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European Journal of Pharmacology 342 (1998) 225–233

ejp

Coronary vasoconstriction produced by vasopressin in anesthetized goats. Role of vasopressin V_1 and V_2 receptors and nitric oxide

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Received 28 July 1997; revised 30 October 1997; accepted 4 November 1997

Abstract

To examine the role of vasopressin V_1 and V_2 receptors, nitric oxide and prostanoids in the coronary vascular effects of [Arg^8]vasopressin, coronary blood flow was measured with an electromagnetic flow transducer placed around the left circumflex (23 goats) or anterior descending (11 goats) coronary artery and vasopressin (0.03–1 μg) was intracoronarily injected in 34 anesthetized, open-chest goats. Basal mean values for coronary blood flow, mean systemic arterial pressure and heart rate, were 34 ± 2.38 ml/min, 89 ± 3.34 mmHg and 80 ± 3.06 beats/min, respectively. Vasopressin produced dose-dependent decreases in coronary blood flow and the maximal reduction of this flow, attained with 1 μg of vasopressin, was 14 ± 1.49 ml/min (42 ± 2.64% of basal flow) ($P < 0.01$). Desmopressin (0.03–1 μg; 8 goats) did not affect significantly coronary blood flow. The intracoronary infusion of the antagonist for vasopressin V_1 receptors d(CH_2)$_5$Tyr (Me) arginine vasopressin (2 μg/min per kg, 6 animals) significantly diminished the effects of vasopressin on coronary blood flow (the effects of 1 μg of vasopressin were reduced by 28%, $P < 0.05$). The mixed antagonist for vasopressin V_1 and V_2 receptors desGly-d(CH_2)$_5$-D-Tyr(Et)Val arginine vasopressin (0.2, 0.7 and 2 μg/min per kg, 9 animals) decreased in a dose-dependent manner the effects of vasopressin on coronary blood flow (the effects of 1 μg of vasopressin were decreased by 61% with 2 μg/min per kg, $P < 0.01$). Intracoronary infusion of saline (vehicle, 3 goats) did not change the effects of vasopressin on coronary blood flow. Intravenous administration of the inhibitor of nitric oxide synthesis N^{ω}-nitro-L-arginine methyl ester (L-NAME, 47 mg/kg, 9 animals) decreased resting coronary blood flow by 10% ($P < 0.01$) and augmented mean systemic arterial pressure by 20% ($P < 0.01$), without changing heart rate. During this treatment the reduction in coronary blood flow produced by vasopressin was higher than under control (the effects of 1 μg of vasopressin were increased by 28%, $P < 0.01$). Intravenous administration of the inhibitor of cyclooxygenase, meclofenamate (5 mg/kg, 7 animals), neither modified resting coronary blood flow, arterial pressure and heart rate nor the effects of vasopressin on this flow. These data indicate that vasopressin produces marked coronary vasoconstriction and suggest that: (a) it may be mediated by vasopressin V_1 receptors, without involvement of vasopressin V_2 receptors, (b) it is probably inhibited by nitric oxide under normal conditions and (c) it may be not modulated by prostanoids. © 1998 Elsevier Science B.V.

Keywords: Coronary circulation; Coronary blood flow; Vasopressin receptor; N^{ω}-nitro-L-arginine methyl ester (L-NAME); Prostanoid

1. Introduction

Arginine vasopressin is present in the plasma of normal humans and animals, its plasma concentrations can increase in some stressful situations and it could be involved in the regulation of the cardiovascular system (Cowley and Liard, 1987). Although [Arg^8]vasopressin can induce powerful vasoconstrictor effects in a variety of vascular beds, a common finding is the heterogeneity of vascular reactivity depending on the vascular bed and the species (Altura and Altura, 1984; Cowley and Liard, 1987; García-Villalón et al., 1996). Arginine vasopressin can interact with at least two types of receptors, V_1 and V_2 receptors. Vasopressin V_1 receptors mediate the vasoconstriction elicited by this peptide (Cowley and Liard, 1987), and vasopressin V_2 receptors may mediate vasodilatation in some vascular beds (Schwartz et al., 1985; Walker, 1986; Abboud et al., 1990). The modulator role of nitric oxide and prostaglandins in the vascular actions of arginine vasopressin has been also suggested (Randall et al., 1988; Gardiner et al., 1991).

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With regard to the coronary circulation, although there are studies showing that arginine vasopressin can produce coronary vasodilatation (Berde, 1965; Altura, 1966; Turlapaty and Altura, 1982), most studies indicate that this peptide produces coronary vasoconstriction (Green et al., 1942; Nakano, 1967; Corliss et al., 1968; Heyndrickx et al., 1976; Khayyal et al., 1985; Myers et al., 1989; Maturi et al., 1991) and some of these studies suggest that this vasoconstriction could be severe enough to cause myocardial ischemia (Khayyal et al., 1985; Maturi et al., 1991). These observations, together with data showing that high levels of plasma arginine vasopressin are present in myocardial infarction (Hart and Gokal, 1977; Raya et al., 1990; Donald et al., 1994), suggest that this peptide could be of importance in the physiology and pathophysiology of the coronary circulation.

Studies of the mechanism of action of arginine vasopressin in the coronary vasculature have provided conflicting results and more investigations are required for an understanding of these mechanisms. Some experiments suggest that the coronary vasoconstriction produced by arginine vasopressin in rabbits (Serradeil-Le-Gal et al., 1995) and in isolated rat hearts (Walker et al., 1988) as well as in isolated arteries from rabbit hearts (García-Villalón et al., 1996) is mediated by vasopressin V_1 receptors. Experiments where agonists for vasopressin V_2 receptors have been used provide contradictory results because these agonists had no effect on the coronary vasculature of isolated rat hearts (Walker et al., 1988) or caused coronary vasodilatation in dogs (Liard, 1986).

With regard to the role of the endothelium and nitric oxide in the coronary vascular effects of arginine vasopressin, the results reported are controversial (Katusic et al., 1984; Myers et al., 1989; Liard, 1994; Pohl et al., 1994; Bax et al., 1995; Rapps et al., 1997). In relation to the role played by nitric oxide in these effects, it has been reported that inhibition of nitric oxide synthesis does not affect the coronary vasoconstriction in isolated hearts from rats (Pohl et al., 1994) and isolated coronary arteries from dogs (Rapps et al., 1997), that inactivation of endothelium-derived relaxing factor (nitric oxide) with hemoglobin increases the contraction of isolated coronary arteries from pigs (Myers et al., 1989) and that inhibition of nitric oxide synthesis diminishes in vivo coronary vasodilatation in dogs (Liard, 1994). With regard to the role of prostanoids, it has been reported that these substances may be (Lee et al., 1991) and may not be (Maturi et al., 1991; Rapps et al., 1997) involved in the coronary vasoconstriction in response to arginine vasopressin.

The present study was performed to examine the effects of arginine vasopressin on the coronary vasculature and especially to analyze the role played by vasopressin V_1 and V_2 receptors, nitric oxide and prostanoids in these effects. The experiments were carried out in anesthetized goats in which the effects of intracoronary injections of arginine vasopressin and desmopressin (selective agonist for vasopressin V_2 receptors) on blood flow through the left circumflex or left descending coronary arteries (electromagnetically measured) were recorded under control conditions, after intracoronary administration of a specific antagonist for vasopressin V_1 receptors or a combined antagonist for vasopressin V_1 and V_2 receptors, after i.v. administration of the inhibitor of nitric oxide synthesis N^{ω}-nitro-L-arginine methyl ester (L-NAME) and after i.v. administration of the cyclooxygenase inhibitor meclofenamate. To examine the role of the endothelium in the coronary vascular effects of arginine vasopressin, a series of in vitro experiments was also performed.

2. Methods

2.1. Experimental preparation

In this study 34 female goats (35–61 kg) were used. The animals were anesthetized with intramuscular injection of 10 mg/kg ketamine hydrochloride and i.v. administration of 2% thiopental sodium; supplemental doses were given as necessary for maintenance. After orotracheal intubation, artificial respiration with room air was instituted by use of a Harvard respirator.

A left thoracotomy in the fourth interspace was performed and the pericardium was opened. The proximal segment of the left circumflex coronary artery (23 animals) or the anterior descending coronary artery (11 animals) was dissected and an electromagnetic flow transducer (Biotronex) was placed on one of these arteries to measure blood flow. A snare-type occluder was also placed around the artery, just distal to the probe, to obtain baseline flow.

Systemic arterial pressure was measured through a polyethylene catheter placed in one temporal artery and connected to a Statham transducer. Blood flow, systemic arterial pressure and heart rate were simultaneously recorded on a Grass model 7 polygraph.

2.2. Experimental protocol

2.2.1. In vivo studies

Arginine vasopressin (34 goats) and desmopressin (selective agonist for vasopressin V_2 receptors; 8 of the 34 goats), prepared in isotonic saline, were directly injected through a catheter into the coronary artery where blood flow was measured at doses of 0.03, 0.1, 0.3 and 1 μg, using volumes of 0.3 ml injected over 5–10 s. Arginine vasopressin was injected in the animals under control conditions and under the following conditions: (a) during intracoronary infusion of d$(CH_2)_5$Tyr(Me) arginine vasopressin (selective vasopressin V_1 receptor antagonist); (b) during intracoronary infusion of desGly-d$(CH_2)_5$-D-Tyr(Et)Val arginine vasopressin (combined vasopressin V_1 and V_2 receptor antagonist); (c) during i.v. administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME, inhibitor of

nitric oxide synthesis) and (d) after i.v. administration of meclofenamate (inhibitor of cyclooxygenase). Desmopressin was injected under control conditions (5 goats) and during a state of moderate coronary vasoconstrictor tone induced by intracoronary infusion of endothelin-1 (0.04–0.06 nmol/min) (3 goats). Desmopressin was not studied during administration of the antagonists for vasopressin receptors because it did not produce any effect on resting coronary blood flow. The antagonists for vasopressin receptors were dissolved in saline (250 μg/ml) and the antagonist for vasopressin V_1 receptors was infused at rate of 2 μg/min per kg body weight in 6 goats and the combined antagonist for vasopressin V_1 and V_2 receptors was infused in 9 goats at rates of 0.2 (4 goats), 0.7 (5 goats) and 2 (4 goats) μg/min per kg body weight (the 4 goats that received the combined antagonist at a dose of 0.2 μg/min/kg were from the group of 5 goats that received the combined antagonist at a dose of 0.7 μg/min per kg). The rate of infusion of these antagonists ranged from 0.1–0.3 ml/min and they were infused through the same catheter used for injecting arginine vasopressin. This peptide was injected 10 min after the start of the administration of the antagonists; during the injection of arginine vasopressin the infusion of the antagonist was stopped. For testing the action of these antagonists, we first recorded the effects of arginine vasopressin under control conditions and then 30–40 min after this control dose–response curve was completed, we recorded in the same animal the effects of arginine vasopressin after treatment with the selective vasopressin V_1 receptor antagonist or the mixed vasopressin V_1 and V_2 receptor antagonist. Two dose–response curves for arginine vasopressin were consecutively determined under control conditions in 3 of the animals that then received the intracoronary infusion of the vasopressin V_1 receptor antagonist, and in 2 of the animals that then received the mixed vasopressin V_1 and V_2 receptors antagonist. Arginine vasopressin was also tested in 3 other animals before (control) and during intracoronary infusion of isotonic saline (vehicle) at rate of 0.3 ml/min. L-NAME, prepared in isotonic saline at a concentration of 10 mg/ml, was administered in an i.v. bolus (35 mg/kg), followed by i.v. infusion at 0.05–0.1 mg/kg per min in 9 animals (in total each animal received 47 mg/kg of L-NAME); arginine vasopressin was injected during this i.v. infusion of L-NAME when the hemodynamic variables reached a steady state. Meclofenamate, prepared in isotonic saline at a concentration of 10 mg/ml, was i.v. administered by hand at a dose of 5 mg/kg over 5–8 min in 7 goats; arginine vasopressin was administered 20–30 min after the end of injection of meclofenamate.

Blood samples from the temporal artery were taken periodically to measure pH, pCO_2 and pO_2 by standard electrometric methods (Radiometer, ABL 300, Copenhagen, Denmark). After termination of the experiments, the goats were euthanized with an overdose of i.v. thiopental sodium and potassium chloride.

2.2.2. In vitro studies

The hearts of 8 goats were removed after the animals were anesthetized with an i.v. injection of 2% thiopental sodium and killed by injection of a saturated solution of potassium chloride. Branches of the left anterior descending and circumflex coronary arteries were dissected free and cut into cylindrical segments, 3 mm in length and 0.7–1 mm in external diameter and then mounted in organ baths for isometric tension recording. Two stainless steel pins, 150 μm in diameter, were introduced through the arterial lumen; one pin was fixed to the organ wall and the other was connected to a strain gauge. The recording system included a Universal transducing cell (UC 3), a Statham microscale accessory (U15) and a Beckman type RS recorder. Each artery segment was set up in a 4 ml organ bath containing modified Krebs–Henseleit solution of the following composition (in mM): 115 NaCl, 4.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 25 $NaHCO_3$, 11.1 glucose and 0.01 disodium EDTA. The solution was equilibrated with 95% O_2–5% CO_2 to give a pH of 7.3–7.4. The temperature was held at 37°C.

A resting tension of 1 g was applied to each vascular segment and equilibrated for 60–90 min before experiments began. Concentration–response curves for arginine vasopressin (10^{-11} to 10^{-7} M) were determined in a cumulative manner in resting coronary arteries under control conditions, after endothelium removal and after treatment with L-NAME (10^{-4} M). L-NAME was applied to the organ bath 20–25 min before arginine vasopressin was tested. Removal of the endothelium was performed by gently rubbing the internal surface of the arteries with a roughened steel rod. The presence of the endothelium or the adequacy of endothelium removal was tested functionally at the end of the experiments by recording the relaxatory response to acetylcholine (10^{-7} and 10^{-6} M) in the arteries after contraction with arginine vasopressin.

The geometric effective concentrations eliciting 50% of the maximal response (EC_{50}) to arginine vasopressin were obtained by a graphic method for the different conditions tested.

2.3. Chemicals

The drugs used were: [Arg^8]vasopressin acetate; [deamino-Cys^1, D-Arg^8]vasopressin (desmopressin) acetate; the combined vasopressin V_1 and V_2 receptor antagonist des-Gly^9-(b-mercapto-b,b-cyclopenta-methylenepropionyl^1, O-Et-Tyr^2, Val^4, Arg^8)-vasopressin [desGly-d(CH_2)$_5$-D-Tyr(Et)Val arginine vasopressin]; the vasopressin V_1 receptor antagonist (b-mercapto-b,b,-cyclopenta-methylenepropionyl^1, O-Me-Tyr^2, Arg^8)-vasopressin [d(CH_2)$_5$Tyr(Me) arginine vasopressin] and N^w-nitro-L-arginine methyl ester hydrochloride (L-NAME), all from Sigma and sodium meclofenamate was from Parke Davis.

2.4. Data analysis

The data are expressed as means ± S.E.M. The effects of the different doses of arginine vasopressin and desmopressin on the hemodynamic variables recorded under control conditions were analyzed by using an analysis of variance, followed by Dunnett's test. The effects of d(CH_2)$_5$Tyr(Me) arginine vasopressin, desGly-d(CH_2)$_5$-D-Tyr(Et)Val arginine vasopressin, L-NAME and meclofenamate on the hemodynamic variables recorded and on blood gases and pH, as well as the action of arginine vasopressin on the hemodynamic variables recorded before (control) and after the treatments used (the antagonist for vasopressin V_1 receptors, the mixed antagonist for vasopressin V_1 and V_2 receptors, L-NAME and meclofenamate) were evaluated by using the Student's *t*-test for paired data; in each case the same animal was used as its own control.

For the different conditions tested, the changes in coronary blood flow, measured during the maximal effects, were considered both as percentages and as absolute values. The changes in the other hemodynamic variables recorded and blood gases and pH were considered only in absolute values. Results for the in vitro studies were analyzed by means of Student's *t*-test for unpaired data. In each case, $P < 0.05$ was considered statistically significant.

3. Results

3.1. In vivo studies

3.1.1. Control conditions

Arginine vasopressin (0.03–1 μg), injected directly into the left anterior descending (11 goats) or left circumflex (23 goats) coronary artery, reduced blood flow in the corresponding artery in a dose-dependent manner. The effects of arginine vasopressin on blood flow were comparatively similar in both coronary arteries and consequently the results obtained in these two arteries were pooled. For 34 animals, the reduction in coronary blood flow averaged 10 ± 1.09% (0.03 μg), 17 ± 1.28% (0.1 μg), 32 ± 2. 34% (0.3 μg) and 42 ± 2.64% (1 μg) (all $P < 0.01$).

The effects of arginine vasopressin on coronary blood flow were evident at about 5 s and they were maximal at about 25–30 s after the start of drug administration. The doses of 0.3 and 1 μg of this peptide also increased significantly systemic arterial pressure and this effect began to be evident at about 2 min for 0.3 μg and at about 1 min for 1 μg after drug administration (these effects have not been considered in the present work). Heart rate was not significantly changed after arginine vasopressin administration.

The two dose–response curves for arginine vasopressin obtained consecutively under control conditions in 5 animals showed that the effects of this peptide on coronary blood flow were not significantly different (not shown). The effects of arginine vasopressin on coronary blood flow recorded during intracoronary infusion of isotonic saline (vehicle) in 3 goats were not significantly different from those recorded under control conditions in the same animals (not shown).

Desmopressin (agonist for vasopressin V_2 receptors, 0.03–1 μg) did not affect significantly coronary blood flow, systemic arterial pressure and heart rate in 5 goats under control conditions. Also, desmopressin did not modify these hemodynamic parameters in 3 goats in which coronary vasoconstrictor tone had been induced previously by intracoronary infusion of endothelin-1 (in these animals endothelin-1 decreased resting coronary blood flow by about 25% of basal values, without changing systemic arterial pressure and heart rate) (these data are not shown).

Table 1 summarizes the hemodynamic effects of arginine vasopressin and desmopressin in anesthetized goats under control conditions.

3.1.2. Effects of the antagonists for vasopressin receptors

Intracoronary administration of the specific antagonist for vasopressin V_1 receptors, d(CH_2)$_5$Tyr(Me) arginine vasopressin (2 μg/min per kg body weight; 6 goats), did not affect resting coronary blood flow, systemic arterial pressure and heart rate. During this treatment, the reduction in coronary blood flow produced by arginine vasopressin (0.03–1 μg) both in percentage and in absolute value, was significantly lower than under control conditions (Fig. 1). In 3 of these goats we found that 30–40 min after the intracoronary administration of this vasopressin V_1 antagonist was stopped, the effects of arginine vasopressin on coronary blood flow had normalized as they were not significantly different from those obtained under

Table 1
Hemodynamic effects of arginine vasopressin (AVP) and desmopressin in anesthetized goats under control conditions

	CBF (ml/min)	MAP (mm Hg)	HR (beats/min)
Basal ($n = 34$)	34 ± 2.38	89 ± 3.34	80 ± 3.06
AVP, μg ($n = 34$)			
0.03	31 ± 2.17	89 ± 3.33	81 ± 3.24
0.1	29 ± 2.15	91 ± 3.63	81 ± 3.23
0.3	24 ± 2.09[a]	92 ± 3.73	81 ± 3.18
1	20 ± 1.85[a]	93 ± 3.86	80 ± 3.20
Desmopressin, μg ($n = 8$)			
0.03	33 ± 4.10	91 ± 5.20	82 ± 4.17
0.1	33 ± 5.09	91 ± 4.68	82 ± 5.07
0.3	32 ± 4.97	90 ± 4.18	80 ± 4.88
1	32 ± 4.05	91 ± 4.77	80 ± 5.93

Values are means ± S.E.M. These data correspond to measurements obtained during the maximal effects of AVP on CBF, where there were no significant changes in systemic arterial pressure and heart rate.
CBF = coronary blood flow; MAP = mean systemic arterial pressure; HR = heart rate.
n = number of animals.
[a] $P < 0.01$ compared with the control.

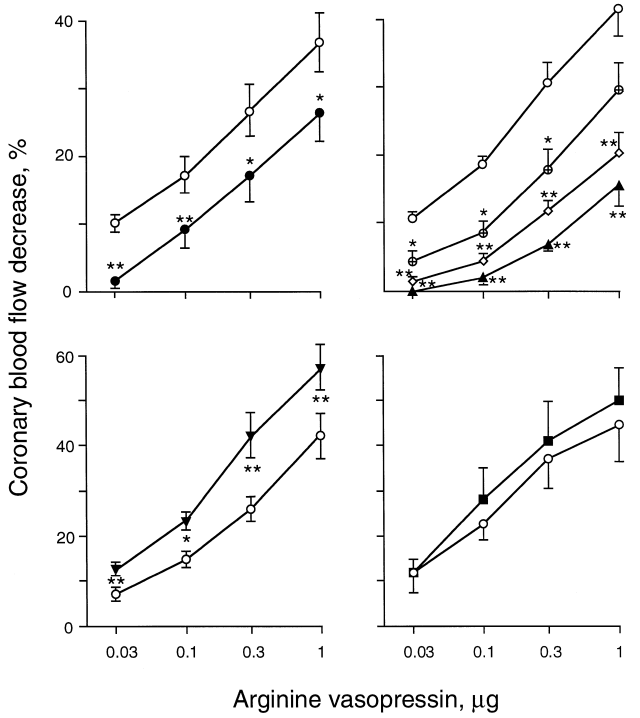

Fig. 1. Summary of decreases in coronary blood flow produced by arginine vasopressin in anesthetized goats under control conditions (– ∘ –) and after treatment with the antagonist for vasopressin V_1 receptors $d(CH_2)_5$Tyr(Me) arginine vasopressin (2 μg/min per kg, – • –, 6 animals, top, left), with the combined antagonist for vasopresin V_1 and V_2 receptors desGly-$d(CH_2)_5$-D-Tyr(ET)Val arginine vasopressin (0.2 μg/min per kg, – ⊕ –, 4 animals; 0.7 μg/min per kg, – ◇ –, 5 animals; 2 μg/min per kg, – ▲ –, 4 animals, top, right), with L-NAME (47 mg/kg, – ▼ –, 9 animals, bottom, left) and with meclofenamate (5 mg/kg, 7 animals, – ■ –, bottom, right). $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the corresponding control.

control conditions. This antagonist did not affect significantly the coronary blood flow reduction produced by intracoronary injections of endothelin-1 (0.01–0.3 nmol) in the same goats (these particular results are not shown).

Intracoronary administration of the mixed antagonist for vasopressin V_1 and V_2 receptors, desGly-$d(CH_2)_5$-D-Tyr(Et)Val arginine vasopressin 0.2 (4 goats), 0.7 (5 goats) and 2 (4 goats) μg/min per kg body weight, did not change resting coronary blood flow, systemic arterial pressure and heart rate. After treatment at these doses, the reduction in coronary blood flow produced by arginine vasopressin (0.03–1 μg), both in percentage and absolute value, was diminished in a dose-dependent way (Fig. 1). This mixed antagonist infused at the rate of 2 μg/min per kg did not modify significantly the reduction in coronary blood flow caused by intracoronary injections of endothelin-1 (0.01–0.3 nmol) in 4 goats (these results are not shown).

3.2. Effects of treatment with L-NAME

Intravenous administration of L-NAME (47 mg/kg; 9 goats) reduced slightly but significantly resting coronary blood flow by 10% ($P < 0.05$) and augmented significantly mean systemic arterial pressure by 20% ($P < 0.01$%), without changing significantly heart rate and blood gases and pH (Table 2).

Table 2
Hemodynamic variables and blood gases and pH values obtained from 9 anesthetized goats under control conditions and after treatment with N^{w}-nitro-L-arginine methyl ester (L-NAME, 47 mg/kg)

	Control	L-NAME
CBF (ml/min)	34 ± 2.59	30 ± 2.28[a]
MAP (mm Hg)	89 ± 4.22	107 ± 6.07[a]
HR (beats/min)	92 ± 5.88	87 ± 3.84
pO_2 (mm Hg)	101 ± 5.73	107 ± 6.04
pCO_2 (mm Hg)	29 ± 4.36	28 ± 4.80
pH	7.40 ± 0.02	7.39 ± 0.03

Values are means ± S.E.M.
CBF = coronary blood flow; MAP = mean systemic arterial pressure; HR = heart rate.
[a] $P < 0.01$ compared with the control.

After L-NAME, the reduction in coronary blood flow elicited by arginine vasopressin (0.03–1 μg) was significantly higher than under control conditions, both in absolute value and in percentage. Fig. 1 summarizes the effect, as percentages, of the effects of arginine vasopressin, on coronary blood flow measured under control conditions and during L-NAME treatment. In absolute values (ml/min) the reduction in coronary blood flow produced by arginine vasopressin under control conditions and during L-NAME treatment, respectively, was for 0.03 μg, 2 ± 0.44 versus 3.7 ± 0.49 ($P < 0.05$); for 0.1 μg, 5 ± 0.51 versus 7.3 ± 1.05 ($P < 0.01$); for 0.3 μg, 9 ± 1.24 versus 13 ± 1.85 ($P < 0.01$) and for 1 μg, 13.4 ± 1.74 versus 19 ± 2.66 ($P < 0.01$).

The effects of arginine vasopressin on systemic arterial pressure and heart rate were not significantly different under control conditions and after L-NAME treatment.

3.2.1. Effects of treatment with meclofenamate

Intravenous administration of meclofenamate (5 mg/kg; 7 goats) did not change significantly basal values for the hemodynamic variables recorded, or blood gases and pH (Table 3). The effects of arginine vasopressin (0.03–1 μg)

Table 3
Hemodynamic variables and blood gases and pH values obtained from 7 anesthetized goats under control conditions and after treatment with meclofenamate (5 mg/kg)

	Control	Meclofenamate
CBF (ml/min)	31 ± 3.72	29 ± 2.01
MAP (mm Hg)	88 ± 5.85	87 ± 5.64
HR (beats/min)	77 ± 4.28	75 ± 5.74
pO_2 (mm Hg)	98 ± 5.58	97 ± 5.17
pCO_2 (mm Hg)	30 ± 4.15	31 ± 4.93
pH	7.38 ± 0.03	7.39 ± 0.02

Values are means ± S.E.M.
CBF = coronary blood flow; MAP = mean system arterial pressure; HR = heart rate.

on coronary blood flow as well as on systemic arterial pressure and heart rate were similar under control conditions and after treatment with meclofenamate (Fig. 1).

3.3. In vitro studies

In control coronary arteries, arginine vasopressin (10^{-11}–10^{-7} M, 16 segments from 8 goats) produced dose-dependent contractions and the EC_{50} values averaged 7.61 (5.42–10.63) $\times 10^{-10}$ M and the maximal contraction averaged 412 ± 42 mg. After endothelium removal, the response of coronary arteries to arginine vasopressin (10^{-11}–10^{-7} M, 16 segments from 8 goats) showed a similar sensitivity and a maximal contraction that tended to be higher, but not significantly, than that recorded in the control arteries. For these de-endothelized arteries, the EC_{50} values averaged 5.38 (2.37–12.40) $\times 10^{-10}$ M and the maximal contraction averaged 472 ± 42 mg ($P > 0.05$ compared with the control arteries). In coronary arteries after 10^{-4} M L-NAME treatment (15 segments from 7 goats), the sensitivity was similar ($P > 0.05$) and the maximal contraction was higher ($P < 0.02$) than that in control arteries in response to arginine vasopressin. For these arteries treated with L-NAME, the EC_{50} values averaged 6.08 (4.47–8.27) $\times 10^{-10}$ M and the maximal contraction averaged 722 ± 88 mg.

4. Discussion

The present results for anesthetized goats show that arginine vasopressin injected intracoronarily produces a marked reduction in coronary blood flow, which was independent of its effects on systemic arterial pressure. They also indicate that the vascular territory of the left circumflex and that of left anterior descending coronary arteries exhibit similar reactivity to this peptide. This was confirmed by our in vitro results. Therefore, our results suggest that arginine vasopressin is a coronary vasoconstrictor, which agrees with findings reported by others on the basis of studies of the coronary circulation of different species (Green et al., 1942; Nakano, 1967; Corliss et al., 1968; Heyndrickx et al., 1976; Khayyal et al., 1985; Myers et al., 1989; Maturi et al., 1991). Some of these studies (Khayyal et al., 1985; Myers et al., 1989) suggest that the effects of arginine vasopressin on coronary blood flow are a consequence of its vasoconstrictor effect on distal coronary arteries.

In the present study, the levels of arginine vasopressin reached in coronary artery blood, estimated from the injected doses of this peptide and the measured coronary blood flow, ranged from 0.9 to 29 pg/l and in plasma, estimated from the injected doses of vasopressin and the total plasma volume, they ranged from 0.02 to 0.8 pg/l. The daily plasma levels of arginine vasopressin in normal humans and animals range from 0.0003 to 0.03 pg/l and they can increase to levels of 0.1–0.5 pg/l during certain stressful events (Cowley and Liard, 1987). After myocardial infarction in patients plasma levels of arginine vasopressin are increased (about 0.027 versus 0.002 pg/l) and in some of these patients they were of 0.1–0.2 pg/l (Donald et al., 1994). It is apparent that the levels of vasopressin reached in coronary artery blood in our study were higher than those described in plasma after hypotension and surgery (Cowley and Liard, 1987) and after myocardial infarction (Donald et al., 1994). However, we can consider that the estimated pharmacologic concentrations of vasopressin reached in coronary circulation in the present study were probably very transient, given the washout effect of blood flow and that the equilibrium levels of this peptide may be lower than those estimated. With regard to plasma, the levels of arginine vasopressin reached in the present study may be in the range of those found in patients during certain stressful events (Cowley and Liard, 1987; Donald et al., 1994) and they could affect peripheral vasculature and renal function.

Although there are many data indicating that arginine vasopressin produces marked coronary vasoconstriction, the mechanisms involved in these effects of this peptide are less clear. The present data show that both the specific antagonist for vasopressin V_1 receptors and the combined antagonist for vasopressin V_1 and V_2 receptors used blocked the effects of arginine vasopressin on coronary blood flow. Also, we found that desmopressin, a specific agonist for vasopressin V_2 receptors, did not affect coronary blood flow under basal conditions or under a moderate coronary vasoconstrictor tone induced with endothelin-1. These observations suggest that the reduction in coronary blood flow produced by arginine vasopressin is related to activation of vasopressin V_1 receptors by this peptide, with no or only slight involvement of vasopressin V_2 receptors. This is in agreement with results obtained for the coronary circulation of rabbits (Serradeil-Le-Gal et al., 1995) and for isolated rat hearts (Walker et al., 1988) as well as for isolated human (Bax et al., 1995) and rabbit (García-Villalón et al., 1996) coronary arteries. Our data, obtained with the selective vasopressin V_1 receptor antagonist, support the suggestion that vasopressin V_2 receptors do not play a role in the effects of arginine vasopressin in the coronary circulation. As it is accepted that vasopressin V_2 receptors mediate vasodilatation, if vasopressin V_2 receptors were present in our experimental preparation we would have expected that arginine vasopressin would increase coronary blood flow when it was tested in the presence of the selective antagonist for vasopressin V_1 receptors. The data of Walker et al. (1988) obtained for isolated rat hearts also suggest that vasopressin V_2 receptors are not involved in the effects of arginine vasopressin on the coronary vasculature.

Several investigations indicate that arginine vasopressin can produce vasodilatation, which may be ascribable to activation of vasopressin V_2 receptors (Schwartz et al.,

1985; Walker, 1986; Abboud et al., 1990). Walker (1986) observed systemic vasodilatation in response to arginine vasopressin in rats after blockade of vasopressin V_1 receptors. Liard (1986) found an increase in renal blood flow after vasopressin V_1 but not after combined vasopressin V_1 and V_2 receptor blockade and that vasopressin V_2 receptor activation increased blood flow in several vascular beds, including the coronary circulation, under conditions of a sustained increase in circulating arginine vasopressin concentration provoked by 48 h of dehydration in conscious dogs. In spite of these observations, the role of vasopressin V_2 receptors in mediation of the vacular effects of arginine vasopressin remains, however, controversial as there is no definitive evidence for the presence of these receptors in blood vessels (Lolait et al., 1992; Sharif and Hanley, 1992). The discrepancy between the present results and those of Liard (1986) with regard to the role of vasopressin V_2 receptors in the coronary circulation may be related, in part, to differences in the experimental approach and conditions used in two studies. Species differences may be an important factor in explaining the differences in the vascular effects and action mechanisms of arginine vasopressin (Cowley and Liard, 1987).

Knowledge about the role of vasopressin V_1 and V_2 receptors in the vascular effects of arginine vasopressin has been complicated by recent results that suggest that vasopressin V_1 receptors located in the endothelium may mediate relaxation in response to arginine vasopressin in the coronary and cerebral (Katusic et al., 1984) and pulmonary (Russ et al., 1992) circulations. Also, not only the contribution of vasopressin V_1 and V_2 receptors (Cowley and Liard, 1987), but also the role of the endothelium (García-Villalón et al., 1996) in mediating the vascular response to arginine vasopressin may differ between vascular beds and perhaps species. The present data for awake goats agree with those of Serradeil-Le-Gal et al. (1995) for the rabbit coronary circulation, with those of Walker et al. (1988) for the rat coronary circulation, with those of Bax et al. (1995) for human coronary arteries and with those of García-Villalón et al. (1996) for rabbit coronary arteries. These results suggest that vasopressin V_1 receptors mediate the coronary vasoconstriction elicited by arginine vasopressin, and that vasopressin V_2 receptors, if present, are not of functional significance for the coronary vascular effects of this peptide.

Experiments for examining the role of nitric oxide on the coronary vascular reactivity to arginine vasopressin are relatively sparse and the results reported are contradictory (Myers et al., 1989; Liard, 1994; Pohl et al., 1994). Some of these studies suggest that inactivation of endothelium-derived relaxing factor (nitric oxide) with hemoglobin potentiates the in vitro contraction of canine coronary resistance arteries elicited by arginine vasopressin (Myers et al., 1989), whereas inhibition of the synthesis of nitric oxide with N^G-nitro-L-arginine (L-NNA) does not affect the coronary vasoconstriction produced by arginine vasopressin in isolated hearts from rabbits (Pohl et al., 1994). Our data show that L-NAME reduced basal coronary blood flow, which suggests the presence of a basal vasodilator tone produced by nitric oxide. This confirms previous observations from our laboratory (García et al., 1992) and agree with data reported by others (Chu et al., 1991). Our experiments with L-NAME also show that the reduction in coronary blood flow produced by arginine vasopressin was augmented during treatment with this inhibitor of nitric oxide synthesis, which is in line with the results of Myers et al. (1989). Thus, it is suggested that, under normal conditions, nitric oxide may modulate the coronary vascular reactivity to arginine vasopressin by inhibiting its vasoconstrictor action. The present in vitro results support this hypothesis because L-NAME potentiated the maximal contraction of isolated coronary arteries. These in vitro studies also showed that endothelium removal tended to increase, but not significantly, the arterial response to arginine vasopressin. With these in vitro experiments we can not identify with confidence the source of the nitric oxide released in response to arginine vasopressin. It is possible that the procedure used for removing the endothelium damaged the underlying smooth musculature and this decreased the ability of the arteries to contract, thus masking the possible potentiating effects of endothelium removal upon the response to arginine vasopressin. Therefore, we can not exclude the endothelium as a source of nitric oxide released in response to this peptide.

In relation to the role of nitric oxide, our in vivo results differ from those of Pohl et al. (1994) and this discrepancy may be related to the different experimental preparations and animal species used in the two studies because Pohl et al. (1994) used isolated hearts from rabbits. Gardiner et al. (1991) also found that lower doses of L-NAME increased the vasoconstriction of renal, mesenteric and hindquarter vascular beds of conscious rats in response to arginine vasopressin. Therefore, nitric oxide release may oppose the vasoconstrictor effects of arginine vasopressin in different vascular beds, including the coronary vasculature, under normal conditions.

The present data for meclofenamate show that this substance did not affect the action of arginine vasopressin on coronary blood flow, suggesting that cyclooxygenase products are probably not involved in the coronary vasoconstriction produced by this peptide. This is in line with the results obtained in dog coronary vasculature in vivo (Maturi et al., 1991) and in vitro (Rapps et al., 1997). Lee et al. (1991) suggest that prostanoids modulate the coronary vasoconstriction induced by vasopressin in perfused rat hearts. The discrepancy between this study (Lee et al., 1991) and that of Maturi et al. (1991), that of Rapps et al. (1997) and the present study may be related to the different experimental preparations and animal species used in these studies, as was also evidenced for the role of nitric oxide. Studies performed in conscious rats indicate that indomethacin enhanced the renal vasoconstriction induced

by arginine vasopressin, whereas it attenuated the hindquarter vasoconstriction in response to arginine vasopressin (Gardiner et al., 1991). These observations of Gardiner et al. (1991) suggest that the role of cyclooxygenase products in the vascular response to arginine vasopressin may differ between vascular beds. The present results suggest that clooxygenase products do not have a role in the coronary vasculature.

In conclusion, the present data show that arginine vasopressin produces a marked coronary vasoconstriction in vivo which may be mediated by activation of vasopressin V_1 receptors without involvement of vasopressin V_2 receptors. Nitric oxide, but not cyclooxygenase products, may modulate the coronary vascular effects of arginine vasopressin by inhibiting its coronary vasoconstrictor effects.

Acknowledgements

The authors are grateful to H. Fernández-Lomana and M.E. Martínez for their technical assistance. This work was supported, in part, by FIS (96/0474), DGICYT (PM 95/0032) and CAM (AE 263/95).

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