Urol. Res. 9, 5-11 (1981)

Urological Research

Effect of Dopamine on Renal Haemodynamics in the Denervated Dog Kidney

H. Huland, H. J. Augustin, H. G. Baumgarten and S. Jenner

Department of Internal Medicine, Department of Functional Anatomy, Department of Urology, University Hospital Hamburg-Eppendorf, Federal Republic of Germany

Accepted: November 6, 1979

Summary. The aim of the present study was to explore the potential modulating role of the renal sympathetic nerves in the dopamine-induced vasodilatation in the dog kidney, using the 133-Xenon washout technique.
Immediately before and 48 h after combined surgical and local chemical destruction of the sympathetic nerves innervating the left kidney, renal and intrarenal blood flow was monitored in both kidneys. Catecholamine content was determined in control kidneys from untreated dogs, and in the denervated and contralateral, non-denervated kidney 48 h after unilateral sympathectomy.
By 48 h after destruction of the sympathetic nerves innervating the left kidney, total renal and particularly cortical flow rates were significantly increased when compared to the right, innervated kidney. Destruction of the sympathetic nerves also resulted in a disappearance of noradrenaline and adrenaline from the kidney and a significant reduction in the content of dopamine in the denervated but an increased level in the innervated kidney.
The dopamine-induced increase in renal blood flow was similar in the denervated and in the innervated kidney, suggesting that the sympathetic nerves do not significantly alter the vascular effects of dopamine. The persistence of the dopamine-induced vasodilatation in the sympathectomized kidney supports the concept that dopamine acts directly on postsynaptic, specific dopamine receptors.

Key words: Dopamine, Renal haemodynamics, Kidney denervation, Catecholamine content, Fluorescence histochemistry of adrenergic nerves, Dog kidney.

INTRODUCTION

Among the naturally occurring catecholamines, dopamine (DA) is unique for its specific vasodilatatory action in the kidney of certain species (6, 12, 13, 15, 17), said to be mediated by dopamine receptors in the renal vascular system (7, 12). We have previously shown (2) that dopamine fails to induce vasodilatation in the isolated, perfused, catecholamine-depleted kidney of the rat. This finding may be explained by the absence of modulatory autonomic reflexes in the isolated kidney (implying that dopamine inhibits sympathetic activity at the level of the pre- or postganglionic neurones or fibres) or by the lack of specific DA receptors in the renal vascular system of this species.

The potential role of sympathetic nerves in DA-induced vasodilatation receives some support from the findings of McDonald et al. (15) that vascular resistance decreased in the isolated hindleg of the dog during DA infusion by a neurogenic mechanism, and from the findings of Bogaert et al. (5) that the dopamine – induced decrease in flow resistance of the femoral artery was reversed by prior denervation. Such findings may tentatively be explained by assuming a preferential action of DA on presynaptic autoreceptors which inhibit release of NA from the sympathetic nerve endings (4). Whether such observations obtained on isolated, perfused organs tissues reflect the situation in the intact animal remains questionable.

The aim of the present study was to explore the in vivo effect of dopamine on renal haemodynamics of the anaesthetized dog comparing flow patterns in the innervated (right) with those in the denervated (left) kidney. Of particular interest was the question whether the extent of DA-induced vasodilatation was changed in kidneys when their autonomic reflexes were interrupted by combined

0300-5623/81/0009/0005/$ 01.40

surgical and local chemical destruction of the sympathetic renal efferent fibres.

METHODS

a. In vivo Measurement of Blood Flow in the Innervated and in the Denervated Dog Kidney

10 Mongrel dogs of either sex ranging in weight from 15 to 25 kg were used to study the influence of dopamine on haemodynamics of innervated and denervated kidneys. Another 3 dogs served as untreated controls for catecholamine determinations and histochemical localization of the catecholamines. Anaesthesia was started with 30 mg . kg^{-1} pentobarbital, iv. After intubation, the animals were connected to an open respirator system (Bird respirator, Marck 8, Bird corp.) and a continuous level of anaesthesia was obtained by injecting another bolus of pentobarbital (60 mg . kg^{-1}, iv.). The left kidney was exposed through a left lateral rectus incision. The nerves travelling with the renal vessels were carefully prepared. After this procedure, the measurement of renal and intrarenal blood flow, as described below, was performed in duplicate prior to and during dopamine infusion. Following these control measurements, all visible nerve fibres along the renal artery, vein and ureter were stripped for a distance of one to two cm. Additionally, 6-hydroxy-dopamine (6-OH-DA) was injected into the adventitial tissue of the renal artery proximal to the renal pelvis (usually 3 mg 6-OH-DA in 3 ml saline containing 3 mg 1-ascorbic acid). At the end of this procedure, the left rectus incision was closed. 48 h later, the left kidney was exposed through the same incision. Renal and intrarenal haemodynamics were measured again after cannulating the renal artery. Following these control measurements, dopamine was administered by infusion. Blood flow through the right kidney served as a control and was monitored immediately after denervation of the contralateral kidney (see above) as well as 48 h later.

Renal and intrarenal haemodynamics were measured by means of the 133 Xenon washout technique. The renal artery of the investigated kidney was catheterized via a femoral artery using an Odman X-ray opaque catheter no. 5. Under fluoroscopic control, the catheter tip was carefully moved a few mm into the ostium of the renal artery. A bolus of 0, 5 mCi 133 Xenon (Radiochemical Center, Amersham, England), dissolved in 2 ml saline at 37°C, was injected rapidly into the renal artery via the indwelling catheter. The rate of disappearance of the 133 Xenon was externally monitored with a gamma ray scintillation detector placed over the flank of the recumbent dog. The data were plotted by means of a digital ratemeter connected to a digital printer. The washout of the gas from the kidney was monitored for 25 min.

The analysis of the 133 Xenon washout curve permits the calculation of total renal flow as well as estimation of blood flow of the renal cortex (component I), of juxtamedullary cortex plus outer medulla (component II) and of the inner medulla (component III). The computer programme of Dell et al. (9) was applied for the evaluation of total renal and compartmental blood flow rates.

Dopamine, diluted in a 5% laevulose solution was administered by continous infusion in a concentration of 4 µg . kg^{-1} . min^{-1}.

b. Chemical Analysis of Dog Kidney Catecholamine Content

Pieces of dog kidney were frozen in liquid nitrogen and stored in a freezer (at -80°C) until homogenization in 0.1 N $HClO_4$ (1:2, w:v). The homogenized samples were centrifuged at 10.000 x g for 30 min. 30 µl of the supernatant were used for radioenzymatic determination of noradrenaline, adrenaline and dopamine (NA, A, DA) following the procedure devised by Engelman et al. (10). Extraction and separation of the tritiated, O-methylated derivatives of the three catecholamines were carried out by a modifcation of the method of Engelman et al. (Jenner and Baumbarten, to be published). This modification comprises use of a thin-layer chromatographic step. The sensitivity of the assay was: 50 pg for DA and 100 pg for NA and A, respectively. The observed differences in sensitivity depend mainly on the differing solubility of the O-methylated catecholamines in the organic extraction medium. The values given in Tables 3 and 4 are corrected for recovery.

c. Histochemical Demonstration of Tissue Catecholamine

An unpublished modification of the method of Falck and Owman (11) was used to visualize tissue monoamines in the kidneys of untreated and DA-perfused dogs. This modification consists of supravital perfusion of the kidneys with an ice-cold Tyrode solution containing 10 g aluminium sulphate (x 18 H_2O) per 150 ml Tyrode (Björklund, to be published). Small tissue pieces were rapidly dissected from the perfused kidneys and quenched in propane/propylene (8:2) cooled by liquid nitrogen. The frozen samples were freeze-dried, reacted with gaseous formaldehyde, embedded in paraffin, sectioned at 10 µ, mounted on glass slides and analyzed in a fluorescence microscope. Part of the sections were treated with sodium borohydride (Corrodi et al., 8) in

order to establish the nature of the fluorophores as catecholamine derivatives and to differentiate them from autofluorescent substances of similar colour.

RESULTS

a. Blood Flow Measurements

Total renal and regional intrarenal haemodynamics are different in kidneys of dogs with intact and with denervated sympathetic input. These differences were apparent in preinfusion control measurements as well as during dopamine infusions (Table 1 and Table 2). During infusion of 4 $\mu g.kg^{-1}.min^{-1}$ dopamine both the right and left kidneys revealed a significant increase in blood flow. This increase in blood flow occurred during anaesthesia and also following operative exposure of the left kidney (i.e. prior to denervation).

Measurements of the intrarenal haemodynamics indicated that the cortical fraction of the blood flow (expressed as % of total RBF) was similar in the right and left (exposed) kidney (about 78%; cf. Table 1) and that the cortical perfusion rate increased to a similar degree in both kidneys following DA infusion.

48 h following surgical and chemical denervation of the left kidney, the perfusion rate was elevated when compared to the innervated control kidney (Table 2). While DA infusion resulted in vasodilatation in both kidneys, the % increase in flow rate was greater in the innervated than in the denervated kidney (Table 2).

By 48 h after unilateral denervation, cortical blood flow was significantly higher in the left kidney than in the right, innervated kidney (Table 2). In the denervated kidney, the cortical fraction of total RBF was thus signficantly higher (Table 2).

DA infusion increased the blood flow in both the control kidney and the denervated one concomitant with a corresponding percentage increase in the cortical fraction (compare Table 1 and 2). The denervated left kidney, which showed an elevated rate of blood flow (when compared to the right one), responded to DA infusion with a slightly lower increase in blood flow than the control kidney.

Prior to denervation, the perfusion rate of the juxtamedullary cortical component and of the external and internal medullary component (CII and CIII) reacted to DA infusion in a comparable manner in both the right and left (exposed) kidney. Due to a restriction of blood flow through the cortical component (CI), the medullary fraction (CII %) and (CIII %) was found to be increased in the kidneys before DA infusion. However, under the influence of DA, the medullary fraction, expressed as % of total RBF, was decreased

Table 1. Total renal blood flow (RBF) and blood flow of individual components (I, II, III) in ml/100 g tissue/min before (= control) and during dopamine infusion (4 $\mu g.kg^{-1}.min^{-1}$) in dogs with intact innervation of the kidneys. The values in brackets represent the component flow rates expressed as percentages of total renal blood flow

	RBF	CI	CII	CIII
Renal blood flow in anaesthetized dogs after laparatomy				
Left kidney	304.6±50.8	501.2±79.0 (78.1±15.6)	62.4±21.1 (15.8± 4.2)	2.3±1.0 (6.0±1.3)
Right kidney	283.8±50.7	465.6±90.6 (77.7±11.2)	60.8±17.2 (18.0± 1.1)	2.0±0.8 (4.3±1.9)
Renal blood flow in anaesthetized dogs infused with dopamine				
Left kidney	406.5±89.0 p<0.05	649.2±99.7 (88.4± 4.3) p<0.01	88.4±19.6 (9.0± 1.1) p<0.05	3.0±0.8 (3.5±1.2) ns
Right kidney	371.3±67.8 p<0.05	608.2±69.8 (88.1± 9.7) p<0.01	82.4± 12.4 (9.1± 5.0) p<0.05	2.9±2.3 ns

whereas the cortical fraction appeared increased. 48 h later, the denervated kidney behaved differently in that the perfusion of the medullary compartment was significantly higher ($p < 0,01$) than that of the right kidney and also when compared to the situation prior to denervation in the left kidney. Following DA infusion, the % increase in flow rate was lower in face of an elevated renal blood flow. The medullary fraction of the denervated kidney was lower (at the same time as an enhanced cortical fraction) and it became further reduced during infusion of DA.

b. Catecholamine Content

The concentrations of catecholamines in the whole kidney and in different regions are given in Table 3 and in Table 4. The predominating catecholamine in the untreated dog kidney and its major subdivisions (cortex and medulla) is noradrenaline (NA), adrenaline (A) and dopamine (DA) amounting to only four to six percent of the NA concentration. As can be seen from Table 3, the amount of A (when expressed as % of NA content) was somewhat higher in the dopamine - perfused animals (22%). The concentration of DA was increased in the kidney of experimental animals having an intact sympathetic innervation (twice the level noted in kidneys of untreated dogs) but strongly decreased in the sympathectomized kidneys of DA-perfused dogs. Denervation of the sympathetic input to the kidney resulted in a near-total loss of NA and A (measurable levels were obtained in only two out of the four kidneys analysed: cf. Table 3) indicating that both catecholamines are exclusively contained in adrenergic fibres. Though denervation also resulted in a lowering of DA content, small amounts of this primary catecholamine could still be detected in the whole kidney (Table 3, bottom line).

c. Fluorescence Histochemical Findings

The mongrel dog has elaborate plexuses of intensely green fluorescent adrenergic fibres running in company with large arteries and veins, afferent and efferent glomerular arterioles,

Table 2. Total renal blood flow (RBF) and blood flow of components (I, II, III) in ml/100 mg tissue/min before (= control) and during dopamine infusion (see legend to Table 1) in dogs, 48 h after combined surgical and local chemical denervation of the kidney. The values in brackets represent the component flow rates expressed as percent of total renal blood flow

	RBF	C I	C II	C III
Renal blood flow in anaesthetized dogs after laparatomy, 2 days following denervation of the left kidney				
Left kidney	336.4±68.2	553.2±48.0 (88.0± 4.7)	76.9±27.5 (9.9± 4.2)	2.7±0.7 (2.0)
Right kidney	273.7±64.5	445.5±90.6 (76.1± 2.3)	64.8±27.3 (19.2± 3.0)	2.0±2.1 (4.8±1.8)
Renal blood flow in anaesthetized dogs after laparatomy, 2 days following denervation of the left kidney, infused with dopamine				
Left kidney	416.5±32.4 p<0.05	638.4±86.5 (90.2± 3.3) p<0.05	86.1±25.5 (8.2± 5.0) ns	3.3±0.1 (1.5±0.6) ns
Right kidney	363.8±52.4 p<0.05	608.2±99.7 (87.6± 8.4) p<0.01	80.2±24.1 (10.3± 1.1) p<0.05	3.1±1.1 (2.1±1.9) ns

particularly the epitheloid granular cells of the afferent arteriole, and the arteriolae rectae of the medulla. Some perivascularly arranged adrenergic fibres have a close association with renal tubules, especially the small diameter tubules interconnecting the proximal and distal tubules.

Two days following combined surgical and local chemical interruption of the sympathetic fibres running in company with the renal artery and its main branches, there was a near-total loss of formaldehyde-induced catecholamine fluorescence throughout the whole kidney, suggesting that most adrenergic fibres were involved in antegrade degeneration. The medium and small sized portions of the renal tubules and parts of the distal tubules which are characterized by 1) a low columnar or flat epithelium and 2) a moderately intense, diffuse cytoplasmic autofluorescence, showed an enhanced fluorescence following DA-perfusion. This was also noted in the kidneys of sympathectomized, dopamine-perfused animals. Application of the sodium borohydride test to the tissue sections of experimental animals revealed that part of the enhanced tubular fluorescence was due to the presence of a catecholamine, most probably DA. It is uncertain whether also the large sized portions of the proximal and distal tubules store DA since their epithelia contain intensely autofluorescent granules of varying colour thus obscuring the potential presence of catecholamines.

DISCUSSION

a. Renal Haemodynamics in Intact and Sympathectomized DA Perfused Dog Kidneys

The main finding in the present study is a reduction in total RBF and particularly that of the cortical component during anaesthesia and after laparatomy. Accordingly, the cortical fraction of the total renal blood flow is decreased in fa-

Table 3. Catecholamine content in dog kidney (ng. mg^{-1} wet tissue weight)

Treatment	Noradrenaline	Adrenaline	Dopamine
no treatment (controls) n = 3	0.403±0.004	0.02515±0.008	0.0254±0.0038
DA-perfused dogs with intact innervation of the kidney[b] n = 4	0.132±0.0959	0.0291±0.0172	0.0499±0.0381
DA-perfused dogs with sympathectomized kidney (n = 4)[b]	0.0148[a]	0.0022[a]	0.0078±0.0055

[a] NA and A were detected in only two out of the four kidneys analyzed for their catecholamine content, i.e. the levels of NA and A were below the sensitivity of the radioenzymatic assay used (cf. Material and Methods)

[b] the right kidney served as the control, the left kidney as the sympathectomized experimental kidney

n = number of determinations

Table 4. Regional catecholamine content in the kidney of untreated dogs (ng.mg^{-1} wet tissue weight); n = 3

Region analysed	Noradrenaline	Adrenaline	Dopamine
Cortex	0.345±0.037	0.0194±0.0066	0.0264±0.003
Medulla	0.7085±0.164	0.029 ±0.0084	0.0288±0.0063
Inner medulla, sinus renalis and pelvic mucosa	0.0232[c]	0.0030[c]	0.011[c]

[c] results from a single determination (material from 3 animals pooled)

vour of the medullary fraction. 48 h after denervation, total RBF and the cortical fraction of the denervated kidney are increased when compared to the pre-denervation condition and compared to the blood flow pattern in the right, innervated kidney. Similar results have been reported by Rosen et al. (19) in kidneys with intact innervation. Following autotransplantation of the kidney (e.g. in a situation with surgical denervation of the kidney), the distribution of blood flow was not altered by laparatomy. Taken together, these findings indicate that total and regional blood flow in the dog kidney are significantly altered by increased sympathetic activity during stressful situations (e.g. anaesthesia and laparatomy).

That the cortical fraction of the renal perfusion rate was preferentially affected can be explained by the exceptionally dense adrenergic innervation of the cortical afferent and efferent arterioles in the dog kidney. Renal nerve stimulation results in changes of intrarenal blood flow (Pomeranz et al. (18)) that closely resemble those registered in anaesthetized dogs subjected to laparatomy.

Our results confirm previous observations (13, 14, 15, 17) that iv. administration of DA (4 μg. kg^{-1} . min^{-1}) induces vasodilatation in the dog kidney. The concept that the DA-induced vasodilatation may be mediated by specific DA receptors and not by mechanisms involving the sympathetic nerve terminals is supported by the persistence of vasodilatation in the sympathectomized kidney. The DA-induced increase in total RBF and of cortical and medullary fraction is similar (though not identical) in the innervated and in the denervated kidney. We conclude that DA acts directly on DA receptors of the kidney vasculature and that the modulating influence of the sympathetic fibres on the DA-mediated perfusion changes is negligible, in contrast to the situation in the intact hind leg of the dog (12) where vascular DA receptors seem to be lacking and DA may have a presynaptic site of action. The doses of DA required to induce vasodilatation in the denervated dog kidney may be used as a guide-line for therapeutic doses of DA necessary to improve flow rate in human transplanted kidneys with hypoxic impairment of renal function.

b. Catecholamine Content and Cellular Localization of Catecholamines

Our study confirms earlier findings of Anton and Sayre (1) that the dog kidney contains noradrenaline, adrenaline and dopamine. The regional differences in the concentration of NA as reported here agree principally with the variations in NA levels found in subdivisions of the cortex and medulla of the dog by McKenna and Angelakos (16). When expressed as % of NA content, both A and DA amount to only 4 to 8% in the kidneys of untreated dogs. These low DA levels in relation to NA content suggest that they merely reflect stationary concentrations of precursor DA in NA biosynthesis, and they are not consistent with the assumption of a separate dopaminergic innervation of the dog kidney. The presence of A in the dog kidney raises the question as to its origin. Since all dogs used in this study were anaesthetized for variable periods of time before removal of the kidneys it seems reasonable to assume that the amounts of adrenaline found in the kidney reflect uptake of this amine from the circulating blood into which A is released from the adrenal medulla in response to the anaesthesia and operation stress. This assumption is also supported by the absence of chromaffin – like cells in the dog kidney as disclosed in our histochemical studies.

In the kidney of the DA-perfused animals, NA content was found to be reduced when compared to that in untreated animals. The reason for this is obscure at present but it is at least conceivable that the longer duration of the anaesthesia in combination with the stress of DA infusion results in an acceleration of NA turnover (due to increased firing of the renal sympathetic nerves) but insufficient de novo synthesis of neuronal NA or impaired storage of NA. This finding deserves further investigation.

The results presented here indicate that NA and A have a neuronal localization since sympathectomy (by a combination of surgical and local chemical destruction) caused both amines to disappear from the kidney. The results of our chemical and histochemical analysis show that DA is partly taken up by and stored in the sympathetic nerves and partly confined to non-neural structures (e.g. the renal tubules) of the kidney. At least under the conditions of our experiment, the major fraction of DA taken up into the dog kidney from the circulating DA of exogenous origin is retained by the adrenergic fibres, since only about 15% of the level of DA stored in the right, innervated kidney was contained in the contralateral, denervated kidney (Table 3).

REFERENCES

1. Anton, A.H., Sayre, D.F.: The distribution of dopamine and dopa in various animals and method for their determination in diverse biological material. Journal of Pharmacology and Experimental Therapeutics 145, 326 (1964)
2. Augustin, H.J., Baumgarten, H.G., Huland, H., Leichtweiss, H.-P.: The vasoconstrictive effect of dopamine in the isolated, perfused rat kidney after catecholamine depletion. Research in Experimental Medicine 170, 1 (1977)

3. Barajas, L.: Innervation of the renal cortex. Federation Proceedings, Federation of American Societies for Experimental Biology 37, 1192 (1978)
4. Bevan, J.A.: Norepinephrine and the presynaptic control of adrenergic transmitter release. Federation Proceedings; Federation of American Societies for Experimental Biology 37, 187 (1978)
5. Bogert, M.G., De Schaepryver, A.F., Willems, J.L.: Dopamine-induced neurogenic vasodilatation in the intact hindleg of the dog. British Journal of Pharmacology 59, 283 (1977)
6. Breckenbridge, A., Orme, M., Dollery, C. T.: The effects of dopamine on renal blood flow in man. Journal of Clinical Pharmacology 3, 131 (1971)
7. Brotzu, G.: Inhibition by chlorpromazine of the effects of dopamine on the dog kidney. Journal of Clinical Pharmacology 22, 664 (1970)
8. Corrodi, H., Hillarp, N.A., Jonsson, G.: Fluorescence methods for the histochemical demonstration of monoamines. 3. Sodium borohydride reduction of the fluorescent compounds as a specificity test. Journal of Histochemistry and Cytochemistry 12, 582 (1964)
9. Dell, R.B., Sciacca, R., Liebermann, K., Case, D.B., Cannon, P.J.: A weighted least-squares technique for the analysis of kinetic data and its application to the study of renal 133-Xenon washout in dogs and man. Circulation Research 31, 71 (1973)
10. Engelman, K., Portnoy, B., Lovenberg, W.: A sensitive and specific double-isotope derivative method for the determination of catecholamine in biological specimens. American Journal of medical Sciences 225, 259 (1968)
11. Falck, B., Owman, Ch.: A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta Universitatis Lundensis, Sectio 2, 7, 1 (1965)
12. Goldberg, L.I., Yeh, B.K.: Specific block of dopamine receptors in the renal vascular bed by chlorpromazine. In: Abstracts IV, p. 359. International Congress of Pharmacology. Basel 1969
13. Goldberg, L.I.: Cardiovascular and renal actions of dopamine. Potential clinical applications. Pharmacological Reviews 248, 24 (1972)
14. Hardaker, W.T., Wechsler, A.S.: Redistribution of renal intracortical blood flow during dopamine infusion in dogs. Circulation Research 33, 437 (1973)
15. McDonald, R.H., Goldberg, L.I.: Analysis of the cardiovascular effects of dopamine in the dog. Journal of Pharmacology and Experimental Therapeutics 140, 60 (1963)
16. McKenna, O.C., Angelakos, E.T.: Adrenergic innervation of the canine kidney. Circulation Research 22, 345 (1968)
17. Meyer, M.B., McNay, J.L., Goldberg, L.I.: Effects of dopamine on renal function and hemodynamics in the dog. Journal of Pharmacology and Experimental Therapeutics 156, 186 (1967)
18. Pomeranz, B.H., Birtek, A.G., Barger, A. C.: Neural control of intrarenal blood flow. American Journal of Physiology 215 (S), 1067 (1968)
19. Rosen, S.M., Truniger, B.P., Krick, H.R., Murray, J.E., Merrik, J.P.: Intrarenal distribution of blood flow in the transplanted dog kidney: effect of denervation and rejection. Journal of Clinical Investigation 46, 1239 (1967)

Priv. Doz. Dr. H. Huland
Urologische Universitätsklinik
Hamburg-Eppendorf
D-2000 Hamburg 20
Federal Republic of Germany