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Intratracheal and subcutaneous liposomal VIP normalizes arterial pressure in spontaneously hypertensive hamsters

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Abstract

We determined whether a single intratracheal and subcutaneous administration of biocompatible and biodegradable vasoactive intestinal peptide self-associated with sterically stabilized liposomes (VIP-SSL) normalizes mean arterial pressure (MAP) in spontaneously hypertensive hamsters (SHH). We found that VIP-SSL (0.1 nmol) administered by either routes normalizes MAP ($p < 0.05$). Maximal effect was observed within 10–20 min and lasted for 6 h. VIP-SSL had no significant effects on heart rate. VIP alone (0.1 nmol) and empty SSL had no significant effects on MAP. VIP-SSL (0.1 nmol) had no significant effects on MAP and heart rate in age/genetically-matched control hamsters. Given these data, we suggest that pulmonary and subcutaneous delivery of VIP-SSL should be further developed as peptide nanomedicine for essential hypertension.

Keywords: Formulation; Drug delivery; DSPE-PEG; Sterically stabilized liposomes

Despite recent advances in medical therapeutics, essential hypertension still represents an unmet medical need (Cosentino and Volpe, 2005). Hence, there is an ongoing need to develop and test new drugs to treat this condition (Henning and Sawmiller, 2001). To this end, we sought to determine whether a single intratracheal and subcutaneous administration of self-associated VIP with biocompatible and biodegradable sterically stabilized liposomes (VIP-SSL) normalizes systemic arterial pressure in spontaneously hypertensive hamsters.

Egg yolk phosphatidylcholine, egg yolk phosphatidylglycerol, cholesterol and polyethylene glycol (molecular mass, 1,900) grafted to distearoyl-phosphatidylethanolamine (molar ratio, 5:1:3.5:0.5; total phospholipid content, 17 mmol) were mixed and dissolved in chloroform as previously described in our laboratory (Séjourné et al., 1997). The solvent was evaporated at 45 °C in a rotary evaporator followed by vacuum drying overnight. The dry lipid film was then hydrated in 250 μl saline, vortexed, bath-sonicated for 5 min and extruded through stacked polycarbonate filters (pore size, 200, 100, 50 nm). Human VIP (0.4 mg) was added to the extruded suspension which was then frozen in acetone-dry ice bath and lyophilized overnight at −46 °C under constant pressure. Thereafter, the lyophilized "cake" was resuspended in 250 μl deionized water. VIP associated with SSL was separated from free VIP by column chromatography and stored at 4 °C for up to 15 days. Size of SSL was 250 ± 10 nm (mean ± S.D.). VIP/phospholipids mole ratio in the formulation was 0.004.

Adult male hamsters with spontaneous hypertension and age/genetically-matched normotensive hamsters (120–140 g body weight) were anesthetized with pentobarbital sodium (6 mg/100 g body weight, i.p.) (Séjourné et al., 1997). A tracheostomy was performed to facilitate spontaneous breathing and for drug administration. A femoral vein was cannulated to inject supplemental anesthesia during the experiment (2–4 mg/100 g body weight/h) and saline. A femoral artery was cannulated to record systemic arterial blood pressure and heart rate. Body temperature was monitored and kept constant (37–38 °C) throughout the experiment using a heating pad. Arterial blood pressure and heart rate were recorded continuously during the experiment using a pressure transducer and a strip-chart recorder. Mean arterial pressure (MAP) was calculated as diastolic pressure plus one-third pulse pressure.

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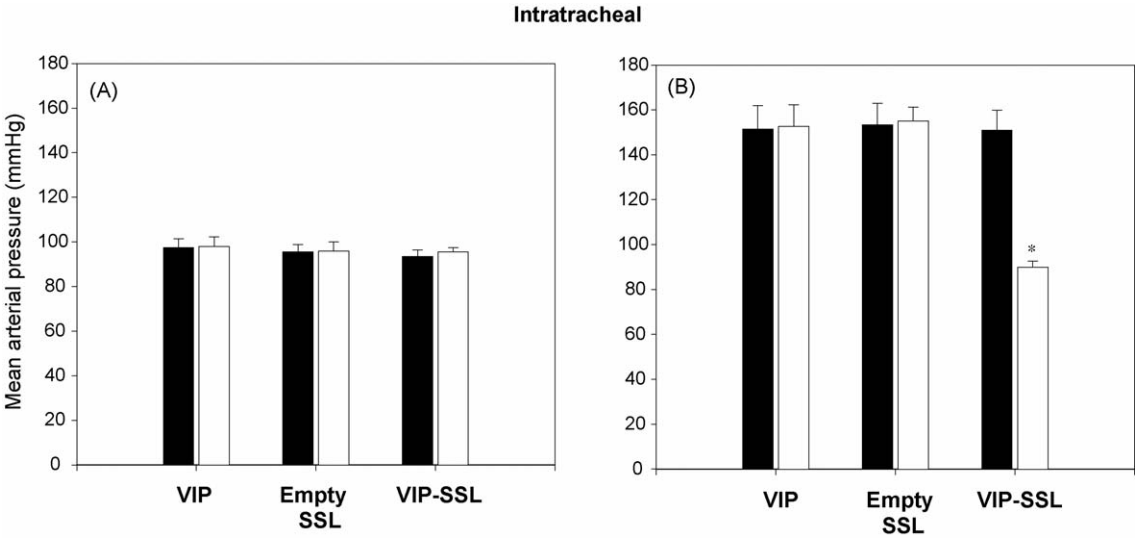

Fig. 1. Effects of a single intratracheal administration of self-associated VIP with sterically stabilized liposomes (VIP-SSL), VIP alone (each, 0.1 nmol) and empty SSL on mean arterial pressure in control hamsters (A) and spontaneously hypertensive hamsters (B). Closed bars, baseline; open bars, conclusion of the 6-h observation period. Data are means ± S.D. Each group, $n = 4$ animals; $p < 0.05$ in comparison to baseline.

VIP-SSL, VIP alone (each, 0.1 nmol) or empty SSL diluted in phosphate-buffered saline (final volume, 1.0 ml) was injected intratracheally through a PE-90 tubing inserted into the tracheostomy tube of hamsters followed by 1.0 ml room air at a rate of 1.0 ml/min using an infusion pump. MAP and heart rate were recorded before and for 6 h after administration of drugs. This time point was chosen because it conforms to rodent welfare guidelines during general anesthesia.

VIP-SSL, VIP alone (each, 0.1 nmol) or empty SSL diluted in phosphate-buffered saline (final volume, 0.25 ml) was injected subcutaneously at the inter-scapular region of hamsters over 1 min using an infusion pump. MAP and heart rate were recorded in before and for 6 h after administration of drugs as outlined above.

Data are expressed as means ± S.D. Statistical analysis was performed using ANOVA and Neuman–Keuls test. A $p < 0.05$ was considered statistically significant.

Intratracheal VIP-SSL, VIP alone (each, 0.1 nmol) and empty SSL had no significant effects on MAP and heart rate in normotensive hamsters (Figs. 1A and 2A; $p > 0.5$). By contrast, intratracheal VIP-SSL elicited a significant decrease in MAP in hypertensive hamsters that was observed within 10 min and lasted for 6 h (Fig. 1B; $p < 0.05$). Intratracheal VIP-SSL (0.1 nmol) had no significant effects on heart rate (Fig. 2B; $p > 0.5$). Intratracheal VIP alone (0.1 nmol) and empty SSL had no significant effects on MAP and heart rate in hypertensive hamsters (Figs. 1B and 2B; $p > 0.5$).

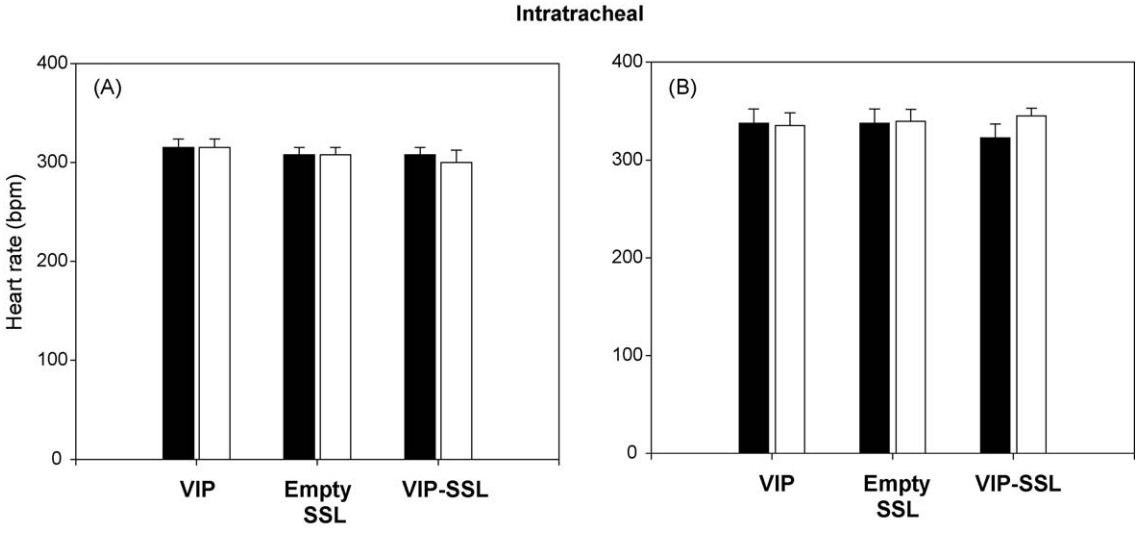

Fig. 2. Effects of a single intratracheal administration of self-associated VIP with sterically stabilized liposomes (VIP-SSL), VIP alone (each, 0.1 nmol) and empty SSL on heart rate in control hamsters (A) and spontaneously hypertensive hamsters (B). Closed bars, baseline; open bars, conclusion of the 6-h observation period. Data are means ± S.D. Each group, $n = 4$ animals.

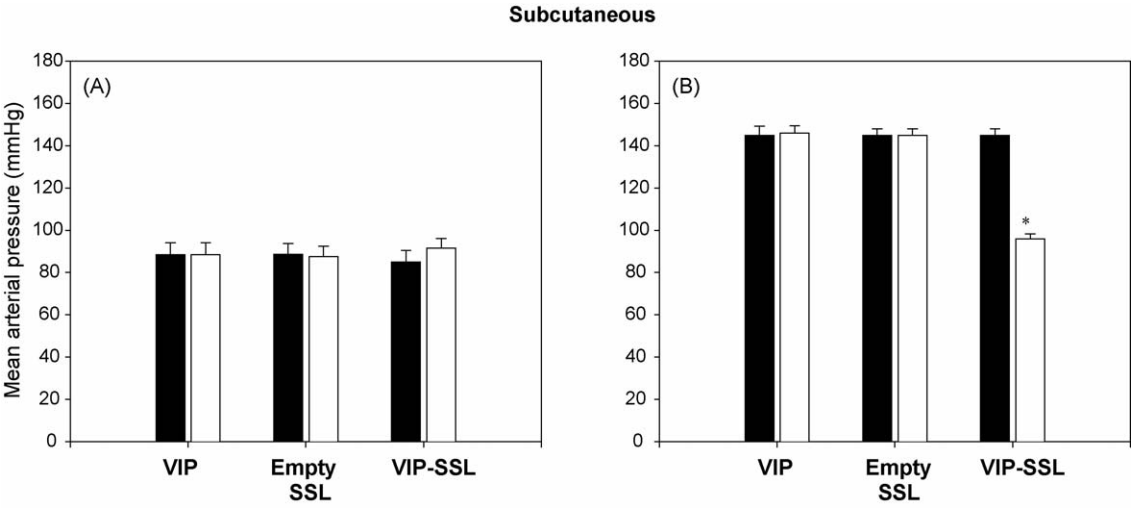

Fig. 3. Effects of a single subcutaneous administration of self-associated VIP with sterically stabilized liposomes (VIP-SSL), VIP alone (each, 0.1 nmol) and empty SSL on mean arterial pressure in control hamsters (A) and spontaneously hypertensive hamsters (B). Closed bars, baseline; open bars, conclusion of the 6-h observation period. Data are means ± S.D. Each group, $n = 4$ animals; $p < 0.05$ in comparison to baseline.

Subcutaneous VIP-SSL, VIP alone (each, 0.1 nmol) and empty SSL had no significant effects on MAP and heart rate in normotensive hamsters (Figs. 3A and 4A; $p > 0.5$). By contrast, subcutaneous VIP-SSL elicited a significant decrease in MAP in hypertensive hamsters that was observed within 20 min and lasted for 6 h (Fig. 3B; $p < 0.05$). Subcutaneous VIP-SSL (0.1 nmol) had no significant effects on heart (Fig. 4B; $p > 0.5$). Subcutaneous VIP alone (0.1 nmol) and empty SSL had no significant effects on MAP and heart rate in hypertensive hamsters (Figs. 3B and 4B; $p > 0.5$).

The new finding of this study is that a single, low-dose intratracheal and subcutaneous administration of biocompatible and biodegradable VIP-SSL normalizes MAP in spontaneously hypertensive hamsters for 6 h. The onset of VIP-SSL-induced responses was within 10–20 min with no further decline in MAP observed once the normative range was reached. VIP-SSL had no significant effects on heart rate suggesting its salutary effects are not related to depressed cardiac function. Importantly, VIP-SSL had no significant effects on MAP and heart rate in normotensive hamsters implying its effects are selective.

The onset of action, magnitude and duration of VIP-SSL-induced responses in spontaneously hypertensive hamsters were similar whether the drug was administered intratracheally or subcutaneously. This implies that liposomal VIP is absorbed rapidly and, most likely, intact from the lung and subcutaneous tissue into the systemic circulation where it evades degradation and uptake by the reticuloendothelial system (Suzuki et al., 1996; Séjourné et al., 1997; Gololobov et al., 1998). This, in turn, improves VIP bioavailability and amplifies its vasoactive effects over a prolonged period of time (Séjourné et al., 1997; Gololobov et al., 1998). Due to its relatively large size (∼250 nm), liposomal VIP does not extravasate through the intact resistant arteriolar

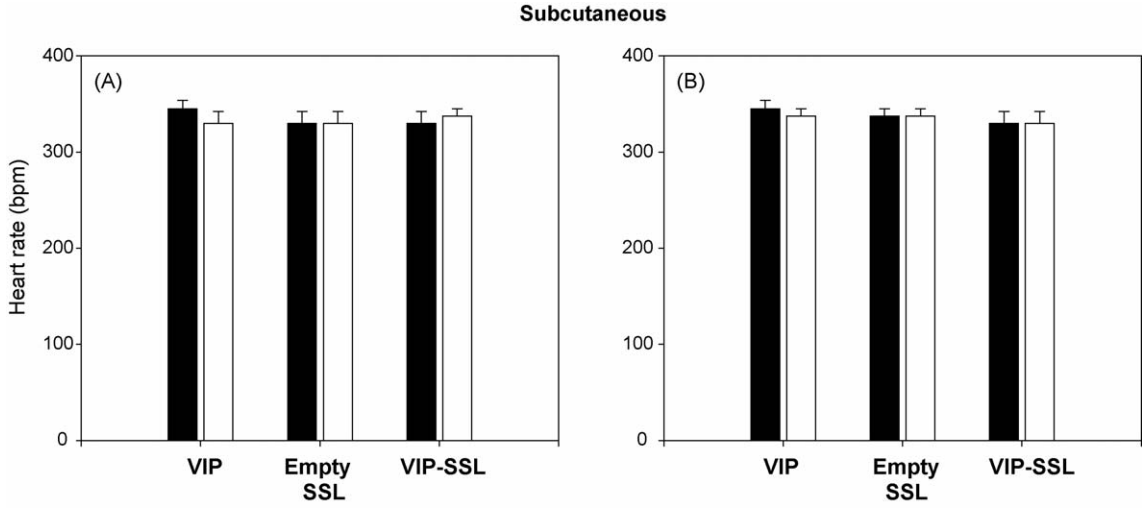

Fig. 4. Effects of a single subcutaneous administration of self-associated VIP with sterically stabilized liposomes (VIP-SSL), VIP alone (each, 0.1 nmol) and empty SSL on heart rate in control hamsters (A) and spontaneously hypertensive hamsters (B). Closed bars, baseline; open bars, conclusion of the 6-h observation period. Data are means ± S.D. Each group, $n = 4$ animals.

wall in normotensive hamsters thereby mitigating its vasorelaxant effects.

In summary, a single intratracheal and subcutaneous administration of low-dose, long-circulating biocompatible and biodegradable liposomal formulation of VIP normalizes MAP in spontaneously hypertensive hamsters for several hours. We suggest that pulmonary and subcutaneous delivery of VIP-SSL should be further developed as peptide nanomedicine for essential hypertension.

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