Role of endogenous PACAP in catecholamine secretion from the rat adrenal gland

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Although catecholamine secretion from chromaffin cells of the adrenal medulla is controlled by ACh released from the splanchnic nerve endings, noncholinergic neurotransmitters or neuromodulators are released with ACh to participate in the neural control of adrenal catecholamine secretion (8). Vasoactive intestinal peptide (VIP) has been regarded as one of these substances (27). Pituitary adenylate cyclase-activating polypeptide (PACAP) is also reported to act as a noncholinergic neurotransmitter or neuromodulator in the adrenal gland (11, 21).

PACAP was first isolated from ovine hypothalamus and named for its ability to activate adenylate cyclase in rat anterior pituitary cells (14). PACAP shows 68% homology with VIP in the amino acid sequence and is classified into the secretin/glucagon/VIP family (14). PACAP has two biologically active forms, PACAP-38 and its NH2-terminal residues PACAP-27 (15). There are at least two types of receptors for PACAP, PACAP type I receptors, for which PACAP has a higher affinity than VIP, and PACAP type II receptors, for which PACAP and VIP have similar affinities (2, 25). The PACAP-binding sites are widely found not only in the brain (13, 20) but also in the peripheral tissues (23, 25).

Among rat peripheral tissues, the adrenal medulla contains a high concentration of PACAP (1). Autoradiography shows abundant PACAP-specific binding sites in rat adrenal chromaffin cells (25). Moreover, PACAP induces catecholamine secretion from rat (28), porcine (10), and bovine (19) adrenal chromaffin cells. The potency of PACAP to induce adrenal catecholamine secretion is higher than that of VIP (8, 28).

However, it has not been fully understood whether endogenous PACAP participates in the neural control of adrenal catecholamine secretion. To clarify this issue, in the present study we examined the effects of PACAP-(6–38), a PACAP type I receptor antagonist, and [Lys1,Pro2,5,Ara3,4,Tyr6]-vasoactive intestinal peptide (LPAT-VIP; 30–3,000 nM). Transmural electrical stimulation (ES; 1–10 Hz) or infusion of ACh (6–200 nM) increased adrenal epinephrine and norepinephrine output. The PACAP-induced catecholamine output responses were inhibited by the PACAP type I receptor antagonist PACAP-(6–38) (30–3,000 nM) but were resistant to the PACAP type II receptor antagonist [Lys1,Pro2,5,Ara3,4,Tyr6]-vasoactive intestinal peptide (LPAT-VIP; 30–3,000 nM). Transmural electrical stimulation (ES; 1–10 Hz) or infusion of ACh (6–200 nM) increased adrenal epinephrine and norepinephrine output. PACAP-(6–38) (3,000 nM), but not LPAT-VIP, also inhibited the ES-induced catecholamine output responses. However, PACAP-(6–38) did not affect the ACh-induced catecholamine output responses. PACAP at low concentrations (0.3–3 nM), which had no influence on catecholamine output, enhanced the ACh-induced catecholamine output responses, but not the ES-induced catecholamine output responses. These results suggest that PACAP is released from the nerve endings to facilitate the neurally evoked catecholamine secretion through PACAP type I receptors in the rat adrenal gland.

transmural electrical stimulation; acetylcholine; pituitary adenylate cyclase-activating polypeptide receptor antagonists; pituitary adenylate cyclase-activating polypeptide (6–38); adrenal chromaffin cells

Materials and Methods

Preparation. All procedures for handling animals were approved by the Animal Experimentation Committee of Tohoku University Graduate School of Pharmaceutical Sci-

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ences. Male Wistar rats, weighing 200–320 g, were housed at 21–24°C and maintained on a standard diet and water ad libitum. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The surgical procedure used in the present study was described previously (18). A polyethylene cannula for perfusion of the adrenal gland was inserted into the adrenal vein through the renal vein. Then the adrenal gland was carefully removed, and a small slit was made into the adrenal cortex just opposite the entrance of the adrenal gland. Perfusion of the adrenal gland was started to ensure that the perfusate escaped only from the slit of the adrenal gland. The adrenal gland was put on a platinum bipolar electrode, and the adrenal vein through the renal vein. Then the adrenal gland was killed by exsanguination.

**Perfusion of the adrenal gland.** The adrenal gland was perfused by means of a peristaltic pump (MP-3A, EYELA) at a rate of 0.2 ml/min with Krebs-Henseleit solution of the following composition (mM): 118 NaCl, 4.7 KCl, 1.2 MgSO4, 2.6 CaCl2, 1.2 KH2PO4, 24.9 NaHCO3, 11.1 glucose; Krebs-Henseleit solution was maintained at 37°C by the thermostat controlled water circulator (NTT-1200, EYELA, Tokyo, Japan). After extraction of the adrenal gland, the animal was cut on the 37°C by the thermostat controlled water circulator (NTT-1200, EYELA, Tokyo, Japan). After extraction of the adrenal gland, the animal was killed by exsanguination.

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the experimental periods in each group (data not shown). Infusion of PACAP (100 nM) into the adrenal gland increased Epi and NE output in the first trial (groups 1 and 2, Fig. 1). ES (1–10 Hz) or infusion of ACh (6–200 μM) into the adrenal gland frequency or concentration dependent increased Epi and NE output (groups 3–7, Figs. 2–4).

Effects of PACAP receptor antagonists on PACAP-induced catecholamine output responses. The PACAP type I receptor antagonist PACAP-(6–38) at 300 nM significantly inhibited the PACAP-induced increases in Epi and NE output (group 1, Fig. 1A). The highest concentration (3,000 nM) of PACAP-(6–38) abolished the PACAP-induced output responses. The PACAP-induced increases in Epi and NE output were not affected by the PACAP type II receptor antagonist LPAT-VIP at 30 and 300 nM and inhibited by LPAT-VIP at 3,000 nM (group 2, Fig. 1B).

Effects of PACAP on ES- or ACh-induced catecholamine output responses. PACAP (0.3, 1, and 3 nM) did not affect the ES-induced increases in Epi and NE output (group 6, Fig. 4A). In contrast, the ACh-induced increases in Epi and NE output were significantly enhanced by PACAP (group 7, Fig. 4B). Potencies of the enhancement were almost the same among 0.3, 1, and 3 nM PACAP.

DISCUSSION
In the present study, infusion of PACAP (100 nM) into the isolated perfused rat adrenal gland increased
Epi and NE output, as observed in the bovine (19, 26), porcine (10), and rat (28) cultured adrenal chromaffin cells or in the isolated perfused rat adrenal gland (8). PACAP-(6–38), a PACAP type I receptor antagonist (22), at 300 nM inhibited and at 3,000 nM abolished the PACAP-induced catecholamine output responses. PACAP-(6–38) at the highest concentration (3,000 nM) abolished the catecholamine output responses. On the other hand, the PACAP-induced catecholamine output responses were resistant to LPAT-VIP, a PACAP type II receptor antagonist (7). LPAT-VIP only at the highest concentration (3,000 nM) inhibited the PACAP-induced catecholamine output responses by ~40%. PACAP type II receptors are reported to occupy only 7% of total PACAP-binding sites in rat adrenal chromaffin cells (25). Taken together, PACAP type I receptors may predominantly mediate the PACAP-induced adrenal catecholamine secretion.

To elucidate a role of endogenous PACAP in the adrenal gland, we examined whether the blockade of PACAP receptors could affect neurally evoked adrenal catecholamine secretion. We applied ES to the isolated adrenal gland, the method of which has been confirmed to be adequate to evaluate drug actions on adrenal catecholamine secretion in response to neural release of endogenous ACh (16–18). PACAP-(6–38) (3,000 nM), but not LPAT-VIP, inhibited the increases in Epi and NE output induced by ES. Our present study is the first that demonstrates an inhibitory effect of the selective PACAP type I receptor antagonist on endogenous ACh-induced adrenal catecholamine secretion. On the other hand, PACAP-(6–38) did not affect the increases in Epi and NE output induced by ACh infusion. It is therefore likely that PACAP released from the nerve endings together with ACh plays a facilitory role in neurally evoked catecholamine secretion via PACAP type I receptors in the rat adrenal gland.

Whereas the ES-induced catecholamine output responses in the rat adrenal gland are mainly mediated by nicotinic receptors (19), the inhibition by PACAP-(6–38) of the ES-induced responses was ~60%. This large inhibition indicates that neurally released PACAP may not only directly induce catecholamine secretion but also modulate the cholinergic mechanisms of adrenal catecholamine secretion. It is unlikely that PACAP-(6–38) blocks or PACAP activates the cholinergic receptors, because PACAP-(6–38) failed to affect the exogenous ACh-induced catecholamine output responses in this study (the responses are also mediated by nicotinic receptors as well as muscarinic receptors, Ref. 18), and neither nicotinic nor muscarinic receptor antagonist inhibits exogenous PACAP-induced catecholamine secretion from the rat adrenal medulla in vivo (29). It has been reported that exogenous PACAP enhances the cardiac parasympathetic neurotransmitter release (9, 24) and the ACh-induced...
adrenal catecholamine secretion (11) in anesthetized dogs. Taken together, we postulated that neurally released PACAP acts on the nerve endings (presynaptic site) to accelerate neural ACh release or acts on the chromaffin cells (postsynaptic site) to amplify processes of the ACh-induced responses in the rat adrenal gland.

To confirm the modulation by PACAP of the postsynaptic events, we examined whether PACAP at concentrations that do not induce catecholamine secretion affects exogenous ACh-induced catecholamine secretion. PACAP at 0.3 nM enhanced the ACh-induced catecholamine output responses, 0.3 nM of PACAP may be a supramaximal concentration to modulate the cholinergic mechanisms. Although the possibility that the presynaptic action of PACAP affects the postsynaptic events still remains, the neurally released PACAP may act on the postsynaptic site to amplify the processes of cholinergic adrenal catecholamine secretion. Because PACAP at higher concentrations (1 and 3 nM) did not further enhance the ACh-induced catecholamine output responses, 0.3 nM of PACAP may be a supramaximal concentration to modulate the cholinergic mechanisms. Although the possibility that the presynaptic action of PACAP affects the postsynaptic events still remains, the neurally released PACAP may act on the postsynaptic site to amplify the processes of cholinergic adrenal catecholamine secretion. On the other hand, PACAP (0.3–3 nM) did not affect the ES-induced adrenal catecholamine secretion. The failure of exogenous PACAP to enhance the ES-induced responses would imply that the neurally released PACAP maximally facilitates the catecholamine secretion.

It has been suggested that VIP is released from the nerve endings and contributes to neurally evoked adrenal catecholamine secretion (12, 27). Exogenous VIP causes catecholamine secretion, and [Ac-Tyr₁,D-Phe₂]-VIP nerve endings and contributes to neurally evoked adrenal catecholamine secretion. PACAP at 0.3 nM enhanced the ACh-induced catecholamine output responses without affecting basal catecholamine output. Similar enhancement was observed by administration of PACAP at a dose that increased basal catecholamine secretion in the dog adrenal gland in vivo (11). Our finding indicates that the increased adrenal PACAP level, even though it does not directly induce catecholamine secretion, can influence cholinergic mechanisms of adrenal catecholamine secretion. Because PACAP at higher concentrations (1 and 3 nM) did not further enhance the ACh-induced catecholamine output responses, 0.3 nM of PACAP may be a supramaximal concentration to modulate the cholinergic mechanisms. Although the possibility that the presynaptic action of PACAP affects the postsynaptic events still remains, the neurally released PACAP may act on the postsynaptic site to amplify the processes of cholinergic adrenal catecholamine secretion. On the other hand, PACAP (0.3–3 nM) did not affect the ES-induced adrenal catecholamine secretion. The failure of exogenous PACAP to enhance the ES-induced responses would imply that the neurally released PACAP maximally facilitates the catecholamine secretion.

A recent report suggests that PACAP type I receptors also mediate exogenous VIP-induced adrenal catecholamine secretion in dogs in vivo (4). However, binding affinity of VIP for PACAP type I receptors is 500–1,000 times less than that for PACAP type II receptors (6), and micromolar levels of VIP are required to induce catecholamine secretion from the isolated perfused rat adrenal gland (27). It is therefore unlikely that the PACAP type I receptor antagonist PACAP-(6–38) inhibited the facilitatory action of endogenous VIP in the present study. In our experimental condition, VIP may play little or no role in adrenal catecholamine secretion.

In conclusion, the present study suggests that neurally released PACAP modulates cholinergic mechanisms via PACAP type I receptors and thereby plays a facilitatory role in the peripheral neural control of catecholamine secretion in the rat adrenal gland. The facilitation by PACAP may occur at the postsynaptic site, but the possibility still remains that PACAP also acts on the presynaptic site to enhance neural ACh release.

Perspectives

The present study suggests that neurally released PACAP facilitates cholinergic adrenal catecholamine secretion. To obtain direct evidence for the neural PACAP release, we measured PACAP concentration in the perfusate by radioimmunoassay. A tendency that ES increased adrenal PACAP output was observed, but precise output values were not obtained due to low sensitivity of the assay method we used.

PACAP activates adenylate cyclase and also evokes the calcium influx from extracellular fluid or the calcium release from intracellular stores in adrenal chromaffin cells (19, 21, 26). Our recent study has also demonstrated the roles of adenylate cyclase and extracellular calcium in the PACAP-induced catecholamine secretion from the isolated perfused rat adrenal gland (3). One or more of these cellular events may modulate pathways involved in the ACh-induced adrenal catecholamine secretion. As we noted in the DISCUSSION, however, the present study cannot rule out the possibility that PACAP acts on the presynaptic site and thereby facilitates the postsynaptic response. Elevation by PACAP of cytosolic cAMP or free calcium level at the nerve endings would enhance the ACh release.

Determination of neural PACAP and ACh release in the adrenal gland will be required to clarify the role of endogenous PACAP in the control of adrenal catecholamine secretion.

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