Anesthesiology 2005; 102:85–92

Comparative Cardiac Effects of Terlipressin, Vasopressin, and Norepinephrine on an Isolated Perfused Rabbit Heart

Alexandre Ouattara, M.D., Marc Landi, M.D.,* Yannick Le Manach, M.D.,† Patrick Lecomte, M.D.,† Morgan Leguen, M.D.,† Gilles Boccara, M.D., Ph.D.,* Pierre Coriat, M.D.,‡ Bruno Riou, M.D., Ph.D.§*

Background: **Terlipressin, a synthetic analog of arginine-vasopressin (AVP), has been proposed as an effective vasopressive therapy in catecholamine-resistant vasodilatory shock. Although beneficial effects of terlipressin on systemic arterial pressure have been clearly demonstrated, its intrinsic effects on coronary circulation and myocardial performances remain unknown.**

Methods: **The authors compared the coronary and myocardial effects of terlipressin (1–100 nM, n = 10), AVP (10–1000 pM, n = 10), and norepinephrine (1–100 nM, n = 10) on an erythrocyte-perfused isolated rabbit heart. The cardiac effects of terlipressin were also assessed in erythrocyte-perfused hearts in which the myocardial oxygen delivery was maintained constant and buffer-perfused hearts. Finally, the cardiac effects of terlipressin and AVP were studied in hearts pretreated by $[d(CH_2)_5Tyr(Me)]AVP$ (0.1 μM), a selective V_{1a} receptor antagonist.**

Results: **Norepinephrine induced a biphasic coronary effect associated with a concentration-dependent increase in myocardial performances. AVP and terlipressin significantly decreased coronary blood flow and impaired myocardial performances from 30 pM and 30 nM, respectively ($P < 0.05$). The cardiac side-effects of terlipressin were confirmed in buffer-perfused hearts but the maintenance of a constant myocardial oxygen delivery constant abolished its effects on myocardial performances. The cardiac effects induced by terlipressin and AVP were nearly completely abolished on hearts pretreated by $[d(CH_2)_5Tyr(Me)]AVP$.**

Conclusions: **On isolated rabbit heart, terlipressin induced a coronary vasopressor effect and in turn myocardial depression only at supratherapeutic concentrations (≥30 nM). Its effects are mainly mediated *via* V_{1a} receptors. However, these potential negative side effects on the heart were less pronounced than were those of AVP.**

THE effectiveness of low-dose of exogenous arginine vasopressin (AVP) in restoring blood arterial pressure in patients suffering from catecholamine-resistant vasodilatory shock has been reported in various clinical settings.[1–5] Nonetheless, its ultrashort half-life of several minutes necessitates administration by continuous infusion. In addition, a hypotension rebound at the discontinuation of infusion enforces a careful stepwise withdrawal, which may take several days.[6,7] For these reasons, some authors have suggested the preferential use of terlipressin (tricyl-lysine vasopressin), a long-acting synthetic analog of AVP.[8–10] Terlipressin was initially used for the treatment of ruptured esophageal varices and hepatorenal syndrome in patients suffering from cirrhosis with portal hypertension.[11,12] It is a drug precursor that must be metabolized by endopeptidases into the vasoactive lysine-vasopressin.[13] Its specific metabolism determines that terlipressin has a longer biologic half-life than AVP (approximately 6 h), which allows it to be given in intermittent intravenous boluses. Like AVP, terlipressin exerts its vasopressor effects predominantly *via* vasopressin receptors but with a higher affinity for the vascular type V_{1a}. V_{1a}/V_2 receptor ratio is 2.2 for terlipressin but only 1 for AVP.[10] Despite the fact that AVP restores global hemodynamics, the fact remains that its potent vasopressor effect could compromise regional circulation.[14,15] Some *in vitro* studies have reported that AVP provokes a coronary vasoconstriction,[16,17] which may even be responsible for myocardial ischemia.[16] In addition, a direct negative inotropic effect could contribute to the decrease in the cardiac output observed during its clinical use.[17] Although some studies reported that the restoration of arterial pressure by terlipressin infusion is also associated with a decrease in cardiac output,[8] no previous study has evaluated its intrinsic cardiac effects. In addition, adverse myocardial ischemic events provoked by the potent vasopressive effects of terlipressin have been previously reported.[18] Thus, the aim of this study was to assess intrinsic cardiac effects of increasing concentrations of terlipressin (1 to 100 nM) on an erythrocyte-perfused and isolated rabbit heart. These effects were compared to those of AVP (10 to 1,000 pM) and norepinephrine (1 to 100 nM). In addition, the hypothesis of the implication of vasopressin receptors in terlipressin-induced cardiac effects was tested in additional hearts pretreated with $[d(CH_2)_5Tyr(Me)]AVP$, a potent selective antagonist of V_{1a} receptors.

Materials and Methods

A total of 55 hearts from New Zealand albino female rabbits weighing 2.75–3.25 kg were used in this study. Animals were fasted for 12 h before the experiment with free access to water. Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regu-

* Assistant Professor, † Fellow of Anesthesiology, ‡ Professor of Anesthesiology, Chairman, Department of Anesthesiology, § Professor of Anesthesiology, Director of the Laboratory of Anesthesiology, Chairman, Department of Emergency Medicine and Surgery, CHU Pitié-Salpêtrière, Paris.

Received from Laboratoire d' Anesthésiologie, Département d' Anesthésie-Réanimation, Service d'Accueil des Urgences, Centre Hospitalier Universitaire (CHU) Pitié-Salpêtrière, Université Pierre et Marie Curie, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France. Submitted for publication June 4, 2004. Accepted for publication August 27, 2004. Support was provided solely from institutional and/or departmental sources. Presented in part at the 46th Annual Congress of the French Society of Anesthesiologists, Paris, France, April 17–18, 2004.

Address correspondence and reprint requests to Dr. Ouattara: Département d' Anesthésie Réanimation, Groupe Hospitalier Pitié-Salpêtrière, 47 boulevard de l' Hôpital, 75651 Paris Cedex 13, France. Address electronic mail to: alexandre.ouattara@psl.ap-hop-paris.fr.

lations of the official edict of the French Ministry of Agriculture.

Perfusate Preparation

The perfusion medium was reconstituted by mixing human erythrocytes (Etablissement Français du Sang, Paris, France) and a modified Krebs-Henseleit bicarbonate buffer containing 118 mM NaCl, 5.9 mM K^+, 2.5 mM free Ca^{2+}, 0.5 mM $MgSO_4$, 1.17 mM NaH_2PO_4, 28 mM $NaHCO_3$, 11 mM glucose, 0.9 mM lactate, and 0.5% bovine serum albumin (Sigma-Aldrich; Saint-Quentin Fallavier, France). The human erythrocytes were stored at 4°C in our laboratory for no longer than 1 week. They were centrifuged and washed with saline (Cell-Saver 4; Hemonetics, Braintree, MA). The mixture allowed us to obtain a hemoglobin value of around 8 g/dl. The reconstituted blood was filtered, then continuously oxygenated with a gas mixture comprising 20% oxygen, 5% carbon dioxide, and 75% nitrogen using a membrane oxygenator (Optima; Cobe Cardiovascular, Arvada, CO). After rewarming to 37°C, electrolyte concentrations were adjusted to achieve physiologic concentrations and sodium bicarbonate was added to obtain a pH between 7.35 and 7.45.

Heart Preparation

Each of New Zealand rabbit was anesthetized with pentobarbital sodium (25–35 mg/kg intravenously). After thoracotomy, the heart and aorta arch were rapidly excised and placed in cold (4°C) isotonic saline solution. Under immersion, the pericardium was quickly removed and the aorta was cannulated. Then, a retrograde perfusion was begun according to the Langendorff's technique with a hydrostatic perfusion pressure of 80 mmHg. The coronary driving pressure was computed from the signal pressure obtained from a pressure transducer that was positioned above the aortic valves. The apparatus was modified to enable the continuous recording of speed of peristaltic pump, which reflected coronary blood flow[19,20] and was electronically regulated by the signal of coronary driving pressure to maintain it at 80 mmHg throughout the experiment. Whenever possible, the heart rate was maintained constant during each experiment by atrial pacing. Nevertheless, when the heart developed arrhythmia during pacing or when the spontaneous heart rate was faster than 150 bpm, the heart was not paced. The coronary sinus was drained by a small catheter inserted into the pulmonary artery. The drained venous blood was not recycled. A cannulated fluid-filled balloon connected to a pressure transducer by a rigid catheter was inserted into the left ventricle through a left atrial incision. A 2-ml graduated syringe was connected to this pressure transducer and allowed to increase the intraventricular volume. The latter was then adjusted to obtain a left ventricular end-diastolic pressure of approximately 10 mmHg.[21] Left ventricular end-systolic and end-diastolic pressures and heart rate were recorded, and the maximal positive and negative left ventricular pressure derivatives were electronically derived from the left ventricular pressure signal. Because intraventricular volume and heart rate were held constant, the maximal positive and negative left ventricular pressure derivatives reflected inotropic and lusitropic properties, respectively. The whole apparatus was enclosed in a thermostatic chamber at 37.5°C.

Blood Gas and Electrolytes Measurements

Arterial and venous coronary oxygen tension, arterial and venous coronary carbon dioxide tension, and pH were measured with standard electrodes at 37°C (GEM 3000; Instrumentation Laboratory, Saint-Mandé, France) and the arterial hemoglobin concentration and arterial and venous coronary saturations were measured with a hemoximeter (Model IL 682, Instrumentation Laboratory). Arterial and venous coronary oxygen content and myocardial oxygen consumption were derived from standard formulae. At the onset of each experiment, a sample of the reconstituted blood was withdrawn to determine the concentration of main electrolytes (Na^+, K^+, Cl^-, $NaHCO_3$, and Ca^{2+}).

Experimental Protocol

Cardiac Effects of Terlipressin, AVP, and Norepinephrine on Erythrocyte-perfused Hearts. A 10-min period was allowed for stabilization of ventricular performances and coronary blood flow. After baseline measurements, terlipressin (Glypressine, Ferring, Limhamn, Sweden) was added to the perfusion medium just above the aortic cannula in 10 hearts. Intracoronary infusions were used to produce target concentrations of 1, 3, 10, 30, and 100 nM. The infusion was maintained until a new steady state was obtained (approximately 5 min). After the end of each infusion of terlipressin, a recovery period was allowed to return to baseline values. Before and after each terlipressin infusion, arterial and coronary sinus samples were collected for blood gas analysis. Intrinsic cardiac effects of increased concentrations of [Arg^8]vasopressin (Sigma-Aldrich) from 10 to 1000 pM and norepinephrine (Aguettant, Lyon, France) from 1 to 100 nM were studied under similar conditions in additional hearts (n = 10 in each group). All these concentrations were chosen to encompass the therapeutic range. It was found that an intravenous bolus of 7.5 μg/kg of terlipressin induced a maximum plasma terlipressin concentration of approximately 17 nM.[13] In critically ill patients, Tsuneyoshi *et al.*[7] reported that a continuous infusion of vasopressin at 0.04 U/min induced a plasma vasopressin concentration of approximately 300 nM. Finally, a norepinephrine infusion of 0.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in anesthetized and ventilated dogs induced a mean plasma concentration of free norepinephrine of 44 nM.[22]

Cardiac Effects of Terlipressin on Erythrocyte-perfused Heart with Constant Myocardial Oxygen Delivery. To eliminate changes in myocardial performances induced by ischemic coronary vasopressor effect, we also assessed terlipressin in five hearts in which coronary blood flow was maintained constant. The coronary effect of the drug was reflected by changes of coronary perfusion pressure.

Coronary and Myocardial Effects of Terlipressin on Buffer-perfused Hearts. Because erythrocytes may be involved in metabolism of drugs, intrinsic cardiac effects of terlipressin were assessed in eight additional hearts perfused with Krebs-Henseleit bicarbonate buffer.

Role of V_{1a} Receptor in Cardiac Effects Induced by Terlipressin and AVP. The involvement of the V_{1a} receptor was assessed in six hearts after pretreatment with a potent and specific V_{1a} receptor antagonist [d(CH_2)$_5$Tyr(Me)]AVP (Sigma-Aldrich).[23,24] Therefore, after an initial 10-min recovery period and baseline measurements, an intracoronary infusion of [d(CH_2)$_5$Tyr(Me)]AVP (0.1 μM) was initiated and maintained throughout the whole experiment. Ten minutes later, intracoronary infusion of 100 nM of terlipressin was started. To ensure that a large number of vasopressin receptors were efficiently blocked by this pretreatment, we also assessed the intrinsic cardiac effects of 1,000 pM of AVP in six additional hearts pretreated under similar conditions.

All drugs were freshly prepared and dissolved in distilled water excepted for the V_{1a} receptor antagonist [d(CH_2)$_5$Tyr(Me)]AVP, which was dissolved in dimethyl sulfoxide and distilled water as a stock solution. The final intracoronary concentration of dimethyl sulfoxide was 0.001%. Dimethyl sulfoxide concentrations of up to 0.01% have been shown to be devoid of significant effects on our experimental model.[25] Dilutions of drugs used were adapted so that the volume of intracoronary infusion never exceeded 5% of the resting coronary flow rate. Finally, all syringes were protected from light throughout the study.

Statistical Analysis

Data are expressed as mean ± SD. Comparison of several means were performed using repeated-measures analysis of variance and the Newman-Keuls test. Comparison of two means was performed using a nonpaired Student *t* test. A *P* value less than 0.05 was required to reject the null hypothesis. All statistical analysis were performed using NCSS 6.0 software (Statistical Solutions Ltd., Cork, Ireland).

Results

There were no significant differences in pH and electrolyte composition of the reconstituted perfusate between terlipressin, vasopressin, and norepinephrine groups (pH, 7.42 ± 0.06; Na^+, 136 ± 5 mM, Cl^-, 98 ± 4 mM; K^+, 5.2 ± 1.2 mM, $NaHCO_3$, 23 ± 3 mM; and Ca^{2+}, 2.1 ± 0.3 mM). Similarly, the left ventricular weight was comparable between all groups (4.91 ± 0.68 g)

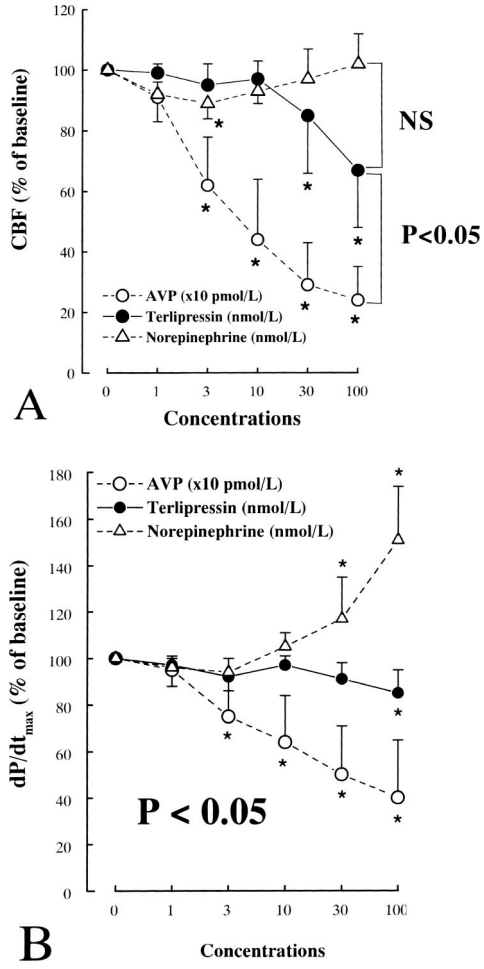

Fig. 1. (*A*) Effects of increased concentrations of terlipressin, arginine-vasopressin (AVP), and norepinephrine on coronary blood flow (CBF) and (*B*) maximal positive intraventricular pressure derivative (dP/dt$_{max}$) of erythrocyte-perfused hearts (n = 10 in each group). Data are expressed as mean ± SD. *P* value refers to between-groups comparison. **P* < 0.05 *versus* baseline values.

Cardiac Effects of Terlipressin, AVP, and Norepinephrine on Erythrocyte-perfused Hearts

Baseline values of coronary blood flow were comparable between groups (3.9 ± 1.0 *versus* 3.1 ± 0.7 *versus* 3.5 ± 1.1 ml · min^{-1} · g^{-1}, NS). Return to baseline values was obtained before each perfusion of drugs (data not shown). Norepinephrine induced a biphasic coronary effect with a maximal and significant decrease at 3 nM and then increased above basal at 30 and 100 nM concentrations (fig. 1A). AVP and terlipressin induced a concentration-dependent decrease in coronary blood flow starting from 30 pM AVP concentration and 30 nM concentrations of terlipressin (fig. 1A).

Table 1. Effects of Terlipressin (T, nmol/L, n = 10), Vasopressin (V, ×10 pmol/L, n = 10), and Norepinephrine (N, nmol/L, n = 10) on Myocardial Performances

Variables	Baseline Value	Concentrations of Vasoactive Drugs (% of Baseline Value)					Between-Groups Comparison
		1	3	10	30	100	
LVESP (mmHg)							
Terlipressin	114 ± 20	97 ± 2	95 ± 3	99 ± 2	92 ± 13	86 ± 13*	
Vasopressin	107 ± 23	97 ± 3	85 ± 9	74 ± 18*	63 ± 21*	57 ± 19*	T ≠ V ≠ N
Norepinephrine	125 ± 21	97 ± 3	97 ± 4	104 ± 4	113 ± 8*	126 ± 13*	
dP/dt_{min} (mmHg/s)							
Terlipressin	1303 ± 268	100 ± 3	92 ± 6	98 ± 5	84 ± 19*	80 ± 21*	
Vasopressin	1342 ± 506	96 ± 7	73 ± 16*	58 ± 23*	43 ± 21*	34 ± 23*	T ≠ V ≠ N
Norepinephrine	1587 ± 445	97 ± 6	97 ± 4	104 ± 9	120 ± 14*	144 ± 17*	
LVEDP (mmHg)							
Terlipressin	11 ± 3	94 ± 7	109 ± 23	96 ± 13	115 ± 21	110 ± 19	
Vasopressin	11 ± 4	98 ± 7	118 ± 19	132 ± 33	123 ± 37	151 ± 67*	V ≠ T, N
Norepinephrine	11 ± 2	98 ± 3	99 ± 5	99 ± 2	100 ± 11	96 ± 14	

Data are mean ± SD.

dP/dt_{min} = maximal negative intraventricular pressure derivative; LVEDP = left ventricular end-diastolic pressure; LVESP = left ventricular end-systolic pressure.

* $P < 0.05$ versus baseline value.

Baseline values of myocardial performances were comparable among the groups (table 1). Similarly, heart rate was similar among the three groups (116 ± 13 *versus* 120 ± 10 *versus* 128 ± 13 bpm, not significant) and remained stable throughout all experiments (data not shown). The intracoronary infusion of norepinephrine induced a concentration-dependant increase in myocardial performance that was significant from 30 nM (table 1 and fig. 1B). Intracoronary infusions of AVP (≥30 pM) and terlipressin (≥30 nM) were associated with significant decreases in myocardial performances of hearts (table 1 and fig. 1B). Only an intracoronary infusion of 100 pM of vasopressin provoked a significant increase in left ventricular end-diastolic pressure (table 1).

Baseline values of myocardial oxygen consumption were comparable between groups (table 2). Return to baseline value of oxygenation variables was obtained before each new concentration in three groups (data not shown). Norepinephrine induced a significant increase in myocardial oxygen consumption from a concentration of 30 nM. AVP produced concentration-dependent decreases in myocardial oxygen consumption that reached significance as early as 100 pM concentrations (table 2). Although there was a trend towards a decrease

Table 2. Effects of Terlipressin (T, nmol/L, n = 10), Vasopressin (V, ×10 pmol/L, n = 10) and Norepinephrine (N, nmol/L, n = 10) on Myocardial Oxygen Consumption

	Baseline value	Concentrations of Vasoactive Drugs					Between-Groups Comparison
		1	3	10	30	100	
Hemoglobin (g/dl)							
Terlipressin	7.8 ± 0.3	7.8 ± 0.3	7.8 ± 0.3	7.8 ± 0.3	7.8 ± 0.3	7.8 ± 0.3	
Vasopressin	7.8 ± 0.7	7.8 ± 0.7	7.8 ± 0.7	7.8 ± 0.7	7.8 ± 0.7	7.8 ± 0.7	NS
Norepinephrine	8.0 ± 0.5	8.0 ± 0.5	8.0 ± 0.5	8.0 ± 0.5	8.0 ± 0.5	8.0 ± 0.5	
Pa_{O_2} (mmHg)							
Terlipressin	147 ± 10	147 ± 10	145 ± 15	144 ± 19	142 ± 22	142 ± 23	
Vasopressin	136 ± 19	134 ± 23	137 ± 18	134 ± 19	127 ± 22	134 ± 21	NS
Norepinephrine	149 ± 7	149 ± 7	147 ± 9	140 ± 15	137 ± 16	135 ± 19	
Pv_{O_2} (mmHg)							
Terlipressin	25 ± 2	25 ± 2	26 ± 3	26 ± 2	24 ± 3	22 ± 4*	
Vasopressin	26 ± 3	25 ± 3	25 ± 6	21 ± 4*	20 ± 5*	18 ± 3*	NS
Norepinephrine	25 ± 3	25 ± 4	25 ± 3	24 ± 2	24 ± 4*	23 ± 3*	
Cv_{O_2} (ml/dl)							
Terlipressin	8.0 ± 0.9	8.0 ± 0.8	8.0 ± 0.5	7.8 ± 0.7	7.7 ± 0.7	7.0 ± 0.9*	
Vasopressin	8.3 ± 1.2	8.1 ± 1.2	7.6 ± 1.3	6.9 ± 1.6*	6.6 ± 1.9*	6.1 ± 1.3*	NS
Norepinephrine	8.2 ± 0.8	8.1 ± 0.9	8.1 ± 1.0	7.8 ± 0.8	7.6 ± 1.0*	7.4 ± 1.0*	
MV_{O_2} ($ml/min^{-1}/100\ g^{-1}$)							
Terlipressin	8.0 ± 1.7	8.2 ± 1.9	7.1 ± 2.5	7.7 ± 2.3	7.1 ± 2.5	6.6 ± 2.3	
Vasopressin	9.1 ± 2.5	7.2 ± 2.6	6.3 ± 3.7	6.3 ± 3.1*	4.3 ± 3.1*	3.7 ± 3.0*	N ≠T, V
Norepinephrine	8.8 ± 2.7	8.2 ± 2.8	7.8 ± 3.1	9.0 ± 2.8	10.0 ± 3.3*	11.7 ± 3.3*	

Data are mean ± SD.

Cv_{O_2} = coronary venous oxygen content; MvO_2 = myocardial oxygen consumption; Pa_{O_2}, Pv_{O_2} = coronary arterial and venous oxygen pressure.

* $P < 0.05$ versus baseline value, NS = not significant.

Table 3. Comparison of Coronary and Myocardial Effects of Terlipressin on Erythrocyte-perfused (n = 10) and Krebs-Henseleit Buffer-perfused (n = 8) Isolated Hearts

	Baseline Value	Concentrations of Terlipressin (% of baseline) nmol/L					Between-groups comparison
		1	3	10	30	100	
CBF (ml/min^{-1}/g^{-1})							
Blood-perfused	3.1 ± 0.7	99 ± 3	91 ± 7	97 ± 6	82 ± 19*	69 ± 19*	NS
Buffer-perfused	7.5 ± 1.3†	97 ± 3	90 ± 6	88 ± 9*	83 ± 8*	66 ± 20*	
LVESP (mmHg)							
Blood-perfused	114 ± 20	97 ± 2	95 ± 3	99 ± 2	92 ± 13*	86 ± 13*	P < 0.05
Buffer-perfused	100 ± 18	97 ± 3	93 ± 6	91 ± 7	87 ± 10*	73 ± 19*	
dP/dt_{max} (mmHg/s)							
Blood-perfused	2124 ± 478	97 ± 3	92 ± 4	98 ± 3	88 ± 13*	82 ± 14*	P < 0.05
Buffer-perfused	2051 ± 568	97 ± 4	92 ± 8	91 ± 9	87 ± 11*	71 ± 19*	
dP/dt_{min} (mmHg/s)							
Blood-perfused	1303 ± 268	100 ± 3	92 ± 6	98 ± 5	84 ± 19*	80 ± 21*	NS
Buffer-perfused	1694 ± 541	100 ± 7	96 ± 11	93 ± 10	87 ± 14	62 ± 27*	
LVEDP (mmHg)							
Blood-perfused	11 ± 3	94 ± 2	109 ± 23	96 ± 13	115 ± 21	110 ± 19	NS
Buffer-perfused	9 ± 4	96 ± 20	102 ± 20	101 ± 21	102 ± 23	107 ± 22	

Data are mean ± SD.

CBF = coronary blood flow; dP/dt_{max}, dP/dt_{min} = maximal positive and negative intraventricular pressure derivatives; LVEDP = left ventricular end-diastolic pressure; LVESP = left ventricular end-systolic pressure.

* $P < 0.05$ versus baseline value; NS = not significant.

† $P < 0.05$ versus blood-perfused (baseline value only).

in myocardial oxygen consumption with terlipressin, it did not reach statistical significance.

Cardiac Effects of Terlipressin during Constant Myocardial Oxygen Delivery

Intracoronary infusion of terlipressin induced a significant increase in coronary perfusion pressure from a concentration of 30 nM. However, this coronary vasopressor effect was associated with no significant changes in myocardial performances up to 100 nM where maximal positive and negative left ventricular pressure derivatives were 95 ± 2% and 95 ± 6% of baseline values, respectively (not significant).

Cardiac Effects of Terlipressin on Buffer-perfused Hearts

As expected, the baseline value of coronary blood flow was significantly higher in buffer-perfused hearts (table 3). Before each concentration, return to baseline values was obtained (data not shown). The effects of terlipressin on coronary blood flow and lusitropic properties were not significantly affected by the type of perfusate. However, terlipressin impaired the inotropic parameters in buffer-perfused hearts significantly more (table 3).

Implications of V_{1a} Receptors in Terlipressin-induced Cardiac Effects

The intracoronary infusion of 0.1 μM of [d(CH_2)$_5$Tyr(Me)]AVP affected neither resting coronary blood flow nor myocardial performances of erythrocyte-perfused hearts (data not shown). The cardiac effects of AVP (1000 pM) and terlipressin (100 nM) were nearly completely abolished in hearts pretreated with [d(CH_2)$_5$Tyr(Me)]AVP 0.1 μM (fig. 2).

Discussion

The principle findings of the current study are that terlipressin, a synthetic analog of AVP, causes a reduction in coronary blood flow and, consequently, depression of myocardial performance only at supra-therapeutic concentration (30 nM), terlipressin's intrinsic cardiac effects are significantly less pronounced that those observed by AVP, and, like AVP, its cardiac effects are essentially mediated *via* V_{1a} vasopressin receptors.

AVP, an endogenous nonapeptide of the neurohypophysis, and its long-acting synthetic analog, terlipressin, have been proposed as efficient alternative therapies to restore arterial blood pressure in refractory shock from various origins.[1–9,26] The physiologic rationale of this treatment is based on low catecholamine effectiveness and inappropriate low vasopressin concentrations in these clinical settings.[1–3,7] This deficiency in AVP seems to be related to an impaired synthesis because exogenous administration allows restoration of a high plasma concentration of AVP in these patients.[1,7] Although devoid of vasopressor effect in healthy patients, a vasopressin pressor hypersensitivity has been reported in patients suffering from vasodilatory septic shock.[6] This discrepancy could be explained by an increase in vascular receptor affinity to norepinephrine by AVP or a septic-induced autonomic insufficiency.[6] Like AVP, terlipressin vasopressor effect is principally mediated *via* V_{1a} vasopressin receptors.[10] However, their potent vaso-

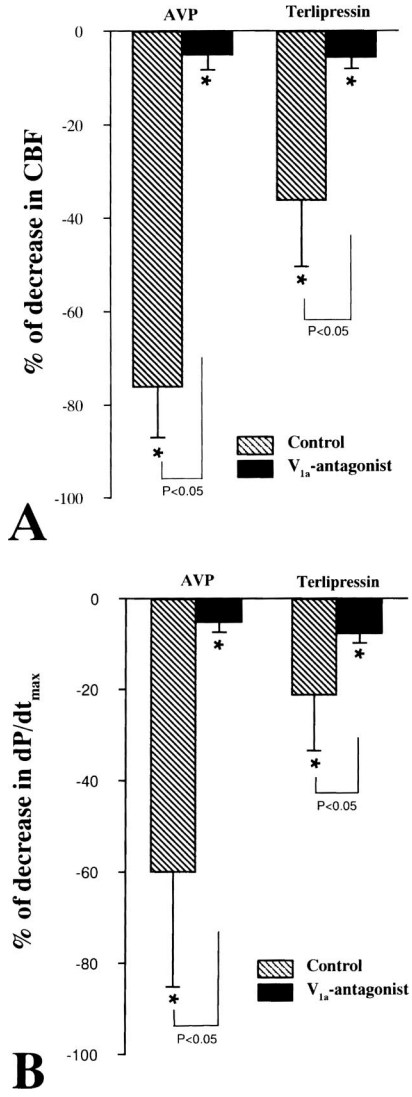

Fig. 2. (*A*) Effects of arginine-vasopressin (AVP, 1,000 pM) and terlipressin (100 nM) on coronary blood flow (CBF) and (*B*) maximal positive intraventricular pressure derivative (dP/dt_{max}) in control groups (n = 10) and after a pretreatment with the V_{1a}-antagonist $d(CH_2)_5Tyr(Me)AVP$ 0.1 μM (n = 6). *P* value refers to between-groups comparison. **P* < 0.05 *versus* baseline value.

pressor effect could compromise regional blood flow and, thus, tissue oxygenation.[15,16,27,28] In the coronary circulation, some *in vitro* studies reported that AVP produces a significant coronary vasoconstriction.[16,17] In the current study, we assessed intrinsic cardiac effects of vasopressinergic system agonists by using an isolated and erythrocyte-perfused rabbit heart. The clear advantage of this model is the presence of erythrocytes in perfusate medium, which avoids using a high degree of arterial oxygen tension, which, in turn, induces coronary vasoconstriction *via* ATP-sensitive K^+ channels.[25] Because experimental studies suggest that these ATP-sensitive K^+ channels are involved in the vasoconstrictor effect of AVP,[29,30] we thought that previous closure of these channels by high degrees of oxygen tension might interfere with the vascular effects of a vasopressinergic system agonist. In erythrocyte-perfused rabbit hearts, we showed that AVP induces a significant coronary vasoconstriction from a concentration of 30 pM. This concentration is lower than those measured by Tsuneyoshi *et al.*[7] in plasma (up to 500 pM) of patients with vasodilatory septic shock and receiving a continuous infusion of low dose of exogenous AVP. Although myocardial ischemia could complicate these adverse coronary vasopressive effects, no clinical studies have reported ischemic myocardial side effects using these two drugs to correct a refractory hypotensive state in critically ill patients. However, in most of these studies, patients with coronary disease were excluded. In the current study, coronary vasoconstrictor effects of vasopressin were associated with depression of myocardial performances. Our results are consistent with a previous *in vitro* study that demonstrated that AVP induced significant depressive myocardial effects.[17] However, the fact that myocardial depression was the only consequence of ischemic coronary vasoconstrictor effect of AVP cannot be completely ruled out in the current study. Our findings suggest that a negative inotropic effect could also be involved in the decrease in cardiac output that is usually observed when sepsis-induced hypotension is corrected by using AVP.[1,27] However, although the assessment of intrinsic properties of myocardium remains difficult *in vivo*, some authors have reported that AVP had no negative effect on myocardial performances in patients suffering from catecholamine-resistant septic shock.[31] Because terlipressin has more attractive pharmacokinetic properties, some authors have used it preferentially to correct refractory hypotension.[8–10,32] Classically, terlipressin is described as inactive itself, meaning that it should be metabolized by peptidase into lysine-vasopressin, which is significantly detectable only after 40 min.[13] However, some authors previously reported a short onset of vasopressor effect of terlipressin, suggesting that it could exert intrinsic vasoactive effects.[12,33] Our findings suggest that terlipressin is not acting simply as a prodrug but rather, in an isolated rabbit heart, induces an intrinsic coronary vasoconstrictor effect from a concentration of 30 nM. In addition, the fact that these coronary intrinsic effects persisted in buffer-perfused hearts eliminated a potential metabolism of tricyl-lysine vasopressin into lysine-vasopressin by the erythrocytes present in the medium perforate. On the other hand, as previously reported by our group,[34] we observed that the inotropic effects of a drug can be significantly modified in buffer-perfused hearts. However, a metabolism by tissular peptidase cannot be completely ruled out. Our study is the first to report an intrinsic coronary effect of terlipressin. This coronary vasoconstrictor effect was significantly lower than that of AVP. The fact that terlipressin provokes a lower coronary vasoconstriction should be taken into account for its use in patients with coronary disease.

No previous study has compared coronary vasopressor effects between terlipressin and AVP. Although terlipressin-induced coronary vasopressor effects occurred only in supra-therapeutic concentrations, they were powerful enough to provoke ischemic side effects. We showed that terlipressin-induced myocardial dysfunction disappeared when the myocardial oxygen delivery was maintained constant, suggesting the lack of direct negative inotropic effect. These findings are consistent with those obtained by Eyraud et *al*.,[35] who observed that terlipressin is devoid of significant negative inotropic effects in patients suffering from anesthesia-induced hypotension. On erythrocyte-perfused and isolated heart, norepinephrine induced a biphasic effect on coronary circulation characterized by an initial vasoconstriction up to 3 nM followed by a concentration-dependant increase. This latter effect is likely to be related to the increase in myocardial performances from a concentration of 3 nM. This well-known positive cardiac effect of norepinephrine[36] explains that, unlike AVP or terlipressin, its clinical use preserves cardiac output in patients.[27] These beneficial cardiac effects could be a serious advantage in maintaining the global oxygen transport, which can be significantly altered during clinical use of AVP.[28] The precise mechanism of the vasopressor effect induced by AVP remains unclear. AVP induces a contraction of the vascular smooth cells *via* V_{1a} vasopressin receptor.[17] However, the ATP-sensitive K^+ channels and nitric oxide both play a role in AVP-induced vasoconstriction.[30,37] In the current study, we found that intrinsic cardiac effects of AVP, like terlipressin, are principally mediated by the V_{1a} vasopressin receptor. Nevertheless, we observed a residual effect induced by both drugs after V_{1a} receptor blockade. Our results are consistent with a previous *in vitro* study[17] which reported residual negative cardiac side effects induced by AVP despite a pretreatment of 0.5 10^{-7} M [d(CH_2)$_5$Tyr(Me)]AVP, a concentration similar to that used in the current study. These results confirm that several mechanisms are involved in AVP-mediated coronary vasoconstriction. Although the involvement of the vasopressin receptors in AVP-induced cardiac effects has been previously reported,[17,37] no previous study has reported the mechanisms involved in terlipressin-induced cardiac effects.

The following points must be considered in the assessment of the clinical relevance of our study. First, our findings have been obtained from an isolated rabbit heart preparation. Consequently, any clinical extrapolation must be made with caution. Second, these experiments were performed with constant coronary perfusion pressure. However, when vasopressor agents are used, systemic blood pressure and thus coronary perfusion pressure are increased. Third, although the perfusion medium did not affect the intrinsic cardiac effect of terlipressin, a metabolism by tissular peptidase could be completely abolished. Fourth, we observed a residual cardiac effect induced by AVP and terlipressin after the pretreatment by [d(CH_2)$_5$Tyr(Me)]AVP 0.1 μM. Although the involvement of other mechanisms could probably explain this residual effect, we did not perform a curve response to eliminate that a larger concentration of V_{1a} antagonist could completely abolish cardiac effects induced by vasopressinergic system agonists. Finally, these experiments were performed in a normal beating heart model with intact coronary circulation. Therefore, further investigations will be necessary to confirm our results during different pathologic states, such as septic shock, in which the studied vasopressors are usually employed.

In conclusion, terlipressin is not only a precursor drug but also exerts intrinsic cardiac effects on an isolated heart model only at supra-therapeutic concentrations ($\geq$30nM). These effects included coronary vasoconstriction and, in turn, myocardial depression. As with AVP, the cardiac effects induced by terlipressin are principally mediated by V_{1a} receptors. However, in concentrations at the lower end of the clinically therapeutic range, terlipressin could have a better cardiac hemodynamic profile.

The authors thank David J. Baker, M.D., F.R.C.A. (Staff Anesthesiologist, Department of Anesthesiology, Centre Hospitalier Universitaire Necker-Enfants Malades, Paris, France), for reviewing the manuscript.

References

1. Landry DW, Levin HR, Gallant EM, Ashton RC, Seo S, D'Alessandro D, Oz MC, Oliver JA: Vasopressin deficiency contributes to the vasodilation of septic shock. Circulation 1997; 95:1122–5
2. Morales D, Madigan J, Cullinane S, Chen J, Heath M, Oz M, Oliver JA, Landry DW: Reversal by vasopressin of intractable hypotension in the late phase of hemorrhagic shock. Circulation 1999; 100:226–9
3. Chen JM, Cullinane S, Spanier TB, Artrip JH, John R, Edwards NM, Oz MC, Landry DW: Vasopressin deficiency and pressor hypersensitivity in hemodynamically unstable organ donors. Circulation 1999; 100(suppl II):II-244–6
4. Morales DLS, Gregg D, Helman DN, Williams MR, Naka Y, Landry DW, Oz MC: Arginine vasopressin in the treatment of 50 patients with postcardiotomy vasodilatory shock. Ann Thorac Surg 2000; 69:102–6
5. Patel BM, Chittock DR, Russell JA, Walley KR: Beneficial effects of short-term vasopressin infusion during severe septic shock. ANESTHESIOLOGY 2002; 96: 576–82
6. Landry DW, Levin HR, Gallant EM, Seo S, D'Alessandro D, Oz MC, Oliver JA: Vasopressin pressor hypersensitivity in vasodilatory septic shock. Crit Care Med 1997; 25:1279–82
7. Tsuneyoshi I, Yamada H, Kakihana Y, Nakamura M, Nakano Y, Boyle WA: Hemodynamic and metabolic effects of low-dose vasopressin infusions in vasodilatory septic shock. Crit Care Med 2001; 29:487–93
8. O'Brien A, Clapp L, Singer M: Terlipressin for norepinephrine-resistant septic shock. Lancet 2002; 359:1209–10
9. Rodriguez-Nunez A, Fernandez-Sanmartin M, Martinon-Torres F, Gonzalez-Alonso N, Martinon-Sanchez JM: Terlipressin for catecholamine-resistant septic shock in children. Intensive Care Med 2004; 30:477–80
10. Asfar P, Pierrot M, Veal N, Moal F, Oberti F, Croquet V, Douay O, Gallois Y, Saumet JL, Alquier P, Calès P: Low-dose terlipressin improves systemic and splanchnic hemodynamics in fluid-challenged endotoxic rats. Crit Care Med 2003; 31:215–20
11. Chiu KW, Sheen IS, Liaw YF: A controlled study of glypressin versus vasopressin in the control of bleeding from oesophageal varices. J Gastroenterol Hepatol 1990; 5:549–53
12. Escorsell A, Bandi JC, Moitinho E, Feu F, Garcia-Pagan JC, Bosch J, Rodes J: Time profile of the haemodynamic effects of terlipressin in portal hypertension. J Hepatol 1997; 26:621–7
13. Forsling ML, Aziz LA, Miller M, Davies R, Donovan B: Conversion of triglycylvasopressin to lysine-vasopressin in man. J Endocrinol 1980; 85:237–44
14. Westphal M, Freise H, Kehrel BE, Bone HG, Van Aken H, Sielenkämper

AW: Arginine vasopressin compromises gut mucosal microcirculation in septic rats. Crit Care Med 2004; 32:194–200

15. Dünser MW, Mayr AJ, Tür A, Pajk W, Barbara F, Knotzer H, Ulmer H, Hasibeder WR: Ischemic skin lesions as a complication of continuous vasopressin infusion in catecholamine-resistant vasodilatory shock: Incidence and risk factors. Crit Care Med 2003; 31:1394–8

16. Maturi MF, Martin SE, Markle D, Maxwell M, Burruss CR, Speir E, Greene R, Ro YM, Vitale D, Green MV, Goldstein SR, Bacharach SL, Patterson RE: Coronary vasoconstriction induced by vasopressin: Production of myocardial ischemia in dogs by constriction of nondiseased small vessels. Circulation 1991; 83:2111–21

17. Walker BR, Childs ME, Adams EM: Direct cardiac effects of vasopressin: Role of V_1-and V_2-vasopressinergic receptors. Am J Physiol 1988; 255:H261–5

18. Rosario R, Lalanne B, Lebre P, Lepesan D, Martelet JP, Dupont M, Camatte R, Lambot G, Bourlière M: Myocardial infarction after injection of terlipressin for digestive hemorrhage. Gastroenterol Clin Biol 1996; 20:712–3

19. Duruble M, Duvelleroy M, Gauduel Y, Martin JL, Teisseire B: Transient responses of coronary flow in the blood-perfused isolated rat heart submitted to changes in oxygen content. J Physiol (Lond) 1985; 358:321–34

20. Duvelleroy MA, Duruble M, Martin JL, Teisseire B, Droulez J, Cain M: Blood-perfused working isolated rat heart. J Appl Physiol 1976; 41:603–7

21. Ouattara A, Langeron O, Souktani R, Mouren S, Coriat P, Riou B: Myocardial and coronary effects of propofol in rabbits with compensated cardiac hypertrophy. ANESTHESIOLOGY 2001; 95:699–707

22. Cuche JL, Jondeau G, Ruget G, Selz F, Piga JC, Harboun C: Effects of an intravenous infusion of noradrenaline on the plasma concentration of the free and sulfoconjugated catecholamines in anesthetized dogs. Pharmacology 1986; 32:90–100

23. Laszlo FA, Laszlo F, De Wied D: Pharmacology and clinical perspectives of vasopressin antagonists. Pharmacol Rev 1991; 43:73–108

24. Kruszynski M, Lammek B, Manning M, Seto J, Haldar J, Sawyer WH: [1(beta-mercapto-beta-cyclopentamethylenepropionic acid)2-(O-methyl) tyrosine] arginine-vasopressin and [1-(beta-mercapto-beta, beta-cyclopentamethylenepropionic acid)] arginine-vasopressin, two highly potent antagonists of the vasopressor response to arginine-vasopressin. J Med Chem 1980; 23:364–80

25. Mouren S, Souktani R, Beaussier M, Abdenour L, Arthaud M, Duvelleroy M, Vicaut E: Mechanisms of coronary vasoconstriction induced by high arterial oxygen tension. Am J Physiol 1997; 272:H67–75

26. Wenzel V, Krismer AC, Arntz R, Sitter H, Stadlbauer KH, Lindner KH, for the European Resuscitation Council Vasopressor during Cardiopulmonary Resuscitation Study Group: A comparison of vasopressin and epinephrine for out-of-hospital cardiopulmonary resuscitation. N Engl J Med 2004; 350:105–13

27. Martikainen TJ, Tenhunen JJ, Uusaro A, Ruokonen E: The effects of vasopressin on systemic and splanchnic hemodynamics and metabolism in endotoxin shock. Anesth Analg 2003; 97:1756–63

28. Westphal M, Stubbe H, Sielenkämper AW, Borgulya R, Van Haken H, Ball C, Bone HG: Terlipressin dose response in healthy and endotoxemic sheep: Impact on cardiopulmonary performance and global oxygen transport. Intensive Care Med 2003; 29:301–8

29. Tsuchiya M, Tsuchiya K, Maruyama R, Takemura G, Minatoguchi S, Fujiwara H: Vasopressin inhibits sarcolemmal ATP-sensitive K^+ channels via V_1 receptors activation in the guinea pig heart. Cir J 2002; 66:277–82

30. Wakatsuki T, Nakaya Y, Inoue I: Vasopressin modulates K^+-channel activities of cultured smooth muscle cells from porcine coronary artery. Am J Physiol 1992; 263:H491–6

31. Dünser MW, Mayr AJ, Ulmer H, Ritsch N, Knotzer H, Pajk W, Luckner G, Mutz NJ, Hasibeder WR: The effects of vasopressin on systemic hemodynamics in catecholamine-resistant septic and postcardiotomy shock: A retrospective analysis. Anesth Analg 2001; 93:7–13

32. Scharte M, Meyer J, Van Aken H, Bone HG: Hemodynamic effects of terlipressin (a synthetic analog of vasopressin) in healthy and endotoxemic sheep. Crit Care Med 2001; 29:1756–60

33. Boccara G, Ouattara A, Godet G, Dufresne E, Bertrand M, Riou B, Coriat P: Terlipressin versus norepinephrine to correct refractory arterial hypotension after general anesthesia in patients chronically treated with renin-angiotensin system inhibitors. ANESTHESIOLOGY 2003; 98:1338–44

34. Mouren S, Baron JF, Albo C, Szekely B, Arthaud M, Viars P: Effects of propofol and thiopental on coronary blood flow and myocardial performance in an isolated rabbit heart. ANESTHESIOLOGY 1994; 80:634–41

35. Eyraud D, Brabant S, Dieudonne N, Fléron MH, Godet G, Bertrand M, Coriat P: Treatment of intraoperative refractory hypotension with terlipressin in patients chronically treated with an antagonist of the renin-angiotensin system. Anesth Analg 1999; 88:980–4

36. Irlbeck M, Mühling O, Iwai T, Zimmer HG: Different response of the rat left and right heart to norepinephrine. Cardiovasc Res 1996; 31:157–62

37. Fernandez N, Garcia JL, Garcia-Villalon AL, Monge L, Gomez B, Dieguez G: Coronary vasoconstriction produced by vasopressin in anesthetized goats: Role of vasopressin V_1 and V_2 receptors and nitric oxyde. Eur J Pharmacol 1998; 342:225–33