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ACTIONS AND INTERACTIONS OF ALDOSTERONE MONO-ACETATE AND NEUROHYPOPHYSIAL HORMONES ON THE ISOLATED CAT KIDNEY

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Intravenous infusions of quantities of aldosterone too small to affect renal function raised the concentration of potassium in the plasma of cats under chloralose anaesthesia (Davey & Lockett, unpublished). This observation indicated extra-renal action of the hormone and raised the question whether the effects of aldosterone on renal function were predominantly the result of the direct action of this compound within the kidney itself or were, in large part, the consequence of its extra-renal effects. The existence of extra-renal actions of mineralocorticoids is well known; they have been described by Wilson (1957) and Flanagan, Davis & Overmann (1950) amongst others. There has, however, been no direct demonstration of the intrarenal effects of aldosterone under conditions which exclude the possibility of extra-renal effects. The nearest approach to such conditions is found in the work of Barger, Berlin & Tulenka (1958) and of Ganong, Mulrow & Hollinger (1957). Barger and his collaborators studied the effect of infusions of aldosterone into the left renal artery of the dog. Unilateral effects were observed in some of their experiments, but these were preceded by a long latent period, and no observations were made of the effect of their infusions on the concentrations of electrolytes in plasma. Ganong and his colleagues used larger doses of aldosterone and were unable to show that the kidney which received the intra-arterial injection of hormone responded either more markedly or more rapidly than did the other.

The object of our experiments was therefore to study the renal actions of aldosterone in kidneys perfused with blood and isolated from all nervous and hormonal continuity with the animal body.

METHODS

The cats used were of male, female or neuter sex and weighed from 1·3 to 4·6 kg. Young animals were commonly selected to provide the heart–lung–kidney preparations and the older animals were used as blood donors. Only lactating females or those in advanced stage

of pregnancy were judged unsuitable for use. In all but two experiments the kidney and the heart–lung preparation were taken from the same cat (Lockett, 1959).

Anaesthesia was induced in the heart–lung and blood-donor cats with a mixture of ether and chloroform (1:4 (v/v)) and was maintained in sixteen experiments by the intravenous injection of 8·0 ml. 1% chloralose in 0·9% NaCl per kilogram. In all other experiments anaesthesia was induced with the same mixture of chloroform and ether, but the animals were then all made 'spinal' by passing a blunt rod upwards through the foramen magnum to destroy the medulla, pons, mid-brain and thalamus. This procedure tears the pituitary stalk.

Each experiment began with the collection of heparinized blood (heparin 1500 u./100 ml. blood) for the perfusion circuits from donor cats. Most commonly approximately half of this blood was stored at 7° C during the night and next day until the heart–lung–kidney preparation was made. The first step in this preparation was the cannulation of the left ureter with No. 46 polythene tubing inserted into the renal pelvis through the upper 1–2 cm of the ureter. Next, the kidney was mobilized by ligature and section of any branches of the renal artery or vein which supplied extra-renal tissue and of any small vessels making anastomosis through the renal capsule. Then the aorta and inferior vena cava were prepared for cannulation. All branches except those to and from the left kidney were divided between ligatures from a point 0·5 cm above to 3·0 cm below the left renal branches. Finally, loose ligatures were placed around the abdominal aorta and the inferior vena cava to assist in the very rapid cannulation of these vessels later in the experiment. A heart–lung preparation was then made in the same cat (Lockett, 1957). Immediately after the external circuit of the heart–lung preparation had been established by ligature of the third part of the aortic arch and the inferior vena cava in the thorax, the abdominal aorta and cava were ligated above and cannulated below the origins of the left renal vessels. Then the kidney was swiftly freed by appropriate sections in the ureter, aorta and cava and was perfused through a branch in the external circuit of the heart–lung preparation which arose from a point 5 cm below the peripheral resistance, between it and the brachiocephalic cannula. The renal venous blood was returned by gravity through a 2 mm wide polythene tube to the heart–lung reservoir (Fig. 1). Perfusion of the kidney had begun within 3 min of the ligation of the thoracic aorta which rendered it ischaemic. The preparation was completed in 1–2 hr, according to the time taken for the ureteric cannulation.

There were five types of experiment:

Group I (14 expts.). Anaesthesia was maintained by chloralose, as described above, during the bleeding of the cats and the making of the heart–lung–kidney preparation.

Group II (2 expts.). Anaesthesia as in Group I, but the bleeding of the donor cats was preceded by ligature of the brachiocephalic and left subclavian arteries and was thus effected without there being a circulation through the head.

Group III (5 expts.). After the induction of anaesthesia with chloroform and ether the animals were made spinal as described above after the ligation of the brachiocephalic and left subclavian arteries. Bleeding of the donors and the heart–lung–kidney preparation was thus effected without a circulation through the head.

Group IV (4 expts.). These were prepared as in Group III, except that the brachiocephalic and subclavian arteries were not tied. At the most, the carotid arteries were only temporarily occluded for the moment of spinalization. There was therefore circulation in the head during the bleeding of donors and during the operative preparation.

Group V (4 expts.). The preparations were made under chloralose anaesthesia as in Group I, but the hypophyses were removed from both donor and heart–lung–kidney cats immediately the chloralose had been administered.

Hypophysectomy was begun by the exposure and trephining of the right temporal bone. Then the right side of the vault of the cranium was removed with nibbling forceps, and bleeding from the bone and sinuses was arrested by gentle pressure and bone-wax substitute.

Next the dura was opened to expose the brain and the temporal lobe was lifted so that the optic chiasma and the hypophysis could be seen clearly. The latter was removed with forceps and the resultant bleeding was stopped by gentle pressure before the temporal lobe was lowered into its normal position. Ten minutes later, blood donors were bled from a carotid artery or the operative procedure was begun.

The temperature of the blood and of the kidney chamber varied from 36 to 38° C and the arterial pressure from 126 to 138 mm Hg on different occasions, but these remained constant throughout each experiment. Urine was collected during measured serial periods of time; blood samples were taken at the mid points of these periods from the blood returning to the reservoir through the peripheral resistance (Fig. 1), and were therefore specimens of the

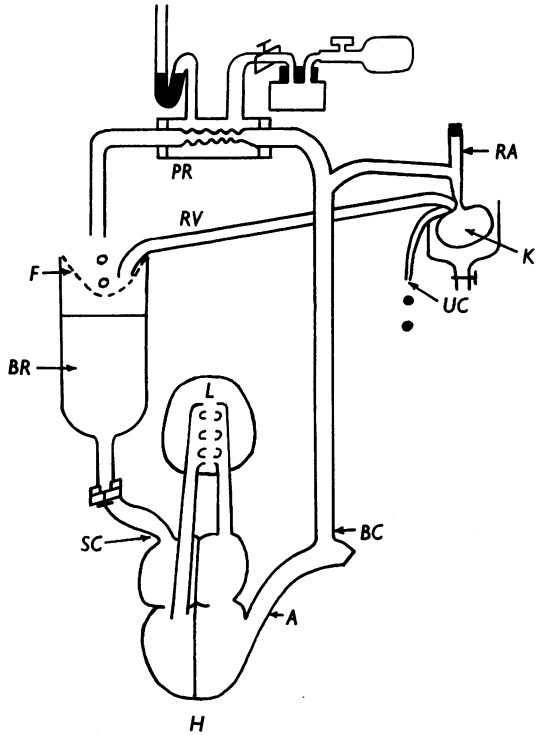

Fig. 1. Diagram of the circuit used to perfuse the kidney from a heart–lung preparation. *A* Aorta, *BC* brachiocephalic cannula, *BR* blood reservoir, *F* filter, *H* heart, *K* kidney, *L* lungs, *PR* peripheral resistance, *RA* renal arterial cannula, *RV* renal vein, *SC* superior caval cannula, *UC* ureteric cannula.

arterial blood supplying the kidney. Hormones were added to the blood in the reservoir 1 min before the end of a period of urine collection. Renal blood flow was measured directly by collecting the venous return from the kidney into a measuring cylinder for a period of time determined by stopwatch.

Creatinine (British Drug Houses, Ltd) was mixed with the blood before the perfusion circuit was filled. Protein-free filtrates of plasma were prepared by the modified tungstate method of Folin & Wu (Smith, 1956) and concentrations of creatinine were estimated both in these filtrates and in urine by the method of Brod & Sirota (1948). The concentration of chloride ions in the urine was determined by the micromethod of Brun (1949) and of sodium and potassium in plasma and urine by means of an EEL flame photometer.

The DL-aldosterone monacetate used was a gift from Ciba Laboratories, Ltd. Synthetic oxytocin (Syntocinon; Sandoz Products, Ltd), natural oxytocin (Pitocin; Parke, Davis, Ltd) and natural vasopressin (Pitressin; Parke, Davis, Ltd) were obtained commercially.

RESULTS

The renal blood flow (RBF) always increased, sometimes fourfold, in the first 20–60 min of an experiment, to reach a plateau level (Fig. 2). The minute volume of urine also increased, becoming constant with or before the RBF in the presence of chloralose (Fig. 2) and with or after the RBF in its absence. This plateau was maintained without great change for several hours (Fig. 2) and during this time the actions of hormones were

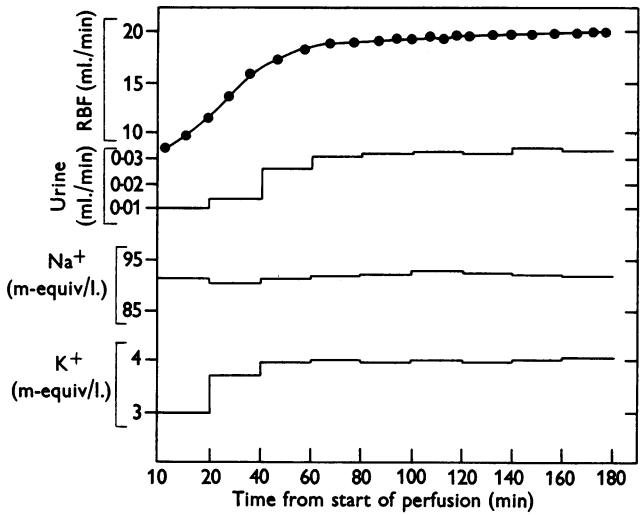

Fig. 2. Renal blood flow, urine volume, and concentrations of sodium and potassium in urine of a kidney perfused without addition of hormones. Kidney weight 7·4 g; an experiment of Group I.

tested. Table 1 shows the values reached in the early stage of the plateau by preparations of the various groups (see above). The effect of aldosterone on renal function depended on the manner of the preparation, but was never accompanied by change in the electrolyte concentrations of plasma.

Actions of aldosterone in experiments of Group I and Group IV

In each case blood was passing through the head during bleeding of the donor animal and until the heart–lung preparation was made; in Group IV the animals were spinal and in Group I under chloralose anaesthesia.

Figure 3 shows the typical result in an experiment of Group IV. Fifty minutes after the experiment began, when the RBF and the rate of urine excretion had reached the plateau, 0·5 μg DL-aldosterone monoacetate was added to the blood in the reservoir to mix with 150 ml. of circulating blood.

After a latent period, which varied from 3 to 5 min in different experiments, the RBF increased to reach a maximum in 10–15 min. Reduction in the minute volume and in the concentrations of sodium and chloride in the urine became apparent in the first 15 min after the addition of aldosterone and reached a maximum in the subsequent period of urine collection. The concentration of potassium in the urine either fell slightly, as in

TABLE 1. Comparison of the function of kidneys perfused with blood from chloralosed, spinal and hypophysectomized (chloralosed) cats, in the absence of added hormone: mean ± S.E. of mean, no. of expts. in brackets

Type	Kidney weight (g)	Renal blood flow (ml./min)	G.F.R. (ml./min)	Urinary excretion (μequiv/min) Na+	K+	Cl−	Urine vol. (ml./min)
Chloralosed							
Group I: with head circulation	9·6±0·43 (12)	22·2±2·64 (12)	1·61±0·21 (9)	13·3±3·11 (10)	2·4±0·48 (10)	15·2±3·48 (10)	0·08±0·02 (12)
Group V: hypophysectomized	9·2±0·65 (4)	20·0±2·53 (4)	2·25±0·47 (4)	31·5±5·79 (4)	3·9±0·69 (4)	38·0±7·09 (4)	0·22±0·08 (4)
Spinal							
Group III: no head circulation	9·9±0·64 (5)	22·6±3·09 (5)	1·76±0·04 (3)	11·2±4·36 (5)	2·0±0·32 (5)	16·8±3·41 (5)	0·05±0·04 (5)
Group IV: with head circulation	8·5±0·53 (4)	23·0±2·08 (4)	1·70±0·05 (3)	13·5±1·37 (4)	2·3±0·63 (4)	17·0±1·73 (4)	0·03±0·01 (4)

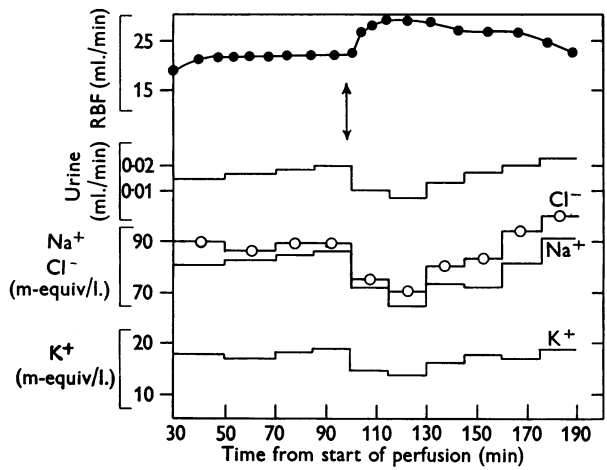

Fig. 3. Effect in an experiment of Group III of aldosterone 0·5 μg at arrow.

this experiment, or remained unchanged; there was always a reduction in the output of potassium ions per minute. In Fig. 3 there was recovery in the composition of the urine in parallel with that in the RBF. More often, however, changes in the RBF have been outlasted by those in the composition of the urine.

The chloralosed preparations were relatively insensitive to aldosterone, so that the doses used in experiments of Group I were five to ten times those in Group IV. The changes induced in renal function were, however, similar in the two groups of experiments.

Figure 4 presents results from an experiment of Group I which shows that a second dose of aldosterone may produce a recognizable effect when the effects of the first dose have not disappeared. Usually about 5–10 μg aldosterone produced maximal changes. There were in this experiment rather transient increases in glomerular filtration rate after each addition of aldosterone; this was a common but not a constant finding. Some

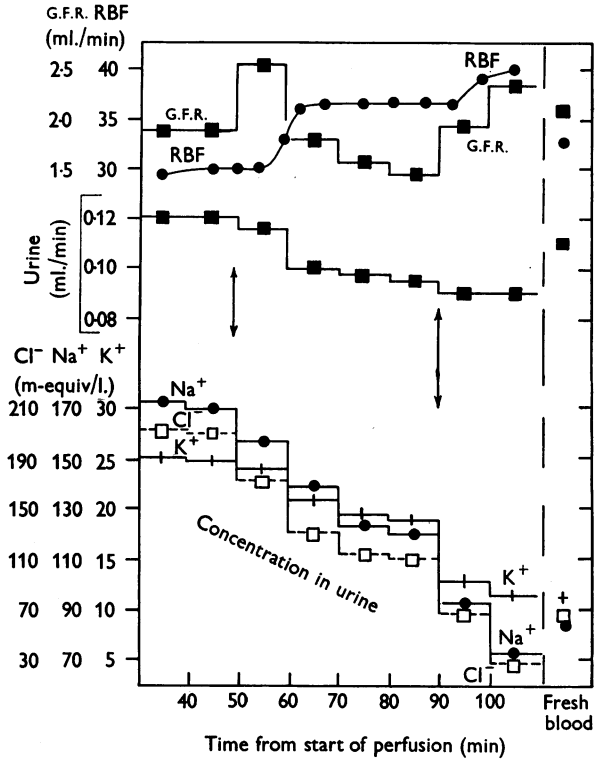

Fig. 4. Effect in an experiment of Group IV of aldosterone 2·5 μg at first arrow, 5 μg at second. From 110 min the kidney was perfused with blood to which no hormone had been added.

recovery in RBF, the minute volume and the concentrations of sodium and chloride of urine was observed 10 min after circulation of fresh blood to which no hormone had been added.

Effects of aldosterone in experiments of Groups II and III

These were experiments in which cats were used with the head circulation excluded during bleeding or preparation. The cats of Group II were under chloralose anaesthesia, those of Group III were spinal. Figure 5 shows the effect of aldosterone in a Group III preparation. There was an increase in RBF in this and in all other experiments. Transient increases in

creatinine clearance were seen in three experiments out of five. The minute volume of urine always increased. Although there was decrease in the concentrations of sodium and chloride in the urine after aldosterone, the diuresis was usually enough to increase the amount of these ions lost per minute. The concentration of potassium in the urine often increased, as in Fig. 5, but sometimes remained unchanged. This diuretic response to aldosterone accompanied by increase in the rate of potassium excretion was characteristic of all experiments of Groups II and III, in which the circulation through the head was excluded during collection of the blood and the operative preparation. It was not solely a response to an initial

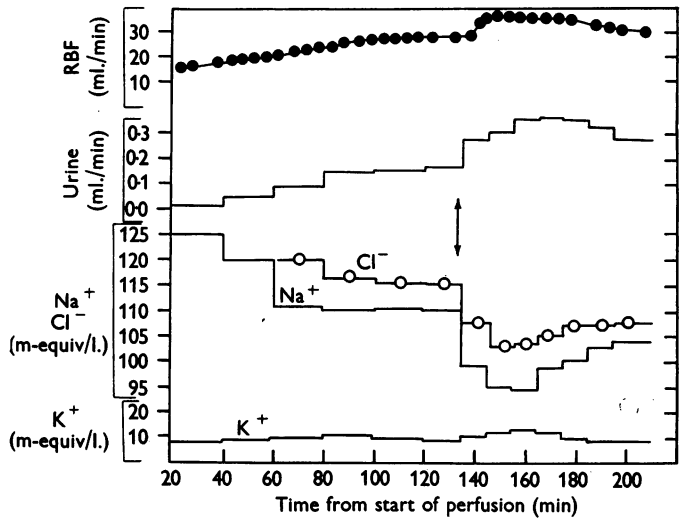

Fig. 5. Effect in an experiment of Group III of aldosterone 5 μg at arrow.

dose of aldosterone, nor to a critical concentration of aldosterone, for it remained apparent throughout the 5 hr of an experiment in which the concentration of circulating aldosterone was steadily raised by the addition of 2·5–5 μg aldosterone monacetate to 160 ml. of circulating blood at intervals of 20–40 min.

The response typical of experiments in Groups II and III (Fig. 5) could be converted to that of experiments belonging to Groups I and IV (Figs. 3 and 4) by oxytocin, as is shown in Fig. 6. The first response to DL-aldosterone monoacetate 2 μg/150 ml. blood produced an increase in the minute volume of urine and in potassium excretion. However, after the addition of 4 m-u. Syntocinon to the circulating 150 ml. of blood, 2 μg aldosterone monacetate caused reduction in the minute volume of urine and in the amount of potassium excreted per minute. In other words, the effect of aldosterone on the kidney perfused with blood that had not recently circu-

lated through the head was converted, by the addition of oxytocin, to an action of aldosterone typical of this hormone on kidneys perfused with blood which had recently circulated through the head. The naturally occurring oxytocin, Pitocin, was as effective as the synthetic preparation in this respect.

Effects of aldosterone in experiments of Group V

Four experiments were made in which the kidney from a cat hypophysectomized under chloralose anaesthesia was perfused with blood

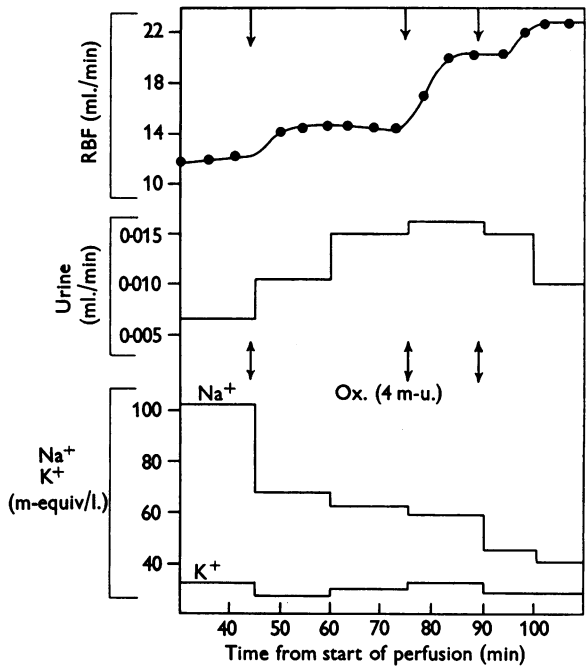

Fig. 6. Effect in an experiment of Group III of aldosterone, 2 μg at first and third arrows, and its modification by oxytocin 4 m-u. at second arrow.

collected from other cats which had been hypophysectomized under chloralose shortly before being bled. In every case the addition of 2–7 μg aldosterone monoacetate to 150 ml. of circuit blood caused an increase in RBF, a diuresis and decrease in the concentrations of sodium and chloride with increase or little change in that of potassium in the urine (Fig. 7). The response was like that in preparations of Groups II and III, which were made without a circulation through the head (Fig. 5). Moreover, synthetic oxytocin 10 m-u./150 ml. blood converted this diuretic action of aldosterone to one of antidiuresis accompanied by potassium retention, characteristic of the action of this hormone in preparations made in the

presence of a circulation through the head (Groups I and IV, Figs. 3 and 4). This conversion could also be effected by 50 m-u. of vasopressin, as was well shown by the experiment in Fig. 8, in which the circuit blood was divided into two portions. One part was used for the study of the effect of vasopressin on the responses of the preparation of aldosterone. The blood used for this part of the experiment was then replaced by the second portion of the same blood, and later, when the kidney was fully acclimatized to the change, examination was made of the effect of oxytocin. After 5 m-u. of oxytocin, aldosterone produced antidiuretic effect.

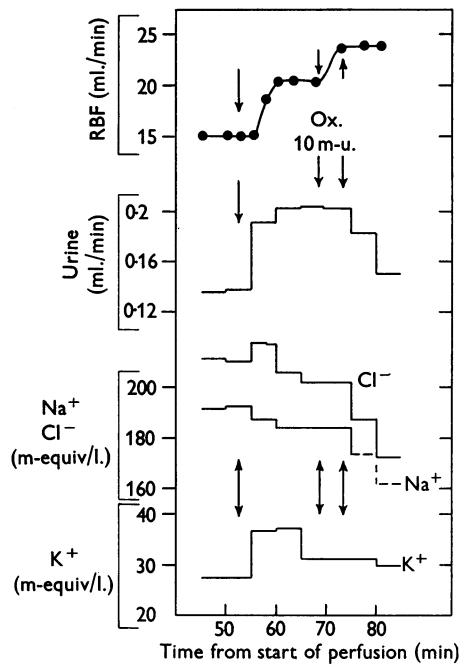

Fig. 7. Effect in an experiment of Group V of aldosterone 7 μg at first and third arrows, and its modification by oxytocin 10 m-u. at second arrow.

Actions of oxytocin and vasopressin

The actions of oxytocin and vasopressin alone have also been investigated. It should be noted that the heart–lung–kidney preparations in which the effects of the neurohypophysial hormones were studied had already been used for investigation of the actions of aldosterone, and that the long-lasting changes induced in the concentrations of urinary electrolytes by the adrenal hormone had only rarely been fully reversed when the pituitary hormones were administered.

Oxytocin. Since oxytocin has been shown to be released during haemorrhage in rats (Ginsburg & Brown, 1957) the direct actions of oxytocin on

the kidney were examined only in preparations of Groups II, III and V, in which the initial concentrations of oxytocin were probably low. Oxytocin (4–25 m-u.) added to 150 ml. of such blood increased the RBF and the clearance of creatinine but failed to influence either the concentrations of electrolytes or the minute volume of urine (Table 2 and Fig. 9). There

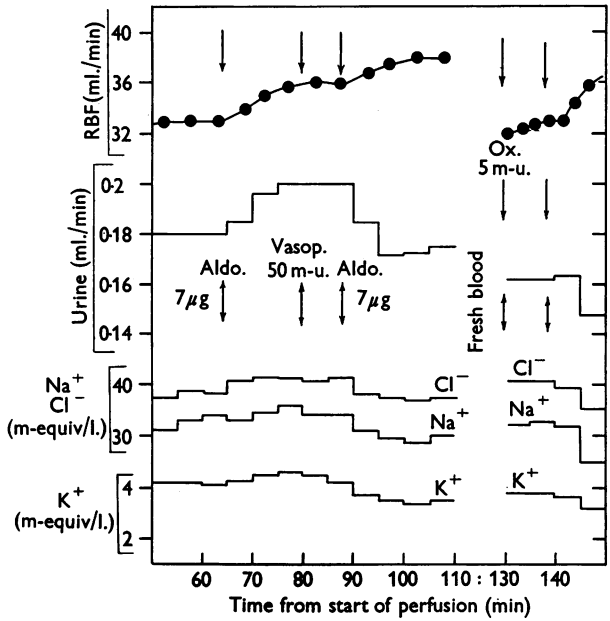

Fig. 8. Effect in an experiment of Group V of aldosterone 7 μg at first, third and fifth arrows, and its modification by vasopressin 50 m-u. at second arrow. The kidney was perfused with fresh blood at 120 min. At fourth arrow, oxytocin 5 m-u.

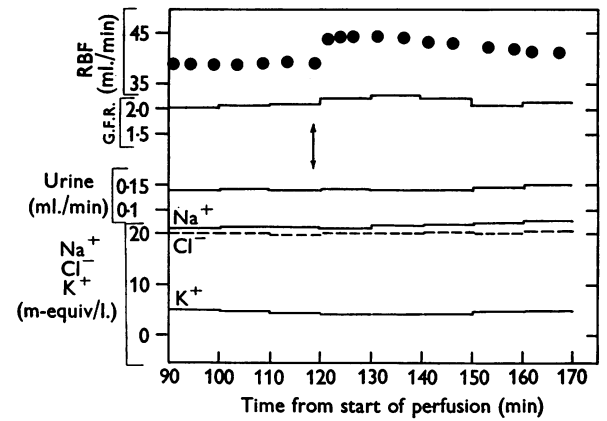

Fig. 9. Effect in an experiment of Group V of oxytocin 10 m-u. at arrow. Plotting as in Fig. 2.

was no apparent difference in the effect of these concentrations of oxytocin in the presence and absence of chloralose anaesthesia.

Vasopressin. In preparations of Group III (spinal, without a head circulation) small doses of vasopressin produced decrease in urine flow,

TABLE 2. Effect of oxytocin on the isolated perfused cat kidney

Type of preparation	Oxytocin (m-u./150 ml.)	Urine (min vol)	RBF (ml./min)	G.F.R. (ml./min)	Na^+ (μequiv/min)	K^+ (μequiv/min)
Group III	4	0·016	14·4	1·63	93·0	5·1
		0·017	19·0	1·69	98·0	3·4*
Group III	20	0·056	28·0	2·20	57·0	3·0
		0·060	33·5	2·59	65·0	3·3*
Group II	4	0·36	46·0	2·41	37·5	6·5
		0·36	48·0	3·00	37·5	6·9*
Group V	4	0·66	35·2	3·14	87·5	6·8
		0·76	42·4	3·62	89·2	6·9*
Group V	5	0·13	32·8	1·40	20·7	3·7
		0·14	36·6	1·70	21·8	3·7*
Group V	10	0·22	20·4	3·17	40·2	7·3
		0·22	24·0	3·49	40·8	7·3*
Group II	25	0·17	26·4	1·60	20·4	4·6
		0·18	30·6	1·93	22·5	4·6*

* In an observation period of 20–30 min after oxytocin had been added to the circulating blood.

TABLE 3. The effect of chloralose anaesthesia on the sensitivity of the kidney to antidiuretic hormone

Type of preparation	Vasopressin for threshold effect (m-u./150 ml.)	Vol. urine (ml./min)	RBF (ml./min)	Urinary electrolytes (μequiv/min)		
				Na^+	K^+	Cl^-
Group II	4	0·032	16·2	50	2·0	56
		0·037	16·2	50	2·0	58*
Group II	20	0·014	28·0	14	2·0	15
		0·016	30·0	16	2·3	18*
Group V	8	0·050	22·6	60	1·4	73
		0·035	22·6	50	1·0	65*
Group V	10	0·040	21·8	12	1·1	—
		0·060	21·8	23	1·2	—*
Group V	10	0·025	24·0	24	1·2	27
		0·040	23·5	26	1·4	31*
	(μu./150 ml.)					
Group III	20	0·36	24·4	106	9·5	—
		0·20	24·4	95	4·6	—*
Group III	20	0·31	20·0	37	3·0	38
		0·28	21·0	35	3·0	36*
Group III	25	0·24	22·6	62	2·8	65
		0·18	23·0	60	3·0	62*

* After vasopressin had been added to the circulating blood.

20–25 μu./150 ml. blood evoking threshold effect (Table 3). In the presence of chloralose anaesthesia in preparations of Groups V and II much larger doses failed to produce antidiuresis (Table 3). Chloralose anaesthesia therefore very greatly reduced the sensitivity of the kidney to vasopressin.

DISCUSSION

The classical effect of aldosterone in the intact animal is one of sodium retention and potassium loss (Mach, Fabre, Duckert, Borth & Decommun, 1954). It has not been possible to demonstrate this on the isolated kidney in any of the selected groups of experimental circumstances. In experiments of Groups I and IV, in which the blood was drawn and the preparation made whilst blood circulated through the head, aldosterone caused

TABLE 4. Changes induced by aldosterone in the excretion of sodium and potassium by the isolated perfused cat kidney: mean ± S.E. of mean, no. of expts. in brackets

Type of preparation	Aldosterone (μg/150 ml. blood)	Percentage change in urinary electrolytes: Amount excreted per minute — Na	Amount excreted per minute — K	Concentration per litre — Na	Concentration per litre — K
Group I	10	−53 ± 3·5 (4)	−34 ± 5·2 (4)	−18 ± 3·9 (4)	−11 ± 3·3 (4)
	5	−37 ± 3·0 (4)	−37 ± 4·8 (4)	−23 ± 6·5 (4)	−15 ± 3·2 (4)
Group III	2	36 ± 7·1 (4)	52 ± 9·8 (4)	−21 ± 7·9 (4)	17 ± 4·7 (4)
Group IV	2	−30 ± 9·0 (3)	−44 ± 3·5 (3)	−20 ± 1·1 (3)	−22 ± 6·8 (3)
Group V	7	3 ± 1·1 (4)	39 ± 5·3 (4)	−9 ± 1·2 (3)	21 ± 3·7 (3)

Only data derived from renal responses to the first addition of hormone to the perfusing blood have been used in the compilation of this table.

antidiuresis and reduction in the rate of excretion of both sodium and potassium. When the head was excluded from the circulation (Group III) or the hypophysis was removed (Group V) before bleeding and preparation, aldosterone caused diuresis and increased the rate of excretion of both potassium and sodium (Table 4). The difference in the action of aldosterone in Groups I and IV on the one hand and Groups II, III, and V on the other is most probably to be attributed to differences in the concentrations of an oxytocin-like substance present, since the response given by preparations of Groups III and V was regularly converted to that characteristic of Groups I and IV by the addition of oxytocin to the circulating blood. The nearest approach to the action of aldosterone in the whole animal was seen in preparations of Groups III and V. In these the hormone caused a fall in the sodium and a rise in the potassium concentrations of the urine (Table 4) but this was accompanied by diuresis. No such diuresis accompanies the action of physiological doses of aldosterone in the intact cat (unpublished observations).

Chloralose failed to alter the sensitivity of the isolated kidney to

oxytocin (Table 2), caused some decrease in sensitivity to aldosterone without effecting qualitative change in the actions of this hormone (Table 4) and grossly decreased renal sensitivity to vasopressin (Table 3). It may therefore be concluded that chloralose, in the concentrations used, had little effect on the renal blood vessels, only slightly depressed the active reabsorption of sodium and potassium excretion, but profoundly inhibited the facultative reabsorption of water in the distal nephron by preventing those changes in cellular permeability to water brought about by vasopressin (Wirz, 1953).

SUMMARY

1. The actions of DL-aldosterone monoacetate 0·5–10 μg/150 ml. of circulating blood have been examined in cats' kidneys perfused from heart–lung preparations.

2. If either in spinal cats or under chloralose anaesthesia blood was circulating through the head during the operative preparation of the heart–lung–kidney preparation and during collection of the blood for the circuit, aldosterone caused an increase in renal blood flow, decrease in the urine volume, and decreases in the urinary concentrations of Na, K, Cl.

3. If either in spinal cats or under chloralose anaesthesia the circulation through the head was excluded during the bleeding and operative preparation, aldosterone caused an increase in renal blood flow, an increase in the urine volume, little change in K and decreases in Na and Cl concentrations in the urine. In consequence there was an increase in Na and Cl excretion, and a smaller increase in K excretion. Aldosterone produced a similar effect when hypophysectomized animals were used.

4. The effect of aldosterone was changed from that in (3) to that in (2) above by oxytocin 10 m-u./150 ml. blood. Vasopressin was less effective.

5. Oxytocin (1–20 m-u./150 ml. blood) did not alter the volume or electrolyte composition of the urine.

6. Chloralose reduced the sensitivity of the kidneys to vasopressin. In the absence of chloralose vasopressin (15–20 μu./150 ml.) reduced urine flow; in the presence of chloralose, 4–20 m-u. had no effect. There was no effect on electrolyte excretion.

7. Chloralose made the kidneys only slightly less sensitive to aldosterone and did not influence the character of the response.

This work was undertaken whilst one of us, M.J.D., was receiving a grant for personal maintenance from the Pharmaceutical Society of Great Britain for Training in Research.

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