Journal of Physiology (1991), **444**, *pp*. 303–315
With 5 *figures*
Printed in Great Britain

RENOVASCULAR EFFECTS OF NEUROPEPTIDE-Y IN THE SPLIT HYDRONEPHROTIC RAT KIDNEY: NON-UNIFORM PATTERN OF VASCULAR REACTIVITY

BY M. S. DIETRICH, M. FRETSCHNER, R. NOBILING, P. B. PERSSON AND M. STEINHAUSEN
From the I. Physiologisches Institut der Universität Heidelberg, Germany

(*Received 26 November 1990*)

SUMMARY

1. The renovascular effects of neuropeptide-Y (NPY) were examined in the split hydronephrotic rat kidney.

2. Systemic infusion of low non-pressor doses of NPY (0·2 μg kg^{-1} up to 5·0 μg kg^{-1}) produced a non-uniform pattern of vascular reactivity. In general, a significant constriction of the proximal and distal arcuate artery was seen at all doses. No constriction was seen at the interlobular artery or the larger part of the afferent arteriole. These segments initially dilated during the lower dose infusions. The very distal part of the afferent arteriole adjacent to the glomerulus and the proximal efferent arteriole responded in a similar way to the arcuate arteries.

3. NPY, locally applied into the tissue bath at concentrations of 1 nmol l^{-1} up to 25 nmol l^{-1}, produced non-uniform vascular reactions similar to those of intravenously infused NPY. At the considerably higher local dosage of 1·14 μmol l^{-1}, all vascular segments revealed vasoconstriction.

4. NPY application did not attenuate effects of acetylcholine. This observation suggests that the mechanism of NPY-induced vasoconstriction does not rely upon antagonism of endothelium-derived vasodilatation.

5. The pattern of vascular reactivity to NPY was substantially different from that known for the vasoconstrictors noradrenaline and angiotensin II in our preparation.

INTRODUCTION

The general physiological properties of neuropeptide-Y (NPY) are well established (see the Introduction of the preceding paper by Persson, Ehmke, Nafz, Lang, Hackenthal, Nobiling, Dietrich & Kirchheim (1991) for a summary). However, several more detailed aspects, like a differential regional mode of action of NPY on the peripheral vascular system, or a possible interaction with the vascular endothelium, are still subjects of controversy.

Very little is known about the reactivity of different segments of the renal arterioles in response to NPY application, as no direct observation of vascular responses is possible in most experimental models. An overall reduction of both renal

MS 8958

blood flow (RBF) and glomerular filtration rate (GFR) has been reported by Hackenthal, Aktories, Jakobs & Lang (1987) in the isolated perfused rat kidney and Echtenkamp & Dandridge (1989) in uninephrectomized primates, but no precise evidence about pre- and postglomerular vascular reactivity has been reported up to now. Furthermore, some authors report an intact endothelium to be necessary for NPY-induced vascular effects (Daly & Hieble, 1987; Fallgren, Ekblad & Edvinsson, 1989; Hieble, Duesler & Daly, 1989; Pernow, 1989; Kwan, Wadsworth & Kane, 1990). However, others, such as Mejia, Pernow, von Holst, Rudehill & Lundberg (1988), Budai, Vu & Duckles (1989) and Gustafsson & Nilsson (1990), describe an endothelium-independent action of NPY.

Electrophysiological studies on the *in vitro* mouse kidney (Nobiling, Gabel, Persson, Dietrich & Bührle (1991), accompanying paper) and whole-animal experiments on the dog (Persson *et al.* (1991), accompanying paper) suggest a non-uniform renovascular response to NPY. The present paper investigates NPY effects in the split hydronephrotic kidney of rats. The model of the split hydronephrotic rat kidney was developed to permit assessment of differential vascular reactivity in the renal arteriolar system (Steinhausen, Snoei, Parekh, Baker & Johnson, 1983). Whereas the tubular system atrophies during development of hydronephrosis, the vascular system remains remarkably intact. No histological or ultrastructural changes in the vasculature can be observed, and the electrical properties of the afferent arterioles (resting membrane potentials and depolarization) are similar to those of normal kidneys (Nobiling, Bührle, Hackenthal, Helmchen, Steinhausen, Whalley & Taugner, 1986). The vascular response to various substances (for a review see: Steinhausen, Endlich & Wiegman, 1990) and autoregulatory responses (Steinhausen, Fleming, Holz, Parekh & Wiegman, 1989) are preserved. All segments of the renal microvasculature from the arcuate artery up to the welling point of the efferent arteriole are accessible to direct observation. Furthermore, drugs may be added locally to the tissue bath at defined concentrations, thereby avoiding systemic effects.

METHODS

Induction of hydronephrosis

In this study nineteen female Wistar rats (body weight ranging from 190 to 250 g) were used. Preparation and experimental procedures have been described in detail previously (Steinhausen *et al.* 1983, 1989). Briefly, the rats were anaesthetized with pentobarbitone (40 mg kg^{-1} I.P., Nembutal, Ceva, Bad Segeberg, Germany) and a permanent ligation of the left ureter was carried out. In the next 8–12 weeks, a unilateral hydronephrosis developed.

Preparation of the hydronephrotic kidney

Rats were anaesthetized with thiobutabarbitone (100 mg kg^{-1} I.P., Inactine, Byk Gulden, Konstanz, Germany). A rectal temperature probe and a heating table were used to maintain the body temperature at 37 °C. The trachea was intubated and the left jugular vein was cannulated for the continuous infusion of isotonic saline (60 μl min^{-1}) and for infusion of drugs. A catheter was inserted into the left carotid artery for measuring systemic blood pressure continuously.

The kidney was exposed by a left subcostal incision and split carefully along the greater curvature with a thermal cautery. The ventral half of the kidney was sutured to a semicircular-shaped wire frame. Blood flow and innervation remained intact after this preparation. The wire frame with the fixed and spread kidney was placed in a plexiglass chamber suitable for *in vivo* translumination microscopy. The entry of the renal hilus into the chamber was kept watertight with silicone grease. The chamber was filled with 50 ml of an isotonic, isocolloidal solution

(Haemaccel, E. Behring AG, Marburg, Germany). By means of a feedback control system, the tissue bath was kept at a constant temperature of 37 °C. After the preparation, each kidney was allowed to adapt to the tissue bath conditions for 1 h before the experiments were started.

Microscopy

The spread kidney was visualized with a Leitz Ultropac objective UO-55 (water immersion). The microscopical image was recorded with a television camera and projected on a calibrated monitor and recorded on videotape. The luminal vessel diameters were measured directly from the monitor,

TABLE 1. Experimental protocols

First series ($n = 6$)	Second series ($n = 6$)	Single experiment ($n = 1$)	Third series ($n = 6$)
Control	Control	Control	Control
NPY 0·2 μg kg^{-1} I.V.	Solvent	NPY 1 nmol l^{-1} I.B.	ACh 10^{-6} mol l^{-1} I.B.
NPY 0·7 μg kg^{-1} I.V.	Solvent	NPY 3·5 nmol l^{-1} I.B.	ACh washed out
NPY 2·0 μg kg^{-1} I.V.	Solvent	NPY 10 nmol l^{-1} I.B.	NPY 0·14 μmol l^{-1} I.B.
NPY 5·0 μg kg^{-1} I.V.	(At the same rate of infusion as in the first series)	NPY 25 nmol l^{-1} I.B.	ACh 10^{-6} mol l^{-1} I.B.
ACh 10^{-6} mol l^{-1} I.B.		ACh 10^{-6} mol l^{-1} I.B.	Prazosin 10^{-6} mol l^{-1} I.B.
Nipr 10^{-5} mol l^{-1} I.B.		Nipr 10^{-5} mol l^{-1} I.B.	Nipr 10^{-5} mol l^{-1} I.B.

NPY, neuropeptide-Y; ACh, acetylcholine; Nipr, sodium nitroprusside; I.B., in bath; I.V., intravenous

and the *in vivo* diameters were calculated using the magnification parameters (linear magnification 3000-fold). Diameter changes of 1 μm or less (3 mm on the screen) could be measured with this magnification. In order to assess renal blood flow, a dye bolus (3% Lissamine Green) was injected into the jugular vein. A dye arrival time at a selected glomerulus of 2·5 s or less was considered to represent an adequate tissue perfusion, based on a similar procedure carried out on normal rat kidneys (Steinhausen, 1963). The microcirculatory parameters of the split hydronephrotic kidney have been demonstrated to be stable for more than 3 h (Steinhausen *et al.* 1989).

Renal vascular segments

Measurements of the following vessel segments were carried out, the vessels being identified according to the branching pattern of the vessels from the selected glomerulus: (1) Proximal arcuate artery (near the interlobular artery); (2) distal arcuate artery (near the interlobular artery); (3) proximal interlobular artery (near the arcuate artery); (4) distal interlobular artery (near the afferent arteriole); (5) the largest part of the afferent arteriole (nearer the interlobular artery); (6) the distal arteriole (at the narrowest segment before entering the glomerulus; *ca* 5% of total length); (7) proximal efferent arteriole (within 50 μm of the glomerulus); (8) distal efferent arteriole (near the welling point).

Glomerular blood flow (GBF)

The glomerular blood flow was determined in the efferent arteriole by an RBF velocity-tracking correlator (IPM Inc. model 102B, San Diego, CA, USA) (Wayland & Johnson, 1967; Intaglietta & Tompkins, 1973; Intaglietta, Silverman & Tompkins, 1975). Two photodiodes obtained photometric signals from the moving red blood cells. The average time delay between similar

events in upstream and downstream signals indicates the transit time the cells need to traverse the interdiode space. In order to obtain the glomerular blood flow, the measured red cell velocity was multiplied by the luminal diameter of the efferent arteriole and corrected for the Fahraeus effect (Gaehtgens, 1980).

Experimental protocols

The different experimental protocols are represented in Table 1. Every protocol was divided into periods lasting 15 min each, if not otherwise indicated. They started with a control period (period 0). The results from the following periods were represented as relative percentage changes *versus* the period 0 value. Drugs were added locally to the tissue bath at the beginning of a period to produce the required concentration, or they were infused in the jugular vein. If referred to below, they were washed out at the end of the period by draining and refilling the bath twice. Measurements of vessel diameters and glomerular blood flow were made between the 10th and 14th minute of a period, if not otherwise indicated.

The *first series* of experiments was done on six rats with the following interventions: period 1, control; periods 2–4, intravenous infusion of NPY ($0{\cdot}2\ \mu g\ kg^{-1}$) and measurements of vessel diameters after 2, 20 and 40 min; periods 5–7, intravenous infusion of NPY ($0{\cdot}7\ \mu g\ kg^{-1}$) and measurements of vessel diameters after 2, 20 and 40 min; periods 8–10, intravenous infusion of NPY ($2{\cdot}0\ \mu g\ kg^{-1}$) and measurements of vessel diameters after 2, 20 and 40 min; periods 11–13, intravenous infusion of NPY ($5{\cdot}0\ \mu g\ kg^{-1}$) and measurements of vessel diameters after 2, 20 and 40 min; period 14, additional local administration of 10^{-6} mol l^{-1} acetylcholine; period 15, additional local administration of 10^{-5} mol l^{-1} sodium nitroprusside to confirm persisting vascular reactivity and basal tone.

The *second series* of experiments was done on six rats in order to check for non-specific effects of the solvent of NPY. The interventions were the same as in the first series: 1 ml of the solvent of NPY was intravenously infused at the same rate as during the first series. Measurements of vessel diameters were carried out after 2, 20 and 40 min.

In a *single experiment* ($n = 1$), NPY was locally administered in dosages corresponding to those intravenously infused in the first series of experiments. The experimental procedures were: period 1, control, periods 2–3, local administration of NPY (1 nmol l^{-1}) and measurements of vessel diameters after 2 and 20 min; periods 4–5, local administration of NPY ($3{\cdot}5$ nmol l^{-1}) and measurements of vessel diameters after 2 and 20 min; periods 6–8, local administration of NPY (10 nmol l^{-1}) and measurements of vessel diameters after 2, 20 and 40 min; periods 9–11, local administration of NPY (25 nmol l^{-1}) and measurements of vessel diameters after 2, 20 and 40 min; period 12, additional local administration of 10^{-6} mol l^{-1} acetylcholine; period 13, additional local administration of 10^{-5} mol l^{-1} sodium nitroprusside to confirm persisting vascular reactivity.

In the *third series*, NPY was locally applied into the tissue bath. Experiments were performed on six rats with the following interventions: period 1, control; period 2, local administration of 10^{-6} mol l^{-1} acetylcholine; period 3, acetylcholine washed out; periods 4–11, local administration of NPY ($0{\cdot}14\ \mu mol\ l^{-1}$), measurements being repeated every 15 min up to 2 h after application of NPY; period 12, additional local administration of 10^{-6} mol l^{-1} acetylcholine; period 13, additional local administration of 10^{-6} mol l^{-1} prazosin; period 14, additional local administration of 10^{-5} mol l^{-1} sodium nitroprusside.

At the end of the experimental protocols, the rats were killed by an intravenous overdose of the anaesthetic.

Statistics

Results are given as means ± S.E.M. Changes of vascular diameters and glomerular blood flow are given as percentage changes from the control value. The statistical significance of multiple interventions was calculated by analysis of variance and a post-hoc *t* test, and, additionally, by Wilcoxon's signed-rank test.

Percentage values with $P < 0{\cdot}05$ were considered significant. The abbreviation n.s. is used to denote no significance.

RESULTS

Control values

The absolute values for different parameters during the initial control period are given in Table 2. No drug application had any significant effect on systemic blood pressure in any experimental protocol.

TABLE 2. Control values of vessel diameters, glomerular blood flow, blood pressure and body weight

	Series 1 ($n = 6$)	Series 2 ($n = 6$)	Series 3 ($n = 6$)
Arcuate artery proximal (μm)	68·9 ± 2·6	72·7 ± 4·4	57·9 ± 8·4
Arcuate artery distal (μm)	46·1 ± 3·2	47·4 ± 4·5	43·5 ± 4·0
Interlobular artery proximal (μm)	25·6 ± 1·6	27·1 ± 1·4	27·9 ± 2·7
Interlobular artery distal (μm)	13·9 ± 1·3	15·2 ± 1·2	14·2 ± 2·0
Afferent arteriole proximal (μm)	9·8 ± 0·6	9·5 ± 0·8	10·0 ± 0·9
Afferent arteriole distal (μm)	8·3 ± 0·5	7·1 ± 0·6	7·3 ± 0·6
Efferent arteriole proximal (μm)	11·8 ± 0·8	11·6 ± 0·5	9·5 ± 0·6
Efferent arteriole distal (μm)	19·3 ± 1·0	15·8 ± 1·0	14·4 ± 1·1
GBF (nl min^{-1})	57·7 ± 6·0	45·9 ± 4·9	38·4 ± 13·0
Blood pressure (mmHg)	116·7 ± 3·1	110·0 ± 2·6	106·7 ± 4·6
Body weight (g)	209·2 ± 10·8	235·0 ± 3·4	233·3 ± 9·2

Intravenous NPY application (series 1)

Vessel diameters

Changes in vessel diameters in the first and second (controls) series are depicted in Fig. 1.

Infusion of the solvent of NPY induced a slight, mostly preglomerular vasodilatation that persisted throughout the whole period of observation. At the lowest dosage (0·2 $\mu g\ kg^{-1}$), NPY produced transient increases in vascular diameters after 2 min at the distal arcuate artery and the proximal and distal interlobular artery only. These vessels were no longer significantly dilated 20 and 40 min after NPY application.

A significant constriction of both the proximal and distal arcuate artery occurred at all three higher concentrations 40 min after intravenous NPY application. At the two highest dosages, these observations were constant during the whole period of observation. At the three highest dosages, a significant constriction of the proximal efferent arteriole occurred constantly during the whole period of observation.

At the lowest dosage (0·2 $\mu g\ kg^{-1}$), NPY produced transient increases in vascular diameters after 2 min at the distal arcuate artery and the proximal and distal interlobular artery only. These vessels were no longer significantly dilated 20 and 40 min after NPY application.

Glomerular blood flow

Percentage changes in the glomerular blood flow in the first experimental protocol are depicted in Fig. 2 as percentage changes of the first control value. The solvent of NPY produced slight non-significant increases of GBF. The lowest intravenous dose

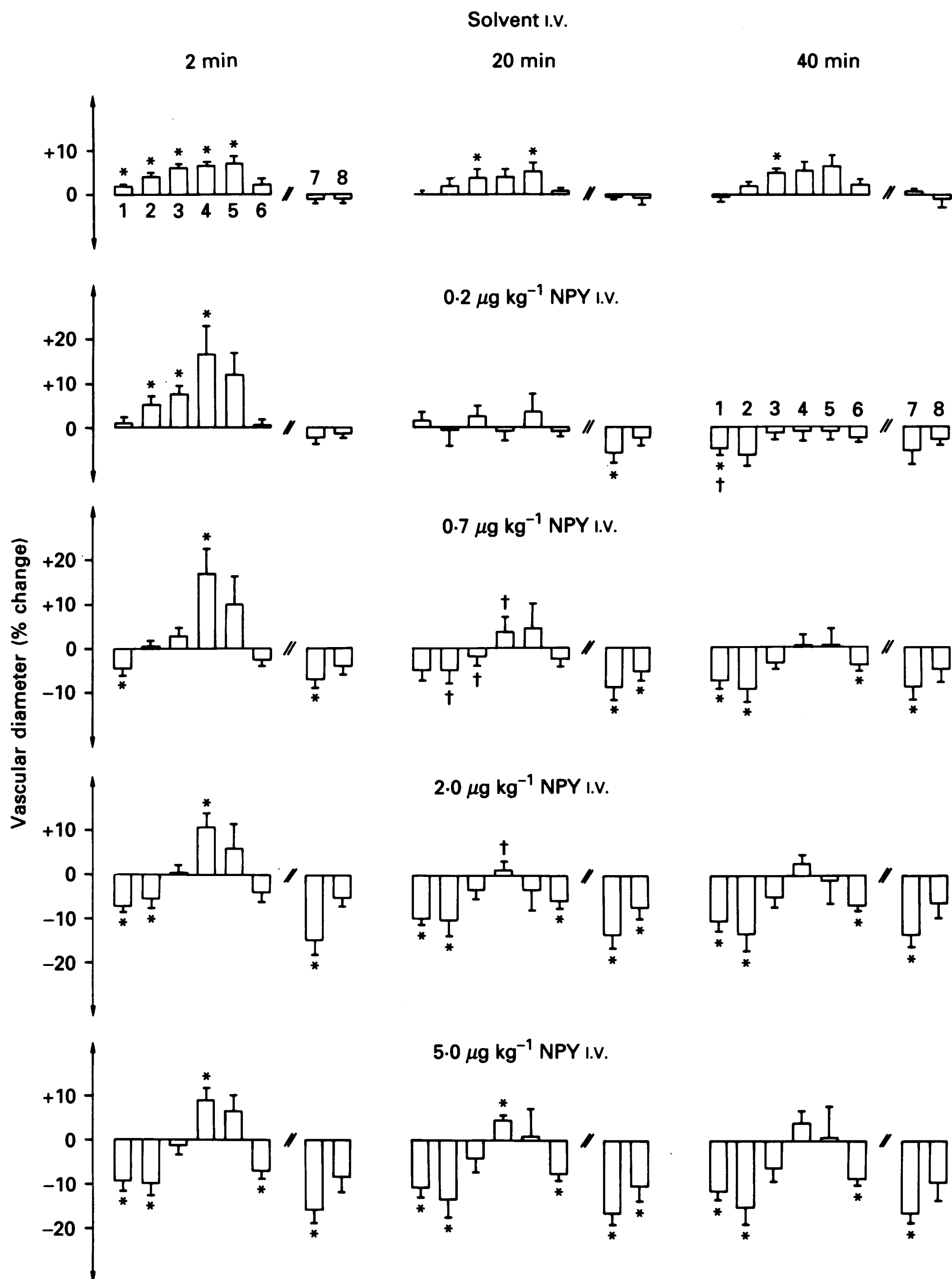

Fig. 1. Percentage changes in vascular diameters (mean ± S.E.M., $n = 6$) in the first and second (controls) experimental series. $*P < 0.05$ against control; $\dagger P < 0.05$ against previous value. Preglomerular: 1, arcuate arteries (proximal); 2, arcuate arteries (distal); 3, interlobular arteries (proximal); 4, interlobular arteries (distal); 5, afferent arterioles (near interlobular arteries); 6, afferent arterioles (near glomeruli). Postglomerular: 7, efferent arterioles (near glomeruli); 8, efferent arterioles (near welling point).

of NPY (0·2 μg kg^{-1}) produced a significant increase of GBF after 2 min, and non-significant decreases after 20 and 40 min. Higher doses of NPY produced significant decreases of GBF during the whole period of observation.

Local application of NPY

Changes in vessel diameters and glomerular blood flow in the single experiment are depicted in Fig. 3. Vascular reactivity was predominant in the proximal and distal arcuate artery, similar to the reactions to intravenous infusion in the first series.

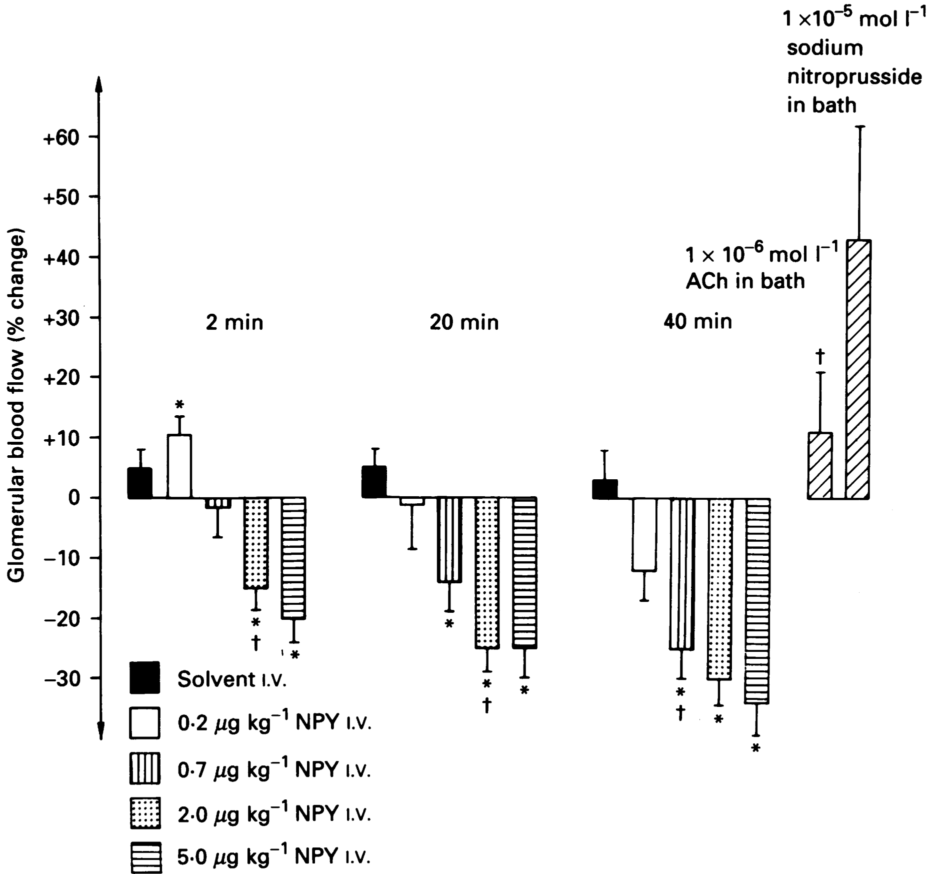

Fig. 2. Percentage changes in glomerular blood flow (mean ± S.E.M., $n = 6$) in the first and second (controls) experimental series. * $P < 0.05$ against control; † $P < 0.05$ against previous value. ACh, acetylcholine.

Third series (high local NPY doses)

Changes in vessel diameters in the third series of experiments are depicted in Fig. 4; the corresponding percentage changes in the glomerular blood flow (GBF) are given in Fig. 5.

Application of acetylcholine led to a significant vasodilatation and increase of GBF ($P < 0.05$ against the previous value). By wash-out of acetylcholine, GBF and most

vessel diameters were normalized. NPY application in a very high local dose significantly reduced vessel diameters and GBF, this effect being constant during 2 h of observation. Additional application of acetylcholine produced significant increases in vessel diameters and GBF, whereas the additional application of prazosin led to

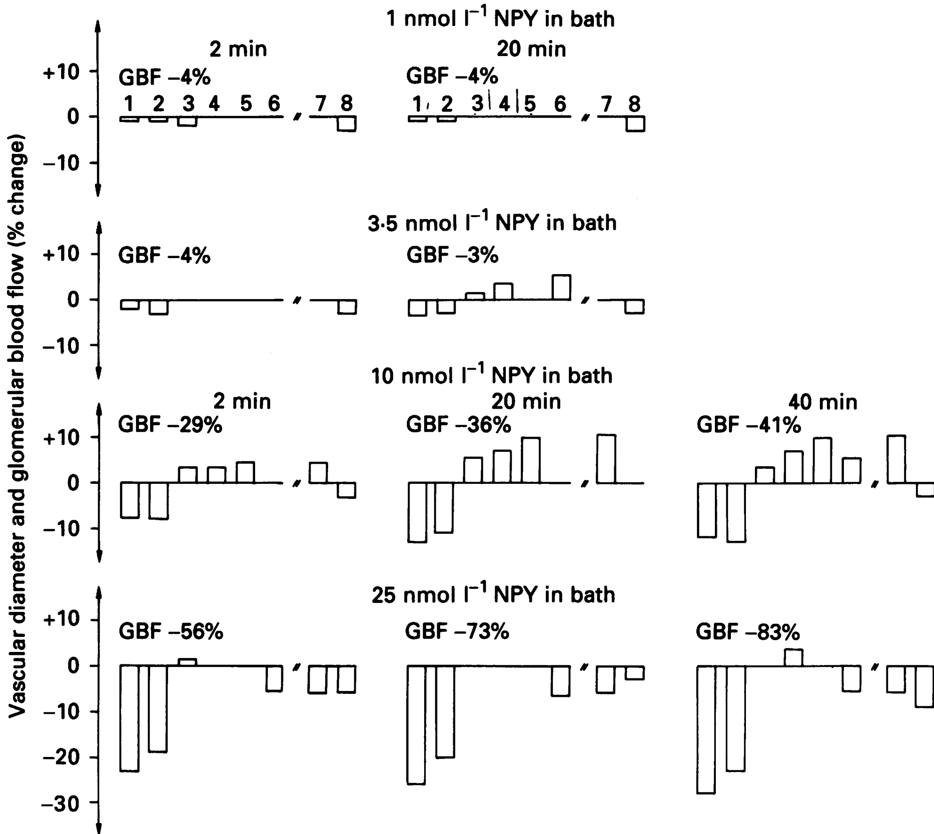

Fig. 3. Percentage changes in vascular diameters and in glomerular blood flow (GBF) in a single experiment. Vessel segments 1–8 as indicated in Fig. 1.

no significant vascular effects. Nitroprusside led to non-significant increases in GBF in most preglomerular vessels.

DISCUSSION

This study was designed to directly observe the pattern of vascular responses to NPY in different segments of the renal arteriolar system. Furthermore, we investigated the influence of NPY on the endothelium-dependent vasodilatation. Our most striking observation was the non-uniform reactivity of the renal arterioles to NPY: vasoconstriction was confined to the proximal and distal segments of the arcuate artery, the short part of the distal afferent arteriole adjacent to the glomerulus and the proximal efferent arteriole (except for the lowest intravenous

concentration immediately 2 min after application). The above-described pattern of vascular reactivity to NPY contrasts with the actions of other hormones like angiotensin II, noradrenaline and adrenaline in our preparation. Vasoconstriction induced by these substances follows an entirely different pattern (Steinhausen *et al.*

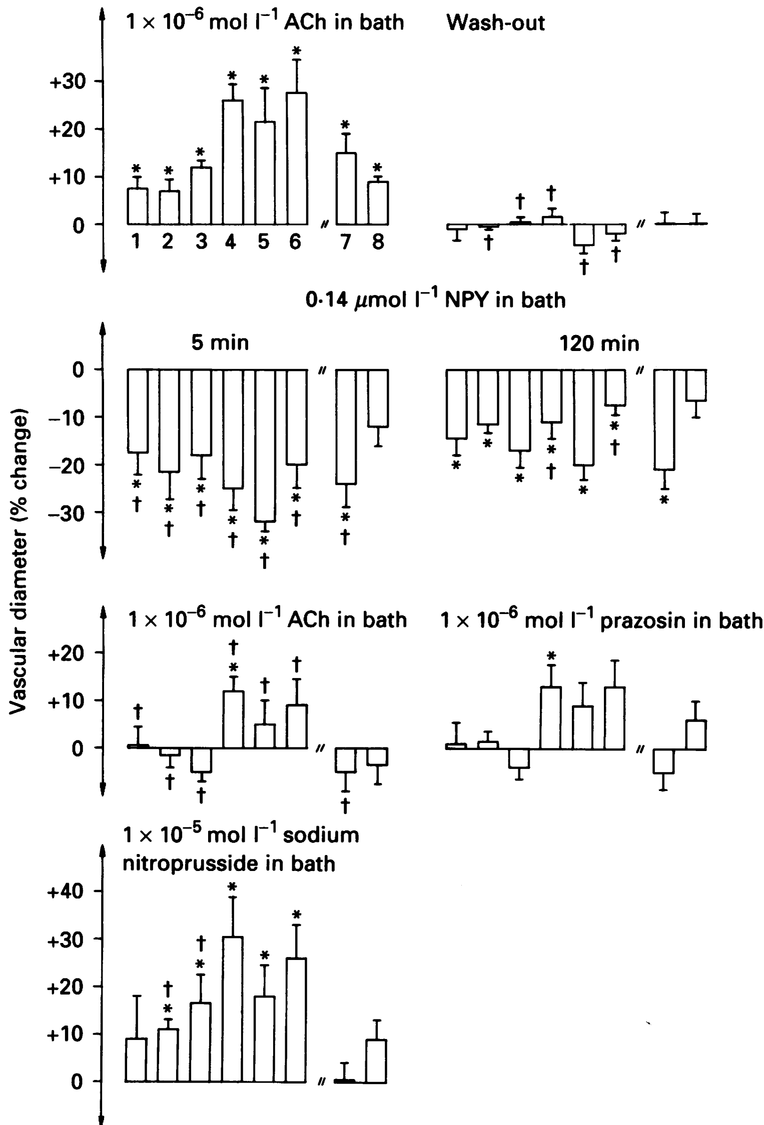

Fig. 4. Percentage changes in vascular diameters (means ± S.E.M., $n = 6$) in the third experimental series. ACh, acetylcholine. $*P < 0{\cdot}05$ against control; $\dagger P < 0{\cdot}05$ against previous value. 1–8, as Fig. 1 legend.

1990). In the case of intravenous infusion of angiotension II, all vascular segments observed in our preparation show significant vasoconstriction, the effect being largest in the distal interlobular artery and the proximal afferent arteriole.

Noradrenaline constricts all preglomerular vessels except for the distal segment of the afferent arteriole. Only very high local doses of NPY led to vasoconstriction of all preglomerular vessels. These doses were not applied by systemic infusion because they induced circulatory disturbances. Observations in the conscious dog (Persson *et*

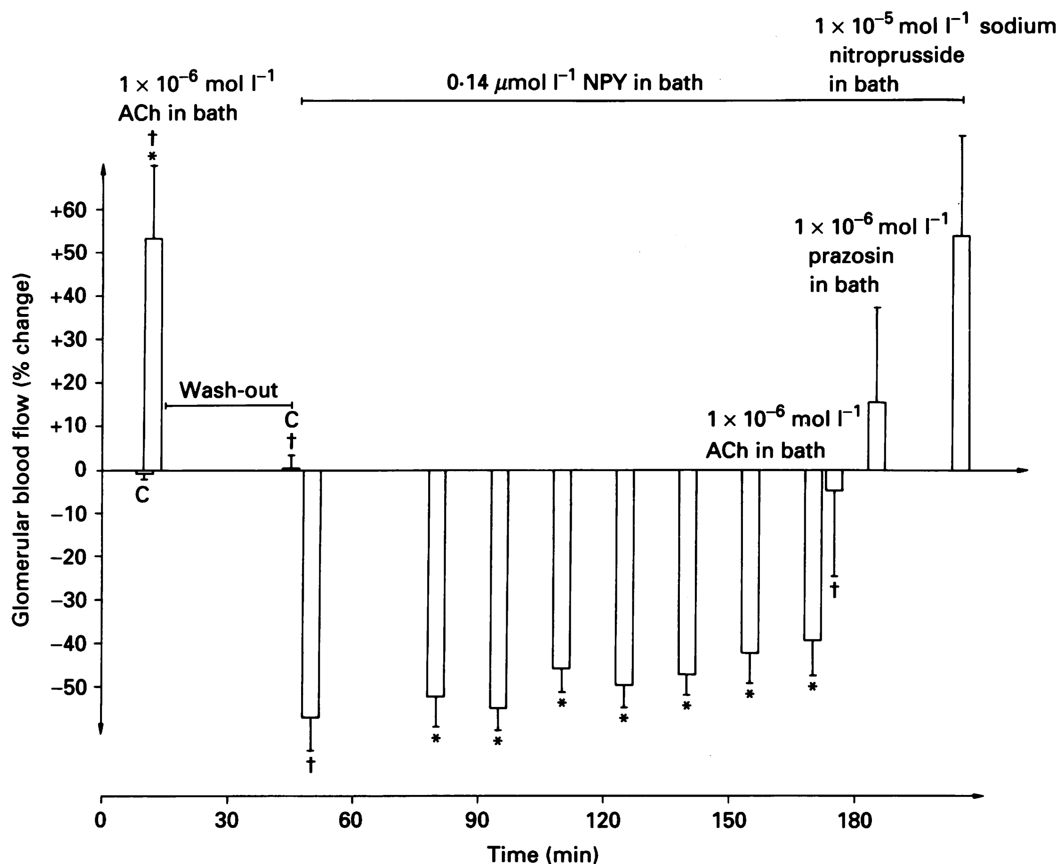

Fig. 5. Percentage changes in glomerular blood flow (mean ± S.E.M., $n = 6$) in the third experimental series. ACh, acetylcholine. * $P < 0{\cdot}05$ against control; † $P < 0{\cdot}05$ against previous value.

al. 1991; see accompanying paper) and *in vitro* electrophysiological studies in the mouse kidney (Nobiling *et al.* 1991; see accompanying paper) also support a non-uniform pattern of renovascular reactivity to NPY application.

The pattern of renin secretion observed in the conscious dog during servo-control of blood pressure suggest different reactions to NPY along the renal vascular tree. Electrophysiological data obtained by *in vitro* studies in the hydronephrotic kidney of mice indicate reactivity of the vessel segments that lie at a distance of 300 μm from the glomerulus, whereas, in contrast, no depolarization induced by NPY could be observed at a distance of 50 μm from the glomerulus. It is not possible to directly compare the numerical distances of vessel segments from the glomerula in the kidneys of rats and mice due to differences in size in the two species (Taugner,

Hackenthal, Nobiling, Harlacher & Reb, 1981), but the pattern of reactivity observed in the two preparations has obvious similarities: no reaction in the vessel segments in proximity to the glomerulus, but reaction of the larger preglomerular vessels; the vascular segments corresponding to our distal afferent arteriole not being accessible to investigation in the electrophysiological studies.

At lower intravenous concentrations, a transient vasodilatation was observed in the interlobular artery and in the greater part of the afferent arteriole. These vascular reactions were no longer present 20 and 40 min after NPY infusion. A similar transient pattern of vasodilatation could be obtained, to a lesser degree, by infusion of the solvent of NPY at the same rate and volume. Given the difference in the amount of reactivity in these vascular segments to the solvent and the NPY infusions, an initial vasodilator effect of NPY may contribute to these observations.

In our third series of experiments, the effects of acetylcholine were not significantly attenuated by NPY. The effects of acetylcholine are dependent on the presence of an intact endothelium and the endothelium-derived relaxing factor (EDRF) released from it (Furchgott, 1983), although stimulation of prostacyclin synthesis has also been reported as a result of acetylcholine administration (Förstermann, Alheid & Hohlfeld, 1990). As the vascular effects of acetylcholine were not significantly attenuated in our third series of experiments, a major influence by NPY on endothelium-derived dilator effects, either due to EDRF or prostacyclin, is unlikely.

In the same series, application of prazosin, effecting an α-adrenoreceptor blockade, did not cause any significant vascular reaction after NPY had been applied. According to this observation, and the above-described non-similarity of NPY effects to those of other vasoconstrictors, the effects of NPY may be caused either by a more complex modulation by NPY of the two above-mentioned substances or a direct effect by NPY on these vascular segments, or a combination of both mechanisms.

An observation in accordance with these suggestions was reported in 1989 by Minson *et al.* for conscious rabbits: the rise of total peripheral resistance induced by NPY proved to be independent of autonomic blockade, therefore suggesting a direct vasoconstrictor action of NPY independent of neural mechanisms. Pernow & Lundberg (1989) reported evidence for adrenergic as well as non-adrenergic components in sympathetic vascular control of skeletal muscle, NPY being a factor of the non-adrenergic component. Therefore, NPY might be a component of non-adrenergic control of glomerular function.

We are grateful to R. Dussel for technical assistance. This work was supported by the DFG (Deutsche Forschungsgemeinschaft, Forschergruppe Niere).

REFERENCES

Budai, D., Vu, H. Q. & Duckles, S. P. (1989). Endothelium removal does not affect potentiation by neuropeptide-Y in rabbit ear artery. *European Journal of Pharmacology* **168**, 97–100.

Daley, R. N. & Hieble, J. P. (1987). Neuropeptide-Y modulates adrenergic neurotransmission by an endothelium-dependent mechanism. *European Journal of Pharmacology* **138**, 445–446.

Echtenkamp, S. F. & Dandridge, P. F. (1989). Renal actions of neuropeptide-Y in the primate. *American Journal of Physiology* **256**, F524–531.

FALLGREN, B., EKBLAD, E. & EDVINSSON, L. (1989). Co-existence of neuropeptide-Y and differential inhibition of vasodilatory responses by neuropeptide-Y in guinea-pig uterine arteries. *Neuroscience Letters* **100**, 71–76.

FÖRSTERMANN, U., ALHEID, U. & HOHLFELD, J. (1990). Endothelial production of prostacyclin and endothelium-derived relaxing factor is controlled by separate mechanisms. In *Endothelium-Derived Relaxing Factors*, ed. RUBANYI, G. M. & VANHOUTTE, P. M., pp. 291–302. Karger, Basel.

FURCHGOTT, R. F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circulation Research* **53**, 557–573.

GAEHTGENS, P. (1980). Flow of blood through narrow capillaries: rheological mechanisms determining capillary hematocrit and apparent viscosity. *Biorheology* **17**, 183–189.

GUSTAFSSON, H. & NILSSON, H. (1990). Endothelium-independent potentiation by neuropeptide-Y of vasoconstrictor responses in isolated arteries from rat and rabbit. *Acta Physiologica Scandinavica* **138**, 503–507.

HACKENTHAL, E., AKTORIES, K., JAKOBS, K. H. & LANG, R. E. (1987). Neuropeptide-Y inhibits renin release by a pertussis toxin-sensitive mechanism. *American Journal of Physiology* **252**, F543–550.

HIEBLE, J. P., DUESLER, J. G. JR & DALY, R. N. (1989). Effects of neuropeptide-Y on the response of isolated blood vessels to norepinephrine and sympathetic field stimulation. *Journal of Pharmacology and Experimental Therapeutics* **250**, 523–528.

INTAGLIETTA, M., SILVERMAN, N. R. & TOMPKINS, W. R. (1975). Capillary flow velocity in vivo and in situ by television methods. *Microvascular Research* **10**, 165–179.

INTAGLIETTA, M. & TOMPKINS, W. R. (1973). Microvascular measurements by video image shearing and splitting. *Microvascular Research* **5**, 309–312.

KWAN, Y. W., WADSWORTH, R. M. & KANE, K. A. (1990). Effects of neuropeptide-Y and calcitonin gene-related peptide on sheep coronary artery rings under oxygenated, hypoxic and simulated myocardial ischaemic conditions. *British Journal of Pharmacology* **99**, 774–778.

MEJIA, J. A., PERNOW, J., VON HOLST, H., RUDEHILL, A. & LUNDBERG, J. M. (1988). Effects of neuropeptide-Y, calcitonin gene-related peptide, substance P, and capsaicin on cerebral arteries in man and animals. *Journal of Neurosurgery* **69**, 913–918.

MINSON, R., MCRITCHIE, R. & CHALMERS, J. (1989). Effects of neuropeptide-Y on the renal, mesenteric and hindlimb vascular beds of the conscious rabbit. *Journal of the Autonomic Nervous System* **27**, 139–146.

NOBILING, R., BÜHRLE, C. P., HACKENTHAL, E., HELMCHEN, U., STEINHAUSEN, M., WHALLEY, A. & TAUGNER, R. (1986). Ultrastructure, renin status, contractile and electrophysiological properties of the afferent glomerular arteriole in the rat hydronephrotic kidney. *Virchows Archiv A* **410**, 31–42.

NOBILING, R., GABEL, M., PERSSON, P. B., DIETRICH, M. S. & BÜHRLE, C. P. (1991). Differential effect of neuropeptide-Y on membrane potential of cells in renal arterioles of the hydronephrotic mouse. *Journal of Physiology* **444**, 317–327.

PERNOW, J. (1989). Actions of constrictor (NPY and endothelin) and dilator (substance P, CGRP and VIP) peptides on pig splenic and human skeletal muscle arteries: involvement of the endothelium. *British Journal of Pharmacology* **97**, 983–989.

PERNOW, J. & LUNDBERG, J. M. (1989). Release and vasoconstrictor effects of neuropeptide-Y in relation to non-adrenergic sympathetic control of renal blood flow in the pig. *Acta Physiologica Scandinavica* **136**, 507–517.

PERSSON, P. B., EHMKE, H., NAFZ, B., LANG, R., HACKENTHAL, E., NOBILING, R., DIETRICH, M. S. & KIRCHHEIM, H. R. (1991). Effects of neuropeptide-Y on renal function and its interaction with sympathetic stimulation in conscious dogs. *Journal of Physiology* **444**, 289–302.

STEINHAUSEN, M. (1963). Eine Methode zur Differenzierung proximaler und distaler Tubuli der Nierenrinde von Ratten in vivo und ihre Anwendung zur Bestimmung tubulärer Strömungsgeschwindigkeiten. *Pflügers Archiv* **277**, 23–35.

STEINHAUSEN, M., ENDLICH, K. & WIEGMAN, D. L. (1990). Glomerular blood flow. *Kidney International* **38**, 769–784.

STEINHAUSEN, M., FLEMING, J. T., HOLZ, F. G., PAREKH, N. & WIEGMAN, D. L. (1989). Visualization of renal autoregulation in the split hydronephrotic kidney of rats. *Kidney International* **35**, 1151–1160.

STEINHAUSEN, M., SNOEI, H., PAREKH, N., BAKER, R. & JOHNSON, P. (1983). Hydronephrosis: a new method to visualize vas afferents, efferents and glomerular network. *Kidney International* **23**, 794–806.

TAUGNER, R., HACKENTHAL, E., NOBILING, R., HARLACHER, M. & REB, G. (1981). The distribution of renin in the different segments of the renal arterial tree. Immunocytochemical investigation in the mouse kidney. *Histochemistry* **73**, 75–88.

WAYLAND, H. & JOHNSON, P. (1967). Erythrocyte velocity measurement in microvessels by a two-slit photometric method. *Journal of Applied Physiology* **22**, 333.