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Short communication

Inhibition of catecholamine synthesis by proadrenomedullin N-terminal 20 peptide in cultured bovine adrenal medullary cells

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Abstract

In cultured bovine adrenal medullary cells, proadrenomedullin N-terminal 20 peptide (PAMP), at concentrations ≥ 3 μM, inhibited carbachol-induced [^{14}C]catecholamine synthesis from [^{14}C]tyrosine. Carbachol-induced activation of tyrosine hydroxylase was also attenuated by PAMP. These results suggest that PAMP is a novel endogenous peptide that regulates catecholamine synthesis via the suppression of its rate-limiting enzyme in adrenal medullary cells.

Keywords: PAMP (proadrenomedullin N-terminal 20 peptide); Catecholamine synthesis; Tyrosine hydroxylase; Nicotinic receptor; Adrenal medulla

1. Introduction

Proadrenomedullin N-terminal 20 peptide (PAMP) is processed from proadrenomedullin (Ishimitsu et al., 1994; Kitamura et al., 1994a), and its carboxy terminus is amidated as in other biologically active peptides (Kitamura et al., 1994b; Washimine et al., 1994). The immunoreactivity and mRNA for PAMP (Kitamura et al., 1994a; Washimine et al., 1994) are much more abundant in the adrenal medulla, and PAMP is co-secreted with catecholamines by nicotinic receptor-mediated Ca^{2+}-dependent exocytosis from adrenal medullary cells (Katoh et al., 1994). Although intravenous administration of PAMP lowers blood pressure in rats (Kitamura et al., 1994b) and PAMP inhibits catecholamine secretion from cultured adrenal medullary cells (Katoh et al., 1994), little is known about the biological effects of PAMP and the signal transduction of the putative receptors of PAMP.

In adrenal medullary cells, the stimulation of nicotinic receptors increases catecholamine synthesis in a Ca^{2+}-dependent manner (Haycock et al., 1882), which is associated with the activation of tyrosine hydroxylase (EC 1.14.16.2). This enzyme catalyzes the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), the rate-limiting step in the biosynthesis of catecholamines. To clarify the physiological role of PAMP, we investigated the effects of PAMP on the synthesis of [^{14}C]catecholamines and the activity of tyrosine hydroxylase in cultured bovine adrenal medullary cells.

2. Materials and methods

2.1. Adrenal medullary cells

Bovine adrenal medullary cells were isolated and cultured (4×10^6 cells/dish, Falcon 35 mm) in Eagle's minimum essential medium containing 10% calf serum and antibiotics for 3–5 days (Wada et al., 1985).

2.2. Synthesis of [^{14}C]catecholamines from [^{14}C]tyrosine

Cultured cells were preincubated at 37°C for 10 min in the absence or presence of various concentrations of PAMP in 1.0 ml of oxygenated Krebs-Ringer phosphate (KRP) buffer (mM):NaCl 154, KCl 5.6, $MgSO_4$ 1.1, $CaCl_2$ 2.2, Na_2HPO_4 2.15, NaH_2PO_4 0.85, glucose

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5; and 0.5% bovine serum albumin, pH 7.4. After aspiration of the medium, the cells were incubated with L-[U-^{14}C]tyrosine in the absence or presence of PAMP and 300 μM carbachol at 37°C for 15 min. [^{14}C]Catechol compounds formed in the cells and secreted into the medium were extracted with 0.4 M perchloric acid, separated from [^{14}C]tyrosine, and counted in a scintillation counter (Yanagihara et al., 1987).

2.3. Assay of tyrosine hydroxylase activity

After the preincubation (see above), cells were incubated at 37°C for 30 s without or with 300 μM carbachol in the absence or presence of 3 μM PAMP, washed with ice-cold phosphate buffered saline, and immediately frozen on dry ice. The cells were harvested with 400 μl of 30 mM potassium phosphate buffer (pH 6.8) containing 50 mM NaF and 1 mM EDTA. After centrifugation at 48000 $\times g$ for 10 min, the supernatant was applied to a Sephadex G-50 column to remove catecholamines and other small molecules. The activity of tyrosine hydroxylase in the effluent was determined by a modified decarboxylase-coupled assay using L-[1-^{14}C]tyrosine (Uezono et al., 1989). The reaction mixture (final volume, 100 μl) consisted of 100 mM potassium phosphate buffer (pH 6.8), 5 mM ascorbic acid, 6500 units of catalase, 5 mM EDTA, 250 μM D,L-2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine-HCl, 100 μM L-[1-^{14}C]tyrosine and enzyme. The specific activity of tyrosine hydroxylase was expressed as nmol $^{14}CO_2$ formed per minute per 4×10^6 cells.

2.4. Chemicals

L-[U-^{14}C]Tyrosine and L-[1-^{14}C]tyrosine were from Amersham Japan. Human synthetic PAMP was prepared by solid phase methods in the Peptide Institute, Osaka, Japan.

2.5. Statistical analysis

The data are expressed as means ± S.E.M. One-way analysis of variance was used to test the overall statistical significance. Scheffé's test was used when three or more groups were to be compared.

3. Results

3.1. Effect of PAMP on [^{14}C]catecholamine synthesis

Basal as well as carbachol-induced synthesis of [^{14}C]catecholamines from [^{14}C]tyrosine were both increased linearly with the duration of incubation for at least 15 min (data are not given). Over a period of 15 min, carbachol (300 μM) increased [^{14}C]catecholamine synthesis about 2-fold over the basal values (Fig. 1). PAMP (10 μM) did not alter the basal [^{14}C]catecholamine synthesis, whereas the peptide, at 3 and 10 μM, significantly inhibited the carbachol-stimulated [^{14}C]-catecholamine synthesis by 33 and 48%, respectively. The uptake of [^{14}C]tyrosine by the cells was similar between non-treated (3.30 ± 0.04 nmol/4 × 10^6 cells/15 min) and PAMP-treated (3.15 ± 0.01 nmol/4 × 10^6 cells/15 min) cells even in the presence of carbachol ($n = 4$).

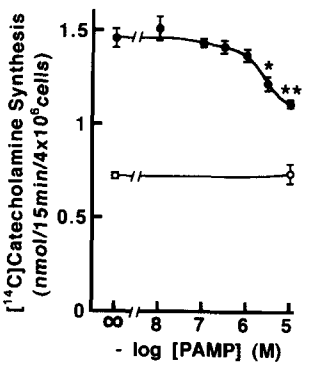

Fig. 1. Effect of PAMP on [^{14}C]catecholamine synthesis from [^{14}C]tyrosine in adrenal medullary cells. The cells were incubated without (○) or with (●) 300 μM carbachol (CCh) in the absence or presence of various concentrations of PAMP at 37°C for 15 min. Means ± S.E.M. ($n = 4$). $^*P < 0.05$, $^{**}P < 0.01$ compared with the value in the absence of PAMP.

3.2. Effect of PAMP on the activity of tyrosine hydroxylase

The effect of PAMP on the activity of tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis, was investigated. As shown in Fig. 2, the activity of tyrosine hydroxylase was increased

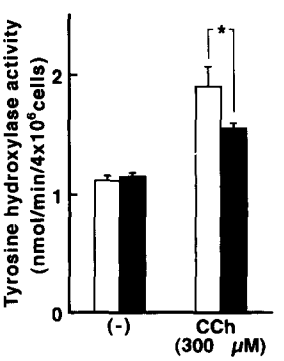

Fig. 2. Effect of PAMP on the activity of tyrosine hydroxylase in adrenal medullary cells. The cells were incubated without or with 300 μM carbachol in the absence (open column) or presence (closed column) of 3 μM PAMP at 37°C for 30 s. Means ± S.E.M. ($n = 8$). $^*P < 0.05$ compared with the value in the absence of PAMP.

1.7-fold by 300 μM carbachol. PAMP (3 μM) did not affect the basal activity of tyrosine hydroxylase, but significantly inhibited carbachol-induced tyrosine hydroxylase activation by 45%.

4. Discussion

The present study showed that PAMP reduces the synthesis of catecholamines by inhibition of tyrosine hydroxylase activation by carbachol, suggesting that PAMP is a regulator of catecholamine synthesis in the adrenal medulla. The lack of effect of PAMP on the basal activity of tyrosine hydroxylase indicates that PAMP specifically inhibits carbachol-induced events but does not directly interact with the tyrosine hydroxylase molecule. The concentration-response curve of PAMP in the present study was quite similar to that for carbachol-induced catecholamine secretion (Katoh et al., 1994). PAMP, even at 3 and 10 μM, did not completely inhibit carbachol-stimulated catecholamine synthesis, indicating that PAMP plays a role in the fine regulation of catecholamine synthesis. The concentrations of PAMP used in the present study were much higher than those in the plasma (Washimine et al., 1994). The PAMP content is, however, extremely high in the adrenal medulla (13.8 fmol/mg wet weight in human), and a significant amount of PAMP (16% of cell content) is co-secreted with catecholamines from the cells in response to nicotinic receptor stimulation (Washimine et al., 1994; Katoh et al., 1994), suggesting that the concentration of PAMP in the vicinity of the cell membrane may reach a high level.

In adrenal medullary cells, the synthesis of catecholamines is increased by nicotinic receptor agonists (Haycock et al., 1982) through the influx of Ca^{2+} which stimulates the Ca^{2+}-dependent phosphorylation and activation of tyrosine hydroxylase. The mechanism by which PAMP inhibits catecholamine synthesis is unknown; however, it could be related to interference with nicotinic receptor function (Katoh et al., 1994).

In anesthetized rats, an intravenous bolus injection of PAMP caused a rapid and remarkable hypotension in a dose-dependent manner (Kitamura et al., 1994b). Inhibition of the synthesis and secretion of catecholamines will be useful for the better understanding of the cellular mechanism(s) whereby PAMP regulates the functions of the cardiovascular system.

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