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THE EFFECTS OF VASOPRESSIN IN ISOLATED RAT HEARTS

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Abstract : The roles of cGMP, prostaglandins, the entry of extracellular Ca^{2+} through slow channels, endothelium and V_1 receptors in the negative inotropic, chronotropic and coronary vasoconstrictor responses to arginine vasopressin (AVP) have been investigated in isolated perfused rat hearts. The bolus injection of 5×10^{-5} M AVP produced a significant decrease in contractile force, heart rate and coronary flow. AVP also significantly decreased contractile force, heart rate and coronary flow in hearts pretreated with an inhibitor of soluble guanylate cyclase methylene blue (10^{-6} M), an effective drug for removing endothelium saponin (500 µg/ml), an inhibitor of cyclooxygenase indomethacin (10^{-5} M) or a calcium channel antagonist verapamil (5×10^{-7} M). The potent V_1 receptor antagonist [Deamino-Pen^1, Val^4, D-Arg^8]-vasopressin (9×10^{-5} M) did not alter effects of AVP but the very potent V_1 receptor antagonist [β-Mercapto-β, β-cyclopentamethylene-propionyl^1, O-Me-Tyr^2, Arg^8]-vasopressin (8×10^{-5} M) abolished these effects. Our results suggest that AVP produces negative inotropic, chronotropic and coronary vasoconstrictor effects in isolated perfused rat hearts. cGMP, prostaglandin release and Ca^{2+} entry does not involve in the effects of AVP. These effects are endothelium independent and mediated by V_1 receptors. The use of V_1 receptor antagonist [β-mercapto-β, β-cyclopentamethylene-propionyl^1, O-Me-Tyr^2, Arg^8]-vasopressin may be beneficial for preventing the negative inotropy, chronotropy and coronary vasoconstriction induced by AVP.

Key words : vasopressin, isolated perfused rat heart, myocardial contractility, coronary flow, heart rate, vasopressin receptor antagonists

INTRODUCTION

AVP is a polypeptide hormone released from the posterior pituitary (1). Apart from its principal antidiüretic action (1), AVP has been reported to produce coronary vasoconstriction (2–4), a reduction in myocardial contractility (2, 5) and heart rate (6). It is known that at least two types of receptors which are termed V_1 and V_2 mediate the actions of this hormone (7). V_1 receptors on vascular smooth muscle and

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hepatocytes and V_2 receptors on collecting duct cells of kidney are located (5). The activation of V_1 receptors lead to vasoconstriction, whereas V_2 receptors mediate the antidiüretic action of vasopressin (8, 9).

The mechanisms responsible for the coronary vasoconstrictor, negative inotropic and chronotropic effects of vasopressin have not been known exactly. It has been suggested that vasopressin directly decreases myocardial contraction (5, 10) or coronary vasoconstriction evoked by the peptide reduces contractility (11–13). On the other hand, it has been concluded that vasopressin induces the release of prostaglandins which contributed to the coronary vasoconstrictor action (14). The experiments on isolated arteries have been demonstrated that vasopressin-induced contraction is not endothelium dependent (15) and the entry of external Ca^{2+} through voltage-dependent Ca^{2+} channels involves in contraction (16).

The role of extracellular Ca^{2+} entry, cGMP and endothelium in the negative inotropic, chronotropic and coronary vasoconstrictor actions of the vasopressin and the role of prostaglandins in the negative inotropic and chronotropic effect have not been examined. Therefore, we have investigated the possible involvement of the entry of extracellular Ca^{2+}, cGMP, prostaglandin release and endothelium in the negative inotropic, chronotropic and vasoconstrictor responses to vasopressin in the isolated perfused rat hearts. In addition we have investigated whether this effects are mediate by V_1 receptors.

METHODS

In the aged of 9–10 months Wistar rats of either sex weighing between 350–450 g were used in all the experiments. One hour after the administration of heparin (1000 IU, i.p.), the chest was opened under light ether anesthesia and heart was rapidly removed and placed in ice-cold (0–4°C) modified Krebs-Henseleit solution (mKHs) until contractions ceased. After the heart was cleaned off surrounding fat and other tissues, aorta was immediately tied to a stainless steel cannula of the perfusion apparatus and heart was perfused retrogradely under constant pressure (70 mmHg) with mKHs by the nonrecirculating langendorff technique. The pulmonary artery was incised to facilitate complete coronary drainage in the ventricles. The perfusion solution was mKHs of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. mKHs was continuously gassed with 95% O_2 and 5% CO_2 using a disposable infant oxygenator and pH of the solution was 7.4. The temperature was continuously measured in aorta cannula and kept at 37°C. Contractile force was measured by attaching one end of a piece of silk suture to the apex of the heart and other end to a force displacement transducer (Nihon Kohden TB 611T, Tokyo). A resting tension of 5 g was applied and developed isometric tension of the heart was displayed on a polygraph (Nihon Kohden RM 6000). Heart rate was determined from the tracings of the contractile force at a paper speed of 2.5 mm/s. Coronary flow was measured by collecting the amount of perfusate leaving the heart every minute with the aid of a graduated cylinder.

The hearts were allowed to equilibrate for 30 min before the administration of drugs. AVP at the concentration of 5×10^{-5} M was given to a group of the hearts. 5×10^{-5} M AVP significantly decreased contractile force, coronary flow and heart rate. Therefore, this dose was used in all other experiments concerning with mechanism of cardiac effects of this peptide. In other group of the hearts simultaneous bolus infections of AVP and V_1 receptor antagonist (B-mercapto-β, β-cyclopentamethylene-propionyl[1], 0-Me-Tyr[2], Arg[8]-vasopressin (8×10^{-5}M] or AVP and other V_1 receptor antagonist (Deamino-Pen[1], Val[4], D-Arg[8]-vasopressin (9×10^{-5}M) were made. Saponin (500 µg/ml), verapamil (5×10^{-7}M) or indomethacin (10^{-5}M) were infused to different sets of the hearts for 3, 5 and 6 min, respectively. AVP was injected in the presence of the infusion. In a separate group methylene blue (10^{-6}M) was infused for 1 min. After methylene blue infusion was stopped AVP was administrated. The drugs were infused into aortic perfusion line using an infusion pump (B, Braun-Melsungen AG, Bayern]. AVP and V_1 receptor antagonists were given as a bolus in a volume of 0.1 ml into the perfusate, 2 cm proximal to the aortic cannula. The dose of the drugs used was calculated to a final concentration in the perfusion medium.

[Arg[8]]-vasopressin, [Deamino-Pen[1], Val[4], D-Arg[8]]-vasopressin, [β-Mercapto-β, β-cyclopentamethylene-propionyl[1], O-Me-Tyr[2], Arg[8]]-vasopressin, indomethacin, verapamil hydrochloride and methylene blue were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Saponin crudum was obtained from Merck (Darmstadt). mKHs was prepared as daily and verapamil solution was protected from the light to avoid photodecomposition.

Results are presented as the means and standard errors of the means. Statistical analysis of the data was performed by two-way analysis of variance (ANOVA) followed by the Tukey-HSD multiple comparisons test. A P value less than 0.05 was considered to be significant.

RESULTS

The injection of AVP (Fig. 1, 2 and 3) produced a significant decrease in contractile force, coronary flow and heart rate (n = 6). V_1 receptor antagonist [Deamino-Pen[1], Val[4], D-Arg[8]]-vasopressin did not alter the cardiac effects of AVP when the antagonist and AVP were administered

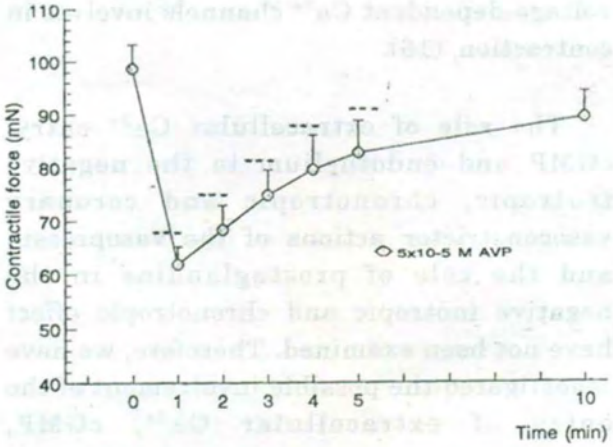

Fig. 1: Time course of the effects of AVP on contractile force. Time 0 represents control values. Vertical bars show standard errors. ***P<0.001 significantly different from the respective control.

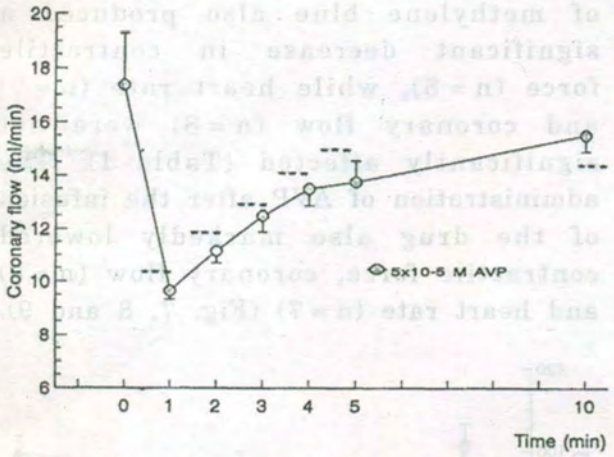

Fig. 2: Time course of the effects of AVP on coronary flow.

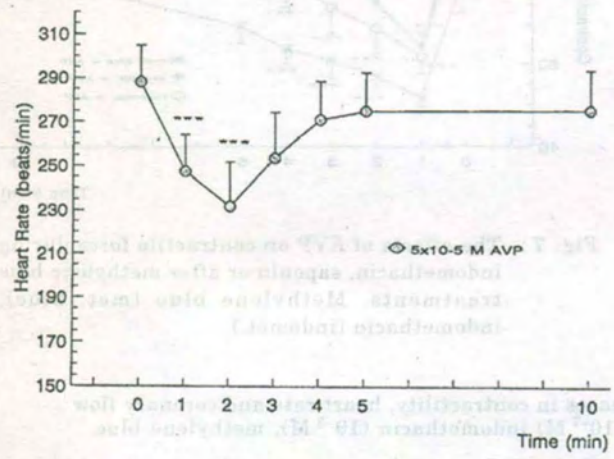

Fig. 3: Time course of the effects of AVP on heart rate.

to the hearts. In this group AVP caused a significant decrease in contractile force, heart rate (n = 5) and coronary flow (n = 6) (Fig. 4, 5 and 6). In contrast, when the hearts were treated with other V_1 receptor antagonist [β-Mercapto-β, β-cyclopentamethylene-propionyl^1, O-Me-Tyr^2, Arg^8]-vasopressin and AVP, the antagonist almost completely abolished the myocardial and coronary effects of AVP. In the presence of this antagonist AVP caused only a small and insignificant decrease in contractile force, coronary flow and heart rate (n = 6, Fig. 4, 5 and 6).

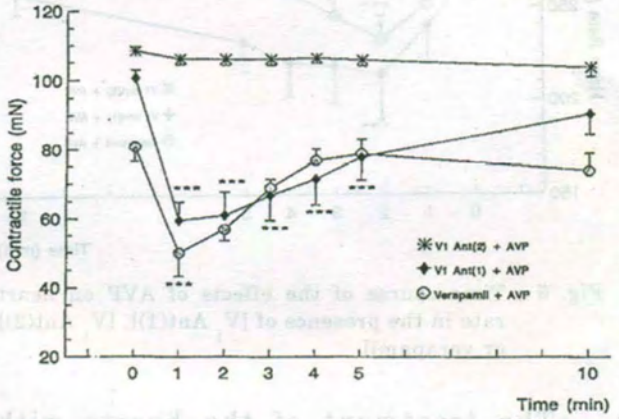

Fig. 4: Time course of the effects of AVP on contractile force in the presence of potent V_1 receptor antagonist [V_1 Ant(1)], very potent V_1 receptor antagonist [V_1 Ant(2)] or verapamil. V_1 Ant(1) [Deamino-Pen^1, Val^4, D-Arg^8]-vasopressin, V_1 Ant(2) [β-Mercapto-β, β-cyclopentamethylene-propionyl^1, O-Me-Tyr^2, Arg^8]-vasopressin.

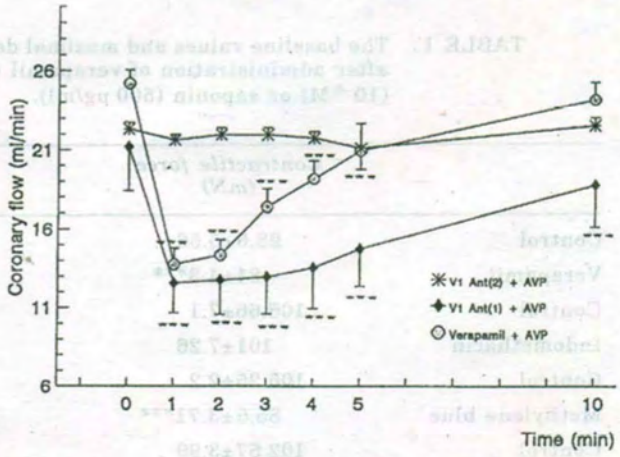

Fig. 5: Time course of the effects of AVP on coronary flow in the presence of [V_1 Ant(1)], [V_1 Ant(2)] or verapamil.

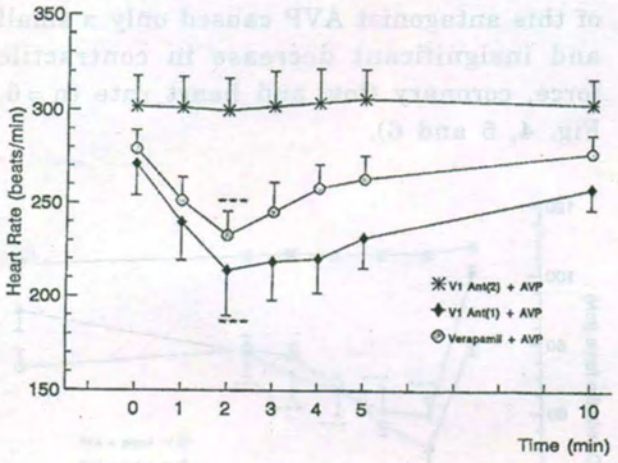

Fig. 6: Time course of the effects of AVP on heart rate in the presence of [V_1 Ant(1)], [V_1 Ant(2)] or verapamil.

The treatment of the hearts with verapamil did not exert any significant effect on coronary flow and heart rate, but significantly reduced contractile force (n = 5, Table I). During this treatment AVP markedly reduced contractile force, coronary flow and heart rate (n = 5, Fig. 4, 5 and 6). The infusion of methylene blue also produced a significant decrease in contractile force (n = 8), while heart rate (n = 7) and coronary flow (n = 8) were not significantly affected (Table I). The administration of AVP after the infusion of the drug also markedly lowered contractile force, coronary flow (n = 8) and heart rate (n = 7) (Fig. 7, 8 and 9).

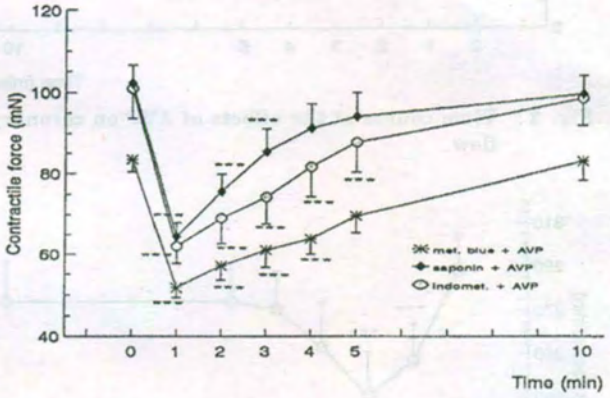

Fig. 7: The effects of AVP on contractile force during indomethacin, saponin or after methylene blue treatments. Methylene blue (met. blue), indomethacin (indomet.)

TABLE I: The baseline values and maximal decreases in contractility, heart rate and coronary flow after administration of verapamil (5×10^{-7} M) indomethacin (10^{-5} M), methylene blue (10^{-6} M) or saponin (500 µg/ml).

	Contractile force (mN)	*Heart rate (beats/min)*	*Coronary flow (ml/min)*
Control	98.6±5.56	285.2±10.3	24±1.09
Verapamil	81±4.3***	279.4±10.02	25.2±0.86
Control	106.66±7.1	319.5±13.14	20.66±0.33
Indomethacin	101±7.26	307±12.56	20.33±0.21
Control	105.25±2.2	276.42±12.88	19.75±0.67
Methylene blue	85.5±3.71***	259.42±9.77	18.75±0.72
Control	102.57±3.99	236±12.88	18.14±0.45
Saponin	102.28±4.26	226±12.26	17.85±0.5

***P<0.001 significantly different from respective control.

As illustrated in Table I saponin alone had no significant effect on contractile force coronary flow (n = 7) and heart rate (n = 5). On the other hand, AVP in the presence of this agent significantly decreased contractile force, coronary flow (n = 7) and heart rate (n = 6) (Fig. 7, 8 and 9). Indomethacin alone also did not affect significantly contractile force, coronary flow and heart rate (n = 6, Table I). In the presence of this drug AVP markedly reduced contractile force, coronary flow and heart rate (n = 6, Fig 7, 8 and 9).

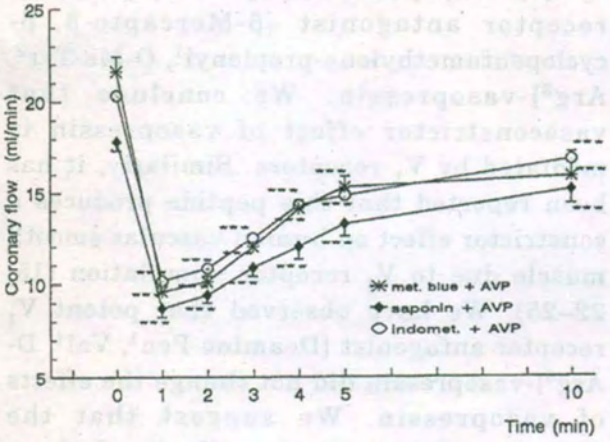

Fig. 8: Time course of the effects of AVP on coronary flow during indomethacin, saponin or after methylene blue treatments.

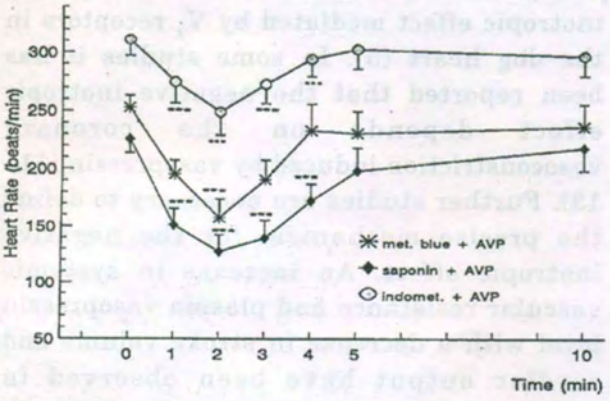

Fig. 9: The effects of AVP on heart rate during indomethacin, saponin or after methylene blue treatments.

DISCUSSION

Our results demonstrate that vasopressin markedly decreases myocardial contractility heart rate and coronary flow in the isolated perfused rat hearts. Our findings are in agreement with the results from previous studies showing that vasopressin produces coronary vasoconstrictor (2, 3, 17, 18), negative inotropic (2, 3, 5, 17) and chronotropic (6) effect.

We have used saponin in order to disrupt the endothelium. In isolated perfused guinea pig hearts electron microscopic examination confirmed that saponin treatment destroyed the endothelium while the vascular smooth muscle was left intact (19). Our data show that the negative inotropic, chronotropic and coronary vasoconstrictor effects of vasopressin may not depend on the presence of an intact endothelium because vasopressin after removal of endothelium by saponin treatment produced same effects. We suggest that substances released from endothelial cells may not responsible for mediating these cardiac effects of vasopression. We also suggest that vasopressin receptors which mediates contraction may be located predominantly on smooth muscle cells but not endothelial cells.

It has been demonstrated that vasopressin increases guanosine 3', 5'-monophosphate (cGMP) by interacting with oxytocin receptors coupled to soluble

guanylate cyclase in LLC-PK1 kidney epithelial cells (20). Accordingly the possibility that cGMP may mediate the effect of vasopressin on contractility, heart rate and coronary tone has been investigated in the present study. We have found that an inhibitor of soluble guanylate cyclase did not change the negative inotropic, chronotropic and vasoconstrictor effects of vasopressin. These findings suggest that cGMP does not involve in these effects.

We have observed that in the presence of indomethacin vasopressin still caused a marked decline in contractility, heart rate and coronary flow. That fact that the effects of vasopressin were not modified by indomethacin suggests that the release of prostaglandins does not play a role in the effects of the peptide on heart. It has been reported that vasopressin increases coronary vascular resistance and PGI_2 production in perfused nonbeating (A-V node suppressed) rat hearts and the inhibition of cyclooxygenase by indomethacin causes a fall in coronary vascular resistance (21). However, it has been demonstrated that indomethacin increases renal vasoconstrictor response to vasopressin in conscious rats (6). On the other hand, we have observed that indomethacin treatment had no effect on vasopressin-induced coronary vasoconstriction. The differences in preparations used in these studies may be responsible for the different results.

We have also considered the possibility that the effects of vasopressin may depend on an increase in extracellular Ca^{2+} entry through slow channels. We have observed that the calcium channel antagonist verapamil did not change the effects of this peptide. Similarly, the experiments with human mesenteric arteries have shown that the other calcium antagonist nifedipine did not significantly change the constrictor responses induced by vasopressin (16). Our results suggest that an influx of extracellular Ca^{2+} through slow channels does not play a role in the cardiac effects of vasopressin.

In the present study changes induced by AVP were prevented by a very potent V_1 receptor antagonist [β-Mercapto-β, β-cyclopentamethylene-propionyl[1], O-Me-Tyr[2], Arg[8]]-vasopressin. We conclude that vasoconstrictor effect of vasopressin is mediated by V_1 receptors. Similarly, it has been reported that this peptide produces a constrictor effect on human vascular smooth muscle due to V_1 receptor stimulation (15, 22–25). We have observed that potent V_1 receptor antagonist [Deamino-Pen[1], Val[4], D-Arg[8]]-vasopressin did not change the effects of vasopressin. We suggest that the difference between the effects of these antagonists may be due to their different potencies. It has been suggested that vasopressin produces a direct negative inotropic effect mediated by V_1 receptors in the dog heart (5). In some studies it has been reported that the negative inotropic effect depends on the coronary vasoconstriction induced by vasopressin (11–13). Further studies are necessary to define the precise mechanism for the negative inotropic effect. An increase in systemic vascular resistance and plasma vasopressin level with a decrease in stroke volume and cardiac output have been observed in patients with burn injury (26). The blockade of V_1 receptors by using [β-Mercapto-β, β-

cyclopentamethylene-propionyl[1], O-Me-Tyr[2], Arg[8]]-vasopressin may be useful in pathophysiological conditions where vascular resistance are increased and contractility are decreased by high plasma vasopressin levels. It has been suggested that the V_1 receptor blockade decreases vascular resistance in clinical conditions such as hypertension and congestive heart failure with increased plasma vasopressin concentration (27, 28).

In the present study vasopressin-induced vasoconstriction may depend on the increase in cytosolic Ca^{2+} concentration. It has been found that vasopressin rises cytosolic free Ca^{2+} concentration in neonatal rat cardiomyocyte (29, 30) and A7r5 aortic smooth muscle cells (31). It is also possible that vasopressin may cause the Ca^{2+} mobilization from intracellular stores and induce an increase in intracellular Ca^{2+} concentration.

We have observed that AVP produces a negative chronotropic effect. In isolated guinea pig hearts it has been reported that the decrease in coronary flow induced by AVP causes a negative chronotropic effect (32). Therefore, the bradycardia observed in our experiments may depend on the AVP-induced decrease in coronary flow.

In conclusion, our result demonstrate that vasopressin produces negative inotropic, chronotropic and coronary vasoconstrictor effects in the isolated rat hearts. These effects are mediated by V_1 receptors and independent of endothelium. cGMP, prostaglandin release or an influx of extracellular calcium through slow channels does not play a role in the cardiac effects of this peptide. [β-Mercapto-β, β-cyclopentamethylene-propionyl[1], O-Me-Tyr[2], Arg[8]]- vasopressin may exert beneficial effects by preventing vasopressin-induced negative inotropic, chronotropic and coronary vasoconstrictor actions.

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