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ANALYSIS OF DIRECT RENAL ACTIONS OF ALPHA AND BETA ADRENERGIC STIMULATION UPON SODIUM EXCRETION COMPARED TO ACETYLCHOLINE

BY

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The renal actions of adrenergic drugs have been widely studied, but because of their profound cardiovascular effects, their direct renal actions are still not understood (Blake, 1955; Kruhoffer, 1960). Lees & Lockett (1963) have shown β-adrenergic receptors in rat kidneys. Isoprenaline, a β-adrenergic drug, produces general vasodilator effects similar to those of acetylcholine.

Cholinergic drugs injected into the renal artery of the dog have been shown to produce saluresis by a direct renal action (Vander, 1964; Pinter, O'Morchoe & Sikand, 1964; Lavender, Aho & Pullman, 1965; Williams, Pearson & Carter, 1965). This saluresis is effected chiefly by increasing the tubular rejection fraction of the filtered load of sodium (Williams *et al.*, 1965).

The effects of adrenaline on renal function were shown by Blake (1955) to be both direct and indirect; the quality of the renal effects was highly dose dependent. The infusion of noradrenaline into one kidney of conscious dogs invariably resulted in sodium retention although the accompanying haemodynamic changes varied (Barger, Muldowney & Liebowitz, 1959). The purpose of the present report was to compare direct renal actions of α and β adrenergic drugs in the same experimental conditions and to give reasons for the tremendous qualitative differences produced by different dosage levels and different routes of administration. Acetylcholine was used as a reference because its effects are consistent and documented.

METHODS

Female mongrel dogs were anaesthetized with intravenous injections of pentobarbital sodium, 30 mg/kg. Both ureters were cannulated through an abdominal midline incision, and the cannulae were positioned approximately 0.5 in. below the ureteral pelvic junction. A femoral vein and artery were cannulated and the arterial cannula was connected to a E & M linear transducer with a three-way stopcock for recording blood pressure with a polygraph. Arterial blood samples were obtained through the three-way stopcock. Solutions containing appropriate amount of creatinine, *para*-aminohippurate (PAH), and normal saline were infused at a rate of 5 ml./min through the venous system by means of a dual-syringe constant-flow infusion pump. After exposing the left renal artery

by the retroperitoneal approach, a stay suture was placed in the renal artery near the aortic junction. A 27-gauge hypodermic needle, attached to number 10 polyethylene tubing, was placed into the left renal artery in the direction of blood flow. Through this renal arterial system a solution of isotonic sodium chloride was continuously infused at a rate of 0.1 ml./min. Solutions of drugs were also infused at the same rate through the system by changing the renal arterial infusate to one containing test drugs dissolved in normal saline. The patency of this system was checked by infusing 10% glucose solution and testing for unilateral spillage with Diabetic Test paper. One to two hours were allowed for equilibration and then collections of 10 min urine samples from each kidney were begun. Blood samples, drawn every 20 min, were heparinized, centrifuged, and the plasma immediately removed. At least three control urine samples were collected before test agents were infused into the kidney.

Analytical

Chloride concentrations in urine and plasma were determined using a Buchler-Cotlove chloridometer, sodium and potassium concentrations in urine and plasma were determined using a Baird-Atomic flame photometer with an internal lithium standard, a modification of the Bonsnes & Taussky (1945) method was used in the determination of plasma and urine creatinine, and PAH determinations were made using a modification of the method of Bratton & Marshall (1939). The osmolalities were determined with a Fiske osmometer. In addition to the electrolyte excretion rates, the glomerular filtration rates (GFR), the effective renal plasma flow (ERPF), and the tubular rejection fraction (TRF) of sodium were calculated. The tubular rejection fraction is the ratio of the rate of sodium excretion to the filtered load of sodium, and represents the tubular rejection of a part of the filtered load. The TRF can vary independently of changes in filtered load (Simmons, Harvey & Hoshiko, 1954). Because only concentrations are used in the calculation of TRF, its magnitude is independent of the size of the animal and facilitates comparisons between dogs. The absolute rate of tubular reabsorption (T_{Na}) is less useful because it is markedly affected by filtered load, which in turn is a product of extrarenal as well as renal factors.

$$TRF_{Na} = \frac{U_{Na}V}{P_{Na} \cdot GFR} = \frac{U_{Na} \cdot P \text{ creatinine}}{P_{Na} \cdot U \text{ creatinine}}$$

$$TRF_{H_2O} = \frac{V}{GFR} = \frac{P \text{ creatinine}}{U \text{ creatinine}}$$

where U is the urine concentration, P is the plasma concentration, V is the urine flow in ml./min and GFR is the clearance of creatinine.

Student's t test was used in the statistical analysis of the data in this paper (Snedecor, 1956).

All drug doses are based on the respective salts: acetylcholine bromide, isoprenaline sulphate, *l*-noradrenaline bitartrate monohydrate, pentolinium tartrate, and atropine sulphate. MA-1277 is a new α-adrenergic blocking agent from Miles Laboratories Inc., Elkhart, Indiana. The chemical formula is 4-phenyl-l-[2-(5-tetrazolyl) ethyl] piperazine trihydrochloride.

RESULTS

Direct renal effects

Beta-adrenergic stimulation. The administration of isoprenaline sulphate (0.01 μg/kg/min) by infusion into the left renal artery resulted in a saluresis similar to that of acetylcholine bromide (0.1 μg/kg/min) (Fig. 1). The natriuresis was caused by an increased tubular rejection fraction (TRF_{Na})—compare A, B, and C in Fig. 1. Potassium excretion was increased but this increase was very small in comparison with that of sodium excretion. In this dog the effective renal plasma flow (ERPF) was increased on the left side by acetylcholine during the first 10 min and returned to control levels during the next 10 min of infusion (Fig. 1D). The ERPF on the control side dropped during the first 10

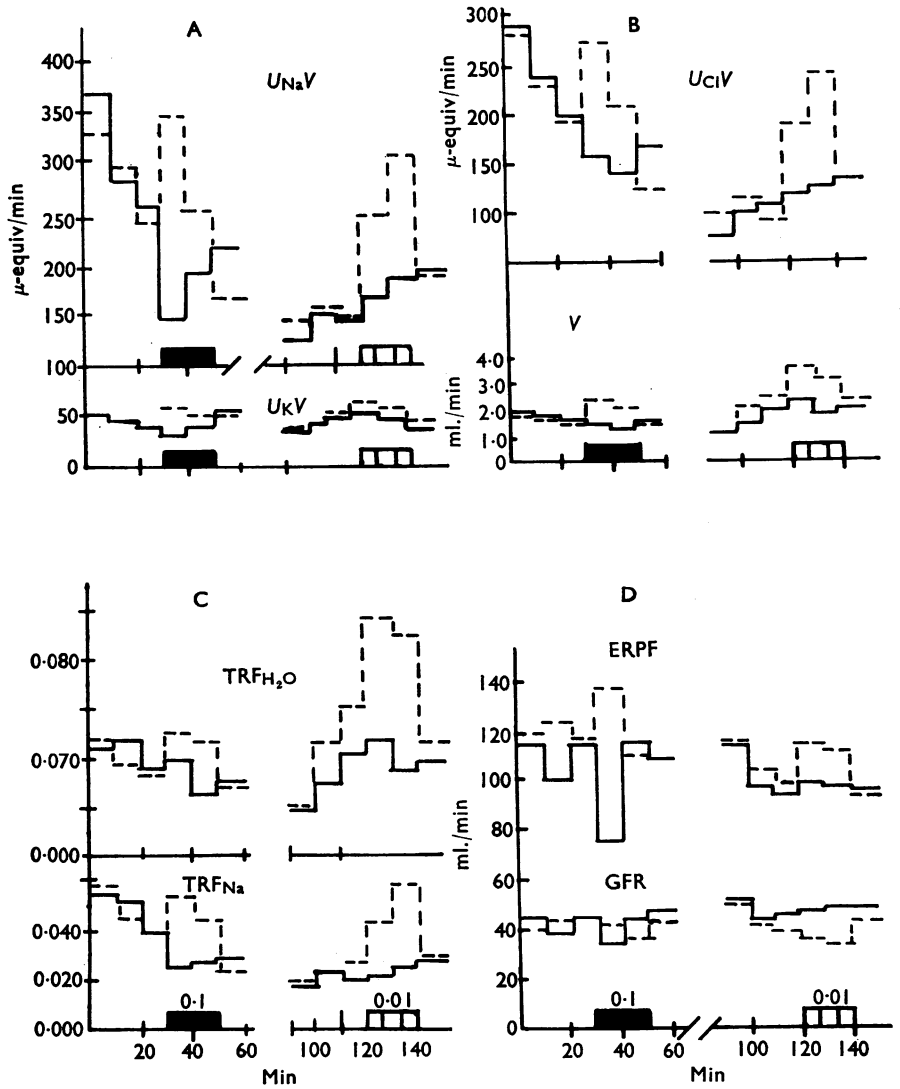

Fig. 1. Effects of unilateral infusion of acetylcholine and isoprenaline into the left renal artery of a dog (female) weighing 18 kg. A: Both result in a marked, unilateral increase in the rate of sodium excretion ($U_{Na}V$). The rate of potassium excretion (U_kV), although increased, is only slightly affected in comparison with the sodium excretion. B: The pattern and the magnitude of the change in chloride excretion ($U_{Cl}V$) is very close to that of sodium. The changes in urinary flow (V) are unilateral and seem to be a result of the saluresis. C: The TRF_{H2O}, which is the fraction of the filtered fluid volume that is excreted, and the TRF_{Na}, which is the tubular rejection fraction of the filtered sodium, are both pure numbers. The TRF_{Na} has a similar pattern to that of the sodium excretion. D: The effective renal plasma flow (ERPF) increased in the left kidney during the first 10 min of acetylcholine infusion, but returned below control during the second 10 min period. Infusion of isoprenaline resulted in a unilateral increase in the ERPF during the full 20 min period. The glomerular filtration rate (GFR) was decreased slightly during the infusion of both agents. - - - Experimental (left) kidney; ——, control (right) kidney; ▥, isoprenaline (μg/kg/min, left renal artery); ■, acetylcholine (μg/kg/min, left renal artery).

minutes and returned during the next period of infusion in a manner nearly reciprocal to that of the infused side. In contrast, the unilateral natriuresis was clearly maintained. Infusion of isoprenaline produced a rise in ERPF while the control side stayed the same. Although infusion of acetylcholine produced a variable change in GFR, infusion of isoprenaline produced an apparent fall in GFR (Fig. 1D). The tubular rejection of water was very similar to the tubular rejection of sodium. A unilateral effect of larger doses of isoprenaline was never as large as that of acetylcholine in subsequent experiments.

Alpha-adrenergic stimulation. When noradrenaline (0.05–0.12 μg/kg/min) was infused into the left kidney there was a unilateral decrease in sodium excretion (Fig. 2A). In the same dog, infusion of acetylcholine resulted in the usual unilateral saluresis. At this lower dose-level the salt retention produced by noradrenaline was the result of decreased tubular rejection of sodium (Fig. 2C). Figure 2D shows that the changes in GFR are negligible during the infusion of noradrenaline. The tubular rejection of water closely follows that of sodium (compare Figs. 2B and C). Neither the urine flow nor the tubular rejection of water are determined by the free water clearance in this experiment (Fig. 2B and D). The potassium excretion was slightly decreased by noradrenaline, but was slightly increased by acetylcholine.

The direct, renal sodium-retaining effect of noradrenaline (0.07 μg/kg/min, left renal artery) is further shown in Fig. 3A. In this experiment, the dog was infused with a 5% solution of sodium chloride to magnify bilaterally the level of saluresis during the infusion of noradrenaline. The effect of noradrenaline shown in Fig. 3A is the same as that previously shown in Fig. 2. When the 5% sodium chloride solution was intravenously infused at a rate of 5 ml./min, noradrenaline retained its unilateral, salt-retaining action (Fig. 3A). Decreased urine flow was not accompanied by decreased clearance of free water (Fig. 3B). The chloride excretion was decreased more than the effective renal plasma flow (Fig. 3B) as shown by the decreased renal rejection fraction (RRF_{Cl}), where

$$RRF_{Cl} = \frac{U_{Cl}V}{P_{Cl} \cdot ERPF} = \frac{U_{Cl} \cdot P_{PAH}}{P_{Cl} \cdot U_{PAH}}$$

RRF_{Cl} is that fraction of the effective plasma load of chloride which was excreted.

Comparison of the direct renal effects of isoprenaline and noradrenaline in the same dog

Infusion of isoprenaline (0.1 μg/kg/min) produced direct effects similar to those already described. Systemic effects were, however, also produced (Fig. 4). The mean arterial blood pressure fell, renal plasma flow fell, excretion of sodium and total solutes from both kidneys fell while only the left kidney was being infused. Both kidneys excreted less sodium than in control periods, but the infused kidney excreted more sodium than the other. The values of the left infused kidney, which had been lower than the right kidney before infusion of isoprenaline, became higher than those of the right kidney during infusion of the drug. There was no effect on the osmolality of the urine during the first period of infusion but there was a marked increase on both sides during and for 20 min after the second period of infusion. With this concentration of isoprenaline, the ERPF and GFR in both kidneys rose above previous control levels while the sodium excretion remained depressed on both sides during the last 10 min of the infusion.

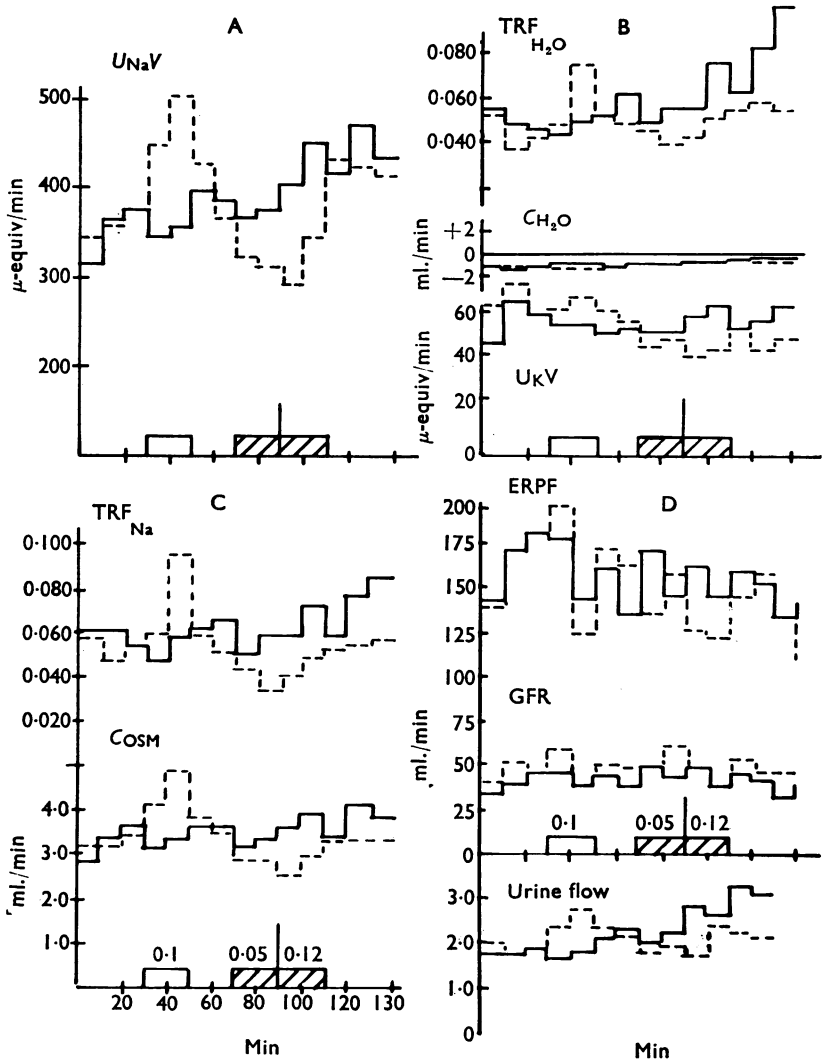

Fig. 2. Comparison of the direct renal effects of acetylcholine (□) and noradrenaline (▨) in a dog (female, 19 kg). A: The direct, unilateral effect of acetylcholine increased the sodium excretion and the direct unilateral effect of noradrenaline decreased the sodium excretion. The right kidney(—) seems to show a reciprocal effect in the opposite direction for each drug. Left kidney (- - -) B: Changes in the tubular rejection fraction of water were identical in pattern to those for sodium. There was no observable change in the free water clearance (C_{H_2O}). Potassium excretion was increased during infusion of acetylcholine and decreased during infusion of noradrenaline. C: Infusion of acetylcholine increased the tubular rejection of sodium from the left kidney while noradrenaline decreased the TRF_{Na}. The osmolal clearance was identical in pattern to that of the sodium excretion rates. D: The effects (left renal artery) of acetylcholine and noradrenaline on ERPF and GFR were variable, but the urine flow was unilaterally increased by acetylcholine and unilaterally decreased by noradrenaline.

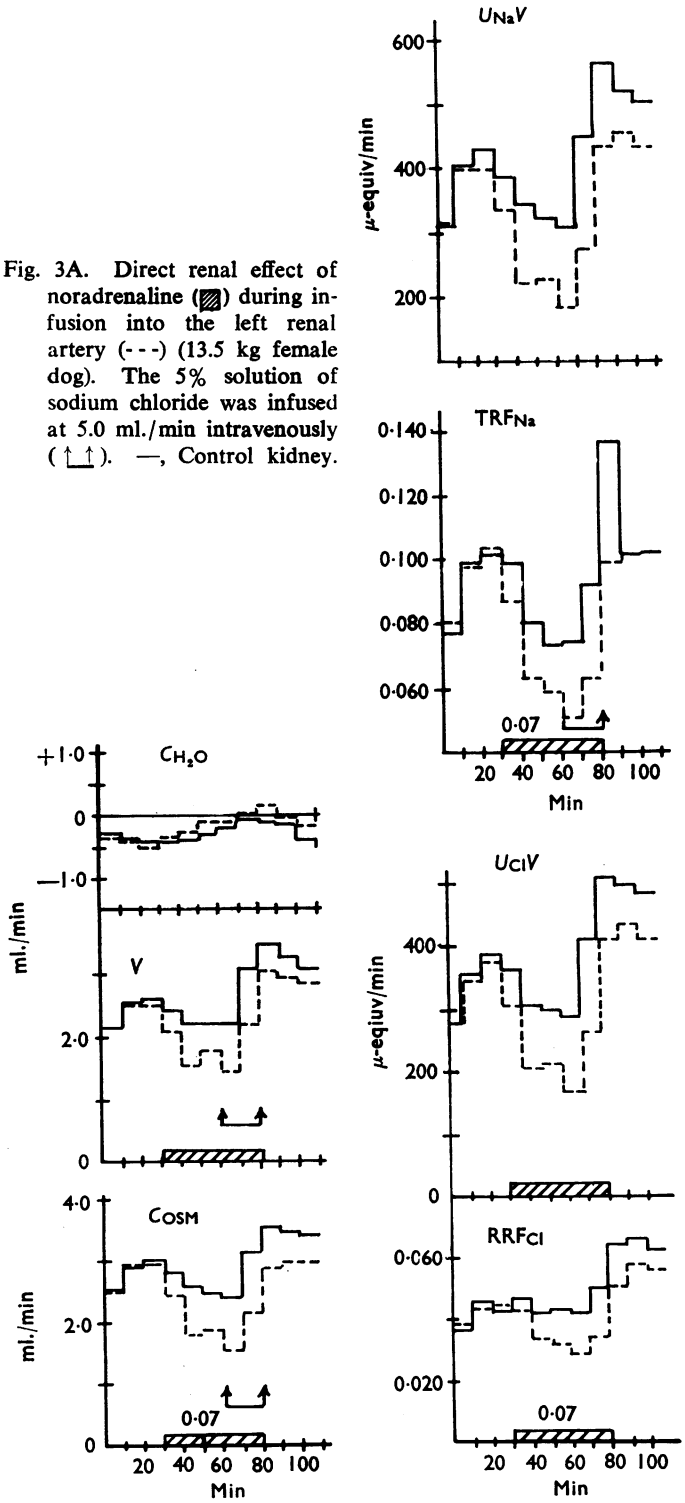

Fig. 3A. Direct renal effect of noradrenaline (▨) during infusion into the left renal artery (- - -) (13.5 kg female dog). The 5% solution of sodium chloride was infused at 5.0 ml./min intravenously (↑ ↑). —, Control kidney.

Fig. 3B. Direct renal effect of noradrenaline, same experiment as shown in Fig. 3A. The RRF_{Cl} represents the renal rejection fraction which $= \frac{U_{Cl}V}{P_{Cl} \times ERPF}$; it is that fraction of the effective total renal plasma load of chloride which is excreted.

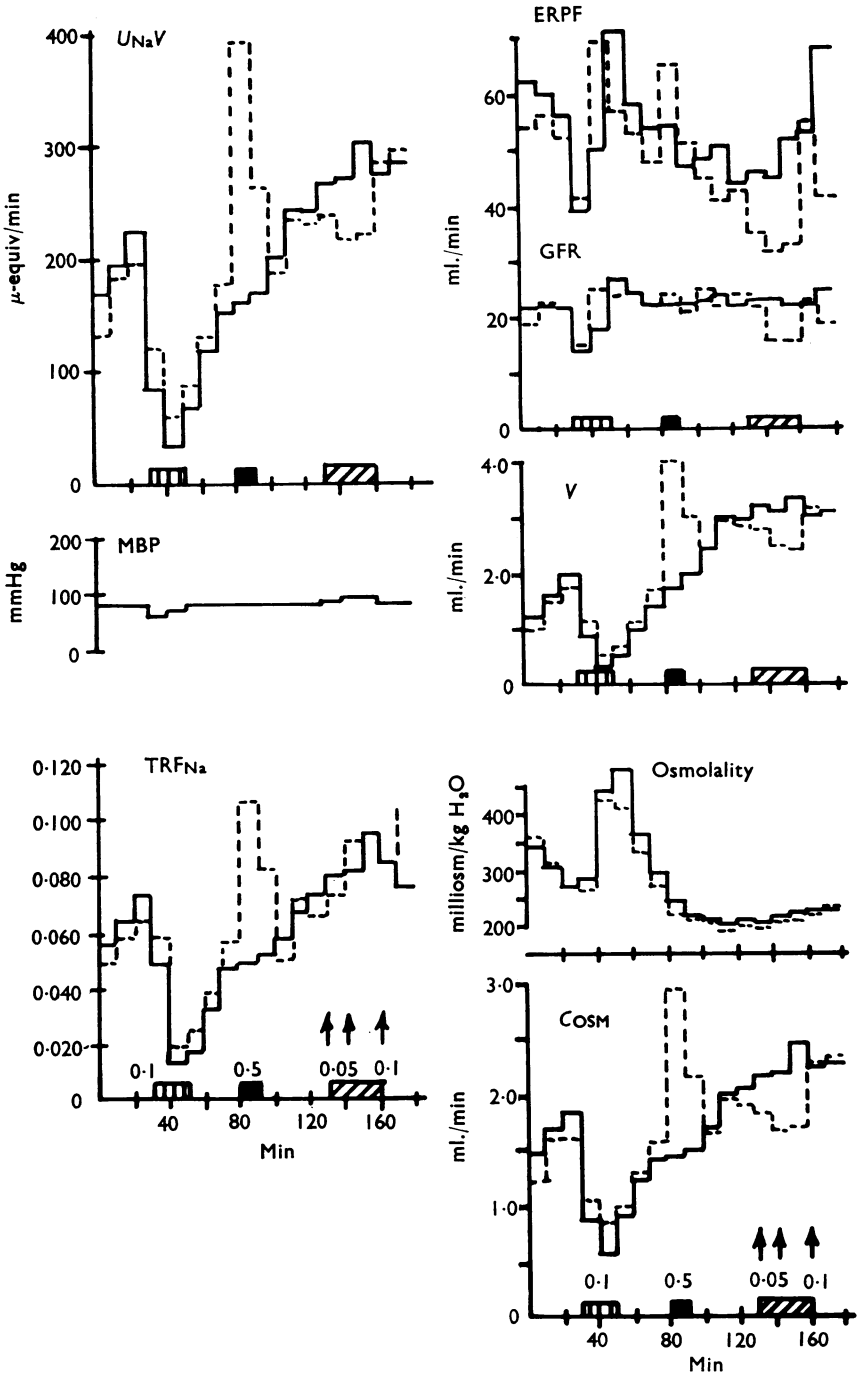

Fig. 4. Unilateral renal arterial infusion of isoprenaline (▥), acetylcholine (■), and noradrenaline (▨) in a dog (female, 12 kg). In this dog the systemic effects of isoprenaline are predominant. Although the unilateral effects can be observed by the cross-over of the left values (- - -) above the control side (—), there was a bilateral decrease in salt excretion. This decrease was accompanied by a fall in mean blood pressure (MBP), but the ERPF and GFR fell only during the first 10 min and rose above control during the next 10 min.

Infusion of acetylcholine (0.5 μg/kg/min) produced marked unilateral increases in sodium excretion, tubular rejection fraction, urine flow, osmolar clearance, and in ERPF (Fig. 4). There was, however, no effect on the osmolality of the urine or GFR. The systemic blood pressure was not affected with this dose of acetylcholine in this dog. Infusion of noradrenaline (0.05 μg/kg/min) produced a slight unilateral decrease in sodium excretion, ERPF, urine flow, and in osmolar clearance during the first 10 min. The TRF of sodium, urinary osmolality, or GFR were apparently not changed. With a higher concentration of noradrenaline (0.1 μg/kg/min) sodium excretion, GFR, ERPF, urine flow, and osmolar clearance were decreased in the infused kidney. Osmolality was unchanged. The mean blood pressure was increased by about 20 mm Hg. In this experiment, the tubular rejection fraction of sodium was apparently not changed by noradrenaline.

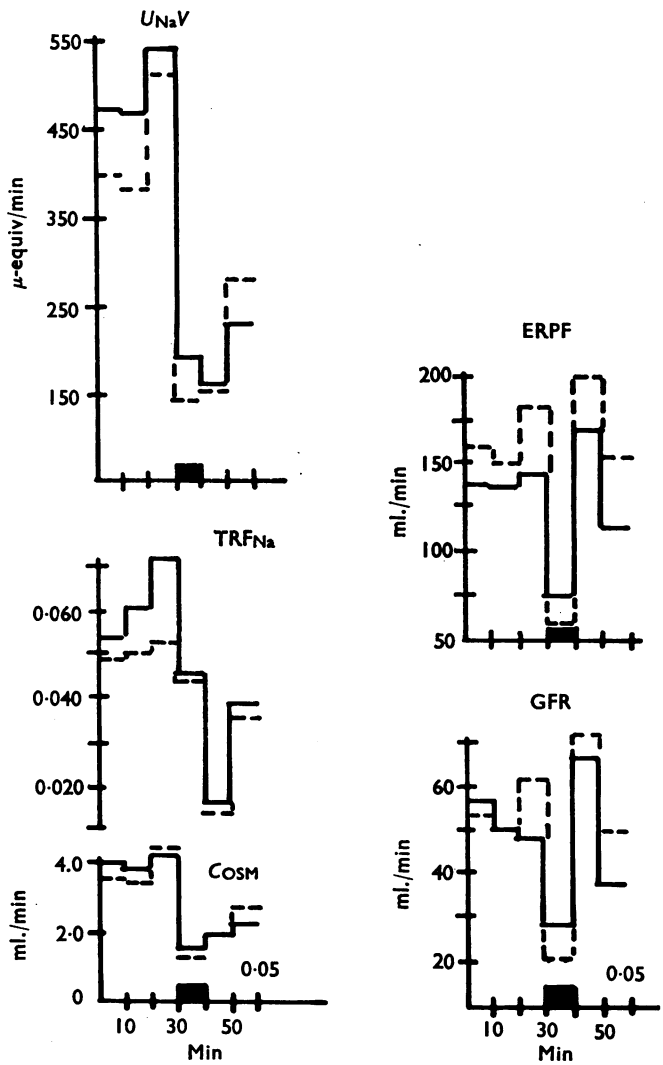

Fig. 5. Renal effect of intravenous infusion of isoprenaline (■) (male dog, 19 kg). The GFR, ERPF, $U_{Na}V$ and osmolal clearance (C_{osm}) fell promptly and markedly during the intravenous infusion of the drug. All effects were bilateral. Although the TRF of sodium fell during the drug infusion, the fall was most apparent when the infusion was stopped. Concomitantly, after the infusion was stopped, GFR and ERPF rose above previous control levels, however, the excretion of sodium and total solutes remained the same as during the drug infusion, - - -, Left kidney; —, right kidney.

Systemic effects

Isoprenaline and pentolinium

In order to demonstrate the systemic effects of isoprenaline it was infused intravenously (Fig. 5). Intravenous infusion (0.05 μg/kg/min) produced an immediate fall in sodium excretion, TRF of sodium, ERPF, osmolar clearance, and in GFR. The mean blood pressure was reduced about 20 mm Hg (not shown in Fig. 5). When the infusion was stopped, however, the ERPF and GFR increased to nearly four times higher than during the drug infusion while the sodium excretion remained decreased. As a consequence, the calculated tubular rejection fraction of sodium fell markedly.

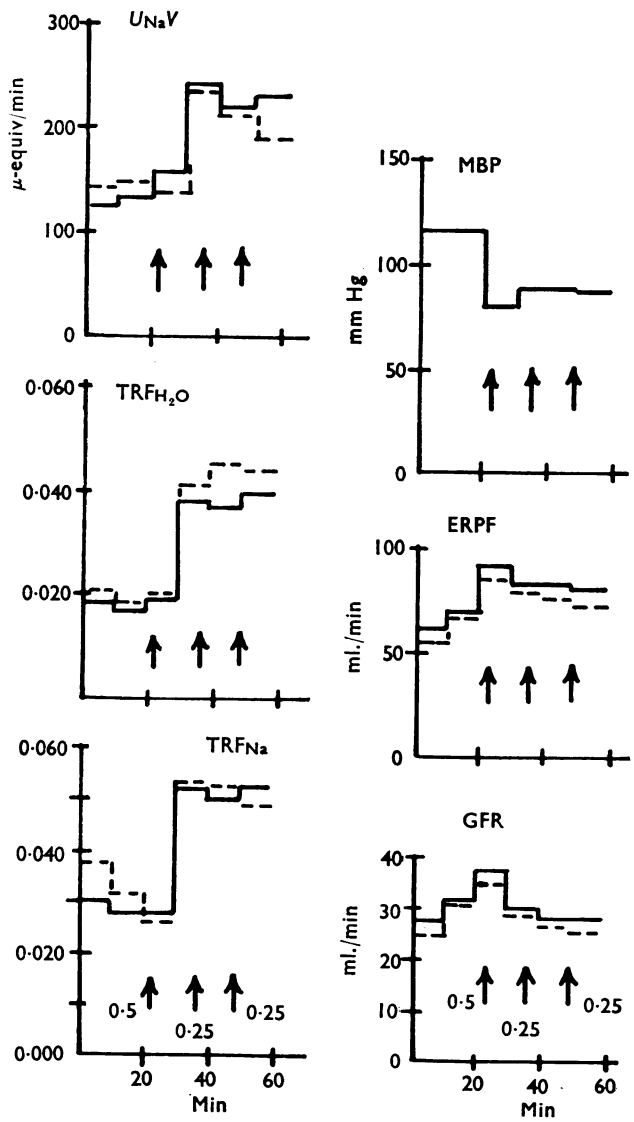

Fig. 6. Renal effects of intravenous injection (at arrow) of pentolinium tartrate (1 mg/kg) (female dog, 14.5 kg). In this experiment when the mean arterial blood pressure (MBP) was lowered by ganglionic blocking activity, the tubular rejection of sodium and water increased. The GFR and ERPF increased greatly at first then fell toward, but not to, the level of previous controls. - - -, Left kidney; —, right kidney.

In contrast, when the arterial blood pressure was lowered by the administration of pentolinium tartrate, a ganglionic blocking agent, the GFR and ERPF initially rose (Fig. 6). Also, during the subsequent decline in the ERPF and GFR the salt excretion increased. The ERPF never returned to control levels in spite of a sustained fall in mean arterial blood pressure. This confirms our opinion that systemic effects of isoprenaline were the result of an autonomic reflex.

Noradrenaline

The intravenous, systemic effects of noradrenaline can either decrease or increase salt excretion depending on the dose. Figure 7 shows that when a dog was given noradrenaline (8.3 μg/kg/min), infused intravenously, a sharp bilateral fall in both the GFR and ERPF and a slight fall in TRF_{Na} resulted. The renal sodium chloride excretion was markedly reduced. When the drug infusion was stopped, the renal haemodynamics returned to control levels (above control for GFR), but the salt excretion remained low because the tubular rejection fraction of sodium sharply fell. On the other hand the infusion of one-tenth the previous level of noradrenaline (0.83 μg/kg/min, intravenously) resulted in an increase in the sodium chloride excretion, in spite of a slight fall in GFR, by increasing the TRF_{Na}.

MA-1277, an α-adrenergic blocking agent, infused at 42 μg/kg/min into the left renal artery completely abolished the vasoconstrictor effects of noradrenaline in the same dog. The MA-1277 was in the systemic circulation, although it was infused into the left kidney, as shown by a fall of 20–30 mm Hg in the arterial blood pressure.

Statistical analysis of the direct renal effects

Twelve dogs were studied during forty-six different experimental infusion periods and fifty control periods. All drugs were infused unilaterally into the left renal artery. The right kidney served as the control. Interpretation and design of the statistical procedure were based on the following assumptions: (*a*) were a drug to act directly on the kidney, the response would be limited to or be greater in the infused kidney than in the contralateral kidney ; (*b*) any extrarenal, systemic action, whether hormonal or nervous, should be of equal magnitude in both kidneys ; (*c*) extrarenal responses could either augment or counteract the direct renal effects ; (*d*) the numerical values utilized for Student's *t* test were normally distributed.

Thus only the differences (Δ) between changes in the left and right kidneys were used for statistical analysis. These differences were obtained as follows: the pre-infusion values were subtracted from values obtained during drug infusion for each kidney. Then the changes in the control kidney were subtracted from the changes in the infused kidney (Table 1). For example

$$\Delta\chi=(\chi_{dl}-\chi_{cl})-(\chi_{dr}-\chi_{cr})$$

where *d* is the drug period, *c* the control period, *l* the left kidney and *r* the right kidney. Student's *t* distribution was used throughout to test the significance of paired differences. The level of significance was taken at $P<0.05$.

It is exceedingly difficult to obtain renal responses limited to the infused kidney by infusing adrenergic amines, because they are usually not destroyed or completely retained in that kidney (Inouye & Tanaka, 1965). The end result depends upon the renal mass and the rate of infusion. Even acetylcholine, which is more rapidly destroyed in blood and kidney, will produce contralateral effects if the rate of infusion is too fast. A dog

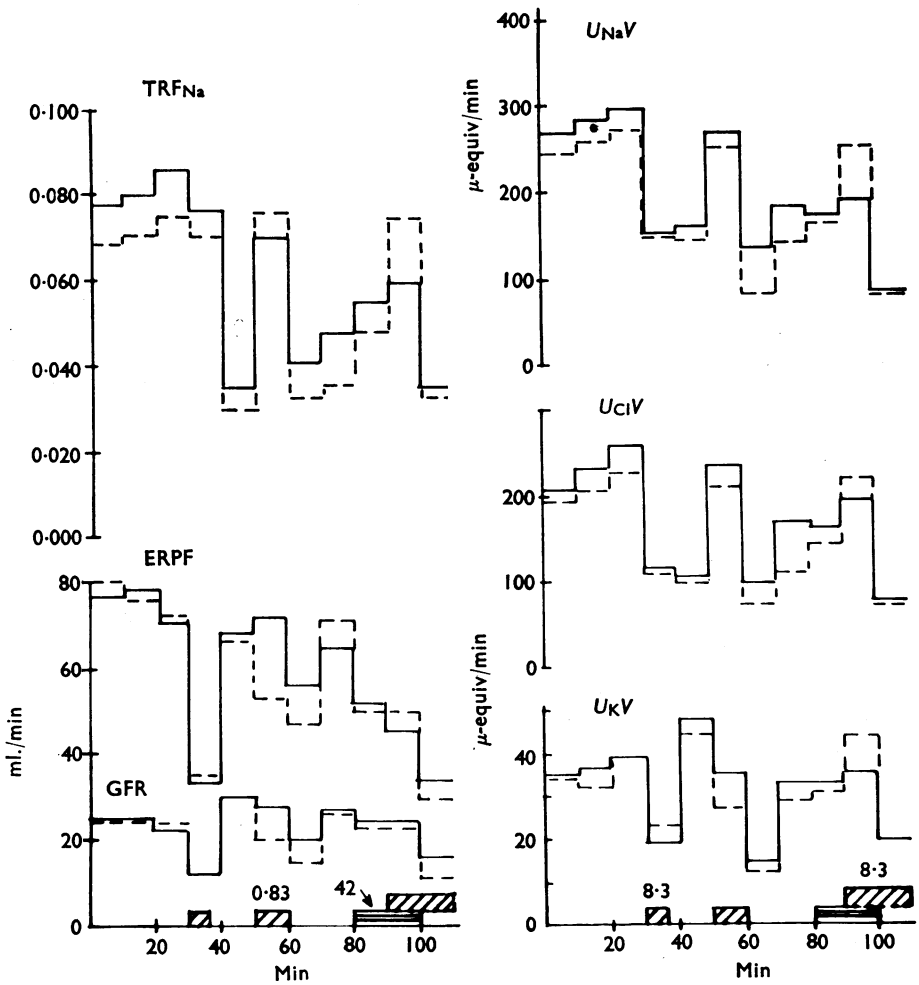

Fig. 7. Renal effects of intravenous infusion of noradrenaline (▨) at two dose levels (female dog, 12 kg). The intravenous infusion of noradrenaline at 8.3 μg/kg/min was stopped after 5 min because of a dangerously high arterial blood pressure (over 200 mm Hg, not shown). At this dose there was a marked fall in GFR and ERPF and a marked fall in sodium, chloride, and potassium excretion. The fall in TRF of sodium was only slight. Upon recovery of the ERPF and GFR to control levels, however (above control level for GFR), the sodium and chloride excretion remained depressed, and the TRF of sodium was greatly reduced. Note that the potassium excretion returned with the GFR. When noradrenaline was again infused at one-tenth the previous dose, the TRF_{Na} was increased along with an increase in sodium chloride excretion. Infusion of MA-1277 (an α-adrenergic blocker) (▤) blocked the vasoconstrictor and salt retaining action of the high dose of noradrenaline.

weighing 10 kg may have a kidney weighing between 29 and 76 g (see McDonald & deWardener, 1965). Our purpose was to characterize the renal effect produced by a dosage range of each drug and not to establish the dose-response curve. The logarithmic transformation of the renal effects was used in the statistical analysis for the following reasons. (*a*) The standard deviations of all values used appeared to vary directly as the means. (*b*) The tubular rejection fractions (TRF) are ratios which have a lower limit of zero with no sharply defined upper limit. (*c*) Renal sodium excretion is a product of a ratio times the filtered load ($TRF_{Na} \times FL_{Na} = U_{Na}V$). (*d*) The arithmetic mean is not representative of proportional effects. (*e*) With the exception of GFR, all values changed in one direction.

The geometric mean, and assumption of a normal distribution of log effects has long been used in biological assays for drug tolerances (Finney, 1964). With the exception of GFR and filtered loads, which did not change in one direction, all the means presented are therefore geometric means. The geometric mean of the differences was compared with zero by direct application of the *t* test on the logarithms. The logarithmic transformation should be useful and efficient for any data concerning direct renal effects. All renal changes lent themselves to the use of logarithms except the changes in GFR. We believe this indicates that changes in GFR may belong to a different kind of distribution from that of the other values (see Tables 1 and 2, especially for acetylcholine and isoprenaline).

Relative importance of tubular rejection and filtered loads

Nearly all the effect on sodium excretion was determined by the change in tubular rejection (ΔTRF_{Na}). There was no correlation with changes in filtered loads (Table 1). The changes in sodium excretion were always in the same direction while the changes in filtered loads were variable. As shown in Table 1, acetylcholine and isoprenaline produce a unilateral natriuresis while noradrenaline produces a unilateral decrease in sodium excretion. Isoprenaline was about one fourth as effective as acetylcholine in the dose ranges used. The difference between the theoretical changes in sodium excretion and the actual changes in sodium excretion was highly significant ($P<0.001$). The theoretical changes of sodium excretion represent that amount of sodium that would have been excreted, based on changes of filtered load alone. For each individual kidney:

Theoretical $\Delta U_{Na}V = \Delta Fl \times TRF_{Na_d}$

For statistics, the differences ($\Delta\chi$) between changes in the left and right kidneys

Theoretical $\Delta U_{Na}V = (\Delta Fl_l \times TRF_{Na_{dl}}) - (\Delta Fl_r \times TRF_{Na_{dr}})$

Actual $\Delta U_{Na}V = \Delta U_{Na}V_l - \Delta U_{Na}V_r$

The actual $\Delta U_{Na}V$ were simply obtained by subtracting sodium values from each kidney which were determined by analysis of the urinary samples by using a flame-photometer.

Where FL = filtered load

$\Delta FL = \Delta FL_l - \Delta Fl_r$

All values shown in Table 1.

As shown in Table 2, acetylcholine and isoprenaline produced significant increases in urine flow, osmolal clearance, ERPF, sodium excretion, and potassium excretion. They both exerted a variable effect on the GFR which was not significant. It should, however,

TABLE 1

RELATION BETWEEN CHANGES IN TUBULAR REJECTION FRACTIONS AND CHANGES IN FILTERED LOADS UPON SODIUM EXCRETION DURING INFUSION OF DRUGS INTO THE LEFT RENAL ARTERY*

* Each value represents a mean of from two to six 10-min collection periods. † Level of significance ($P<0.05$), Student's t test, paired comparison. ‡ All △T means are arithmetic means.

Dog No.	Tubular rejection fractions of sodium (TRF_{Na}): Left kidney, Control period	Left kidney, Drug period	Right kidney, Control period	Right kidney, Drug period	Left (d-c)	Right (d-c)	$\triangle TRF_{Na^+}$ (L-R)	Filtered loads, △FL: Left (d-c)	Right (d-c)	△FL (L-R)	Theoretical $\triangle U_{Na}V$ (μ-equiv/min): Left	Right	△T (L-R)	Actual (△A) $\triangle U_{Na}V$ (μ-equiv/min): Left (d-c)	Right (d-c)	△A (L-R)	(△A-△T)
Acetylcholine (0·1–1·1 μg/kg/min, left renal artery)																	
13	0·029	0·044	0·028	0·026	0·018	−0·002	0·020	1,050	75	975	46	2	44	137	−13	150	106
14	0·037	0·050	0·040	0·029	0·024	−0·002	0·026	−613	−416	−197	−31	−12	−19	57	−81	138	157
15	0·086	0·144	0·070	0·085	0·068	0·015	0·053	237	237	0	34	20	14	157	48	109	95
22	0·029	0·050	0·032	0·025	0·021	−0·007	0·028	−912	608	−1,520	−46	15	−61	55	−14	69	130
24	0·054	0·076	0·060	0·057	0·022	−0·003	0·025	−98	392	−490	−7	22	−29	119	0	119	148
27	0·065	0·116	0·065	0·035	0·051	−0·030	0·081	0	−1,514	1,514	0	−53	53	266	−200	466	413
35	0·048	0·094	0·046	0·051	0·046	0·005	0·041	−174	−149	−25	−16	−7	−9	164	10	154	163
Geometric mean	0·046	0·075	0·046	0·040			0·035						1‡			145	154
S.E.	±0·007	±0·013	±0·006	±0·007			±0·006									±32	±29
P†							$P<0·001$									$P<0·001$	$P\ll0·001$
Isoprenaline (0·01–0·2 μg/kg/min, left renal artery)																	
14	0·025	0·053	0·024	0·023	0·028	−0·001	0·029	−882	0	−882	−46	0	−46	114	−15	129	175
15	0·070	0·069	0·059	0·053	−0·001	−0·006	0·005	−154	−154	0	−11	−8	−3	−14	−21	7	10
18	0·058	0·052	0·061	0·049	−0·006	−0·012	0·006	441	294	147	23	14	9	−16	−66	50	41
20	0·042	0·035	0·055	0·037	−0·007	−0·018	0·011	0	−620	620	0	−23	23	−26	−76	50	27
22	0·027	0·026	0·025	0·020	−0·001	−0·005	0·004	456	0	456	12	0	12	9	−20	29	17
35	0·057	0·039	0·064	0·031	−0·018	−0·033	0·015	−149	−894	745	−6	−28	22	−77	−137	60	38
Geometric mean	0·043	0·043	0·044	0·033			0·009						3‡			40	33
S.E.	±0·008	±0·006	±0·008	±0·005			±0·002									±16	±13
P							$P<0·001$									$P<0·001$	$P<0·001$
Noradrenaline (0·05–0·12 μg/kg/min, left renal artery)																	
24	0·057	0·048	0·062	0·061	−0·009	−0·001	−0·008	405	74	331	19	5	14	−69	4	−73	−87
25	0·055	0·064	0·077	0·098	0·001	0·036	−0·035	−830	−729	−101	−57	−71	14	−15	21	−36	−50
26	0·037	0·038	0·033	0·069	−0·013	−0·005	−0·008	−664	−415	−249	−25	−29	4	−18	8	−26	−30
27	0·027	0·014	0·029	0·024	0·009	0·021	−0·012	484	1,142	−658	7	27	−20	−50	−16	−34	−14
35	0·081	0·086	0·075	0·085	0·005	0·010	−0·005	−596	−87	−509	−51	−7	−44	−36	14	−50	−6
37	0·099	0·065	0·106	0·084	−0·034	−0·022	−0·012	−95	231	−326	−6	19	−25	−156	−92	−64	−39
Geometric mean	0·054	0·046	0·058	0·064			−0·011						−10‡			−44	−28
S.E.	±0·011	±0·012	±0·012	±0·013			±0·003									±7	±10
P							$P<0·001$									$P<0·001$	$P<0·001$

TABLE 2

A STATISTICAL ANALYSIS OF THE DIFFERENCE BETWEEN THE LEFT AND RIGHT KIDNEYS DURING INFUSION INTO THE LEFT ARTERY*

* Each value in the table represents a mean of from two to six 10-min collection periods.
† Every change (△)=(△ left kidney)—(△ right kidney).
‡ All △GFR means are the arithmetic means.
§ Level of significance ($P<0.05$), Student's t test, paired comparison.

Dog No.	$\triangle V$† ml./min	$\triangle C_{OSM}$ ml./min	△GFR‡ ml./min	△ERPF ml./min	$\triangle U_{Na}V$ μ-equiv/min	$\triangle U_{K}V$ μ-equiv/min
Acetylcholine (0·1–1·1 μg/kg/min)						
13	0·99	1·13	7	25	150	7
14	0·98	1·09	−2	20	138	17
15	0·87	0·73	0	8	109	2
22	0·55	0·39	−2	10	69	—
24	0·62	0·79	−4	1	119	5
27	2·84	3·17	10	39	466	42
35	1·49	1·06	0	12	154	9
Geometric mean	1·03	0·99	1·3‡	10·9	145	8·8
±S.E.	±0·22	±0·24	±1·8	±5·0	±32	±3·8
P value§	*P*<0·001	*P*<0·001	*P*<0·4	*P*<0·01	*P*<0·001	*P*<0·01
Isoprenaline (0·01–0·20 μg/kg/min)						
14	1·39	0·82	−6	17	129	14
15	0·04	0·02	0	4	7	2
18	0·45	0·54	1	1	50	4
20	0·24	0·36	4	13	50	3
22	0·30	0·17	3	1	29	—
35	0·49	0·47	5	15	60	5
Geometric mean	0·31	0·25	2·2‡	4·9	40	4·4
S.E.	±0·15	±0·14	±1·4	±2·7	±16	±1·5
P value	*P*<0·001	*P*<0·01	*P*<0·2	*P*<0·05	*P*<0·001	*P*<0·02
Noradrenaline (0·05–0·12 μg/kg/min)						
24	−0·42	−0·52	1	−27	−73	−18
25	−0·34	−0·27	−1	−8	−36	−4
26	−0·52	−0·36	−2	−1	−26	−4
27	−0·24	−0·37	−5	−4	−34	−6
35	−0·64	−0·50	−4	−8	−50	−9
37	−0·42	−0·45	−3	−5	−64	−4
Geometric mean	−0·41	−0·40	−2·3‡	−5·7	−44	−6·3
S.E.	±0·06	±0·04	±0·84	±2·3	±7	±1·6
P value	*P*<0·001	*P*<0·001	*P*<0·05	*P*<0·02	*P*<0·001	*P*<0·01

be noted that with the ΔGFRS statistics were performed on the absolute units. In this circumstance these arithmetic means cannot be simply compared with the other data because the others are given as the geometric means. In the dose ranges used, isoprenaline was about one-fourth as active as acetylcholine on the urine flow, osmolal clearance, and sodium excretion; and about one-half as active on the ERPF and potassium excretion.

Noradrenaline produced a decrease, compared with control, in all the values presented (Table 2). The decrease in GFR does not account for the decrease in sodium excretion (Table 1).

Considering the means given in Table 2, none of the drugs affected a change in free water clearance (C_{H_2O}) where: $\Delta C_{H_2O} = \Delta V - \Delta C_{OSM}$.

DISCUSSION

The interpretation of results from standard renal clearance experiments concerning sodium excretion is now easier, with respect to the influence of filtered loads, than it was. In the past, data of rapid changes in renal haemodynamics and in renal sodium excretion was considered not to be meaningful because it was thought that undetected changes in GFR would greatly affect the sodium excreted. We now know that this is not so (Levinsky, 1966 ; Lindheimer, Lalone & Levinsky, 1967 ; see also Harvey's work (1966) in which acetylcholine did not change the GFR despite generalized vasodilation). The percentage changes of sodium excretion are of the same order of magnitude as those of changes of GFR, if fractional tubular reabsorption does not change. The natriuresis of saline loading is chiefly a result of increased tubular rejection of sodium because the net tubular reabsorption of sodium follows in parallel with the filtered load (Stein, Bercovitch & Levitt, 1964). Dirks, Cirksena & Berliner (1965) and Watson (1966) have further shown that this natriuresis of saline loading in the dog is accompanied by an increased tubular rejection fraction of sodium in the proximal tubule during micropuncture studies. Watson (1966) concluded that all the increased sodium excreted could be accounted for by the decreased fractional reabsorption of sodium in the proximal tubule. The mechanism of this increased proximal tubular rejection of sodium as a result of saline loading is unknown (Rector, Sellman, Martinez-Maldonado & Seldin, 1967). The above evidence gives some basis for the validity of our use of the changes in tubular rejection fractions to explain the renal tubular effect of these drugs. The relationship between vascular changes and tubular changes is not known. Early & Friedler (1965) postulate that the haemodynamic alterations are the chief cause for the natriuresis. We postulate that the renal tubular effect of autonomic agents is a direct action upon the tubules. The site and mechanism of such an action cannot be determined from our procedure, but it is tempting to speculate that this site may be the proximal tubule, because there were very slight changes in free water clearance in our dogs. Shuster, Alexander, Lalone & Levinsky (1966) demonstrated that the natriuresis of saline loading is not caused by increased total or non-cortical renal plasma flow. Neither was the natriuresis caused by washout of the countercurrent gradient. May & Carter (1967) have shown that arecoline, infused unilaterally into the renal portal system of hens, produces a saluresis by direct renal action. They concluded that the small increase in plasma flowing through the portal system in their experiments could not be the cause of the saluresis and postulated a direct tubular mechanism of action.

Effects of isoprenaline

The renal vasodilator response to isoprenaline at the lower infusion rates are consistent with reports of Aviado, Wnuck & De Beer (1958) and McNay & Goldberg (1966). We had hoped to separate changes in renal plasma flow from changes in sodium excretion by the use of isoprenaline, because the renal vasodilatation was produced by stimulation of a different receptor from that stimulated by acetylcholine. The increased sodium excretion was, however, the result of increased tubular rejection of sodium during the infusion of each drug, as shown in Fig. 1. In this experiment the systemic blood pressure was not changed during the infusion of either drug. In subsequent experiments using higher

infusion rates of isoprenaline, the direct renal effect of isoprenaline was always complicated by the systemic effects which accompanied small reductions in mean blood pressure (10–20 mm Hg). This is illustrated in Fig. 4 in which the infusion of isoprenaline resulted in a bilateral decrease in sodium excretion from both kidneys, while simultaneously the direct renal effect can still be shown because the difference between the left and the right kidneys increased in the positive direction. In those instances in which the right kidney was excreting more sodium than the left kidney during control clearances, the left kidney values rose above the right kidney values during the infusion of isoprenaline. The profound fall in sodium excretion produced during the intravenous infusion of the same doses of isoprenaline exemplifies the antagonism of systemic reflexes against the direct saluretic effect. This antagonism is a result of compensatory reflex actions which we suggest are principally increased sympathetic nervous stimultion to the kidney during the fall in blood pressure. Here, we are reasoning from the fact that a fall in blood pressure during ganglionic blockade resulted in an increase rather than a decrease in sodium excretion. A similar effect was reported by Gill, Carr, Fleischmann, Casper & Bartter (1967).

The relationship between changes in mean renal plasma flows and changes in sodium excretion was not simple in individual dogs.

In Fig. 5 it was shown that during the systemic infusion of isoprenaline the ERPF and GFR fell simultaneously with the excretion of sodium, but when the infusion of isoprenaline was stopped the excretion of sodium remained depressed while the ERPF and GFR returned to levels slightly above control. This apparent increase in GFR after a period of reduced urine flow has been shown by Brewin, Ekins, Nashat & Portal (1966) to be the result of concealed filtration. Their results indicate that there is more of a tubular effect than is apparent because of the existence of concealed filtration during slow urine flows. The direct tubular effect of the proposed increased sympathetic nervous activity apparently lasts longer than renal vascular effects.

The decrease in the excretion of electrolytes as a result of systemic responses to the administration of isoprenaline is in accord with the results of Botting, Farmer & Lockett (1961) in conscious rats after subcutaneous injections of isoprenaline (2–4 μg/150 g body weight). In contrast to our results with direct natriuretic effects of isoprenaline in the dog, when Lees & Lockett (1965) administered isoprenaline (0.3–1.0 μg/150 ml.) to the circulating blood of isolated perfused cat kidneys an increased renal blood flow was accompanied by decreased urine flow, decreased rates of filtration, and decreased urinary excretion of sodium and potassium. Apparently the vascular tissue maintained its characteristic response to β-receptor stimulation but the response of the renal parenchyma was altered. This may have been due to a species difference.

Effects of noradrenaline

Noradrenaline infusion results in different qualitative renal electrolyte excretion effects depending upon the dose. The infusion rate determines the degree of systemic extrarenal effects, as well as the degree of renal vasoconstriction. An intense oliguria can obscure tubular effects (Brewin *et al.*, 1966). These authors presented good evidence for the continuation of filtration during anuria produced by a noradrenaline infusion.

If the renal arterial infusion of noradrenaline is in the range of 0.05 μg/kg/min and below, the direct renal effect of salt retention can be demonstrated by a decreased tubular rejection of sodium. In this range the systemic effects are slight. A relative saluretic effect can be observed in both kidneys when noradrenaline is infused intravenously (0.83 μg/kg/min) as shown in Fig. 7. This was reported by Hernandez & Coulson (1956) in the alligator. This systemic effect of noradrenaline was also shown by the work of Handley & Moyer (1954). Natriuretic effects of intravenously infused noradrenaline were made apparent when the direct vasoconstrictor effects were blocked by the infusion of phenoxybenzamine into the left renal artery. Using the data of Handley & Moyer (1954) and assuming that the serum sodium concentration was 150 m-equiv/l., it can be shown that the tubular rejection fraction of the left kidney, which was infused with phenoxybenzamine, was markedly increased during the intravenous infusion of noradrenaline (50 to 100 μg/min).

The renal effect of noradrenaline alone (100 μg/min/12 kg dog), given systemically in our experiments (Fig. 7) produced a marked fall in ERPF, GFR, and salt excretion in spite of large increase in systemic blood pressure. This observation was also made in the right kidney of the experiments of Handley & Moyer (1954) which was not blocked by phenoxybenzamine. The most apparent mode of action at this dose is vasoconstriction.

Natriuretic effects of noradrenaline therefore seem to depend upon at least three requirements: increased arterial blood pressure, the intravenous route, and a concentration with minimal direct renal effects. In 1961, Green & Sim reported that adrenaline and noradrenaline (0.5 mg/kg and 0.25 mg/kg subcutaneously) produced saluresis in rats. Noradrenaline produced a relatively smaller loss of sodium than did adrenaline. This can now be possibly explained by the additional direct renal action possessed by the beta-adrenergic effects of adrenaline. Pretreatment with phenoxybenzamine greatly depressed the diuretic effects in their rats. This was probably because of the absence of pressor activity caused by alpha-adrenergic blockade.

Mitchell (1950, 1951, 1953) has shown that the kidney of man is profusely innervated by autonomic nerves. The importance of the complex actions of autonomic drugs upon renal function will probably be understood when we know more about the function of this innervation and its response to cardiovascular changes (Williams, 1967). The possibility of its importance in controlling salt excretion is amplified by the fact that direct renal action of acetylcholine produces saluresis while that of noradrenaline produces sodium retention.

SUMMARY

1. Autonomic drugs were infused unilaterally during standard renal clearance periods in dogs.

2. Beta-adrenergic stimulation, isoprenaline infusion, results in a unilateral saluresis by a direct renal action similar to that of cholinergic stimulation in dogs. The magnitude of this action is approximately one-fourth that of acetylcholine.

3. Although the direct action of isoprenaline is one of natriuresis, the systemic effect results in a bilateral sodium retention which usually produced a net sodium retention in the dog.

4. Alpha-adrenergic stimulation, infusion of noradrenaline, results in a retention of sodium by a direct renal action.

5. The principal effect on sodium excretion, produced by all three drugs, was determined by changes in tubular rejection fractions. Changes in GFR could not account for the changes in sodium excretion.

6. The patterns of the changes in ERPF were not congruous with those of sodium excretion. The direct renal actions of these autonomic agents are believed to be produced mainly through an action on the tubules.

7. The net effect of autonomic drugs on renal function is the end result of a kind of algebraic summation of direct renal effects and indirect systemic responses. The quality and magnitude of the renal effects are therefore highly dependent upon the dosage and the route of administration. This helps explain the many paradoxical effects of adrenergic amines upon renal sodium excretion.

8. Direct renal actions of autonomic agents implicates the importance of the autonomic innervation to the kidney and would provide intra-renal responses to fluid and electrolyte needs of the body.

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