Biochemical Pharmacology, Vol. 55, pp. 1–7, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00183-4

COMMENTARY

Receptors for Natriuretic Peptides in Adrenal Chromaffin Cells

Hideyuki Kobayashi, Hiromi Niina, Ryuichi Yamamoto and Akihiko Wada*
DEPARTMENT OF PHARMACOLOGY, MIYAZAKI MEDICAL COLLEGE, MIYAZAKI 889-16, JAPAN

ABSTRACT. Atrial, brain, and C-type natriuretic peptides of the atrial natriuretic peptide family are present in adrenal chromaffin cells, and are secreted with catecholamines by exocytosis. These peptides modulate the physiological functions of the cells such as synthesis and secretion of catecholamines in an autocrine manner interacting with natriuretic peptide receptors. BIOCHEM PHARMACOL 55;1:1–7, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. natriuretic peptides; receptor; cGMP; adrenal medulla; chromaffin cells; catecholamine

After the discovery of ANP† (also called ANF) from cardiac atrium [1–3], its related peptides, BNP [4] and CNP [5], were isolated. The wide distributions of these ANP family peptides and of three distinct types of receptors [6, 7] with different affinities for these peptides led to the concept that they play regulatory roles in a variety of physiological and pathological situations.

Adrenal chromaffin cells are endocrine cells embryologically derived from the neural crest, which synthesize and secrete catecholamines and peptides such as the ANP family, enkephalins, and neuropeptide Y [8–10] in catecholamine-containing chromaffin granules. Acetylcholine, a physiological secretagogue liberated from splanchnic nerve terminals, evokes secretion of soluble constituents of chromaffin granules, including these peptides and catecholamines, by a Ca^{2+}-dependent exocytosis. Several lines of evidence have demonstrated the existence of receptors for the natriuretic peptides in adrenal chromaffin cells of various species, suggesting that these peptides secreted from adrenal medulla regulate chromaffin cell functions in an autocrine manner. In this review, we focus on the receptors for natriuretic peptides in relation to their physiological roles in adrenal chromaffin cells.

NATRIURETIC PEPTIDES

ANP, BNP, and CNP in humans consist of 28, 32, and 22 amino acid residues, respectively [7, 11, 12]. They share a common 17 amino acid ring structure formed by a disulfide linkage between two cysteine residues, in which the composition of the amino acids is well conserved among the peptides (11/17 in humans), whereas they differ considerably in the amino- and carboxy-termini. CNP ends at the second cysteine residue with no C-terminal tail [5]. The subsequently discovered N-terminally extended CNP is composed of 53 amino acid residues (CNP-53), and has pharmacological potency similar to that of CNP [13]. The amino acid sequences of ANP and CNP, but not BNP, are highly conserved across species. These peptides are coded in different genes, and are transcribed/translated as precursor (immature) peptides that are proteolytically processed to mature peptides [7, 11, 12].

ANP and BNP are highly concentrated in cardiac atrium and ventricle, respectively, although BNP was identified initially in brain [4]. The concentration of CNP in brain is much higher than those of ANP or BNP, suggesting that CNP may act as a neurotransmitter or neuromodulator [8]. CNP is also produced in vascular endothelial cells [14] and macrophages [15].

The major actions of the natriuretic peptides are diuresis, natriuresis, vasodilatation, and inhibition of aldosterone secretion [6, 10–12]. The potencies of CNP in causing natriuresis and hypotension are much weaker than those of ANP and BNP; however, its relaxing activity on chicken rectal muscle is 3–4 times more potent than that of ANP [5]. The characteristic distributions of these peptides and their receptors, as well as their own pharmacological actions, suggest that each member of the ANP family has distinct functions in various tissues and situations [8].

* Corresponding author: Hideyuki Kobayashi, Ph.D., Department of Pharmacology, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki 889-16, Japan. Tel. (81) (985) 85-1786; FAX (81) (985) 84-2776.

† *Abbreviations:* ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; cAMP, cyclic AMP; cGMP, cyclic GMP; ANPR-A, ANP receptor type A; ANPR-B, ANP receptor type B; ANPR-C, ANP receptor type C; NPR-A, natriuretic peptide A receptor; NPR-B, natriuretic peptide B receptor; GC-A, guanylyl cyclase-A receptor; GC-B, guanylyl cyclase-B receptor; ANF, atrial natriuretic factor; ANF-R_{1A}, ANF receptor subtype 1A; ANF-R_{1C}, ANF receptor subtype 1C; ANF-R_2, ANF receptor subtype 2; K_d, dissociation constant; B_{max}, maximum binding; and DOPA, 3,4-dihydroxyphenylalanine.

CLASSIFICATION OF NATRIURETIC PEPTIDE RECEPTORS

The biological activities of the natriuretic peptides are mediated by cell membrane receptors with a single transmembrane domain [6, 7, 16, 17]. Three types of receptors have been cloned: (1) ANPR-A (also called NPR-A; GC-A; ANF-R_{1A}) containing a cytoplasmic guanylyl cyclase domain that is activated by ANP more potently than BNP, but not by CNP, (2) ANPR-B (NPR-B; GC-B; ANF-R_{1C}) containing a cytoplasmic guanylyl cyclase domain that is fairly selectively activated by CNP, but not by ANP or BNP, (3) ANPR-C (initially called a clearance receptor; ANF-R_2) lacking a guanylyl cyclase domain, which interacts with these three peptides with similar affinities. C-ANF, an analogue of ANP, is a selective agonist for this type of receptor [18]. The clearance receptor was named because of its role in the removal of ANP from the circulation [18], but it may also mediate signal transduction through inhibition of adenylate cyclase [19], activation of phospholipase C [20] or inhibition of mitogen-activated protein kinase [21]. However, cloned human ANPR-C expressed in host cells has failed to modulate any signal transduction pathway [7].

In various tissues, ANPR-A and ANPR-B are single polypeptides of 120–140 kDa, whereas ANPR-C is a disulfide-linked homodimer consisting of 60–70 kDa subunits [6, 7]. It is not clear whether higher molecular forms (140–180 kDa) of ANPR-A are heavily glycosylated ANPR-A or other subtypes unidentified thus far [22].

EXISTENCE OF THE ANP FAMILY IN CHROMAFFIN CELLS

In addition to the mature natriuretic peptides, their precursors are also stored in chromaffin cells [8, 23–27]. The concentrations of the precursors are considerably high, in some cases higher than those of mature peptides [26], suggesting that these peptides are synthesized and processed to mature ones in adrenal chromaffin cells. ANP is the most abundant natriuretic peptide among the ANP family in adrenal chromaffin cells [8].

The contents of ANP, BNP, and CNP in chromaffin cells are differentially regulated by cAMP-dependent protein kinase and protein kinase C. Combined treatment with phorbol ester, an activator of protein kinase C, and forskolin, an activator of adenylate cyclase resulting in the activation of cAMP-dependent protein kinase, increases the levels of these natriuretic peptides to different extents [27–29].

In adrenal medulla, two distinct types of chromaffin cells have been identified: adrenergic cells and noradrenergic cells. Morel *et al.* [25] found in the rat that ANP distributes unevenly in chromaffin cells [25]. The *in situ* hybridization studies indicated the localization of ANP mRNA in about 15% of adrenal chromaffin cells, which was proportional to the population of noradrenergic cells. ANP-like immunoreactivity, on the other hand, was identified in both noradrenergic and adrenergic cell populations. The immunoreactivity was localized almost exclusively in secretory vesicles of the noradrenergic cells, whereas it was widely distributed in the adrenergic cells among plasma membranes and intracellular components. Adrenergic cells also contained immunoreactive ANP in secretory vesicles, but relatively smaller amounts compared with noradrenergic cells. These results suggest that ANP may be synthesized primarily in noradrenergic cells, whereas adrenergic cells primarily bind and internalize the extracellular ANP into cells in rats [25]. However, such differential expression of natriuretic peptides has not been reported thus far in the adrenal medulla of other species.

DISTRIBUTION OF NATRIURETIC PEPTIDE RECEPTORS IN CHROMAFFIN CELLS

As shown in Table 1, the existence of receptors for natriuretic peptides in chromaffin cells was first shown by *in vivo* autoradiography [30]. ^{125}I-ANP, when injected into rats, was localized in noradrenergic as well as adrenergic cells. The number of binding sites of ^{125}I-ANP, however, was much smaller in adrenal medulla than in adrenal cortex. Detailed study of ^{125}I-ANP distribution after its intravenous injection by Morel *et al.* [25] showed a 3-fold preferential and specific radiolabeling of adrenergic cells, compared with noradrenergic cells. In adrenergic cells, plasma membranes as well as intracellular components were labeled, suggesting that extracellular ^{125}I-ANP could be internalized into the cells. The intracellular localization of ^{125}I-ANP seems to be well correlated with the intracellular distribution of guanylate cyclase reaction product stimulated by ANP or BNP from a GTP analogue, guanosine 5′-β, γ-iminotriphosphate [31]; the reaction product was detected only in adrenergic cells but not noradrenergic cells, suggesting that ANPR-A may locate only in adrenergic cells in rat adrenal medulla.

A high density of ^{125}I-ANP binding sites was found, by *in vitro* autoradiography, in adrenal cortex of various species; however, those in adrenal medulla were detected initially only in the guinea pig [32] and tree shrew [33], but not in the rat, mouse, hamster, rhesus monkey, cow, or human [32, 34–36]. In the tree shrew, ^{125}I-ANP binding sites were much more abundant in adrenal medulla than in adrenal cortex [33], suggesting that there is a large species difference in the relative density of the natriuretic peptide receptors between adrenal medulla and cortex. The binding sites may be difficult to identify by *in vitro* autoradiography in tissues such as adrenal medulla, where the density of ANP binding sites may be low, and the endogenous natriuretic peptides are abundant. The subsequent experiments by Konrad *et al.* [37, 38], however, showed the presence of ^{125}I-ANP, ^{125}I-BNP and ^{125}I-[Tyr^0]-CNP binding sites in adrenal medulla of the rat, the species previously shown to have no ANP receptors [32, 34, 36]. In the rat, the numbers of ^{125}I-BNP and ^{125}I-[Tyr^0]-CNP binding sites were quite

TABLE 1. Existence of receptors for natriuretic peptides in adrenal chromaffin cells and pheochromocytoma

Methods	Comments*	Species	Reference
^{125}I-ANP *in vivo* autoradiography	+	Rat	30
	+	Rat	25
	A cell > NA cell		
^{125}I-ANP *in vitro* autoradiography	+	Bovine	50
	Cultured cells		
	+	Guinea pig	32
	−	Mouse, hamster, rhesus monkey, cow, rat	
	−	Rat	34
	−	Guinea pig, rat, human	35
	+	Tree shrew	33
^{125}I-BNP *in vitro* autoradiography	+	Rat	37
^{125}I-ANP *in vitro* autoradiography	+	Rat	38
^{125}I-[Tyr^{0}]-CNP *in vitro* autoradiography	+		
Guanylate cyclase product stimulated by ANP or BNP	+	Rat	31
	Only in A cells		
In situ hybridization		Rhesus monkey	39
mRNA for ANPR-A	+		
ANPR-B	+		
ANPR-C	−		
(ANPR-C)	(+)	(Endothelial cells)	
^{125}I-ANP binding	+	Bovine, membrane fraction	40
^{125}I-ANP binding	+	Bovine, isolated cells	41
and chemical cross-linking	+	120 kDa	
cGMP production by ANP	+	Bovine, cultured cells	42, 44, 45
BNP	+	(CNP ≫ BNP > ANP)	
CNP	+		
^{125}I-ANP binding	+	Human, pheochromocytoma	54
and chemical cross-linking	+	140 kDa in non-reducing conditions	
	+	70 kDa in reducing conditions	
cGMP production by ANP	+		
cGMP production by ANP	+	Rat, PC12 cells	42
CNP	+	(ANP > CNP)	
^{125}I-ANP binding	+	Rat, PC12 cells	55
^{125}I-ANP chemical cross-linking	+	Rat, PC12 cells	56
		130 kDa, 70 kDa in reducing conditions	

* Key: +, present; −, not detected; A, adrenergic; and NA, noradrenergic.

abundant, with the latter binding sites being much more ample in adrenal medulla than in adrenal cortex. The ^{125}I-ANP and ^{125}I-BNP bindings were displaced by ANP and BNP, but not by ANP (106–113)-NH_2, an analogue selective for ANPR-C, unless the analogue was employed at extremely high concentrations. These results suggest that there exist ANPR-A and ANPR-B, but few, if any, ANPR-C in rat adrenal medulla [38].

The distribution of the binding sites seems to be well correlated to those of transcripts of ANP receptor genes. Wilcox *et al.* [39] showed by an *in situ* hybridization technique that mRNAs for ANPR-A and ANPR-B were present in adrenal chromaffin cells of the rhesus monkey. ANPR-C mRNA was localized to discrete and infrequent groupings of cells, suggesting that this mRNA is located in capillary endothelial cells rather than adrenal chromaffin cells.

Heisler and Morrier [40] first demonstrated the existence of ^{125}I-ANP binding sites in the membrane fraction of bovine adrenal medulla. They consisted of a single class of high-affinity sites with a K_d of 94 pM, were saturable with a B_{max} of 1.7 pmol/mg of membrane protein, and were displaced by unlabeled ANP.

We have also shown the existence of ANP binding sites in freshly isolated bovine adrenal chromaffin cells [41]. ^{125}I-ANP specifically bound to the cells with high affinity (K_d of 103 pM) and in a saturable manner (B_{max} of 16.4 fmol/mg cell protein). C-ANF, an agonist for ANPR-C, and CNP did not compete for ^{125}I-ANP binding even at concentrations up to 10 nM. Chemical cross-linking of ^{125}I-ANP to the cells and a subsequent SDS gel electrophoresis in reducing conditions showed a single band at the position of 120 kDa, but not of 60–70 kDa, a molecular size corresponding to the subunits derived from the ho-

TABLE 2. Functions of natriuretic peptides in bovine adrenal chromaffin cells

Response	Effect*	Natriuretic peptides	cGMP	Reference
CA secretion by low concentrations of nicotine (3 μM)	+	ANP	Dependent	52
Acetylcholine (10 μM) induced membrane current	−	ANP	?	50
Nicotinic (10 μM) current	−	CNP, C-ANF	Independent	43
CA secretion by nicotine (10 μM)	−			
CA secretion by histamine (100 μM)	=			
CA secretion by KCl (56 mM)	=			
$[Ca^{2+}]_i$ increase and CA secretion by acetylcholine (50 μM)	−	CNP	Dependent	51
$[Ca^{2+}]_i$ increase and CA secretion by acetylcholine (10 μM)	=	CNP		
$[Ca^{2+}]_i$ increase and CA secretion by KCl (30 mM)	−	CNP	Dependent	
$[Ca^{2+}]_i$ increase and CA secretion by KCl (18 mM)	=	CNP		
TH activation	+	ANP (>1 μM)	Dependent	44
	+	BNP(>1 μM)		
CA synthesis	+	CNP (>100 nM)	Dependent	45
	=	C-ANF		

Abbreviations: CA, catecholamine; and TH, tyrosine hydroxylase.
* Key: −, decreases; =, no change; and +, increases.

modimeric disulfide-linked ANPR-C. These results suggest that the receptors recognized by ^{125}I-ANP in bovine adrenal chromaffin cells are classified as ANPR-A [41].

CNP increased cellular levels of cGMP much more potently than ANP [42, 43], suggesting the existence of ANPR-B in bovine chromaffin cells; however, we have not detected the binding sites for ^{125}I-[Tyr^0]-CNP in freshly isolated or cultured bovine adrenal chromaffin cells (unpublished data).

FUNCTIONS OF NATRIURETIC PEPTIDES IN ADRENAL CHROMAFFIN CELLS

The natriuretic peptides are secreted from chromaffin cells in parallel with catecholamines by stimulation of cholinergic receptors or depolarization of the cells by high concentrations of potassium [26–29]. Therefore, all of the natriuretic peptides secreted may modulate functions of chromaffin cells in an autocrine manner. CNP synthesized and secreted from vascular endothelial cells [14] may also act on chromaffin cells.

The effects of exogenously added natriuretic peptides on synthesis, secretion, and uptake of catecholamines in adrenal chromaffin cells are summarized in Table 2. In cultured bovine adrenal chromaffin cells, the natriuretic peptides increased cGMP with the rank order of potency of CNP ≫ BNP > ANP [42, 44, 45]. These peptides also stimulated catecholamine synthesis through cGMP-dependent activation of tyrosine hydroxylase, an enzyme catalyzing the conversion of tyrosine to DOPA, the rate-limiting step in catecholamine synthesis [46]. ANP and BNP increased phosphorylation of tyrosine hydroxylase molecules in the cells [44], a condition known to raise the activity of tyrosine hydroxylase [47]. CNP stimulated catecholamine synthesis from tyrosine but not from DOPA, suggesting that the stimulation of catecholamine synthesis by either natriuretic peptide is due to the activation of tyrosine hydroxylase [45]. C-ANF failed to stimulate the synthesis of catecholamines, suggesting that the stimulation of catecholamine synthesis by the natriuretic peptides is mediated by ANPR-A and ANPR-B but not by ANPR-C [45].

In adrenal medulla, acetylcholine acts on the nicotinic receptor-ion channel complex, increases influx of Na^+, and gates voltage-dependent Ca^{2+} channels [48, 49], thereby triggering catecholamine secretion. In bovine chromaffin cells, Bormann *et al.* [50] first demonstrated by the patch-clamp technique that simultaneous addition of ANP at high concentrations (>1 μM) inhibited acetylcholine (10 μM)-induced membrane currents in a concentration-dependent manner with an IC_{50} value of 5.2 μM and a Hill coefficient of about 1. ANP did not affect GABA-activated whole-cell current significantly over a wide range of membrane voltages, suggesting that ANP blocks specifically the acetylcholine receptor channels.

Babinski *et al.* [43] have shown that CNP and C-ANF, a selective agonist for ANPR-C, equipotently inhibited DMPP (1,1-dimethyl-4-phenylpiperazinium, a nicotinic receptor agonist)-induced whole-cell currents, and catecholamine secretion caused by nicotine, but not by histamine or KCl (56 mM) in bovine chromaffin cells. The inhibitory effect of CNP and C-ANF on catecholamine secretion was

observed at concentrations (≥10 pM) five orders of magnitude lower than those (>1 μM) reported for ANP [50]. Maximal inhibition (~40%) occurred rapidly, generally within 1 min after peptide administration. The linearized form of C-ANF that was produced by the cleavage of its disulfide-bridge structure had lower affinity for ANPR-C and a weaker inhibitory effect on nicotinic current than did C-ANF. CNP produced its maximal inhibition of nicotine-induced secretion at 0.1 to 1 nM, a concentration range that is far below those able to increase cGMP levels. In addition, 8-bromo-cGMP, a membrane permeable analogue of cGMP, failed to exert an inhibitory effect on nicotine-induced catecholamine secretion. These results suggest that CNP and C-ANF inhibit catecholamine secretion by interfering with nicotinic-receptor mediated events in a cGMP-independent mechanism mediated by ANPR-C.

The inhibition of catecholamine secretion from bovine chromaffin cells by CNP was also reported by Rodrigues-Pascual *et al.* [51] via a mechanism different from that reported by Babinski *et al.* [43]. CNP inhibited a rise in the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$) and catecholamine secretion evoked by either acetylcholine (50 μM), or KCl (30 mM), but not by histamine. Although it is unclear why CNP failed to exert inhibitory effects against a low concentration of acetylcholine (10 μM), these results suggest that CNP interfered with Ca^{2+} influx via voltage-dependent Ca^{2+} channels. In these experiments, the inhibitory effects of CNP developed gradually, and the maximal inhibition of both responses due to either secretagogue occurred only after a 30- to 60-min preincubation with 100 nM CNP. The IC_{50} value (11 nM) of CNP for inhibition of acetylcholine-stimulated Ca^{2+} influx was close to the EC_{50} value (26 nM) of CNP for cellular production of cGMP. Zaprinast, an inhibitor for phosphodiesterase-catalyzing cGMP hydrolysis, increased cGMP and inhibited catecholamine release, as did CNP. In addition, an inhibitor of cGMP-dependent protein kinase, 8-(4-chlorophenylthio)-guanosine 3′,5′-cyclic monophosphorothioate, Rp isomer, reversed the inhibitory effects of CNP. These results suggest that CNP acts on ANPR-B and inhibits voltage-dependent Ca^{2+} channels in a cGMP-dependent pathway, thereby reducing catecholamine secretion.

O'Sullivan and Burgoyne [52], on the contrary, have shown that ANP (>10 nM) potentiated catecholamine secretion due to a low concentration (3 μM) of nicotine in bovine adrenal chromaffin cells. Potentiation of catecholamine secretion was also observed by the addition of 8-bromo-cGMP, or of sodium nitroprusside known to elevate cGMP, suggesting the involvement of a cGMP-dependent signaling pathway.

Thus, the apparent discrepancy between the inhibitory and stimulatory effects of natriuretic peptides, depending on the concentration of secretagogues and the mechanisms in catecholamine secretion [43, 50–52], remains to be resolved.

On the other hand, in slices of rat adrenal medulla, ANP rapidly increased noradrenaline uptake, and modified the intracellular distribution of the amine store; the granular fraction of noradrenaline increased, while the cytosolic fraction of noradrenaline decreased [53]. Thus, natriuretic peptide may also modify the pharmacokinetics of catecholamines.

EFFECTS OF NATRIURETIC PEPTIDES ON HUMAN PHEOCHROMOCYTOMA AND PC12 CELLS

ANPR-A and ANPR-C have been reported to exist in human pheochromocytoma, a tumor derived from adrenal medulla [54]. In tissue slices of pheochromocytoma, basal secretion of catecholamines was inhibited by ANP in a concentration-dependent manner, whereas secretagogue-induced catecholamine secretion has not been examined. ^{125}I-ANP bound to a single class of high-affinity binding sites of the membrane fraction. When the receptors were covalently tagged with ^{125}I-ANP and electrophoresed under non-reducing and reducing conditions, they migrated at positions of 140 and 70 kDa, respectively, suggesting that pheochromocytoma has ANPR-C of disulfide-linked homodimer. An addition of ANP to the membrane prepared from pheochromocytoma resulted in a 2-fold increase in cGMP accumulation, indicating that ANPR-A is also present in this tumor. The presence of ANPR-B has not been examined thus far.

In rat pheochromocytoma PC12 cells, ^{125}I-ANP binding sites [55] may represent ANPR-A of 130 kDa and ANPR-C of 70 kDa, which were analyzed by affinity cross-linking experiments [56]. ANP was much more potent than CNP in generating cGMP [42]. This is in striking contrast to that in bovine adrenal chromaffin cells, where CNP was much more potent than ANP [43], despite the fact that both cells are embryologically derived from the neural crest. Thus, the physiological functions of natriuretic peptides seem to be different between adrenal chromaffin cells and pheochromocytoma.

CONCLUSION AND PROSPECTIVE

The functions of natriuretic peptides in adrenal chromaffin cells are summarized in Fig. 1. ANP, BNP, and CNP are synthesized, stored, and secreted from adrenal chromaffin cells. CNP may also be synthesized and secreted from vascular endothelial cells in adrenal medulla, and may act on chromaffin cells. The natriuretic peptides bind with distinct types of receptors on the cell surface and modify cell functions such as catecholamine synthesis, secretion, and uptake in autocrine/paracrine manners. Furthermore, these peptides may exert paracrine regulation of adrenal cortical cells to inhibit aldosterone secretion and/or local vasorelaxation.

Although the effects of natriuretic peptides on adrenal chromaffin cell functions have been reported in several experimental systems, ANP receptor subtypes and their

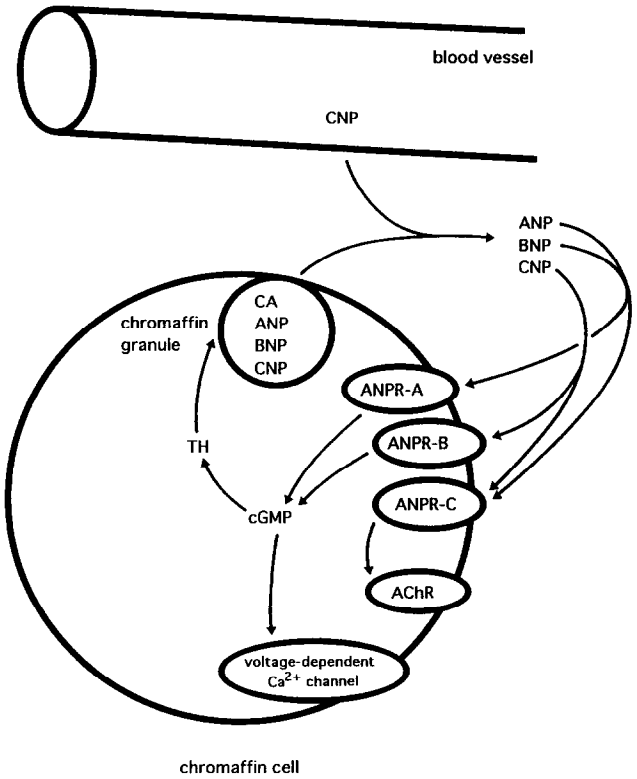

FIG. 1. Schema of the function of natriuretic peptides in adrenal chromaffin cells. The details are shown in the text. Abbreviations not defined previously: CA, catecholamine; TH, tyrosine hydroxylase; and AChR, acetylcholine receptor.

intracellular signal transduction pathways involved remain to be identified, even in bovine adrenal chromaffin cells in which most of the findings have been obtained. The elucidation of these regulatory mechanisms would give us a clue to understanding the importance of autocrine/paracrine regulation by the natriuretic peptides in various physiological and pathological states.

This work was supported, in part, by a Grant for a Special Research Project from Miyazaki Medical College.

References

1. Flynn TG, de Bold ML and de Bold AJ, The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. *Biochem Biophys Res Commun* **117:** 850–865, 1983.
2. Kangawa K and Matsuo H, Purification and complete amino acid sequence of α-human atrial natriuretic polypeptide (α-hANP). *Biochem Biophys Res Commun* **118:** 131–139, 1984.
3. de Bold AJ, Atrial natriuretic factor: A hormone produced by the heart. *Science* **230:** 767–770, 1985.
4. Sudoh T, Kangawa K, Minamino N and Matsuo H, A new natriuretic peptide in porcine brain. *Nature* **332:** 78–81, 1987.
5. Sudoh T, Minamino N, Kangawa K and Matsuo H, C-type natriuretic peptide (CNP): A new member of natriuretic peptide family identified in porcine brain. *Biochem Biophys Res Commun* **168:** 863–870, 1990.
6. Maack T, Receptors of atrial natriuretic factor. *Annu Rev Physiol* **54:** 11–27, 1992.
7. Koller KJ and Goeddel DV, Molecular biology of the natriuretic peptides and their receptors. *Circulation* **86:** 1081–1088, 1992.
8. Ueda S, Minamino N, Aburaya M, Kangawa K, Matsukura S and Matsuo H, Distribution and characterization of immunoreactive porcine C-type natriuretic peptide. *Biochem Biophys Res Commun* **175:** 759–767, 1991.
9. Winkler H and Westhead E, The molecular organization of adrenal chromaffin granules. *Neuroscience* **5:** 1803–1823, 1980.
10. Pelto-Huikko M, Immunocytochemical localization of neuropeptides in the adrenal medulla. *J Electron Microsc Tech* **12:** 364–379, 1989.
11. Yandle TG, Biochemistry of natriuretic peptides. *J Int Med* **235:** 561–576, 1994.
12. Rosenzweig A and Seidman CE, Atrial natriuretic factor and related peptide hormones. *Annu Rev Biochem* **60:** 229–255, 1991.
13. Minamino N, Makino Y, Tateyama H, Kangawa K and Matsuo H, Characterization of immunoreactive human C-type natriuretic peptide in brain and heart. *Biochem Biophys Res Commun* **179:** 535–542, 1991.
14. Suga S, Nakao K, Itoh H, Komatsu Y, Ogawa Y, Hama N and Imura H, Endothelial production of C-type natriuretic peptide and its marked augmentation by transforming growth factor-β. Possible existence of "vascular natriuretic peptide system." *J Clin Invest* **90:** 1145–1149, 1992.
15. Ishizaka Y, Kangawa K, Minamino N, Ishii K, Takano S, Eto T and Matsuo H, Isolation and identification of C-type natriuretic peptide in human monocytic cell line, THP-1. *Biochem Biophys Res Commun* **189:** 697–704, 1992.
16. Koller KJ, Lowe DG, Bennet GL, Minamino N, Kangawa K, Matsuo H and Goeddel DV, Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science* **252:** 120–123, 1991.
17. Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, Arai H, Saito Y, Kambayashi Y, Inouye K and Imura H, Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology* **130:** 229–239, 1992.
18. Maack T, Suzuki M, Almeida FA, Nussenzveig D, Scarborough RM, McEnroe GA and Lewicki JA, Physiological role of silent receptors of atrial natriuretic factor. *Science* **238:** 675–678, 1987.
19. Anand-Srivastava MB, Sairam MR and Cantin M, Ring-deleted analogs of atrial natriuretic factor inhibit adenylate cyclase/cAMP system. Possible coupling of clearance atrial natriuretic factor receptors to adenylate cyclase/cAMP signal transduction system. *J Biol Chem* **265:** 8566–8572, 1990.
20. Hirata M, Chang CH and Murad F, Stimulatory effects of atrial natriuretic factor on phosphoinositide hydrolysis in cultured bovine aortic smooth muscle cells. *Biochim Biophys Acta* **1010:** 346–351, 1989.
21. Prins BA, Weber MJ, Hu R-M, Pedram A, Daniels M and Levin ER, Atrial natriuretic peptide inhibits mitogen-activated protein kinase through the clearance receptor. Potential role in the inhibition of astrocyte proliferation. *J Biol Chem* **271:** 14156–14162, 1996.
22. Paul AK, Marala RB, Jaiswal RK and Sharma RK, Coexistence of guanylate cyclase and atrial natriuretic factor receptor in a 180-kD protein. *Science* **235:** 1224–1226, 1987.
23. De Léan A, Ong H, McNicoll N, Racz K, Gutkowska J and Cantin M, Identification of aldosterone secretion inhibitory factor in bovine adrenal medulla. *Life Sci* **36:** 2375–2382, 1985.
24. Ong H, Lazure C, Nguyen TT, McNicoll N, Seidah N,

Chrétien M and De Léan A, Bovine adrenal chromaffin granules are a site of synthesis of atrial natriuretic factor. *Biochem Biophys Res Commun* **147:** 957–963, 1987.
25. Morel G, Chabot J-G, Garcia-Caballero T, Gossard F, Dihl F, Belles-Isles M and Heisler S, Synthesis, internalization, and localization of atrial natriuretic peptide in rat adrenal medulla. *Endocrinology* **123:** 149–158, 1988.
26. Okazaki M, Yanagihara N, Izumi F, Nakashima Y and Kuroiwa A, Carbachol-induced cosecretion of immunoreactive atrial natriuretic peptides with catecholamines from cultured bovine adrenal medullary cells. *J Neurochem* **52:** 222–228, 1989.
27. Nguyen TT, Babinski K, Ong H and De Léan A, Differential regulation of natriuretic peptide biosynthesis in bovine adrenal chromaffin cells. *Peptides* **11:** 973–978, 1990.
28. Nguyen TT, Ong H and De Léan A, Secretion and biosynthesis of atrial natriuretic factor by cultured adrenal chromaffin cells. *FEBS Lett* **231:** 393–396, 1988.
29. Babinski K, Féthière J, Roy M, De Léan A and Ong H, C-type natriuretic peptide in bovine chromaffin cells. The regulation of its biosynthesis and secretion. *FEBS Lett* **313:** 300–302, 1992.
30. Bianchi C, Gutkowska J, Thibault G, Garcia R, Genest J and Cantin M, Radioautographic localization of ^{125}I-atrial natriuretic factor (ANF) in rat tissue. *Histochemistry* **82:** 441–452, 1985.
31. Rambotti MG, Ercolani P and Spreca A, Ultracytochemical localization of particulate guanylate cyclase in rat adrenal gland exposed to stimulation by porcine brain natriuretic peptide. *Histochem J* **25:** 384–391, 1993.
32. Maurer R and Reubi J-C, Distribution and coregulation of three peptide receptors in adrenals. *Eur J Pharmacol* **125:** 241–247, 1986.
33. Fuchs E, Shigematsu K and Saavedra JM, Binding sites of atrial natriuretic peptide in tree shrew adrenal gland. *Peptides* **7:** 873–876, 1986.
34. Chai SY, Sexton PM, Allen AM, Figdor R and Mendelsohn FAO, *In vivo* autoradiographic localization of ANP receptors in rat kidney and adrenal gland. *Am J Physiol* **250:** F753–F757, 1986.
35. Mantyh CR, Kruger L, Brecha NC and Mantyh PW, Localization of specific binding sites for atrial natriuretic factor in peripheral tissues of the guinea pig, rat, and human. *Hypertension* **8:** 712–721, 1986.
36. Stewart RE, Swithers SE, Plunkett LM and McCarty R, ANF receptors: Distribution and regulation in central and peripheral tissues. *Neurosci Biobehav Rev* **12:** 151–168, 1988.
37. Konrad EM, Thibault G, Pelletier S, Genest J and Cantin M, Brain natriuretic peptide binding sites in rats: *In vitro* autoradiographic study. *Am J Physiol* **259:** E246–E255, 1990.
38. Konrad EM, Thibault G and Schiffrin EL, Autoradiographic visualization of the natriuretic peptide receptor-B in rat tissues. *Regul Pep* **39:** 177–189, 1992.
39. Wilcox JN, Augustine A, Goeddel DV and Lowe DG, Differential regional expression of three natriuretic peptide receptor genes within primate tissues. *Mol Cell Biol* **11:** 3454–3462, 1991.
40. Heisler S and Morrier E, Bovine adrenal medullary cells contain functional atrial natriuretic peptide receptors. *Biochem Biophys Res Commun* **150:** 781–787, 1988.
41. Niina H, Kobayashi H, Yamamoto R, Yuhi T, Yanagita T and Wada A, Receptors for atrial natriuretic peptide in adrenal chromaffin cells. *Biochem Pharmacol* **51:** 855–858, 1996.
42. Féthière J, Graihle R, Larose L, Babinski K, Ong H and De Léan A, Distribution and regulation of natriuretic factor-R1c receptor subtype in mammalian cell lines. *Mol Cell Biochem* **124:** 11–16, 1993.
43. Babinski K, Haddad P, Vallerand D, McNicoll N, De Léan A and Ong H, Natriuretic peptides inhibit nicotine-induced whole-cell currents and catecholamine secretion in bovine chromaffin cells: Evidence for the involvement of the atrial natriuretic factor R_2 receptors. *J Neurochem* **64:** 1080–1087, 1995.
44. Yanagihara N, Okazaki M, Terao T, Uezono Y, Wada A and Izumi F, Stimulatory effects of brain natriuretic peptide in cyclic GMP accumulation and tyrosine hydroxylase activity in cultured bovine adrenal medullary cells. *Naunyn-Schmiedeberg's Arch Pharmacol* **343:** 289–295, 1991.
45. Tsutsui M, Yanagihara N, Minami K, Kobayashi H, Nakashima Y, Kuroiwa A and Izumi F, 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, 1994.
46. Weiner N, Regulation of norepinephrine biosynthesis. *Annu Rev Pharmacol* **10:** 273–390, 1970.
47. Kumer SC and Vrane KE, Intricate regulation of tyrosine hydroxylase activity and gene expression. *J Neurochem* **67:** 443–462, 1996.
48. Burgoyne RD, Control of exocytosis in adrenal chromaffin cells. *Biochim Biophys Acta* **1071:** 174–202, 1991.
49. Burgoyne RD, Morgan A, Robinson I, Pender N and Cheek TR, Exocytosis in adrenal chromaffin cells. *J Anat* **183:** 309–314, 1993.
50. Bormann J, Flügge G and Fuchs E, Effect of atrial natriuretic factor (ANF) on nicotinic acetylcholine receptor channels in bovine chromaffin cells. *Pflügers Arch* **414:** 11–14, 1989.
51. Rodriguez-Pascual F, Miras-Portugal MT and Torres M, Effect of cyclic GMP-increasing agents nitric oxide and C-type natriuretic peptide on bovine chromaffin cell function: Inhibitory role mediated by cyclic GMP-dependent protein kinase. *Mol Pharmacol* **49:** 1058–1070, 1996.
52. O'Sullivan AJ and Burgoyne RD, Cyclic GMP regulates nicotine-induced secretion from cultured bovine adrenal chromaffin cells: Effect of 8-bromo-cyclic GMP, atrial natriuretic peptide, and nitroprusside (nitric oxide). *J Neurochem* **54:** 1805–1808, 1990.
53. Vatta MS, Bianciotti LG and Fernandez BE, Influence of atrial natriuretic factor on uptake, intracellular distribution, and release of norepinephrine in rat adrenal medulla. *Can J Physiol Pharmacol* **71:** 195–200, 1993.
54. Shionoiri H, Hirawa N, Takasaki I, Ishikawa Y, Minamisawa K, Miyajima E, Kinoshita Y, Shimoyama K, Shimonaka M, Ishido M and Hirose S, Presence of functional receptors for atrial natriuretic peptide in human pheochromocytoma. *Biochem Biophys Res Commun* **148:** 286–291, 1987.
55. Boumezrag A, Lyall F and Dow JAT, Characterization of specific binding of atrial natriuretic peptide (ANP) to rat PC12 phaeochromocytoma cells. *Life Sci* **43:** 2035–2042, 1988.
56. Rathinavelu A and Isom GE, Differential internalization and processing of atrial-natriuretic-factor B and C receptors in PC12 cells. *Biochem J* **276:** 493–497, 1991.