British Journal of Pharmacology (1996) 118, 1790–1796

Direct chronotropic effects of atrial and C-type natriuretic peptides in anaesthetized dogs

*,†Pierre Beaulieu, *,†René Cardinal, *André De Léan & [1],*Chantal Lambert

*Department of Pharmacology, Faculty of Medicine, Université de Montréal, C.P. 6128, Succursale Centre-Ville, Montréal, Québec, Canada H3C 3J7 and †Research Centre, Hôpital du Sacré-Coeur, 5400 Boulevard Gouin Ouest, Montréal, Québec, Canada H4J 1C5

1 The chronotropic effects of atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) were investigated using injections (50 μg in 1 ml of Tyrode solution as bolus over 1 min) directly into the sinus node artery of 21 anaesthetized and vagotomized dogs which had been pretreated with a β-adrenoceptor antagonist. The injections were also repeated following: (a) α-adrenoceptor antagonism (prazosin) and muscarinic receptor antagonism (atropine); (b) inhibition of prostaglandin synthesis (indomethacin); (c) angiotensin II AT_1 receptor antagonism (losartan); (d) histamine H_1 (mepyramine) and H_2 (cimetidine) receptor antagonism.

2 The results obtained indicate that ANP had no significant effect on the basal sinus rate, whereas CNP produced a slight but significant increase of 12 ± 2 beats min^{-1}. The effect of CNP was long-lasting (return to pre-injection levels after maximum effect in 17 ± 3 min) and was not influenced by the various antagonists mentioned above.

3 During *in vitro* experiments on spontaneously beating right atria isolated from 6 dogs, the injection of CNP (50 μg in 1 ml of Tyrode solution) into the sinus node artery produced an increase in atrial rate of 14 ± 1 beats min^{-1}.

4 The results of this work indicate that CNP exerts a significant and prolonged positive chronotropic effect both *in vivo* and *in vitro*. Other studies are required to elucidate the mechanism of action of CNP on the heart conduction system, to ascertain the presence of natriuretic peptide receptor B in the region of the sinoatrial node and to determine the role of CNP in the control of heart rate.

Keywords: Atrial natriuretic peptide (ANP); C-type natriuretic peptide (CNP); chronotropic effect; sinoatrial node

Introduction

The natriuretic peptide family is made up of three distinct peptides; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). These peptides are thought to play an important role in cardiovascular homeostasis (Brown *et al.*, 1993). CNP, the most recently identified member of the family, clearly displays distinct features from ANP and BNP (Cargill *et al.*, 1994; Hunt *et al.*, 1994). Indeed, CNP which was originally considered to be primarily a neuropeptide (Sudoh *et al.*, 1990), is widely distributed within the vascular endothelium (Espiner, 1994) and may play a role in the regulation of vascular tone (Clavell *et al.*, 1993). Moreover, CNP is antinatriuretic in anaesthetized dogs (Stingo *et al.*, 1992) and the plasma concentrations of CNP are in the low picomolar concentrations. ANP and CNP bind preferentially to different receptors, namely, the natriuretic peptide receptor A (NPR-A) or guanylate cyclase-A (GC-A), and the natriuretic peptide receptor B (NPR-B) or guanylate cyclase-B (GC-B) (Suga *et al.*, 1992).

The hypotension caused by ANP is often not accompanied by the anticipated reflex tachycardia. The mechanism of this relative negative chronotropic effect is still unclear (Hiwatari *et al.*, 1986; Zeuzem *et al.*, 1990; Atchison & Ackermann, 1990; 1993; Atchison *et al.*, 1993; Imaizumi & Takeshita, 1993) but, among other possibilities, it has been suggested that ANP exerts a direct effect on the sinus node (Lambert *et al.*, 1994). On the other hand, it has been postulated that CNP has a paracrine action within the heart (Espiner, 1994). These two hypotheses are supported by the following findings in the rat: (1) nodal and transitional cells of the sinoatrial node display ANP-containing secretory granules (Cantin *et al.*, 1989); (2) the heart expresses NPR-A and NPR-B receptors (Brown *et al.*, 1993) and (3) the expression of the mRNA for CNP has been documented in atria and ventricles (Vollmar *et al.*, 1993). However, no data are available regarding the presence of natriuretic peptide receptors in the conduction system.

This study was undertaken to investigate the direct chronotropic effects of ANP and CNP on the sinus node of anaesthetized dogs in which baroreceptor-mediated responses had been totally abolished by surgical bilateral vagotomy and β-adrenoceptor blockade.

Methods

In vivo *experiments*

Animal preparation Female adult mongrel dogs ($n=21$) weighing 27 ± 1 kg were anaesthetized with sodium thiopentone (25 mg kg^{-1}, i.v.) and α-chloralose (80 mg kg^{-1}, i.v. followed by 15 mg kg^{-1} h^{-1}) and heparinized (250 iu kg^{-1}, i.v. followed by 1000 iu h^{-1}). The dogs were artificially ventilated with room air through an endotracheal tube using a Harvard pump (model 607). A right thoracotomy was performed at the fourth intercostal space, and the heart was suspended in a pericardial cradle. The sinus node artery and both femoral arteries and veins were cannulated with polyethylene catheters for drug administration, pressure measurement and blood sampling. After careful elective cannulation, the sinus node artery was perfused with fresh arterial blood withdrawn from the femoral artery using a Harvard pump (model 22) at a constant flow rate (1 ml min^{-1}). Preliminary studies have indicated that, at this rate, a stable heart frequency can be maintained for several hours, lower flow rates being associated with a progressive decline in heart rate. The

[1] Author for correspondence.

ligation of the sinus node artery had no effect on sinus node function due to extensive arterial anastomoses (James & Nadeau, 1962). Surgical bilateral cervical vagotomy was performed just before the blockade of the β-adrenoceptors with propranolol (1 mg kg^{-1}, i.v. followed by a second dose 3 h later in cases where the experiment was not finished). This dose of propranolol prevents the activation of β-adrenoceptors by isoprenaline (0.01 μg kg^{-1}, i.v.) in the dog (Lambert, 1995).

Experimental protocol Systolic and diastolic arterial blood pressures, and electrocardiogram (lead II) were monitored on a polygraph system (model RM-6000, Nihon-Kohden, Tokyo, Japan). Heart rate was derived from the electrocardiogram.

After a 30 min period of stabilization following the end of surgical preparation, Tyrode solution (1 ml) was injected (as a bolus over 1 min) into the sinus node artery. Thereafter, ANP or CNP (50 μg dissolved in 1 ml of Tyrode solution) was injected (as bolus over 1 min) into the sinus node artery, keeping the rate of perfusion constant. Each administration of natriuretic peptide was preceded (≈5 min) by an injection of Tyrode solution as a control. An interval of at least 30 min was allowed between each injection of the peptide. Preliminary results, using the same experimental model, demonstrated that under these conditions, the pressor and chronotropic effects of ANP and CNP are maintained for at least 120 min.

Following these baseline determinations, the animals were separated into 4 groups and the pressor and chronotropic effects of ANP and CNP were reassessed: (1) following the blockade of α-adrenoceptors with prazosin (1 mg kg^{-1}, i.v.) (Stanaszek *et al.*, 1983) and muscarinic receptors with atropine (1 mg kg^{-1}, i.v.) (Henning, 1992); (2) 1 h after the inhibition of cyclooxygenase with indomethacin (10 mg kg^{-1}, i.v.) (Ribuot *et al.*, 1993); (3) following the blockade of angiotensin II AT_1 receptors with losartan (50 μg kg^{-1} min^{-1} infused for 2 h) (Lambert, 1995) and finally; (4) following the blockade of histamine H_1 and H_2 receptors with mepyramine (4 mg kg^{-1}, i.v. in 20 min) and cimetidine (8 mg kg^{-1}, i.v. in 10 min), respectively.

The dose of prazosin used in this study prevents the activation of α-adrenoceptors caused by phenylephrine (1 μg kg^{-1}, i.v.) (Lambert, 1995). In order to ensure that the dose of atropine used was sufficient to block muscarinic receptors, we have evaluated the effects of acetylcholine on the heart rate of 4 dogs before and after administration of atropine (25 μg kg^{-1}, i.v.). Indeed, prior to the administration of atropine, acetylcholine caused a decrease of 8±4 beats min^{-1}, whereas it had no effect (0±0 beats min^{-1}) after atropine administration. Similarly, in order to ensure that mepyramine or cimetidine (at the doses used in this study) attenuate the tachycardia elicited by histamine, the alterations in heart rate caused by histamine, either prior to or after the administration of the respective H_1 or H_2-receptor antagonist were investigated in 4 dogs. Prior to administration of these histamine-receptor antagonists, histamine caused a significant increase in heart rate of 28±5 beats min^{-1}, which was abolished after the administration of mepyramine and cimetidine (1±0 beats min^{-1}).

Sodium nitroprusside was injected directly into the sinus node artery (50 μg kg^{-1}, as bolus over 1 min) of 4 dogs in order to evaluate the effect of a potent systemic and coronary arterial dilator on sinus node behaviour (Rowe & Henderson, 1974).

All procedures for animal experimentation were carried out in accordance with the guidelines of the Canadian Council for Animal Care and monitored by an institutional animal care committee.

In vitro *experiments*

Preparation Six adult mongrel dogs of either sex weighing 27±3 kg were anaesthetized with sodium thiopentone (25 mg kg^{-1}, i.v.) and artificially ventilated with room air. The heart was exposed through a left intercostal thoracotomy and the pericardium was opened. One minute after the injection of heparin (1000 iu in 1 ml) into the left ventricle, the heart (with at least 1 cm of each vena cava) was quickly excised and immersed in cooled Tyrode solution. Dissection was carried out according to the method described by Woods *et al.* (1976). The right atrium containing the anterior free wall of the atrium, the right atrial appendage as well as the sinus node and its artery was isolated. The sinus node artery was cannulated with Tyrode-filled polyethylene tubing size-50. This right atrial preparation was then transferred to a chamber (Radnoti, Monrovia, CA., U.S.A.) and pinned to the silastic floor, endocardial side face-up. The preparation was both superfused (20 ml min^{-1}), and perfused through the catheter with Tyrode solution circulating through a warming bath kept at 37±0.5°C and gassed with an O_2:CO_2 mixture (95:5) (pH 7.4±0.1) with a roller pump (peristaltic pump P1, Pharmacia Fine Chemicals, Sweden) at a constant flow (6 ml min^{-1}). The extracellular potentials were recorded with a bipolar electrode (silver-silver chloride rods of 0.25 mm in diameter and insulated). The electrode was connected to a bioelectric amplifier (model RMP-6004M, Nihon Kohden). Endocardial atrial electrograms were recorded on a polygraph (model WI-641G, Nihon Kohden).

Experimental protocol After 1 h period stabilization following the cannulation of the sinus node artery, Tyrode solution (1 ml) was injected over 2 min into the sinus node artery. This injection protocol was selected to minimize any disturbance of atrial rate. Then, CNP (50 μg dissolved in 1 ml of Tyrode) was injected (2 min) into the sinus node artery. After an interval of at least 30 min, this protocol was repeated so that each of the 6 dogs received 2 injections of CNP, each preceded by an injection of Tyrode solution as a control.

Drugs

ANP (human ANP 1–28) and CNP (human CNP-22) (Peninsula Laboratories, Belmont, CA, U.S.A.) were dissolved in 125 μl of water. Prazosin hydrochloride (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was dissolved in dimethylsulphoxide (DMSO): NaCl 0.9% (5:35 ml) immediately before use. Indomethacin (Sigma) was dissolved in Trizma base (0.2 M). Losartan potassium salt (DuP 753) (Du Pont Medical Products, Billerica, Ma., U.S.A.), atropine sulphate (British Drug Houses Ltd, Poole, England), propranolol hydrochloride (Sigma), mepyramine maleate (Sigma) and sodium nitroprusside (Sigma) were dissolved in NaCl 0.9% at room temperature, whereas cimetidine (Sigma) was dissolved in NaCl 0.9% at 37°C. Preliminary experiments have shown that the administration of these vehicles alone in our experimental model had no chronotropic or pressor effect, and did not influence the chronotropic and pressor effect of the natriuretic peptides. The Tyrode solution was prepared daily, oxygenated for 15 min with an O_2: CO_2 mixture (95:5) when used for *in vivo* experiments, and the pH of the solution was kept at 7.4±0.1. The composition of the Tyrode solution used in all experiments was in mmol l^{-1}: NaCl 128, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 20.1, NaH_2PO_4 0.5, $CaCl_2$ 2.3 and dextrose 11.1, all from Sigma. The solution used for control injections (1 ml) into the sinus node artery was made up of 125 μl of water and 875 μl of Tyrode solution.

Statistics

Data are expressed as mean±s.e.mean and were compared by Student's paired *t* tests or one-way (repeated measures as indicated) analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. The critical level of significance was set at $P \leqslant 0.05$.

Results

In vivo *studies*

Effects of bilateral vagotomy and blockage of β-adrenoceptors Bilateral vagotomy and blockage of β-adrenoceptors

caused a significant decrease in basal heart rate (from 135±7 to 122±4 beats min^{-1}) but had no effect on mean systemic arterial pressure (from 107±5 to 104±5 mmHg) ($P>0.05$).

Pressor and chronotropic effects of Tyrode solution The injection of Tyrode solution (1 ml) into the sinus node artery of 21 dogs had no effect on mean systemic arterial pressure. It did, however, cause a slight increase in heart rate (from 121±4 to 123±4 beats min^{-1}). The peak increase in heart rate occurred within 27±4 s after the injection and heart rate returned to pre-injection levels within 72±4 s.

Pressor effects of ANP and CNP The injections of ANP (50 μg in 1 ml of Tyrode solution, $n=16$) and CNP (50 μg in 1 ml of Tyrode solution, $n=19$) into the sinus node artery of dogs caused significant decreases in mean systemic arterial pressure (from 104±4 to 85±5 mmHg and from 108±4 to 98±4 mmHg, respectively) (Figure 1). Both peptides affected the systolic and diastolic blood pressures equally. A maximum degree of hypotension was observed 95±5 and 103±6 s after injection of ANP and CNP. The blood pressure returned to pre-injection values within 13±2 and 12±2 min.

Chronotropic effects of ANP and CNP The injection of ANP (50 μg in 1 ml of Tyrode solution, $n=16$) into the sinus node artery had no significant effect on the heart rate (from 124±5 to 125±5 beats min^{-1}) ($P>0.05$). The injection of CNP (50 μg in 1 ml of Tyrode solution, $n=19$) into the sinus node, however, produced a slight but significant increase in heart rate (from 118±4 to 130±4 beats min^{-1}) (Figure 2). The maximum increase in heart rate was observed 92±5 s after the injection. This positive chronotropic effect was long-lasting with a return to pre-injection levels 17±3 min after the observed maximum effect (Figure 3).

Effects of different specific antagonists and inhibitor on ANP and CNP chronotropic action The changes induced by the specific receptor antagonists or by indomethacin on mean systemic arterial pressure and heart rate are shown in Table 1. Prazosin, atropine and indomethacin had no significant pressor effects. Losartan induced a significant decrease in mean systemic arterial pressure, whereas cimetidine and mepyramine caused significant increases. Mepyramine caused a significant increase in heart rate of 13±1 beats min^{-1}.

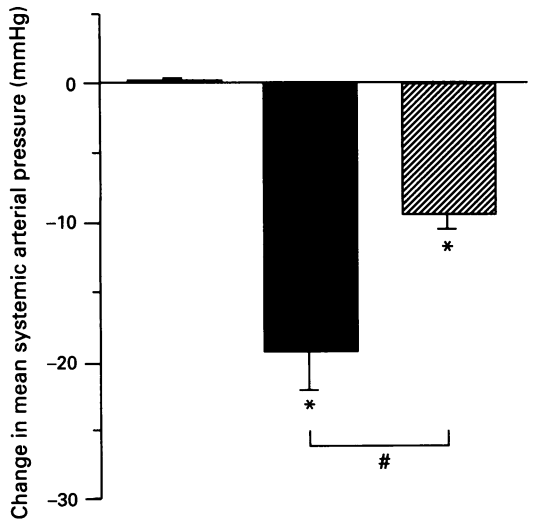

Figure 1 Effects of Tyrode solution (1 ml, $n=21$) (open column), ANP (50 μg in 1 ml of Tyrode solution, $n=16$) (solid column) and CNP (50 μg in 1 ml of Tyrode solution, $n=19$) (hatched column) injected (as bolus over 1 min) directly into the sinus node artery of anaesthetized, vagotomized dogs in the presence of a β-adrenoceptor antagonist on mean systemic arterial pressure. Data are expressed as mean±s.e.mean. *Significant differences compared with pre-injection levels, and (#) represents significant differences between ANP and CNP.

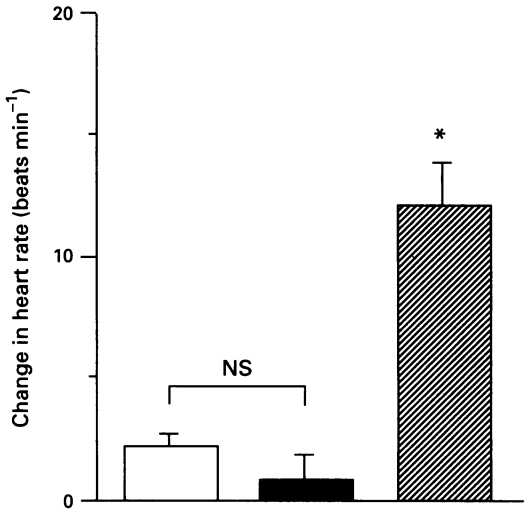

Figure 2 Effects of Tyrode solution (1 ml, $n=21$) (open column), ANP (50 μg in 1 ml of Tyrode solution, $n=16$) (solid column) and CNP (50 μg in 1 ml of Tyrode solution, $n=19$) (hatched column) injected (as bolus over 1 min) directly into the sinus node artery of anaesthetized, vagotomized dogs in the presence of a β-adrenoceptor antagonist on heart rate. Data are expressed as mean±s.e.mean. *Significant differences compared with Tyrode and ANP.

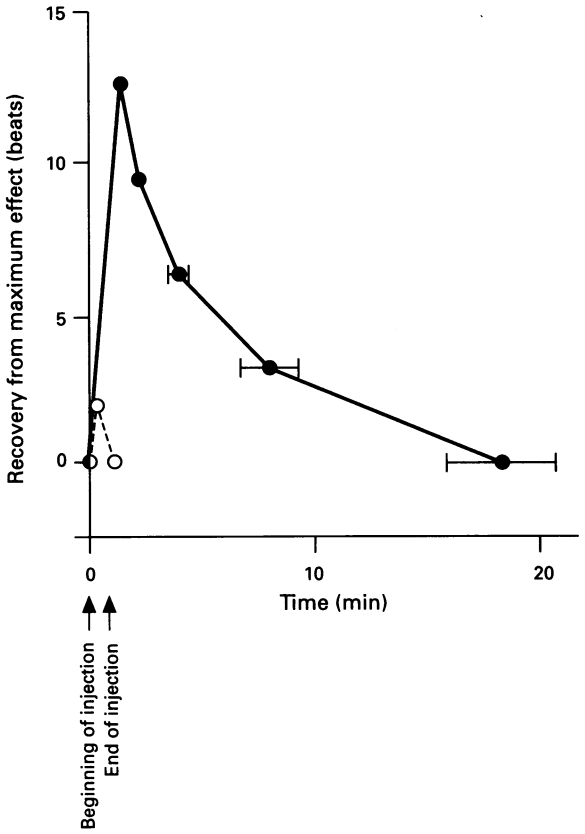

Figure 3 Times for the heart rate to decline from maximum effect to pre-injection levels following the administration of Tyrode solution (1 ml) (- - -) and CNP (50 μg in 1 ml of Tyrode solution) (——) injected (as bolus over 1 min) directly into the sinus node artery of anaesthetized, vagotomized dogs ($n=19$) in presence of a β-adrenoceptor antagonist. Data are expressed as mean±s.e.mean.

Table 1 Mean arterial blood pressure (MAP) and heart rate (HR) before and after i.v. administration of drugs in anaesthetized, vagotomized dogs in presence of a β-adrenoceptor antagonists

Drugs	*MAP* (mmHg)	*HR* (beats min^{-1})
Prazosin ($n=4$)		
before	79±5	102±10
after	65±5	102±11
Atropine ($n=4$)		
before	88±2	104±12
after	87±3	104±12
Indomethacin ($n=4$)		
before	103±8	127±7
after	111±7	128±7
Losartan ($n=4$)		
before	79±7	118±10
after	41±2*	114±9
Cimetidine ($n=5$)		
before	90±12	120±6
after	96±11*	120±6
Mepyramine ($n=5$)		
before	93±11	117±7
after	107±14*	104±6*

Values are expressed as mean±s.e.mean. *Significant differences compared with values prior to drug administration.

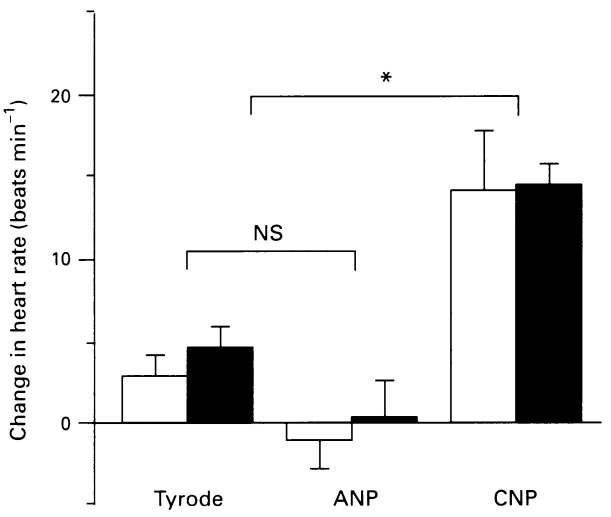

Figure 4 Effects of Tyrode solution (1 ml), ANP (50 μg in 1 ml of Tyrode solution) and CNP (50 μg in 1 ml of Tyrode solution) injected (as bolus over 1 min) directly into the sinus node artery of anaesthetized, vagotomized dogs ($n=4$) in presence of a β-adrenoceptor antagonist before (open columns) and after (solid columns) the bolus i.v. administration of prazosin (1 mg kg^{-1}) and atropine (1 mg kg^{-1}) on heart rate. Data are expressed as mean± s.e.mean. *Significant differences compared with Tyrode and ANP.

Figures 4, 5, 6 and 7 show that blockade of α-adrenoceptors or of muscarinic, angiotensin or histamine receptors or the inhibition of cyclo-oxygenase did not significantly change the chronotropic effect of ANP and CNP.

Chronotropic and pressor effects of sodium nitroprusside The injection of sodium nitroprusside directly into the sinus node artery (50 μg kg^{-1}, in 1 min) produced a marked decrease (48±2%) in mean systemic arterial pressure ($P<0.05$). This hypotension, however, resulted in only a small chronotropic effect (↑2±3 beats min^{-1}).

In vitro *studies*

The injection of Tyrode solution (1 ml, $n=6$) into the sinus node artery of isolated atrial preparation caused a very slight decrease in atrial rate (from 104±4 to 102±5 beats min^{-1}). The maximum peak changes in atrial rate occurred 50±7 s after injection, and heart rate returned to pre-injection levels within 170±18 s. The injection of CNP (50 μg in 1 ml of Tyrode solution) into the sinus node artery, however, produced a slight but significant increase in atrial rate (from 102±5 to 116±6 beats min^{-1}) (Figure 8). The peak of the chronotropic effect elicited by CNP was reached within 77±19 s and heart rate returned to baseline within 20±2 min (following the maximum effect).

Discussion

Numerous previous studies have demonstrated that the decrease in arterial blood pressure elicited by the administration of ANP is not accompanied by a reflex tachycardia (Baum *et al.*, 1986; Goetz *et al.*, 1986; Bie *et al.*, 1988; Ebert *et al.*, 1988; Lambert *et al.*, 1994). These results form the basis of a first hypothesis that ANP interferes with the baroreflex control of the circulation via the autonomic nervous system. Attenuation of sympathetic nervous activity (Sasaki *et al.*, 1986), as well as enhancement of parasympathetic nervous input to the heart (Zeuzem *et al.*, 1990; Atchison & Ackerman, 1990; Franco-Saenz *et al.*, 1992) have been postulated to account for this effect of ANP. Atchison & Ackermann (1993) have suggested that during vagus nerve stimulation, ANP acts as a physiological antagonist interfering with α_1-adrenoceptor modulation of efferent cardiac vagal action. We have already reported, using anaesthetized and vagotomized dogs pretreated with β-adrenoceptor antagonist, that an i.v. infusion of physiological and pharmacological doses of ANP produces a significant negative chronotropic effect (Lambert *et al.*, 1994). We, as well as others (Ackerman *et al.*, 1988; Gisbert & Fischmeister, 1988) have therefore suggested a second hypothesis postulating that ANP has a direct cardiac effect. Unexpectedly, in the

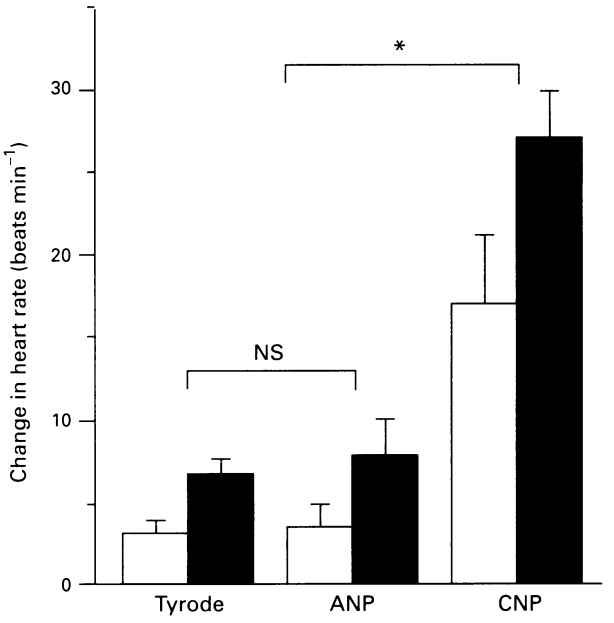

Figure 5 Effects of Tyrode solution (1 ml), ANP (50 μg in 1 ml of Tyrode solution) and CNP (50 μg in 1 ml of Tyrode solution) injected (as bolus over 1 min) directly into the sinus node artery of anaesthetized, vagotomized dogs ($n=4$) in presence of a β-adrenoceptor antagonist before (open columns) and after (solid columns) the bolus i.v. administration of indomethacin (10 mg kg^{-1}) on heart rate. Data are expressed as mean±s.e.mean. *Significant differences compared with Tyrode and ANP.

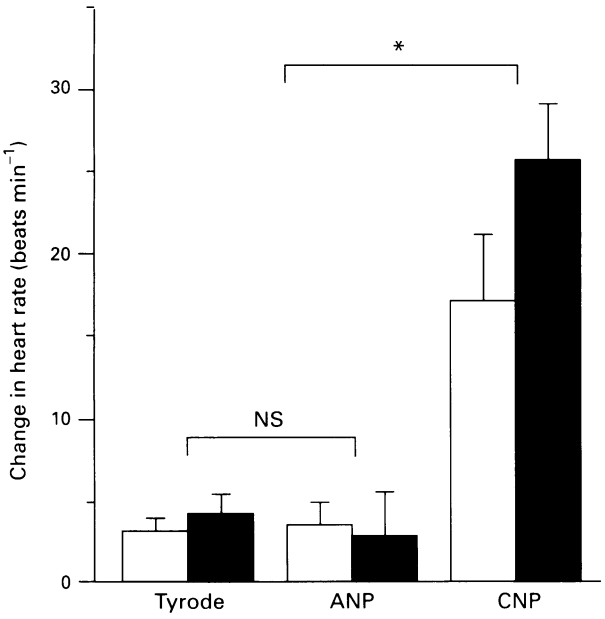

Figure 6 Effects of Tyrode solution (1 ml), ANP (50 μg in 1 ml of Tyrode solution) and CNP (50 μg in 1 ml of Tyrode solution) injected (as bolus over 1 min) directly into the sinus node artery of anaesthetized, vagotomized dogs ($n=4$) in presence of a β-adrenoceptor antagonist before (open columns) and after (solid columns) the i.v. infusion of losartan (50 μg kg^{-1} min^{-1}) for 2 h on heart rate. Data are expressed as mean $\pm$ s.e.mean. *Significant differences compared with Tyrode and ANP.

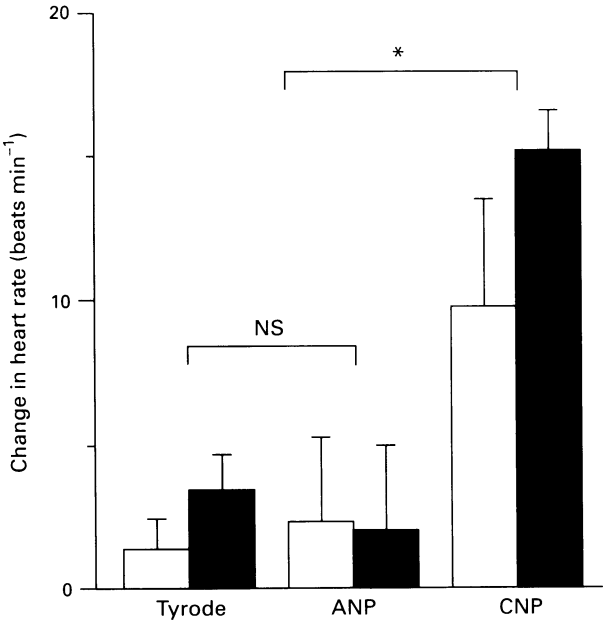

Figure 7 Effects of Tyrode solution (1 ml), ANP (50 μg in 1 ml of Tyrode solution) and CNP (50 μg in 1 ml of Tyrode solution) injected (as bolus over 1 min) directly into the sinus node artery of anaesthetized, vagotomized dogs ($n=5$) in presence of a β-adrenoceptor antagonist before (open columns) and after (solid columns) the bolus i.v. administration of mepyramine (4 mg kg^{-1}) and cimetidine (8 mg kg^{-1}) on heart rate. Data are expressed as mean $\pm$ s.e.mean. *Significant differences compared with Tyrode and ANP.

present study, ANP did not produce bradycardia. These divergent results might be explained by differences in the experimental protocol (dosages, route and mode of administration) and/or by the fact that there are no NPR-A receptors in the region of the sinus node.

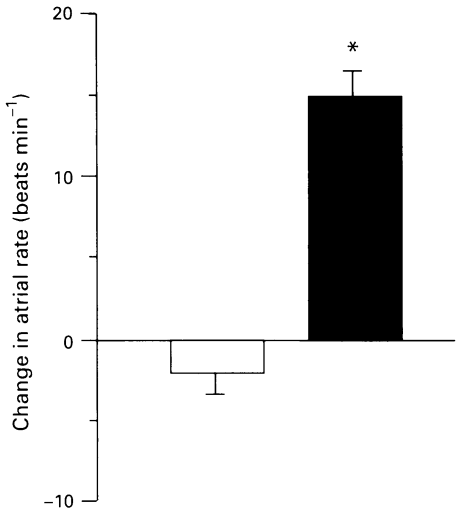

Figure 8 Effects of Tyrode solution (1 ml) (open column) and CNP (50 μg in 1 ml of Tyrode solution) (solid column) injected (as bolus over 2 min) directly into the sinus node artery of isolated atrial preparation of dogs ($n=6$) on the atrial rate. Data are expressed as mean $\pm$ s.e.mean. *Significant differences compared with Tyrode.

We have shown that the direct chronotropic effects of ANP or CNP injected into the sinus node artery anaesthetized dogs are different. CNP induces a prolonged tachycardia whereas ANP has no significant effect on heart rate. CNP was first isolated from porcine brain tissue (Sudoh *et al.*, 1990), but has also been detected in various other tissues (Chrisman *et al.*, 1993). It is widely distributed within the vascular endothelium (Heublein *et al.*, 1992; Komatsu *et al.*, 1992; Suga *et al.*, 1992), and is a potent dilator of peripheral veins and coronary arteries (Wei *et al.*, 1993; 1994). The presence of NPR-A, NPR-B and NPR-C receptor mRNAs has been detected in rat and human cardiac tissues by the technique of cDNA amplification with the polymerase chain reaction (PCR). The A and B receptor transcripts appeared to be homogenously distributed between atria and ventricles (Nunez *et al.*, 1992). CNP-specific sequences have also been detected in rat atria and ventricles of the heart, using the same technique. The relative levels of CNP-mRNA in the atria and ventricles were about 5 and 9% of the ones retrieved in the brain (Vollmar *et al.*, 1993).

Charles *et al.* (1995) have studied the effects of intracerebroventricular infusion of CNP on the neurohumoral response to acute moderate haemorrhage in conscious sheep. They have shown that this peptide induces a transient tachycardia (prior to haemorrhage) with heart rates returning to control levels within 30 min. Nevertheless, the positive chronotropic effect of CNP observed in the present study was not expected and, to our knowledge, has never been reported in the literature. The different *in vivo* protocols that were applied, aimed at demonstrating that CNP does not produce its effect via a stimulation of non-natriuretic peptide receptors or via a release of other substances which have positive chronotropic actions. It is now established that α_1-adrenoceptors participate in the regulation of cardiac rhythm (Terzic *et al.*, 1993). Prazosin, a predominant α_1-adrenoceptor antagonist, did not block the effect of CNP on heart rate demonstrating that these receptors do not mediate the chronotropic effect of CNP. Similarly, the blockade of muscarinic receptors or inhibition of the biosynthesis of prostaglandins did not modify the response to CNP in our model. Interactions between ANP and angiotensin II have been well documented (Schiffrin *et al.*, 1993), but little data are available concerning CNP and angiotensin II. Tsutsui *et al.* (1995) recently reported that angiotensin II attenuates the production of cyclic GMP induced by CNP in bovine cultured adrenal medullary cells. Further-

more, Cargill *et al.* (1994) have shown that the plasma levels of CNP were not affected by the infusion of angiotensin II, whereas the concentrations of ANP were significantly increased. In the present study, the positive chronotropic effect of CNP does not appear to depend on an interaction between CNP and angiotensin II. Finally, in man, histamine-induced tachycardia is mediated by H_2 receptor stimulation (Levi *et al.*, 1982). The situation is less clear in the dog, where both H_1 and H_2 receptors may be involved (Hageman *et al.*, 1979). Both types of receptors were blocked before the injection of CNP. The pretreatment with histamine receptor antagonists did not attenuate the chronotropic response elicited by CNP. Therefore, the tachycardia induced by CNP does not appear to be histamine-dependent, either through a direct stimulation of histamine receptors or through a release of histamine.

When administered *in vivo*, CNP dilates the canine coronary circulation (Wright *et al.*, 1993). To exclude the possibility that the increase in heart rate observed following CNP injection was a reflex tachycardia secondary to coronary vasodilatation, sodium nitroprusside was injected via the sinus node artery. Sodium nitroprusside was selected because of its mechanism of action. Like ANP and CNP, it increases cyclic GMP (Murad *et al.*, 1985). In our protocol, high doses were used which caused a very significant fall in mean arterial pressure. Despite this drastic drop, the heart rate remained practically unchanged. This strongly suggests that the tachycardia caused by CNP is not mediated by a local reflex mechanism due to coronary dilatation.

The magnitude of the tachycardia observed with CNP ($\uparrow 12 \pm 2$ beats min^{-1}) is less than that already reported for angiotensin II ($\uparrow 29 \pm 2$ beats min^{-1}) (Lambert, 1995) or for neuropeptides such as vasoactive intestinal peptide (VIP) ($\uparrow 49 \pm 3$ beats min^{-1}) and peptide histidine isoleucine (PHI) ($\uparrow 58 \pm 4$ beats min^{-1}) (Rigel, 1988) in similar dog models. The effect of CNP, however, was very long-lasting when compared with that of angiotensin II, where heart rate returned to pre-injection levels in 2–3 min (Lambert *et al.*, 1994). The prolonged action of CNP may be a characteristic feature, as suggested for other peptide neurotransmitters, producing a slowly developing and declining time-course effect in several tissues (Saito *et al.*, 1986) or may be the consequence of a sustained second-messenger stimulation. In agreement with this second possibility, Osterode *et al.* (1995) have reported in their study of the kinetics of plasma ANP and cyclic GMP following i.v. injection of 50 μg of ANP in man, that the mean elimination half-lives of immunoreactive-ANP and cyclic GMP were 5.6 ± 1.1 and 28.4 ± 3.1 min, respectively.

The fact that the positive chronotropic effect of CNP can be reproduced in *in vitro* experiments using isolated right atria suggest that this effect of CNP is due to a direct receptor stimulation, presumably of the NPR-B receptor. Moreover, the *in vitro* model demonstrates that all possible central and peripheral nervous system influences that may have been exerted following *in vivo* CNP injections can be discounted.

In summary, we have shown that CNP, but not ANP, causes a sustained slight positive chronotropic effect. This effect was not influenced either by blockade of α-adrenoceptors, muscarinic, angiotensin II AT_1 and histamine (H_1 and H_2) receptors or by blockade of prostaglandin synthesis. *In vitro* experiments testing CNP on isolated spontaneously beating atrial preparations confirmed the positive chronotropic effect observed *in vivo*. Our results suggest that CNP may play a role in the control of heart rate via stimulation of NRP-B receptors. Other studies are necessary to ascertain the presence of NPR-B receptors in the region of the sinoatrial node, to clarify the mechanism of action of CNP on the sinus node function and to evaluate the role of CNP in the regulation of heart frequency. Accordingly, receptor localisation using cDNA amplification with the PCR technique, as well as intracellular recordings of action potentials during CNP injection into the sinus node artery of isolated atrial preparation are in progress. This last issue certainly represents an interesting question especially in the light of the recent reports by Trachte *et al.* (1995) who have suggested that some actions of CNP may be guanylate cyclase-independent and by Wei *et al.* (1994) who have demonstrated that the induced relaxation of isolated coronary arteries related to CNP was caused by K^+ channel activation and membrane hyperpolarization.

The authors wish to thank Mrs Marie-France Legault for her excellent technical assistance and Mrs Elisabeth Pérès for the artwork. The losartan was kindly supplied by Dr Ronald D. Smith from Du Pont Merck Pharmaceutical Company. This work was supported by grants to C.L. and R.C. from the Medical Research Council of Canada. P.B. holds a scholarship from Unimédia and the Faculté des études supérieures of the Faculté de Médecine, Université de Montréal.

References

ACKERMANN, U., KHANNA, J. & IRIZAWA, T. (1988). Atrial natriuretic factor alters autonomic interactions in the control of heart rate in conscious rats. *Can. J. Physiol. Pharmacol.*, **66**, 930–936.

ATCHISON, D.J. & ACKERMANN, U. (1990). Influence of atrial natriuretic factor on autonomic control of heart rate. *Am. J. Physiol.*, **258**, R718–R723.

ATCHISON, D.J. & ACKERMANN, U. (1993). The interaction between atrial natriuretic peptide and cardiac parasympathetic function. *J. Autonom. Nerv. Syst.*, **42**, 81–88.

ATCHISON, D.J., PENNEFATHER, P.S. & ACKERMANN, U. (1993). ANP has no postsynaptic effect on autonomic regulation of cardiac pacemaker rate in the rat. *Am. J. Physiol.*, **265**, H1983–1987.

BAUM, T., SYBERTZ, E.J., WATKINS, R.W., NELSON, S., COLEMAN, W., PULA, K.K., PRIOLI, N., RIVELLI, M. & GROSSMAN, A. (1986). Hemodynamic actions of a synthetic atrial natriuretic factor. *J. Cardiovasc. Pharmacol.*, **8**, 898–905.

BIE, P., WANG, B.C., LEADLEY, Jr, R.J. & GOETZ, K.L. (1988). Hemodynamic and renal effects of low-dose infusions of atrial peptide in awake dogs. *Am. J. Physiol.*, **254**, R161–R169.

BROWN, L.A., NUNEZ, D.J.R. & WILKINS, M.R. (1993). Differential regulation of natriuretic peptide receptor messenger RNAs during the development of cardiac hypertrophy in the rat. *J. Clin. Invest.*, **92**, 2702–2712.

CANTIN, M., THIBAULT, G., HAILE-MESKEL, H., DING, J., MILNE, R.W., BALLAK, M., CHARBONNEAU, C., NEMER, M., DROUIN, J., GARCIA, R. & GENEST, J. (1989). Atrial natriuretic factor in the impulse-conduction system of rat cardiac ventricles. *Cell Tissue Res.*, **256**, 309–325.

CARGILL, R.I., COUTIE, W.J. & LIPWORTH, B.J. (1994). The effects of angiotensin II on circulating levels of natriuretic peptides. *Br. J. Clin. Pharmacol.*, **38**, 139–142.

CHARLES, C.J., ESPINER, E.A., RICHARDS, A.M. & DONALD, R.A. (1995). Central C-type natriuretic peptide augments the hormone response to hemorrhage in conscious sheep. *Peptides*, **16**, 129–132.

CHRISMAN, T.D., SCHULZ, S., POTTER, L.R. & GARBERS, D.L. (1993). Seminal plasma factors that cause large elevations in cellular cyclic GMP are C-type natriuretic peptides. *J. Biol. Chem.*, **268**, 3698–3703.

CLAVELL, A.L., STINGO, A.J., WEI, C-M., HEUBLEIN, D.M. & BURNETT, JR, J.C. (1993). C-type natriuretic peptide: a selective cardiovascular peptide. *Am. J. Physiol.*, **264**, R290–R295.

EBERT, T.J. & COWLEY, JR, A.W. (1988). Atrial natriuretic factor attenuates carotid baroreflex-mediated cardioacceleration in humans. *Am. J. Physiol.*, **254**, R590–R594.

ESPINER, E.A. (1994). Physiology of natriuretic peptides. *J. Intern. Med.*, **235**, 527–541.

FRANCO-SAENZ, R., SOMANI, P. & MULROW, P.J. (1992). Effect of atrial natriuretic peptide (8-33-Met ANP) in patients with hypertension. *Am. J. Hypertens.*, **5**, 266–275.

GISBERT, M-P. & FISCHMEISTER, R. (1988). Atrial natriuretic factor regulates the calcium current in frog isolated cardiac cells. *Circ. Res.*, **62**, 660–667.

GOETZ, K.L., WANG, B.C., GEER, P.G., SUNDET, W.D. & NEEDLEMAN, P. (1986). Effects of atriopeptin infusion versus effects of left atrial stretch in awake dogs. *Am. J. Physiol.*, **250**, R221–R226.

HAGEMAN, G.R., URTHALER, F., ISOBE, J.H. & JAMES, T.N. (1979). Chronotropic and dromotropic effects of histamine on the canine heart. *Chest*, **75**, 597–604.

HENNING, R.J. (1992). Vagal stimulation during muscarinic and β-adrenergic blockade increases atrial contractility and heart rate. *J. Autonom. Nerv. Syst.*, **40**, 121–130.

HEUBLEIN, D., CLAVELL, A., STINGO, A., LERMAN, A., WOLD, L. & BURNETT, Jr, J.C. (1992). C-type natriuretic peptide immunoreactivity in human breast vascular endothelial cells. *Peptides*, **13**, 1017–1019.

HIWATARI, M., SATOH, K., ANGUS, J.A. & JOHNSTON, C.I. (1986). No effect of atrial natriuretic factor on cardiac rate, force and transmitter release. *Clin. Exp. Pharmacol. Physiol.*, **13**, 163–168.

HUNT, P.J., RICHARDS, A.M., ESPINER, E.A., NICHOLLS, M.G. & YANDLE, T.G. (1994). Bioactivity and metabolism of C-type natriuretic peptide in normal man. *J. Clin. Endocrinol. Metab.*, **78**, 1428–1435.

IMAIZUMI, T. & TAKESHITA, A. (1993). Influence of ANP on sympathetic nerve activity and chronotropic regulation of the heart. *J. Cardiovasc. Electrophysiol.*, **4**, 719–729.

JAMES, T.N. & NADEAU, R.A. (1962). Direct perfusion of the sinus node: an experimental model for pharmacologic and electrophysiologic studies of the heart. *Henry Ford Hosp. Med. Bull.*, **10**, 21–25.

KOMATSU, Y., NAKAO, K., ITOH, H., SUGA, S., OGAWA, Y. & IMURA, H. (1992). Vascular natriuretic peptide. *Lancet*, **340**, 622.

LAMBERT, C. (1995). Mechanisms of angiotensin II chronotropic effect in anaesthetized dogs. *Br. J. Pharmacol.*, **115**, 795–800.

LAMBERT, C., RIBUOT, C., ROBICHAUD, A. & CUSSON, J.R. (1994). Negative chronotropic effect of the atrial natriuretic peptide in an anaesthetized dog model. *Eur. J. Pharmacol.*, **252**, 245–252.

LEVI, R., OWEN, D.A.A. & TRZECIAKOWSKI, J. (1982). Actions of histamine on the heart and vasculature. In *Pharmacology of Histamine Receptors*. ed. Ganellin, C.R. & Parsons, M.E. pp. 236–297. Bristol: Wright. PSG.

MURAD, F., RAPOPORT, R.M. & FISCUS, R. (1985). Role of cyclic-GMP in relaxations of vascular smooth muscle. *J. Cardiovasc. Pharmacol.*, **7**, S111–S118.

NUNEZ, D.J.R., DICKSON, M.C. & BROWN, M.J. (1992). Natriuretic peptide receptor mRNAs in the rat and human heart. *J. Clin. Invest.*, **90**, 1966–1971.

OSTERODE, W., NOWOTNY, P., VIERHAPPER, H. & WALDHAUSL, W. (1995). Kinetics of plasma cyclic GMP and atrial natriuretic peptide after intravenous, intramuscular and subcutaneous injection of 50 μg hANP in man. *Horm. Metab. Res.*, **27**, 100–103.

RIBUOT, C., GODIN, D., COUTURE, R., REGOLI, D. & NADEAU, R. (1993). In vivo B_2-receptor-mediated negative chronotropic effect of bradykinin in canine sinus node. *Am. J. Physiol.*, **265**, H876–H879.

RIGEL, D.F. (1988). Effects of neuropeptides on heart rate in dogs: comparison of VIP, PHI, NPY, CGRP, and NT. *Am. J. Physiol.*, **255**, H311–H317.

ROWE, G.G. & HENDERSON, R.H. (1974). Systemic and coronary hemodynamic effects of sodium nitroprusside. *Am. Heart J.*, **87**, 83–87.

SAITO, A., KIMURA, S. & GOTO, K. (1986). Calcitonin gene-related peptide as potential neurotransmitter in guinea pig right atrium. *Am. J. Physiol.*, **250**, H693–H698.

SASAKI, A., KIDA, O., KANGAWA, K., MATSUO, J. & TANAKA, K. (1986). Involvement of sympathetic nerves in cardiosuppressive effects of α-human atrial natriuretic polypeptide (α-hANP) in anaesthetized rats. *Eur. J. Pharmacol.*, **120**, 345–349.

SCHIFFRIN, E.L., GARCIA, R. & KONRAD, E.M. (1993). Interactions of angiotensin II and atrial natriuretic peptide. In *Cellular and Molecular Biology of the Renin-Angiotensin System*. ed. Raizada, M.K., Phillips, M.I. & Sumners, C. pp. 433–468. Boca Raton: CRC Press.

STANASZEK, W.F., KELLERMAN, D., BROGDEN, R.N. & ROMANKIEWICZ, J.A. (1983). Prazosin update: a review of its pharmacological properties and therapeutic use in hypertension and heart failure. *Drugs*, **25**, 339–384.

STINGO, A.J., CLAVELL, A.L., AARHUS, L.L. & BURNETT, Jr, J.C. (1992). Cardiovascular and renal actions of C-type natriuretic peptide. *Am. J. Physiol.*, **262**, H308–H312.

SUDOH, T., MINAMINO, N., KANGAWA, K. & MATSUO, H. (1990). C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. *Biochem. Biophys. Res. Commun.*, **168**, 863–870.

SUGA, S-I., NAKAO, K., HOSODA, K., MUKOYAMA, M., OGAWA, Y., SHIRAKAMI, G., ARAI, H., SAITO, Y., KAMBAYASHI, Y., INOUYE, K. & IMURA, H. (1992). Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology*, **130**, 229–239.

TERZIC, A., PUCÉAT, M., VASSORT, G. & VOGEL, S.M. (1993). Cardiac α_1-adrenoceptors: an overview. *Pharmacol. Rev.*, **45**, 147–175.

TRACHTE, G.J., KANWAL, S., ELMQUIST, B.J. & ZIEGLER, R.J. (1995). C-type natriuretic peptide neuromodulates via 'clearance' receptors. *Am. J. Physiol.*, **268**, C978–C984.

TSUTSUI, M., YANAGIHARA, N., MINAMI, K., KOBAYASHI, H., NAKASHIMA, Y., KUROIWA, A. & IZUMI, F. (1994). C-type natriuretic peptide stimulates catecholamine synthesis through the accumulation of cyclic GMP in cultured bovine adrenal medullary cells. *J. Pharmacol. Exp. Ther.*, **268**, 584–589.

VOLLMAR, A.M., GERBES, A.L., NEMER, M. & SCHULZ, R. (1993). Detection of C-type natriuretic peptide (CNP) transcript in the rat heart and immune organs. *Endocrinology*, **132**, 1872–1874.

WEI, C-M., AARHUS, L.L., MILLER, V.M. & BURNETT, Jr, J.C. (1993). Action of C-type natriuretic peptide in isolated canine arteries and veins. *Am. J. Physiol.*, **264**, H71–H73.

WEI, C-M., HU, S., MILLER, V.M. & BURNETT, Jr, J.C. (1994). Vascular actions of C-type natriuretic peptide in isolated porcine coronary arteries and coronary vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **205**, 765–771.

WOODS, W.T., URTHALER, F. & JAMES, T.N. (1976). Spontaneous action potentials of cells in the canine sinus node. *Circ. Res.*, **39**, 76–82.

WRIGHT, R.S., MILLER, W.L., AARHUS, L.L. & BURNETT, Jr, J.C. (1993). The differential actions of C-type natriuretic peptide and atrial natriuretic peptide on the canine coronary circulation. *Am. J. Hypertens.*, **6**, 16A.

ZEUZEM, S., OLBRICH, H.G., HAAK, T. & JUNGMANN, E. (1990). In vivo evidence that human atrial natriuretic factor-(99-126) (hANF) stimulates parasympathetic activity in man. *Eur. J. Clin. Pharmacol.*, **39**, 77–79.

(Received November 13, 1995
Revised April 10, 1996
Accepted April 12, 1996)