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STEROID SECRETION BY RAT ADRENAL GLANDS DURING THE FIRST WEEK FOLLOWING ADRENAL ENUCLEATION

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SUMMARY

Adrenal venous blood was collected from rats 4 days after adrenal enucleation and from sham operated controls. The amount of regenerated cortical tissue was measured in serial sections of the glands. The newly regenerated adrenal cortex produced per unit adrenal tissue only about 14% of the amount of corticosterone and less than 12% of the amount of 18-OH-DOC secreted by the intact tissue, whereas the production of DOC was not decreased. The relative increase in the amount of DOC available to the rats bearing regenerating adrenal glands might contribute to the sodium retention observed in these rats during the first week after adrenal regeneration.

INTRODUCTION

LITTLE information on the secretion of steroids by enucleated adrenal glands during the first week of regeneration is so far available [1]. During this period the rats show an increased ability to retain sodium [2] which was attributed to the production of a hitherto unidentified steroid by newly regenerated adrenal tissue [1]. In the present experiments, adrenal venous blood was collected on the 4th day following adrenal enucleation and the blood extracts were investigated for the presence of several steroids.

METHODS

Operative procedures. Bilateral adrenal enucleation was carried out on male Wistar rats (90–120 g body weight) from dorsal flank incisions under ether anaesthesia. In a group of sham operated control rats the adrenal glands were exposed and only touched. All rats received 0·9% sodium chloride instead of drinking water. On the fourth day following the operation the rats were anaesthetized with sodium pentobarbitone (30–50 mg/kg) and a cannula inserted into the left renal vein after the renal peduncle, the spermatic vein and the entrance of the renal vein into the cava had been tied. Heparin was injected into the right femoral vein and adrenal venous blood was collected for 15 min. The body temperature was kept between 37 and 38°C and the blood flow maintained by infusing 0·9% sodium chloride into the right femoral vein.

Chemical methods. The tubes in which the adrenal blood was collected contained tracer amounts of the following steroids labelled with carbon-14 at C_4: 18-0H-deoxycorticosterone (18-0H-DOC): 3217 d.p.m. (prepared by incubating rat adrenals with [^{14}C]-labelled progesterone, extracting the labelled 18-0H-DOC

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formed from the incubation medium and isolating it by paper chromatography); pregnenolone: 38525 d.p.m. and progesterone: 8368 d.p.m. (Radiochemical Centre, Amersham, England). The samples of whole blood were diluted with an equal volume of water, extracted three times with double the volume of ethyl acetate and the extracts evaporated to dryness under a stream of nitrogen. [^{14}C]-labelled corticosterone had not been available at the time of the blood collection and was therefore only added before the first paper chromatography. All samples were first chromatographed in the toluene-propylene glycol (TPG) system [3] for 42 h. The regions corresponding to the R_F values of corticosterone and 18-0H-DOC were eluted. Corticosterone was estimated quantitatively by the sulphuric acid fluorescence method. A portion of the eluate containing the 18-0H-DOC was used to carry out the reaction with the Porter–Silber reagent. The major portion was oxidised with sodium periodate to form the γ-lactone of 18-0H-DOC and rechromatographed in the B_3-system of Bush [4] in which it travels more slowly than DOC and faster than 11 β-OH-progesterone. The region of the paper containing the γ-lactone of 18-0H-DOC was located in the radioscanner and eluted. Ten per cent of each eluate were removed for counting in a liquid scintillation spectrometer and the remainder subjected to gas liquid chromatography (GLC). The overflow of the TPG chromatogram which contained deoxycorticosterone (DOC), pregnenolone and progesterone was collected, and rechromatographed in Bush's B_3 system. The region containing DOC was eluted, the eluate acetylated, and rechromatographed in Bush's system A [4].

Progesterone and pregnenolone, which were located near the solvent front in the B_3 system, were eluted together, and the eluate rechromatographed in the E_1 system of Eberlein and Bongiovanni [6] in which the two steroids were separated. They were measured by GLC as described earlier [7]. Gas–liquid chromatography was carried out on an F + M model 402 chromatograph using 120 cm 3·8% SE-30 columns at 230°C, argon as carrier gas and flame ionization detection. For the quantitative estimations internal standards were used and the calculations were based on the straight line relation between the ratio of the concentrations of two steroids in a solution and the ratio of the peak heights on the GLC-tracing [7]. The γ-lactone of 18-0H-DOC had a retention time of 1·58 relative to that of cholestane (retention time 1·00). When it was established that the eluates did not produce any peaks with a retention time similar to that of 11 β-OH-progesterone this steroid was used as internal standard. More than 100 ng of 18-0H-DOC had to be present in the extracts in order to be detected by this method. The eluates of the regions of the A chromatograms which contained DOC-acetate were also subjected to GLC using again 11 β-OH-progesterone as internal standard [5]. The limit of the sensitivity of this method was about 30 ng/sample. All results were corrected for losses as indicated by the recoveries of the radioactive steroids added. As the quantities of some of the steroids estimated were very low, the amounts of steroids added with the radioactive tracers were subtracted from the final results. Eluates from chromatograms on which "blank samples" were developed, which contained no steroids but were otherwise treated in the same manner as the blood extracts, did not produce peaks in the relevant regions of the gas chromatography tracings.

Histology. In order to establish the amount of cortical tissue which was present in the enucleated adrenal glands, serial sections were made of all left glands. They were stained with haematoxylin–eosin, the microscope image projected

onto paper, the areas of cortical tissue and the blood clot enclosed by the capsule drawn, cut out and weighed. In this way it was possible to calculate the percentage of the total adrenal weight which was contributed by actual cortical tissue. This method assumes that the blood clots and the tissue have the same specific weight.

RESULTS AND DISCUSSION

The histological investigation of the left adrenal glands showed that out of 8 rats the glands of only 5 rats had been totally enucleated. The remaining 3 contained remnants of medullary tissue and some islets of organized, intact cortical tissue.

Whereas the sham operated control rats gained about 15 g in body weight in the days following the operation, the rats with enucleated adrenals gained only 4 g. The weight gain of the partially adrenal enucleated rats was within the range of that of the control rats. The mean volume of adrenal venous blood obtained in 15 min from the intact glands was 1·3 ml, from the regenerating glands it was 1·2 ml. Both groups required on average the infusion of 1 ml 0·9% sodium chloride to maintain a constant blood flow.

The results listed in Table 1 show the total amount of steroids secreted by the left adrenal gland of each rat under conditions of operative stress. The amount of corticosterone secreted by the regenerating adrenal glands was only 5% of that secreted by the intact glands. 18-0H–DOC (< 12% of normal) and progesterone (< 8% of normal) could not be detected in the venous blood from the enulceated glands. In contrast, the regenerating glands secreted about 50% of the amount of DOC which was secreted by the normal glands. Consequently, the ratio B/DOC, which was 50 in the rats with intact adrenal glands, was less than 10 in the rats bearing regenerating adrenal glands. Assuming that the relative secretion rates of the steroids in unstressed rats are similar to those measured under maximal stress conditions it can be speculated that the relative increase in the amount of DOC in the circulation contributes to the salt retention which was observed during the first week following adrenal enucleation. This possibility is further supported by the fact that administration of corticosterone during this period antagonizes the sodium retention [1]. The steroid secretion rates from the partially enucleated adrenal glands were between those of the other 2 groups. The corticosterone secretion was smaller than in the sham operated controls, the DOC secretion, if anything, higher.

By serial sectioning the adrenal glands and measuring the amount of regenerated adrenal tissue in the enucleated glands it became also possible to calculate the steroid secretion rates per unit tissue (Table 2). It was of particular interest to see that the ability of the newly regenerated cortical tissue to synthesize corticosterone and 18-OH–DOC was greatly diminished. The newly regenerated tissue could however synthesize DOC at the same rate as the normal tissue. The amounts of pregnenolone secreted from 100 mg normal or regenerating tissue were also not significantly different.

The blood extracts were also tested for steroids other than those listed in the tables by eluting the regions which remained after the known steroids had been removed from the B_3 and A chromatograms, and subjecting the eluates to GLC. No significant peaks with a relative retention time of less than 3·0 (relative to cholestane, 1·0) were found in any of the blood extracts. If the extracts contained

Table 1. *In vivo* secretion of steroids by normal adrenal glands and by enucleated adrenal glands on the 4th day after the operation. Secretion rates in μg/left adrenal gland/h

		Group I. Sham operated control rats						
No. of rats		Total adrenal weight (mg)	Steroid secretion rates (μg/left adrenal gland/h) Corticosterone (B)	DOC	18-OH-DOC	pregnenolone	progesterone	Ratio of secretion B/DOC
8	mean ± S.E.:	15·5 ± 0·85	45·01 ± 7·40	1·03 ± 0·18	25·8 ± 3·0	1·60 ± 0·42	0·84 ± 0·20	55·9 ± 11·6
		Group II. Rats with totally enucleated left adrenal glands.						
Rat No.		Calculated weight of regenerated cortical tissue (mg)	B	DOC	18-OH-DOC	Pregnenolone	Progesterone	B/DOC
9*		10·20	2·48	0·23	n.d.†	0·44	n.d.‡	10·9
4		6·58	2·84	0·64	n.d.	0·12	n.d.	4·4
6		6·14	1·16	0·46	n.d.	1·03	n.d.	2·5
11		6·17	2·48	0·26	n.d.	0·11	n.d.	9·5
18		5·97	3·60	0·77	n.d.	0·08	n.d.	4·7
	mean ± S.E.:	7·01 ± 0·80	2·51 ± 0·40	0·47 ± 0·10	< 3·00+	0·36 ± 0·18	< 0·06§	6·4 ± 1·61
		Group II compared with group I (group I = 100%)	5·6%	45·6%	< 12%	21·9%	< 8%	
		P-values (Student's t-test)	< 0·001	< 0·02		0·02–0·01		0·01–0·001

Table 1. *cont.*

Group III. Rats with partially enucleated left adrenal glands

(Some intact cortical tissue arranged in zones and medullary islets left)

Rat No.	Calculated wight of cortical tissue (mg)	B	DOC	18-OH-DOC	Pregneno-lone	Progeste-rone	B/DOC
22	15·27	24·96	2·02	lost	1·56	0·82	12·4
15	8·94	11·40	1·22	11·11	0·79	0·10	9·4
13	10·10	24·12	1·43	14·76	1·04	n.d.	16·8
mean values:	11·44	20·16	1·56	12·94	1·13	–	12·9

DOC = Deoxycorticosterone; *Adrenal cortex showed very active regeneration, many cells, but no zonation; n.d.: Not detected; †Limit of sensitivity of estimation method: 100 ng/sample; ‡Limit of sensitivity of estimation method: 25 ng/sample; §Calculated from the recoveries of the added radioactive steroids and the limit of the sensitivites of the estimation method.

Table 2. *In vivo* steroid secretion per unit tissue weight by normal and regenerating adrenal glands

No. of rats		Corticosterone	DOC	18OH-DOC	Pregnenolone	Progesterone
		Group I. Sham operated control rats				
		Steroid secretion rates in μg/100 mg adrenal tissue/h				
8	mean ± S.N.:	284·00 ± 36·4	7·28 ± 1·77	161·0 ± 22·7	10·96 ± 3·40	5·75 ± 1·34
		Group II. Rats with totally enucleated left adrenal glands				
		Steroid secretion rates in μg/100 mg regenerated cortical tissue/h				
5	mean ± S.E.:	37·4 ± 7·4	11·01 ± 4·19	< 10·0*	5·23 ± 2·92	< 2·0*
	Group II compared with group I (group I = 100%)	14·0%	151·0%	< 12·0%	48·0%	< 35·0%
	P-values (Student's t-test):	< 0·001	> 0·1		> 0·1	
		Group III. Rats with partially enucleated left adrenal glands (see Table 1)				
		Steroid secretion rates in μg/100 mg cortical tissue/h				
Rat No.						
22		163·5	13·23	Lost	10·22	5·37
15		127·5	13·65	124·3	8·84	1·12
13		238·8	14·16	146·1	10·30	< 0·08*
	Mean values:	176·6	13·68	135·2	9·79	–

*Calculated from the recoveries of the added radioactive steroids and the limit of the sensitivities of the estimation method (see Table 1).

any unknown steroids less polar than corticosterone then they were either destroyed under the conditions of GLC or they were only present in very small quantities.

The aldosterone content of the venous blood from newly regenerated adrenal glands has been measured by Tait (in Ref. 1) and was found to be lower than in the blood from normal adrenal glands.

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