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Renal handling of norepinephrine and epinephrine in the pig*

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Abstract. To investigate the renal handling of catecholamines in the pig, intravenous infusions of ^{51}Cr-EDTA and PAH were performed in 7 animals, and samples for simultaneous measurement of norepinephrine (NE), epinephrine (E), ^{51}Cr-EDTA and PAH were obtained through catheters placed into the aorta, left renal vein and both urethers. For both kidneys together, ^{51}Cr-EDTA clearance [GFR] averaged 48 ± 14 (± SD) ml/min (2.23 ± 0.66 ml/kg/min). In the left kidney, GFR averaged 22 ± 9 ml/min, arteriovenous PAH extraction 0.87 ± 0.09, and calculated total renal plasma flow 91 ± 30 ml/min. Plasma NE and E were lower in renal venous than arterial blood ($P < 0.005$), extraction ratios averaging 0.36 and 0.77, respectively. NE excretion rate in final urine (8.9 ± 4.3 ng/min) exceeded transrenal NE extraction rate (5.2 ± 3.9 ng/min) by 3.7 ± 4.4 ng/min. In contrast, urinary E excretion rate (2.9 ± 2.0 ng/min) was slightly lower than transrenal E extraction rate (3.6 ± 3.8 ng/min). These observations suggest that in pig kidneys, plasma PAH extraction rate and GFR related to body weight are quite similar to values in man. Three quarters of circulating E are extracted for the most part by tubular secretion, and the slightly smaller amount appearing in urine is consistent with some intrarenal metabolism. NE, presumably originating from intrarenal neuronal release and/or de novo production, is secreted into the urine.

Key words: Renal regulation of catecholamines – Norepinephrine – Epinephrine – Renal function – Pig

Introduction

The adrenergic system interacts with a variety of organs, including the kidney. The role of the kidney in the regulation of catecholamines is yet poorly understood. The mechanisms involved in catecholamine clearance and excretion by the kidney have been investigated in various experimental animals, such as chicken, rat, dog and rabbit (Baines et al. 1979; Baines 1982; Ball et al. 1982; Boren et al. 1980; Jones and Blake 1958; Kopp et al. 1983; Lappe et al. 1980, 1982; Oliver et al. 1980; Overy et al. 1967; Rennick and Prior 1965; Silva et al. 1979; Unger et al. 1978). Studies in intact animals have been performed only in dogs and to a limited extent in rats and rabbits.

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The present study was undertaken to investigate the renal handling of norepinephrine and epinephrine, as related to standard indices of kidney function in the domestic pig. The latter is, like man, a carnivorous mammal.

Methods

Preparation of the pigs. Experiments were performed in seven female baby pigs (body weight 22 kg, range 17–26 kg). The pigs had been fasted with free access to water for 24 h previous to the experiment. Anesthesia was induced by ketamine (Parke Davis, Detroit, Michigan), azaperon and metomidate (Janssen Pharmaceutica, Beerse, Belgium). The pigs were intubated by a cuffed endotracheal tube, through which a mixture of 40% oxygen and 60% nitrous oxyde was administered by a respirator. Muscular relaxation was achieved by alloferin (Hoffmann-La Roche, Basel, Switzerland). Pethidine was used to deepen the anesthesia. Solutions of sodium chloride, ringer lactate and gelatine (Physiogel, R. Hausmann, St. Gallen, Switzerland) were administered intravenously (30 ml/h/kg body weight) to prevent a decrease in mean arterial pressure to less than 70–80 mm Hg. Arterial blood gases were determined to optimize ventilation volumes. Blood pressure and heart rate were continuously monitored by a transducer sonde (Statham transducer) inserted into the carotid artery. This sonde was also used to sample aortic blood.

The surgical preparation consisted of a mid-abdominal incision and the cautious preparation of the left renal hilum. The left renal vein was visualized, and it was ascertained that there was no inflow from adrenal veins. The kidney was left untouched in its peritoneal planes. A 6 F opaque polyethylene catheter was introduced into the left renal vein via the left jugular vein and its correct position was confirmed. Both urethers were cut 10 cm distally of the renal hilum, and 7 F polyvinyl catheters were inserted and a ligature was applied to them. The viscera was covered by a damp tissue to prevent exsiccation. At the end of the operative procedure, the pigs were heparinized by liquemine (Hoffmann-La Roche, Basel, Switzerland) until clotting times of 200 to 400 seconds were achieved.

Clearance procedure. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by constant infusion clearance technique using ^{51}Cr-EDTA and para-aminohippuric acid (PAH), respectively (Reubi 1982; Vorburger et al. 1969). A priming dose of 125 μCi ^{51}Cr-EDTA (Farbwerke Hoechst, Frankfurt, FRG) and 0.6 g of

PAH was diluted in 100 cc of saline and administered rapidly intravenously through an ear vein. This was followed by a susteined drip; 0.8 g PAH and 100 μCi of ^{51}Cr-EDTA were diluted in 200 ml of normal saline and applied at a rate of 1 ml/min by a constant infusion pump. After an equilibration period of 30 min, 2 to 3 urine collection periods of 15–20 min with midline blood sampling were performed. For sampling, the blood was allowed to drip freely from the catheters dwelling in the renal vein and aorta. The first two milliliters of each sample were rejected. In all cases, the aortic and renal vein samples were collected simultaneously in heparinized glass tubes. All samples were chilled immediately after sampling, centrifuged and stored at −70° C until processed.

Analytical procedures. Norepinephrine (NE) and epinephrine (E) were measured in plasma and urine by the radioenzymatic method of Da Prada and Zürcher (1976), as reported previously from this laboratory (Weidmann et al. 1978). Samples were processed in triplicate. Aliquots of urine (diluted 1:50 in 0.01 m HCl) or acid deproteinized plasma, to which EGTA and MgCl were added previously, were incubated with ^{3}H-S-adenosyl methionine (Amersham, specific activity 15 Ci/mmol) and catechol-o-methyl transferase obtained from rat liver. Aliquots of external and internal standards were also included in the assay. The methylated catecholamines were separated by thin layer chromatography, oxydized to vanilline, diluted in a toluene based scintillation fluid, and the radioactivity counted in a beta-counter.

PAH concentrations and ^{51}Cr-EDTA activity in plasma and urine were obtained by standard methods (Reubi 1982; Vorburger et al. 1969).

Statistical methods. The results are expressed as means ± SD. The statistical evaluation of the results was made with paired Student-*t*-test.

Calculations

The following additional abbreviations are used.

A = arterial (aortic) plasma concentration; RV = renal venous plasma concentration; U = urinary concentration; UV = urine volume per min; $TRPF$ = total renal plasma flow.

Effective renal plasma flow ($ERPF$) (ml/min)

$$= \frac{U_{PAH} \cdot UV}{A_{PAH}}.$$

Total renal plasma flow ($TRPF$) (ml/min)

$$= \frac{U_{PAH} \cdot UV}{A_{PAH} - RV_{PAH}}.$$

Glomerular filtration rate (GFR) (ml/min)

$$= \frac{U_{^{51}Cr\text{-}EDTA} \cdot UV}{A_{^{51}Cr\text{-}EDTA}}.$$

Filtration fraction

$$= \frac{GFR}{ERPF}.$$

Renal arterio-venous extraction ratio

$$= \frac{A - RV}{A}.$$

Net transrenal plasma extraction rate (ng/min)

$$= TRPF \cdot [A_{NE} - RV_{NE}].$$

Net transrenal plasma NE clearance (ml/min)

$$= TRPF \cdot \left[\frac{A - RV}{A}\right].$$

Urinary NE excretion rate (ng/min)

$$= U_{NE} \cdot UV.$$

Net renal tubular secretion rate (ng/min)

$$= [U_{NE} \cdot UV] - [TRPF \cdot (A_{NE} - RV_{NE})].$$

Results

Blood pressure in the seven pigs averaged 110/84/71 ± 17/15/15 mmHg (systolic/mean/diastolic).

The plasma levels of PAH, ^{51}Cr-EDTA and catecholamines in the aorta and left renal vein are shown in Table 1. In addition to the expected arterio-venous decrease in PAH and ^{51}Cr-EDTA, NE and E concentrations also were consistently lower in renal venous than aortic plasma ($P < 0.005$).

In the left kidney, PAH clearance averaged 79 ml/min, PAH arterio-venous extraction ratio 0.89, calculated TRPF 91 ml/min, GFR 22 ml/min, ^{51}Cr-EDTA arteriovenous extraction ratio 0.21, and filtration fraction 0.24 (Table 2). The slight difference between the two latter parameters was expected (Reubi et al. 1950). Moreover, ERPF and GFR were similar in the left and right kidney during the experiment (Table 2).

The urinary NE excretion rate also did not differ significantly between the two kidneys (Table 3). The arterio-venous extraction ratio and net transrenal plasma clearance of NE exceeded that of ^{51}Cr-EDTA by approximately 50%. Furthermore, urinary NE excretion exceeded transrenal NE extraction; the net renal NE secretion rate averaged 3.7 ng/min.

The arterio-venous extraction ratio and net transrenal plasma clearance of E was three times greater than that of ^{51}Cr-EDTA (Table 4). The urinary excretion rate of E did not differ significantly between the two kidneys. In the left kidney, the excretion rate tended to be slightly lower than the net transrenal plasma extraction rate, but this did not reach statistical significance.

Discussion

The findings of the present study indicate that in addition to glomerular filtration, transrenal extraction of circulating catecholamines and active tubular secretion of NE and E occur in the normal pig kidney. Thus, the net renal arterio-venous extraction ratios of NE and E exceeded that of the glomerular marker ^{51}Cr-EDTA by about 70% and 265%, respectively, and the net renal arterio-venous E extraction averaged 88% of PAH extraction. Moreover, the total

Table 1. Plasma levels of catecholamines, PAH and ^{51}Cr-EDTA[a]

	Aorta	Renal vein
PAH, mg/dl	2.30 ± 0.21	0.27 ± 0.16*
^{51}Cr-EDTA, cpm	590 ± 299	475 ± 257*
Norepinephrine, pg/ml	158 ± 95	106 ± 88*
Epinephrine, pg/ml	46 ± 23	12 ± 12*

[a] Mean values ± SD
* $P < 0.005$ versus aorta

Table 2. Effective and total renal plasma flow, glomerular filtration rate, renal PAH and ^{51}Cr-EDTA extraction, and filtration fraction

	Left kidney	Right kidney
PAH clearance (ERPF), ml/min	79 ± 26	80 ± 30
PAH renal arterio-venous extraction ratio	0.87 ± 0.09	
Total renal plasma flow, ml/min	91 ± 31	
^{51}Cr-EDTA clearance (GFR), ml/min	22 ± 9	26 ± 7
^{51}Cr-EDTA renal arterio-venous extraction ratio	0.21 ± 0.07	
Filtration fraction	0.24 ± 0.06	

Table 3. Renal handling of norepinephrine

	Left kidney	Right kidney
Renal arterio-venous NE extraction ratio	0.36 ± 0.19	
Net transrenal plasma NE clearance, ml/min	36 ± 30	
Net transrenal plasma NE extraction rate, ng/min	5.2 ± 3.9	
Urinary NE excretion rate, ng/min	8.9 ± 4.3	11.2 ± 9.2
Net renal tubular NE secretion rate, ng/min	3.7 ± 4.4	

Table 4. Renal handling of epinephrine

	Left kidney	Right kidney
Renal arterio-venous E extraction ratio	0.77 ± 0.20	
Net transrenal plasma E clearance, ml/min	69 ± 35	
Net transrenal plasma E extraction rate, ng/min	3.6 ± 3.8	
Urinary E excretion rate, ng/min	2.9 ± 2.0	3.4 ± 2.8
Net renal tubular E secretion rate, ng/min	−0.7 ± 2.8	

amounts of NE and E appearing in the final urine exceeded the theoretical amounts delivered by glomerular filtration by 156% and 186%, respectively.

Because of the complexity of the factors involved in renal NE regulation, no direct determination of secretory rates has been possible. In the rat, tubular secretion of NE injected under the renal capsule was estimated to be 13% − 22% (Baines et al. 1979; Baines 1982). From the available data in dogs (Ball et al. 1982), an approximative tubular NE secretion rate can be derived by the formula:

$$\frac{\text{Excretory rate of NE in the final urine-filtered load of NE}}{\text{Total renal plasma flow} \times \text{plasma NE concentration}}.$$

Plasma NE levels in these dogs (Ball et al. 1982) were corrected for a NE-plasma protein binding of 30% (Rennick 1968). The resulting approximative tubular NE secretion of 10% is not influenced by intrarenal tissue metabolism, but could be an overestimation in the case that a relevant amount of NE appearing in final urine is derived from intrarenal neuronal release; the latter possibility is rather improbable in dogs studied under basal conditions (Kopp et al. 1983).

In our pigs, an approximative value for NE passing from the circulation directly into the kidney by ways other than glomerular filtration can be derived from the formula:

$$\frac{\text{Transrenal NE extraction rate-filtered load of NE}}{\text{Total renal plasma flow} \times \text{arterial plasma NE concentration}}.$$

The resulting estimate is 12%. Nevertheless, the occurrence of any metabolic breakdown of NE in the pig kidney tissue would decrease the net tubular NE secretion rate. Conversely, the calculated estimate may tend to be underrated a) by not considering possible NE plasma protein binding, which is not known for the pig, and b) by a modulating influence of certain anaesthetic drugs used in the present study. Ketamine, which was used for the induction of general anaesthesia, may have a stimulatory effect on the central sympathetic system (Traber and Wilson 1969) and induce a dose-dependent inhibition of neuronal NE re-uptake (Miletich et al. 1973; Nedergaard 1973). However, the central nervous influence did not exceed 20 min after bolus injection of therapeutic doses (2 − 4 mg/kg, plasma levels 5 μg/ml), and a blunting of neuronal norepinephrine re-uptake was observed only in isolated organs exposed to relatively high concentrations of ketamine (Miletich et al. 1973; Nedergaard 1973). Since in our experiments catecholamine sampling was always performed 4 − 5 h after the induction of anaesthesia, and considering the relatively fast elimination of ketamine from the circulation (t/2 for the fast phase 11 min, t/2 for the slow phase 2.5 h), this drug might have had a minor influence on our results (Wieber et al. 1975). Pethidine, used for the deepening of the anaesthesia, slightly augmented NE outflow and diminished neuronal re-uptake of NE in isolated rabbit heart (Montel and Starke 1973). However, spontaneous NE outflow caused by pethidine was observed only at pharmacological concentrations (e.g. 28 μg/ml as compared to therapeutic concentrations of 0.5 − 0.7 μg/ml, of which 60% − 80% are bound to plasma glycoproteins) (Edwards et al. 1982; Wieber et al. 1975). While mild inhibition of NE re-uptake (16%) was obtained with pethidine concentrations of 0.28 μg/ml in protein free perfusion medium, the latter concentration, when corrected for protein binding, exceeds by 50% the therapeutic concentration of pethidine. Whatever the exact values may be, it appears probable that only a small amount of NE is removed from the circulation by means of tubular secretion in rat, dog or pig.

NE reaches the kidney not only by the circulation, but is also released locally from renal sympathetic nerves. In

fact, urinary NE excretion in our pigs exceeded transrenal NE extraction by 41%. Apart from neuronal discharge, some of this NE could theoretically also be derived from de novo synthesis in renal tissue. In the rat, the renal nerves were found to contribute 30% of NE excreted in the urine (Baines 1982). Thus, rat and pig kidneys may function again quite similarly in this regard. In rabbit, stimulation of renal nerves caused a 35 to 39% surplus secretion of NE into the urine (Lappe et al. 1982). In the dog, urinary NE excretion also was augmented by mild renal nerve stimulation, but did not respond further to high level renal stimulation (Kopp et al. 1983).

Some of the NE discharged from renal nerves does not appear in the urine, but may undergo neuronal re-uptake, intrarenal degradation and/or, at least in some species, escape into the circulation. Indeed, in the dog a positive renal veno-arterial NE ratio indicated that a relevant fraction of NE leaves the kidney by this route, under basal conditions as well as after nerve stimulation (Ball et al. 1982; Boren et al. 1980; Oliver et al. 1980; Unger et al. 1978). Therefore, renal venous NE concentrations may be a useful and urinary NE excretion a poor indicator of intrarenal sympathetic nerve activity in the dog (Kopp et al. 1983; Oliver et al. 1980). In contrast, our finding of a negative veno-arterial NE ratio in the pig reveals no important spillover of NE from renal tissue into the circulation. Renal nerve stimulation will be required to detect a more discrete renal venous NE secretion. Nevertheless, an apparently minor venous escape of NE may render urinary NE in the pig more dependent on changes in intrarenal NE release than is the case in the dog.

The handling of E may differ less between the various mammalian species. In rat (Baines et al. 1979), tubular secretion of E assessed following subcapsular injection of radioactively labelled E was estimated as 46%. Moreover, arterio-venous extraction amounted to 77% in our pigs and 75% in the dog (Ball et al. 1982), while the ratio between urinary E excretion and plasma E averaged 77% in man (Link et al. 1982; Link 1984). The present study allows only indirect conclusions on renal E metabolism. Average transrenal E extraction rate slightly exceeded urinary E excretion rate (3.6 vs 2.8 ng/min) in the pig. Therefore, metabolism breakdown of E, which was found to be quite pronounced in dog kidneys (Ball et al. 1982), appears to occur also to a certain extent in the renal tissue of the pig.

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