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CATECHOLAMINE METABOLISM AND MONOAMINE OXIDASE ACTIVITY IN ADRENALECTOMIZED RATS

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Abstract—The excretion of 4-hydroxy-3-methoxyphenylglycol (HMPG), 4-hydroxy-3-methoxymandelic acid (VMA), metadrenaline (MA), normetadrenaline (NMA) and homovanillic acid (HVA) has been measured in the urine of adrenalectomized rats. Urinary HMPG was significantly increased compared with the controls, presumably reflecting an increased production of noradrenaline. HVA output was similarly increased although to a lesser extent. Treatment with hydrocortisone (10 mg per kg) for 7 days abolished these increases. A small amount of urinary MA was still detectable in four out of six adrenalectomized rats, providing further evidence for extra-adrenal production of adrenaline. VMA and NMA excretion were not significantly altered by adrenalectomy.

After adrenalectomy, changes in the subcellular distribution of monoamine oxidase (MAO) are complex and vary from tissue to tissue. Total activity in heart, brain and vas deferens of adrenalectomized rats was significantly increased, a reflection of increased mitochondrial activity. An increase in microsomal MAO was observed only in the vas deferens. The heart was the only tissue examined to show an increase in the supernatant fraction. In the duodenum mitochondrial MAO activity was significantly increased, but this rise was offset by a significant decrease in enzyme in the supernatant fraction.

CHANGES in the urinary output of adrenaline and noradrenaline following bilateral adrenalectomy or medullo-adrenalectomy have been described in a variety of animal species.[1–5] Adrenaline excretion is abolished[2, 4] or markedly reduced[1, 3] whereas urinary noradrenaline levels are unchanged[2, 3] or increased.[1, 5] We have now measured a number of quantitatively important catecholamine metabolites in the urine of bilaterally adrenalectomized rats in order to study more closely the effect of this procedure on catecholamine production.

An increase in the monoamine oxidase (EC 1.4.3.4) (MAO) activity in heart tissue homogenates of adrenalectomized rats has been reported.[6] As we have found evidence of an increased production of noradrenaline, one of the substrates of this enzyme, we thought it of interest to try to confirm this observation and determine whether any alteration in subcellular distribution of MAO occurs after adrenalectomy.

METHODS

Animals. Bilaterally adrenalectomized male Wistar rats and control animals of the same age, sex and weight range were used in the study (Scientific Products Farm, Canterbury, England). The rats were housed under warm conditions and the treated animals allowed free access to solid food and 0·9% (w/v) NaCl solution. Control animals were given tap water instead of NaCl solution.

Urine collection. Samples (24 hr) from adrenalectomized or control rats were collected into 6 N HCl (1 ml) under conditions previously described.[7] During the collection period, animals were fasted from solid food but allowed 0·9% NaCl solution or water *ad lib.*

Materials. DL-4-Hydroxy-3-methoxymandelic acid (British Drug Houses, Ltd., Poole, England); *bis* (4-hydroxy-3-methoxyphenylglycol) piperazine salt (Regis Chemical Co., Chicago, Ill.); DL-metanephrine (metadrenaline) hydrochloride and DL-normetanephrine (normetadrenaline) hydrochloride (Calbiochem, Los Angeles, Calif.); homovanillic acid (Calbiochem); DL-normetanephrine-7-^{3}H (DL-normetadrenaline-7-^{3}H) hydrochloride (3700 mc/mM) in 0·01 N acetic acid (New England Nuclear Corp.); "Efcortelan Soluble" (hydrocortisone sodium succinate for injection, B.P.) (Glaxo Laboratories, Ltd., Greenford, England); 4-hydroxyquinoline (Koch–Light Laboratories, Ltd., Colnbrook, England); kynuramine dihydrobromide (Sigma Chemical Company, St. Louis, Mo.); ^{14}C-methoxy-labelled metadrenaline was prepared as described by Caesar *et al.*[7]

Catecholamine metabolite determinations. Total 4-hydroxy-3-methoxyphenylglycol (HMPG), 4-hydroxy-3-methoxymandelic acid (VMA), metadrenaline (MA) and normetadrenaline (NMA) were estimated in 24-hr specimens of adrenalectomized and control rat urine by methods described by Caesar *et al.*[7] Free urinary homovanillic acid (HVA) was estimated by the method of Karoum *et al.*[8] At the time of these estimations the rats weighed from 280 to 350 g.

Later, the adrenalectomized rats, now weighing 372–522 g, were each treated daily for 7 days with a subcutaneous injection of 10 mg/kg body weight hydrocortisone as replacement therapy. Urinary excretion values of HMPG and HVA were again estimated in test and control groups during treatment.

Monoamine oxidase determination. Eight weeks after the conclusion of the metabolite studies the adrenalectomized and control rats were killed. Brain, heart, submaxillary glands, liver, spleen, duodenum and vas deferens were quickly removed, washed with ice-cold 0·9% (w/v) NaCl solution, weighed and stored at 4° until required. After tissue homogenization in ice-cold 0·3 M sucrose, subcellular fractionation was carried out, essentially by the method of Hawkins,[9] at 0–4°. Following centrifugation at 1000 *g* for 30 min to remove cell debris, the supernatant was centrifuged at 17,500 *g* for 1 hr to spin down the mitochondrial fraction and at 108,000 *g* for 1 hr to isolate the microsomal fraction. The fractions were suspended in phosphate buffer (0·05 M, pH 7·4) and, together with the final supernatant, were stored at −15° until required for assay.

The MAO activity of the subcellular fractions was estimated by the method of Kraml,[10] slightly modified to use half quantities of reagents.

RESULTS

Urinary excretion of catecholamine metabolites

There was a highly significant increase in urinary output of HMPG and, to a lesser extent, in that of HVA, in adrenalectomized rats compared with controls (Table 1). VMA and normetadrenaline excretion were not significantly altered by adrenalectomy. Treatment with hydrocortisone for 7 days abolished the rise in urinary HMPG and HVA output (Table 2); indeed when expressed in terms of weight the levels of HMPG and HVA dropped significantly below normal levels. Although the excretion of

TABLE 1. EXCRETION OF TOTAL HMPG, VMA, NMA AND FREE HVA IN THE URINE OF ADRENALECTOMIZED AND CONTROL RATS

	Adrenalectomized (Mean excretion ± S.E.M.)		Controls (Mean excretion ± S.E.M.)	
Metabolite	Expts.	(μg/24 hr)	Expts.	(μg/24 hr)
HMPG*	6	60·63 ± 2·63 (303·7 ± 20·0)	6	34·54 ± 2·55 (187·6 ± 13·8)
VMA	6	11·72 ± 1·43	6	13·50 ± 2·04
NMA	4	3·52 ± 1·45	5	5·10 ± 1·80
HVA†	6	55·92 ± 4·68 (207·0 ± 18·0)	6	44·45 ± 4·05 (160·2 ± 14·0)

* $P < 0·001$.
† $P < 0·05$ (Mann–Whitney test).[11]
Values in parentheses represent μg per kg per 24 hr.

TABLE 2. EXCRETION OF TOTAL HMPG AND FREE HVA IN THE URINE OF ADRENALECTOMIZED RATS AFTER TREATMENT WITH 10 mg/kg HYDROCORTISONE DAILY FOR 7 days

	Adrenalectomized (Hydrocortisone-treated) (Mean excretion ± S.E.M.)			Controls (Mean excretion ± S.E.M.)		
Metabolite	Expts.	(μg/24 hr)	(μg/kg/24 hr)	Expts.	(μg/24 hr)	(μg/kg/24 hr)
HMPG	5	48·50 ± 6·09	90·4 ± 16·4*	6	60·06 ± 5·03	147·9 ± 13·0
HVA	5	29·58 ± 2·21	65·6 ± 5·3*	6	34·77 ± 2·07	86·5 ± 6·6

* $P < 0·05$.

TABLE 3. EXCRETION OF TOTAL METADRENALINE IN THE URINE OF ADRENALECTOMIZED AND CONTROL RATS

Adrenalectomized		Controls*	
Rat	(μg/24 hr)	Rat	(μg/24 hr)
1	0·33	1	0·70
2	—†	2	1·06
3	0·18	3	0·79
4	—†	4	0·33
5	0·24	5	1·77
6	0·11	6	0·89

* Mean ± S.E.M. = 0·92 ± 0·20 μg/24 hr.
† Below limits of sensitivity of the method.

HMPG in the controls increased with age this increase was not significant when expressed in terms of weight. Urinary HVA, however, was significantly reduced ($P < 0·001$) in the older controls when expressed on a weight basis. Metadrenaline excretion was drastically decreased in adrenalectomized rats (Table 3) although measurable amounts could be detected in four out of six animals.

Monoamine oxidase activity

Total MAO activity of heart, brain and vas deferens was significantly increased in

TABLE 4. MAO ACTIVITY OF ADRENALECTOMIZED AND CONTROL MALE RAT TISSUES EXPRESSED AS μg 4-HYDROXYQUINOLINE FORMED PER min PER g WET WEIGHT $\pm$ S.E.M.

		Mitochondrial fraction		Microsomal fraction		Supernatant fraction		Sum	
Heart	Control*	5·21 ± 0·40	$P < 0·001$	2·36 ± 0·32	NS	0·59 ± 0·12	$P < 0·05$	8·15 ± 0·46	$P < 0·001$
	Adrenalectomized†	12·25 ± 2·09		3·16 ± 0·35		1·26 ± 0·29		16·67 ± 1·03	
Brain	Control	3·31 ± 0·43	$P < 0·05$	0·26 ± 0·03	NS	0·10 ± 0·01	NS	3·67 ± 0·27	$P < 0·001$
	Adrenalectomized	5·42 ± 0·63		0·20 ± 0·03		0·18 ± 0·03		5·79 ± 0·40	
Vas deferens	Control	1·38 ± 0·09	$P < 0·01$	0·42 ± 0·05	$P < 0·05$	0·32 ± 0·04	NS	2·11 ± 0·18	$P < 0·01$
	Adrenalectomized	1·96 ± 0·10		0·64 ± 0·05		0·31 ± 0·05		2·92 ± 0·22	
Duodenum	Control	3·75 ± 0·04	$P < 0·01$	0·80 ± 0·03	NS	1·35 ± 0·14	$P < 0·001$	5·90 ± 0·47	NS
	Adrenalectomized	4·61 ± 0·05		0·62 ± 0·06		0·51 ± 0·07		5·74 ± 0·25	
Submaxillary gland	Control	2·54 ± 0·12	NS	0·71 ± 0·06	NS	0·35 ± 0·02	$P < 0·02$	3·59 ± 0·27	NS
	Adrenalectomized	2·67 ± 0·13		0·81 ± 0·08		0·25 ± 0·02		3·73 ± 0·39	
Liver	Control	11·14 ± 1·13	NS	2·69 ± 0·36	NS	2·66 ± 0·22	NS	16·49 ± 1·13	NS
	Adrenalectomized	14·61 ± 2·13		2·16 ± 0·20		3·12 ± 0·33		19·89 ± 1·41	
Spleen	Control	1·80 ± 0·11	NS	0·22 ± 0·02	NS	0·15 ± 0·02	NS	2·17 ± 0·21	NS
	Adrenalectomized	1·99 ± 0·14		0·19 ± 0·02		0·20 ± 0·04		2·38 ± 0·08	

* Six animals per group.
† Five animals per group.

adrenalectomized rats (Table 4) but there was no significant alteration in activity in the other tissues examined. There was a greater than 2-fold increase in mitochondrial MAO activity in the heart and significant increases were detected in the mitochondrial fractions of brain, vas deferens and duodenum. The rise in duodenal mitochondrial activity was offset, however, by a highly significant decrease of MAO activity in the supernatant. Only in the heart supernatant was MAO activity increased after adrenalectomy. Microsomal MAO activity was not significantly affected by adrenalectomy in any tissue examined apart from the vas deferens where an increase was observed.

DISCUSSION

We have shown that the paradoxical increase in urinary noradrenaline output following bilateral adrenalectomy in humans[1] and rats[5] is accompanied by a significantly elevated HMPG output and can thus be viewed as a manifestation of increased production deriving from such sites as the heart.[12] Its cause is unknown. HMPG, the major metabolite of adrenaline and noradrenaline in rat urine,[13] appears to provide a better index of noradrenaline turnover than VMA or normetadrenaline, the urinary excretion of which was unchanged by adrenalectomy. A similar correlation was noted in rats after immunosympathectomy,[7] where a significant fall in urinary noradrenaline output[4, 14, 15] is matched by a parallel change in HMPG excretion without any corresponding alteration in VMA or normetadrenaline output. Urinary noradrenaline excretion does not increase in medullo-adrenalectomized dogs and cats[2, 3] which possess an intact adrenal cortex and indeed, the administration of corticosterone to totally adrenalectomized rats abolishes the increase in noradrenaline output.[5] We have now shown that treatment of adrenalectomized rats with hydrocortisone causes a reversion of the elevated HMPG excretion to control values. There was even a tendency for levels to drop below those of the control group although this did not reach significance in the small groups tested.

Four of the six adrenalectomized rats put out measurable amounts of metadrenaline in the 24-hr urine sample, further proof of the existence of extra-adrenal sources of adrenaline. Other studies have shown that extirpation of the adrenals is not followed by complete disappearance of adrenaline from the urine.[1, 3, 16, 17] Axelrod[18] has detected phenylethanolamine *N*-methyltransferase, the enzyme which converts noradrenaline to adrenaline, in rabbit heart, whilst de Potter *et al.*[19] have shown that labelled noradrenaline can be converted to adrenaline in rat hearts. Other workers have demonstrated that brain tissue can form adrenaline[20–22] and low endogenous concentrations have been identified in the brain.[23–25]

Dopamine forms a small proportion only of the catecholamine content of the adrenal medulla[23] but has been detected in the adrenal venous effluent of some species.[26] Paradoxically adrenalectomy gives rise to an increased urinary output of the major metabolite of dopamine, HVA. This rise, proportionately not as great as that of HMPG, may reflect accelerated production of precursor dopamine involved in the increased turnover of noradrenaline in adrenalectomized animals.[12, 27]

In addition to providing evidence of increased amine turnover we have been able to confirm that one of the two important amine inactivating enzymes, MAO, shows greater activity in certain tissues after adrenalectomy. The other, catechol *O*-methyltransferase, is known to be unaffected.[6] The heart was the organ showing the greatest change and the only one to show a rise in supernatant MAO activity. Although the

microsomes contain a greater fraction of cardiac MAO compared with other tissues and indeed, the major proportion of intracellular enzyme in the different rat species used by de Champlain *et al.*,[28] adrenalectomy only brought about an increase in microsomal MAO in the vas deferens. Whether such increases in enzyme activity and compartment shifts are adaptive responses to facilitated production of substrate or, perhaps, stem from an action of glucocorticoids on the permeability of intracellular membranes to amines is at present unknown.

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