethasone (20 μg/100 ml) in drinking water *ad libitum* overnight. Intravenous injections of 6 ml of plasma or vehicle (2% gelatine–0.01 *N* HCl–saline). Blood collected 18 min after injection.

[b] Vehicle only.

[c] Normal plasma collected from 35 donor animals. Animals fasted 48 hr before ip injection of citrate–phosphate buffer pH 4.00 in doses of 0.1 ml/100 g 72 hr before sacrifice.

[d] Diabetic plasma collected from 34 donor animals. Animals fasted 48 hr before ip injection of 2% alloxan in citrate–phosphate buffer pH 4.00 in doses of 20 mg/100 g 72 hr before sacrifice. Glycosuria 2% minimum.

[e] Response = difference from vehicle treated control animals.

TABLE III. Pituitary ACTH of Normal and Diabetic Male Rats Assayed in Dexamethasone Suppressed Rats.

Recipient groups[a]	*N*	Plasma corticosterone (μg/100 ml)	Response[e]
Controls[b]	3	2.53 ± 0.31	
0.1 Pituitary/100 g			
Normal animals[c]	4	4.75 ± 0.67	+2.22 ± 0.74 ($p < 0.05 > 0.025$)
Diabetic animals[d]	5	4.94 ± 0.63	+2.41 ± 0.70 ($p < 0.02 > 0.01$)
0.2 Pituitary/100 g			
Normal animals[c]	4	20.19 ± 0.60	+17.66 ± 0.67 ($p < 0.001$)
Diabetic animals[d]	5	19.38 ± 2.38	+16.85 ± 2.40 ($p < 0.001$)

[a] Sprague-Dawley female rats (200–250 g) receiving dexamethasone (20 μg/100 ml) in drinking water *ad libitum* overnight. Blood collected 18 min after intravenous injection of 0.2 ml/100 g of pituitary suspensions or vehicle (2% gelatine–0.01 *N* NCl–saline).

[b] Vehicle only.

[c] Normal pituitaries collected from 5 donor animals for each suspension made. Animals fasted 48 hr before ip injection of citrate–phosphate buffer pH 4.00 in doses of 0.1 ml/100 g 72 hr before sacrifice.

[d] Diabetic pituitaries collected from 5 donor animals for each suspension made. Animals fasted 48 hr before ip injection of 2% alloxan in citrate–phosphate buffer pH 4.00 in doses of 20 mg/100 g 72 hr before sacrifice. Glycosuria 2% minimum.

[e] Response = difference from vehicle treated control assay animals.

rapid ACTH synthesis.

Summary. Plasma and adrenal corticosterone as well as ACTH in both plasma and pituitaries of acutely diabetic rats were measured 72 hr after intraperitoneal injection of alloxan (20 mg/100 g). ACTH assays were performed in dexamethasone suppressed rats. A 50% increase in adrenal weights was associated with a 90% increase in total adrenal corticosterone and a 67% increase in adrenal corticosterone concentration. The high level of adrenal activity was associated with an equally high level of plasma corticosterone as well as plasma ACTH. The pituitary ACTH reserve remained unchanged. Experimental diabetes is associated with increased pituitary–adrenal activity without the depletion of the pituitary ACTH reserve. In the basal state of uncontrolled diabetes, ACTH synthesis is capable of keeping pace with the rate of ACTH release.

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