**Vasopressin V₁-Receptor Stimulation Produces a Positive Inotropic Response without Affecting pHᵢ in Guinea Pig Papillary Muscles**

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Received December 9, 1994 Accepted April 6, 1995

**ABSTRACT**—Effects of arginine-vasopressin (AVP) on the contractile force, action potential (AP) and intracellular pH (pHᵢ) were studied in isolated guinea pig papillary muscles using conventional and ion-selective microelectrode techniques. AVP increased the developed tension and the resting tension, and these responses were attenuated by the V₁-receptor antagonist OPC-21268 (1-{1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl}-3,4-dihydro-2(1H)-quinolinone). However, AVP failed to affect AP configuration or pHᵢ. These results suggest that AVP produces a positive inotropy by mechanism(s) other than intracellular alkalinization.

**Keywords:** Arginine-vasopressin, Positive inotropic effect, Intracellular pH

Arginine-vasopressin (AVP) elicits various biological effects through V₁- and V₂-receptors, which couple to the phosphatidylinositol turnover system and adenylate cyclase-cAMP system, respectively (1). In the cardiovascular system, AVP produces vasoconstriction through V₁-receptors in vascular smooth muscles and retains water through V₂-receptors in the kidney. It is established that AVP reduces cardiac output and contractility by its vasoconstricting effect in vivo (1). However, in terms of its direct action on the myocardial contractility, conflicting results have been reported. Positive inotropy was observed in rat (2) and cat myocardium (3), while negative inotropy was observed in dog (4) and rabbit myocardium (5). It has been postulated that the modulation of myocardial contractility is mediated by the V₁-receptors that are coupled to stimulation of phosphoinositide (PI) hydrolysis (5). Activation of endothelin receptors (6) and α₁-adrenoceptors (7), which are also coupled to increased PI hydrolysis, produces intracellular alkalinization in isolated cardiac cells. It has been postulated that the rise in intracellular pH (pHᵢ) plays a role in the establishment of the positive inotropic response by means of increasing myofilament sensitivity to Ca²⁺. Therefore, this study was undertaken to determine the effects of AVP on the contractile force, action potential and pHᵢ in isolated guinea pig papillary muscles using conventional and ion-selective microelectrode techniques.

Female guinea pigs weighing 200 - 400 g were killed by a blow on the head to cause immediate death. The hearts were quickly removed and placed in a bath filled with a bicarbonate-buffered Tyrode solution equilibrated with 95% O₂ and 5% CO₂. Papillary muscles with a diameter less than 1 mm were dissected from the right ventricle of the hearts. The preparations were continuously superfused with the oxygenated Tyrode solution in a tissue chamber. The bicarbonate-buffered Tyrode solution had the following composition: 123 mM NaCl, 5.4 mM KCl, 0.33 mM NaH₂PO₄, 0.5 mM MgCl₂, 1.8 mM CaCl₂, 5.5 mM glucose and 25 mM NaHCO₃. The temperature of the tissue bath was kept constant at 33.0±1.0°C. Transmembrane potentials and pHᵢ were measured using conventional and ion-selective microelectrode techniques, as previously described (8). The hydrogen ion-selective microelectrode (H-ISE) was backfilled with a H⁺-sensitive ion exchanger (Fluka 95291; Buchs SG, Switzerland), and the shaft was filled with a solution containing 100 mM NaCl and 50 mM HEPES-NaOH (pH 7.40). Calibration solutions were of a composition that mimicked the intracellular environment (10 mM NaCl, 140 mM KCl, 0.5 mM MgCl₂ and 5 mM HEPES-KOH) with pHs of 6.4, 6.9, 7.4, 7.9. Each electrode was calibrated both before and after the experiments. The time constant of the electrode response was less than 5 sec and pHᵢ could be measured with an accuracy in a range of

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±0.005 pH unit. Measurements of membrane potential with conventional and ion-selective microelectrodes were performed simultaneously in the same preparation. Data were taken only from preparations in which stable impalements of both electrodes were maintained, and the others were discarded.

In another series of experiments, the developed tension was measured concomitantly with the membrane potential, as previously described (9). One end of the muscle was hooked to the lever arm of a force transducer (TB651T; Nihon Kohden, Tokyo) mounted on a micro-manipulator, and the other was pinned to the bottom of the tissue chamber. Resting tension was progressively increased to 2 mN. The preparations were stimulated through platinum field electrodes at a rate of 0.5 Hz using rectangular pulses of 1-msec duration at 50% above the threshold. In order to avoid tachyphylaxis, one preparation was exposed to only one concentration of AVP.

The following drugs were used (sources in parentheses): Arginine-vasopressin and propranolol hydrochloride (Sigma Chemical, St. Louis, MO, USA); OPC-21268 (1-{1-[4-(3-acetylamino)propoxy]benzoyl}-4-piperidyl)-3,4-dihydro-2(1H)-quinolinone), OPC-31260 (5-dimethylamino-1-{4-(2-methylbenzoylamino)benzoyl}-2,3,4,5-tetrahydro-1H-benzazepine) (generous gifts from Otsuka Pharmaceutical Co., Tokushima); prazosin hydrochloride (generous gift from Pfizer Pharmaceutical, Inc., Tokyo). OPC-21268 was dissolved in dimethyl sulfoxide as a stock solution of 10^{-2} M and then diluted in Tyrode solution. The final concentration of dimethyl sulfoxide was less than 0.1%, and it was confirmed that this concentration of dimethyl sulphoxide alone did not produce any appreciable changes in the measured parameters. Other compounds were dissolved in distilled water.

**Fig. 1.** Electromechanical effects of AVP in guinea pig papillary muscles. A: Typical changes in membrane potential and developed tension produced by 10^{-6} M AVP in a papillary muscle. Upper trace: membrane potential, Middle trace: developed tension, Lower traces: action potential and twitch wave-form recorded at the time points indicated by a, b and c. B: Concentration-response curve for the effect of AVP on developed tension (n=5 for each point, *P<0.05). C: Concentration-response curve for the effect of AVP on resting tension (n=5 for each point, *P<0.05). Changes in DT and RT were measured at 20 min after the introduction of AVP.
All data are presented as means ± standard errors (S.E.). Analysis by Student's t-test was performed for paired and unpaired observations. P values of less than 0.05 were considered significant.

Figure 1A illustrates typical changes in membrane potential and developed tension produced by 10⁻⁶ M AVP in a guinea pig papillary muscle. Although AVP hardly affected the action potential configuration, it produced increases in developed tension (DT) and resting tension (RT). After the introduction of AVP, the increases in RT and DT appeared and reached the plateau level within 10–20 min. These changes returned to the baseline level 20–30 min after the washout. AVP in concentrations higher than 10⁻⁷ M increased DT significantly (Fig. 1B). The increases in DT produced by 10⁻⁷ M and 10⁻⁶ M AVP were 33±9% (n=5, P<0.05) and 35±9% (n=5, P<0.05), respectively. AVP in lower concentrations significantly increased RT (Fig. 1C). In quiescent papillary muscles, AVP still produced a similar increase in RT (data not shown). In a preparation treated with 10⁻⁶ M propranolol and 10⁻⁶ M prazosin, AVP produced increases in DT and RT of a similar magnitude. In contrast, AVP hardly affected the action potential configuration. Baseline values of resting membrane potential, action potential amplitude, action potential duration at 50% repolarization level and 90% repolarization level were −82±0.1 mV, 124±2.9 mV, 245±17 msec, 276±18 msec, respectively (n=5). AVP at a concentration of 10⁻⁶ M failed to affect any of these parameters significantly.

To determine the receptor subtype responsible for the AVP-induced positive inotropic response, influences of OPC-21268 and OPC-31260, selective antagonists for V₁ and V₂ receptors, were examined. The preparations were treated with one of these antagonists from 20 min prior to the introduction of AVP. It was reported that the Kᵢ value

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**Fig. 2.** Effects of AVP antagonists on the mechanical response to AVP. A: Effects of the V₁-receptor antagonist OPC-21268 and the V₂-receptor antagonist OPC-31260 on AVP-induced positive inotropic response. B: Influences of V₁- and V₂-receptor antagonists on AVP (10⁻⁷ M)-induced increase in developed tension (DT) (n=4–7, *P<0.05 vs changes in the control (untreated preparations)). C: Influences of V₁- and V₂-receptor antagonists on the AVP (10⁻⁷ M)-induced increase in resting tension (RT) (n=4–7, *P<0.05 vs changes in control (untreated preparations)). Changes in DT and RT were measured at 20 min after the introduction of AVP.
of OPC-21268 for V1-receptors was $1.4 \times 10^{-7}$ M, and those of OPC-31260 for V1- and V2-receptors were $4.4 \times 10^{-7}$ M and $3.7 \times 10^{-9}$ M, respectively (10, 11). Accordingly, we used $3 \times 10^{-6}$ M or $10^{-5}$ M OPC-21268 and $10^{-7}$ M OPC-31260 in the present study. Neither OPC-21268 ($10^{-5}$ M, n=5) nor OPC-31260 ($10^{-7}$ M, n=4) affected basal DT and RT significantly; DT after the V1- and V2-receptor antagonist was 102.1±1.9% and 100.7±0.2% of the pre-drug values, respectively. As shown in Fig. 2, the V1-receptor antagonist OPC-21268 in concentrations of $3 \times 10^{-6}$ M and $10^{-5}$ M almost completely abolished the AVP ($10^{-7}$ M)-induced increase in DT. However, the AVP-induced increase in RT was more resistant to the V1-antagonist, and $10^{-5}$ M OPC-21268 was needed to attenuate the AVP ($10^{-7}$ M)-induced increase in RT significantly. In contrast, the V2-receptor antagonist OPC-31260 ($10^{-7}$ M) failed to affect the AVP-induced increases in DT and RT. These findings indicate that the AVP-induced positive inotropic response is mediated by V1-receptors.

Basal values of pH$_i$ in quiescent papillary muscles were 7.17±0.03 (n=9) in the bicarbonate-buffered solution. AVP ($10^{-6}$ M) hardly affected the resting membrane potential and pH$_i$, as shown in Fig. 3. Changes in pH$_i$ at 20 min after the introduction of $10^{-7}$ M and $10^{-6}$ M AVP were $-0.004 \pm 0.008$ (n=4) and $0.017 \pm 0.013$ units (n=5), respectively, both of which were statistically insignificant.

In terms of the inotropic action of AVP, conflicting results have been reported. Walker et al. (2) reported that low concentrations of AVP exerted a positive inotropic effect, whereas high concentrations of AVP produced a negative inotropic effect in isolated rat hearts. In contrast, Furukawa et al. (4) demonstrated that in the isolated blood-perfused heart preparations of the dog, V1-receptor stimulation decreased the atrial and ventricular contractile force even if the preparation was perfused with a constant flow. In addition, Endoh et al. (5) demonstrated that V1-receptor stimulation produced a decrease in developed tension and an increase in PI hydrolysis in the isolated rabbit ventricular myocardium. In this study, however, AVP in concentrations higher than $10^{-7}$ M produced a positive inotropic response in isolated guinea pig papillary muscles. Inconsistencies of these results may stem from differences in species and/or experimental conditions.

The AVP-induced inotropic response was associated with an increase in resting tension. The AVP-induced

![Fig. 3. Lack of pH$_i$ change after AVP in quiescent papillary muscles. Representative traces of membrane potential and pH$_i$ during exposure to $10^{-6}$ M AVP are shown in the upper panel. Summarized data of changes in pH$_i$ at 20 min after the application of $10^{-7}$ M and $10^{-6}$ M AVP are shown in the lower panels. Values are expressed as means±S.E. of 4–5 preparations.]
increases in developed tension and resting tension were blocked by the V₁-receptor antagonist OPC-21268 (10) but not by the V₂-receptor antagonist OPC-31260 (11). It is well known that V₁-receptors are coupled to an increase in PI hydrolysis in cardiac and non-cardiac cells (1, 5). Stimulation of various receptors coupled to PI hydrolysis, e.g., endothelin and α₁-receptors, is reported to produce an intracellular alkalosis, potentially due to activation of the Na⁺-H⁺ exchange system (6, 7). Intracellular alkalization is postulated to increase the Ca²⁺ sensitivity of the contractile protein. Indeed, the resting tension was increased by V₁-receptor stimulation in quiescent as well as constantly-stimulated preparations in this study. However, AVP failed to affect pHᵢ significantly. Therefore, factor(s) other than pHᵢ change may be involved in the increase in resting tension and developed tension. In this context, it is noteworthy that V₁-receptor stimulation increased cytosolic free Ca²⁺ in neonatal rat cardiomyocytes (12).

AVP had little influence on action potential configuration, although it increased developed tension and resting tension. Recently Abe et al. (13) reported that AVP increased the delayed rectifier K⁺ current (Iₖ) through the activation of V₁-receptors in guinea pig ventricular cells. However, the action potential shortening could not be readily observed in multicellular preparations in this study. Although endothelin-1 and α₁-agonists increase Iₖ through the activation of protein kinase C (14, 15), they hardly affected APD in guinea pig papillary muscles (H. Nakaya et al., unpublished observations).

In conclusion, AVP produces a positive inotropic response and an increase in resting tension through the activation of V₁-receptors, at least, in guinea pig papillary muscles. However, pHᵢ was not influenced by AVP. The intracellular mechanism(s) responsible for the modulation of cardiac contractility via the activation of the receptors coupled to PI hydrolysis is undoubtedly complex. Further studies are needed to clarify the underlying mechanisms and to define the similarities and dissimilarities in the receptor systems.

REFERENCES

1 Hays RM: Antidiuretic hormone. In The Pharmacological Basis of Therapeutics, Edited by Gilman AG, Rall TW, Nies AS and Taylor P, Eighth Edition, pp 732–740, Pergamon Press, New York (1990)
2 Walker BR, Childs ME and Adams EM: Direct cardiac effects of vasopressin: role of V₁- and V₂-vasopressinergic receptors. Am J Physiol 255, H261–H265 (1988)
3 Shoemaker IE, Meulemans AL, Andries LJ and Brutsaert DL: Role of endocardial endothelium in positive inotropic action of vasopressin. Am J Physiol 259, H1148–H1151 (1990)
4 Furukawa Y, Takayama S, Ren LM, Sawaki S, Inoue Y and Chiba S: Blocking effects of V₁ (OPC-21268) and V₂ (OPC-31260) antagonists on the negative inotropic response to vasopressin in isolated dog heart preparations. J Pharmacol Exp Ther 263, 627–631 (1992)
5 Endoh M, Takaashi M and Norota I: Effects of vasopressin on phosphoinositide hydrolysis and myocardial contractility. Eur J Pharmacol 218, 355–358 (1992)
6 Krämer BK, Smith TW and Kelly RA: Endothelin and increased contractility in adult rat ventricular myocytes: Role of intracellular alkalosis induced by activation of the protein kinase C-dependent Na⁺-H⁺ exchanger. Circ Res 68, 269–279 (1991)
7 Ikawara K, Hori M, Watanabe Y, Kitabatake A, Crague EJ Jr, Yoshida H and Kamada T: α₁-Adrenoceptor stimulation increases intracellular pH and Ca⁹⁺ in cardiomyocytes through Na⁺/H⁺ and Na⁺/Ca²⁺ exchange. Eur J Pharmacol 186, 29–40 (1990)
8 Shida S, Nakaya H, Matsumoto S and Kanno M: β₂ Adrenoceptor mediated decrease in pHᵢ in quiescent ventricular myocardiun. Cardiovasc Res 28, 112–118 (1994)
9 Nakaya H, Hattori Y, Tohsne N, Shida S and Kanno M: β₂ Adrenoceptor-mediated depolarization of the resting membrane in guinea pig papillary muscles: changes in intracellular Na⁺, K⁺ and Cl⁻ activities. Pfiegers Arch 417, 185–193 (1990)
10 Yamamura Y, Ogawa H, Chihara T, Kondo K, Onogawa T, Nakamura S, Mori T, Tominaga M and Yabuuchi Y: OPC-21268, an orally effective, nonpeptide vasopressin V₁ receptor antagonist. Science 252, 572–574 (1991)
11 Yamamura Y, Ogawa H, Yamashita H, Chihara T, Miyamoto H, Nakamura S, Onogawa T, Yamashita H, Hosokawa T, Mori T, Tominaga M and Yabuuchi Y: Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin V₁ receptor antagonist. Br J Pharmacol 105, 787–791 (1992)
12 Xu YJ and Gopalakrishnan V: Vasopressin increases cytosolic free [Ca²⁺] in the neonatal rat cardiomyocyte: Evidence for V₁ subtype receptors. Circ Res 69, 239–245 (1991)
13 Abe T, Fujisawa S and Iijima T: Vasopressin-β₁-receptor-mediated increase in delayed rectifier potassium current in ventricular cells of guinea pig heart. Jpn J Pharmacol 61, Supp I, 100P (1993)
14 Habuchi Y, Tanaka H, Furukawa T, Tsujimura Y, Takahashi H and Yoshimura M: Endothelin enhances delayed potassium current via phospholipase C in guinea pig ventricular myocytes. Am J Physiol 262, H345–H354 (1992)
15 Tohsne N, Nakaya H and Kanno M: α₁-Adrenoceptor stimulation enhances the delayed rectifier K⁺ current through the activation of protein kinase C. Circ Res 71, 1441–1446 (1992)