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STIMULATION BY VASOACTIVE INTESTINAL POLYPEPTIDE OF CATECHOLAMINE SYNTHESIS IN ISOLATED BOVINE ADRENAL CHROMAFFIN CELLS

POSSIBLE INVOLVEMENT OF PROTEIN KINASE C

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Abstract—In isolated bovine adrenal medullary cells, vasoactive intestinal polypeptide (VIP) stimulated ^{14}C-catecholamine synthesis from ^{14}C-tyrosine, but not from ^{14}C-DOPA. This stimulatory effect of VIP on ^{14}C-catecholamine synthesis was not dependent upon extracellular Ca^{2+}. VIP did not affect the intracellular cyclic AMP (cAMP) level. The stimulatory effect of VIP on ^{14}C-catecholamine synthesis was additive with that of carbamylcholine, which was dependent upon extracellular Ca^{2+}, but not with that of phorbol ester 12-O-tetradecanoyl phorbol 13-acetate (TPA), an activator of protein kinase C. Moreover, 1-(isoquinolinyl-sulfonyl)-2-methylpiperazine (H-7), an inhibitor of protein kinase C, inhibited not only TPA-stimulated, but also VIP-stimulated ^{14}C-catecholamine synthesis from ^{14}C-tyrosine. These results suggested that VIP stimulated catecholamine synthesis by activation of tyrosine hydroxylase and that protein kinase C was involved in this stimulatory mechanism.

Recently, vasoactive intestinal polypeptide (VIP)-like immunoreactivity has been demonstrated not only in brain and sympathetic ganglia, but also in chromaffin cells and nerve fibers of the adrenal medulla and in human pheochromocytoma tissue [1–3]. Moreover, bovine adrenal chromaffin cells have been shown to synthesize VIP during primary culture [4]. These results suggest that VIP may modulate the functions of adrenal chromaffin cells. Ip *et al.* [5] and Tischler *et al.* [6] reported that VIP increases the cyclic AMP (cAMP) contents of rat sympathetic ganglion cells and PC12 cells, and thereby the formation of DOPA from tyrosine.

In the present study, we examined the effect of VIP on the synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine in isolated bovine adrenal chromaffin cells. Results showed that VIP stimulates the synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine without affecting the level of cAMP in the cells and suggested that this stimulatory effect of VIP may be mediated by activation of protein kinase C.

MATERIALS AND METHODS

Bovine adrenal medullary cells were isolated by sequential digestion of adrenal medullary slices with collagenase as reported previously [7]. For most experiments, the freshly isolated cells ($\sim 2 \times 10^6$ cells/ml) were incubated at 37° with or without test compounds in 2 ml of medium consisting of 154 mM NaCl, 5.6 mM KCl, 1.1 mM $MgCl_2$, 2.2 mM $CaCl_2$, 10 mM Tris–HCl (pH 7.4) and 10 mM glucose. For some experiments, calcium-free medium was prepared by adding 1 mM EGTA to this medium instead of 2.2 mM $CaCl_2$.

For determination of catecholamine synthesis, isolated cells were incubated with ^{14}C-tyrosine (final concentration, 2×10^{-5} M, 175×10^4 cpm) as described in the footnote of tables. In some experiments, L-^{14}C-DOPA (2×10^{-5} M, 340×10^4 cpm) was used as substrate instead of ^{14}C-tyrosine. After incubation, the tubes were rapidly chilled in ice, and the cells were separated from the medium and homogenized in 5 ml of 0.4 N perchloric acid (PCA). The ^{14}C-labelled catecholamine in the supernatant was measured by ion-exchange chromatography on a Duolite C-25 column (H^+ form, 0.4 × 7.0 cm) as reported previously [8].

For determination of cAMP, cells were incubated in the medium in the presence of 3-isobutyl-1-methylxanthine (IBMX, 0.5 mM). The reaction was stopped by addition of 0.5 ml of ice-cold 25% trichloroacetic acid, and the cAMP was separated on an ion-exchange column (AG 500W × 8, H-type 200–400 mesh) and measured by the protein binding method [9].

For determination of catecholamine release, the catecholamine contents of the cells and medium were determined by a fluorometric method [10].

12-O-Tetradecanoyl phorbol 13-acetate (TPA) was dissolved in dimethyl sulfoxide (DMSO). All solutions contained 0.5% (v/v) DMSO. The sources of the materials used were as follows: L-^{14}C-tyrosine, ^{14}C-DOPA and [^{3}H]cAMP (Radiochemical Centre, Amersham, U.K.); VIP, TPA, DB·cAMP and IBMX (Sigma); carbamylcholine (Nakarai Chemical

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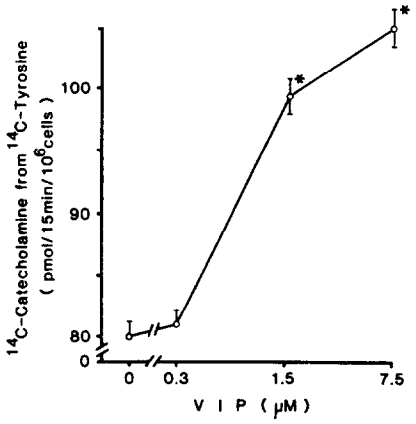

Fig. 1. Dose–response curve for VIP-stimulated ^{14}C-catecholamine synthesis from ^{14}C-tyrosine. Cells were incubated for 15 min with or without VIP (0.3–7.5 μM) in the normal medium containing ^{14}C-tyrosine (2×10^{-5} M). Values are means ± SEM of 4–6 experiments. *P < 0.005 relative to basal.

Co.); forskolin (Calbiochem-Behring). 1-(5-Isoquinolinylsulfonyl)-2-methylpiperazine (H-7) was a gift from Dr H. Hidaka, Department of Pharmacology, Mie University School of Medicine, Japan.

RESULTS AND DISCUSSION

Figure 1 shows the effect of VIP at concentrations of up to 7.5 μM on the synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine in isolated bovine chromaffin cells on incubation for 15 min. Stimulation of ^{14}C-catecholamine synthesis by VIP was marked at 1.5 μM and even greater at 7.5 μM, the maximum concentration tested. This stimulation by VIP was observed on incubation for at least 45 min. VIP did not stimulate the release of catecholamine from the cells. Other active peptides, such as secretin and substance P at concentrations of 10 μM did not affect the synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine (data not shown). VIP did not stimulate ^{14}C-catecholamine synthesis when ^{14}C-DOPA was used as a substrate instead of ^{14}C-tyrosine (Table 1), indicating

Table 1. Effect of VIP on ^{14}C-catecholamine synthesis from ^{14}C-DOPA

	^{14}C-catecholamine synthesis
Control	440 ± 25
VIP	435 ± 25

Cells were incubated for 15 min in medium containing ^{14}C-DOPA (2×10^{-5} M) with or without VIP (7.5 μM). ^{14}C-Catecholamine synthesis from ^{14}C-DOPA is expressed in pmol/15 min per 10^6 cells. Values are means ± SEM of four experiments.

that VIP stimulated catecholamine synthesis through an effect on the hydroxylation of tyrosine to DOPA, the rate-limiting step in catecholamine synthesis.

Stimulation of catecholamine synthesis from tyrosine by nicotinic agonists or depolarizing agents is known to depend on extracellular Ca^{2+}. Therefore, we examined whether the stimulatory effect of VIP on catecholamine synthesis was dependent on extracellular Ca^{2+}. As shown in Table 2, the increase in ^{14}C-catecholamine synthesis caused by VIP was not affected by omission of Ca^{2+} from the medium, unlike the increase in ^{14}C-catecholamine synthesis by carbamylcholine, which was dependent on extracellular Ca^{2+}. Therefore, the stimulation of ^{14}C-catecholamine synthesis by VIP did not seem to be mediated by an extracellular Ca^{2+}-dependent mechanism and increased uptake of Ca^{2+} into the cells as in stimulation by carbamylcholine.

The synthesis of catecholamine from tyrosine is also known to be stimulated by cAMP [11]. Therefore, the effect of VIP on the cAMP level in the cells was examined. As shown in Table 3, VIP did not increase the intracellular cAMP level. Forskolin, an activator of adenylate cyclase, increased the cAMP level and stimulated the synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine [12]. Furthermore, VIP did not affect the increase of the cAMP level caused by forskolin. Therefore, the stimulation of catecholamine synthesis by VIP was apparently not mediated by increase in the intracellular cAMP level.

Recently, protein kinase C has been shown to participate in the regulation of catecholamine synthesis from tyrosine [13–16]. Therefore, we examined whether protein kinase C was involved in the

Table 2. Effect of extracellular Ca^{2+} on VIP-, and carbamylcholine-stimulated catecholamine synthesis

	Increase in ^{14}C-catecholamine synthesis	
	VIP	Carbamylcholine
Ca^{2+} (2.2 mM)	25 ± 2	85 ± 3
Ca^{2+} (0 mM)	24 ± 2	1 ± 1*

Cells were incubated for 15 min with or without VIP (7.5 μM) or carbamylcholine (100 μM) in normal or Ca^{2+}-free medium. The increase in synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine in the presence of VIP or carbamylcholine is expressed in pmol/15 min per 10^6 cells. The basal level of ^{14}C-catecholamine synthesis was 80 ± 3 pmol/15 min per 10^6 cells. Values are means ± SEM of 4–6 experiments.

* P < 0.005.

Table 3. Effects of VIP and forskolin on the intracellular cAMP level

	cAMP (pmol/10^6 cells)
Control	3.6 ± 0.2
VIP	3.7 ± 0.2
Forskolin	32.4 ± 1.5*
VIP + Forskolin	33.1 ± 1.7*

Cells were incubated for 15 min with or without VIP (7.5 μM) or forskolin (10 μM) in normal medium containing 0.5 mM IBMX. Values are means ± SEM of 4–6 experiments. Increase in the cAMP level caused by forskolin (10 μM) reached a maximum after 5–10 min incubation, and then decreased slowly. The level of cAMP was still high after 15 min.

* $P < 0.005$ relative to control.

stimulation of catecholamine synthesis by VIP. Table 4 shows the effects of VIP on the stimulation of ^{14}C-catecholamine synthesis from ^{14}C-tyrosine caused by carbamylcholine and the phorbol ester TPA, an activator of protein kinase C. The stimulation of catecholamine synthesis by carbamylcholine (acetylcholine) is known to be dependent on the presence of Ca^{2+} in the medium (Table 2) and is thought to be mediated by Ca^{2+}-calmodulin dependent protein kinase [17–19], while stimulation of catecholamine synthesis by TPA is thought to be mediated by activation of protein kinase C [20–22]. It is reported that 4α-phorbol 12,13-didecanoate does not activate protein kinase C [20]. The synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine was not increased by 4α-phorbol 12,13-didecanoate (10^{-5}–10^{-7} M) (data not shown). As shown in Table 4, the increase in the

Table 4. Stimulation by VIP of catecholamine synthesis and its inhibition by H-7

	Increase in ^{14}C-catecholamine synthesis (pmol/15 min per 10^6 cells)
VIP	25 ± 2
Carbamylcholine	85 ± 3
TPA	86 ± 3
VIP + Carbamylcholine	118 ± 5*
VIP + TPA	89 ± 4
H-7	0 ± 1†
VIP + H-7	1 ± 1†
TPA + H-7	4 ± 1†
VIP + TPA + H-7	5 ± 1†

Cells were incubated in normal medium for 15 min with or without VIP (7.5 μM), carbamylcholine (100 μM), TPA (100 nM) and H-7 (100 μM). The concentrations of carbamylcholine and TPA used were the lowest that produced maximal increase in ^{14}C-catecholamine synthesis from ^{14}C-tyrosine. The increase in synthesis of ^{14}C-catecholamine from ^{14}C-tyrosine is expressed in pmol/15 min per 10^6 cells. The basal level of ^{14}C-catecholamine synthesis was 80 ± 3 pmol/15 min per 10^6 cells. Values are means ± SEM of 4–6 experiments.

* $P < 0.01$ compared to VIP or carbamylcholine alone.
† $P < 0.05$ compared to basal, VIP or TPA alone.

synthesis of ^{14}C-catecholamine by VIP was additive with that by carbamylcholine, but not with that by TPA. These results suggest that the stimulation of catecholamine synthesis by VIP is mediated by a similar mechanism to that by TPA. Moreover, H-7, an inhibitor of protein kinase C [23, 24], was found to inhibit the stimulation of ^{14}C-catecholamine synthesis caused by not only TPA but also VIP (Table 4). Therefore, provided that the effect of H-7 at the concentrations used in this study is specific for protein kinase C, the stimulation of catecholamine synthesis by VIP is probably mediated by a mechanism involving protein kinase C.

We conclude from the present results that VIP stimulates catecholamine synthesis from tyrosine by an effect on tyrosine hydroxylation and suggest that this stimulatory effect of VIP may be mediated by activation of protein kinase C. Further investigations are required on the mechanism by which VIP activates the protein kinase C system. We are studying whether phosphoinositide hydrolysis is induced in response to VIP in adrenal chromaffin cells.

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