Synergistic Effects of Muscarinic Agonists and Secretin or Vasoactive Intestinal Peptide on the Regulation of Tyrosine Hydroxylase Activity in Sympathetic Neurons

N. Y. Ip,* R. E. Zigmond**

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

Received 2 February 1999; accepted 15 June 1999

ABSTRACT: Cholinergic agonists and certain peptides of the glucagon-secretin family acutely increase tyrosine hydroxylase activity in the superior cervical ganglion *in vitro*. The present study was designed to investigate possible interactions between these two classes of agonists in regulating catecholamine biosynthesis. Synergistic effects were found between carbachol and either secretin or vasoactive intestinal peptide in the regulation of DOPA (dihydroxyphenylalanine) synthesis. In addition, synergism was found at the level of the accumulation of cyclic adenosine monophosphate, the likely second messenger in the peptidergic regulation of tyrosine hydroxylase activity. The synergism seen with carbachol was blocked by a muscarinic, but not by a nicotinic, antagonist. Synergism was also found between bethanechol, a muscarinic agonist, and secretin, but not between secretin and dimethylphenylpiperazinium, a nicotinic agonist. Since previous immunohistochemical results suggest that vasoactive intestinal peptide and acetylcholine are colocalized in some preganglionic sympathetic neurons, the present data raise the possibility that the two might act synergistically *in vivo* in regulating catecholamine biosynthesis. Synergistic postsynaptic actions may be a common feature at synapses where peptides of the secretin-glucagon and acetylcholine are colocalized.

 J Neurobiol 42: 14–21, 2000

Keywords: **cholinergic; muscarinic; secretin; tyrosine hydroxylase; vasoactive intestinal peptide**

Tyrosine hydroxylase (TH) (EC 1.14.16.2) catalyzes the rate-limiting step in catecholamine biosynthesis, and its activity is highly regulated (Zigmond, 1985; Zigmond et al., 1989). In the superior cervical ganglion (SCG) of the rat, a number of agonists have been shown to elevate this enzyme activity acutely. For example, carbachol acting via both nicotinic and muscarinic receptors produces an increase in TH activity that has a rapid onset and short duration (Ip et al., 1982a; Horwitz and Perlman, 1984). In addition, certain members of the secretin-glucagon family of peptides increase the activity of this enzyme. These peptides include secretin, vasoactive intestinal peptide (VIP), PHI [a 27-amino-acid peptide (P) containing an NH_2-terminal histidine (H) and a COOH-terminal isoleucine amide (I)], rat growth hormone releasing factor, and helospectin, a component of Gila monster venom (Ip et al., 1982b, 1985; Schwarzschild et al., 1989).

Electrical stimulation of the predominantly preganglionic cervical sympathetic trunk also leads to an acute activation of TH (Ip et al., 1983). The pharma-

* *Present address:* Department of Biology and Biotechnology Research Institute, Hong Kong University Science and Technology, Clear Water Bay, Hong Kong, People's Republic of China

** *Present address:* Case Western Reserve University, School of Medicine, Department of Neurosciences, 10900 Euclid Avenue, Cleveland, OH 44106-4975

Correspondence to: R. E. Zigmond

Contract grant sponsor: NIH; contract grant number: NS 12651

Contract grant sponsor: USPHS; contract grant number: NS 07009

cology of this transsynaptic effect can be both cholinergic and noncholinergic depending on the pattern of nerve stimulation. We have proposed that the noncholinergic transmitter is a member of the secretin-glucagon family of peptides, perhaps VIP (Zigmond, 1998). Neural processes exhibiting VIP-like immunoreactivities (IR) are present in the SCG (Sasek and Zigmond, 1989), as are a population of preganglionic neuronal cell bodies in the thoracic spinal cord that project to the SCG (Baldwin et al., 1991).

In a number of instances where both VIP and acetylcholine elicit tissue responses, the two agents produce synergistic effects (e.g., Gardner and Jackson, 1977; Lundberg, 1981). We therefore looked for possible synergism between the effects of cholinergic and peptidergic agonists in the regulation of ganglionic TH activity. Preliminary reports of some of these results were presented at the International Conference on VIP and Related Peptides (Ip et al., 1984).

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) (weight 160–200 g) were housed in individual plastic cages under controlled lighting (12:12 h light/dark cycle) for 1 week prior to each experiment. In experiments in which decentralized ganglia were used, animals were anesthetized with chloral hydrate (640 mg/kg, subcutaneously) 4 days prior to the experiment, and a short section of each cervical sympathetic trunk was removed proximal to the ganglion. In these experiments, sham-operated animals were used as controls.

The animals were killed by cervical dislocation, and both SCG were removed and desheathed. Individual ganglia were incubated at 37°C in Earle's balanced salt solution (Grand Island Biological Co., Grand Island, NY) supplemented with 0.1 m*M* tyrosine and 0.1 m*M* ethylenediaminetetracetic acid (EDTA) and equilibrated with 95% O_2/5% CO_2. In most of the experiments, ganglia were preincubated for 60 min in control medium or in medium containing a cholinergic agonist, a peptide, or both. Brocresine (an inhibitor of DOPA decarboxylase; 150 μM) was then added to the medium, and the incubation was continued for a further 15 min. Bovine serum albumin (1 mg/mL, crystallized form; Miles, Naperville, IL) was present in both the preincubation and incubation media. This incubation protocol was modified slightly in some experiments, as indicated in the figure legends. TH activity was assayed by measuring the accumulation of DOPA in ganglia and media in the presence of brocresine. Individual ganglia were homogenized, combined with their respective incubation media, and centrifuged. The DOPA content of an aliquot of the supernatant fraction was measured following high-performance liquid chromatography (HPLC) by electrochemical detection as previously described (Ip et al., 1982a). All data are expressed as the mean rate of DOPA accumulation per ganglion per 15 or 30 min [± standard error of the mean (S.E.M.)]. In experiments in which both DOPA and cyclic adenosine monophosphate (cAMP) were measured in the same samples, an aliquot of the supernatant was extracted with water-saturated diethyl ether. The samples were then dried in a Speed-Vac centrifuge (Savant, Hicksville, NY) and the pellets resuspended and assayed for cAMP using the competitive protein binding assay of Brown et al. (1971). In experiments in which synergism between a peptidergic and a cholinergic agonist was examined, one of the agonists was used at a concentration which produced either no effect or a submaximal effect.

Secretin and VIP were purchased from Peninsula Laboratories (Belmont, CA). Carbachol, dimethylphenylpiperazinium (DMPP) iodide, bethanechol chloride, atropine sulfate, and hexamethonium bromide were purchased from Sigma Chemical Co. (St. Louis, MO). Brocresine was a gift from Dr. David N. Ridge (Lederle Laboratories, Pearl River, NY).

RESULTS

To examine possible interactions between carbachol and secretin in their regulation of TH activity, the concentration–response relationship for the effects of secretin on TH activity was examined in the presence and absence of a low concentration of carbachol. Carbachol, at a concentration of 3 μM, produced no significant increase in DOPA synthesis by itself (Fig. 1). However, at all doses of secretin examined (1 n*M* to 1 μM), a larger increase in DOPA synthesis was produced in the presence of carbachol than in its absence (Fig. 1). The maximal fold increase was 2.8 in the presence of secretin alone and 3.4 in the presence of both carbachol and secretin. Similarly, when studies were performed with VIP (0.1–10 μM), a larger effect on DOPA synthesis was seen when peptide was present together with carbachol (3 μM) than when only VIP was present (Fig. 2).

These results raised the question of whether synergism between cholinergic and peptidergic agonists could also be seen at a biochemical step prior to activation of TH. Therefore, we looked at the level of accumulation of cAMP, the second messenger most likely to be involved in the peptidergic regulation of TH (Ip et al., 1985; Waymire et al., 1991). When ganglia were incubated at a low concentration of carbachol (3 μM) together with either secretin or VIP (1 μM), synergism was seen both at the level of DOPA synthesis [Fig. 3(A)] and at the level of cAMP accumulation [Fig. 3(B)].

To investigate whether the effects of carbachol under these conditions are mediated via nicotinic or

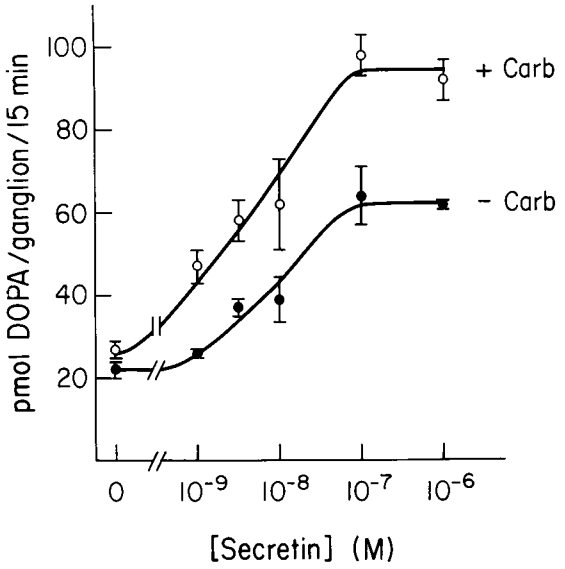

Figure 1 Synergistic effect of carbachol and secretin on DOPA synthesis. Ganglia were preincubated with various concentrations of secretin in the presence or absence of carbachol (Carb, 3 μM) for 60 min. DOPA synthesis during the subsequent 15 min was measured. Each data point represents the mean ± S.E.M. of three ganglia. The apparent EC_{50} for the stimulation of DOPA synthesis by secretin (with or without addition of carbachol) was 5–10 n*M*, as previously reported (Ip et al., 1984).

muscarinic receptors, the ability of hexamethonium and atropine to block this synergism was examined. Since it has been proposed that preganglionic nerve terminals in the SCG contain nicotinic receptors that facilitate the release of acetylcholine (Volle and Koelle, 1961), decentralized ganglia were used in these studies. As in intact ganglia, the effects of secretin (1 μM) on DOPA synthesis [Fig. 4(A)] and on cAMP accumulation [Fig. 4(B)] were potentiated by 3 μM carbachol in decentralized ganglia. These synergistic effects were not altered by the addition of the nicotinic antagonist hexamethonium (3 m*M*) but were completely blocked by the addition of the muscarinic antagonist atropine (6 μM) [Fig. 4(A,B)].

In addition to the use of selective antagonists, the effects of selective nicotinic and muscarinic agonists were examined. Synergism between the muscarinic agonist bethanechol (10 μM to 1 m*M*) and a low concentration of secretin (3 n*M*) was found both at the level of DOPA synthesis [Fig. 5(A)] and at the level of cAMP accumulation [Fig. 5(B)]. On the other hand, no synergism was found between the effects of the nicotinic agonist DMPP and secretin. DMPP (10–100 μM) produced a small increase in DOPA synthesis by itself, but failed to enhance the effect of secretin (1 n*M*) (Fig. 6).

DISCUSSION

Tyrosine hydroxylase activity in the rat SCG is regulated acutely by multiple neurotransmitters (Zigmond et al., 1989). Both cholinergic agonists such as carbachol and peptides such as secretin and VIP increase the activity of this enzyme (Ip et al., 1982a,b). The present study demonstrates that the activation of TH produced by secretin and VIP can be potentiated by the addition of a low concentration of carbachol. Whereas the effect of carbachol by itself on TH activity is mediated in part via nicotinic and in part via muscarinic receptors (Ip et al., 1982a), the ability of carbachol to potentiate the effects of secretin and VIP appears to be mediated entirely via muscarinic receptors. In agreement with these findings, secretin produces synergistic actions on TH activation with the muscarinic agonist bethanechol but not with the nicotinic agonist DMPP.

Interestingly, in electrophysiological studies VIP has been found to enhance the slow ganglionic depolarization produced by acetylcholine acting via muscarinic receptors in the cat SCG (Kawatani et al., 1985) and guinea pig inferior mesenteric ganglion

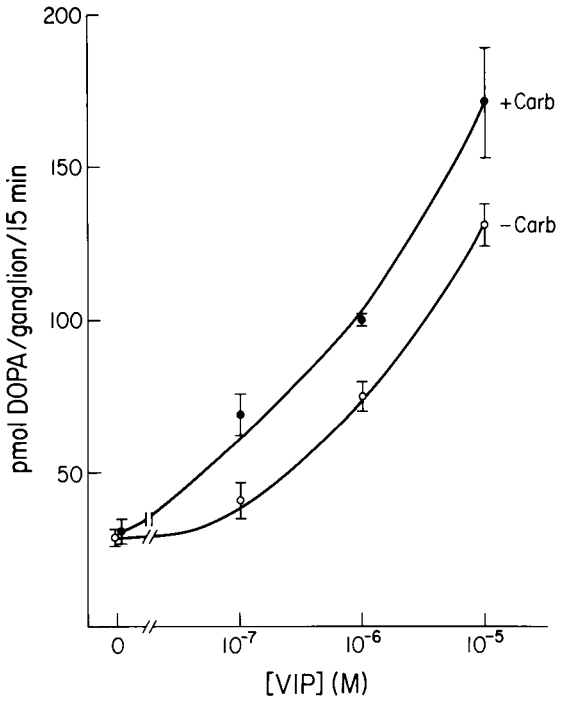

Figure 2 Synergistic effect of carbachol and VIP on DOPA synthesis. Ganglia were preincubated with various concentrations of VIP in the presence or absence of carbachol (Carb, 3 μM) for 60 min. DOPA synthesis during the subsequent 15 min was measured. Each data point represents the mean ± S.E.M. of four ganglia.

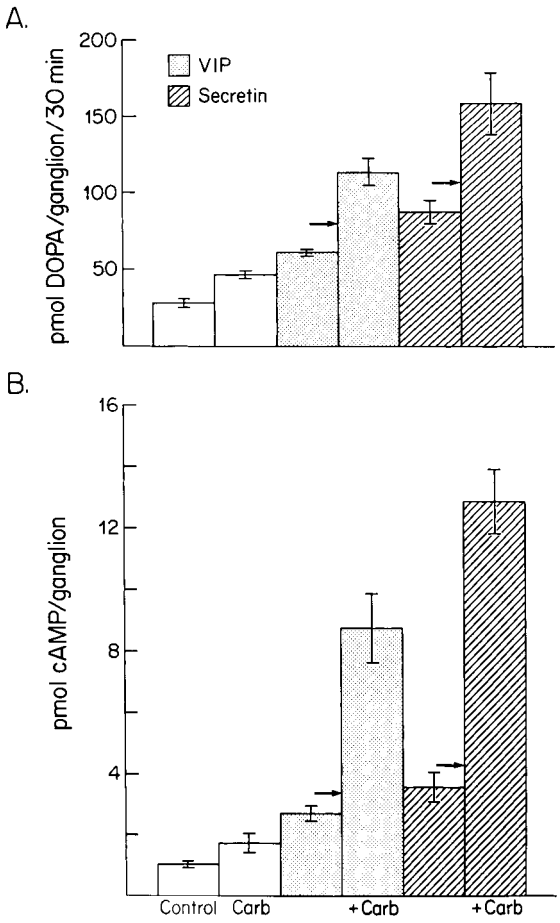

Figure 3 Synergistic effect of carbachol (3 μM) and VIP (1 μM) or secretin (1 μM) on DOPA synthesis (A) and cAMP accumulation (B). Arrows point to the predicted values for DOPA synthesis when the effects of the cholinergic and peptidergic agonists were strictly additive. For measurement of DOPA synthesis, all groups included four ganglia except the control group in which $n = 3$. For measurement of cAMP accumulation, all groups had three to four ganglia except the control group and the secretin plus carbachol groups, where $n = 2$.

(Mo and Dun, 1984), without affecting the rapid nicotinically mediated discharge. Carbachol is also known to augment the actions of VIP and secretin on amylase secretion by pancreatic acinar cells (Collen et al., 1982; Gardner and Jackson, 1977), enhance glucagon secretion by VIP (Ahren and Lundquist, 1982), and potentiate the cAMP response to VIP in both the submandibular gland (Enyedi et al., 1982; Fredholm and Lundberg, 1982) and the olfactory bulb (Olianas and Onali, 1993; Onali and Olianas, 1995). In each case, these actions of carbachol are mediated via muscarinic receptors. One exception to the interaction of peptides of the secretin-glucagon family with acetylcholine occurring via a muscarinic mechanism is the report that VIP potentiates the action of a nicotinic agonist on TH gene expression in bovine chromaffin cells (Olasmaa et al., 1992).

The submandibular gland has been a particularly interesting organ for studying interactions between VIP and acetylcholine, since the two neurotransmitters are colocalized in that gland in terminals of postganglionic parasympathetic neurons and both are released during nerve stimulation (Lundberg, 1981). Considerable evidence indicates that VIP (or a closely related peptide) and acetylcholine are also cotransmitters in the rat SCG. Previous studies on the acute

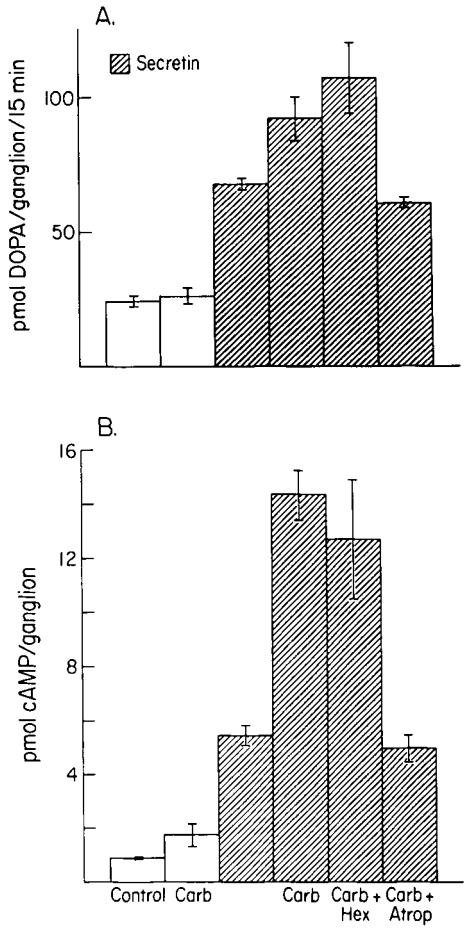

Figure 4 Inhibition of the synergistic effect of carbachol and secretin by a muscarinic antagonist. Decentralized ganglia were preincubated for 60 min with either control medium (Control), carbachol (Carb, 3 μM), secretin (1 μM), or both agonists. Some of the ganglia in the latter group were also exposed to hexamethonium (Hex, 3 mM) or atropine (Atrop, 6 μM). Ganglia that were exposed to these cholinergic antagonists were preincubated with the antagonists for 10 min prior to the addition of carbachol and secretin. DOPA synthesis (A) and cAMP accumulation (B) during the subsequent 15 min were measured. Each bar represents the mean ± S.E.M. of three to four ganglia.

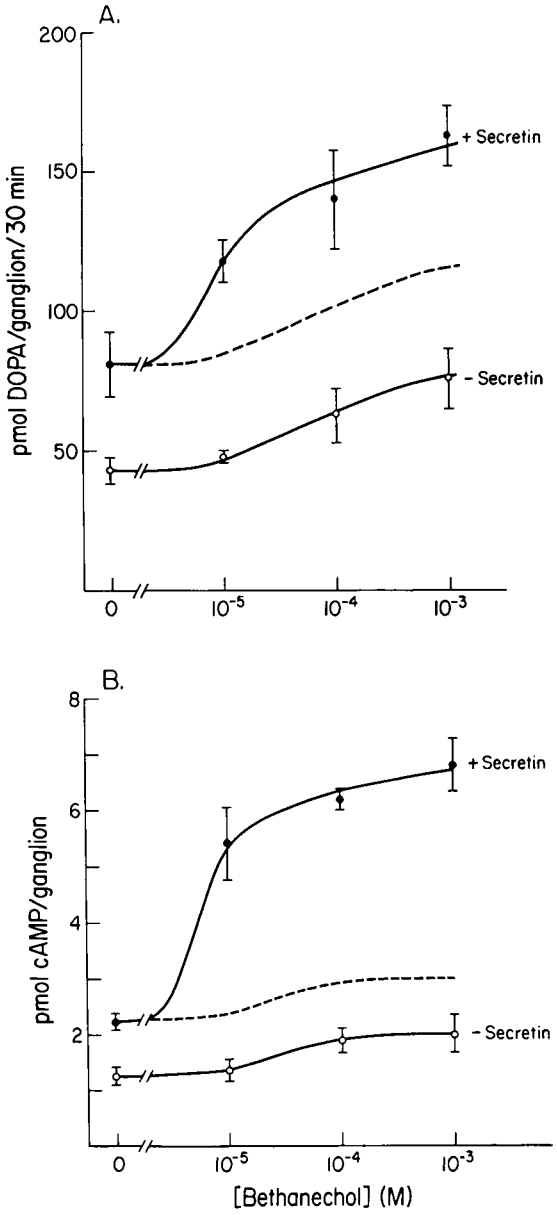

Figure 5 Concentration–response curve for the effects of bethanechol in the presence or absence of a low concentration of secretin. Previously decentralized ganglia were preincubated with control medium for 60 min prior to incubation with various concentrations of bethanechol for 30 min. Other groups of ganglia were preincubated with bethanechol in the presence of secretin (3 n*M*) for 30 min. DOPA synthesis (A) and cAMP accumulation (B) during the incubation period were measured. Each data point represents the mean ± S.E.M. of three to four ganglia. The dotted line indicates the predicted values when the effects were strictly additive.

increase in TH activity in the SCG following preganglionic nerve stimulation have demonstrated the existence of a noncholinergic preganglionic neurotransmitter in the rat SCG (Ip et al., 1983; Ip and Zigmond, 1984). Secretin and VIP are candidates for this noncholinergic transmitter as both of them increase TH activity acutely in the presence of high concentrations of cholinergic antagonists (Ip et al., 1982b). Pituitary adenylate cyclase activating peptide (PACAP), a more recently identified member of the secretin-glucagon family, is another candidate. Whereas the acute effects of PACAP on TH activity have not yet been reported in sympathetic neurons, this peptide acutely increases TH activity in adrenal chromaffin cells (Houchi et al., 1994; Haycock, 1996) via an increase in cAMP levels (Marley et al., 1996). Both VIP-IR and PACAP-IR have been detected in this ganglion by immunohistochemical techniques (Hokfelt et al., 1977; Sasek and Zigmond, 1989; Klimaschewski, 1996) and by radioimmunoassay together with HPLC (Hyatt-Sachs et al., 1993; Brandenburg et al., 1997). In addition, VIP-IR and PACAP-IR have also been found in the thoracic spinal cord in neuronal cell bodies that project to the SCG (Baldwin et al., 1991; Beaudet et al., 1998). No evidence for the presence of

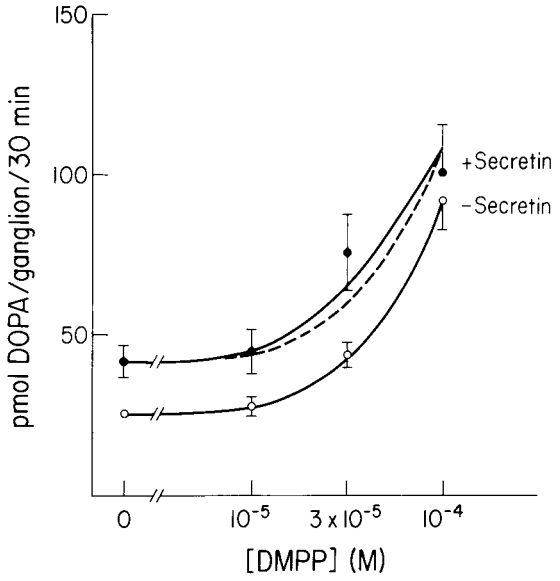

Figure 6 Concentration–response curves of DMPP in the presence or absence of a low concentration of secretin. Previously decentralized ganglia were preincubated with control medium for 60 min prior to incubation with various concentrations of DMPP for 30 min. Other groups of ganglia were preincubated with secretin (3 n*M*) for 60 min prior to incubation with DMPP in the presence of secretin (3 n*M*) for 30 min. DOPA synthesis during the incubation period was measured. Each data point represents the mean ± S.E.M. of four ganglia. The dotted line indicates the predicted values when the effects of DMPP and secretin were strictly additive.

secretin-IR in the SCG or thoracic spinal cord has been reported.

Most of the existing data indicate that the ability of peptides of the secretin-glucagon family to activate TH is mediated via cAMP-dependent phosphorylation (Ip et al. 1985; Roskoski et al., 1989; Schwarzschild et al., 1989; Schwarzschild and Zigmond, 1991; Waymire et al., 1991; Haycock, 1996; but see Houchi et al., 1987). The amino acid residue at which TH becomes phosphorylated in response to different ligands has been studied in adrenal chromaffin cells. VIP leads predominantly to the phosphorylation of Ser^{40}, which is also the primary site of phosphorylation in response to other agents that increase cAMP levels (Waymire et al., 1991; Haycock and Wakade, 1992). Less is known about the signalling pathway used by muscarinic agonists to activate TH (Horwitz and Perlman, 1984; Horwitz et al., 1985; Haycock, 1996). Muscarinic agonists primarily lead to the phosphorylation of TH at Ser^{19} and Ser^{31}, known to be sites of phosphorylation by Ca^{++}/calmodulin-dependent protein kinase II and mitogen-activated protein kinases, respectively (Haycock, 1996). Whereas phosphorylation of Ser^{40} has been shown to cause TH activation, the relationship of phosphorylation of the other two sites to changes in enzyme activity is not clearly established (Haycock, 1996). It would, of course, be interesting to determine whether the synergism between VIP (and secretin) was also seen at the level of phosphorylation of a specific serine residue.

Several biochemical mechanisms have been suggested for the synergism between VIP and muscarinic agonists. In the submandibular gland, for example, VIP has been reported to increase the affinity of muscarinic ligands for their receptors (Lundberg et al., 1982). In the olfactory bulb, the interaction between VIP and muscarinic agonists occurs at the level of adenylate cyclase (Olianas and Onali, 1993; Onali and Olianas, 1995), with an increase in maximal adenylate cyclase activity, with no change in the potency of either agonist. On the other hand, in the adrenal gland, the interaction of the two agents seems to occur at least in part via a post–adenylate cyclase step, as muscarinic agonists can potentiate the response to cAMP analogues in this system (Anderova et al., 1998). These apparent differences may relate to the subtypes of muscarinic receptors and the type of G-proteins involved in each case.

The physiological significance of the synergism between peptides and cholinergic agonists in regulating TH activity remains to be determined. Assuming that under certain physiological circumstances VIP (and/or PACAP) and acetylcholine are coreleased in the SCG during increased sympathetic nerve activity, their ability to act synergistically in regulating TH activity may be an effective mechanism to increase catecholamine synthesis and maintain normal levels of the neurotransmitter norepinephrine (Zigmond et al., 1989; Zigmond, 1998). In addition, the synergism seen at the level of cAMP may lead to increased phosphorylation of other key proteins, in addition to TH.

This research was supported by a grant from the National Institutes of Health (NS 12651). NYI was a postdoctoral trainee of the United States Public Health Service (NS 07009). The authors thank Dr. William C. de Groat for suggesting the use of decentralized ganglia, and Tom Ellenberger for help with the experiments.

REFERENCES

Ahren B, Lundquist I. 1982. Interaction of vasoactive intestinal peptide (VIP) with cholinergic stimulation of glucagon secretion. Experientia 38:405–406.

Anderova M, Duchene AD, Barbara JG, Takeda K. 1998. Vasoactive intestinal peptide potentiates and directly stimulates catecholamine secretion from rat adrenal chromaffin cells. Brain Res 809:97–106.

Baldwin C, Sasek CA, Zigmond RE. 1991. Evidence that some preganglionic sympathetic neurons in the rat contain vasoactive intestinal peptide- or peptide histidine isoleucine amide-like immunoreactivities. Neuroscience 40:175–184.

Beaudet MM, Braas KM, May V. 1998. Pituitary adenylate cyclase activating polypeptide (PACAP) expression in sympathetic preganglionic projection neurons to the superior cervical ganglion. J Neurobiol 36:325–336.

Brandenburg CA, May V, Braas KM. 1997. Identification of endogenous sympathetic neuron pituitary adenylate cyclase-activating polypeptide (PACAP): depolarization regulates production and secretion through induction of multiple propeptide transcripts. J Neurosci 17: 4045–4055.

Brown BL, Albano JDM, Ekins RP, Sgherzi AM. 1971. A simple and sensitive saturation assay method for the measurement of adenosine 3′:5′-cyclic monophosphate. Biochem J 121:561–562.

Cahill AL, Perlman RL. 1984. Electrical stimulation increases phosphorylation of tyrosine hydroxylase in superior cervical ganglion of rat. Proc Natl Acad USA 81: 7243–7247.

Collen, MJ, Sutliffe VE, Pan G-Z, Gardner JG. 1982. Postreceptor modulation of action of VIP and secretin on pancreatic enzyme secretion by secretagogues that mobilize cellular calcium. Am J Physiol 242:423–428.

Durroux T, Barberis C, Jard S. 1987. Vasoactive intestinal polypeptide and carbachol act synergistically to induce

the hydrolysis of inositol containing phospholipids in the rat superior cervical ganglion. Neurosci Lett 75:211–215.

Enyedi P, Fredholm BB, Lundberg JM, Anggard A. 1982. Carbachol potentiates the cyclic AMP-stimulating effect of VIP in cat submandibular gland. Eur J Neurosci 79: 39–143.

Fredholm BB, Lundberg JM. 1982. VIP-induced cyclic AMP formation in the cat submandibular gland: potentiation by carbacholine. Acta Physiol Scand 114:157–159.

Gardner JD, Jackson MJ. 1977. Regulation of amylase release from dispersed pancreatic acinar cells. J Physiol 270:439–454.

Haycock JW. 1996. Short- and long-term regulation of tyrosine hydroxylase in chromaffin cells by VIP and PACAP. Ann NY Acad Sci 805:219–230.

Haycock JW, Wakade AR. 1992. Activation and multiple-site phosphorylation of tyrosine hydroxylase in perfused rat adrenal glands. J Neurochem 58:57–64.

Hokfelt T, Elfin L-G, Schultzberg M, Fuxe K, Said SI, Mutt V, Goldstein M. 1977. Immunohistochemical evidence of vasoactive intestinal polypeptide-containing neurons and nerve fibers in sympathetic ganglia. Neuroscience 2:885–896.

Horwitz J, Perlman RL. 1984. Activation of tyrosine hydroxylase in the superior cervical ganglion by nicotinic and muscarinic agonists. J Neurochem 43:546–552.

Horwitz J, Tsymbalov S, Perlman R. 1985. Muscarine stimulates the hydrolysis of inositol-containing phospholipids in the superior cervical ganglion. J Pharmacol Exp Ther 233:235–241.

Houchi H, Oka M, Misbahuddin M, Morita K, Nakanishi A. 1987. Stimulation by vasoactive intestinal polypeptide of catecholamine synthesis in isolated bovine adrenal chromaffin cells: possible involvement of protein kinase C. Biochem Pharmacol 36:1551–1554.

Houchi H, Hamano S, Masuda Y, Ishimura Y, Azuma M, Ohuchi T, Oka M. 1994. Stimulatory effect of pituitary adenylate cyclase-activating polypeptide on catecholamine synthesis in cultured bovine adrenal chromaffin cells: involvements of tyrosine hydroxylase phosphorylation caused by Ca2+ influx and cAMP. Jpn J Pharmacol 66:323–330.

Hyatt-Sachs H, Schreiber RC, Bennett TA, Zigmond RE. 1993. Phenotypic plasticity in adult sympathetic ganglia in vivo: effects of deafferentation and axotomy on the expression of vasoactive intestinal peptide. J Neurosci 13:1642–1653.

Ip NY, Baldwin C, Zigmond RE. 1984. Acute stimulation of ganglionic tyrosine hydroxylase activity by secretin, VIP, and PHI. Peptides 5:309–312.

Ip NY, Baldwin C, Zigmond RE. 1985. Regulation of the concentration of cAMP and the activity of tyrosine hydroxylase in the rat superior cervical ganglion by three neuropeptides of the secretin family. J Neurosci 5:1947–1954.

Ip NY, Perlman RL, Zigmond RE. 1982a. Both nicotinic and muscarinic agonists acutely increase tyrosine 3-monooxygenase activity in the superior cervical ganglion. J Pharmacol Exp Ther 223:280–283.

Ip NY, Ho CK, Zigmond RE. 1982b. Secretin and vasoactive intestinal peptide acutely increase tyrosine 3-monooxygenase activity in the rat superior cervical ganglion. Proc Natl Acad USA 79:7566–7569.

Ip NY, Perlman RL, Zigmond RE. 1983. Acute transsynaptic regulation of tyrosine 3-monoxygenase activity in the superior cervical ganglion: evidence for both cholinergic and noncholinergic mechanisms. Proc Natl Acad USA 80:2081–2085.

Ip NY, Zigmond RE. 1984. Pattern of presynaptic nerve activity can determine the type of neurotransmitter regulating a postsynaptic event. Nature 311:472–474.

Kawatani M, Rutigliano M, de Groat WC. 1985. Depolarization and muscarinic excitation induced in a sympathetic ganglion by vasoactive intestinal polypeptide. Science 229:879–881.

Klimaschewski L, Hauser C, Heym C. 1996. PACAP immunoreactivity in the rat superior cervical ganglion in comparison to VIP. Neuroreport 7:2797–2801.

Lundberg JM. 1981. Evidence for coexistence of vasoactive intestinal polypeptide (VIP) and acetylcholine in neurons of cat exocrine glands: morphological, biochemical, and functional studies. Acta Physiol Scand 496(Suppl):1–57.

Lundberg JM, Hedlund B, Bartfai T. 1982. Vasoactive intestinal polypeptide enhances muscarinic ligand binding in cat submandibular salivary gland. Nature 295:147–149.

Marley PD, Cheung CY, Thomson KA, Murphy R. 1996. Activation of tyrosine hydroxylase by pituitary adenylate cyclase- activating polypeptide (PACAP-27) in bovine adrenal chromaffin cells. J Auton Nerv Syst 60:141–146.

Mo N, Dun NJ. 1984. Vasoactive intestinal polypeptide facilitates muscarinic transmission in mammalian sympathetic ganglia. Neurosci Lett 52:19–23.

Olianas MC, Onali P. 1993. Synergistic interaction of muscarinic and opioid receptors with GS-linked neurotransmitter receptors to stimulate adenylyl cyclase activity of rat olfactory bulb. J Neurochem 61:2183–2190.

Onali P, Olianas MC. 1995. Bimodal regulation of cyclic AMP by muscarinic receptors: involvement of multiple G proteins and different forms of adenylyl cyclase. Life Sci 56:973–980.

Olasmaa M, Guidotti A, Costa E. 1992. Vasoactive intestinal polypeptide facilitates tyrosine hydroxylase induction by cholinergic agonists in bovine adrenal chromaffin cells. Mol Pharmacol 41:456–464.

Roskoski R Jr, White L, Knowlton R, Roskoski LM. 1989. Regulation of tyrosine hydroxylase activity in rat PC12 cells by neuropeptides of the secretin family. Mol Pharmacol 36:925–931.

Sasek CA, Zigmond RE. 1989. Localization of vasoactive intestinal peptide- and peptide histidine isoleucine amide-like immunoreactivities in the rat superior cervical ganglion and its nerve trunks. J Comp Neurol 280:522–532.

Schwarzschild MA, Zigmond RE. 1991. Effects of peptides of the secretin-glucagon family and cyclic nucleotides on

tyrosine hydroxylase activity in sympathetic nerve endings. J Neurochem 56:400–406.

Schwarzschild MA, Vale W, Corigliano-Murphy AC, Pisano JJ, Ip NY, Zigmond RE. 1989. Activation of ganglionic tyrosine hydroxylase by peptides of the secretin-glucagon family: structure-function studies. Neuroscience 31:159–167.

Volle RL, Koelle GB. 1961. The physiological role of acetylcholinesterase (AChE) in sympathetic ganglia. J Pharmacol Exp Ther 133:223–240.

Waymire JC, Craviso GL, Lichteig K, Johnston JP, Baldwin C, Zigmond RE. 1991. Vasoactive intestinal peptide stimulates catecholamine biosynthesis in isolated adrenal chromaffin cells: evidence for a cyclic AMP-dependent phosphorylation and activation of tyrosine hydroxylase. J Neurochem 57:1313–1324.

Zigmond RE. 1985. Biochemical consequences of synaptic stimulation: the regulation of tyrosine hydroxylase by multiple transmitters. Trends Neurosci 8:63–69.

Zigmond RE. 1998. Regulation of tyrosine hydroxylase by neuropeptides. Adv Pharmacol 42:21–25.

Zigmond RE, Schwarzschild MA, Rittenhouse AR. 1989. Acute regulation of tyrosine hydroxylase by nerve activity and by neurotransmitters via phosphorylation. Annu Rev Neurosci 12:415–461.