Natriuretic peptide-induced cyclic GMP accumulation in adult guinea-pig cerebellar slices

¹Félix Hernández, ²Stephen P.H. Alexander & David A. Kendall

Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 Second messenger responses to natriuretic peptides were studied in guinea-pig cerebellar slices by use of radioactive precursors.

2 The rank order of potency of the different natriuretic peptides in generating [³H]-guanosine 3':5'-cyclic monophosphate (cyclic GMP) was atrial natriuretic peptide (ANP) > brain natriuretic peptide (BNP) >> C-type natriuretic peptide (CNP) with EC₅₀ values of 19.5 ± 8.8 nM for ANP and 169 ± 41 nM for BNP. CNP induced [³H]-cyclic GMP accumulation only at concentrations greater than 1 µM.

3 An additive response to ANP (1 µM) was observed in the presence of the adenosine receptor agonist, 5'-N-ethylcarboxamido-adenosine (NECA, 10 µM) or the soluble guanylyl cyclase activator, sodium nitroprusside (SNP, 100 µM) for [³H]-cyclic GMP accumulation.

4 ANP, BNP and CNP (all at 1 µM) failed to alter significantly either basal-, forskolin- (10 µM), isoprenaline- (100 µM), or NECA- (10 µM) induced [³H]-cyclic AMP generation. Natriuretic peptides also did not change the [³H]-cyclic AMP steady-state reached after 10 min of treatment with 10 µM forskolin.

5 Natriuretic peptides failed to elicit significant accumulation of [³H]-inositol phosphates at concentrations up to 10 µM.

6 These data are consistent with the presence of ANP_A, rather than ANP_B or clearance receptors (C-receptors), linked to second messenger cascades in guinea-pig cerebellar slices.

Keywords: ANP_A receptor; cyclic GMP; guinea-pig cerebellum; natriuretic peptides

Introduction

The natriuretic peptide family are considered to play a major role in cardiovascular homeostasis. However, the role of the natriuretic peptides in the central nervous system is comparatively poorly understood. The presence of natriuretic peptides in the brain has been clearly demonstrated by immunological and molecular biological approaches, e.g. atrial natriuretic peptide (ANP)-like immunoreactivity has been reported in different brain areas (Morii *et al.*, 1985; Quirion *et al.*, 1986; Quirion, 1988) and ANP mRNA transcripts have been detected in the brain (Gardner *et al.*, 1987). ANP can be released, at least in hypothalamic slices, in a Ca²⁺-dependent manner (Shibasaki *et al.*, 1986). Brain natriuretic peptide (BNP), the second member of the natriuretic peptide family, first isolated from porcine brain (Sudoh *et al.*, 1988), exhibits a high diversity between species in both its amino acid sequence (Ogawa *et al.*, 1990; Suga *et al.*, 1992) and sites of synthesis (Saito *et al.*, 1989; Kojima *et al.*, 1990; Ogawa *et al.*, 1990). C-type natriuretic peptide (CNP), the last member of the natriuretic peptide family discovered (Sudoh *et al.*, 1990), is found almost exclusively in the brain (Ueda *et al.*, 1991) and CNP mRNA also seems to be restricted to brain (Kojima *et al.*, 1990).

Molecular cloning studies have identified three natriuretic peptide receptor subtypes (for reviews see Maack, 1992; Garbers, 1992; Levin, 1993). ANP_A (or GC-A) and ANP_B (GC-B) are monomeric receptors with constitutive guanylyl cyclase activity. At ANP_A receptors, ANP is the most potent for displacing [¹²⁵I]-ANP binding, followed by BNP, while CNP is very much less potent. In contrast, CNP appears to

be a specific ligand for ANP_B receptors (Koller *et al.*, 1991). The third member of the natriuretic peptide family, called the clearance receptor (C-receptor), is dimeric in nature with an unclear physiological role other than the clearance of circulating natriuretic peptides (Levin, 1993). Any signal transduction system associated with this receptor has yet to be clearly demonstrated. [¹²⁵I]-ANP binding to the C-receptor is most potently inhibited by ANP, while BNP and CNP appear to be equipotent (Maack, 1992). In rat brain, mRNA for both guanylyl cyclase receptors has been detected (Talerico-Melnik *et al.*, 1992), although there is evidence for binding of natriuretic peptides to sites resembling ANP_A and C-receptors, but not ANP_B receptors (Brown & Zuo, 1993). However, a high degree of variability exists between species. Thus, neither binding of ANP (Quirion & Delpé, 1988) nor an increase in guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Takayanagi *et al.*, 1986) could be observed in rat cerebellum, while a high density of binding sites was apparent in guinea-pig cerebellum (Quirion *et al.*, 1986).

It has been proposed that natriuretic peptides can signal through transduction systems other than cyclic GMP generation. Thus, a decrease in adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels has been observed either through an inhibition of adenylyl cyclase activity (Pandey *et al.*, 1985; Resink *et al.*, 1988; Anand-Srivastava *et al.*, 1990; Tseng *et al.*, 1990) or through activation of cyclic GMP-stimulated phosphodiesterases (MacFarland *et al.*, 1991). Activation of phosphoinositide turnover has been reported in vascular smooth muscle cells (Resink *et al.*, 1988; Hirata *et al.*, 1989) and in inner medullary collecting tubule cells (Berl *et al.*, 1991).

The purpose of this study was to use guinea-pig cerebellum, an area which exhibits high density of [¹²⁵I]-ANP binding sites (Quirion & Delpé, 1988), to investigate functional receptors to natriuretic peptides using second messenger cascades as an assay for receptor function.

¹ Present Address: Departamento de Biología Molecular, Centro de Biología Molecular 'Severo Ochoa' (CSIC-UAM), Universidad Autónoma de Madrid, E-28049 Madrid, Spain.

² Author for correspondence.

Methods

Tissue preparation and cyclic nucleotides accumulation

Preparation and incubation of slices were essentially the same as described previously (Hernández *et al.*, 1993). Briefly, cross-chopped cerebellar slices ($350 \times 350 \mu\text{m}$) were prepared from guinea-pigs (Dunkin-Hartley, either sex, weighing 200–300 g) and incubated in a shaking water bath for 60 min at 37°C in Krebs-bicarbonate medium (mM): NaCl 118, KCl 4.7, MgSO_4 1.2, KH_2PO_4 1.2, glucose 11.7, NaHCO_3 25, CaCl_2 1.2, equilibrated with 95% O_2 :5% CO_2 to pH 7.4. The slices were then suspended in fresh Krebs medium and either [^3H]-guanine or [^3H]-adenine was added to a final concentration of 74 kBq ml^{-1} . After an additional 60 min of incubation, the slices were washed and aliquots ($25 \mu\text{l}$ —ca. 1 mg protein) transferred into flat-bottomed plastic vials containing Krebs buffer to give a final volume of $300 \mu\text{l}$. Slices were allowed to equilibrate for 15 min prior to addition of agents in $10 \mu\text{l}$; the tubes were resealed under 95% O_2 :5% CO_2 after each addition. Unless otherwise stated, peptidase inhibitors were not included in the assays of natriuretic peptide receptor function. Incubations were terminated at the indicated times by addition of $200 \mu\text{l}$ of HCl (1 M), followed by $750 \mu\text{l}$ of ice-cold water. [^3H]-cyclic GMP and [^3H]-cyclic AMP were extracted by the Dowex-alumina method of Salomon *et al.* (1974), monitoring recovery of cyclic nucleotides through the addition of [^{14}C]-cyclic AMP and [^{14}C]-cyclic GMP (ca. 30 Bq/tube) to the samples. Data are expressed as d.p.m. per $25 \mu\text{l}$ slices.

Inositol phosphates accumulation

Slices were equilibrated with Krebs-bicarbonate buffer for 60 min and distributed as $25 \mu\text{l}$ aliquots into flat-bottomed vials in the presence of [^3H]-inositol (40 kBq ml^{-1}) and LiCl (5 mM) to a final volume of $300 \mu\text{l}$, as previously described (Alexander *et al.*, 1989). After 40 min, histamine or natriuretic peptides was added in $10 \mu\text{l}$ aliquots and the incubation continued for 45 min, before termination with $100 \mu\text{l}$ of 7.5% perchloric acid. After neutralization, [^3H]-inositol phosphates were resolved by chromatography on Dowex-1 (chloride form) columns. Data are expressed as d.p.m. per $25 \mu\text{l}$ slices.

Chemicals

[8- ^3H]-guanine ($324 \text{ GBq mmol}^{-1}$) was purchased from Rotem Industries Ltd., Beer-Sheva, Israel. [8- ^3H]-adenine ($962 \text{ GBq mmol}^{-1}$) and [adenine- ^{14}C]-adenosine 3',5'-cyclic phosphate ($11.4 \text{ GBq mmol}^{-1}$) were obtained from Amersham International, plc, UK. [8- ^{14}C]-guanosine 3':5'-cyclic phosphate ($1.9 \text{ GBq mmol}^{-1}$) was from Moravsek Biochemicals Inc., California, U.S.A. [^3H]-inositol ($455.1 \text{ GBq mmol}^{-1}$) was purchased from DuPont NEN, 5'-N-ethylcarboxamido-adenosine (NECA) was from RBI SEMAT, Herts. 3-Isobutyl-1-methylxanthine (IBMX), sodium nitroprusside (SNP), thiorphan, histamine, synthetic rat ANP (A2288), BNP (B9901) and CNP (N8768) were all from Sigma. All other compounds were from standard commercial sources. The natriuretic peptides were dissolved to $300 \mu\text{M}$ in purified water (Waters Milli-Q system) and stored as aliquots at -20°C for up to 2 weeks.

Calculations and statistical analysis

In the text, values represent means $\pm$ s.e.mean of n experiments conducted in triplicate. The results were analysed by Student's unpaired t test to determine the significant difference between means. The computer programme InPlot (GraphPad, California, U.S.A.) was used to generate EC_{50} values from concentration-response data.

Results

Effects of natriuretic peptides on [^3H]-cyclic GMP accumulation

Incubation of [^3H]-guanine-prelabelled adult guinea-pig cerebellar slices in the presence of ANP ($1 \mu\text{M}$) led to an increase in [^3H]-cyclic GMP levels. In the absence of IBMX, a significant stimulation of [^3H]-cyclic GMP accumulation in response to $1 \mu\text{M}$ ANP was observed within 2 min, and remained elevated for at least 20 min (Figure 1). In the presence of the phosphodiesterase inhibitor, IBMX (0.5 mM), the response to ANP was much larger and reached an elevated steady-state level within 15 min. Concentration-response curves for natriuretic peptide-stimulated [^3H]-cyclic GMP production at 5 min in the presence of 0.5 mM IBMX are shown in Figure 2. ANP and BNP increased [^3H]-cyclic GMP production in a concentration-dependent manner with EC_{50} values of $19.5 \pm 8.8 \text{ nM}$ ($n = 5$) and $169 \pm 41 \text{ nM}$ ($n = 3$), respectively. At $1 \mu\text{M}$, [^3H]-cyclic GMP levels were 273 ± 15.5 ($n = 7$, $P < 0.01$) and 208 ± 10 ($n = 3$, $P < 0.01$) % over the control levels, respectively. CNP significantly stimulated [^3H]-cyclic GMP production only at concentrations greater than $10 \mu\text{M}$ in the presence of 0.5 mM IBMX (at $10 \mu\text{M}$, $80 \pm 10\%$ over control, $n = 3$, $P < 0.05$).

Preliminary experiments suggested that the natriuretic peptides were not subject to endopeptidase 24-11-mediated hydrolysis during the incubation period, since thiorphan ($10 \mu\text{M}$) failed to alter significantly the potency of either ANP or BNP to generate [^3H]-cyclic GMP when added 5 min prior to a 5 min incubation with the natriuretic peptides (data not shown).

We have recently described the cyclic GMP response elicited by activation of the soluble guanylyl cyclase activity in guinea-pig cerebellar slices by sodium nitroprusside (Hernández *et al.*, 1994), and so we compared the time course of cyclic GMP responses elicited by soluble and particulate guanylyl cyclases. Although the magnitude of the cyclic GMP response to SNP was greater than the ANP response, the two agents elicited similar time course profiles when assayed in the presence of 0.5 mM IBMX (Figure 3). When responses to ANP (Figure 1) and SNP (Hernández *et al.*, 1994) are compared in the absence of IBMX, however, it is apparent that the response to ANP is smaller, but appears to

Figure 1 Time course profiles for atrial natriuretic peptide (ANP)-stimulated [^3H]-cyclic GMP accumulation in the presence or absence of 3-isobutyl-1-methylxanthine (IBMX). [^3H]-guanine prelabelled slices were preincubated in the presence (□, ■) or in the absence (○, ●) of 0.5 mM IBMX for 5 min, prior to addition of $1 \mu\text{M}$ ANP (filled symbols) or vehicle (open symbols) for varying incubation periods. Data are means $\pm$ s.e.mean of triplicate determinations from a single experiment repeated on two additional occasions with essentially identical results. In this and in subsequent figures, where error bars are not shown, they are smaller than the symbol used. Basal [^3H]-cyclic GMP accumulation was $1135 \pm 41 \text{ d.p.m. per } 25 \mu\text{l}$ aliquot.

be better maintained. The response to a combination of $1\ \mu\text{M}$ ANP and $100\ \mu\text{M}$ SNP appeared to be additive when assayed in the presence of $0.5\ \text{mM}$ IBMX (Figure 3), implying compartmentalisation of soluble and particulate guanylyl cyclases in the guinea-pig cerebellum.

Adenosine and related analogues acting through A_{2b} adenosine receptors are able to potentiate the cyclic GMP response to SNP in guinea-pig cerebellar slices (Hernández *et al.*, 1993). In the absence of IBMX (since the phosphodiesterase inhibitor is a potent adenosine receptor antagonist, Smellie *et al.*, 1979), NECA elicited an increase in [^3H]-cyclic GMP levels (Figure 4), as previously noted (Hernández *et al.*, 1994). However, NECA failed to potentiate the [^3H]-cyclic GMP accumulation elicited by ANP (Figure 4).

Effects of natriuretic peptides on cyclic AMP generation

We examined the potential interaction of natriuretic peptides with cyclic AMP generating systems using two approaches. Firstly, we investigated whether simultaneous application of natriuretic peptides with different adenylyl cyclase activators led to a modified cyclic AMP response. ANP, BNP and CNP (all at $1\ \mu\text{M}$) failed to alter significantly either basal-, forskolin- ($10\ \mu\text{M}$), isoprenaline- ($100\ \mu\text{M}$) or NECA- ($10\ \mu\text{M}$) induced [^3H]-cyclic AMP accumulation (data not shown). Secondly, we studied the effects of natriuretic peptides on elevated levels of [^3H]-cyclic AMP. Forskolin ($10\ \mu\text{M}$) stimulated an increase in [^3H]-cyclic AMP levels with a maximal response at about 10 min, stable for up to 30 min. This maintained response was unchanged by the addition of ANP, BNP, or CNP (all at $1\ \mu\text{M}$, for example at 10 min, responses were 104 ± 8 , 105 ± 5 and $107 \pm 7\%$ control, respectively, $n = 3$).

Effects of natriuretic peptides on phosphoinositide turnover

Since ANP has been observed to stimulate phosphoinositide turnover in vascular smooth muscle cells (Resink *et al.*, 1988; Hirata *et al.*, 1989) and in inner medullary collecting tubule cells (Berl *et al.*, 1991), we investigated whether this response might also be elicited in the guinea-pig cerebellum. All three natriuretic peptides, in the presence of $5\ \text{mM}$ LiCl, failed to alter significantly basal levels of [^3H]-inositol phosphates accumulation over the range of concentrations tested (0.01

nM to $10\ \mu\text{M}$). For example, at $1\ \mu\text{M}$ the response to ANP, BNP and CNP was 89 ± 19 , 82 ± 22 , and $122 \pm 15\%$ control, respectively ($n = 3$). In contrast, $1\ \text{mM}$ histamine elicited a large accumulation of [^3H]-inositol phosphates from basal levels of $969 \pm 100\ \text{d.p.m.}$ to $5678 \pm 650\ \text{d.p.m.}$ per $25\ \mu\text{l}$ aliquot.

Discussion

Cyclic GMP generation in guinea-pig cerebellum by natriuretic peptides is mediated by ANP_A receptors

In this paper, we present evidence that natriuretic peptides elicit increases in cyclic GMP accumulation in guinea-pig

Figure 3 Time course profiles for atrial natriuretic peptide (ANP)- and sodium nitroprusside (SNP)-stimulated [^3H]-cyclic GMP accumulation in the presence of 3-isobutyl-1-methylxanthine (IBMX). [^3H]-guanine prelabelled slices were preincubated in the presence of $0.5\ \text{mM}$ IBMX for 10 min, prior to addition of $1\ \mu\text{M}$ ANP ($\square$), $100\ \mu\text{M}$ SNP (Δ), $1\ \mu\text{M}$ ANP plus $100\ \mu\text{M}$ SNP ($\bullet$) or vehicle ($\diamond$) for varying incubation periods. Data are means $\pm$ s.e. mean of triplicate determinations from a single experiment repeated on two additional occasions with essentially identical results.

Figure 2 Concentration-response curves for stimulation of [^3H]-cyclic GMP induced by atrial natriuretic peptide ($\square$), brain natriuretic peptide (Δ) or C-type natriuretic peptide ($\circ$). [^3H]-guanine prelabelled slices were preincubated in the presence of $0.5\ \text{mM}$ 3-isobutyl-1-methylxanthine (IBMX), for 5 min, prior to addition of natriuretic peptides. The incubations were continued for an additional 5 min. Basal [^3H]-cyclic GMP levels in the absence and presence of $0.5\ \text{mM}$ IBMX were 2120 ± 164 and $8137 \pm 213\ \text{d.p.m.}$, respectively. Data are means $\pm$ s.e. mean of triplicate determinations from a single experiment repeated on two additional occasions with essentially identical results.

Figure 4 Time course profiles for atrial natriuretic peptide (ANP)-stimulated [^3H]-cyclic GMP accumulation in the presence or absence of 5'-N-ethylcarboxamidoadenosine (NECA). [^3H]-guanine prelabelled slices were preincubated in the presence ($\square$, $\blacksquare$) or in the absence ($\circ$, $\bullet$) of $10\ \mu\text{M}$ NECA for 10 min, prior to addition of $1\ \mu\text{M}$ ANP (filled symbols) or vehicle (open symbols) for varying incubation periods. Data are means $\pm$ s.e. mean of triplicate determinations from a single experiment repeated on two additional occasions with essentially identical results.

cerebellar slices, without any contemporary change in cyclic AMP accumulation or phosphoinositide turnover. The rank order of agonist potency (ANP > BNP >> CNP) is indicative of the involvement of ANP_A receptors in this response (Schulz *et al.*, 1989). However, the divergence of BNP molecular weight between species, together with the variable affinity of BNP from different species, means that some caution must be exercised when viewing data regarding natriuretic peptide potency (Ogawa *et al.*, 1990; Suga *et al.*, 1992). In an homologous assay system (natriuretic peptides and receptors of the same species), the ratio of EC₅₀ values (EC₅₀ of rat-ANP/EC₅₀ of rat-BNP) for cyclic GMP production through ANP_A receptors in PC12 cells is 0.17 (Suga *et al.*, 1992). In our experimental conditions (a heterologous system comprising synthetic rat natriuretic peptides and guinea-pig receptors), the ratio of EC₅₀ values is 0.12. Taking into account the lack of effect of CNP in the present study, therefore, it is likely that ANP increases cyclic GMP through an ANP_A receptor.

Other natriuretic peptide receptors and signal transduction mechanisms

We find no evidence for the suggested routes of coupling of the C-receptor (inhibition of cyclic AMP and stimulation of phosphoinositide turnover – see Introduction), implying either the absence of C-receptors in guinea-pig cerebellum or, more likely, that the C-receptors are not coupled to these signal transduction mechanisms in this tissue. The lack of effect of CNP at concentrations up to 1 µM on cyclic GMP generation suggests that ANP_B receptors do not exist or are not functionally active in guinea-pig cerebellum. Similar results might be expected if the peptides were subject to peptidase activity or removal from the biophase by the C-receptor. The former eventuality appears unlikely since there was no effect of thiorphan (an inhibitor of endopeptidase 24–11, Trapani *et al.*, 1989) on natriuretic peptide-induced cyclic GMP generation. Similarly the latter can be effectively dismissed since the C-receptor exhibits higher affinity for ANP than for CNP (Suga *et al.*, 1992), and thus would be expected to influence the responses to both peptides. ANP_B receptors have been detected in brain microvessel endothelial cells, but not in aortic endothelial cells, which express ANP_A receptors (Vigne & Frelin, 1992). The possible role of CNP (apparently a brain-restricted peptide, Kojima *et al.*, 1990; Komatsu *et al.*, 1991) in the cerebellum and whether CNP interacts with the cerebral microvasculature require further investigation.

Although there is evidence for ANP and/or the C-receptor coupling to phosphoinositide turnover in other systems (see Introduction), we were unable to observe an increase in [³H]-inositol phosphates induced by ANP, BNP or CNP in the guinea-pig cerebellum, although 1 mM histamine genera-

ted a substantial increase in phosphoinositide turnover (ca. 6 fold). We were also unable to find evidence for coupling of natriuretic peptide receptors to modulation of cyclic AMP generation in guinea-pig cerebellum. This indicates that ANP- (and SNP-, Hernández *et al.*, 1994) induced cyclic GMP elevations do not activate type II (cyclic GMP-stimulated cyclic AMP) or type III (cyclic GMP-inhibited cyclic AMP) phosphodiesterase activity, and therefore one potential route of ANP modulation of cyclic AMP accumulation in guinea-pig cerebellum can be disregarded (cf. MacFarland *et al.*, 1991).

Cellular localizations of particulate and soluble guanylyl cyclases

The particulate (ANP_A receptor) and soluble (nitric oxide-stimulated) guanylyl cyclase activities are likely to be in distinct cellular populations, since accumulations of [³H]-GMP were additive in the presence of both these agents. The particulate guanylyl cyclase activity and A_{2b} adenosine receptor also appear to be in distinct cellular populations, since NECA failed to enhance ANP-evoked cyclic GMP elevations. In contrast, the NECA-induced elevation of SNP-evoked cyclic GMP accumulation suggests co-localization of these two entities to some extent (Hernández *et al.*, 1993). ANP seems to be generally associated with cerebral structures involved in fluid and cardiovascular control (Quirion *et al.*, 1986; Brown & Czarnecki, 1990), but the physiological relevance of ANP in the cerebellum is less obvious. ANP binding sites are present in guinea-pig cerebellum, but not in the rat (Quirion & Dalpé, 1988) and the potential consequences of this variability are at present unknown, as is the physiological role of natriuretic peptide-induced cyclic GMP generation.

Concluding remarks

In summary, we have observed functional receptors for natriuretic peptides in guinea-pig cerebellum, linked to cyclic GMP formation through ANP_A receptors. No activation through ANP_B receptors could be detected. In addition, we have not been able to furnish evidence for other transduction pathways (cyclic AMP or phosphoinositide systems) for natriuretic peptides in this tissue. Cyclic GMP induced by nitric oxide through activation of soluble guanylyl cyclase has been implicated in the phenomenon of long-term depression in the cerebellum (Shibuki & Okada, 1991) and the question of whether a similar role exists for the particulate guanylyl cyclases requires further investigation.

We are grateful for the financial support of the Medical Research Council and the Wellcome Trust.

References

- ALEXANDER, S.P.H., KENDALL, D.A. & HILL, S.J. (1989). Differences in the adenosine receptor modulating inositol phosphates and cyclic AMP accumulation in mammalian cerebral cortex. *Br. J. Pharmacol.*, **98**, 1241–1248.
- ANAND-SRIVASTAVA, M.B., SAIRAM, M.R. & CANTIN, M. (1990). Ring-deleted analogs of atrial natriuretic factor inhibit adenylate cyclase/cAMP system. *J. Biol. Chem.*, **265**, 8566–8572.
- BERL, T., MANSOUR, J. & TEITELBAUM, I. (1991). ANP stimulates phospholipase C in cultured RIMCT cells: roles of protein kinases and G protein. *Am. J. Physiol.*, **260**, F590–F595.
- BROWN, J. & CZARNECKI, A. (1990). Autoradiographic localisation of atrial and brain natriuretic peptide receptors in rat brain. *Am. J. Physiol.*, **258**, R57–R63.
- BROWN, J. & ZUO, Z. (1993). C-type natriuretic peptide and atrial natriuretic peptide receptors of the rat brain. *Am. J. Physiol.*, **264**, R513–R523.
- GARBERS, D.L. (1992). Guanylyl cyclase receptors and their endocrine, paracrine, and autocrine ligands. *Cell*, **71**, 1–4.
- GARDNER, D.G., VLASUK, G.P., BAXTER, J.D., FIDDES, J.C. & LEWICKI, J.A. (1987). Identification of atrial natriuretic factor gene transcripts in the central nervous system of the rat. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 2175–2179.
- HERNÁNDEZ, F., ALEXANDER, S.P.H. & KENDALL, D.A. (1994). Forskolin potentiates the effects of sodium nitroprusside on cyclic GMP levels in adult guinea-pig cerebellar slices. *J. Neurochem.*, **62**, 2212–2218.
- HERNÁNDEZ, F., KENDALL, D.A. & ALEXANDER, S.P.H. (1993). Adenosine receptor-induced second messenger production in adult guinea-pig cerebellum. *Br. J. Pharmacol.*, **110**, 1085–1090.
- HIRATA, M., CHANG, C.-H. & MURAD, F. (1989). Stimulatory effects of atrial natriuretic factor on phosphoinositide hydrolysis in cultured aortic smooth muscle cells. *Biochim. Biophys. Acta*, **1010**, 346–351.

- KOJIMA, M., MINAMINO, N., KANGAWA, K. & MATSUO, H. (1990). Cloning and sequence analysis of a cDNA encoding a precursor for rat C-type natriuretic peptide (CNP). *FEBS Lett.*, **276**, 209–213.
- KOLLER, K.J., LOWE, D.G., BENNET, G.L., MINAMINO, N., KANGAWA, K., MATSUO, H. & GOEDDEL, D.V. (1991). Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science*, **252**, 120–123.
- KOMATSU, T., NAKAO, K., SUGA, S., OGAWA, Y., MUKOYAMA, M., ARAI, H., SHIRAKAMI, G., HOSODA, K., NAKAGAWA, O., HAMA, N., KISHIMOTO, I. & IMURA, H. (1991). C-type natriuretic peptide (CNP) in rats and humans. *Endocrinology*, **129**, 1104–1106.
- LEVIN, E.R. (1993). Natriuretic peptide C-receptor: more than a clearance receptor. *Am. J. Physiol.*, **264**, E483–E489.
- MAACK, T. (1992). Receptors of atrial natriuretic factor. *Annu. Rev. Physiol.*, **54**, 11–27.
- MACFARLAND, R.T., ZELUS, B.D. & BEAVO, J.A. (1991). High concentrations of a cGMP-stimulated phosphodiesterase mediate ANP-induced decreases in cAMP and steroidogenesis in adrenal glomerulosa cells. *J. Biol. Chem.*, **266**, 136–142.
- MORII, N., NAKAO, K., SUGAWARA, A., SAKAMOTO, M., SUGA, M., SHIMOKURA, M., KISO, Y., KIHARA, M., YAMORI, Y. & IMURA, H. (1985). Occurrence of atrial natriuretic polypeptide in brain. *Biochem. Biophys. Res. Commun.*, **127**, 413–419.
- OGAWA, Y., NAKAO, K., MUKOYAMA, M., SHIRAKAMI, G., ITOH, H., HOSODA, K., YOSHIHIKO, S., ARAI, H., SUGA, S.-I., JOUGASAKI, M., YAMADA, T., KAMBAYASHI, Y., INOUE, K. & IMURA, H. (1990). Rat brain natriuretic peptide, tissue distribution and molecular form. *Endocrinology*, **126**, 2225–2227.
- PANDEY, K.N., KOVACS, W.J. & INAGAMI, T. (1985). The inhibition of progesterone secretion and the regulation of cyclic nucleotides by atrial natriuretic factor in gonadotropin responsive murine Leydig tumor cells. *Biochem. Biophys. Res. Commun.*, **133**, 800–806.
- QUIRION, R. (1988). Atrial natriuretic factor and the brain: an update. *Trends Neurosci.*, **11**, 58–62.
- QUIRION, R. & DALPÉ, M. (1988). Characterization, distribution, and plasticity of atrial natriuretic factor binding sites in brain. *Can. J. Physiol. Pharmacol.*, **66**, 280–287.
- QUIRION, R., DALPÉ, M., DAM, T.V. & DAM, T.V. (1986). Characterization and distribution of receptors for the atrial natriuretic peptides in mammalian brain. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 174–178.
- RESINK, T.J., SCOTT-BURDEN, T., BAUR, U., JONES, C.R. & BÜHLER, F.R. (1988). Atrial natriuretic peptides induce breakdown of phosphatidylinositol phosphates in cultured vascular smooth-muscle cells. *Eur. J. Biochem.*, **172**, 499–505.
- SAITO, Y., NAKAO, K., ITOH, H., YAMADA, T., MUKOYAMA, M., ARAI, H., HOSODA, K., SHIRAKAMI, G., SUGA, S.-I., MINAMINO, N., KANGAWA, K., MATSUO, H. & IMURA, H. (1989). Brain natriuretic peptide is a novel cardiac hormone. *Biochem. Biophys. Res. Commun.*, **158**, 360–368.
- SALOMON, Y., LONDOS, C. & RODBELL, M. (1974). A highly sensitive adenylate cyclase assay. *Anal. Biochem.*, **58**, 541–548.
- SCHULZ, S., SINGH, S., BELLET, R.A., SINGH, G., TUBB, D.J., CHIN, H. & GARBERS, D.L. (1989). The primary structure of a plasma membrane guanylate cyclase demonstrates diversity within this new receptor family. *Cell*, **58**, 1155–1162.
- SHIBASAKI, T., NARUSE, M., NARUSE, K., MASUDA, A., KIM, Y.S., IMAKI, T., YAMAUCHI, N., DEMURA, H., INAGAMI, T. & SHIZUME, K. (1986). Atrial natriuretic is released from rat hypothalamus in vitro. *Biochem. Biophys. Res. Commun.*, **136**, 590–595.
- SHIBUKI, K. & OKADA, D. (1991). Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. *Nature*, **349**, 326–328.
- SMELLIE, F.W., DAVIS, C.W., DALY, J.W. & WELLS, J.N. (1979). Alkylxanthines: inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. *Life Sci.*, **24**, 2475–2482.
- SUDOH, T., KANGAWA, K., MINAMINO, N. & MATSUO, H. (1988). A new natriuretic peptide in porcine brain. *Nature*, **332**, 78–81.
- 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., INOUE, K. & IMURA, H. (1992). Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinol.*, **130**, 229–239.
- TAKAYANAGI, R., GRAMMER, R.T. & INAGAMI, T. (1986). Regional increase of cyclic GMP by atrial natriuretic factor in rat brain: markedly elevated response in spontaneously hypertensive rats. *Life Sci.*, **39**, 573–580.
- TALLERICO-MELNYK, T., YIP, C.C. & WATT, V.M. (1992). Widespread co-localization of mRNAs encoding the guanylate cyclase-coupled natriuretic peptide receptors in rat tissues. *Biochem. Biophys. Res. Commun.*, **189**, 610–616.
- TRAPANI, A.J., SMITS, G.J., MACGRAW, D.E., SPEAR, K.L., KOEPKE, J.P., OLINS, G.M. & BLAINE, E.H. (1989). Thiorphan, an inhibitor of endopeptidase 24.11, potentiates the natriuretic activity of atrial natriuretic peptide. *J. Cardiovasc. Pharmacol.*, **14**, 419–424.
- TSENG, Y.-C.L., LAHIRI, S., SELITI, D.F., BURMAN, K.D., D'AVIS, J.C. & WARTOFISKY, L. (1990). Characterization by affinity cross-linking of a receptor for atrial natriuretic peptide in cultured human thyroid cells associated with reductions in both adenosine 3',5'-monophosphate production and thyroglobulin secretion. *J. Clin. Endocrinol. Metab.*, **70**, 528–533.
- UEDA, S., MINAMINO, N., ABURAYA, M., KANGAWA, K., MATSUKURA, S. & MATSUO, H. (1991). Distribution and characterization of immunoreactive porcine C-type natriuretic peptide. *Biochem. Biophys. Res. Commun.*, **175**, 759–767.
- VIGNE, P. & FRELIN, C. (1992). C-type natriuretic peptide is a potent activator of guanylate cyclase in endothelial cells from brain microvessels. *Biochem. Biophys. Res. Commun.*, **183**, 640–644.

(Received February 3, 1994

Revised May 9, 1994

Accepted May 16, 1994)