A Novel Formulation of VIP in Sterically Stabilized Micelles Amplifies Vasodilation *In Vivo*

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Purpose. To determine whether human vasoactive intestinal peptide (VIP)-poly(ethylene glycol) (PEG)-grafted distearoyl-phosphatidylethanolamine (DSPE) micelles elicit potent and stable vasodilation *in vivo*.
Methods. PEG-DSPE micelles were prepared by co-precipitation. VIP was loaded into micelles by incubation at room temperature. Vasoactivity of VIP in SSM was determined by monitoring changes in diameter of resistance arterioles in the *in situ* hamster cheek pouch using intravital microscopy.
Results. VIP easily undergoes self-assembly into small PEG-DSPE micelles (mean [±SEM] size, 18 ± 1 nm) in a time-dependent fashion. This generates a potent vasoactive matrix at nanomole concentrations of VIP as manifested by ~3-fold potentiation and prolongation of vasodilation relative to that evoked by aqueous VIP alone ($p < 0.05$). This response is specific and mediated by the L-arginine/nitric oxide (NO) biosynthetic pathway. Micellar VIP dispersion remains vasoactive for at least 14 days after preparation and storage at 4°C.
Conclusions. A novel, self-associated, small and stable PEG-DSPE micellar formulation of VIP amplifies vasodilation in the *in situ* peripheral microcirculation in a specific fashion by elaborating NO. An optimized formulation could be considered for certain cardiovascular disorders associated with L-arginine/NO biosynthetic pathway dysfunction.

KEY WORDS: microcirculation; vasomotor tone; amphipathic peptides; nitric oxide; PEG-DSPE micelles.

INTRODUCTION

Vasoactive intestinal peptide (VIP) is a pleiotropic 28-amino acid amphipathic mammalian neuropeptide co-localized with nitric oxide (NO) synthase in perivascular nerves (1,2). Upon its release, VIP elicits potent, albeit short-lived, vasodilation in the peripheral circulation (3–5). Recent work from our laboratory showed that this response is amplified by self-assembling VIP on liposomes which activates the L-arginine/NO biosynthetic pathway in the endothelium (6–10). This cascade of biologic responses may be related, in part, to phospholipid-induced conformational transition of VIP molecule from predominantly random coil in aqueous phase to helix, which is optimal for peptide-receptor interaction(s) (11–16).

The unique physico-chemical properties of VIP in lipid environment could potentially be exploited to develop liposomal formulations of VIP to restore L-arginine/NO biosynthetic pathway responsiveness in certain cardiovascular disorders, such as systemic and pulmonary hypertension, peripheral vascular disease, impotence and heart failure (17). However, preparation of such formulations is time-consuming and, once prepared, reproducibility and long-term stability of batches are uncertain thus limiting clinical applicability (7). These considerations led us to develop self-assembled VIP in sterically stabilized phospholipid micelles (VIP in SSM).

Linear polymeric chains composed of an hydrophobic phospholipid, such as distearoyl-phosphatidylethanolamine (DSPE), and an hydrophilic polymer, such as poly(ethylene glycol) (PEG), have a tendency to form micelles in aqueous phase (18–21). The core-shell structure of the micelle is stable due to low critical micellar concentration (CMC) of DSPE and maintains water solubility due to the outer hydrophilic shell of PEG. It is expected that loading of VIP on PEG-DSPE micelles would proceed passively and consistently with VIP assuming helix conformation in the complex because of robust electrostatic and hydrophobic interactions between cationic and hydrophobic poles of VIP molecule and respective anionic phosphate headgroup and hydrophobic tail of DSPE moiety (13,15,18,19).

Moreover, the solid-like core of these micelles equilibrates slowly with phospholipid chain unimers thereby promoting uniform particle size, consistent loading of VIP and reproducibility between batches (18–21). Importantly, the expected small size of PEG-DSPE micelles compared to liposomes, which lack bulk phospholipids, and the presence of a PEG "brush" on the surface will provide steric stabilization of these colloidal particles and reduce exchange of VIP in the micellar core with bulk water resulting in amplified vasoreactivity.

The purpose of this study was to begin to address this paradigm by determining whether human VIP-PEG-DSPE micelles elicit potent and stable vasodilation *in vivo*.

METHODS

Chemicals and Drugs

Human VIP was obtained from American Peptide Company (Sunnyvale, CA). PEG-DSPE was obtained from Avanti Polar Lipids Inc. (Alabaster, AL). *Escherichia coli* nitrate reductase, $NaNO_2$, $NaNO_3$, 3-isobutyl-1-methylxanthine, N^G-nitro-L-arginine (L-NNA), L-arginine and D-arginine were obtained form Sigma Chemical Co. (St. Louis, MO). D-NNA was obtained from Alexis Biochemicals (San Diego, CA). All drugs were prepared before each experiment and diluted in saline to the desired concentrations on the day of the experiment.

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ABBREVIATIONS: VIP, vasoactive intestinal peptide; NO, nitric oxide; PEG, poly(ethylene glycol) (PEG); DSPE, distearoyl-phosphatidylethanolamine; CMC, critical micellar concentration; SSM, sterically stabilized micelles; L-NNA, N^G-nitro-L-arginine; D-NNA, N^G-nitro-D-arginine.

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Preparation of VIP in SSM

In preliminary studies, we determined that CMC of PEG (mol mass, 2,000)-DSPE in saline is 0.8 μM by fluorescence and surface tension determinations. We then used 1.0 μM PEG-DSPE in all subsequent experiments to ascertain formation of sterically stabilized micelles (SSM). PEG-DSPE was dissolved in chloroform and mixed in a round bottom flask. The organic solvent was evaporated using a rotoevaporator (Labconco, Kansas City, MO) at 45°C and desiccation under vacuum overnight. The dry lipid film was then hydrated with saline and the resulting SSM suspension was incubated with different concentrations of human VIP for predetermined time intervals at room temperature before use. Size of VIP in SSM was 18 ± 1 nm as determined by quasi-elastic light scattering (Model 270, Nicomp submicron particle sizer; Pacific Scientific, Menlo Park, CA).

Preparation of Animals

The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23 revised 1985). Adult, male golden Syrian hamsters (n = 45) weighing 130 ± 3 g were anesthetized with pentobarbital sodium (6 mg/100 g body weight, i.p.). A tracheostomy was performed to facilitate spontaneous breathing. A femoral vein was cannulated to inject supplemental anesthesia during the experiment (2–4 mg/100 g body weight/h). A femoral artery was cannulated to record systemic arterial blood pressure and heart rate. Body temperature was monitored and kept constant (37–38°C) via a feedback controller and heating pad throughout the experiment.

To visualize the microcirculation of the cheek pouch, we used a method previously described in our laboratory (7–10,22–25). Briefly, the left cheek pouch was spread over a plastic base plate and an incision was made in the overlying skin to expose the cheek pouch membrane. The avascular connective tissue layer of the membrane was carefully removed and an upper plastic chamber was positioned over the base plate. This arrangement forms a triple-layered complex: the base plate, the upper chamber, and the cheek pouch membrane exposed between the two plates. The chamber contains the suffusion fluid and is connected via a three-way valve to a reservoir that allows continuous suffusion of the cheek pouch with warm (37–38°C) bicarbonate buffer (pH 7.4) bubbled continuously with 95% N_2-5% CO_2 at a rate of 2 ml/min. The chamber is also connected via a three-way valve to an infusion pump (Sage Instruments, Boston, MA) for controlled administration of drugs into the suffusate.

Determination of Arteriolar Diameter

The cheek pouch microcirculation was visualized with a microscope (Nikon, Tokyo, Japan) coupled to a 100−W mercury light source at a magnification of ×40. The microscope image was projected through a low-light TV camera (Panasonic TR-124 MA, Matsushita Communication Industrial Co., Ltd., Yokohama, Japan) onto a video screen (Panasonic). The inner diameter of second order arterioles (48–55 μm), which regulate vascular resistance in the cheek pouch, was determined during the experiment from the video display of the microscope image using a videomicrometer (VIA 100; Boeckler Instruments, Tucson, AZ). In each animal, the same arteriolar segment was used to measure changes in diameter during the experiment.

Nitrites Assay

The concentration of nitrites in the suffusate was determined by a modified Griess reaction as previously described in our laboratory (26). Briefly, samples (1 ml) of the suffusate were incubated with *E.* coli nitrate reductase (0.5 U/ml) at room temperature for 10 min to convert nitrates to nitrites. Thereafter, total nitrites was measured in duplicate by mixing each sample with an equal volume (400 μl) of the Griess reagent. The mixture was incubated at room temperature for 10 min and absorbency was determined at 540 nm using a spectrophotometer (SpectraMAX 340; Molecular Devices, Palo Alto, CA). The concentration of nitrites in each sample, expressed as μM, was determined from a standard curve obtained using known concentrations of $NaNO_2$ and $NaNO_3$ in distilled water. Nitrites concentration in buffer was subtracted from experimental values.

Experimental Protocols

Effects of VIP in SSM on Arteriolar Diameter

We used 3 strategies to address this issue. First, the effect of length of incubation of VIP with SSM *in vitro* on subsequent vasodilation in the cheek pouch was determined. VIP (0.1 nmol) was incubated in 1.4 ml saline alone or in saline containing SSM *in vitro* at room temperature (23 ± 1°C) for 1, 5, 15, 30, 60, 120, 240, and 360 min. At the conclusion of the incubation period, bicarbonate buffer was suffused on the cheek pouch for 45 min (equilibration period) followed by suffusion of each formulation of VIP in SSM for 7 min in an arbitrary fashion. At least 45 min elapsed between subsequent suffusions. Arteriolar diameter was determined before, every minute during suffusion of VIP and VIP in SSM and thereafter until arteriolar diameter returned to baseline. Second, we determined the concentration-dependency of VIP in SSM-induced vasodilation. Increasing concentrations of VIP (0.005, 0.01 and 0.1 nmol) were incubated in saline alone or in saline containing SSM *in vitro* for 120 min at room temperature and then suffused for 7 min each in an arbitrary fashion. The 120-min incubation period was chosen based on results of the studies outlined above. Third, VIP (0.1 nmol) was incubated in saline containing SSM *in vitro* for 120 min at room temperature and stored at 4°C for 44 days. At predetermined time intervals thereafter, a sample of the stock solution was equilibrated at room temperature and then suffused on the cheek pouch for 7 min. In preliminary studies, we determined that repeated suffusions of VIP (0.1 nmol) incubated in saline alone or in saline containing SSM *in vitro* at room temperature for 1, 5, 15, 30, 60, 120, 240, and 360 min were associated with reproducible vasodilation. Suffusion of SSM alone for 7 min had no significant effect on arteriolar diameter (−0.1 ± 1.7% increase from baseline; n = 4; $p > 0.5$). Likewise, suffusion of saline (vehicle) for the entire duration of the experiment had no significant effects on arteriolar diameter. The concentrations of VIP and VIP in SSM used in these studies are based on previous and preliminary studies in our laboratory (6–10,14,22,25).

Effects of NO Synthase Inhibition on VIP in SSM-Induced Responses

The purpose of this study was to determine whether VIP in SSM-induced vasodilation in the cheek pouch is mediated,

in part, by the L-arginine/NO biosynthetic pathway. After the equilibration period, VIP (0.1 nmol) incubated in saline containing SSM *in vitro* at room temperature for 120 min was suffused for 7 min. Once suffusion was stopped and arteriolar diameter returned to baseline, L-NNA, an NO synthase inhibitor (17), or D-NNA (each, 10 μM) was suffused for 30 min before and during suffusion of VIP in SSM (0.1 nmol). In some experiments, L-arginine, the substrate for NO synthase, or D-arginine (each, 100 μM) was suffused together with L-NNA (10 μM) before and during suffusion of VIP in SSM (0.1 nmol). In other groups of animals, 2 concentrations of VIP (0. 1 and 1.0 nmol) were incubated in saline *in vitro* at room temperature for 120 min and then suffused for 7 min each before and during suffusion of L-NNA (10 μM). At least 45 min elapsed between subsequent suffusions of agonists. Arteriolar diameter was determined during each intervention. In preliminary studies, we determined that suffusion of L-NNA, D-NNA (each, 10 μM), L-arginine and D-arginine (each, 100 μM) for 37 min had no significant effects on arteriolar diameter. In addition, suffusion of 1.0 nmol VIP incubated in saline *in vitro* at room temperature for 120 min elicited vasodilation similar to that evoked by 0.1 nmol VIP incubated in saline containing SSM *in vitro* at room temperature for 120 min. The concentrations of L-NNA, D-NNA, L-arginine and D-arginine used in these studies are based on previous studies in our laboratory (8,23).

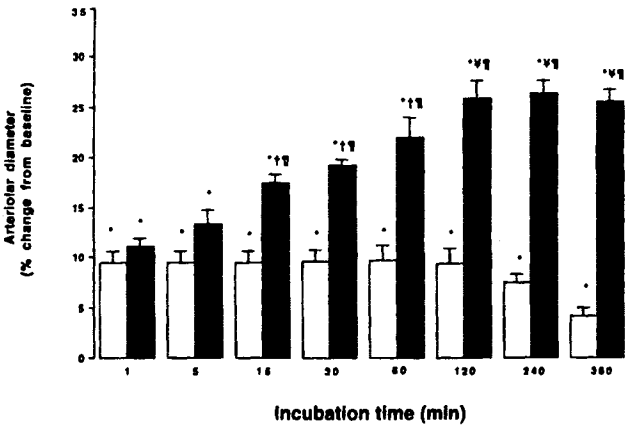

Fig. 1. Effects of duration of incubation of vasoactive intestinal peptide (VIP; 0.1 nmol) in saline (open bars) or in saline containing sterically stabilized poly(ethylene glycol)(PEG)-distearoyl-phosphatidylethanolamine (DSPE) micelles (SSM; 1.0 μM phospholipid; closed bars) *in vitro* at room temperature on arteriolar diameter elicited by 7-min suffusion of these formulations on the *in situ* hamster cheek pouch. Values are means ± SEM; each group, n = 4. *p < 0.05 in comparison to baseline. †p < 0.05 in comparison to VIP incubated in saline containing SSM for 15 min. ¥p < 0.05 in comparison to VIP incubated in saline containing SSM for 30 min. ¶p < 0.05 in comparison to VIP incubated in saline.

Data and Statistical Analyses

When a drug was suffused on the cheek pouch, we determined the maximal change in arteriolar diameter and used it as the response to that drug 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 inter-animal variability. Data are expressed as means ± SEM except for the size of VIP in SSM and body weight which are expressed as mean ± SD because these data are not used for comparison between experimental groups. Statistical analysis was performed using repeated-measures analysis of variance with Neuman-Keuls multiple-range post hoc test to detect values that were different from control values. A p value <0.05 was considered statistically significant. *n* is given as the number of experiments, with each experiment representing a separate animal.

RESULTS

Mean arterial pressure was 102 ± 3 mmHg at the start and 98 ± 4 mmHg at the conclusion of the experiments (n = 45; p > 0.5).

Effects of VIP in SSM on Arteriolar Diameter

Incubation of VIP (0.1 nmol) in saline *in vitro* at room temperature elicited a significant, incubation time-independent increase in arteriolar diameter when suffused on the cheek pouch (Fig. 1; each group, n = 4; p < 0.05). Vasodilation elicited by VIP (0.1 nmol) incubated in saline for 360 min tended to be lower than that evoked by VIP (0.1 nmol) incubated for shorter periods of time (Fig. 1). By contrast, incubation of VIP (0.1 nmol) in saline containing SSM *in vitro* at room temperature elicited a significant incubation time-dependent potentiation of vasodilation relative to that evoked by VIP (0.1 nmol) incubated in saline (Fig. 1; each group, n = 4; p < 0.05). This response was evident within 15 min of incubation and was maximal at 120 min. The latter time point was, therefore, chosen for all subsequent experiments.

Suffusion of VIP in saline elicited a significant increase in arteriolar diameter only at the highest concentration used (Fig. 2; n = 4; p < 0.05). Arteriolar diameter increased by 1.7 ± 1.0%, 3.3 ± 1.4% and 9.4 ± 1.5% from baseline during suffusion of 0.005, 0.01, and 0.1 nmol VIP in saline, respectively (Fig. 2). However, vasodilation elicited by VIP in SSM

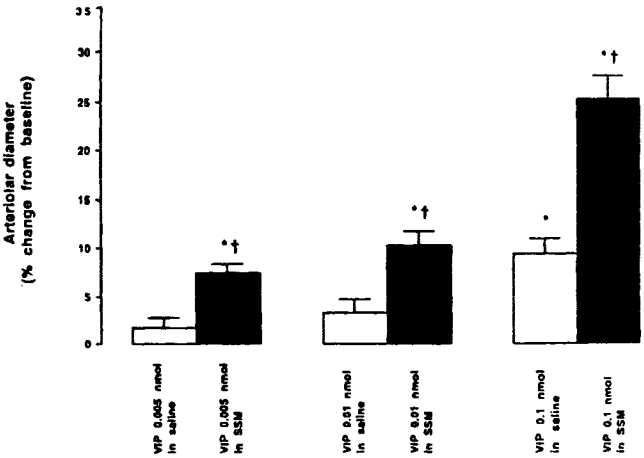

Fig. 2. Effects of 7-min suffusion of vasoactive intestinal peptide (VIP) incubated in saline (open bars) or in saline containing sterically stabilized sterically stabilized poly(ethylene glycol) (PEG)-distearoyl-phosphatidylethanolamine (DSPE) micelles (SSM; 1.0 μM phospholipid; closed bars) *in vitro* at room temperature for 120 min on arteriolar diameter in the *in situ* hamster cheek pouch. Values are means ± SEM; each group, n = 4. *p < 0.05 in comparison to baseline. †p < 0.05 in comparison to VIP incubated in saline.

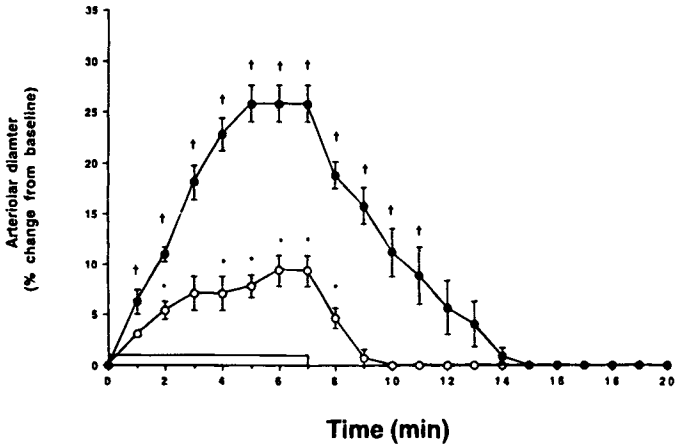

Fig. 3. Effects of 7- min suffusion of vasoactive intestinal peptide (VIP; 0.1 nmol) incubated in saline (open circles) or in saline containing sterically stabilized poly(ethylene glycol) (PEG)-distearoyl-phosphatidylethanolamine (DSPE) micelles (SSM; 1.0 μM phospholipid; closed circles) *in vitro* at room temperature for 120 min on arteriolar diameter in the *in situ* hamster cheek pouch. Open bar, duration of suffusion. Values are means ± SEM; each group, n = 4. *p < 0.05 in comparison to baseline; †p < 0.05 in comparison to VIP incubated in saline.

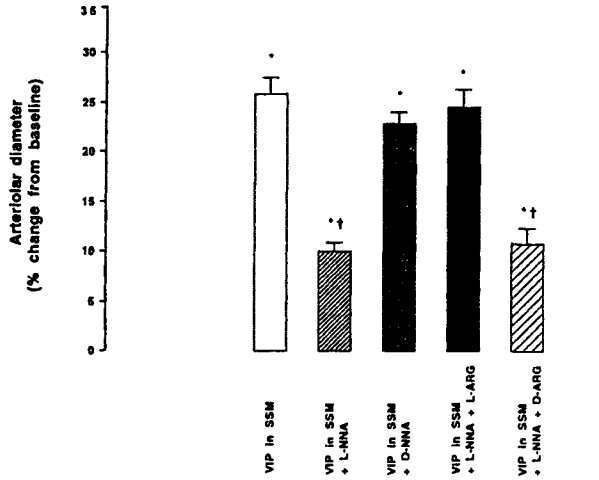

Fig. 5. Effects of 7-min suffusion of vasoactive intestinal peptide (VIP; 0.1 nmol) incubated in saline containing sterically stabilized poly(ethylene glycol) (PEG)-distearoyl-phosphatidylethanolamine (DSPE) micelles (SSM; 1.0 μM phospholipid) *in vitro* for 120 min at room temperature on arteriolar diameter in the *in situ* hamster cheek pouch in the absence and presence of N^G-nitro-L-arginine (L-NNA; 10 μM), D-NNA (10 μM) and L-NNA (10 μM) together with L-arginine or D-arginine (100 μM). Values are means ± SEM; each group, n = 4. *p < 0.05 in comparison to baseline. †p < 0.05 in comparison to VIP incubated in saline containing SSM.

was amplified relative to that evoked by VIP in saline in a concentration-dependent fashion (Fig. 2; each group, n = 4; p < 0.05). Arteriolar diameter increased by 7.4 ± 0.9%, 10.3 ± 1.4%, and 25.2 ± 2.4% during suffusion of 0.005, 0.01, and 0.1 nmol VIP in SSM, respectively (Fig. 2). Importantly, vasodilation evoked by VIP (0.1 nmol) in SSM lasted 7.0 ± 0.4 min after suffusion was stopped whereas that of VIP (0.1 nmol) in saline lasted only 2.5 ± 0.5 min (Fig. 3; each group, n = 4; p < 0.05). Vasodilation elicited by VIP (0.1 nmol) in SSM after 4, 8, 15, 22, and 44 days of storage at 4°C was 86%, 83%, 78%, 69%, and 47% of the initial response evoked by the fresh formulation, respectively (Fig. 4). Suffusion of VIP in SSM (0.1 nmol) for 7 min was not associated with leaky site formation in the cheek pouch (n = 4; [7–9]).

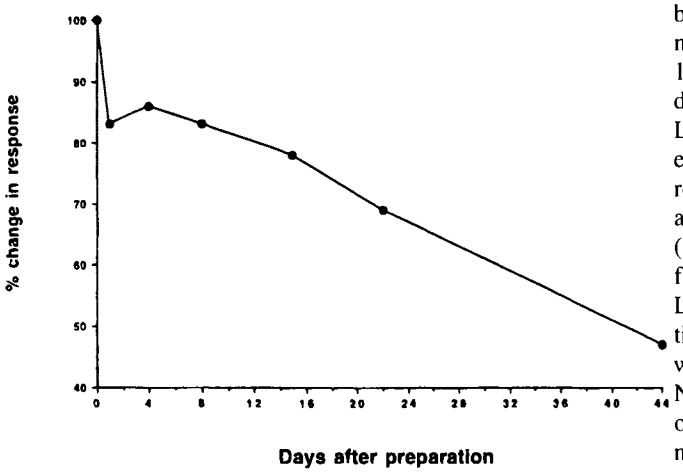

Fig. 4. Effects of 7-min suffusion vasoactive intestinal peptide (VIP; 0.1 nmol) incubated in saline containing sterically stabilized poly-(ethylene glycol) (PEG)-distearoyl-phosphatidylethanolamine (DSPE) micelles (SSM; 1.0 μM phospholipid) *in vitro* for 120 min at room temperature and stored at 4°C for 44 days on arteriolar diameter in the *in situ* hamster cheek pouch. Data are expressed as per cent change from initial response evoked by this formulation on the day of preparation (Day 0).

Effects of NO Synthase Inhibition on VIP in SSM-Induced Responses

Suffusion of VIP in SSM (0.1 nmol), but not of VIP (0.1 nmol) or SSM, was associated with a significant increase in nitrites concentration in the suffusate from 0.21 ± 0.08 μM during suffusion of saline (vehicle) to 9.65 ± 0.33 μM during suffusion of VIP in SSM (n = 3; p < 0.05). L-NNA (10 μM), but not D-NNA (10 μM), significantly attenuated VIP (0.1 nmol) in SSM-induced increase in arteriolar diameter (25.8 ± 1.8%, 10.0 ± 1.0% and 22.8 ± 1.2% increase from baseline during suffusion of VIP in SSM alone, VIP in SSM with L-NNA and VIP in SSM with D-NNA, respectively; Fig. 5; each group, n = 4; p < 0.05 for VIP in SSM with L-NNA relative to VIP in SSM alone). L-NNA-induced responses were abrogated by excess L-arginine (100 μM) but not D-arginine (24.5 ± 1.9% and 10.8 ± 1.6% increase in arteriolar diameter from baseline after suffusion of VIP in SSM, L-NNA and L-arginine, and VIP in SSM, L-NNA and D-arginine, respectively; Fig. 5; each group, n = 4; p > 0.5 for VIP in SSM with L-NNA and L-arginine relative to VIP in SSM with L-NNA). By contrast, L-NNA (10 μM) had no significant effects on VIP induced responses (Fig. 6; each group, n = 4; p > 0.5).

DISCUSSION

There are three new findings of this study. First, we found that human VIP, a potent vasoactive amphipathic neuropeptide (11–15), readily undergoes self-assembly into small PEG-DSPE micelles at room temperature in a time-dependent fashion. This interaction generates a potent vasoactive matrix at

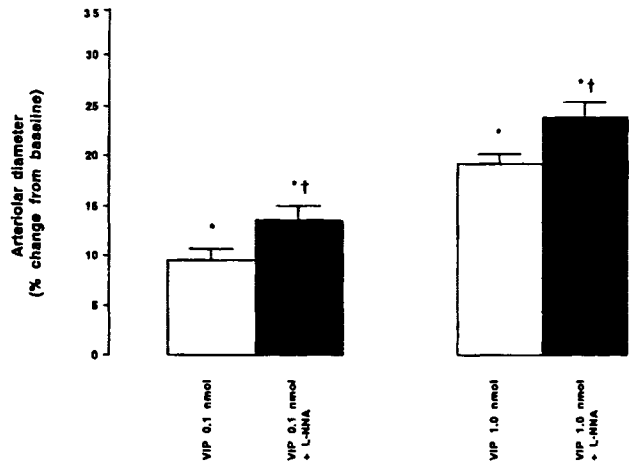

Fig. 6. Effects of 7-min suffusion of vasoactive intestinal peptide (VIP) on arteriolar diameter in the *in situ* hamster cheek pouch in the absence and presence of N^G-nitro-L-arginine (L-NNA; 10 μM). Values are means ± SEM; each group, n = 4. *p < 0.05 in comparison to baseline. † < 0.05 in comparison to VIP above.

nanomole concentrations of VIP as manifested by ~3-fold potentiation and prolongation of vasodilation relative to that evoked by aqueous VIP in the *in situ* hamster cheek pouch. Second, vasodilation elicited by VIP in SSM is reproducible and mediated, in part, by activation of the L-arginine/NO biosynthetic pathway. This response is not related to non-specific effects on microvascular endothelium because suffusion of empty PEG-DSPE micelles has no significant effects on arteriolar diameter, and because arteriolar diameter returns to baseline once suffusion of VIP in SSM is stopped. Third, micellar VIP dispersion remains vasoactive for at least 14 days after preparation and storage at 4°C.

Although the mechanisms underlying self-assembly, amplified vasoreactivity and stability of VIP in SSM were not elucidated in this study, they may have been related, in part, to hydrophobic self-insertion of VIP into the micellar core which stabilizes the matrix, protects the peptide from degradation by shielding susceptible peptide bonds and shifts peptide conformation from random coil to helix, which is optimal for VIP-receptor interaction(s) (11–16,19–21,27). To this end, Ikezaki et al (22) showed that a monoclonal anti-VIP antibody that binds VIP with high affinity and specificity and catalyzes its cleavage *in vitro* attenuates VIP-induced vasodilation in the cheek pouch. Insertion of the peptide on sterically stabilized liposomes curtails this response. On balance, these data suggest that self-association of VIP on PEG-DSPE micelles coupled with bioavailability and stability of the resulting formulation are governed by complex molecular interactions between VIP and hydrophobic core and outer hydrophilic shell of the micelles. Additional studies characterizing these interactions are presently underway in our laboratory.

Séjourné et al (8) showed that vasodilation elicited by aqueous VIP in the cheek pouch is NO-independent whereas that evoked by VIP on sterically stabilized liposomes is NO-dependent. The results of this study support and extend these observations by showing that self-assembly of VIP into PEG-DSPE micelles elicits NO-dependent vasodilation. Conceivably, this response could be related, in part, to binding of micellar VIP to membrane-bound calmodulin, an integral component of NO synthase (28), in microvascular endothelium leading to activation of the L-arginine/NO biosynthetic pathway (28–30). This notion is supported by the study of Ikezaki et al (25) who showed recently that calmodulin selectively amplifies vasodilation elicited by VIP on sterically stabilized liposomes in the cheek pouch through activation of the L-arginine/NO biosynthetic pathway. Alternatively, micellar VIP may be delivered into target cells through peptide exchange between PEG-DSPE micelles and other phospholipid components of the microvascular endothelium and interact directly with an NO synthase-activating G protein(s) (31).

Elaboration of NO in the cheek pouch during suffusion of VIP in SSM was documented by an increase in nitrites concentration in the suffusate, and by stereospecific pharmacologic inhibition and restoration of NO synthase activity (4,5,17,23,25,26). To this end, Séjourné et al (8) showed that pharmacologic inhibition of NO synthase has no significant effects on vasodilation elicited by adenosine, a receptor- and endothelium-dependent, NO-independent agonist, in this organ attesting to the specificity of the pharmacologic approach used in this study. Clearly, further studies using cellular, biochemical and molecular biology techniques are indicated to determine the role of the L-arginine/NO biosynthetic pathway in modulating VIP in SSM-induced vasodilation in the cheek pouch.

Sterically stabilized micelles composed of biocompatible, biodegradable and non-toxic amphiphilic block copolymers have recently been introduced as particulate carriers for delivering antineoplastic drugs and contrast agents (18–20). Application of VIP to treat certain cardiovascular disorders has previously been attempted (7,10). However, rapid degradation of VIP is being perceived as compromising the development of adequate formulations for clinical use (24,27). Nonetheless, Séjourné et al (7) showed that intravenous injection of nanomole concentrations of VIP on sterically stabilized liposomes normalizes systemic arterial pressure in spontaneously hypertensive hamsters for hours while having no significant effects in normotensive hamsters. Unlike liposomes, VIP in SSM lack internal aqueous compartments and equilibrate at a faster rate than do metastable bilayer structures (18–21). Hence, VIP in SSM could be readily stored in a freeze-dried form for a long period of time. In view of ease of preparation, amplified vasoreactivity and improved stability of VIP in SSM, further optimization of this formulation intended to restore L-arginine/NO biosynthetic pathway responsiveness in certain cardiovascular disorders is warranted.

In summary, we developed and tested a novel, self-associated, small and stable PEG-DSPE micellar formulation of VIP. This formulation amplifies VIP vasorelaxation in the *in situ* peripheral microcirculation in a specific fashion by elaborating NO. We suggest that an optimized PEG-DSPE micellar formulation of VIP could be considered for certain cardiovascular disorders associated with L-arginine/NO biosynthetic pathway dysfunction.

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