Br. J. Pharmac. (1985), **86**, 447–453

Atrial natriuretic factor causes specific relaxation of rat renal arcuate arteries

C. Aalkjaer, M.J. Mulvany[1] & N.C.B. Nyborg*

Biophysics and Pharmacology* Institutes, Aarhus University, 8000 Aarhus C, Denmark

1 We have investigated the effect of a synthetic 'atrial natriuretic factor' (ANF) on induced tone in rat isolated renal arcuate arteries (lumen diameter ca. 250 μm), and compared this with the effects of synthetic ANF on resistance vessels of similar size taken from the mesenteric, femoral, cerebral and coronary vasculature.

2 Synthetic ANF was found to cause relaxation of the renal vessels when these were sub-maximally activated with K^+, noradrenaline or 5-hydroxytryptamine, but had no effect on the responses of the other vessels to these agonists. Synthetic ANF had a near maximal effect (65% relaxation) at 100 nM, with an IC_{50} of 7.9 nM. The relaxant effect of synthetic ANF on the renal vessels was fully maintained for at least 15 min.

3 Hydrallazine (100 μM) caused relaxation of renal vessels (47%) and coronary vessels (42%), but had no effect on the other vessel types. By contrast, sodium nitroprusside (1 μM) relaxed all vessel types.

4 The relaxant action of synthetic ANF on the renal vessels was seen in the presence of ouabain (1 mM), propranolol (1 μM), phentolamine (1 μM), atropine (1 μM) and felodipine (1 nM).

5 In the renal vessels, synthetic ANF had no effect on membrane potential, measured with intracellular electrodes, despite the simultaneously measured relaxation.

6 Synthetic ANF had no effect on the efflux of $^{22}Na^+$ in either renal or mesenteric vessels.

7 The results demonstrate that synthetic ANF has a specific and prolonged relaxant effect on renal small arteries, and are consistent with this effect being mediated through specific receptors.

Introduction

The natriuretic effect of atrial extracts is now well documented (DeBold *et al.*, 1981; Keeler, 1982; Trippodo *et al.*, 1983). Furthermore, the factor producing the natriuretic effect ('atrial natriuretic factor' (ANF), a peptide containing an active portion of about 26 amino acid groups) has now been synthesized (Currie *et al.*, 1984; Thibault *et al.*, 1984; Kangawa & Matsuo, 1984; Seidman *et al.*, 1984). However, despite this detailed knowledge of the effect and structure of ANF, its mechanism of action is not known (Needleman *et al.*, 1984), and at least 3 possible mechanisms have been proposed to explain its natriuretic effect. One possibility is that ANF may act by causing decreased re-absorption of sodium in the distal tubuli (DeBold *et al.*, 1981; Sonnenberg *et al.*, 1982), although more recent findings that ANF does not inhibit either renal Na K-ATPase (Thibault *et al.*, 1983) or the vascular Na K-pump (Pamnani *et al.*, 1984) do not support it. A second possible mechanism is based on the finding that aldosterone synthesis is inhibited by ANF (Kudo & Baird, 1984; Atarashi *et al.*, 1984). However, the action of infused ANF is too rapid for the natriuretic effect to be due solely to its effect on aldosterone synthesis (Chartier *et al.*, 1984). The third possible mechanism is suggested by the finding that ANF has a relaxant effect on vascular smooth muscle (Winquist *et al.*, 1984a, Currie *et al.*, 1984). Furthermore since it causes a decrease in the resistance of the renal vasculature, but has no effect on the resistance of the femoral vasculature (Oshima *et al.*, 1984), the natriuretic effect might be associated with altered renal haemodynamics. This possibility is supported by recent experiments which show that purified or synthetic ANF can cause increases in glomerular filtration rate (GFR) (Briggs *et al.*, 1982; Maack *et al.*, 1984).

In the present investigation we have investigated the mechanism behind this renal vasodilatation and have examined the effect of ANF on isolated arcuate arteries (lumen diameter ca. 250 μm). The effect has been compared with the responses of resistance vessels taken from other vascular beds. The work was performed using synthetic ANF.

[1]Author for correspondence at Biophysics Institute, Aarhus Univ., Universitetsparken 185, 8000 Aarhus C, Denmark.

Methods

Preparations

The vessels were taken from male Wistar-Kyoto rats (15–200 g) which had been killed with CO_2. All vessels were ca. 2 mm long segments of small arteries taken from the mesenteric vasculature (Mulvany & Halpern, 1977), the femoral vasculature (Nilsson & Mulvany, 1981), the coronary vasculature (Nyborg, 1985) or the renal or cerebral beds. The renal vessels were arcuate arteries, taken close to the renal cortex and the cerebral vessels were taken from the first branch of the middle cerebral artery. For each animal, not more than 2 vessels of any one type were used, apart from the sodium flux experiments where up to 8 vessels per animal were used.

Mechanical experiments

The small artery segments were mounted as ring preparations in a myograph (Mulvany & Halpern, 1977) by threading them on two stainless steel wires (diameter 40 μm) which were attached to a force transducer and a micrometer, respectively, and placed in an organ bath. The vessel internal circumference was then set to a normalized value corresponding to 90% of the internal circumference which the vessel would reach when relaxed and under a transmural pressure of 100 mmHg. With this extension the active response of the vessels is close to maximal (Mulvany & Halpern, 1977). The mechanical characteristics of the vessels are shown in Table 1. The mean diameters of the 5 vessel types ranged from 168 μm to 243 μm. All vessels, as determined by their response to 125 mM K^+ salt solution, were viable although the responses of the coronary vessels were considerably less than those of the other vessel types.

Membrane potential measurements

In these experiments vessels were mounted on the myograph in the usual way and membrane potential was measured simultaneously with the mechanical responses. Glass microelectrodes (resistance ca. 100 MΩ when filled with 3 M KCl) were used as described previously (Mulvany *et al.*, 1982). A suffusion system was used to allow solution changes without disturbing the electrodes.

$^{22}Na^+$-efflux

A detailed description of determination of $^{22}Na^+$-efflux has been given elsewhere (Aalkjaer & Mulvany, 1983). In brief, vessels were preincubated for 1 h in physiological salt solution, loaded in $^{22}Na^+$ salt solution for 30 min and then washed through a series of vials containing physiological salt solution at 37°C. After 9 min washout the efflux was considered to represent efflux from a cellular pool. The efflux

Table 1 The characteristics of vessels used to investigate the effects of atrial natriuretic factor

	Vessel type				
	Renal	*Mesenteric*	*Femoral*	*Cerebral*	*Coronary*
Number of vessels	29	11	10	11	8
Normalized lumen diameter (μm)	243 ± 2	201 ± 12	168 ± 7	213 ± 10	211 ± 16
Response to 125 mM K^+ salt solution ($N\,m^{-1}$)	1.42 ± 0.12	2.14 ± 0.15	2.12 ± 0.2	1.64 ± 0.12	0.77 ± 0.13

Values show mean ± s.e.

Table 2 Relaxation responses of small arteries of the rat to various agents

	Vessel type				
	Renal	*Mesenteric*	*Femoral*	*Cerebral*	*Coronary*
ANF (10^{-7} M)	59 ± 7* (10)	− 2 ± 5 (7)	7 ± 8 (6)	−14 ± 5 (8)	− 7 ± 6 (8)
SNP (10^{-6} M)	56 ± 6* (11)	64 ± 6* (6)	62 ± 12* (3)	32 ± 7* (7)	77 ± 8* (8)
Hyd (10^{-4} M)	47 ± 7* (5)	11 ± 7 (5)	4 ± 1 (5)	− 9 ± 4 (7)	37 ± 14* (8)

Vessels were first activated with 40 mM K^+ salt solution and then exposed to synthetic atrial natriuretic factor (ANF), sodium nitroprusside (SNP) or hydrallazine (Hyd). The data show relaxation responses (mean ± s.e. of number of vessels in parentheses) measured after 2 min exposure to the agent, expressed as the percentage drop in tension (where 100% represents the wall tension immediately before exposure to the agent).
*Indicates that relaxation is significant ($P < 0.05$).

medium was then modified as indicated and the vessels washed for a further 4 min. The mean $^{22}Na^+$-efflux rate constant was determined between 9 and 13 min after initiation of the washout.

Statistics

In the text the values are generally given as arithmetic mean ± s.e. (number of vessels). For $^{22}Na^+$ efflux the values are given as geometric mean with the 95% confidence limits in square parentheses. Differences were tested for significance using the 2-tailed *t* test and $P < 0.05$ was considered significant.

Solutions

Physiological salt solution contained (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, ethylene-diaminetetraacetic acid (EDTA) 0.026, glucose 5.5. K^+ salt solution was as for physiological salt solution, but with an equimolar exchange of NaCl for KCl to give the concentration of K^+ indicated. In the experiments where ^{22}Na was used, the $CaCl_2$ concentration was only 1.6 mM. The solution used for loading with $^{22}Na^+$ ($^{22}Na^+$ salt solution) was physiological salt solution containing $^{22}Na^+$ (Radiochemical Centre, Amersham) with a specific activity of 1.56 Ci mol^{-1}. All solutions were bubbled with 5% CO_2 in O_2.

The synthetic atrial natriuretic factor used (synthetic ANF; 8–33 amino acid fragment, C-terminal acid, Department of Medicinal Chemistry, Merck) was a gift from Dr R.J. Winquist. The synthetic ANF was made up into stock aqueous solutions (100 μM) and stored at −20°C. The storage vials and mixing test-tubes were precoated with albumin. Other drugs used were sodium nitroprusside (Merck), hydrallazine (Sigma), ouabain (Merck), propranolol (DAK, Denmark), phentolamine (DAK), atropine (DAK), felodipine (Hässle), (−)-noradrenaline (Sigma) and 5-hydroxytryptamine (5-HT) (Sigma).

The responses of vessels to synthetic ANF were obtained by first activating vessels submaximally with 40 mM (or 60 mM) K^+ salt solution, 1 μM noradrenaline or 0.1 μM 5-HT, as indicated, and then adding synthetic ANF cumulatively for 2 min per concentration. IC_{50} values were determined by geometric interpolation.

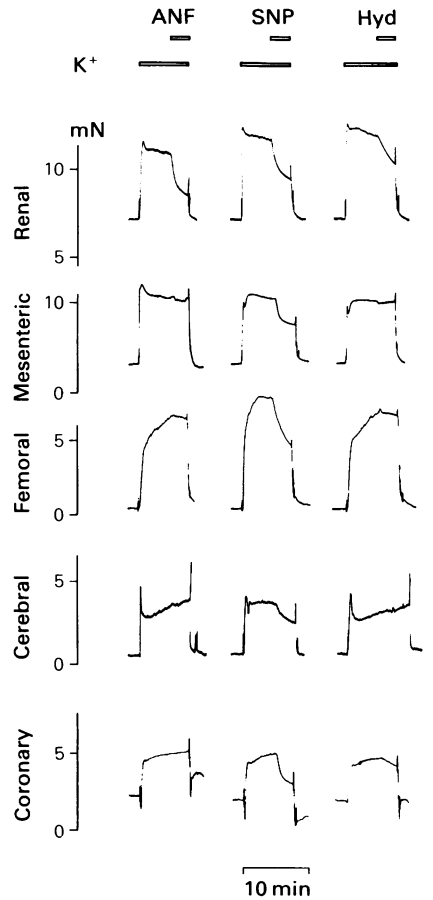

Figure 1 Records of relaxation responses of renal arcuate arteries and of mesenteric, femoral, cerebral and coronary small arteries to synthetic atrial natriuretic factor (ANF), sodium nitroprusside (SNP) and hydrallazine (Hyd). Records show measured wall force. Vessels were activated with 40 mM K^+ salt solution and then exposed either to synthetic ANF (100 nM), SNP (1 μM), or hydrallazine (100 μM), as indicated. Between each activation, vessels were kept in physiological salt solution for about 30 min. Normalized internal diameters (l_o) were: renal vessel, 246 μm; mesenteric vessel, 259 μm; femoral vessel, 132 μm; cerebral vessel, 152 μm; coronary vessel, 276 μm. Note that only the renal vessel relaxed in response to synthetic ANF, that only the renal and coronary vessels relaxed to hydrallazine, but that all vessels relaxed in response to SNP.

Results

Synthetic ANF (100 nM) was found to cause rapid relaxation of the renal resistance vessels when they were activated with K^+ salt solution, but to have no effect on the mesenteric, femoral, cerebral or coronary vessels (Figure 1, Table 2). Hydrallazine (100 μM) was found to cause relaxation of both renal and coronary vessels. By contrast, nitroprusside (11 μM) caused relaxation of all the resistance vessels. Cumulative dose-response experiments in the renal vessels showed that, as regards relaxation of the response to the 40 mM K^+ salt solution, synthetic ANF had an IC_{50} of

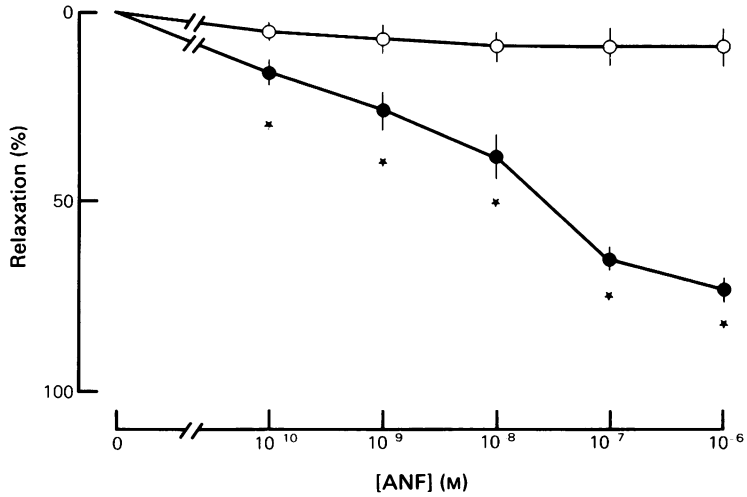

Figure 2 Concentration-response characteristics of 8 renal arcuate arteries from 4 rats to synthetic atrial natriuretic factor (ANF). Vessels were first activated with 40 mM K^+ salt solution (in some cases 60 mM K^+ salt solution), to give half maximal response, and were then cumulatively exposed to the concentrations of synthetic ANF indicted, 2 min per concentration. Closed symbols show mean % relaxation (vertical lines represent s.e.), where 100% = the initial K^+ salt solution response, which for these vessels was $1.48 \pm 0.16\ N\ m^{-1}$. $l_o = 271 \pm 12\ \mu m$. Open symbols show tension in these vessels at corresponding times during control responses without exposure to synthetic ANF. $*P < 0.05$, significantly different from control.

Table 3 Relaxation responses to synthetic atrial natriuretic factor (ANF) in renal interlobular arteries

Agonist	*−log (IC_{50})*	*Relaxation with 10^{-7}M ANF (%)*
40 mM K^+	8.1 ± 0.1 (11)	64 ± 7 (12)
Noradrenaline (10^{-6}M)	7.8 ± 0.1 (13)	47 ± 4 (13)
5-HT (10^{-7}M)	8.0 ± 0.1 (13)	73 ± 3 (13)

Vessels were first exposed to 40 mM K^+ salt solution, noradrenaline or 5-hydroxytryptamine (5-HT), as indicated; they were then exposed to increasing concentrations of synthetic ANF. Data show means ± s.e. of number of vessels in parentheses.

7.9 nM, with a near maximal effect (65% relaxation) at 100 nM (Figure 2). Similar effects were seen for the relaxation of sub-maximal responses to noradrenaline and 5-HT (Table 3). The relaxant effect of synthetic ANF in renal vessels was fully maintained for at least 15 min (4 vessels).

The relaxant effect of synthetic ANF on the renal resistance vessels was seen in the presence of both propranolol and ouabain (Figure 3), indicating that the effect was mediated neither by β-adrenoceptors nor by the Na K-pump. Evidence against the involvement of α-adrenoceptors or cholinoceptors was obtained in 4 other experiments, where the relaxant effect of synthetic ANF in renal vessels was found to be unaffected by the presence of phentolamine (1 μM) or atropine (1 μM). The lack of effect of synthetic ANF

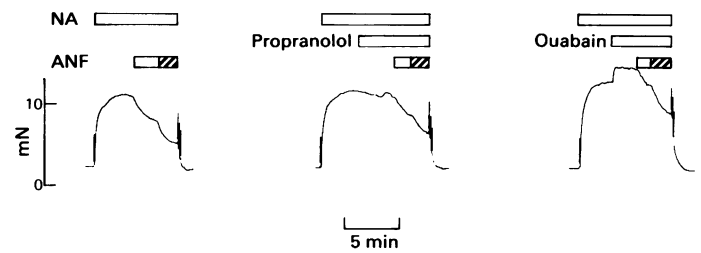

Figure 3 Record showing lack of effect of ouabain or propranolol on relaxation response of renal arcuate artery to synthetic atrial natriuretic factor (ANF). Vessel was first activated with noradrenaline (NA) 1 μM and then exposed to synthetic ANF (open bar, 10 nM; hatched bar, 100 nM) under control conditions. The protocol was then repeated first with addition of propranolol (1 μM), and then with ouabain (1mM), as indicated. $l_o = 188\ \mu m$.

Table 4 Effect of synthetic atrial natriuretic factor (ANF) and ouabain on sodium efflux rate from renal and mesenteric small arteries

Washout solution	*^{22}Na efflux rate* (min^{-1})	
	Renal vessels	*Mesenteric vessels*
Control	0.092 [0.083–0.102] (6)	0.123 [0.117–0.131] (6)
Control + ANF (10^{-7} M)	0.095 [0.092–0.098] (6)	0.112 [0.106–0.119] (6)
Control + ouabain (10^{-3} M)	0.064 [0.059–0.069]* (7)	0.071 [0.067–0.075]* (5)

The control solution was standard physiological salt solution, 37°C. Values show means, with 95% confidence limits, of number of vessels in parentheses.
*Significantly different from control ($P < 0.05$).

on the Na K-pump was confirmed by measurements of the efflux rate of $^{22}Na^+$ from renal and mesenteric vessels (Table 4). Although ouabain (1 mM) reduced the $^{22}Na^+$-efflux rate constant, synthetic ANF (100 nM) had no effect.

The relaxant effect was also seen (4 vessels) in the presence of felodipine (1 nM), a concentration which we have previously found to inhibit responses resulting from depolarization (Nyborg & Mulvany, 1984). This suggested that the relaxation response is not due to a hyperpolarizing effect and this was confirmed by direct measurements of membrane potential (Figure 4). These showed that, in renal vessels, synthetic ANF (200 nM) caused no change in membrane potential (hyperpolarization = 1.3 ± 1.4 mV, $n = 4$) despite a large relaxation response (51 ± 9%).

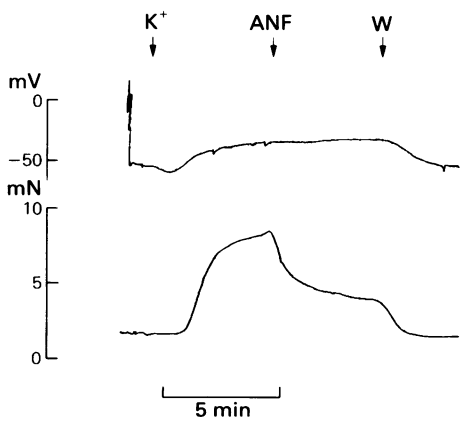

Figure 4 Record showing simultaneous measurements of membrane potential (top record) and wall force (lower record) in a renal arcuate artery when the suffusion solution was first changed to 40 mM K^+ salt solution (K^+) and then synthetic atrial natriuretic factor (ANF; 200 nM) was added to the chamber. K^+ salt solution and synthetic ANF were washed out with physiological salt solution at W. Note that despite the large relaxation, synthetic ANF had no effect on the membrane potential. Note too that the depolarizing effect of the K^+ salt solution was reversible. $l_o = 352\ \mu m$.

Discussion

Previous investigations of the effects of ANF on isolated vessels have been confined to large vessels, such as the rabbit aorta (Currie *et al.*, 1984) and rabbit facial vein (Winquist *et al.*, 1984a). These experiments have confirmed that ANF can have a direct relaxation effect on the vasculature, but have not been able to provide information about the possible effects of ANF on vascular resistance. The present experiments performed using vessels small enough to participate significantly in the determination of peripheral resistance (see Mulvany, 1985) suggest that it is only in the renal circulation that ANF will have a direct effect on the resistance. The results therefore support the findings of Oshima *et al.* (1984) who observed that, *in vivo*, the renal, but not the femoral, resistance was decreased by ANF. Furthermore, the present results show that this action is probably due to a direct effect on the vascular smooth muscle.

However, the mechanism of action of ANF still remains unclarified, although as indicated in Results, the vascular action of synthetic ANF does not appear to be mediated either through β-adrenoceptor activation, or through the Na K-pump (Figure 3, Table 4). Furthermore, since neither phentolamine nor atropine affected the response to synthetic ANF, it seems that this action is not mediated either by adrenergic or cholinergic mechanisms. Recent evidence concerning the rabbit facial vein suggests that the action of synthetic ANF is not mediated through the endothelium either (Winquist *et al.*, 1984a). Also, our results fail to support the recent suggestion (Winquist *et al.*,1984a,b) that the mechanism of action of ANF is similar to that of sodium nitroprusside, as nitroprusside relaxed all vessel types, while synthetic ANF relaxed only the renal vessels. The mechanism of action of synthetic ANF seems to differ from hydrallazine too. First, although both hydrallazine and synthetic ANF had a powerful relaxant effect on renal vessels, in contrast to synthetic ANF hydrallazine also had a relaxant effect on the coronary vessels. Second, hydrallazine is thought to act by hyperpolarization of

the membrane (Khayyal *et al.*, 1981), while the present experiments show that synthetic ANF had no effect on membrane potential. Other recent experiments have suggested that synthetic ANF acts by increasing cellular levels of cyclic GMP (Winquist *et al.*, 1984b; Hamet *et al.*, 1984); though it remains an open question whether or not the relaxation is a consequence of the increased level of cyclic GMP. Taken together our experiments do not therefore provide positive information as to the mechanism of action of synthetic ANF, but they are consistent with the recent indications that synthetic ANF acts through specific synthetic ANF receptors (De Lean *et al.*, 1984; Napier *et al.*, 1984).

The sensitivity of the renal vessels to synthetic ANF (IC_{50} = 7.9 nM) was somewhat less than that previously found for the corresponding sensitivity of rat aorta (IC_{50} = 0.85 nM, Napier *et al.*, 1984). Since we took the precaution of precoating the synthetic ANF storage vials and mixing test-tubes with albumin, we do not believe that the difference is due to binding of synthetic ANF to these. Although we cannot completely exclude the possibility that the difference is due to synthetic ANF binding to parts of our myograph chamber, it seems to us more probable that the difference reflects a difference between aorta and resistance vessels; a difference we have also noted with respect to noradrenaline sensitivity (Mulvany *et al.*, 1982).

The cause of the natriuretic effect of ANF is also still unknown. As indicated in the Introduction, there is evidence that reduced sodium uptake in the distal tubules and inhibition of aldosterone synthesis may contribute to the natriuretic effect of ANF but it is likely that other mechanisms are also involved. The present experiments support the previous indications (Borenstein, *et al.*, 1983; Oshima *et al.*, 1984) that one of the mechanisms of this effect of ANF is associated with its ability to cause relaxation of renal small arteries, a possibility also supported by the close relation which has been described between decreased renal vascular resistance and the natriuretic effect Needleman *et al.*, 1984). How such relaxation could cause increased natriuresis is still a matter for speculation. However, if the relaxation were specifically targeted on the afferent renal arterioles, then it would cause an increase in glomerular pressure which in turn would increase GFR and hence, probably, natriuresis. Such a differentiated effect of ANF on pre- and postglomerular vessels could also explain why the decrease in vascular resistance was observed to be transitory *in vivo* (Maack *et al.*, 1984), while we found it to be maintained: afferent vasodilatation and subsequent efferent vasoconstriction could return renal resistance to normal, but maintain increased glomerular pressure. We believe that this possibility deserves further investigation.

This work was supported by the Danish Medical Research Council. We thank Jørgen Andresen, Tove Jakobsen and Kirsten Olesen for their expert technical assistance.

References

AALKJAER, C. & MULVANY, M. (1983). Sodium metabolism of rat resistance vessels. *J. Physiol.*, **343**, 105–116.

ATARASHI, K., MULROW, P.J., FRANCO-SAENZ, R., SNAJDAR, R. & RAPP, J. (1984). Inhibition of aldosterone production by an atrial extract. *Science*, **224**, 992–993.

BORENSTEIN, H.B., CUPPLES, W.A., SONNENBERG, H. & VERESS, A.T. (1983). The effect of natriuretic atrial extract on renal haemodynamics and urinary excretion in anaesthetized rats. *J. Physiol.*, **334**, 133–140.

BRIGGS, J.P., STEIPE, B., SCHUBERT, G. & SCHNERMANN, J. (1982). Micropuncture studies of the renal effects of atrial natriuretic substance. *Pflügers Arch.*, **395**, 271–276.

CHARTIER, L., SCHIFFRIN, E., THIBAULT, G. & GARCIA, R. (1984). Atrial natriuretic factor inhibits the stimulation of aldosterone secretion by angiotensin II, ACTH and potassium *in vitro* and angiotensin II-induced steroidogenesis *in vivo*. *Endocrinology*, **115**, 2026–2028.

CURRIE, M.G., GELLER, D.M., COLE, B.R., SIEGEL, N.R., FOK, K.F., ADAMS, S.P., EUBANKS, S.R., GALLUPPI, G.R. & NEEDLEMAN, P. (1984). Purification and sequence analysis of bioactive atrial peptides (Atriopeptins). *Science*, **223**, 67–69.

DE BOLD, A.J., BORENSTEIN, H.B., VERESS, A.T. & SONNENBERG, H. (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci.*, **28**, 89–94.

DE LEAN, A., GUTKOWSKA, J., McNICOLL, N., SCHILLER, P.W., CANTIN, M. & GENEST, J. (1984). Characterization of specific receptors for atrial natriuretic factor in bovine adrenal zone glomerulosa. *Life Sci.*, **35**, 2311–2318.

HAMET, P., TREMBLAY, J., PANG, S.C., GARCIA, R., THIBAULT, G., GUTKOWSKA, J., CANTIN, M. & GENEST, J. (1984). Effect of native and synthetic atrial natriuretic factor on cyclic GMP. *Biochem. biophys. Res. Comm.*, **123**, 515–527.

KANGAWA, K. & MATSUO, H. (1984). Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (alpha-hANP). *Biochem. biophys. Res. Comm.*, **118**, 131–139.

KEELER, R. (1982). Atrial natriuretic factor has a direct, prostaglandin-independent action on kidneys. *Can. J. Physiol. Pharmac.*, **60**, 1078–1082.

KHAYYAL, M., GROSS, F. & KREYE, V.A.W. (1981). Studies on the direct vasodilator effect of hydralazine in the isolated rabbit renal artery. *J. Pharmac. exp. Ther.*, **216**, 390–394.

KUDO, T. & BAIRD, A. (1984). Inhibition of aldosterone production in the renal glomerulosa by atrial natriuretic factor. *Nature*, **312,** 756–757.

MAACK, T., MARION, D.N., CAMARGO, M.J.F., KLEINERT, H.D., LARAGH, J.H., VAUGHAN, E.D., ATLAS, S.A. (1984). Effects of auriculin (atrial natriuretic factor) on blood pressure, renal function, and the renin-aldosterone system in dogs. *Am. J. Med.*, **77,** 1069–1075.

MULVANY, M.J. (1985). Pathophysiology of vascular smooth muscle in hypertension. *J. Hypertension*, **2** (Suppl. 3), 413–420.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circulation Res.*, **41,** 19–26.

MULVANY, M.J., NILSSON, H. & FLATMAN, J.A. (1982). Role of membrane potential in the response of rat small mesenteric arteries to exogenous noradrenaline stimulation. *J. Physiol.*, **332,** 363–373.

NAPIER, M.A., VANDLEN, R.L., ALBERS-SCHONBERG, G., NUTT, R.F., BRADY, S., LYLE, T., WINQUIST, R., FAISON, E.P., HEINEL, L.A. & BLAINE, E.H. (1984). Specific membrane receptors for atrial natriuretic factor in renal and vascular tissues. *Proc. natn. Acad. Sci. U.S.A.*, **81,** 5946–5950.

NEEDLEMAN, P., CURRIE, M.G., GELLER, D.M., COLE, B.R. & ADAMS, S.P. (1984). Atriopeptins: potential mediators of an endocrine relationship between heart and kidney. *Trends pharmac. Sci.*, **12,** 506–509.

NILSSON, H. & MULVANY, M.J. (1981). Prolonged exposure to ouabain eliminates the greater noradrenaline-dependent calcium sensitivity of resistance vessels in spontaneously hypertensive rats. *Hypertension*, **3,** 691–697.

NYBORG, N.C.B. (1985). Effects of calcium antagonists on resistance vessels. A dihydropyridine derivative, BAY K 8644, with agonistic properties on isolated coronary resistance vessels from WKY rats. *Adv. appl. Microcirculation*, **8,** 53–58.

NYBORG, N.C.B. & MULVANY, M.J. (1984). Effect of felodipine, a new dihydropyridine vasodilator, on contractile responses to potassium, noradrenaline, and calcium in mesenteric resistance vessels of the rat. *J. cardiovasc. Pharmac.*, **6,** 499–505.

OSHIMA, T., CURRIE, M.G., GELLER, D.M. & NEEDLEMAN, P. (1984). An atrial peptide is a potent renal vasodilator substance. *Circulation Res.*, **54,** 612–616.

PAMNANI, M.B., CLOUGH, D.L., CHEN, J.S., LINK, W.T. & HADDY, F.J. (1984). Effects of rat atrial extract on sodium transport and blood pressure in the rat. *Proc. Soc. exp. Biol. Med.*, **176,** 123–131.

SEIDMAN, C.E., DUBY, A.D., CHOI, E., GRAHAM, R., HABER, R.M., HOMCY, C., SMITH, J.A. & SEIDMAN, J.G. (1984). The structure of rat preproatrial natriuretic factor as defined by a complementary DNA clone. *Science*, **225,** 324–327.

SONNENBERG, H., CUPPLES, W.A., DE BOLD, A.J. & VERESS, A.T. (1982). Intrarenal localization of the natriuretic effect of cardiac atrial extract. *Can. J. Physiol. Pharmac.*, **60,** 1149–1152.

THIBAULT, G., GARCIA, R., CANTIN, M. & GENEST, J. (1983). Atrial natriuretic factor. Characterization and partial purification. *Hypertension*, **5,** (suppl. I), I-75–I-80.

THIBAULT, G., GARCIA, R., CANTIN, M., GENEST, J., LAZURE, C., SEIDAH, N.G. & CHRETIEN, M. (1984). Primary structure of a high M_r form of rat atrial natriuretic factor. *FEBS Letts*, **167,** 352–357.

TRIPPODO, N.C., MACPHEE, A.A. & COLE, F.E. (1983). Partially purified human and rat atrial natriuretic factor. *Hypertension*, **5** (suppl I), I-81–I-88.

WINQUIST, R.J., FAISON, E.P. & NUTT, R.F. (1984a). Vasodilator profile of synthetic atrial natriuretic factor. *Eur. J. Pharmac.*, **102,** 169–173.

WINQUIST, R.J., FAISON, E.P., WALDMAN, S.A., SCHWARTZ, K., MURAD, F. & RAPOPORT, R.M. (1984b). Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. *Proc. natn. Acad. Sci., U.S.A.*, **81,** 7661–7664.

(Received March 19, 1985.
Revised May 25, 1985.
Accepted June 6, 1985.)