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Mechanisms of vasoactive intestinal peptide-elicited coronary vasodilation in the isolated perfused rat heart

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Abstract

The present study investigated the potential role of vasoactive intestinal peptide (VIP) receptors, VPAC1 and VPAC2, in VIP-elicited coronary vasodilation of the isolated perfused rat heart. Additional studies determined the role of ATP-sensitive (K_{ATP}) and voltage-gated K^+ (K_V) channels in the VIP-elicited coronary vasodilation. Both the selective VPAC1 agonist, K15,R16,L27VIP1-7GRF8-27, and the selective VPAC2 agonist, RO25-1553, decreased coronary vascular resistance (CVR) in a dose-dependent manner, with EC_{50} values of 1.67×10^{-9} M and 7.11×10^{-9} M, respectively (VPAC1 vs VPAC2 agonist, $P < 0.05$). K15,R16,L27VIP1-7GRF8-27 and RO25-1553 maximally reduced CVR by $-42 \pm 4\%$ and $-39 \pm 6\%$ at 1×10^{-8} and 3×10^{-8} M, respectively. VIP at 1×10^{-10} M decreased CVR by $-14 \pm 2\%$ in the absence (vehicle), by $-11 \pm 3\%$ in the presence of the nonselective VIP receptor antagonist VIP10-28 (1×10^{-7} M; $P > 0.05$ vs. vehicle) and by only $-4 \pm 2\%$ in the presence of the selective VPAC2 receptor antagonist PACAP6-38 (1×10^{-7} M; $P < 0.05$ vs. vehicle). In additional studies, VIP at 1×10^{-10} M decreased CVR by $-22 \pm 1\%$ in the absence (control) and by only $-10 \pm 2\%$ in the presence of the nonselective K^+ channel blocker tetrabutylammonium (3×10^{-4} M; $P < 0.05$ vs. control). VIP reduced CVR by $-4 \pm 1\%$ in the presence of the K_{ATP} channel blocker glibenclamide (3×10^{-6} M; $P < 0.05$ vs control) and by $-28 \pm 2\%$ in the presence of the K_V channel blocker 4-aminopyridine (3×10^{-4} M; $P > 0.05$ vs control). Thus, selective VPAC1 and VPAC2 receptor activation in the coronary circulation produces vasodilation and the VIP-elicited coronary vasodilation involves activation of VPAC2 receptors and K_{ATP} but not K_V channels. In addition, VIP10-28 does not effectively block coronary vascular VIP receptors.

Keywords: Vasoactive intestinal peptide; Coronary vascular resistance; Vasodilation; VPAC1 receptor; VPAC2 receptor; ATP-sensitive K^+ channel; Voltage-gated K^+ channel; Heart; Rat

1. Introduction

Vasoactive intestinal peptide (VIP) is a 28 amino acid neuropeptide discovered by Said and Mutt in 1970 that has diverse effects in the cardiovascular system (Sawmiller and Henning, 2006), including coronary vasodilation (Popma et al., 1990; Sawmiller et al., 2004; Smitherman et al., 1982). This peptide is widely distributed throughout the intrinsic and extrinsic preganglionic and postganglionic neurons of the heart, innervating coronary arteries and arterioles as well as the myocardium (Forssmann et al., 1988; Weihe et al., 1984). The neural release of VIP increases during vagal stimulation (Anderson et al., 1993; Feliciano and Henning, 1998a,b; Hill et al., 1993) or myocardial ischemia (Kalfin et al., 1994), producing coronary vasodilation and providing some protection against ischemia-reperfusion injury.

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The basal concentration of VIP in the left ventricular coronary arteries varies between 0.7 and 2.2 pmol g^{-1} and a decrease in the coronary artery VIP concentration might contribute to the development of coronary spasm (Brum et al., 1986).

The coronary vasodilatory effect of VIP is mediated by a direct action on coronary resistance arteries and arterioles (Anderson et al., 1988; Popma et al., 1990; Sawmiller et al., 2004). In rats and humans, this vasodilatory effect can occur without increasing heart rate, myocardial contractility or myocardial oxygen consumption, indicating that it is not a metabolic response to increases in myocardial work (Popma et al., 1990; Sawmiller et al., 2004). Recent studies utilizing immunohistochemistry and RT-PCR showed that VPAC1 and VPAC2 receptors, two VIP receptor subtypes (Gozes and Furman, 2003; Laburthe et al., 2002), are expressed in coronary arteries and arterioles of the left ventricle (Sawmiller et al., 2004). However, it has not been determined if these receptors are functionally coupled to coronary vasodilation. Additional studies have shown that the VIP-elicited relaxation of isolated porcine epicardial coronary arteries involves activation of two types of K^+ channel, large conductance Ca^{2+}-activated and voltage-gated K^+ channels, but does not involve activation of small conductance Ca^{2+}-activated or ATP-sensitive K^+ channels (Kawasaki et al., 1997). No study has determined the role of these channels in VIP-elicited vasodilation of the intact coronary circulation. The objective of the present study was to determine if VPAC1 and VPAC2 receptors are functionally coupled to the regulation of coronary vascular resistance, utilizing selective VPAC1 and VPAC2 receptor agonists and antagonists. A second objective was to determine the role of voltage-gated (K_V) and ATP-sensitive K^+ (K_{ATP}) channels in VIP-elicited vasodilation of the intact coronary circulation.

2. Methods and materials

2.1. Experimental protocols

These studies utilized 84 adult male Sprague–Dawley rats (380 ± 6 g body weight). Each rat was anesthetized with sodium pentobarbital (40 mg kg^{-1} ip) and the heart was rapidly removed, rinsed in ice-cold physiological saline solution (PSS) and perfused via an aortic cannula with nonrecirculating PSS, as previously described (Sawmiller et al., 2004). The coronary flow was set at a constant rate of 12 ± 0.3 ml min^{-1} (9.2 ± 0.2 ml min^{-1} g^{-1}) with a Masterflex peristaltic pump (Cole Parmer, Vernon Hills, IL). The PSS contained (in mM): NaCl 118, KCl 4.2, $CaCl_2$ 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11, pH 7.4, gassed with 95% O_2/5% CO_2 and maintained at 37 °C. After 10 min, the heart was perfused with the same physiological solution containing 1.5 mM KCl, which reduced the total potassium concentration to 2.7 mM and yielded a perfusion pressure of 96 ± 2 mm Hg. This ensured the development of a sufficient and sustained increase in coronary vascular resistance, which facilitated observing a vasodilatory response (Sawmiller et al., 2004). A drain was placed into the left ventricular apex via the mitral valve to prevent accumulation of arterial and thebesian drainage and the heart was placed into an organ chamber maintained at 37 °C. The heart was allowed to contract spontaneously throughout all experimental procedures. Perfusion pressure was continuously monitored via a strain-gauge manometer (Statham P23ID, Medex MX860) through a side port in the aortic cannula and displayed with a Gould thermal array recorder (Valley View, OH). Coronary vascular resistance was calculated by dividing the perfusion pressure by the coronary flow rate (mm Hg min g ml^{-1}). Heart rate was determined by counting the number of contractions per min from the aortic pressure pulse waveform. All procedures conformed to the Guide for the Care and Use of Laboratory Animals prepared by the National Research Council and were approved by the Institutional Animal Care and Use Committee of the University of South Florida School of Medicine.

2.1.1. Series 1. Coronary vasodilatory effect of selective VPAC1 and VPAC2 receptor agonists

After a stabilization period of approximately 40 min, the selective VPAC1 agonist K15,R16,L27VIPl-7GRF8-27 (Gourlet et al., 1997a) or the selective VPAC2 agonist RO25-1553 (Gourlet et al., 1997b) was infused into the aortic cannula with a Harvard infusion pump (Holliston, MA) set at 100 µl min^{-1}. Each infusion lasted approximately 9 min, yielding a final concentration of 1×10^{-9} to 1×10^{-8} M for K15,R16,L27VIP1-7GRF8-27 and 3×10^{-9} to 3×10^{-8} M for RO25-1553 administered randomly, and sufficient time was allowed after each infusion before a subsequent infusion was begun. These concentrations are within the range required to reduce ^{125}I-VIP binding or increase adenylyl cyclase activity in CHO ovaries, LoVo epithelial cells or SUP Tl lymphoblasts selectively expressing VPAC1 or VPAC2 receptors (Gourlet et al., 1997a,b). After determining the vasodilatory response to agonist, 1×10^{-6} mol adenosine or sodium nitroprusside was injected into the aortic cannula to determine total vasodilatory capacity (Sawmiller et al., 2004). The vasodilatory response to three or four different concentrations of agonist was determined utilizing 4–11 hearts per concentration and only one agonist at 1 or 2 different concentrations per heart.