Liposomal VIP attenuates phenylephrine- and ANG II-induced vasoconstriction in vivo

HIROYUKI IKEZAKI, HAYAT ÖNYÜKSEL, AND ISRAEL RUBINSTEIN
Departments of Medicine and Pharmaceutics and Pharmacodynamics, University of Illinois at Chicago, and West Side Department of Veterans Affairs Medical Center, Chicago, Illinois 60612

Ikezaki, Hiroyuki, Hayat Önyüksel, and Israel Rubinstein. Liposomal VIP attenuates phenylephrine- and ANG II-induced vasoconstriction in vivo. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R588–R595, 1998.—The purpose of this study was to determine whether vasoactive intestinal peptide (VIP) modulates vasoconstriction elicited by phenylephrine and ANG II in vivo and, if so, to begin to elucidate the mechanisms underlying this phenomenon. Using intravital microscopy, we found that suffusion of phenylephrine and ANG II elicits significant vasoconstriction in the in situ hamster cheek pouch that is potentiated by phenylephrine and ANG II in vivo and, if so, to begin to elucidate the mechanisms underlying this phenomenon. Using intravital microscopy, we found that suffusion of phenylephrine and ANG II in vivo and, if so, to begin to elucidate the mechanisms underlying this phenomenon. Vasoactive intestinal peptide (VIP) modulates vasoconstriction elicited by phenylephrine and ANG II in vivo and, if so, to begin to elucidate the mechanisms underlying this phenomenon.

METHODS

General Methods

Preparation of animals. Adult male Golden Syrian hamsters (n = 46; 132 ± 6 g body wt) were anesthetized with pentobarbital sodium (6 mg/100 g body wt ip). A tracheostomy was performed to facilitate spontaneous breathing. A femoral vein was cannulated to administer supplemental pentobarbital sodium (2–4 mg; 100 g body wt–1 h–1) during the experiment. A femoral artery was cannulated to monitor and record systemic arterial pressure. Body temperature was monitored and maintained constant (37–38°C) throughout the experiment by using a heating pad.

To visualize the microcirculation of the cheek pouch, we used an established method in our laboratory (9, 30, 32–35). Briefly, the left cheek pouch was spread over a small plastic base plate, and an incision was made in the outer skin to expose the cheek pouch membrane. The avascular connective tissue layer of the membrane was removed, and a plastic chamber was positioned over the base plate and secured in place by suturing the skin around the upper chamber. This chamber was connected to a reservoir containing warmed bicarbonate buffer (37–38°C), which allowed sustained continuous infusion of drugs into the suffusate.

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Preparation of VIP on sterically stabilized liposomes. To prepare VIP on sterically stabilized liposomes (SSL), we used a method previously described in our laboratory (31, 32). Briefly, egg yolk phosphatidylcholine, egg yolk phosphatidylethanolamine, and cholesterol were mixed in chloroform. The solvent was evaporated at 45°C in a rotary evaporator under vacuum overnight. The resulting lipid film was rehydrated in 250 µl of saline, vortexed, bath sonicated for 5 min, and extruded through stacked polycarbonate filters using the LiposoFast apparatus (A-5m, Bio-Rad Laboratories, Richmond, CA) and stored at 4°C for 15 days. The size of SSL was 246 ± 6 nm, as determined by quasi-elastic light scattering (Ntech model 29 nm, A-5m, Bio-Rad Laboratories, Richmond, CA) and stored at 4°C for 15 days. The size of SSL was 246 ± 29 nm, as determined by semi-elastic light scattering (Ntech model 270 submicron particle sizer, Pacific Scientific, Menlo Park, CA). The phospholipid concentration in SSL was determined by the Barlett inorganic phosphate assay. VIP concentration in SSL was determined by a commercially available ELISA kit (Peninsula Laboratories, Belmont, CA) after SSL was dissolved with 1% SDS. The recovery was 30% for VIP and 50% for phospholipids, resulting in 0.004 mol VIP/mol phospholipids.

Experimental Protocols

Effects of a VIP receptor antagonist on vasconstriction. The purpose of these studies was to determine whether VIP-(1–28), a VIP receptor antagonist that abrogates VIP-induced vasodilation in the cheek pouch (32), potentiates phenylephrine and ANG II-induced vasconstriction. Bicarbonate buffer was suffused for 45 min (equilibration period). Then phenylephrine (0.01 µM) or ANG II (0.01 nM) was suffused on the cheek pouch for 7 min. Arteriolar diameter was determined during each intervention. In preliminary studies, we determined that arteriolar diameter returns to baseline within 2 and 18 min after suffusion of VIP and SSL on SSL is stopped, respectively. In addition, repeated suffusions of phenylephrine (0.1 µM), ANG II (0.1 nM), VIP, and SSL on SSL (0.1 nmol) were associated with reproducible results. The concentrations of phenylephrine, ANG II, VIP, and SSL on SSL used in these studies are based on previous and preliminary studies in our laboratory and reported in the literature (22, 25, 32–36, 39).

Duration of VIP on SSL-induced responses. The purpose of these studies was to determine the duration of VIP on SSL attenuation of phenylephrine- and ANG II-induced vasconstriction. The experimental design was similar to that outlined above, except that phenylephrine (0.1 µM) or ANG II (0.1 nM) was now suffused 30, 45, or 60 min after suffusion of VIP on SSL (0.1 nmol for 7 min) was stopped. Arteriolar diameter was determined during each intervention.

Mechanisms of responses evoked by VIP on SSL. To begin to probe the mechanisms by which VIP on SSL attenuates phenylephrine- and ANG II-induced vasconstriction, we determined the role of vasodilator prostaglandins and intracellular effector systems in mediating this response.

Role of prostaglandins. The purpose of these studies was to determine whether vasodilator prostaglandins, because VIP has been previously shown to elicit these mediators (15, 17). After the equilibration period, phenylephrine (0.1 µM) or ANG II (0.1 nM) was suffused on the cheek pouch for 7 min. Thirty minutes after suffusion of phenylephrine or ANG II was stopped and arteriolar diameter returned to baseline, indomethacin (10 mg/kg) was infused intravenously over a 30-min period using an infusion pump (Sage Instruments; final volume 1 ml), and suffusion of phenylephrine or ANG II was repeated. In another series of experiments, phenylephrine (0.1 µM) or ANG II (0.1 nM) was suffused on the cheek pouch for 7 min. Thirty minutes after suffusion of phenylephrine or ANG II was stopped and arteriolar diameter returned to baseline, indomethacin (10 mg/kg) was infused intravenously, then VIP on SSL (0.1 nmol for 7 min) was suffused for 7 min. Thirty minutes thereafter, suffusion of phenylephrine or ANG II was stopped and arteriolar diameter returned to baseline, indomethacin (10 mg/kg) was infused intravenously, then VIP on SSL (0.1 nmol) was suffused for 7 min. Thirty minutes thereafter, suffusion of phenylephrine or ANG II was stopped. Arteriolar diameter was determined during each intervention. In preliminary studies, we determined that intravenous infusion of indomethacin (10 mg/kg) for 30 min has no significant effects on arteriolar diameter. The concentration of indomethacin used in these studies has been previously shown to inhibit cytochrome oxidase- and arachidonic acid-induced vasodilation in the cheek pouch (28–30).

Role of NO-, cGMP-, and cAMP-dependent intracellular effector pathways. The purpose of these studies was to determine whether NO-, cGMP-, or cAMP-dependent intracellular
signal transduction pathways that mediate VIP vasorelaxation in the cheek pouch and other vascular beds are involved in attenuation of phenylephrine- and ANG II-induced vasoconstriction by VIP on SSL (2, 10, 15, 16, 32). To accomplish this goal, we determined whether other vasodilators that activate NO-, cGMP-, and cAMP-dependent intracellular signal transduction pathways in the peripheral microcirculation also attenuate phenylephrine- and ANG II-induced vasoconstriction (7, 9, 15, 18, 19, 30, 32–38). The experimental design was similar to that outlined above, except that phenylephrine (0.1 µM) or ANG II (0.1 nM) was suffused for 7 min before and 30 min after suffusion of ACh (1.0 µM), an endothelium- and NO-dependent vasodilator; nitroglycerin (0.1 µM), an endothelium-independent, NO-dependent vasodilator; calcium ionophore A-23187 (1.0 µM), a receptor-independent, endothelium- and NO-dependent vasodilator; 8-bromo-cGMP (8-BrcGMP, 20 µM), a receptor-, endothelium-, and NO-independent vasodilator; or isoproterenol (0.01 µM), an endothelium-independent, NO-dependent vasodilator (10.0 nmol), a receptor-independent vasodilator for 7 min each. Arteriolar diameter was determined during each intervention. In preliminary studies, we determined that the magnitude of vasodilation elicted by the vasodilators at concentrations used in these studies is similar to that elicited by VIP (1.0 nmol) and VIP on SSL (0.1 nmol).

Drugs. Egg yolk phosphatidylcholine, egg yolk phosphatidylglycerol, cholesterol, trehalose, indomethacin, ACh, isoproterenol, calcium ionophore A-23187 hemimagnesium salt, and 8-BrcGMP were purchased form Sigma Chemical (St. Louis, MO). Human VIP was purchased from American Peptide (Sunnyvale, CA). Nitroglycerin was purchased from American Reagent Laboratories (Shirley, NY). Indomethacin was dissolved in sodium bicarbonate. All drugs were diluted in saline to the desired concentrations on the day of the experiment.

Data and statistical analyses. When a compound was suffused on the cheek pouch, we determined the maximal change in arteriolar diameter and used it as the response to that compound in each animal. Arteriolar diameter was expressed as the ratio of experimental to control diameter, with control diameter normalized to 100%, to account for intra- and interanimal variability. Values are means ± SE, except for the size of VIP on SSL and body weight, which are means ± SD, because these data are not used for comparison between experimental groups. Statistical analysis was performed on actual values using repeated-measures ANOVA with Newman-Keuls multiple-range post hoc test to detect values that were different from control values. P < 0.05 was considered significant; n is the number of experiments, with each experiment representing a separate animal.

RESULTS

Mean arterial pressure was 97 ± 2 mmHg at the start and 96 ± 1 mmHg at the conclusion of the experiments (n = 46, P > 0.5).

Effects of a VIP Receptor Antagonist on Vasoconstriction

Suffusion of phenylephrine and ANG II elicits a significant, concentration-dependent decrease in arteriolar diameter from baseline (Figs. 1–3; n = 4/group, P < 0.05). Moreover, phenylephrine (0.01 µM)- and ANG II (0.01 nM)-induced vasoconstriction is significantly potentiated by VIP-(10—28) (10.0 nmol) (Fig. 1; n = 4/group, P < 0.05). Arteriolar diameter decreased by 8.1 ± 0.8 and 8.7 ± 1.1% from baseline during suffusion of phenylephrine (0.01 µM) and ANG II (0.01 nM) alone, respectively, and by 14.1 ± 0.9 and 13.2 ± 0.6% from baseline during suffusion of phenylephrine (0.01 µM) and ANG II (0.01 nM) with VIP-(10—28) (10.0 nmol), respectively (Fig. 1; P < 0.05). Suffusion of VIP-(1—12) (10.0 nmol) had no significant effects on phenylephrine- and ANG II-induced responses (Fig. 1; n = 4/group, P > 0.5).

Effects of VIP on Vasoconstriction

Suffusion of aqueous VIP (1.0 nmol) and VIP on SSL (0.1 and 1.0 nmol) elicits a significant increase in arteriolar diameter from baseline (23.7 ± 1.2, 25.6 ± 0.6, and 39.6 ± 1.4%, respectively, n = 4/group, P < 0.05). Suffusion of empty SSL has no significant effects on arteriolar diameter (n = 4, P > 0.5). Vasoconstriction elicited by phenylephrine (0.1 µM) and ANG II (0.1 nM) was similar before (15.5 ± 0.6 and 15.5 ± 1.0% decrease from baseline, respectively) and after (15.6 ± 1.7 and 14.5 ± 0.7% decrease from baseline, respec-
tively) suffusion of aqueous VIP (1.0 nmol; Fig. 2A; n = 4/group, P > 0.5). By contrast, suffusion of VIP on SSL (0.1 nmol) significantly attenuates phenylephrine- and ANG II-induced vasoconstriction in a concentration-dependent fashion (Fig. 3; n = 4/group, P < 0.05).

Arteriolar diameter decreased by 15.8 ± 0.7 and 16.2 ± 0.6% from baseline during suffusion of phenylephrine (0.1 µM) and ANG II (0.1 nM) before and by 6.7 ± 1.3 and 6.2 ± 0.2% after suffusion of VIP on SSL (0.1 nmol), respectively (Fig. 3; n = 4/group, P < 0.05). Empty SSL

Fig. 2. Effects of suffusion of phenylephrine (A) and ANG II (B) for 7 min each on arteriolar diameter in in situ hamster cheek pouch before and 30 min after suffusion of aqueous VIP for 7 min. Values are means ± SE; n = 4 in each group. *P < 0.05 vs. baseline.

Fig. 3. Effects of suffusion of phenylephrine (A) and ANG II (B) for 7 min each on arteriolar diameter in in situ hamster cheek pouch before and 30 min after suffusion of VIP on sterically stabilized liposomes (SSL) or empty SSL for 7 min. Values are means ± SE; n = 4 in each group. *P < 0.05 vs. baseline; †P < 0.05 vs. phenylephrine or ANG II alone; ‡P < 0.05 vs. 0.1 nmol VIP on SSL.
had no significant effects on responses evoked by phenylephrine (0.1 µM) and ANG II (0.1 nM; Fig. 3; n = 4/group, P > 0.5).

Duration of VIP on SSL-Induced Responses

Attenuation of phenylephrine (0.1 µM)- and ANG II (0.1 nM)-induced vasoconstriction by VIP on SSL (0.1 nmol) was related to the time interval between conclusion of VIP-on-SSL suffusion and subsequent suffusions of both vasoconstrictors. Maximal attenuation was observed within 30 min and was no longer present 60 min after suffusion of VIP on SSL (0.1 nmol) was stopped (Fig. 4; n = 4/group, except for initial response groups to phenylephrine and ANG II, where n = 12).

Mechanisms of Responses Evoked by VIP on SSL

Role of prostaglandins. Pretreatment with indomethacin had no significant effects on VIP-on-SSL (0.1 nmol) attenuation of phenylephrine (0.1 µM)- and ANG II (0.1 nM)-induced vasoconstriction (Fig. 5; n = 4/group, P > 0.5). Role of NO-, cGMP-, and cAMP-dependent intracellular effector pathways. Suffusion of ACh (1.0 µM), nitroglycerin (0.1 µM), calcium ionophore A-23187 (1.0 µM), 8-BrcGMP (20 µM), and isoproterenol (0.01 µM) elicits a significant increase in arteriolar diameter from baseline (20.3 ± 0.5, 20.9 ± 0.8, 21.1 ± 0.3, 21.7 ± 0.6, and 20.2 ± 0.8% increase from baseline, respectively, n = 4/group, P < 0.05). However, suffusion of phenylephrine (0.1 µM) and ANG II (0.1 nM) elicits similar vasoconstriction before and after suffusion of ACh, nitroglycerin, calcium ionophore A-23187, 8-BrcGMP, and isoproterenol (Table 1; n = 4/group, P > 0.5).

DISCUSSION

There are two new findings of this study. First, we found that VIP-(10—28), a VIP receptor antagonist...
that abrogates VIP-induced vasodilation in the cheek pouch (32), but not VIP-(1-12), an inactive peptide fragment, significantly potentiates phenylephrine- and ANG II-induced vasconstriction in the in situ hamster cheek pouch. These data suggest that phenylephrine and ANG II modulate VIP release from nerve endings in this organ. Whether this process is modulated by other vasconstrictors in different microvascular beds and species remains to be determined.

Second, suffusion of aqueous VIP, which assumes a predominantly random coil conformation (11, 27), has no significant effects on phenylephrine- and ANG II-induced vasconstriction. By contrast, association of VIP with SSL, which alters peptide conformation to a predominantly α-helix (11, 27), elicits significant and reversible attenuation of phenylephrine- and ANG II-induced responses. Maximal effect is observed within 30 min and is no longer seen after 60 min. These effects are not mediated by the phospholipid moiety of SSL, because empty SSL are inactive.

The concentration of VIP in the in situ cheek pouch microcirculation during suffusion of aqueous VIP and VIP on SSL was not determined in this study. However, it is not feasible to determine VIP concentration directly in the vicinity of in situ cheek pouch arterioles during suffusion of VIP on SSL, because the peptide remains associated with liposomes for a relatively prolonged period of time (8, 11, 26, 27, 31). Rather, isolated cheek pouch arterioles exposed to aqueous VIP and VIP on SSL in the absence and presence of a detergent to dissolve SSL would have to be used to address this issue (27, 31, 32, 39).

The hamster cheek pouch is an established model to study regulation of vasomotor tone in the peripheral microcirculation under normal and pathophysiological conditions (7, 18, 21, 22, 25, 27-35). Xia et al. (39) showed that phenylephrine and KCl, two potent vasocostrictors, elicit similar transient local and conducted depolarization in smooth muscle and endothelium of isolated cheek pouch arterioles. Importantly, Suzuki et al. (35) showed that VIP-induced vasodilation is blunted in the in situ cheek pouch of hamsters with spontaneous hypertension relative to normoten-

sive hamsters. Partitioning of VIP into phospholipids amplifies and restores VIP vasorelaxation relative to aqueous VIP in the in situ cheek pouch of normotensive and spontaneously hypertensive hamsters, respectively (11, 26, 27, 32, 35, 36). By contrast, Myers et al. (25) showed that ANG II-induced vasconstriction is amplified in the cheek pouch of hamsters with experimentally induced renovascular hypertension. These data, coupled with the results of this study, suggest that VIP regulation of vasomotor tone during phenylephrine- and ANG II-induced vasconstriction is dependent, in part, on secondary structure of the peptide. Further studies are warranted to determine the role of α-helix VIP in modulating vasodilation elicited by other agonists.

The mechanisms underlying the salutary effects of VIP on SSL in the cheek pouch were not identified in this study. Nonetheless, they are not related to local elaboration of vasodilator prostaglandins, because indo-

methacin, at a concentration that inhibits cyclooxygenase- and arachidonic acid-induced vasodilation in the cheek pouch (28-30), has no significant effects on attenuation of phenylephrine- and ANG II-induced vasconstriction by VIP on SSL (17). In addition, the effects of VIP on SSL are not mediated by NO-, cGMP-, or cAMP-dependent intracellular effector mechanisms in the peripheral microcirculation, because selected vasodilators that activate these pathways have no significant effects on phenylephrine- and ANG II-induced vasconstriction.

On balance, these data suggest that α-helix VIP opposes α-adrenergic- and ANG II-induced vasconstriction in the in situ peripheral microcirculation by activating specific plasma membrane-dependent process(es) rather than by eliciting vasodilation that overpowers vasconstriction or by nonspecific effects on vascular smooth muscle contractility (13, 18, 20, 26). Clearly, additional studies using isolated microvascular smooth muscle cells and plasma membranes are indicated to characterize this process(es).

Perspectives

This study suggests that α-adrenergic- and ANG II-dependent metabolic pathways interact with VIP to regulate vasomotor tone in the in situ peripheral microcirculation. This process may be related, in part, to vasodilation-induced release of VIP from nerve endings. The peptide thus released may interact with plasma membrane phospholipids in target cells, change its conformation from predominantly random coil in interstitial fluid to α-helix in phospholipid bilayer, and activate a specific plasma membrane-dependent process(es) that promotes vasodilatoty.

In summary, the results of this study suggest that vasodilation modulates VIP release from nerve endings in the in situ hamster cheek pouch. They indicate that α-helix VIP, the optimal conformation for ligand-receptor interaction (3, 16, 26, 32), opposes α-adrenergic- and ANG II-induced vasconstriction in this organ in a reversible, prostaglandin-, NO-, cGMP-, and cAMP-independent fashion.
REFERENCES


