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Cerebral vasoconstriction produced by vasopressin in conscious goats: role of vasopressin V_1 and V_2 receptors and nitric oxide

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1 To examine the role of vasopressin V_1 and V_2 receptors, nitric oxide and prostanoids in the cerebrovascular effects of arginine vasopressin, cerebral blood flow was electromagnetically measured in awake goats.

2 In 16 animals, vasopressin (0.03–1 μg), injected into the cerebral circulation, caused increments of resting cerebrovascular resistance which ranged from 18% (0.03 μg, $P<0.01$) to 79% (1 μg, $P<0.01$). Desmopressin (0.03–1 μg, four goats) did not affect significantly cerebrovascular resistance.

3 The cerebrovascular resistance increases by vasopressin were reduced significantly by the antagonist for vasopressin V_1 receptors d(CH_2)$_5$Tyr(Me)-AVP in a rate depending way (five (six goats) and 15 (four goats) μg min^{-1}), and by the mixed antagonist for vasopressin V_1 and V_2 receptors desGly-d(CH_2)$_5$-D-Tyr(Et)Val-AVP (5 μg min^{-1}, four goats), and they were not significantly affected by the antagonist for vasopressin V_2 receptors d(CH_2)$_5$, D-Ile^2, Ile^4-AVP (5 μg min^{-1}, four goats).

4 The inhibitor of nitric oxide synthesis N^w-nitro-L-arginine methyl ester (L-NAME, 47 mg kg^{-1} i.v., five goats) augmented cerebrovascular resistance by 130% ($P<0.01$), and for 24 h after this treatment the cerebrovascular effects of vasopressin were potentiated.

5 The inhibitor of cyclo-oxygenase meclofenamate (6 mg kg^{-1} i.v., five goats) did not modify significantly resting haemodynamic variables measured or the cerebrovascular effects of vasopressin.

6 Therefore, the vasopressin-induced cerebral vasoconstriction may be mediated by vasopressin V_1 receptors, without involvement of vasopressin V_2 receptors, and may be modulated by nitric oxide but not by prostanoids.

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Abbreviations: d(CH_2)$_5$,D-Ile^2,Ile^4-AVP, [d(CH_2)$_5$,D-Ile^2,Ile^4,Arg^8]-vasopressin; des-(CH_2)$_5$Tyr(Me)-AVP, [b-mercapto-b,b-cyclopenta-methylenepropionyl^1,O-Me-Tyr^2,Arg^8]-vasopressin; des-Gly-d(CH_2)$_5$-D-Tyr(Et)Val-AVP, des-Gly^9-[b-mercapto-b,b-cyclopenta-methylenepropionyl^1,O-Et-Tyr^2,Val^4,Arg^8]-vasopressin; desmopressin, [deamino-Cys^1,D-Arg^8]-vasopressin acetate; L-NAME, N^w-nitro-L-arginine methyl ester hydrochloride

Introduction

Experimental observations suggest that arginine vasopressin produces vasoconstriction, but the vascular response may differ between vascular beds (Cowley & Liard, 1987). In the cerebral circulation, most of the studies show that arginine vasopressin produces vasoconstriction in different species, including humans (Lluch *et al*., 1984; Nakai, 1987; Faraci *et al*., 1988; Onoue *et al*., 1994), and it has been reported that the magnitude and the sensitivity of the constriction of pial arteries in response to this peptide are higher than those exhibited by coronary, renal, saphenous, mesenteric and pulmonary arteries from rabbits (García-Villalón *et al*., 1996). These studies, together with observations showing that concentrations of arginine vasopressin in cerebrospinal fluid are higher than in plasma (Luerssen *et al*., 1977), and that this peptide has been identified by immunoreactive procedures in cerebral arteries and veins (Feuerstein & Miller, 1997), suggest that arginine vasopressin may play a role in the regulation of the cerebral circulation.

In relation to the action mechanisms of arginine vasopressin on cerebral vasculature, it has been reported that vasopressin V_1 receptors may mediate vasoconstriction and vasodilatation (Suzuki *et al*., 1992) and that vasopressin V_2 receptors may mediate vasodilatation (Kozniewska & Szczepanska-Sadowska, 1990) or may not have a functional role (García-Villalón *et al*., 1996). On the other hand, experiments for examining the role of nitric oxide and prostanoids in the cerebrovascular effects of arginine vasopressin are sparse, and the results reported are contradictory, as nitric oxide (Martínez *et al*., 1994; García-Villalón *et al*., 1996) and prostanoids (Toda *et al*., 1993; Tsuji & Cook, 1994) may, or may not be involved in these effects. Therefore, the mechanisms involved in the cerebrovascular effects of arginine vasopressin are not clear, and more studies are needed to elucidate this issue.

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The present study was performed to examine the role played by vasopressin V_1 and V_2 receptors, and also the role of nitric oxide and prostanoids in the cerebrovascular effects of arginine vasopressin. The experiments were carried out in awake goats where blood flow to one brain hemisphere can be electromagnetically measured on a beat-to-beat basis, and the effects of relatively small doses of drugs on the cerebral circulation can be evaluated without interference of their systemic effects (Reimann *et al*., 1972). Using this experimental preparation, it has been previously reported that arginine vasopressin produces cerebral vasoconstriction (Lluch *et al*., 1984).

Methods

The experimental procedure used in the present study was approved by the Animal Research Committee of the Facultad de Medicina, Universidad Autónoma, Madrid (Spain). In this study 16 adult female goats, ranging in weight from 37–65 kg were used. In this species, each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each cerebral hemisphere *via* the rete mirabile. The vertebral arteries do not contribute to brain blood flow, and the extracranial internal carotid artery is absent (Daniel *et al*., 1953; Reimann *et al*., 1972). The circle of Willis in the goat is similar to that of humans, except that the blood flows in a caudal direction in the basilar artery (Daniel *et al*., 1953; Reimann *et al*., 1972). Analysis of the distribution of radioactively labelled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann *et al*. (1972) indicates that nearly all the blood carried by the internal maxillary artery passes directly to cerebral tissue (Miletich *et al*., 1975). Extracerebral blood flow is minimal, $<5\%$ of total flow.

The operative procedure, performed in anaesthetized animals, has been described elsewhere (Reimann *et al*., 1972). Briefly, the extracerebral vessels from one of the internal maxillary arteries were ligated and thrombosed with 1000 NIH units of thrombin (Thrombostat, Parke Davis, Detroit, MI, U.S.A.) dissolved in 1 ml of 0.9% NaCl solution. This manoeuver produces an almost immediate obliteration of the ethmoidal, ophthalmic and buccinator arteries, and thus eliminates blood flow to the eye and other facial structures. This is confirmed on recovery from surgery by the presence of ipsilateral blindness. However, obliteration of the extracerebral vessels from the internal maxillary artery does not cut off vascular supply to half of the face. The areas supplied by the ethmoidal, buccinator, dental, and temporal arteries are nourished by anastomotic channels that are normally in a state of dynamic balance, but in which the direction of blood flow can be quickly changed, depending on the pressure differential from one side of the union to the other (Daniel *et al*., 1953; Reimann *et al*., 1972). There is no necrosis, and the functions related to these areas such as eating, drinking, and rumen are intact. Obliteration and thrombosis of the ophthalmic artery permanently cuts off vascular supply to the ipsilateral eye. This procedure becomes necessary for the successful isolation of the cerebral circulation (Daniel *et al*., 1953; Reimann *et al*., 1972). The ipsilateral blindness that ensues does not seem to alter normal behaviour and physical condition of the animals.

An electromagnetic flow transducer (Biotronex) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere. A polyethylene catheter (PE-90) inserted in the temporal artery and permanently fixed permitted the injection of drugs directly into the internal maxillary artery in the awake goat; the same catheter was used to measure arterial blood pressure with a Statham transducer (P23 ID), and to obtain samples of arterial blood in order to measure arterial blood pO_2, pCO_2 and pH. A snare-type occluder was placed on the external carotid artery to obtain zero flow baseline values. The external connecting leads from the flow transducer and occluder, and the temporal artery catheter were led out subcutaneously and secured to a horn of the goat.

Heart rate was measured from the arterial pressure pulse with a ratemeter. Cerebral blood flow measurements were made with a Biotronex electromagnetic flowmeter (model BL-610). Cerebral blood flow, arterial blood pressure, and heart rate were recorded on a Dynograph Recorder R611 (SensorMedics).

The experiments on the unanaesthetized animal started 2–3 days after the operative procedure, at which time the goat had fully recovered and was in good condition. The various measurements were made with the goat unrestrained in a large cage, except for a Lucite stock, fitting loosely around the neck, that limited forward and backward motion. Once placed in the cage the animal stood quietly during the experiments and showed no signs of disturbance.

Arginine vasopressin (16 goats) and [deamino-Cys^1,D-Arg^8]-vasopressin (desmopressin, selective agonist for vasopressin V_2 receptors; four of the 16 goats) were prepared in isotonic saline, and injected at doses of 0.03, 0.1, 0.3 and 1 μg directly into the internal maxillary artery of the unanaesthetized animals, in which cerebral blood flow, systemic arterial pressure, and heart rate were simultaneously and continuously recorded. Each dose was administered using volumes of <0.5 ml injected over 7–10 s at 6–40 min intervals. Arginine vasopressin was injected in the animals under control conditions, and during treatment with the antagonist for vasopressin V_1 receptors [b-mercapto-b,b-cyclopentamethylenepropionyl^1,O-Me-Tyr^2,Arg^8]-vasopressin (d(CH_2)$_5$-Tyr(Me)-AVP), the antagonist for vasopressin V_2 receptors [d(CH_2)$_5$,D-Ile^2, Ile^4,Arg^8]-vasopresssin (d(CH_2)$_5$,D-Ile^2,Ile^4-AVP), the combined antagonist for vasopressin V_1 and V_2 receptors 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-AVP), the inhibitor of nitric oxide synthesis N^w-nitro-L-arginine methyl ester (L-NAME), as well as the cyclooxygenase inhibitor meclofenamate.

Desmopressin was tested only under control conditions (four goats) because it did not produce any effect on resting cerebral blood flow. The antagonists for vasopressin receptors used were dissolved in physiological saline (25 μg ml^{-1}), and the selective antagonist for vasopressin V_1 receptors was infused at a rate of 5 μg min^{-1} in a volume of 2 ml min^{-1} (six goats) or 15 μg min^{-1} in a volume of 6 ml min^{-1} (four goats; these goats are among the six goats that received the rate of 5 μg min^{-1}). The antagonist for vasopressin V_2 receptors (four goats) and the mixed antagonist for vasopressin V_1 and V_2 receptors (four goats) were infused only at rate of 5 μg min^{-1} in a volume of 2 ml min^{-1}. These antagonists were administered through the

catheter placed into the internal maxillary artery. In these animals, arginine vasopressin was injected during the infusion of each of these antagonists, beginning 10 min after starting the administration of each antagonist; during the injections of arginine vasopressin the infusion of the antagonist was momentarily stopped because the same route was used. For testing the actions of these antagonists, we first recorded the effects of arginine vasopressin under control conditions and then, at least 12 h after the control dose–response curve was completed, we recorded in the same animal the effects of arginine vasopressin during treatment with the vasopressin V_1 and V_2 receptor antagonists used. Arginine vasopressin was also tested in three animals before (control) and during infusion of physiological saline (vehicle) through the internal maxillary artery at a rate of 0.6 ml min^{-1}. L-NAME, prepared in physiological saline at a concentration of 10 mg ml^{-1}, was first administered by i.v. bolus of 35 mg kg^{-1} and then an additional i.v. infusion was administered at a rate of 0.15–0.20 mg kg^{-1} min^{-1} (in total the animals received 47 mg kg^{-1} of L-NAME). Arginine vasopressin was first tested under control conditions and then, at least 24 h later, it was tested during the i.v. infusion of L-NAME, and periodically after this treatment in five animals. Meclofenamate, prepared in physiological saline at a concentration of 10 mg ml^{-1}, was i.v. administered by hand at a dose of 6 mg kg^{-1} over 5–8 min in five goats. In these animals, arginine vasopressin was first tested under control conditions and then, at least 12 h later, it was tested again 20–30 min after the end of meclofenamate injection.

The cerebrovascular effects of arginine vasopressin under the control conditions and under the different treatments used were measured at the maximal response on cerebral blood flow, and they were evaluated as changes in cerebrovascular resistance. Cerebrovascular resistance was calculated by dividing mean systemic arterial pressure in mmHg to cerebral blood flow in ml min^{-1}.

In each animal, the experiments were performed throughout 7–16 days. During this period, at least three control tests for arginine vasopressin were determined in each animal on different days, and the effects of this peptide after each treatment were also examined. For each animal only one control dose–response curve for arginine vasopressin was considered, which was obtained by averaging the results from the different control tests, and this average response was then used for comparisons with the results obtained in the corresponding animal in the different experimental conditions tested.

Blood samples from the temporal artery were taken before and under the various experimental conditions used to measure pH, pCO_2 and pO_2 by standard electrometric methods (Radiometer, ABL 5, Copenhagen, Denmark).

After termination of the experiments the goats were killed with an overdose of i.v. thiopental sodium and potassium chloride.

Data analysis

The data are expressed as means ± s.e.mean. The effects of the different doses of arginine vasopressin and desmopressin on cerebrovascular resistance under control conditions and the different experimental conditions tested were analysed in each case by applying two-way, repeated measures analysis of variance followed by the Student's *t*-test for paired data; in each case the animal was used as its own control. The effects of the vasopressin receptors antagonists, L-NAME and meclofenamate on the haemodynamic variables recorded and on blood gases and pH were evaluated by using one-way, repeated measures analysis of variance followed by the Student's *t*-test for paired data; in each case the animal was used as own control.

For the different conditions tested, the effects of arginine vasopressin on cerebrovascular resistance, calculated during the maximal effects on cerebral blood flow, were considered as absolute values taken from the corresponding basal value. In each case, $P<0.05$ was considered statistically significant.

Drugs

[Arg^8]-vasopressin acetate; [deamino-Cys^1,D-Arg^8]-vasopressin (desmopressin) acetate; the vasopressin V_1 receptor antagonist [b-mercapto-b,b-cyclopenta-methylenepropionyl^1,-O-Me-Tyr^2,Arg^8]-vasopressin; 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 and N^w-nitro-L-arginine methyl ester hydrochloride (L-NAME), all from Sigma, sodium meclofenamate was from Parke Davis and the vasopressin V_2 receptor antagonist [$d(CH_2)_5$,D-Ile^2,Ile^4,Arg^8]-vasopressin was from Peninsula Laboratories.

Results

The basal values for haemodynamic paremeters recorded and blood gases and pH in 16 awake goats were: cerebral blood flow = 62 ± 3 ml min^{-1}, mean systemic arterial pressure = 104 ± 2 mmHg, cerebrovascular resistance = 1.67 ± 0.04 mmHg ml^{-1} min^{-1}, heart rate = 84 ± 6 beats min^{-1}, pO_2 = 79 ± 3, pCO_2 = 31 ± 2, and pH = 7.40 ± 0.01.

Control conditions

Arginine vasopressin (0.03–1 μg, 16 goats), injected into the internal maxillary artery, produced dose-dependent decreases in cerebral blood flow and increases in cerebrovascular resistance. For the 16 goats the increases in cerebrovascular resistance ranged from 18 ± 2% (for 0.03 μg) ($P<0.01$) to 79 ± 9% (for 1 μg) ($P<0.01$). The effects of this peptide on cerebral blood flow were evident at about 10 s and persisted for 3–20 min depending on the doses used, and they were maximal before systemic effects were evident, when these occurred. The doses of 0.3 and 1 μg of this peptide also produced slight hypertension without changing significantly the heart rate (these systemic effects have not been considered in the present work).

Desmopressin (agonist for vasopressin V_2 receptors, 0.03–1 μg) produced no effect on resting cerebral blood flow, systemic arterial pressure and heart rate in four goats (these data are not shown).

Effects of the antagonists for vasopressin receptors

The specific antagonist for vasopressin V_1 receptors, $d(CH_2)_5$Tyr(Me)-AVP, infused at the rate of 5 and

15 μg min^{-1} per kg body weight (six and four goats, respectively), did not affect resting cerebral blood flow or the other variables measured. During these two rates of the antagonist, the increases in cerebrovascular resistance produced by every dose tested of arginine vasopressin (0.03 – 1 μg) were significantly lower than under control conditions, and this inhibition expressed in percentage was more pronounced ($P<0.05$) during the rate of 15 μg min^{-1} than during the rate of 5 μg min^{-1} of the antagonist (Figure 1).

The specific antagonist for vasopressin V_2 receptors, d(CH$_2$)$_5$, D-Ile^2, Ile-^{4}-AVP (5 μg min^{-1} per kg body weight; four goats), did not affect resting cerebral blood flow or the other variables measured. This treatment did not modify significantly the control increase in cerebrovascular resistance produced by 0.03 – 1 μg of arginine vasopressin (Figure 1).

The mixed antagonist for vasopressin V_1 and V_2 receptors, desGlyd-(CH$_2$)$_5$-D-Tyr(Et)Val-AVP (5 μg min^{-1} per kg body weight; four goats) did not change resting cerebral blood flow or the other variables measured. During this treatment, the increases in cerebrovascular resistance caused by arginine vasopressin (0.03 – 1 μg) were significantly lower than under control conditions for every dose used of this peptide (Figure 1).

In our experiments we found that the cerebrovascular responses to arginine vasopressin obtained about 3 h after the treatment with each of the three antagonists used were not significantly distinct from those obtained before this treatment (control conditions).

The infusion of the vehicle (0.6 ml min^{-1}, three goats) did not affect the resting haemodynamic variables measured or the increases by arginine vasopressin on cerebrovascular resistance as compared with those found under control conditions (these data are not shown).

Effects of treatment with L-NAME and meclofenamate

L-NAME (five goats) reduced resting cerebral blood flow by 40% ($P<0.05$), increased mean systemic arterial pressure by 27% ($P<0.05$) and cerebrovascular resistance by 130% ($P<0.01$), respectively, and decreased heart rate by 39% ($P<0.05$), without changing significantly blood gases or pH. The effects of L-NAME on cerebral blood flow and cerebrovascular resistance disappeared 72 h, and those on mean arterial pressure 48 h after this treatment; the effects on heart rate remained after 72 h post L-NAME infusion (Table 1). The effects of every dose tested of arginine vasopressin on cerebrovascular resistance, in absolute values, were significantly increased during L-NAME treatment, as compared with those found under control conditions (Figure 2). The increases in cerebrovascular resistance induced by arginine vasopressin remained significantly higher 24 h after L-NAME, and they were comparable to those obtained in control conditions 48 h after L-NAME (Figure 2).

Meclofenamate (five goats) did not affect significantly resting cerebral blood flow, systemic arterial pressure, heart rate, blood gases or pH (Table 2). After meclofenamate treatment, the effects of arginine vasopressin (0.03 – 1 μg) on cerebrovascular resistance were not significantly different from those found under control conditions. The increases in cerebrovascular resistance in mmHg ml^{-1} min^{-1} caused by arginine vasopressin, under control conditions and after meclofenamate, respectively, were: for 0.03 μg, 0.29 $\pm$ 0.05 *vs* 0.24 $\pm$ 0.06; for 0.1 μg, 0.53 $\pm$ 0.10 *vs* 0.36 $\pm$ 0.12; for 0.3 μg, 0.84 $\pm$ 0.19 *vs* 0.67 $\pm$ 0.21, and for 1 μg, 1.33 $\pm$ 0.29 *vs* 1.38 $\pm$ 0.24 (all $P>0.05$).

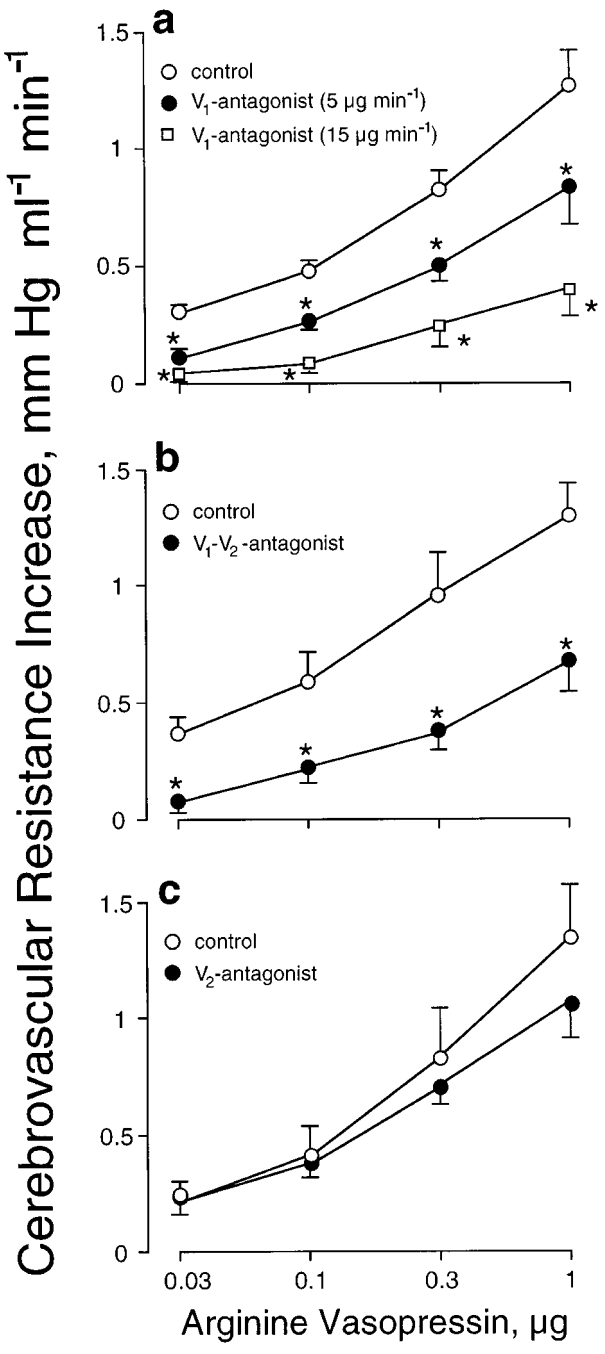

Figure 1 Summary of the cerebrovascular effects of arginine vasopressin in awake goats: (a) under control conditions (six goats) and after treatment with the antagonist for vasopressin V_1 receptors d(CH$_2$)$_5$ Tyr (Me)-AVP at rate of 5 μg min^{-1} (six goats), and of 15 μg min^{-1} (four goats); (b) in four goats under control conditions and after treatment with the mixed antagonist for vasopressin V_1 and V_2 receptors des Gly-d(CH$_2$)$_5$-D-Tyr (Et)Val-AVP, and (c) in four goats under control conditions and after treatment with the antagonist for vasopressin V_2 receptors d(CH$_2$)5, D-Ile^2, Ile^4-AVP.

Table 1 Values for haemodynamic variables and blood gases and pH obtained in awake goats before (control) and after L-NAME

	Control (n=5)	*Immediate* (n=5)	*L-NAME at 24 h* (n=5)	*at 48 h* (n=4)
CBF (ml min^{-1})	63±4	35±4**	41±3**	50±5*
MAP (mmHg)	102±3	128±4**	116±4*	100±6
CVR (mmHg ml min^{-1})	1.65±0.08	3.77±0.58**	2.79±0.23**	2.03±0.33*
HR (beats min^{-1})	82±5	50±6**	55±5**	54±4**
pO_2 (mmHg)	75±4	78±3	79±3	76±5
pCO_2 (mmHg)	34±2	32±1	33±2	32±4
pH	7.44±0.02	7.43±0.01	7.41±0.02	7.43±0.03

Values are means±s.e.mean. CBF=cerebral blood flow; MAP=mean systemic arterial pressure; CVR=cerebrovascular resistance; HR=heart rate; n=number of animals. *$P<0.05$; **$P<0.01$ compared with its corresponding control.

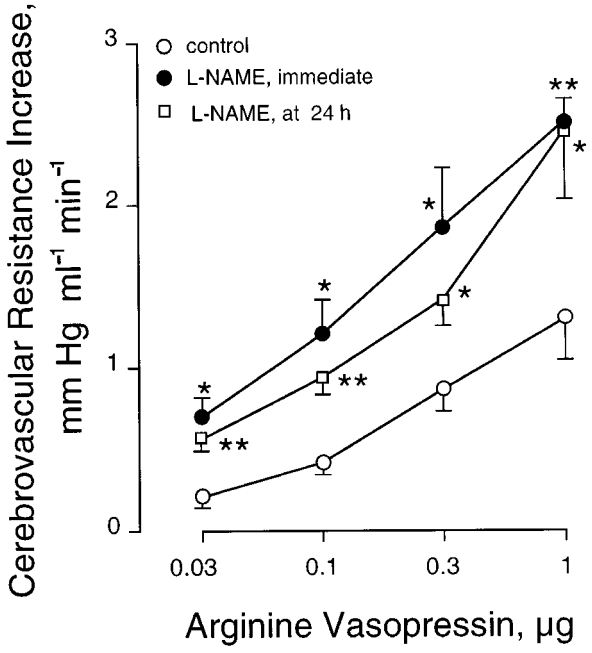

Figure 2 Summary of the cerebrovascular effects of arginine vasopressin in five awake goats under control conditions and after treatment with N^{w}-nitro-L-arginine methyl esther (L-NAME).

Table 2 Values for haemodynamic variables and blood gases and pH obtained in awake goats before (control) and after meclofenamate

	Control	*Meclofenamate*
CBF (ml min^{-1})	60±4	60±5
MAP (mmHg)	103±4	104±3
CVR (mmHg ml min^{-1})	1.70±0.11	1.74±0.14
HR (beats min^{-1})	75±8	72±9
pO_2 (mmHg)	76±3	73±2
pCO_2 (mmHg)	32±2	33±2
pH	7.40±0.03	7.40±0.03

Values are means±s.e.mean from five goats. CBF=cerebral blood flow; MAP=mean systemic arterial pressure; CVR=cerebrovascular resistance; HR=heart rate.

Discussion

The present results under control conditions confirm previous observations using the same experimental preparation (Lluch *et al*., 1984), and show that arginine vasopressin produces cerebral vasoconstriction as also occurs in cerebral vessels from different species, including humans (Lluch *et al*., 1984; Nakai, 1987; Faraci *et al*., 1988; Onoue *et al*., 1994). Nevertheless, although most of the studies may show that arginine vasopressin produces cerebral vasoconstriction, there are also studies showing that this peptide can produce cerebral vasodilatation (Katusic *et al*., 1984; Suzuki *et al*., 1992; Onoue *et al*., 1994). This discrepancy may be related to differences between species and between regions of brain vessels (Katusic *et al*., 1984; Suzuki *et al*., 1992; Onoue *et al*., 1994), and also the vial used to inject this peptide (Suzuki *et al*., 1992). The design of the present experiments does not discern whether or not there are regional differences between brain vessels in response to arginine vasopressin, as global blood flow to only one brain hemisphere was measured. Previous studies indicate that isolated pial small arteries (Lluch *et al*., 1984) and pial veins (Diéguez *et al*., 1983) from goats exhibit constriction in response to arginine vasopressin, and therefore, cerebral arteries and veins may be involved in the observed decreased cerebral blood flow after arginine vasopressin, although the role of veins should be smaller than that of arterial bed where the main changes in vascular resistance should occur.

As the blood supply to the goat brain occurs *via* an intracranial network of medium-sized muscular arteries (carotid rete), it raises the question of whether or not the observed effects of arginine vasopressin on cerebral blood flow in the awake goat could reflect the overall response of retial and brain vasculature to this peptide. It has been reported earlier that isolated retial arteries exhibit a much lower sensitivity and contractile response than isolated arteries of similar caliber from goats to arginine vasopressin (Lluch *et al*., 1984), thus suggesting that cerebral vessels rather than retial vessels should be the main site where this peptide acts for reducing cerebral blood flow in the awake goat.

The cerebral vasoconstriction induced by arginine vasopressin in our experiments may be mainly related to activation of vasopressin V_1 receptors by this peptide, with no involvement of vasopressin V_2 receptors. This suggestion is based on the fact that the specific antagonist for vasopressin V_1 receptors and the combined antagonist for

vasopressin V_1 and V_2 receptors used, blocked the cerebral vasoconstrictor effects of arginine vasopressin, and on that desmopressin, a specific agonist for vasopressin V_2 receptors, did not affect cerebral blood flow, and the specific antagonist for vasopressin V_2 receptors did not affect the cerebral vasoconstriction induced by arginine vasopressin. This is in agreement with results obtained in the cerebral circulation of dogs (Suzuki *et al*., 1992), and in isolated pial arteries from rabbits (García-Villalón *et al*., 1996) and humans (Martín de Aguilera *et al*., 1990). Martínez *et al*. (1994) report that vasopressin is primarily a constrictor of human cerebral arteries *via* vasopressin V_1 receptors located in smooth musculature.

The antagonist for V_1 receptors (Kruszynski *et al*., 1980) and the mixed antagonist for V_1-V_2 receptors (Jard *et al*., 1986) used in the present study have proved to be effective for blocking the *in vivo* vasopressor effects of arginine vasopressin, and this V_1 receptor antagonist has become one of the most widely used for blocking specifically vasopressin V_1 receptors (László *et al*., 1991). In the present study we cannot obtain with confidence the pK values for the antagonism against the cerebrovascular response to vasopressin, but the approximate estimates of these values for the used V_1, and mixed V_1-V_2 receptors antagonists may be about 7.5 and 8, respectively. This suggests that the efficacy for blocking this response may be similar for these two antagonists, and the estimated pK values are consistent with the hypothesis that the observed cerebral vasoconstriction by vasopressin is mainly mediated by the V_1 subtype of vasopressin receptors (Jard *et al*., 1986 report a pA_2 value of 8.2 for the mixed V_1-V_2 antagonist; Kruszynski *et al*., 1980 report a pA_2 value of 8.6 for the V_1 antagonist). Desmopressin is considered to be a specific agonist for V_2 vasopressin receptors, a property that has been proved *in vivo* (Manning *et al*., 1977), and the antagonist for V_2 receptors used in the present study has also proved to be effective for inhibiting the *in vivo* effects of vasopressin (Manning *et al*., 1984). Therefore, our results with these two substances suggest that vasopressin V_2 receptors may have little or no role in the cerebrovascular effects of arginine vasopressin. The role played by vasopressin receptors, presumably of the subtype V_1, in mediating the contraction of the isolated middle cerebral artery (Lluch *et al*., 1984) and isolated pial veins (Diéguez *et al*., 1983) from goats has been previously reported using an antagonist of the vasopressor action of vasopressin ((1-Deaminopenicillamine,4-valine)-8-D-arginine vasopressin, dPVDAVP, Manning *et al*., 1977). The data reported by Lluch *et al*. (1984) suggest that the sensitivity, in terms of the EC_{50} values, of the receptors that mediate the contraction to vasopressin is greater in the human than in the goat middle cerebral artery, and this sensitivity may be similar in the goat middle cerebral artery (Lluch *et al*., 1984) than in the rabbit basilar artery (García-Villalón *et al*., 1996). Based in estimations of the pK values, the affinity of the antagonists for V_1 receptors and V_1-V_2 receptors in the goat cerebral vasculature (present study) may be similar to that in the goat coronary vasculature (Fernández *et al*., 1998). As the estimated pK values for these two antagonists in the present study are distinct from those reported elsewhere (Kruszynski *et al*., 1980; Jard *et al*., 1986; László *et al*., 1991; García-Villalón *et al*., 1996), it is possible that the population of vasopressin V_1 receptors in the goat cerebral vasculature differ from that in other tissues. Data using the same antagonist for V_1 receptors as in the present study suggest that the human middle cerebral artery (pK $\sim$9.3, Martín de Aguilera *et al*., 1990), and the rabbit basilar artery ($pA_2 = 9.3$, García-Villalón *et al*., 1996) exhibit a higher affinity for this antagonist than goat cerebral vasculature (present study). Thus, population of vasopressin V_1 receptors in cerebral blood vessels may vary between species, and perhaps between regions of brain blood vessels. In the canine cerebral vasculature, the antagonist for vasopressin V_1 receptors used in our study also inhibited in a dose-dependent way the vasopressin-induced effects on the vertebral artery territory, suggesting that these receptors also mediate the *in vivo* cerebrovascular action of vasopressin in this species (Suzuki *et al*., 1992). This particular study does not permit the estimation of the degree of affinity of this antagonist for vasopressin V_1 receptors, but the authors suggest that vasopressin reduces vertebral artery flow by activation of vasopressin V_1 receptors located in small vessels of the vertebral artery territory, and that vasopressin produces dilatation of major arteries of this territory which is also mediated by these receptors, probably located in the endothelium (Suzuki *et al*., 1992).

Our results with L-NAME alone confirm previous observations from our laboratory (Fernández *et al*., 1993; Diéguez *et al*., 1998) and agree with the idea that cerebral vasculature has a basal vasodilator tone mediated by nitric oxide (Faraci & Brian, 1994). The cerebrovascular effects of L-NAME persisted for about 48 h, and we found that in these conditions the cerebral vasoconstrictor effects of arginine vasopressin were increased during about 24 h. This suggests that under normal conditions, the basal release or the stimulated release of nitric oxide after arginine vasopressin may blunt the response of cerebral vessels to this peptide, and that the potentiating effects on the cerebrovascular action of arginine vasopressin after inhibition of nitric oxide release may reverse when nitric oxide production normalizes. These effects of inhibition of nitric oxide synthesis on the cerebrovascular effects of arginine vasopressin may be specific for this peptide as we have previously found that the cerebral vasoconstriction by endothelin-1 is not altered after this inhibition using the same experimental procedure as in the present study (Fernández *et al*., 1998). It has been reported from *in vitro* (Katusic *et al*., 1984) and *in vivo* (Suzuki *et al*., 1993) experiments that activation of vasopressin V_1 receptors located in the endothelium stimulates the release of nitric oxide in canine cerebral vessels. Experiments using cerebral (Takayasu *et al*., 1993; García-Villalón *et al*., 1996) and non-cerebral (Gardiner *et al*., 1991; García-Villalón *et al*., 1996) arteries also suggest that endothelial nitric oxide modulates the vasoconstrictor effects of arginine vasopressin.

Experiments for examining the role of prostanoids in the cerebrovascular effects of vasopressin are relatively sparse, and the results reported are inconclusive (Toda *et al*., 1993; Martínez *et al*., 1994; Tsuji & Cook, 1994). It has been reported that the vasopressin-induced vasodilation observed after vasopressin V_1 receptor blockade in human cerebral arteries may be mediated by the release of vasodilator prostaglandins (Martínez *et al*., 1994), and that in canine cerebral arteries with damaged endothelium, thromboxane A_2 production from smooth muscle cells potentiate the cerebral vasoconstriction induced by arginine vasopressin (Tsuji &

Cook, 1994). On the other hand, isolated cerebral arteries from dogs exhibit relaxation to arginine vasopressin, which is not influenced by blockade of cyclo-oxygenase with indomethacin (Toda *et al*., 1993). Our present results show that meclofenamate did not affect the cerebrovascular action of arginine vasopressin, suggesting that cyclo-oxygenase products are probably not involved in the cerebral vasoconstriction produced by this peptide under normal conditions. The dose of meclofenamate administered in the present experiments may be effective to inhibit cyclo-oxygenase as in rats a lower dose of this drug was effective to modify the vascular response to arginine vasopressin (Walker *et al*., 1988).

In conclusion, the present data show that arginine vasopressin produces cerebral vasoconstriction, and suggest that this vasoconstriction may be mediated by activation of vasopressin V_1 receptors, without involvement of vasopressin V_2 receptors, and may be modulated by nitric oxide, but not by prostanoids.

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