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DIFFERENTIAL EFFECT OF NEUROPEPTIDE-Y ON MEMBRANE POTENTIAL OF CELLS IN RENAL ARTERIOLES OF THE HYDRONEPHROTIC MOUSE

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SUMMARY

1. The effects of neuropeptide-Y (NPY) on the membrane potential of vascular smooth muscle cells were studied in renal arterioles of hydronephrotic mouse kidneys.

2. Kidney vessels are only weakly coupled with length constants of less than 10 μm and are most probably 'multiunit' vessels.

3. The vasoconstrictor peptide NPY reversibly depolarizes only smooth muscle cells in arterioles at distances > 200 μm from the glomerulus, whereas no changes of the membrane potential can be evoked close to the glomerulus (distance < 50 μm).

4. The depolarizations, when present, are dose dependent.

5. Regardless of distance from the glomerulus cells respond uniformly to application of the vasoconstrictor angiotensin II.

INTRODUCTION

The neurotransmitter neuropeptide-Y (NPY) is co-stored and co-released with noradrenaline in sympathetic nerve terminals. Although the vascular smooth muscle cell constitutes a preferential binding location (Leys, Schachter & Sever, 1987; Ballesta, Lawson, Pals, Ludens, Lee, Bloom & Polak, 1987; Reinecke & Forssmann, 1988; Knight, Fabre & Beal, 1989), the nerves supplying the juxtaglomerular apparatus (Ballesta, Polak, Allen & Bloom, 1984; Ballesta *et al.* 1987) also reveal NPY staining, and proximal convoluted tubules have been shown to contain NPY receptors (Leys *et al.* 1987).

In the accompanying papers by Persson, Ehmke, Nafz, Lang, Hackenthal, Nobiling, Dietrich & Kirchheim (1991) and by Dietrich, Fretschner, Nobiling, Persson & Steinhausen (1991) a preferential action of NPY on larger renal vessels is proposed.

In the juxtaglomerular apparatus, renin-containing epithelioid cells (in the media of the afferent arteriole) close to the glomerulus (JG cells) are most probably metaplastic transformed vascular smooth muscle cells (VSMC) (Cantin, Araujo-Nascimento, Benchimol & Desormeaux, 1977; Taugner, Kirchheim & Forssmann,

1984). The electrophysiological parameters membrane potential and input resistance of epithelioid cells and VSMC are similar, and respond in a similar manner to stimulation (Bührle, Nobiling & Taugner, 1985). Although vasoconstrictors such as noradrenaline or angiotensin II (Ang II) act on both types of media cells, the observed preference of NPY for larger vessels may have electrophysiological correlates, i.e. there might exist regionally different reactions in the membrane response of VSMCs to application of vasoconstrictors.

A prerequisite for such regionally different vascular reactions is weak or even no coupling between VSMC in kidney arterioles, a point that has been previously made (Barajas, 1981). However, as yet there is no convincing electrophysiological evidence in favour of this idea. On the other hand, anatomical findings indicate a possible metabolic and/or electrotonical coupling in kidney arterioles, (Boll, Forssmann & Taugner, 1976; Taugner *et al.* 1984).

The objective of the present study was to examine whether or not a regionally differentiated response of the membrane potential of vascular smooth muscle cells in renal arterioles to the vasoconstrictor NPY occurs. Because this finding would only be possible in weakly coupled vessels, we also studied the electrotonic spread of signals along these vessels.

We find that the concept of regional differential actions of NPY in the renal vascular bed has electrophysiological correlates.

METHODS

Unilateral hydronephrosis was produced in female NMRI mice (20–25 g body weight) under sodium pentobarbitone anaesthesia (Nembutal, 50 mg kg^{-1} I.P.) as described previously (Bührle, Nobiling, Mannek, Schneider, Hackenthal & Taugner, 1984); 8–12 weeks post-operatively, the mice were again anaesthetized as described above. After a mid-line incision, the abdominal aorta was cannulated with a flexible polyethylene catheter, the vena cava opened by an incision, and the vascular system flushed free of blood by perfusing the animals with 10 ml of Tyrode solution (concentrations in mM: 118 NaCl, 4·8 KCl, 2·5 $CaCl_2$, 1·2 $MgSO_4$, 25 $NaHCO_3$, 1·2 KH_2PO_4, 11 glucose, and 0·57 ascorbic acid; the pH was 7·4; cooled to 4 °C) at a pressure of 100–150 mmHg. Subsequently, about 3 ml of a suspension of India ink particles were injected under pressure control to visualize the renal vasculature and the glomeruli, the injection being stopped as the glomeruli became distinctly black as seen through a dissecting microscope. Prior to infusion, the India ink (Rotring Zeichentusche) had been centrifuged in a desk-top centrifuge at 12000 revolutions min^{-1} for 15 min and the pellet of India ink particles washed 3 times, centrifuged and resuspended in Tyrode solution by sonification.

The hydronephrotic kidney was then rapidly decapsulated *in situ*, removed from the animal, and transferred to oxygenated (95% O_2–5% CO_2, pH 7·4) Tyrode solution cooled to 4 °C. After removal of the hilar region and adherent connective tissue pieces from the hydronephrotic kidney were mounted in a holder and transferred to the recording chamber.

The recording chamber had a volume of 3 ml and was continuously perfused with oxygenated Tyrode solution. The bath solution was exchanged at a rate of 4 ml min^{-1} by means of a peristaltic pump, the pulsations being dampened by an air vessel. The superfusate was heated immediately before the chamber and the temperature was controlled by an electronic feedback system.

The recording chamber had a cover-slip as its bottom and rested on the stage of a compound microscope (Ernst Leitz, Wetzlar). Selection of the structures to be impaled and electrode positioning were done using a low-power dry objective (×6·3, Ernst Leitz, Wetzlar), whereas final impalement was done using a water immersion lens (×22, Ernst Leitz, Wetzlar) at a total magnification of ×275.

To obtain stable impalements of the small (max. diameter: 5 μm) spindle-shaped cells it was necessary to use fast step motor-driven micromanipulators (Marcinowski, Heidelberg). These

devices were mounted on sliding-type, micrometer-fed manipulators which were rigidly attached to the microscope (Fig. 1).

When impaling a vascular structure, the electrode was first positioned in the horizontal plane and then advanced in steps ranging between 10 and 0·5 μm. Reliable impalements were only possible when the electrodes had resistances in excess of 90 MΩ. For these experiments, single

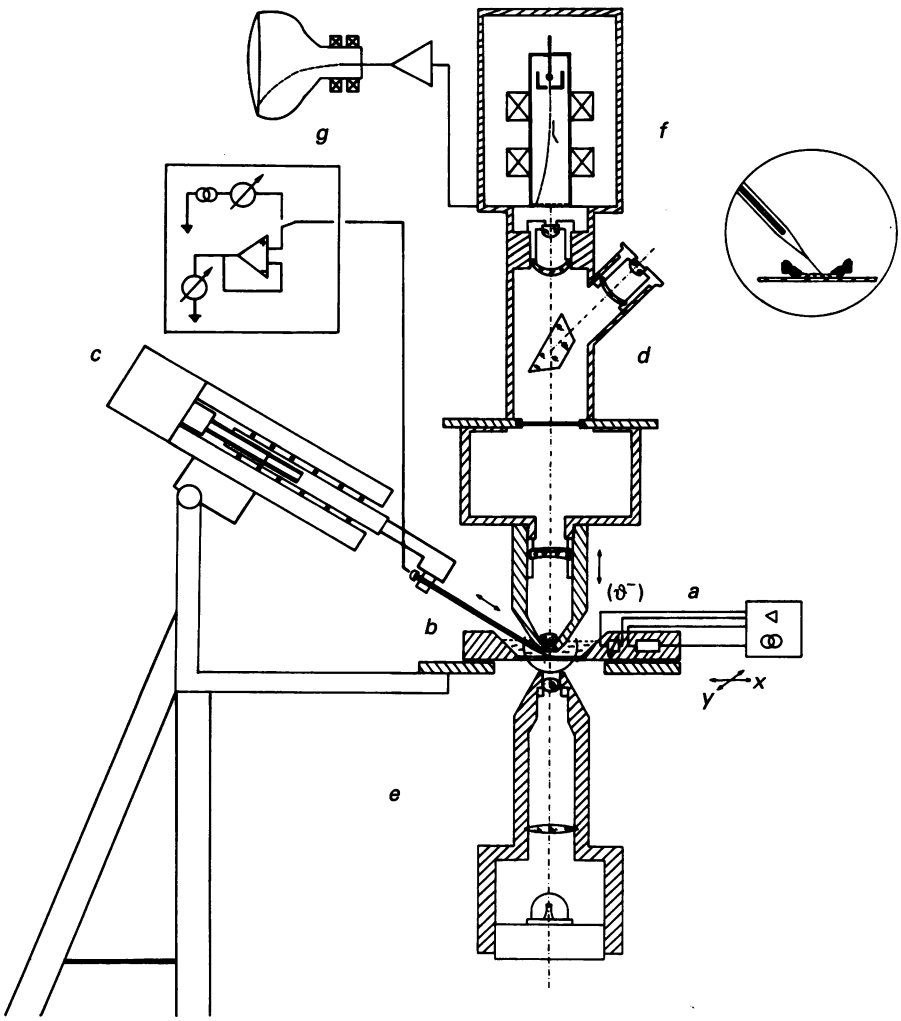

Fig. 1. Schematic view of the experimental set-up for intracellular recordings under microscopic control: *a*, feedback temperature regulator for the superfusion chamber with sensor (ϑ^-); *b*, microelectrodes(s); *c*, stepping motor(s) (8 steps μm^{-1}); *d*, eyepiece for visual control; *e*, illumination: lamp and condenser; *f*, TV camera and monitor for sample control without mechanical disturbance of the recording set-up during experiments; *g*, intracellular amplifier or current injection, respectively. The inset shows the recording geometry at higher magnification.

electrodes of the filament type (Clark Electromedical Instruments, GC 150 TF) with an outer tip diameter of about 0·2 μm, as measured in a scanning electron microscope, were used. When filled with 3 M-potassium acetate their resistances were between 100 and 150 MΩ. Since the electrode tips

tended to break after a few attempts at impalement, the micropipettes had to be changed frequently.

The electrodes were connected to the input stages of a high-impedance, capacitance-compensated amplifier (Axoclamp 2, Axon Instruments, Burlingame, MA, USA) via Ag–AgCl wires. The signals were displayed on a digital oscilloscope (Iwatsu 6440) and a potentiometric writer (Linseis 2045).

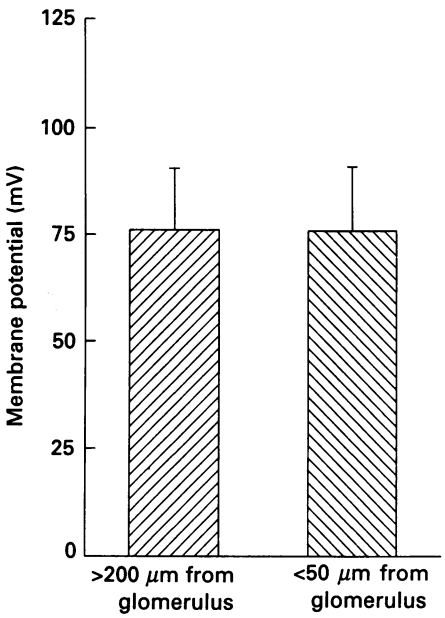

Fig. 2. Resting membrane potential of media cells in kidney vessels at two distances (d) from the glomerulus ($-75{\cdot}9 \pm 15{\cdot}1$ mV, $n = 41$ at $d < 50$ μm; $-76{\cdot}1 \pm 14{\cdot}5$ mV, $n = 27$ at $d > 200$ μm).

For two-electrode measurements, the second impalement had to be performed extremely carefully, because mechanical disturbances by the second electrode often caused loss of the first intracellular recording. The minimum distance between the two electrodes for successful two-electrode measurements was about 25 μm.

All pharmacological agents were applied in the superfusing medium. When substances which evoked a contractile response in vascular smooth muscles were applied for periods longer than 2 min most (*ca* 90%) cells were lost. Presumably the electrode was dislodged by the contraction. We limited the duration of application therefore to less than 30 s.

The substances used in the experiments were angiotensin II (Ciba-Geigy, Basel) and NPY at concentrations between 10^{-6} and 10^{-9} mol l^{-1}. NPY was a generous gift of Dr R. E. Lang, Department of Physiology, University of Marburg.

RESULTS

Successful impalement of the cells was indicated by a sudden negative deflection of the potential recording. In most cases this initial deflection, to about -40 to -50 mV, was followed by a further depolarization, indicating a stabilization of the intracellular measurement over the next few minutes. A stable value of membrane potential in most cases was reached after 5–10 min, when the leak caused by the

microelectrode impalement had sealed. Such impalements were often maintained for up to 4 h without signs of deterioration of the cells, such as decrease in membrane potential or changes in the responsiveness of the cells to pharmacological agents. Upon withdrawal of the electrodes from the cells the potential returned immediately to the extracellular value.

The membrane potentials measured were virtually the same at different distances from the glomeruli (Fig. 2): −75·9 mV close to glomeruli (distance < 50 μm) and −76·1 mV at distances exceeding 200 μm.

As described previously (Bührle, Scholz, Nobiling & Taugner, 1986*c*), in renin-containing (JG) cells as well as in VSMCs spontaneous depolarizing transients of small amplitude superimposed on the resting membrane potential were usually observed. These transients occurred in a random manner at frequencies between 30 and 200 min^{-1}, and their amplitudes ranged between 0·5 and 15 mV (noise level of the recording system with a typical 100 MΩ electrode: 0·2–0·5 mV peak to peak). With their fast depolarizing phase, rounded maximum and exponential decay these transients closely resembled the spontaneous excitatory junction potentials observed in other vascular smooth muscle preparations (Hirst & Neild, 1980; Cheung, 1982).

As with membrane potential, there was also no difference between VSMCs close to the glomerulus (< 50 μm) and at distances > 200 μm in relation to the frequency and amplitude distribution of these depolarizing transients. In addition, it became evident during the course of the experiments that the responses of all cell types to angiotensin II (Ang II) were very much the same. Hence, Ang II served as a control agent for cells that did not respond to other peptides (Figs 4 and 5).

During measurements with two microelectrodes in afferent arterioles one of the electrodes served as 'transmitter': de- and hyperpolarizing rectangular or sinusoidal current injection of up to 300 pA evoked a shift in the membrane potential of about ±50 mV from the resting potential (Fig. 3). In twenty-four experiments with afferent arterioles close to the glomerulus and in some ten more than 100 μm apart, we observed no shift of the membrane potential of the second cell, serving as 'receiver'. The distances of the two electrodes ranged from 20 to 100 μm. To ensure that these negative findings were not the result of a faulty recording technique, we repeated the simultaneous recordings with two intracellular microelectrodes of Hirst & Neild (1978) who had demonstrated an electrotonic coupling in the model of arterioles of the guinea-pig ileum submucosus plexus. The experiment was performed as described by Hirst & Neild (1978) and the procedures for simultaneous recording were identical to those used in the kidney arterioles. Also the microelectrodes, the amplifier used, and all experimental parameters were the same as in the kidney experiments. In contrast to the kidney vessels, the cells of the plexus submucosus arterioles were coupled electrotonically: eleven cell pairs with distances between 20 and 200 μm in arterioles with diameters between 20 and 100 μm were studied. These cell pairs fulfilled the same criteria for stable recordings as described for the kidney arterioles. As described above, sinusoidal or rectangular current injection (1 Hz, 100–1000 pA amplitude) led to membrane potential shifts between 2 and 20 mV in the 'transmitter' cell. In these control experiments, length constants of more than 20 μm were determined. Consequently, a falsely negative result in the kidney vessels could be ruled out.

Because the signal-to-noise ratio of our system allowed the detection of changes of membrane potential of ± 1 mV, an upper limit for the length constant along afferent arterioles of about 5 μm can be estimated.

Similar to the results of Bührle, Scholz, Hackenthal, Nobiling & Taugner (1986*b*), NPY did not affect the membrane potential of JG and VSMCs at distances

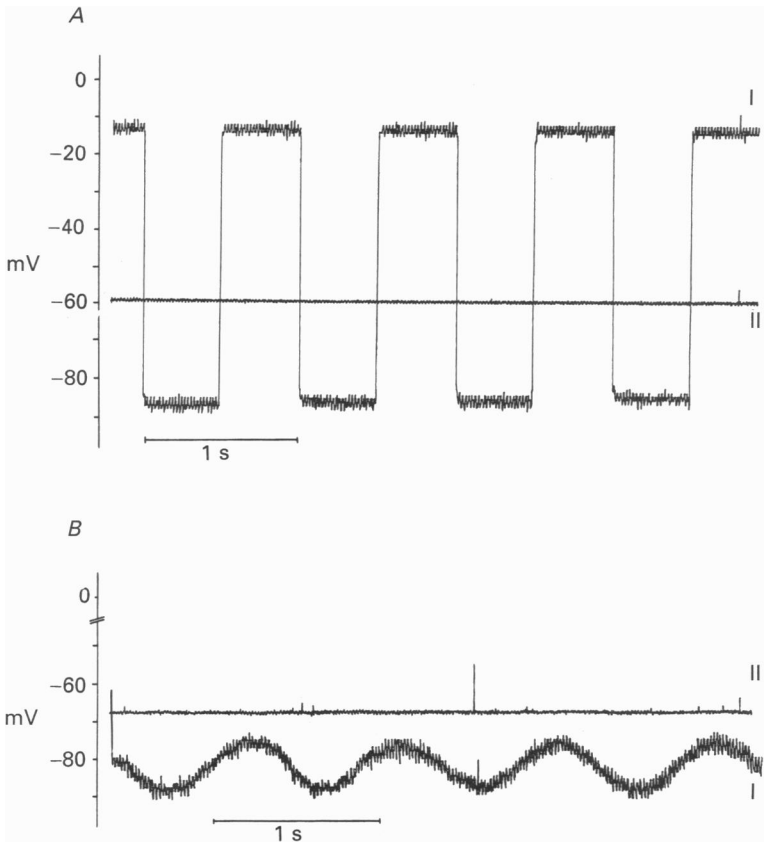

Fig. 3. Simultaneous recordings from two media cells of the afferent arteriole. *A*, recording from two cells in the proximal part of the afferent vessel; distance from the glomerulus 100 μm, interelectrode distance 40 μm. Membrane potential of the 'transmitter cell' (I) $= -52$ mV; membrane potential of the 'receiver cell' (II) $= -59$ mV. Membrane potential deviation in the 'transmitter cell' = 82 mV (41 mV symmetrical, constant current = 280 pA, rectangular, 1 Hz). There are no changes of the membrane potential in the 'receiver cell'. *B*, recording from two cells in the juxtaglomerular part of the afferent arteriole; distance from the glomerulus 30 μm, interelectrode distance 25 μm. Membrane potential of the 'transmitter cell' (I) $= -80$ mV, membrane potential of the 'receiver cell' (II) $= -67$ mV. Membrane potential deviation in the transmitter cell: 13 mV (constant current 100 pA, sinusoidal, 1 Hz). As in *A*, there are no changes of the membrane potential in the 'receiver cell'.

< 50 μm from the glomerulus ($n = 41$ and 9, Fig. 4). At larger distances (200–400 μm), however, NPY mostly (in eight of ten cells) induced a depolarizing action. The reaction was reversible and the pattern of de- and repolarization was very much

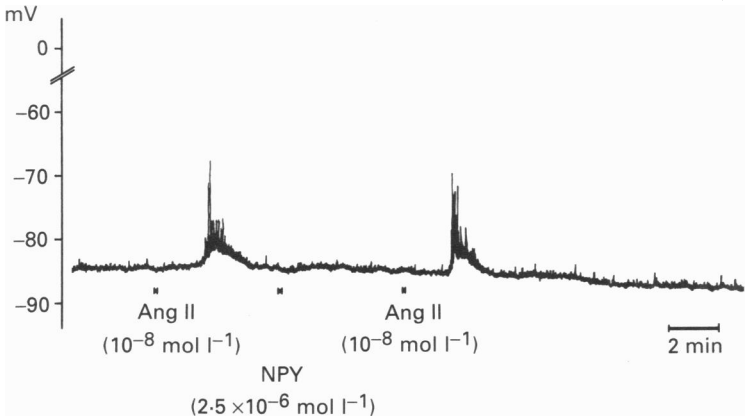

Fig. 4. Depolarizing action of angiotensin II (Ang II) on the membrane potential of a cell in the media of an afferent arteriole 40 μm apart from the glomerulus. During Ang II application a higher frequency of depolarizing junctional transients can also be observed. Application of NPY has no effect.

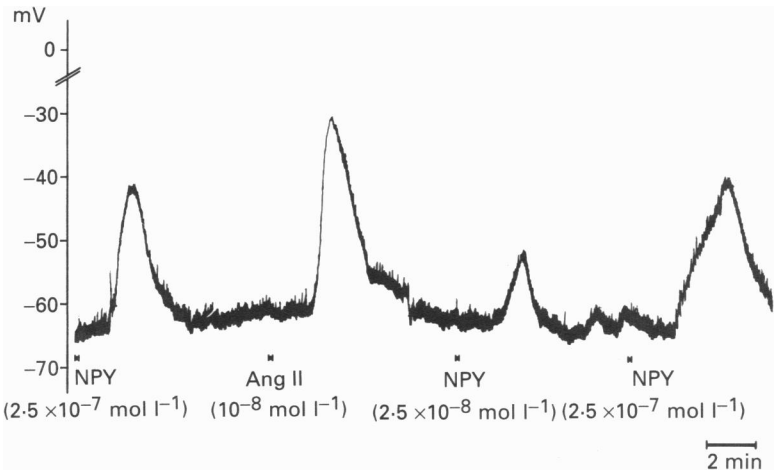

Fig. 5. Depolarizing action of NPY and Ang II on a cell in the media of an interlobular arteriole, 300 μm apart from the glomerulus. The NPY-evoked depolarization is higher at higher concentrations of the agonist.

the same as observed with other vasoconstrictors (Fig. 5). The reaction, as with Ang II, was dose dependent and could not be blocked by the calcium antagonist verapamil.

The impalement was often lost in larger distances from the glomerulus during the application of NPY, probably as a result of vasoconstriction. But also at distances of 200–400 μm from the glomerulus the depolarizing action of NPY could not be observed in all cells: applications of NPY in two of ten cells were without response, whereas all of these cells reacted upon application of Ang II (Fig. 6).

DISCUSSION

Renal arteries and arterioles accomplish the autoregulation of renal blood flow by several mechanisms. In addition, the production and secretion of renin takes place in modified vascular smooth muscle cells (VSMC) situated in the distal afferent

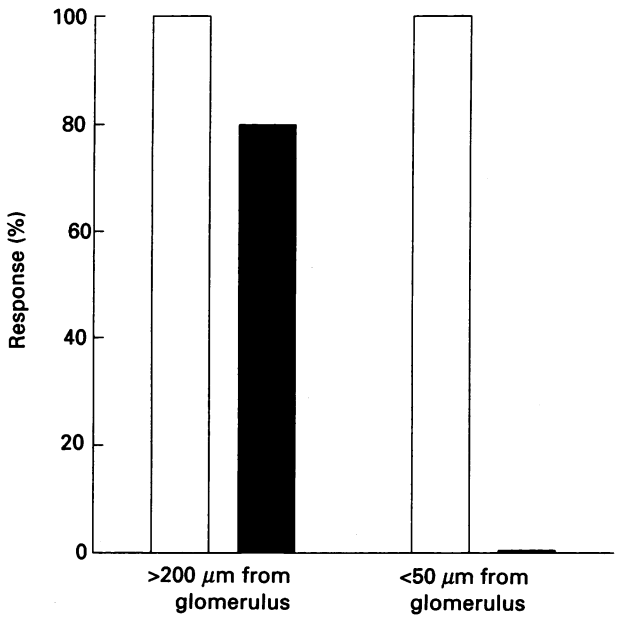

Fig. 6. Membrane potential responses of VSMC in renal arterioles upon application of Ang II and NPY. All values of n exceed 40 except NPY applications at large distances ($n = 10$); here a loss of the recording frequently occurred, most probably due to cellular contraction. □, Ang II; ■, NPY.

arterioles close to the glomerulus (JG cells). Many stimuli are believed to influence both autoregulation and renin release (Hackenthal & Taugner, 1986): wall tension of the afferent glomerular arteriole acting via the so-called intrarenal vascular baroreceptor (Tobian, 1960; Nobiling, Münter, Bührle & Hackenthal, 1990); tubulo-glomerular feedback, i.e. the distal tubular sodium or chloride load converted into the still unknown macula densa signal (Briggs & Schnermann, 1987); activity of the postganglionic sympathetic nerves innervating all parts of the afferent glomerular arteriole (Ehmke, Persson, Fischer, Hackenthal & Kirchheim, 1989; Persson *et al.* 1991, the accompanying paper); and circulating humoral factors.

The renin-containing JG cells are depolarized by most vasoconstrictors in the same manner as the VSMC in the proximal part of the afferent arteriole. Vasoconstrictors generally inhibit and dilators stimulate renin secretion. Thus, besides morphological similarities, functional homologies between these two cell types are observed (Bührle *et al.* 1984; Hackenthal & Taugner, 1986).

Sometimes renin is found more than 100 μm from the glomerulus, and its distribution is rather inhomogeneous (Taugner, Marin-Grez, Keilbach, Hackenthal &

Nobiling, 1982). These and other observations point to a differential reaction of the renal vessels and, most probably, single cells to various stimuli. Thus, it is not surprising that Persson *et al.* (1991), after studies in conscious dogs, also suggest differences in the vascular response to the neurotransmitter NPY. Studies on microcirculation in the hydronephrotic kidney of the rat (Steinhausen, Endlich & Wiegman, 1990) demonstrate different reactions of the various parts of renal arterioles to various vasoactive stimuli and, in particular, to NPY (Dietrich *et al.* 1991, the accompanying paper).

The wall tension of blood vessels is controlled by smooth muscle cells, and vessels can be classified into two different functional types (Bozler, 1948): multiunit structures that are primarily controlled by their dense autonomic innervation (Hirst, 1977), and single-unit structures with inherent cable properties that are controlled by electrotonic spread and, on occasion, exhibit automaticity (Ito & Kuriyama, 1971). Therefore, the question arises, whether the arterioles in the kidney can be regarded as coupled vessels in analogy to mesenteric arterioles (Hirst & Neild, 1980). Such coupling may constitute the basis for transmission of the macula densa signal to more remotely located parts of the vascular tree, i.e. more than 20–30 μm from the glomerulus.

This background must be kept in mind when vasoconstrictor stimuli are automatically linked to all parts of resistance vessels including the inhibition of renin secretion. Some apparently contradictory observations and measurements have to be reconsidered in the light of the present results: although NPY is known to be a potent vasoconstrictor, it did not depolarize media cells close to the glomerulus including JG cells (Bührle *et al.* 1986*b*). Hence, it may not necessarily act in the same manner at all segments of renal arterioles, and the above statement that every vasoconstrictor inhibits renin secretion may only be true in combination with a constrictor-evoked membrane depolarization. This conclusion is an important prerequisite for the concept of a regionally differentiated reaction of the vascular bed to NPY which is developed by Persson *et al.* (1991) in the accompanying paper. This concept is a consequence of surprising findings of the NPY influence on pressure-dependent renin release. Although speculative in the intact animal model, the concept is supported by our electrophysiological findings, that kidney arterioles are nearly uncoupled vessels. Additionally, the regionally differentiated vasoconstriction, as reported by Dietrich *et al.* (1991) supports the concept of a regionally differentiated reaction of renal arterioles to vasoactive substances. This similarity, however, should not be discussed in a micrometre-by-micrometre analogy due to possible species variations in the histology and the distribution of the various cell types. In particular, some characteristic differences between rat and mouse have been observed with the distribution of renin-positive cells along the afferent arteriole (Bührle *et al.* 1986*a*; Nobiling, Bührle, Hackenthal, Helmchen, Steinhausen, Whalley & Taugner, 1986), and species-dependent variations of NPY binding (Leys *et al.* 1987). On the other hand, the large degree of correspondence between the findings of Dietrich *et al.*(1991) and this study leads to the conclusion that different responses to humoral and/or neural stimuli are possible according to the receptor equipment of individual cells without much influence from cells some micrometres apart.

In the present study, we provide the direct experimental evidence of weak or even

non-existing electrotonic coupling in kidney arterioles of mouse, which is the prerequisite for the observed differences in cell membrane responses to NPY.

Consequently, the very short length constant of the kidney vessels favours the concept of a regional differential response to vasoactive stimuli in a 'multiunit' structure. The concept is compatible with morphological, electrophysiological, and functional findings: renin-positive cells are found sometimes in a scattered, non-continuous manner along the afferent arteriole. The basis for the frequently observed inhibition of renin secretion by vasoconstrictors may depend on the depolarization of the outer cell membrane. Hence our observations of a lack of depolarization upon constrictor (NPY) application can be correlated with a lack of a direct effect of NPY on renin secretion, as described by Persson *et al.* (1991). Similarly, the differences in the degree of vessel contraction in the distal and proximal afferent arteriole upon the application of NPY, as described by Dietrich *et al.* (1991), can be correlated with the concept of individually responding cells in this 'multiunit' vessel.

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