J. Physiol. (1964), **172**, *pp.* 138–149
With 2 text-figures
Printed in Great Britain

THE PART PLAYED BY ACTH IN DETERMINING THE RATE OF ALDOSTERONE SECRETION DURING OPERATIVE STRESS

BY MARGARETHE HOLZBAUER

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

(*Received* 16 *January* 1964)

In order to interpret results of experiments on the control of aldosterone secretion in the dog (Holzbauer & Vogt, 1959, 1960, 1963) information on two questions was needed. First, information was required on the rate of secretion of adrenocorticotrophic hormone (ACTH) in a dog subjected to adrenal vein cannulation in chloralose anaesthesia, and secondly, on the effect of acute hypophysectomy and infusions of ACTH on the production of aldosterone.

The experiments described in this paper answer the second question by direct measurements of aldosterone in adrenal vein blood; the first question, requiring an estimate of ACTH secretion which cannot at present be measured directly, has been dealt with by comparing the rates of production of steroids by the adrenal before hypophysectomy with the rates found after hypophysectomy, during infusions of ACTH. A preliminary report on this work has been published (Holzbauer, 1963).

METHODS

Operative procedures

The experiments were carried out on mongrel dogs of different ages and both sexes. They weighed between 8·8 and 20 kg and were kept in the animal house for 2–52 days. All dogs except no. 334, which was kept for 2 days only, were vaccinated on their admission against distemper, either with non-specific γ-globulin, or with Epivax-plus (Burroughs Wellcome). Their daily ration consisted of 600 g dog biscuits and 300 g fresh meat, 4 g of a yeast extract and 4 g of a sheep-bone extract. This mixture contained approximately 30 m-equiv Na^+ and 65 m-equiv K^+ and was supplemented with a further 70 m-equiv Na^+ in the form of NaCl. Drinking water was unrestricted.

The anaesthesia used was ether followed by chloralose (BDH, 70 mg/kg as 0·67 % solution in 0·9 % NaCl) injected into the femoral vein. In some experiments further doses of 0·2 or 0·3 g of chloralose were required. The dogs were kept warm by an electric heating pad. A tracheal cannula was inserted, avoiding any interference with the blood supply to the thyroid gland. The region of the pituitary gland was exposed through the roof of the mouth. A dental drill was used and great care taken to avoid damage to the dural covering of the gland. A piece of sterispon (absorbable gelatin sponge B.P.) was lightly placed into the

bone cavity and the mouth was closed. During this part of the experiment the dog was put on artificial respiration (Starling pump, 20 rev/min, stroke 15 ml./kg body wt.).

The left adreno-lumbar vein was then exposed by an incision in the flank and a siliconed glass cannula inserted close to the lateral edge of the adrenal gland after accessible tributaries coming from other tissues had been tied. The cannula was connected by a silicone rubber tube to a T-piece, one limb of which would divert the blood flow into a measuring cylinder while the other was connected to a cannula tied into the central end of a femoral vein. After injection of heparin (350 u./kg) and ligation of the adreno-lumbar vein at its junction with the vena cava, the adrenal vein blood was either returned through the femoral vein shunt into the dog or drained into a cylinder standing in ice water. The blood pressure was recorded from the right femoral artery with a mercury manometer on a smoked drum.

Blood losses from adrenal vein blood collection or from wound bleeding were replaced by infusions of dog's blood. Mepyramine maleate, 1 mg/kg, was given intravenously in order to try to antagonize ill effects on the circulation caused by infusions of homologous blood (Bliss, Johns & Burgen, 1959, and Remington & Baker, 1959). The donor blood was obtained from non-hypophysectomized dogs which were exsanguinated under ether anaesthesia from a carotid artery on the day before the experiment. Heparin (6500 u./l.) and in some cases glucose (0·2 g/l.) was added. Glucose was reported to decrease the spontaneous haemolysis which occurs in dog's blood on standing (Swisher & Young, 1961). The blood was kept at +4° C for at least 24 hr, was filtered through gauze before use and warmed to 39° C in a water-bath in which it was kept in a slow rhythmic motion. The donor blood was obtained so far in advance of the experiment in order to ensure complete disappearance of any ACTH it contained. According to Nelson & Hume (1955) little or no ACTH can be found in dog's blood as early as 3 hr after withdrawal from the body.

Half an hour after the shunt flow had been established a sample of adrenal venous blood was collected over a period of 20–30 min. Artificial respiration was then resumed, the dural covering of the pituitary gland split and the gland removed by suction. A piece of sterispon was put in place of the pituitary, the cavity in the bone was filled with bone wax, the mouth closed and the artificial respiration discontinued. Another injection of heparin (150 u./kg) was given and the dog was allowed to rest for 1 hr. After this interval one or two adrenal blood samples were collected. Then ACTH solutions were infused into the left jugular vein at a rate of 0·7 ml./min by means of a Palmer slow infusion apparatus. Usually three concentrations differing by a factor of 10 were used in ascending order. Adrenal vein blood collections were started when the infusions had been running for 5 min and were continued for 5 min beyond the end of each infusion. Usually 1 min elapsed between the end of a collection period and the start of the next infusion. At the end of each experiment the skull was opened and the base of the brain searched for pituitary remnants.

Adrenocorticotrophic hormone

The ACTH used in these experiments was 'cortrophin' (Organon) of porcine origin for intramuscular injections, 1·7 i.u./mg. As the preparation was several years old, its potency was tested against a more recently manufactured batch of 'cortrophin' (Organon) of porcine origin prepared for intravenous use and containing 31 i.u./mg. The comparison was carried out on a hypophysectomized dog according to the method for ACTH assay described by Nelson & Hume (1955). The test showed that the older preparation had not lost activity: the ratio of the stimulating effect of the two batches on the secretion of cortisol and corticosterone in the hypophysectomized dog was about the same as the ratio of the activities given by the manufacturers for intravenous administration. The pressor activity of the preparation used was tested on the blood pressure of the pithed rat. It was found that 100 m-u. of ACTH were equivalent to about 0·8 m-u. of pitressin. The concentrations used for the experiments did not have any pressor effect in the dogs.

Chemical procedures

The basic outlines for the methods used for the estimation of aldosterone, cortisol and corticosterone have been described (Holzbauer & Vogt, 1961). Some modifications were made.

(1) The ethanolic phase obtained after defatting the extracts with petroleum ether was not evaporated in vacuo but was taken to dryness in a water bath at 40° C by means of a stream of compressed air.

(2) The aldosterone region in the first chromatogram was located by its position relative to cortisol. In a number of trial runs the ratio between the distance from the origin of the centre of the cortisol spot and the centre of the aldosterone spot was found to be 0·78. A rectangular piece of paper including a region 4 cm above and below the calculated centre of the aldosterone spot was eluted. In samples collected after hypophysectomy 40 μg cortisol was applied with the extract in order to make the cortisol region visible.

(3) The second chromatogram was run on 4 cm lanes, so that four samples could be applied to each paper.

(4) Washing of the papers used for the third chromatogram was restricted to the second washing for 48 hr in ethyl acetate-methanol.

(5) The chromatograms for the estimation of cortisol and corticosterone were run on papers washed as described under 4.

(6) Elution was often found to be incomplete if the size of the eluted rectangles was large. Therefore periods of 3 hr were allowed for papers 4×9 cm, and at least 4 hr for papers 8×9 cm.

(7) The paper blanks against which the colour formed by the steroids when allowed to react with blue tetrazolium was read were reduced by distilling the petroleum ether and the benzene used in the chromatography. The mean value for the blanks was reduced to the equivalent of 0·35 μg aldosterone (S.D. $\pm$ 0·1).

(8) The cortisol and corticosterone contained in the adrenal blood samples which were collected after hypophysectomy were not estimated by the 'macromethod' (Vogt, 1955) but were allowed to react with the small quantities of reagents used for the aldosterone estimations and the colour was read in the microcells.

(9) The recovery of aldosterone was estimated in each individual sample with the aid of 7-^{3}H-aldosterone (specific activity 20 μc/μg) which was apparently radiochemically pure. A solution containing 0·013 μc in 0·1 ml. of ethanol was prepared and kept at $-14°$ C. Immediately at the end of a period in which adrenal vein blood was collected 0·1 ml. of this solution was added to the blood. Recovery was determined from the tritium counts given by the final solution used for colorimetry. The procedure was as follows: after the aldosterone content of each sample had been estimated by its colour reaction with blue tetrazolium, the solution was transferred quantitatively into a counting vial with the help of 2 ml. ethanol and a Pasteur pipette. When 15 ml. of scintillator (4 g 2,5-diphenyloxazole and 0·1 g 1,4-bis-2-(5-phenyloxazolyl)-benzene in 1 l. toluene) was added a clear solution was obtained. The radio-activity was determined in a TRI-CARB liquid scintillation spectrometer. In order to calculate the recovery, the number of counts obtained from each sample was compared with that obtained from the quantity of 7-^{3}H-aldosterone originally added and measured in the presence of the same chemicals. For this purpose 0·1 ml. of the 7-^{3}H-aldosterone solution and 3 μg of cold aldosterone were pipetted into a counting vial. The volume was made up to 0·45 ml. with 95 % ethanol. Tetraethylammonium hydroxide and blue tetrazolium were added and the vial incubated as if it were a genuine sample. Acetic acid, 2 ml. of ethanol and the scintillator were then added. The number of counts obtained was regarded as 100 % recovery. The presence of ethanol and the other chemicals in the scintillator caused quenching by about 50 %. Variations of the degree of quenching in samples containing different amounts of cold or hot aldosterone or eluates from paper strips of different sizes were found to lie within the range of the counting error. Background counts

TABLE 1. Secretion rates of aldosterone, cortisol and corticosterone during stress, after hypophysectomy and during infusions of ACTH. Observations obtained in 6 representative experiments. S = adrenal blood sample

Dog no.	Body wt. (kg)			S_1 before hypophysectomy	S_2 started 60 min after hypophysectomy	S_3 started 90–120 min after hypophysectomy	S_4 During infusion of ACTH 0·03 (m-u./min/kg body wt.)	S_5 During infusion of ACTH 0·3 (m-u./min/kg body wt.)	S_6 During infusion of ACTH 3·0 (m-u./min/kg body wt.)
331 (female)	10·2	Secretion rates (μg/g adrenal/hr)	Aldosterone	9·5	4·0	2·8	6·7	10·6	11·4
			Cortisol	—	46·0	4·0	762·0	1493·0	1493·0
			Corticosterone	—	61·0	23·0	244·0	435·0	435·0
		Mean blood pressure (mm Hg)		160	130	137	140	140	140
		'Adrenal' blood flow (ml./hr)		180	106	108	103	86	62
334 (female)	16·0	Secretion rates (μg/g adrenal/hr)	Aldosterone	11·9	7·6	3·3	8·8	15·1	22·4
			Cortisol	1228·0	109·0	65·0	573·0	960·0	1189·0
			Corticosterone	436·0	55·0	44·0	218·0	404·0	431·0
		Mean blood pressure (mm Hg)		150	135	135	130	135	130
		'Adrenal' blood flow (ml./hr)		255	276	278	312	274	300
340 (female)	12·8	Secretion rates (μg/g adrenal/hr)	Aldosterone	16·8	1·9	0·6	7·2	20·2	26·3
			Cortisol	1268·0	72·0	27·0	960·0	1467·0	1368·0
			Corticosterone	725·0	22·0	24·0	444·0	797·0	769·0
		Mean blood pressure (mm Hg)		135	123	130	130	132	140
		'Adrenal' blood flow (ml./hr)		130	91	82	82	94	101
341 (female)	16·5	Secretion rates (μg/g adrenal/hr)	Aldosterone	21·3	18·4	21·3	18·4	27·0	26·1
			Cortisol	788·0	69·0	33·0	1216·0	1327·0	1309·0
			Corticosterone	299·0	44·0	48·0	552·0	735·0	713·0
		Mean blood pressure (mm Hg)		165	150	150	148	150	150
		'Adrenal' blood flow (ml./hr)		144	115	110	137	158	182
342 (male)	14·5	Secretion rates (μg/g adrenal/hr)	Aldosterone	14·5	7·1	13·2	18·3	14·7	29·3
			Cortisol	1418·0	106·0	89·0	1227·0	1362·0	1384·0
			Corticosterone	826·0	96·0	82·0	647·0	726·0	949·0
		Mean blood pressure (mm Hg)		140	135	135	140	140	140
		'Adrenal' blood flow (ml./hr)		156	168	120	120	120	139
343 (male)	15·3	Secretion rates (μg/g adrenal/hr)	Aldosterone	18·8	12·7	7·2	16·1	—	22·5
			Cortisol	908·0	750·0	127·0	285·0	773·0	1448·0
			Corticosterone	608·0	300·0	90·0	135·0	593·0	847·0
		Mean blood pressure (mm Hg)		152	148	150	150	150	140
		'Adrenal' blood flow (ml./hr)		187	153	153	163	156	168

were not affected by the same chemicals. In the present experiments a mean recovery of 35·1 % with a standard deviation of ±4·2 % was obtained. All figures for secretion rates given in the Tables are corrected for 100 % recovery.

The figures obtained for cortisol and corticosterone have not been corrected for losses. The method for their estimation is less involved and recoveries average 80 % (Holzbauer & Vogt, 1961).

RESULTS

The results are summarized in Tables 1 and 2 and Figs. 1 and 2.

Secretion rate of ACTH during stress. Before hypophysectomy, the mean secretion rate of glucocorticoids (sum of cortisol and corticosterone) in eleven dogs was found to be 1814 μg/g adrenal/hr and the range 1087 to

TABLE 2. Adrenal steroid secretion, mean blood pressure and adrenal blood flow during stress, after hypophysectomy and during infusions of ACTH. Means ± standard errors calculated from results obtained in experiments on eleven dogs, including the results given in Table 1.

n = number of observations in each case. S = adrenal blood sample.

		S_1	S_2	S_3	S_4	S_5	S_6
			started 60 min after	started 90–120 min after	During infusion of ACTH		
		before hypophysectomy			0·03 (m-u./min/kg body wt.)	0·3 (m-u./min/kg body wt.)	3·0 (m-u./min/kg body wt.)
Secetion rates (μg/g adrenal/hr) ± S.E.	Aldosterone	14·5 ±1·28 (n = 11)	8·2 ±1·65 (n = 11)	7·3 ±2·41 (n = 8)	12·2 ±1·72 (n = 8)	17·9 ±2·21 (n = 8)	23·5 ±2·21 (n = 7)
	Cortisol	1200·0 ±91·0 (n = 9)	187·0 ±71·5 (n = 10)	71·0 ±23·4 (n = 8)	864·0 ±117·0 (n = 8)	1527·0 ±204·1 (n = 9)	1475·0 ±116·3 (n = 7)
	Corticosterone	614·0 ±55·4 (n = 9)	93·0 ±27·4 (n = 10)	54·0 ±11·6 (n = 8)	351·0 ±65·0 (n = 8)	682·0 ±60·0 (n = 9)	713·0 ±77·1 (n = 7)
Mean blood pressure (mm Hg) ± S.E.		149 ±4·8 (n = 11)	134 ±4·2 (n = 11)	142 ±3·6 (n = 8)	139 ±2·6 (n = 8)	135 ±6·0 (n = 9)	134 ±6·1 (n = 7)
'Adrenal' blood flow (ml./hr) ± S.E.		233 ±27·9 (n = 11)	202 ±33·1 (n = 11)	195 ±41·1 (n = 8)	180 ±33·5 (n = 8)	181 ±25·7 (n = 9)	168 ±29·8 (n = 7)

2483. One hour after hypophysectomy it had decreased by 85 %, and after a further hour by 93 %. An infusion of 0·03 m-u. ACTH/min/kg body wt. increased the mean secretion rate to 66 % of the prehypophysectomy value. During the subsequent infusion of 0·3 m-u. ACTH/min/kg body wt. the secretion was increased to 120 % of the prehypophysectomy value. The infusion of 3·0 m-u. ACTH/min/kg body wt. did not cause a further rise. In Fig. 1 the infusion rates of ACTH are plotted against the secretion rates of the glucocorticoids on a semi-logarithmic scale. The diagram includes a value for the glucocorticoid secretion during an infusion of 0·01 m-u. ACTH/min/kg body wt. It was obtained in a different set of

experiments on five dogs in which the infusion of ACTH was started 2 hr after hypophysectomy and 10 min later adrenal venous blood was collected for 20–30 min whilst the ACTH infusion was continued. This value suggests that a straight-line relation between the logarithm of the infusion rate of ACTH and the glucocorticoid secretion exists up to a rate of 0·3 m-u./min/kg body wt. This straight line may be used for the estimation of the natural ACTH secretion rate before hypophysectomy. The mean glucocorticoid secretion rate observed before hypophysectomy (1814μg/g

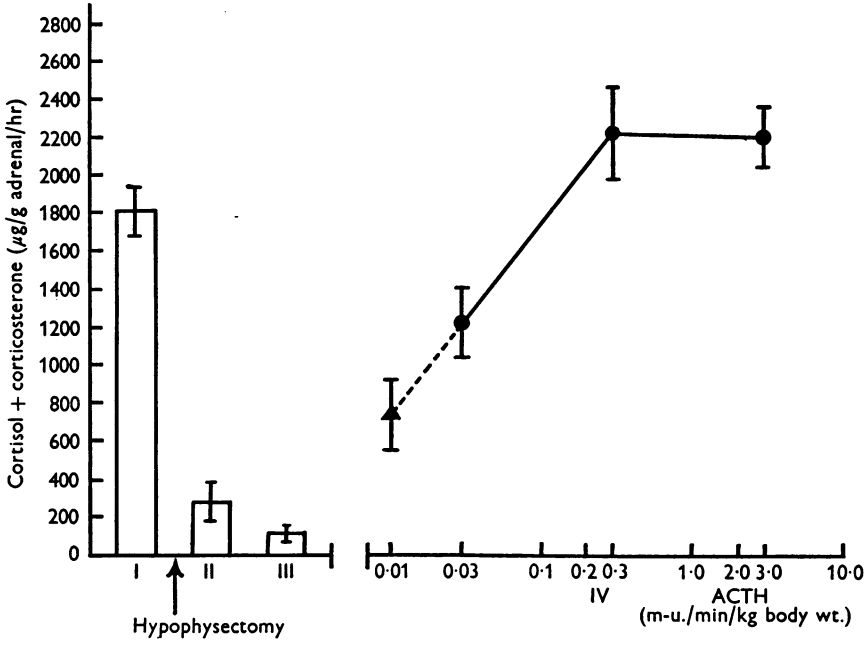

Fig. 1. Means of secretion rates of the sum of cortisol and corticosterone with standard errors. I, under operative stress; II, started 60 min after hypophysectomy; III, started 90–120 min after hypophysectomy; IV, dose–response curve between rates of glucocorticoid secretion and ACTH infusion. ▲, Point obtained in a different set of experiments (see text) from those marked with ●.

adrenal/hr) would correspond to a secretion rate of ACTH in the range of 0·03–0·3 m-u./min/kg body wt., the mean being 0·12 m-u./min/kg body wt.

Before hypophysectomy, the ratio of mean secretion rate of cortisol over mean secretion rate of corticosterone was 1·95; 2 hr afterwards, the ratio was decreased to 1·31. Infusion of 0·03 m-u. ACTH/min/kg body wt. increased it to 2·46.

Aldosterone secretion. The mean secretion rate of aldosterone before hypophysectomy was 14·5 μg/g adrenal/hr, ranging from 9·5 to 21·3 μg.

One hour after hypophysectomy it was significantly decreased to 8·16 μg (range 1·9–18 μg). There was some fall in every animal. Two hours after hypophysectomy the mean secretion rate of aldosterone was further reduced to 7·3 μg. During the infusion of 0·03 m-u. ACTH/min/kg body wt. aldosterone secretion rose in seven out of eight dogs. The mean attained was 84 % of the secretion rate before hypophysectomy. An infusion of 0·3 m-u. ACTH increased aldosterone production to 120 % and an infusion of 3·0 m-u. ACTH to 162 % of the initial value. By plotting the infusion

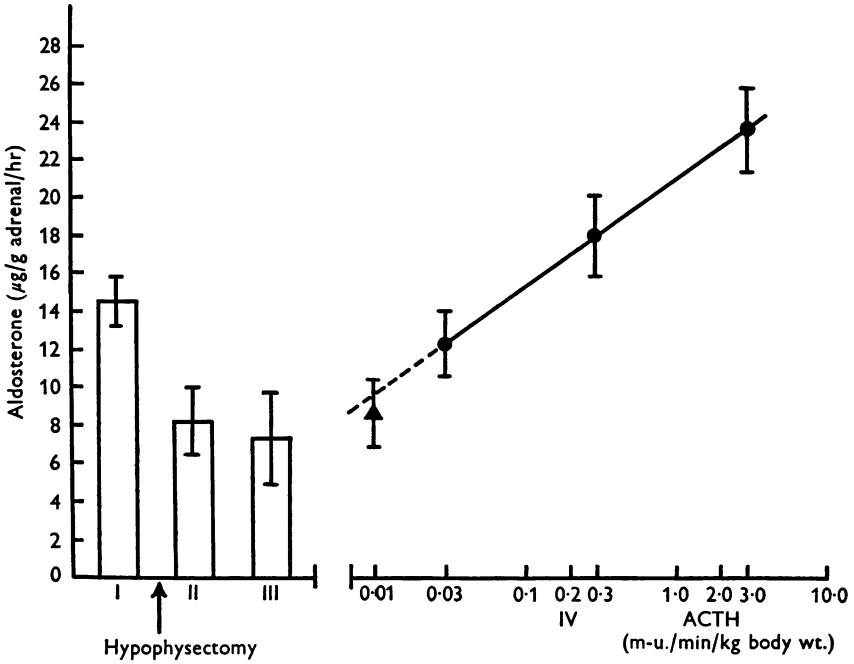

Fig. 2. Means of the secretion rates of aldosterone with standard errors. I–IV, ▲, explanation see Fig. 1.

rates of ACTH against the mean secretion rates of aldosterone on a semi-logarithmic scale (Fig. 2) a straight line dose–response curve was obtained. According to this curve an ACTH secretion rate in the range of 0·03–0·3 m-u./min/kg body wt. (the mean being 0·075 m-u./min/kg body wt.) would be necessary to obtain the mean aldosterone secretion rate observed before hypophysectomy. This estimate is of the same order as that determined from the glucocorticoid responses.

The mean blood pressure before hypophysectomy was 149 mm Hg (± 4·8 S.E.). One hour after hypophysectomy it was significantly decreased to 134 mm Hg (± 4·2) but after another hour it was back to the initial

value ($142 \pm 3{\cdot}6$). During the three infusions of ACTH there was a small but steady drop from $139 \pm 2{\cdot}6$ to $134 \pm 6{\cdot}1$. The mean 'adrenal' blood flow before hypophysectomy was 233 ± 28 ml./hr. After hypophysectomy it fell to 202 ± 33 in 1 hr and to 195 ± 41 in 2 hr. The ACTH infusions had no significant influence on the adrenal blood flow.

DISCUSSION

A direct assessment of the secretion rate of ACTH *in vivo* is not practicable. Therefore an indirect method has been devised for the dog, in which the animal's own adrenal was used as an indicator. Glucocorticoid secretion was measured under conditions of stress. The pituitary gland was then removed and corticoid secretion in response to ACTH infusions of different concentrations determined. By comparing the two sets of figures it was found that the mean secretion rate of ACTH during operative stress under chloralose anaesthesia was about 0·12 m-u./min/kg body wt.

Results obtained in this way are only valid if ACTH is the sole important factor which controls the secretion rate of cortisol and corticosterone. Several other polypeptides have recently been reported to affect steroid secretion in the hypophysectomized animal. One which may be of consequence in the present work is angiotensin. Davis, Ayers & Carpenter (1961) have shown that the already very low secretion rate of corticosterone is further decreased by nephrectomy in hypophysectomized, Na^+-depleted dogs. A detailed study of the response of adrenocortical secretion to infusions of renin and angiotensin was recently published by Slater, Barbour, Henderson, Casper & Bartter (1963). In sodium-loaded, hypophysectomized, nephrectomized dogs infusion of angiotensin II, 3·0 μg/min, caused a rise in the secretion rate of cortisol plus corticosterone by about 100 %. However, in absolute terms, this increase is very small indeed; the levels reached are still within the range of the secretion rates observed 2 hr after hypophysectomy in the present work on dogs which were sodium loaded and not nephrectomized. The effective doses used by Slater *et al.* caused a large rise in blood pressure and were probably far above levels occurring naturally. Thus it is unlikely that under the conditions of the present experiments renin release would have effects on the secretion rates of glucocorticoids which would vitiate the calculations carried out in the preceding paragraphs.

Another factor which could interfere with the assay are changes in the plasma concentrations of Na^+ and K^+ in the course of the experiment. Gann, Cruz, Casper & Bartter (1962) have investigated the effect of intravenous infusions of potassium chloride on the secretion rate of aldosterone and 17-hydroxy-corticosteroids (17-OHCS) in the stressed dog with intact pituitary. Increases in the plasma potassium from an initial

level of between 3 and 3·8 to concentrations up to 10 m-equiv/l. did not have any consistent effect on the secretion of 17-OHCS. Davis, Urquhart & Higgins (1963) infused concentrated solutions of potassium chloride and potassium sulphate into hypophysectomized dogs. They found that increases in the plasma potassium concentration similar to those obtained by Gann *et al.* caused significant rises in the secretion rate of corticosterone, but these changes were quite small compared to the large increase caused by ACTH. In a large series of experiments in which adrenal vein cannulation in the chloralosed dog was carried out (M. Holzbauer & M. Vogt, to be published), plasma concentrations of Na^+ and K^+ varied so little that their influence on secretion of cortisol and corticosterone can be neglected.

Before hypophysectomy, secretion rate of cortisol and corticosterone was submaximal in all dogs except no. 334 (Table 1). This would indicate either that the stress was not maximal, or that the limit of endogenous ACTH production by the pituitary had been reached.

Two hours after hypophysectomy the mean aldosterone secretion was decreased to 50 % whilst the glucocorticoid secretion was reduced to 7 % of its initial value. An infusion of 0·03 m-u. ACTH/min/kg body wt. increased the mean secretion of both aldosterone and the glucocorticoids, but neither reached the secretion rate observed during stress. An infusion of 0·3 m-u. ACTH/min/kg body wt. increased the mean glucocorticoid and the mean aldosterone secretion to 120 % of the stress value. However, when the infusion rate of ACTH was 3 m-u., the mean aldosterone secretion rose further to 162 % whereas the mean glucocorticoid production remained stationary. This indicates that in a dog with intact pituitary an increase in aldosterone secretion due to ACTH release could occur which is not accompanied by a rise in glucocorticoid secretion. This possibility had previously been discussed by Mulrow & Ganong (1961). However, it is not known whether the pituitary gland is capable of releasing these large amounts of ACTH for any length of time.

There is little doubt that ACTH is not the only, and not even the most important, factor in the control of aldosterone secretion. It is therefore to be expected that hypophysectomy or ACTH infusion will only affect aldosterone secretion *pari passu* with glucocorticoid secretion when stronger stimuli of aldosterone secretion, such as lack of sodium in the tissues, or high angiotensin concentration in the blood, are not present. This explains apparent discrepancies in the literature on the effect of hypophysectomy on secretion of aldosterone. A fall in aldosterone secretion after hypophysectomy was observed in stressed dogs by Farrell, Rauschkolb & Royce (1955), Ganong, Lieberman, Daily, Yuen, Mulrow, Luetscher & Bailey (1959), Davis, Bahn, Yankopoulos, Kliman & Peterson (1959) and others. All these authors found that ACTH increased aldosterone secretion, but to

very variable degrees. Slater *et al.* (1963), on the other hand, studied the effect of hypophysectomy on unstressed, Na^+-depleted dogs. In these animals there was no decrease in the secretion rate of aldosterone 18 hr later and little response to ACTH.

In the present experiments the extent of the fall in aldosterone secretion after hypophysectomy varied greatly although all controllable parameters were kept identical. The extremes are represented by dogs 340 and 341 (Table 1). In dog 340 aldosterone secretion was reduced by 97% 2 hr after hypophysectomy. In dog 341 it remained nearly unchanged, although, or perhaps because, the initial secretion rate was higher. An absence of aldosterone stimulating substances other than ACTH in dog 340 and an excess of them in dog 341 would explain the difference.

Differences in the effects can also be caused by the differences in the ACTH preparations. Thus Farrell, Fleming, Rauschkolb, Yatsu, McCally & Anderson (1958) found, in the decerebrated dog, that β- and δ_1- corticotrophin were equally potent in stimulating cortisol secretion but that δ_1-corticotrophin was several times more active than β-corticotrophin in stimulating aldosterone secretion. In the present work a crude preparation of ACTH was used because it was felt that it would resemble the natural secretion of the pituitary more closely than highly purified preparations.

There was a linear relation between aldosterone secretion and the logarithm of the dose of ACTH infused into the hypophysectomized dog. When the amount of ACTH corresponding to the aldosterone secretion observed before hypophysectomy was read from this line the value of ACTH was similar to the estimate of endogenous secretion of ACTH during stress calculated from glucocorticoid secretion. This may indicate that the factors other than ACTH which control aldosterone secretion remained constant in the course of the experiment. They would maintain the background secretion on which the effect of ACTH would be superimposed.

The dose–response curve for aldosterone is not as steep as that for the glucocorticoids. In order to double an initial secretion rate of 10 μg aldosterone/g adrenal/hr the amount of circulating ACTH would have to increase by a factor of 37, whereas only a 10-fold rise would be required to double a glucocorticoid secretion of 1000 μg/g adrenal/hr.

SUMMARY

1. The secretion rate of ACTH during operative stress under chloralose anaesthesia in Na^+-loaded dogs was determined by an indirect method which used glucocorticoid secretion by the dog's own adrenal as an index of circulating ACTH. The rate of release of ACTH was found to lie between 0·03 and 0·3 m-u. ACTH/min/kg body wt.

2. Under the same conditions the mean secretion rate of aldosterone

was 14·5 μg/g adrenal/hr (S.E. ± 1·28). It was decreased by 50 % 2 hr after hypophysectomy. On intravenous infusions of ACTH at rates of 0·03, 0·3 and 3·0 m-u./min/kg body wt. secretion increased linearly when plotted against the log dose of ACTH.

3. The mean secretion rate of cortisol plus corticosterone before hypophysectomy was 1814 μg/g adrenal/hr (S.E. ± 135·5). It was decreased by 93 % 2 hr after hypophysectomy. The secretion was stimulated by infusions up to 0·3 m-u. ACTH/min/kg body wt. but no further increase was achieved by ten times that dose.

My thanks are due to Dr J. B. Dekanski and Dr R. P. Edkins (Organon Labs.) for samples of ACTH. The sample of tritiated aldosterone was a gift to Dr M. Vogt by the Endocrinology Study Section, the National Institutes of Health, Bethesda, through the courtesy of Dr M. M. Graff. I am grateful to Dr M. Vogt, F.R.S., for valuable discussions and the estimation of the pressor activity of the ACTH and to Miss Helen Newport for excellent technical assistance.

REFERENCES

Bliss, J. Q., Johns, D. G. & Burgen, A. S. V. (1959). Transfusion reactions due to plasma incompatibility in dogs. *Circulation Res.* **7**, 79–85.

Davis, J. O., Bahn, R. C., Yankopoulos, N. A., Kliman, B. & Peterson, R. E. (1959). Acute effects of hypophysectomy and diencephalic lesions on aldosterone secretion. *Amer. J. Physiol.* **197**, 380–386.

Davis, J. O., Ayers, C. R. & Carpenter, C. C. J. (1961). Renal origin of an aldosterone-stimulating hormone in dogs with thoracic caval constriction and in sodium-depleted dogs. *J. clin. Invest.* **40**, 1466–1474.

Davis, J. O., Urquhart, J. & Higgins, J. T. Jr. (1963). The effects of alterations of plasma sodium and potassium concentration on aldosterone secretion. *J. clin. Invest.* **42**, 597–609.

Farrell, G. L., Rauschkolb, E. W. & Royce, P. C. (1955). Secretion of aldosterone by the adrenal of the dog. Effects of hypophysectomy and ACTH. *Amer. J. Physiol.* **182**, 269–272.

Farrell, G. L., Fleming, R. B., Rauschkolb, E. W., Yatsu, F. M., McCally, M. & Anderson, C. H. (1958). Steroidogenic properties of purified corticotropins. *Endocrinology*, **62**, 506–512.

Gann, D. S., Cruz, J. F., Casper, A. G. T. & Bartter, F. C. (1962). Mechanism by which potassium increases aldosterone secretion in the dog. *Amer. J. Physiol.* **202**, 991–996.

Ganong, W. F., Lieberman, A. H., Daily, W. J. R., Yuen, V. S., Mulrow, P. J., Luetscher, J. A. Jr. & Bailey, R. E. (1959). Aldosterone secretion in dogs with hypothalamic lesions. *Endocrinology*, **65**, 18–28.

Holzbauer, M. (1963). Aldosterone secretion during operative stress in relation to release of ACTH. *J. Physiol.* **168**, 40 *P*.

Holzbauer, M. & Vogt, M. (1959). Observations on the control of secretion of aldosterone. *J. Physiol.* **148**, 13–14 *P*.

Holzbauer, M. & Vogt, M. (1960). The effect of anoxia produced by removal of red cells on aldosterone secretion from the dog adrenal. *J. Physiol.* **153**, 55 *P*.

Holzbauer, M. & Vogt, M. (1961). Corticosteroids in plasma and cells of adrenal venous blood. *J. Physiol.* **157**, 137–156.

Holzbauer, M. & Vogt, M. (1963). Splanchnotomy and aldosterone secretion. *J. Physiol.* **165**, 21 *P*.

Mulrow, P. J. & Ganong, W. F. (1961). The effect of hemorrhage upon aldosterone secretion in normal and hypophysectomized dogs. *J. clin. Invest.* **40**, 579–585.

Nelson, D. H. & Hume, D. M. (1955). Corticosteroid secretion in the adrenal venous blood of the hypophysectomized dog as an assay for ACTH. *Naval Medical Research Institute*, **13**, 481–490.

REMINGTON, J. W. & BAKER, C. H. (1959). Plasma volume changes accompanying reactions to infusions of blood or plasma. *Amer. J. Physiol.* **197**, 193–200.

SLATER, J. D. H., BARBOUR, B. H., HENDERSON, H. H., CASPER, A. G. T. & BARTTER, F. C. (1963). Influence of the pituitary and the renin-angiotensin system on the secretion of aldosterone, cortisol and corticosterone. *J. clin. Invest.* **42**, 1504–1520.

SWISHER, S. N. & YOUNG, L. E. (1961). The blood grouping systems of dogs. *Physiol. Rev.* **41**, 495–520.

VOGT, M. (1955). Inhibition by hexoestrol of adrenocortical secretion in the rat. *J. Physiol.* **130**, 601–614.