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Renal clearance procedure for the rat: effect of dopamine and standard saluretics

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Four standard diuretic compounds were evaluated for electrolyte output, effects on glomerular filtration rate (GFR), and effective renal plasma flow (RPF) in an anaesthetized rat preparation. Triamterene, hydrochlorothiazide, acetazoleamide and furosemide exerted their characteristic diuretic effects at doses which did not significantly change GFR or RPF. Regulation of the rate of a 3% mannitol-0·9% NaCl infusion to rats permitted the establishment of GFR and RPF values which were approximately in the middle of the reported range for this species. A significant increase in RPF without a concurrent rise in GFR was produced with dopamine. This was effectively blocked by the peripheral dopamine inhibitor, bulbocapnine.

Traditionally, reliable data on glomerular filtration rate (GFR), renal plasma flow (RPF) and electrolyte excretion have come from studies in the unanaesthetized dog (Baer, Russo & Beyer, 1959; Wiebelhaus, Brennan & others, 1967). Reported tests of these functions in the rat have not been numerous, and have often been part of other experiments in which micropuncture (Litchfield & Bott, 1962), volume expansion (Windhager & Giebisch, 1961), or other techniques (Malnic, Klose & Giebisch, 1964) were of primary interest. Recently, however, the appearance of publications on renal clearance (Kau, Sastry & Michelakis, 1974), renal blood flow (Hsu, Kurtz & others, 1975) and glomerular filtration rate in the rat (Huss, Marsh & Kalaba, 1975) has stimulated interest in the desirability of studying these renal functions in this species. The described test has been shown to be a satisfactory procedure for routinely observing two important vascular parameters (GFR and RPF) as well as urinary volume and electrolyte excretion in the anaesthetized rat.

MATERIALS AND METHODS

Materials

Triamterene, hydrochlorothiazide and furosemide were synthesized at SKF laboratories. Bulbocapnine was from Conroy Chem. Inc., acetazoleamide (Diamox powder) from Lederle Laboratories, dopamine from Calbiochem. All other chemicals were of reagent grade and from standard commercial sources. PAH [glycyl-2-^{3}H] and inulin [carboxyl-^{14}C] were obtained from New England Nuclear as a 95% ethanol solution and a crystalline solid, respectively. The purity of radioactivity for these compounds was between 97–99%. Heparin 1000 Units ml^{-1}) was from Elkins-Sinn, Inc., Cherry Hill, New Jersey.

Methods

Experiments were with Charles River (C-D) male rats, 290–335 g. General anaesthesia was induced with 25% urethane at 0·7 ml 100 g^{-1}, half of the dose being given intraperitoneally, the remainder subcutaneously. The abdomen was opened via a midline incision and a small cut made in the bladder to take a glass cannula for urine sampling. The femoral vein was cannulated with PE 10 polyethylene tubing for infusion with a Manostat peristaltic infusion pump. The femoral artery was cannulated with PE 50 polyethylene tubing for the collection of blood samples.

The infusion solution of 3% mannitol in 0·9% NaCl containing 1·5 μCi ml^{-1} [^{14}C]inulin and 2·5 μCi ml^{-1} [^{3}H]PAH was given at 0·08 ml min^{-1}. No prime was administered. A 0·05 ml injection of heparin was given (i.v.) just before the start of the infusion.

After 90 min of infusion, seven 20 min clearance periods (C_1–C_7) were taken. The average of the first three clearances (C_1–C_3 or Phase 1) served as the control for each rat. Test compounds were given during collection of the remaining clearances (C_4–C_7 or Phase 2). The average of these clearances for each rat constituted the test or drug period. Mid-point blood samples (0·25 ml) were collected with heparinized syringes. Urine and plasma samples of 20 μl were pipetted into counting vials containing Instabray and were counted simultaneously for ^{14}C and

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^3H by liquid scintillation. Plasma and urine samples were analysed for Na^+ and K^+ by atomic absorption spectrophotometry, and Cl^- by automatic chloride titrator.

Compounds were given as mg kg^{-1} h^{-1} or μg kg^{-1} min^{-1} of the free base. Doses for standard diuretic compounds were calculated on the basis of the doses found to produce a maximally effective natriuretic response in the dog. Ordinarily a prime of equal dosage would be given with the infusion but this was omitted because of the necessity of isolating another vein for such an injection.

In the computation of statistical significance, differences between the average of control (C_1–C_3) and test (C_4–C_7) for a compound were compared with the difference between (C_1–C_3) *vs* (C_4–C_7) of the separate group of controls. This treatment took into account any increases or decreases in the second (C_4–C_7) phase of the controls. With chloride, where no group of controls was accumulated (assay not used at time of controls), statistical evaluation was done by the paired *t*-test where the individual C_1–C_3 for each compound was compared to the C_4–C_7 phase of that particular experiment.

RESULTS

Standard diuretics

None of the standard diuretic compounds significantly affected GFR or RPF at the doses used (Table 1) which, except for triamterene, resulted in significant increases in urine volume over controls of 77, 209, and 673% for hydrochlorothiazide, acetazoleamide and furosemide respectively. Natriuresis with triamterene was modest, but with reduction in potassium excretion the sodium/potassium ratio rose to 5·25. Hydrochlorothiazide natriuresis was about twice that of triamterene, and kaliuresis was slightly over three times that of the control group. The sodium/potassium ratio was elevated slightly but still remained below one. Administration of acetazoleamide resulted in a marked natriuresis essentially equal to that seen after furosemide; viz approximately 350 μ equiv/20 min. However, this sodium load was excreted in a much lower volume with acetazoleamide than with furosemide (0·068 *vs* 0·170 ml min^{-1}). Kalireses for both compounds also paralleled their natriuretic effects and the sodium/potassium ratios were similar. Furosemide produced transient drops

Table 1. *Effect of four standard diuretic agents in anaesthetized rats.*

Compound	Phase	Urine vol. ml min^{-1}	ml min^{-1}/100 g GFR	ml min^{-1}/100 g RPF	μ equiv excreted in 20 min Na^+	K^+	Cl^-	Na/K
Control	1	0·020 ±0·002	0·92 ±0·02	2·67 ±0·15	4 ±2	21 ±3	Not	0·14 ±0·04
	2	0·024 ±0·003 ↑20%	0·89 ±0·02	2·66 ±0·15	10 ±4	38 ±9	Det.	0·21 ±0·06
Triamterene 4 mg kg^{-1} h^{-1}	1	0·020 ±0·001	0·95 ±0·07	2·67 ±0·07	5 ±2	19 ±3	6 ±2	0·26 ±0·07
	2	0·024 ±0·001 ↑20%	0·98 ±0·09	2·72 ±0·10	42 ±6**	8 ±1*	14 ±6	5·25 ±1·6**
Hydrochlorothiazide 3 mg kg^{-1} h^{-1}	1	0·022 ±0·01	0·93 ±0·05	2·71 ±0·12	3 ±0·5	28 ±5	8 ±2	0·10 ±0·02
	2	0·039 ±0·005* ↑77%	0·91 ±0·05	2·82 ±0·14	84 ±13**	134 ±8**	136 ±25**	0·65 ±0·09**
Acetazoleamide 15 mg kg^{-1} h^{-1}	1	0·022 ±0·004	1·14 ±0·04	2·79 ±0·13	2 ±0·3	41 ±7	13 ±3	0·05 ±0·10
	2	0·068 ±0·002** ↑209%	1·08 ±0·04	2·88 ±0·12	357 ±72*	167 ±23**	29 ±6	2·15 ±0·24**
Furosemide 5 mg kg^{-1} h^{-1}	1	0·022 ±0·002	1·09 ±0·09	2·85 ±0·24	7 ±3	25 ±8	13 ±4	0·24 ±0·07
	2	0·170 ±0·02** ↑673%	0·91 ±0·04	2·44 ±0·10	345 ±75*	154 ±14**	350 ±54**	2·10 ±0·40*

* $P = <0·01$; ** $P = <0·001$. ↑ = increase.
Values are means ± s.e.m. for n = 6.
Phase 1 = C_1–C_3 (20 min collection periods).
Phase 2 = C_4–C_7 (20 min collection periods).

in GFR and RPF which lasted about 20 min and which were not statistically significant.

Chloruresis after triamterene was not significant. Hydrochlorothiazide was moderately chloruretic while the response after furosemide was pronounced and equal to its natriuresic effect. As expected, the carbonic anhydrase inhibitor, acetazoleamide, produced only a slight, insignificant, chloruretic response.

Dopamine

The effects from the administration of dopamine, which has renal vasodilator compound activity (McNay & Goldberg, 1966), on GFR, RPF and electrolyte excretion are described in Table 2. A significant increase in RPF (22%) was produced by an infusion of 4·5 μg kg^{-1} min^{-1}. GFR did not increase significantly. Dopamine also produced a 21% increase in urine volume which was not statistically significant. No significant saluretic response was seen but there was a significant increase in potassium excretion.

Results in preliminary studies indicated that the dose of dopamine required to produce a significant increase in RPF was critical. At 3·0 μg kg^{-1} min^{-1}, the increase in RPF was not statistically significant (16%) but at 6·0 μg kg^{-1} min^{-1} (the maximum effective dose used to increase renal blood flow in dogs, by Meyer, McNay & Goldberg, 1967), urine volume, GFR and RPF were reduced markedly in the third and fourth post-drug clearances.

When the 'optimal' dose of dopamine (4·5 μg kg^{-1} min^{-1}) was infused simultaneously with 50 μg kg^{-1} min^{-1} of the dopamine inhibitor, bulbocapnine (Setler, Pendleton & others, 1975), an almost complete blockade of the dopamine-induced rise in RPF was observed (22 to 2%). The non-significant (9%) increase in GFR produced by dopamine alone, was reduced slightly by bulbocapnine which had no significant effects on urine volume or on the slight saluresis produced by dopamine but it significantly inhibited the kaliuresis produced by the amine.

In experiments where bulbocapnine was given alone, there were no significant effects on GFR, RPF or electrolyte excretion patterns compared with the control responses. Bulbocapnine as a 1 mg kg^{-1} infused over 10 min before the start of C_4, or as 3 mg kg^{-1} (i.v. stat) given 5 min before the beginning of the post-drug clearances (C_4–C_7) had no effect on the increase in RPF produced by dopamine.

DISCUSSION

Several types and rates of infusion were tried before the combination of a rate of 0·08 ml min^{-1} and an infusion of 3% mannitol in 0·9% NaCl was selected. Increasing the infusion rate to 0·12 ml min^{-1}, approximately the rate used by Kau & others (1974) in their clearance tests, resulted in greater control natriuresis (50–100 μ equiv 20 min^{-1}). This elevated level of electrolyte excretion was more desirable for showing the electrolyte response to standard

Table 2. *Effect of dopamine, the combination of dopamine and bulbocapnine and bulbocapnine alone on GFR, RPF and electrolyte excretion in the anaesthetized rat.*

Compound	Phase	Urine vol. ml min^{-1}	ml min^{-1}/100 g GFR	ml min^{-1}/100 g RPF	μ equiv excreted in 20 min Na^+	K^+	Cl^-	Na/K
Dopamine	1	0·023	1·15	2·95	13	44	21	0·25
4·5 μg kg^{-1} min^{-1}		±0·002	±0·05	±0·11	±5	±5	±6	±0·09
	2	0·028	1·25	3·61	29	73	34	0·36
		±0·002	±0·07	±0·12*	±8	±11*	±7	±0·10
		↑21%	↑9%	↑22%				
Dopamine	1	0·022	1·21	3·28	6	30	14	0·23
4·5 μg kg^{-1} min^{-1}		±0·001	±0·10	±0·21	±3	±7	±7	±0·10
plus	2	0·025	1·22	3·36	7	29	18	0·34
Bulbocapnine		±0·002	±0·12†	±0·28‡	±3	±8†	±8	±0·15
50 μg kg^{-1} min^{-1}		↑12%	↑0·8%	↑2%				
Bulbocapnine	1	0·018	1·03	2·69	1	26	7	0·08
50 μg kg^{-1} min^{-1}		±0·002	±0·07	±0·18	±0·3	±6	±3	±0·02
	2	0·019	1·07	2·75	4	30	18	0·15
		±0·001	±0·08	±0·19	±2	±6	±5	±0·05
		↑6%	↑4%	↑2%				

* $P = <0·01$.
† $P = <0·02$; ‡ = $P = 0·001$ compared with effect of dopamine alone.
Values are means ± s.e.m. for n = 6.

diuretics; however, values for GFR and RPF were approximately twice the control values found with the 0·08 ml min^{-1} infusion. Obviously these parameters were functioning near the reported maximum levels possible for the rat and they could not be further influenced by compounds which might normally elevate GFR or RPF. The 0·08 ml min^{-1} infusion was used because our interests were centered around the GFR–RPF relation as well as the patterns and degree of electrolyte excretion.

Infusions of 0·9% NaCl without mannitol were less effective in producing a sustained urinary volume than with the 3% mannitol-0·9% NaCl combination. With the former infusion the rats retained large amounts of sodium for several hours before a marked and rapid natriuresis occurred. The slight osmotic diuretic effect produced by 3% mannitol was thought to aid a more uniform urine flow. The overall consistency of the urine output was also thought to be due to the 45 min infusion of mannitol - 0·9% NaCl before the start of the control (C_1–C_3) clearances. Shorter initial periods of infusion were less effective and resulted in a progressively increasing urine output.

Urethane was used because at the 1·75 g kg^{-1} dose given by the combined intraperitoneal and subcutaneous routes no supplemental anaesthesia was required. The dose was not much greater than the 1·3 g kg^{-1} given to rats intravenously by Volicer & Loew (1971), who found that dose to have no significant effect on the fractional blood flow through the kidneys compared with unanaesthetized controls. Mean blood pressure of several rats monitored during our studies varied between 95–100 mm Hg.

Further confidence that urethane was not having a deleterious effect on renal function was derived from the fact that GFR and RPF values averaged approximately 1·00 and 3·00–4·00 ml min^{-1} min^{-1}/100 g respectively, with the anaesthetics ketamine and, Inactin. Both proved to be too short acting and ketamine also caused some rats to have respiratory failure before the start of the infusion.

The volume and electrolyte excretion patterns of the standard diuretics tested are consistent with the activity of these agents in dogs and man. At the doses used, dopamine significantly increased RPF but was without the natriuretic effect which we and others have seen in the clearance dog (McNay & Goldberg, 1966). It is likely that a greater increase in sodium excretion in the rat could be achieved by increasing the sodium load.

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