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The cardiovascular and renal functional responses to the 5-HT_{1A} receptor agonist flesinoxan in two rat models of hypertension

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1 This study investigated the importance of renal sympathetic nerves in regulating sodium and water excretion from the kidneys of stroke prone spontaneously hypertensive and 2K1C Goldblatt hypertensive rats anaesthetized with chloralose/urethane (17.5/300 mg initially and supplemented at regular intervals), and prepared for measurement of renal function.

2 In stroke prone spontaneously hypertensive rats, flesinoxan, 30-1000 $\mu g\ kg^{-1}$, i.v., caused graded reductions in blood pressure and heart rate of 74 ± 5 mmHg and 63 ± 9 beats min^{-1}, respectively at the highest dose ($P<0.001$). Renal blood flow did not change at any dose of drug while glomerular filtration rate fell by some 20% ($P<0.001$) at the highest dose of drug, absolute and fractional sodium excretions, approximately doubled at 100 $\mu g\ kg^{-1}$, and thereafter fell to below the baseline level at 1000 $\mu g\ kg^{-1}$.

3 This pattern of excretory response was abolished following acute renal denervation when flesinoxan caused dose-related reductions in urine flow and sodium excretion, similar to that obtained by a mechanical reduction of renal perfusion pressure.

4 Flesinoxan administration (30-1000 $\mu g\ kg^{-1}$, i.v.) into 2K1C Goldblatt hypertensive rats caused a maximum decrease in blood pressure and heart rate (both $P<0.001$) of 34 ± 3 mmHg and 20 ± 6 beats min^{-1} and while renal blood flow and glomerular filtration rate were autoregulated, from 160 to 125 mmHg, there were dose-related decreases in urine volume and sodium excretion from the clipped and non-clipped kidneys of approximately 50-60% at the highest dose.

5 These findings suggest that in the stroke prone spontaneously hypertensive rat the renal nerves importantly control sodium and water reabsorption at the level of the tubules, whereas in 2K1C Goldblatt hypertensive rats, they play a minor role.

Keywords: Flesinoxan; stroke prone spontaneously hypertensive rat; Goldblatt hypertensive rat; renal function

Introduction

The sympathetic nervous system has been implicated in many forms of hypertension in man (see Panfilov & Reid, 1994) contributing to either the genesis and/or maintenance of this pathophysiological state. Experimental studies have focused on the role of the renal sympathetic nerves in the generation of hypertension and the work of Wyss *et al.* (1992) have shown that in the spontaneously hypertensive rat, bilateral renal denervation delays the chronic elevation of blood pressure. Furthermore, Katholi *et al.* (1982) found that denervation of the clipped kidney in established hypertension in the 2K1C Goldblatt rat caused a partial normalisation of blood pressure. The mechanisms underlying the renal-nerve mediated hypertension are unclear, but it is possible that both increased efferent renal nerve activity and, potentially, afferent renal nerve activity may be involved.

There is now good evidence that sympathetic nerves innervate all elements of the kidney, that is vasculature, tubules and the renin containing cells (see Barajas *et al.*, 1992). At a functional level, increasing levels of direct renal nerve stimulation leads to increased renin secretion, raised sodium reabsorption and eventually, at high rates of activation, reductions in both renal blood flow and glomerular filtration rate (see Johns, 1991). It is by these regulatory actions of the sympathetic nerves on renal haemodynamic and tubular function that they may have a major impact on fluid volume homeostasis and hence blood pressure. Conversely, there is now good evidence that there are sensory receptors within the kidney itself, sensitive to both intrarenal mechanical stretch and chemical composition of the urine, and these form the afferent arm of renorenal reflexes (see Kopp, 1993). Importantly, in both the genetic and Goldblatt forms of hypertension these reflexes appear to be deficient (Kopp *et al.*, 1987; Kopp & Buckley-Bleiler, 1989) and may also contribute to the chronic elevation of blood pressure.

The level of activity within the renal nerves is dependent upon sensory input from the somatic, visceral, psychological and cardiovascular systems and its processing in the brain (see Panfilov & Reid, 1994). Within the central nervous system, the adrenergic neurones are recognised as exerting an important influence on central sympathetic outflow, but there is growing evidence that central 5-hydroxytryptaminergic pathways play a major role. An early study by Baum & Shropshire (1975) showed that administration of 5-hydroxytryptamine (5-HT) into the lateral ventricles of the cat reduced heart rate and sympathetic nerve activity. Subsequently, a series of studies found that intravenous (Gradin *et al.*, 1985; Dreteler *et al.*, 1990), arterial (Dreteler *et al.*, 1991) and intracisternal (Wouters *et al.*, 1988) administration of selective 5-HT_{1A} receptor agonists, for example 8-hydroxy-2 (di-n-propylamino) tetralin (8-OH-DPAT) or flesinoxan, caused decreases in heart rate and blood pressure reflecting decreased sympathetic outflow. An important observation of Ramage and colleagues (Ramage *et al.*, 1988; Ramage & Wilkinson, 1989) in the cat, was that the central administration of selective 5-HT_{1A} receptor agonists produced decreased sympathetic outflow to most organs, but with sympatho-inhibition to the kidney being most pronounced. The renal functional consequences of the administration of the centrally acting 5-HT_{1A} receptor agonist flesinoxan, was found to be a raised sodium and water excretion, whether renal perfusion pressure was regulated at an unchanged level or not (Chamienia & Johns, 1994a,b), and importantly, the increased fluid excretion was dependent upon intact renal nerves and occurred in the absence of changes in

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renal haemodynamics. These responses were taken to be a consequence of withdrawal of sympathetic tone at the level of the kidney.

The renal functional consequences of the administration of the centrally acting 5-HT_{1A} receptor agonist flesinoxan, was found to be a raised sodium and water excretion which occurred both in the absence of changes in renal haemodynamics and even when renal perfusion pressure was reduced (Chamienia & Johns, 1994a,b). Importantly, this raised fluid excretion was dependent on an intact renal innervation as it did not occur when the renal nerves were sectioned. These excretory responses were taken to be a consequence of an action of flesinoxan within the brain to withdraw sympathetic tone at the level of the kidney.

The stroke-prone spontaneously hypertensive and 2K1C-Goldblatt hypertensive rats are models in which there is evidence of increased sympathetic activity to the kidney (Lundin & Thoren, 1982; Lundin *et al.*, 1984). The question addressed in this investigation was whether in these hypertensive models suppression of renal sympathetic activity by activation of 5-HT_{1A} receptors centrally might induce an enhanced natriuretic and diuretic response. This was done by measuring renal haemodynamic and tubular function when the 5-HT_{1A} receptor agonist, flesinoxan, was given at increasing doses in intact and renally denervated animals.

Methods

All procedures were permitted under the UK Government Home Office Project License No PPL40/00274 and Personal Licence NO PIL40/00371 issued to E.J.J. and PIL40/02600 issued to A.L.C.

Animal preparation

Stroke-prone spontaneously hypertensive rats (SHR-SP), weighing approximately 300 g, were obtained from the in-house breeding colony at the Department of Physiology, University of Birmingham. The two-kidney one-clip (2K1C) Goldblatt hypertensive rats were prepared by the standard procedure in this laboratory. Briefly, male Wistar rats (approx. 130 g) were anaesthetized with halothane and the right kidney exposed via a small cut in the lumbar area, its artery was then dissected from surrounding tissues and a silver clip, 0.25 mm in diameter placed around it. A local antibiotic was applied into the wound (Crystapen benzylpenicillin), (Britannia Pharmaceuticals, Redhill, Surrey) and the wound was then closed in layers. The animals received an analgesic (Temgesic, buprenophine hydrochloride. Reckitt & Colman Pharmaceuticals, Hull; 0.1 ml, i.m.), systemic antiobiotic (Engemycin, oxytretracycline, Mycofarm, Dublin; 0.1 ml i.m.) and were returned to the animal house. The terminal experiments were performed 4 weeks later.

Surgical procedures

Animals (SHR-SP, 295–350 g; 2K1CGoldblatt rats 280–325 g) were anaesthetized with halothane (4%) in an oxygen/nitrous oxide mixture. A cannula was inserted into the right femoral vein and α-chloralose/urethane mixture was given slowly i.v. (up to a dose of 17.5 mg chloralose, 0.3 g urethane over 35 min) and an infusion of 3 ml h^{-1} saline (150 mmol l^{-1} NaCl) was begun. Anaesthesia was maintained throughout the experiment with additional small bolus doses of the same mixture, 0.05 ml i.v. every 30 min. A tracheostomy was carried out and further cannulae inserted into the right carotid artery, for arterial blood pressure monitoring, and in the right femoral artery to allow measurement of renal perfusion pressure and removal of arterial blood samples. Both kidneys were exposed retroperitoneally and their ureters cannulated. The left renal artery was then cleared of surrounding tissue by use of a dissecting microscope and an electromagnetic flow probe (Carolina EP100 series, internal circumference 2–2.5 mm) placed around it for measuring renal blood flow. Stimulating electrodes were applied to the coeliac/aortico-renal ganglia and pulses of 15V, 10 Hz, 0.2 ms were delivered for 10 s which caused a transient blanching of the kidney. Acute denervation was achieved by surgically stripping all tissue around the bifurcation of the renal artery and vein and the kidney was treated as denervated if blanching did not occur. Both arterial catheters were connected to pressure transducer (Statham P23 XL) and the signal fed to a custom built amplifier (Grayden, Birmingham). The flow probe was connected to a square wave electromagnetic flowmeter (Model FM501, Carolina Medical Instruments, U.S.A.). Blood pressure and renal blood flow signals were then presented to an I/O card connected to an Apple Macintosh computer running custom software written in LabVIEW (National Instruments, Austin, TX, U.S.A.) and displayed on the screen and heart rate was derived from the carotid pressure wave signal. Mean values for all parameters were displayed and updated for every 2 s and then meaned over each of the 15 min clearance periods. These meaned data were stored to the hard disk for later off-line analysis. Following completion of the surgery, a priming dose of 2 ml inulin in saline (1.5 g 100 ml^{-1}) was given i.v., the saline infusion replaced by one containing inulin (1.5 g 100 ml^{-1}), and the animals allowed 2 h to establish equilibrium.

Experimental protocols

Five pairs of 15 min urine collections (clearance periods) were taken. Two clearances were taken at the start to generate basal values, thereafter either drug was given over 5 min or renal perfusion pressure was stabilised at a new level for 5 min. A further 10 min was left to allow the drug to have its action then a further pair of clearances were collected which represented the experimental values. Arterial blood samples (300 μl each) were taken before and at the end of each pair of clearances. Blood samples were immediately centrifuged and the plasma aliquoted and thereafter, the red cells were resuspended in an equivalent volume of heparinized saline and reinfused into the animal within 5 min. Urine production was measured gravimetrically. The following groups of animals were studied:

SHR-SP rats: Flesinoxan group (n = 10) The first pair of clearance periods provided basal values. The rats then received flesinoxan as a bolus 0.3 ml i.v. dose 15 min before the start of remaining pairs of clearances at doses of 30, 100, 300 and 1000 μg kg^{-1} in a cumulative fashion. Flesinoxan was injected slowly over 30 s in 300 μl of normal saline.

Pressure reduction group (n = 7) In this group of rats, a loop of surgical thread was placed around the aorta, between the renal arteries and attached to a screw device to allow reduction of left kidney perfusion pressure when tightened (Chamienia & Johns, 1994b). The first two clearances were completed at prevailing pressure and then the perfusion pressure was lowered in steps to achieve the same average values as observed in the group 1 animals at each dose of flesinoxan. Fifteen minutes later the next pair of clearances began. The levels of perfusion pressure achieved were 150, 138, 117 and 94 mmHg, respectively. Only left kidney function was studied in this group.

Renal denervation group (n = 5) In this group of animals, in addition to the surgical preparation described above, the left and right renal sympathetic nerves were identified, carefully dissected and cut. The experimental protocol was identical to that described for the flesinoxan group (above).

2K-1C Goldblatt hypertensive rats: Flesinoxan group (n = 10) The experimental protocol in this group of rats was identical to that of the group of SHR-SP receiving flesinoxan. Both clipped and non-clipped kidney function were studied.

Chemical assays

Urinary and plasma electrolyte concentrations were measured by flame photometry (Ciba Corning 410C). Inulin concentrations of plasma and urine samples were measured as described previously (Johns *et al.*, 1976).

Drugs and chemicals

Flesinoxan was a gift from Solvay-Duphar B.V., Weesp, The Netherlands. Inulin and all other chemicals were purchased from Sigma, St. Louis, MO, U.S.A.

Statistical analysis

The mean values of all variables were calculated for each pair of clearances and were used in the analysis and displayed in the figures. All values were presented as means ± s.e.mean. Statistical analysis was undertaken following the approach of Ludbook (1994). For the groups of SHR-SP, comparisons were undertaken by repeated measures analysis of variance with application of the Greenhouse-Geisser correction testing dose (flesinoxan or pressure) against innervated, denervated or pressure groups and determining whether a significant interaction was present. In the case of 2K1C Goldblatt hypertensive rats, there were no treatment effects but the dose effect was assessed on the difference which existed between right and left kidneys. A SuperANOVA software package (Abbacus Concepts, Berkeley, CA, U.S.A.) was used and significance taken at the 5% level.

Results

SHR-SP rats

Figure 1 presents the mean arterial pressure, renal perfusion pressure, heart rate and renal blood flow in the three groups of rats. The blood pressures in these groups of SHR-SP were all markedly higher than those observed previously in Wistar rats subjected to the same experimental protocols (Chamienia & Johns, 1994a,b). In the first group, flesinoxan caused dose-dependent falls in both mean arterial pressure and renal perfusion pressure, reaching 74±5 and 73±5 mmHg, respectively, at the highest dose (both $P<0.001$). This was accompanied by a progressive decrease in heart rate of some 63±9 beats min^{-1} ($P<0.001$) at the highest dose of the drug. Renal blood flow showed only minimal changes over the dose range of flesinoxan tested although renal conductance would be progressively increased at this time. Figure 2 shows that in the group given flesinoxan, left kidney glomerular filtration rate increased slightly at the two lowest doses, returned to baseline at the third and decreased by 20%, at the highest dose ($P<0.001$). The pattern of urine flow was somewhat 'bell' shaped, increasing by 20% at the first dose, by 44% at the second dose before returning to baseline at the third dose, and falling, by 52% from baseline, at the highest dose. Absolute and fractional sodium excretions (Figure 2) followed a similar pattern to that observed with urine flow, that is increases of 61 and 117% for absolute sodium and 46 and 97% for fractional sodium excretion, with somewhat larger increases at the first two doses. These increases in water and sodium excretion in the animals receiving the flesinoxan were observed despite the concomitant fall in perfusion pressure (by 23 mmHg at the

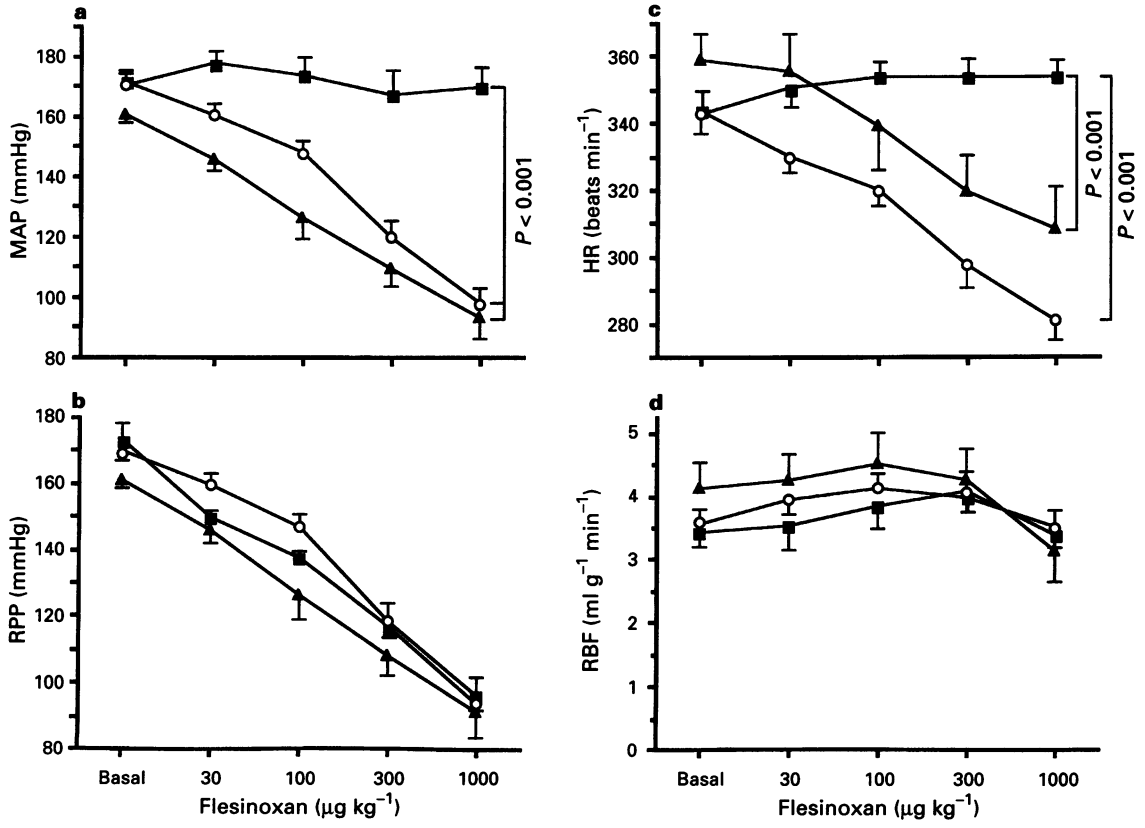

Figure 1 Changes in (a) arterial pressure (MAP), (b) renal perfusion pressure (RPP), (c) heart rate (HR) and (d) renal blood flow (RBF) in three groups of SHR-SP: the first (○, $n=10$) given flesinoxan; the second (■, $n=7$) subjected to reductions in renal perfusion pressure and the third (▲, $n=5$) given flesinoxan and in which both kidneys had been denervated. Each point is the average of the pair of clearance periods collected at each dose of drug given as mean; vertical lines show s.e.mean. The *P* values represent significant interactions between the data from rats with intact kidneys compared to animals in which the intact kidney was subjected to a pressure reduction and the group in which both kidneys were denervated (repeated measures ANOVA).

second dose of flesinoxan). At the same time, right kidney glomerular filtration rate, urine flow, absolute and fractional sodium excretions followed a very similar pattern and there were no statistical differences between the responses observed in the two kidneys (Figure 2).

In the animals in which left renal perfusion pressure was reduced in a stepwise fashion, mean arterial pressure did not change significantly over the 45 min observation period. The levels of perfusion pressure obtained by applying aortic constriction were very similar to those observed at each dose of the flesinoxan (Figure 1) in the animals receiving the drug. Heart rate in the rats subjected to the reduced renal perfusion pressure was maintained at a steady level throughout the experiment which was significantly different from the fall observed in the group of rats given flesinoxan ($P<0.001$). Renal blood flow in the renal perfusion pressure reduction group was not different from that seen in the group of animals given flesinoxan and remained unchanged during the period of renal perfusion pressure reduction (Figure 1). In the rats in which there was reduced renal perfusion pressure, glomerular filtration rate (Figure 3) fell significantly ($P<0.001$) but in a similar pattern to that observed in the flesinoxan-treated rats. During the pressure reduction, urine flow decreased (by 86% at the lowest pressure) which was significantly different from the values observed for both the left and right kidneys in the animals given the flesinoxan ($P<0.001$). At the same time, absolute and fractional sodium excretions (Figure 3) also decreased gradually reaching some 93 and 87%, respectively, at the lowest level of renal perfusion pressure. These decreases in absolute and fractional sodium excretions during reduced renal perfusion pressure were significantly greater than those observed when flesinoxan was given, although at the same pressure ($P<0.001$ for both variables).

In the animals in which both kidneys were surgically denervated, flesinoxan caused dose-dependent decreases (all $P<0.001$) in mean arterial pressure of 67 ± 7 mmHg, renal perfusion pressure of 67 ± 8 mmHg and heart rate of 50 ± 8 beats min^{-1} (Figure 1) at the highest dose of drug, which were not different from those observed in the group given flesinoxan but with innervated kidneys. Renal blood flow (Figure 1) was reduced at the highest dose, by 24%, similar to that observed in the group with innervated kidneys. Left kidney glomerular filtration rate (Figure 3) decreased by 21 and 55% from the baseline at the third and fourth doses of flesinoxan, which was similar to that observed in the innervated group given flesinoxan. In the rats subjected to bilateral renal denervation, left kidney urine flow, absolute and fractional sodium excretions (Figure 3) decreased significantly with increasing dose of flesinoxan (all $P<0.001$) which reached 77,68 and 61%, respectively, at the highest dose. Right kidney glomerular filtration rate, water and sodium excretions in the bilaterally renally denervated rats followed a very similar pattern (Figure 3). The magnitudes of the reductions in urine flow, absolute and fractional sodium excretions from the right and left kidneys in the renally denervated rats were very similar to those observed from the left kidneys of the group in which renal perfusion pressure was reduced mechanically, at each dose of flesinoxan or equivalent time points. However, these reductions in urine and sodium excretion in the rats with denervated kidneys were significantly different from those observed in the group of rats with innervated kidneys given flesinoxan (all $P<0.001$).

2K1C Goldblatt hypertensive rats

The basal mean arterial pressure in these rats given flesinoxan was 162 ± 5 mmHg, which was substantially higher than in normotensive rats in this laboratory (Chamienia & Johns, 1994a,b). Administration of flesinoxan (Figure 4) caused a

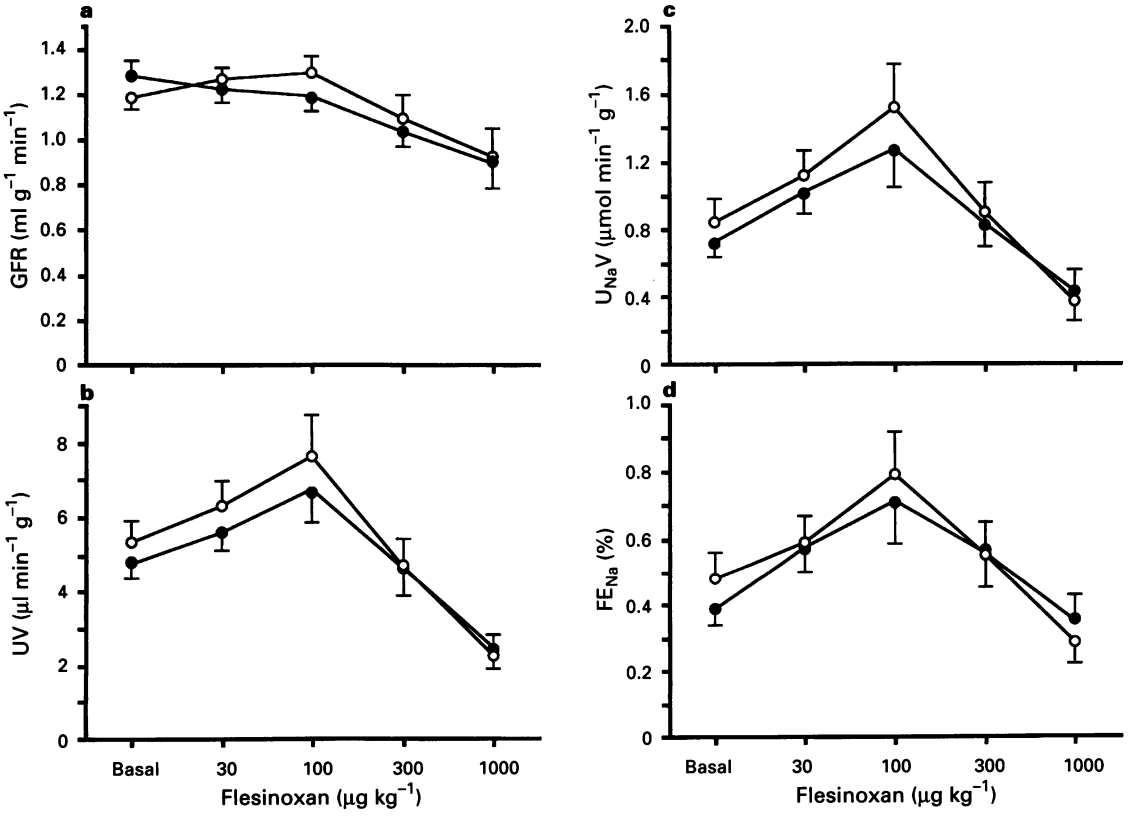

Figure 2 Changes in (a) glomerular filtration rate (GFR), (b) urine flow (UV), (c) absolute sodium excretion ($U_{Na}V$) and (d) fractional sodium excretion (FE_{Na}) in the right (●) and left (○) kidneys of SHR-SP ($n=10$), with innervated kidneys, given increasing doses of flesinoxan. Each point is the average of the pair of clearance periods taken at each dose of flesinoxan with values given as mean and s.e.mean.

dose-dependent decrease in both mean arterial pressure and renal perfusion pressure, of 34±3 and 35±3 mmHg, respectively, at the highest dose (both $P<0.001$). This was accompanied by a fall in heart rate which reached 20±6 beats min^{-1} at the highest dose ($P<0.001$) while renal blood flow, measured in the non-clipped kidney, remained at a constant level throughout the experiment (Figure 4). Figure 5 shows that the non-clipped kidney glomerular filtration rate did not change at any dose of flesinoxan. Urine flow, absolute and fractional sodium excretions of the these renovascular hypertensive rats all decreased in a dose-dependent fashion reaching 71, 73 and 69%, respectively, at the highest dose (Figure 5). This pattern was similar to the SHR-SP animals in which renal perfusion was reduced in a stepwise fashion but different from that observed in the SHR-SP given the same doses of flesinoxan. The clipped kidney glomerular filtration rate was very similar to that of the non-clipped kidney, and did not change significantly throughout the experiment. Basal values of urine flow, absolute and fractional sodium excretions in the clipped kidney were all lower than in the non-clipped kidney and the excretion of water and sodium in the clipped kidney (Figure 5) decreased with the increasing doses of flesinoxan. These changes in urine flow and sodium excretion were significantly different from those observed in the clipped kidney (all $P<0.001$; Figure 5).

Discussion

The aim of the present study was to gain insight into the role of the renal sympathetic nerves in the regulation of haemodynamic and excretory function of the kidney in two models of hypertension, the SHR-SP and the 2K1C Goldblatt rat. There is evidence that in the SHR, of which the SHR-SP is a substrain, the neural control of the kidney is enhanced, as Lundin *et al.* (1984) have shown, by single fibre recordings, that renal nerve activity is elevated compared with the Wistar normotensive control. A similar situation seems to pertain in the 2K1C rat, in that the partial normalisation of blood pressure following renal denervation (Katholi *et al.*, 1982), has been taken to support the notion of increased sympathetic drive to the kidney. The approach taken in the present study was to utilize flesinoxan, a highly selective 5-HT_{1A} receptor agonist (Wouters *et al.*, 1988) which acts within the central nervous system to suppress sympathetic outflow, particularly at the level of the kidney (Ramage *et al.*, 1988; Ramage & Wilkinson, 1989), and to determine how this modulated kidney function. In previous studies in normotensive rats (Chamienia & Johns, 1994a,b) we demonstrated that although the 5-HT_{1A} receptor agonist induced falls in blood pressure and heart rate, there were raised levels of sodium and water excretion which were dependent upon the renal nerves but independent of renal perfusion pressure. These findings were taken to indicate that the drug caused a depression of renal sympathetic outflow with a subsequent decreased reabsorption of sodium along with the nephron and hence increased fluid excretion.

Administration of flesinoxan into the SHR-SP caused decreases in arterial blood pressure, renal perfusion pressure and heart rate in a dose-related fashion, and although the magnitude of the changes were larger, this pattern of response was similar to that described previously by ourselves in the normotensive rat (Chamienia & Johns, 1994a,b) and by others in

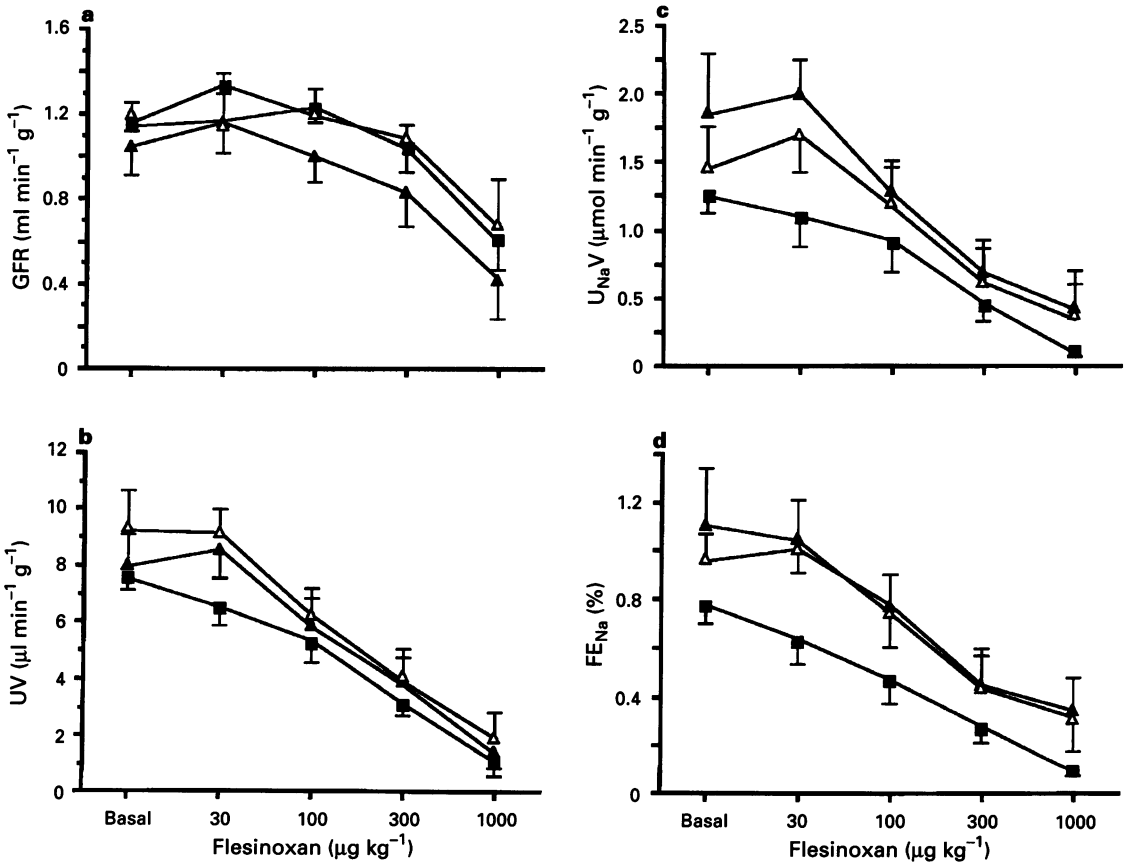

Figure 3 Changes in (a) glomerular filtration rate (GFR), (b) urine flow (UV), (c) absolute sodium excretion ($U_{Na}V$) and (d) fractional sodium excretion (FE_{Na}) of the innervated left kidney of the SHR-SP which were subjected to graded reductions in renal perfusion pressure, (■, $n=7$), and the denervated right (▲) and left (△) kidneys of the denervated group of rats ($n=5$) which were given flesinoxan. Each point is the average of the part of clearance periods taken at each dose of flesinoxan and given as mean and s.e.mean.

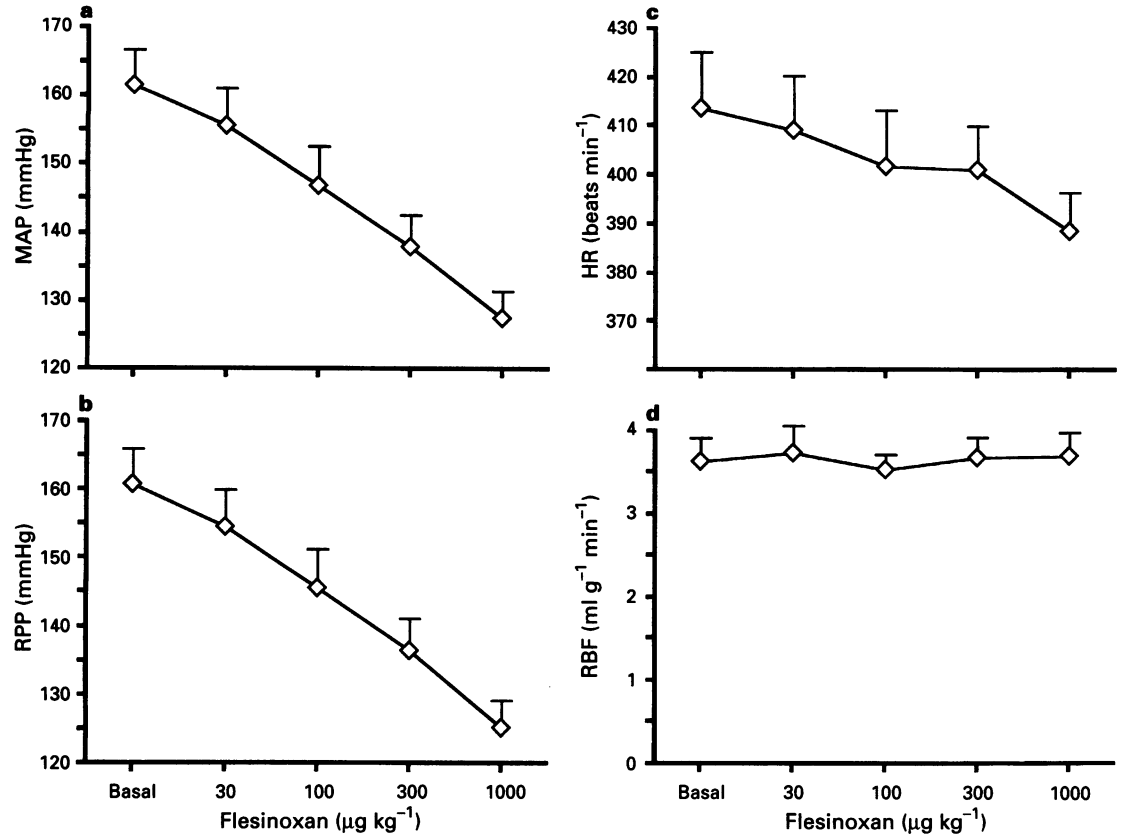

Figure 4 Changes in (a) arterial pressure (MAP), (b) renal perfusion pressure (RPP) (c) heart rate (HR), and (d) left renal blood flow (RBF) in a group of 2K1C Goldblatt hypertensive rats ($n = 10$) given increasing doses of flesinoxan. Each point is the average of the pair of clearance periods taken at each dose of flesinoxan and given as mean and s.e.mean.

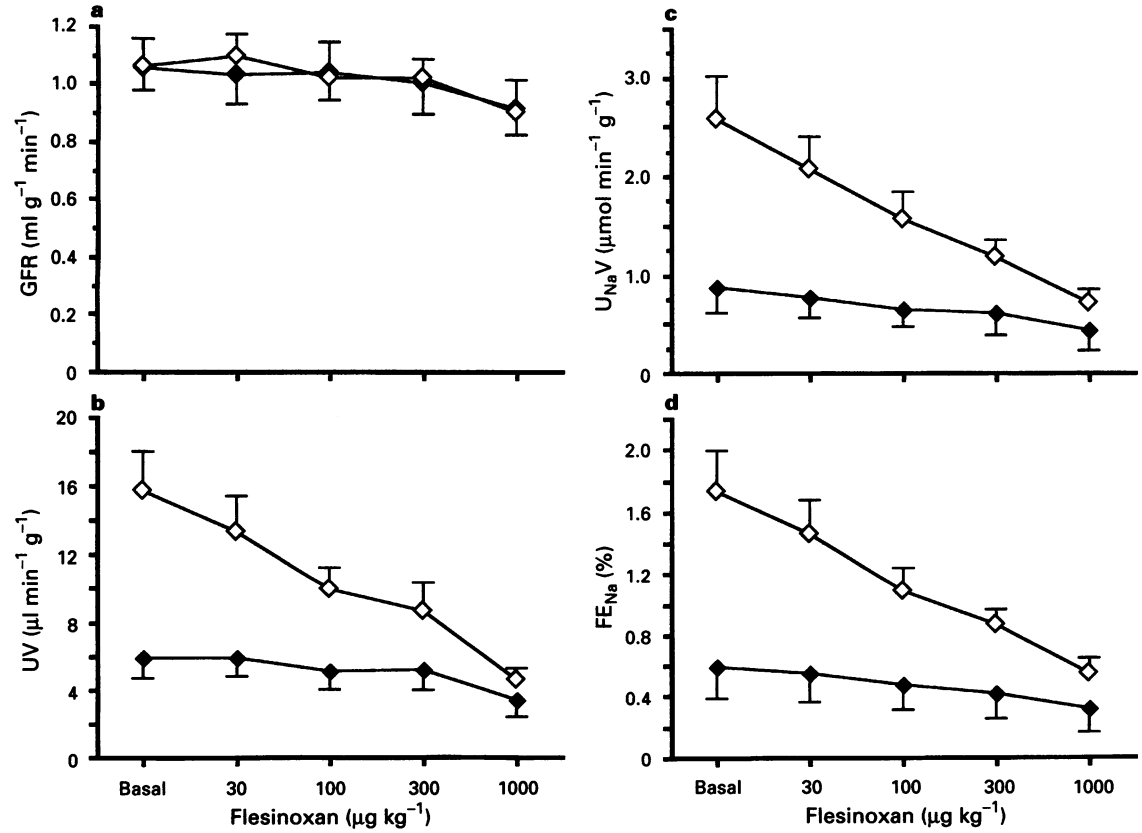

Figure 5 Changes in (a) glomerular filtration rate (GFR), (b) urine flow (UV), (c) absolute sodium ($U_{Na}V$) and (d) fractional sodium (FE_{Na}) excretions in the left non-clipped (◇) and right clipped kidney (◆) in the group of 2K1C Goldblatt hypertensive rats given increasing doses of flesinoxan. Each point is the average of the pair of clearances taken at each dose of flesinoxan and given as mean and s.e.mean.

the cat (Ramage & Wilkinson, 1989), and was probably a consequence of withdrawal of sympathetic tone from the heart and blood vessels of various vascular beds. Despite the large falls in blood pressure, of some 80 mmHg, both renal blood flow and glomerular filtration rate remained at relatively constant levels, demonstrating that there was effective autoregulation of these variables, except at the highest dose of flesinoxan when there was a maximal reduction in renal perfusion pressure of some 80 mmHg. An important observation was that in both the left and right kidneys of the renally innervated rats at the two lower doses of flesinoxan, there were significant increases in urine flow and sodium excretion, an approximate doubling at 100 $\mu g\ kg^{-1}$, even though there was a pressure fall of some 20–30 mmHg. Because it is now recognised that there is a direct relationship between renal perfusion pressure and the level of tubular sodium and water reabsorption (see Roman & Cowley, 1985; Granger, 1992) it is likely that had pressure remained unchanged, the rate of fluid excretion might have risen even further. Moreover, at the higher doses of flesinoxan the reductions in the output of urine and sodium to control levels and below was probably due to the overriding effect of the reduction in blood pressure which was marked at this stage. Interestingly, this bell-shaped pattern of response in water and sodium excretion with the increasing doses of flesinoxan contrasts with that obtained in the normotensive Wistar rats (Chamienia & Johns, 1994a), in which excretion remained constant as blood pressure and renal perfusion pressure fell. This difference could be taken as indicating that the renal nerve modulation of sodium and water handling in the SHR-SP was greater than in the normotensive rats which would be consistent with the observations of raised renal sympathetic nerve traffic in this hypertensive strain of rat (Lundin *et al.*, 1984).

The second group of SHR-SP were animals in which the renal perfusion pressure was mechanically reduced to the levels obtained at each dose of flesinoxan and was an attempt to examine the effect of perfusion pressure itself on both renal haemodynamic and tubular function. It was evident that there was good autoregulation of both renal blood flow and glomerular filtration rate to approximately 110 mmHg, and below this pressure filtration rate tended to fall. Over this pressure range there was a gradual and progressive reduction of urine flow and both absolute and fractional sodium excretion, which was consistent with a direct effect of perfusion pressure, via renal interstitial hydrostatic pressure, on reabsorption of fluid along the nephron (see Roman & Cowley, 1985; Granger, 1992). These findings reinforce the conclusion from the drug treated SHR-SP in that flesinoxan was indeed inducing the natriuresis and diuresis.

In order to establish the importance of the renal nerves in the flesinoxan induced changes in fluid excretion, a third group of SHR-SP were used in which both kidneys were subjected to surgical denervation. Although the cardiovascular variables and renal haemodynamic status of these rats were very comparable to the intact rats, the basal levels of water and sodium excretion from both kidneys were substantially higher than those obtained when the nerves were present, suggesting that the animals were in a state of denervation diuresis and natriuresis. It was clear that flesinoxan induced comparable decreases in blood pressure and heart rate as in the rats with innervated kidneys but under these conditions there was a progressive reduction in urine flow and sodium excretion. This pattern of excretory response was very similar to that obtained in the pressure reduction group but very different from the animals with an intact renal innervation. Thus, these observations provide strong support for the view that the flesinoxan caused a withdrawal of sympathetic tone to the kidney which could be responsible for the elevated water and sodium excretion.

The final study was undertaken in the 2K1C Goldblatt hypertensive rats and over the four week period of renal artery clipping the rats had developed a hypertension as indicated by the mean blood pressure of 160 mmHg, which is comparable to that observed in other studies with these hypertensive rats (Akpogomeh & Johns, 1991; Sattar & Johns, 1994; Davis & Johns, 1994). The renal functional data showed that although glomerular filtration rate was very similar in the clipped and non-clipped kidneys, water and sodium excretion was much higher in the non-clipped compared to the clipped kidney. This was most likely due to the fact that the non-clipped kidney was faced with the raised levels of perfusion pressure, whereas the pressure in the renal artery distal to the clip was probably normal or even low under these experimental conditions of acute anaesthesia. The administration of increasing doses of flesinoxan into the 2K1C rats resulted in a progressive fall in blood pressure and heart rate although to a lesser degree than observed in the SHR-SP, but comparable to that observed previously in normotensive rats (Chamienia & Johns, 1994a,b). This could be interpreted as reflecting a different degree of sympathetic drive between the two hypertensive models, being higher in the SHR-SP than in the 2K1C Goldblatt rats.

Renal blood flow was measured only in the left kidney, but was autoregulated during the flesinoxan-induced falls in perfusion pressure and both right and left glomerular filtration rates were maintained at a relatively constant level down to a pressure of 120 mmHg. However, it was apparent that flesinoxan caused a dose-related decrease in water and sodium output from both kidneys, being more evident in the non-clipped than clipped kidneys because of the higher rates of fluid output. This pattern of excretory response to flesinoxan was very different from that obtained in the intact SHR-SP of this study and from results obtained previously in intact normotensive rats subjected to the same experimental protocol (Chamienia & Johns, 1994a), but was similar to that observed in the renally denervated SHR-SP of this study and normotensive rats (Chamienia & Johns, 1994b). These findings suggest that in this particular model of hypertension the level of sympathetic outflow to the kidney may not be at a sufficient level to exert an important modulating effect on tubular sodium handling. Clearly, such a conclusion supports the view of Wyss *et al.* (1992) showing that renal denervation ameliorates hypertension and directs attention to the role of the renal afferent nerves in contributing to the genesis of elevated blood pressure in this renovascular model of hypertension.

This investigation compared the renal haemodynamic and excretory responses to flesinoxan, a 5-HT_{1A} agonist, which has been shown to suppress sympathetic outflow, particularly at the kidney. In the SHR-SP, flesinoxan dose-dependently decreased blood pressure and heart rate whereas there was a bell-shaped excretory response, with water and sodium output increasing at low doses and falling to below basal levels at the highest dose of drug. This pattern of excretory response was mediated by the renal nerves, as in their absence flesinoxan causes a progressive fall in pressure as well as water and sodium excretion similar to that when perfusion pressure was reduced to equivalent levels mechanically in intact SHR-SP. By contrast, flesinoxan administration into 2K1C Goldblatt rats had minimal effects on renal haemodynamics, but led to a graded reduction in sodium and water output from both clipped and unclipped kidneys which was comparable to that obtained previously in renally denervated normotensive rats (Chamienia & Johns, 1994a) and SHR-SP (present study). These findings are compatible with the suggestion that in the SHR-SP the renal sympathetic nerves exert a major influence on sodium and water reabsorption along the nephron, whereas in the 2K1C Goldblatt hypertensive rats they exert a minimal effect on the tubular reabsorptive processes.

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