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Endogenous vasopressin increases acute endotoxin shock-provoked gastrointestinal mucosal injury in the rat

Csaba Varga [a], Imre Pávó [b], Dominique Lamarque [c], Zoltán Szepes [d], József Kiss [d], Gizella Karácsony [d], Ferenc A. László [a], Ferenc László [d,*]

[a] *Dept. of Comparative Physiology, Attila József University of Sciences, Szeged, Hungary*
[b] *Endocrine Unit, Albert Szent-Györgyi Medical University, Szeged, Hungary*
[c] *Hospital Henri Mondor, University of Paris XII, INSERM U99, Creteil, France*
[d] *First Dept. of Medicine, Albert Szent-Györgyi Medical University, P.B. 469, H-6701 Szeged, Hungary*

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Abstract

Administration of a low dose of endotoxin (from *Escherichia coli*, 3 mg kg^{-1}, i.v.), which does not affect vascular permeability or blood pressure over 1 h, leads to the release of endogenous vasopressin and damage to the mucosal microvasculature. Thus, endogenous vasopressin could be involved in septic shock. In the present study, we investigated the role of endogenous vasopressin in gastrointestinal mucosal injury induced by acute endotoxin shock, which was generated in rats by administering a high dose of *E. coli* endotoxin (50 mg kg^{-1}, i.v.). Tissues were removed 15 min after endotoxin. The vasopressin V_1 receptor antagonist, d[CH_2]$_5$Tyr[Me]arginine–vasopressin (0.2–1 μg kg^{-1}, i.v.), was injected 10 min before endotoxin. Monastral blue (30 mg kg^{-1}, i.v.), which stains damaged vasculature, was injected 10 min before autopsy. Endotoxin reduced systemic arterial blood pressure (from 115 ± 5 to 42 ± 4 mmHg), generated macroscopic and microvascular injury, and elevated plasma vasopressin levels (from 3.4 ± 0.2 to 178 ± 16 pg ml^{-1}). The vasopressin V_1 receptor antagonist reduced this macroscopic injury, and in the vasopressin-deficient Brattleboro rat a similar reduction of gastrointestinal mucosal damage was found. Substantial decreases in endotoxin-induced microvascular damage were observed in each tissue, e.g., the gastric Monastral blue staining was reduced by $47 \pm 3\%$ and $96 \pm 3\%$ ($P < 0.01$) after vasopressin V_1 receptor antagonist treatment and in Brattleboro rats, respectively. Vasopressin, acting through its V_1 receptors, thus appears to be involved in acute endotoxin shock-provoked gastrointestinal injury.

Keywords: Vasopressin; Endotoxin shock; Gastrointestinal mucosa; Microcirculation

1. Introduction

Endotoxin shock is characterised by hypotension, increased vascular permeability and gastrointestinal damage. Bleeding from this gastrointestinal erosions commonly occurs. Once the bleeding becomes manifested, it augments the severe condition and elevates the mortality rate (Robert and Kauffman, 1989; Root and Jacobs, 1991). Although it is important to prevent these stress erosions, it is currently not known how this should be done (Ben-Manachem et al., 1994).

Stress erosions have a multifactorial pathogenesis. Microvascular damage, which leads to hypoxia, has been shown to be an important factor in the generation of gastrointestinal stress erosions (Robert and Kauffman, 1989) as well as in the septic shock (Bone, 1991). In endotoxin shock, the impaired microcirculation may result from a direct injurious action of the lipopolysaccharide component of the bacterial wall on the vascular endothelium (Harlan et al., 1983; Meyrick et al., 1986), or may reflect the formation of tissue-damaging neutrophil- and/or endothelium-dependent vasoactive mediators, such as platelet-activating factor, thromboxanes, leukotrines and/or endothelin (Parillo, 1990; Bone, 1991; Baker et al., 1990; Lefer and Lefer, 1993).

The early release of the potent pituitary nonapeptide, vasopressin, into the plasma has been detected in endotoxaemic states in humans (Dennhardt et al., 1989) and in rats (Baker et al., 1990). Endogenous vasopressin, acting through its pressor (V_1) receptors, has been demonstrated

* Corresponding author. Tel.: +36-62-455-186; fax: +36-62-455-185.

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to have an aggressive action on the gastrointestinal mucosa during the generation of mucosal stress erosion, e.g., lesions induced by ethanol, indomethacin, reserpine, cold-restraint stress and haemorrhagic shock (László et al., 1991a, 1994, 1996; László and Whittle, 1994). It has also been found that in the early phase of endotoxaemia, i.e., following the administration of a low dose of endotoxin (which does not affect vascular permeability or blood pressure), endogenous vasopressin counteracts the actions of endothelium-derived protective factors, such as constitutive nitric oxide (László and Whittle, 1994). This might suggest that endogenous vasopressin can be involved in septic shock, i.e., in the generation of microvascular dysfunction and damage. The aim of our present study was to investigate the effect of vasopressin on the development of acute gastrointestinal mucosal injury induced by endotoxin shock. For the evaluation of the actions of vasopressin released endogenously, we used a vasopressin-deficient rat strain (homozygous Brattleboro rats; for review see Sokol and Valtin, 1982) and a specific vasopressin V_1 receptor antagonist (for review see László et al., 1991b).

Part of this work has been presented to The British Society of Gastroenterology (László et al., 1993) and to The IVth International Vasopressin Conference (Berlin, Germany, 1993 May).

2. Materials and methods

2.1. Experimental protocol

Female Wistar and homozygous Brattleboro rats (from our breeding farm), weighing 200–220 g, were fasted for 24 h, but received water ad libitum. The animals were anaesthetised with Nembutal (40 mg kg^{-1}, i.p.; Serva, Heidelberg, Germany) and *Escherichia coli* endotoxin (serotype 0111:B4; 50 mg kg^{-1}; Sigma) or saline (control) was injected into the tail vein and the rats were killed 15 min later. This high dose of endotoxin was chosen on the basis of previous studies to generate a mild–moderate grossly visible mucosal injury (Hutcheson et al., 1990). For staining of the injured vasculature, all rats were injected with Monastral blue (30 mg kg^{-1}, i.v.; Sigma) 10 min before autopsy. It should be mentioned here that this colloidal stain penetrates only through damaged microvascular endothelial cells, and not through the basal membrane. The vasopressin V_1 receptor antagonist, d[CH_2]$_5$Tyr[Me]arginine–vasopressin (0.2–1 μg kg^{-1}; Bachem, Germany), was injected i.v. 10 min before endotoxin administration. These doses of the vasopressin V_1 receptor antagonist were selected from previous studies on the basis of their potency to reduce the increase in blood pressure provoked by exogenous vasopressin and to protect the gastric mucosa against damage, i.e., against injury provoked by ethanol, indomethacin, reserpine, cold-restraint stress and haemorrhagic shock (László et al., 1991a, 1994, 1996). Blood pressure in the right carotid artery was monitored with an Elcomatic blood pressure transducer connected to a Grass Polygraph.

2.2. Gross evaluation of lesions

The stomach, duodenum and jejunum were removed, stretched out on cork and photographed. The lesions were examined by an investigator unaware of the nature of the experiment and scored on the basis of a semiquantitative scale (from 0 to III), where 0 = no damage; I = 1–5 petechiae on the mucosal surface; II = more than 5 petechiae and/or mild vasocongested parts; III = severe vasocongestion (Hutcheson et al., 1990).

2.3. Microvascular damage

Two standard-size (3 × 10 mm) pieces of tissue were cut out from standard sites of the stomach, duodenum and jejunum, fixed in formalin and embedded in paraffin. For histological staining of vascular elastic fibres, orcein stain was used. In these sections the background was light brown, blood vessels were dark brown, and injured vessels were blue. The distance of damaged blood vessels (stained with Monastral blue) from the serosal parts was measured with an ocular micrometer, and data are expressed as the maximum average distance of injured (i.e., Monastral blue-stained) blood vessels from the serosal surface in μm.

2.4. Plasma vasopressin level

For the evaluation of changes in plasma arginine–vasopressin levels, in a separate study, rats were decapitated and blood was immediately collected from the wound before (0 min) and after (15 min) endotoxin administration. In control studies plasma arginine–vasopressin levels were measured in intact animals that had been fasted for 24 h, but which had access to water ad libitum. Plasma arginine–vasopressin level was determined with a specific radioimmunoassay system as described previously (László et al., 1994).

2.5. Statistics

For statistical comparisons, analysis of variance with the Bonferroni test was used. Differences were taken as significant when the probability was less than 5%.

3. Results

3.1. Gross evaluation of lesions

Administration of endotoxin (50 mg kg^{-1}, i.v.) reduced blood pressure by 63% (Table 1). As described previously

Table 1
Blood pressure (mmHg) before and during acute (15 min) endotoxin (LPS) shock in rats

Groups—treatments	Before treatment	After treatment
Saline (control)	98 ± 6	95 ± 5
LPS (50 mg kg^{-1})	115 ± 5	42 ± 4[a]
LPS + V_1 antagonist (1 μg kg^{-1})	112 ± 5	50 ± 5[a]
LPS in Brattleboro	105 ± 7	43 ± 6[a]

n = 4–8; mean ± S.E.M.
[a] $P < 0.05$ before treatment groups compared to after treatment groups.

(Hutcheson et al., 1990), this acute endotoxin shock generated gross gastric, duodenal and jejunal mucosal injury in normal (Wistar) rats 15 min later (Fig. 1).

Pretreatment with the vasopressin V_1 receptor antagonist (0.2–1 μg kg^{-1}, i.v.) dose dependently attenuated the mucosal damage of the stomach, duodenum and jejunum provoked by endotoxin in normal (Wistar) rats (Fig. 1). Moreover, endogenous vasopressin deficiency (in homozygous Brattleboro rats) also reduced macroscopic mucosal damage (Fig. 1). The vasopressin V_1 receptor antagonist (1 μg kg^{-1}, i.v.) and vasopressin deficiency did not affect the reduced blood pressure caused by endotoxin shock (Table 1).

3.2. Microvascular damage

Acute endotoxin shock injured intra- and submucosal and subserosal blood vessels in the stomach, duodenum and jejunum within 15 min. It was found that endotoxin administration provoked microcirculatory damage that involved almost the entire wall of the gastrointestinal organs investigated. In endotoxin-treated control rats the subserosal vessels were affected the most. This vascular endothelial injury induced by endotoxin shock was significantly and dose dependently attenuated by pretreatment with the vasopressin V_1 receptor antagonist (0.2–1 μg kg^{-1}, i.v.) and by vasopressin deficiency (in homozygous Brattleboro rats) as demonstrated in Table 2.

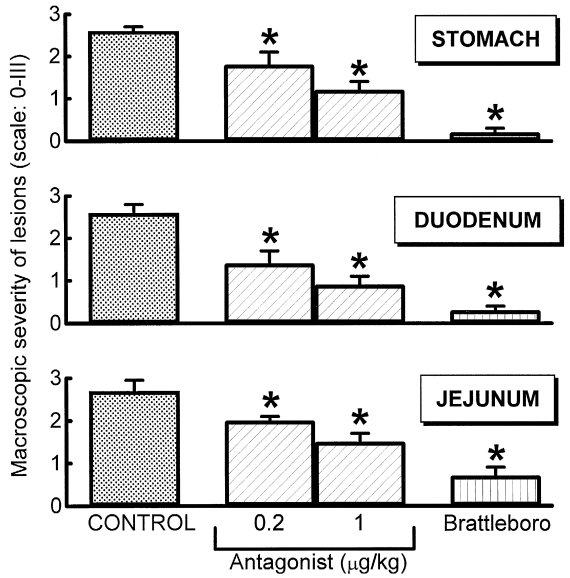

Fig. 1. The protective effect of a vasopressin pressor (V_1) receptor antagonist (Antagonist; d/CH_2/$_5$Tyr/Me/arginine–vasopressin, 0.2–1 μg kg^{-1}, i.v.) and endogenous vasopressin deficiency (in homozygous Brattleboro rats (Brattleboro)) against acute (15 min) endotoxin (LPS, 50 mg kg^{-1}, i.v.) shock-induced gastric, duodenal and jejunal lesions. Macroscopic severity scale: 0–III, determined by an investigator unaware of the nature of the experiment; n = min 4; mean ± S.E.M.; * $P < 0.05$ compared to the LPS-treated (CONTROL) groups.

Table 2
Histological appearance of acute (15 min) vascular damage (assessed by the combination of orcein staining of elastic fibres in blood vessels with the Monastral blue technique for staining damaged vessels) induced by *E. coli* lipopolysaccharide (LPS, 50 mg kg^{-1}), and the protection provided by a vasopressin V_1 receptor antagonist and vasopressin deficiency (homozygous Brattleboro rat)

Groups	Stomach	Duodenum	Jejunum
Saline (control)	0.0	0.0	3.1 ± 0.8[a]
LPS	182.3 ± 7.8	131.5 ± 4.1	56.3 ± 3.2
LPS + V_1 antagonist (0.2 μg kg^{-1})	137.4 ± 4.8[a]	98.0 ± 3.3[a]	37.3 ± 3.8[a]
LPS + V_1 antagonist (1.0 μg kg^{-1})	93.9 ± 5.9[a]	23.9 ± 2.7[a]	13.4 ± 2.2[a]
LPS in Brattleboro	6.1 ± 3.7[a]	1.8 ± 0.9[a]	13.5 ± 4.3[a]

n = min 4; mean ± S.E.M.
[a] $P < 0.05$ compared to LPS-treated groups, data are expressed as the maximum average distance (in μm) of damaged blood vessels (stained with Monastral blue) from the serosal parts of the organs.

3.3. Plasma vasopressin level

Anaesthesia (Nembutal, 40 mg kg^{-1}, i.p.) elevated circulating vasopressin levels. Endotoxin shock caused a further increase in plasma vasopressin. In homozygous Brattleboro rats the plasma vasopressin level was undetectable (Table 3).

4. Discussion

In the present study, the role of endogenous vasopressin in the development of endotoxin shock-provoked acute

Table 3
Changes in plasma arginine vasopressin level during acute (15 min) endotoxin (LPS, 50 mg kg^{-1}, i.v.) shock in rats

Groups	Vasopressin levels (pg ml^{-1})
Control (conscious)	3.4 ± 0.2[a]
Saline (anaesthetised)	43.6 ± 9.0[b]
LPS (anaesthetised)	178.1 ± 16.0[ab]
LPS in Brattleboro (anaesthetised)	> 1.0 ± 0.0

n = 4–8, mean ± S.E.M.
[a] $P < 0.05$ compared to the saline group.
[b] $P < 0.05$ compared to the control group.

gastrointestinal mucosal and microcirculatory damage was evaluated in the stomach, duodenum and jejunum of rats. It was shown that administration of a high dose of endotoxin reduced arterial blood pressure, elevated circulating vasopressin level and generated gastric, duodenal and jejunal macroscopic mucosal and microcirculatory damage in normal (Wistar) rats. Our observations, in relation to changes of plasma vasopressin levels, are in agreement with previous findings in which an increase in circulating vasopressin following anaesthesia or endotoxin administration has been described (Reichlin, 1985; Robertson, 1987; Dennhardt et al., 1989; Baker et al., 1990). Moreover, we found that homozygous Brattleboro rats, which have a congenital deficiency to synthesize vasopressin and consequently have diabetes insipidus (Sokol and Valtin, 1982), were less sensitive to the injurious actions of acute endotoxin shock. Finally, in normal rats, administration of a vasopressin V_1 receptor antagonist dose dependently protected the gastrointestinal microvasculature against the vascular endothelial damage induced by endotoxin. These findings indicate the role of endogenous vasopressin in the generation of acute endotoxin shock-induced gastrointestinal stress erosions.

Microvascular permeability and gastrointestinal damage may respond to initial local changes in blood flow, as vasopressin is a potent vasoconstrictor in the gastrointestinal microcirculation (Burnstock, 1990; Baker et al., 1990; Vanner et al., 1990). Even in endotoxaemic states when the vascular bed is less sensitive to endogenous vasoconstrictor agents, this hormone has been shown to be the most effective vasoconstrictor when compared to other endogenous circulating constrictor agents (Vanner et al., 1990). In addition, vasopressin, via its V_1 receptors, can cause platelet aggregation which may lead the formation of microthrombi in the microcirculation (Filep and Rosenkrantz, 1987). Furthermore, vasopressin may induce gastrointestinal vasoconstriction, vasocongestion or vascular endothelial injury through a thromboxane-dependent mechanism, since it can release thromboxanes from platelets and from vascular tissues (Filep and Rosenkrantz, 1987; Nádasy et al., 1992). The damaging effects of thromboxanes on the gastrointestinal microvasculature following the administration of high doses of endotoxin have been previously reported. In these studies, endotoxin increased the intestinal synthesis of thromboxanes, and the mucosal injury was prevented by thromboxane synthase inhibitors (Boughton-Smith et al., 1989). All of the above described effects of an increased vasopressin formation can lead to an impaired tissue oxygen supply. Indeed, in a recent study vasopressin blockade was shown to improve the impaired oxygen extraction ratio and ketone body availability in the mesenteric circulation following endotoxin administration (Matsuoka and Wisner, 1997).

In conclusion, our current results suggest that endogenous vasopressin has acute injurious actions in the gastrointestinal microcirculation during endotoxin shock. These findings, in conjunction with the beneficial actions of vasopressin deficiency and vasopressin V_1 receptor antagonists in the development of various experimental gastrointestinal lesions (László et al., 1991a, 1994, 1996), indicate the aggressive role endogenous vasopressin has in the generation of gastrointestinal stress erosions in acute septic shock.

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References

Baker, C.H., Sutton, E.T., Zhou, Z., Deitz, J.R.M., 1990. Microvascular vasopressin effects during endotoxin shock in the rat. Circ. Shock 30, 81–95.

Ben-Manachem, T., Fogel, R., Patel, R.V., 1994. Prophylaxis for stress-related gastric hemorrhage in the medical intensive care unit. Ann. Intern. Med. 121, 568–575.

Bone, R.C., 1991. The pathogenesis of sepsis. Ann. Intern. Med. 115, 457–469.

Boughton-Smith, N.K., Hutcheson, I., Whittle, B.J.R., 1989. Relationship between paf-acether and thromboxane A_2 biosynthesis in endotoxin-induced intestinal damage in the rat. Prostaglandins 38, 319–333.

Burnstock, G., 1990. Local mechanisms of blood flow control by perivascular nerves and endothelium. J. Hypertens. 8, S95–S106, Suppl. 7.

Dennhardt, R., Gramm, H.J., Meinhold, K., Voigt, K., 1989. Patterns of endocrine secretion during sepsis. Prog. Clin. Biol. Res. 308, 751–760.

Filep, J., Rosenkrantz, B., 1987. Mechanism of vasopressin-induced platelet aggregation. Thromb. Res. 45, 7–15.

Harlan, J.M., Harker, L.A., Reidy, M.A., Gajdusek, C.M., Schwartz, S.M., Striker, G.E., 1983. Lipopolysaccharide-mediated bovine endothelial cell injury in vitro. Lab. Invest. 48, 269–274.

Hutcheson, I.R., Whittle, B.J.R., Boughton-Smith, N.K., 1990. Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. Br. J. Pharmacol. 101, 815–820.

László, F., Whittle, B.J.R., 1994. Constitutive nitric oxide modulates the injurious actions of vasopressin on rat intestinal microcirculation in acute endotoxaemia. Eur. J. Pharmacol. 260, 265–268.

László, F., Karácsony, G., Szabó, E., Láng, J., Baláspiri, L., László, F.A., 1991a. The role of vasopressin in the pathogenesis of ethanol-induced gastric hemorrhagic erosions in rats. Gastroenterology 101, 1242–1248.

László, F.A., László, F., de Wied, D., 1991b. Pharmacology and clinical perspectives of vasopressin antagonists. Pharmacol. Rev. 43A, 73–108.

László, F., Varga, C., Karácsony, G., Whittle, B.J.R., László, F.A., 1993. Involvement of endogenous vasopressin in endotoxin-induced acute gastrointestinal injury in the rat. Gut 34, S61.

László, F., Karácsony, G., Pávó, I., Varga, C., Rojik, I., László, F.A., 1994. Aggressive role of vasopressin in development of different gastric lesions in rats. Eur. J. Pharmacol. 258, 15–22.

László, F., Szepes, Z., Karácsony, G., Varga, C., László, F.A., 1996. Endogenous vasopressin damages duodenal mucosa during haemorrhagic shock in rats. Inflammopharmacololgy 4, 379–385.

Lefer, A.M., Lefer, D.J., 1993. Pharmacology of the endothelium in ischaemia-reperfusion and circulatory shock. Annu. Rev. Pharmacol. Toxicol. 33, 71–90.

Matsuoka, T., Wisner, D.H., 1997. Hemodynamic and metabolic effects of vasopressin blockade in endotoxin shock. Surgery 121, 162–173.

Meyrick, B.O., Ryan, U.S., Brigham, K.L., 1986. Direct effects of *E. coli* endotoxin on structure and permeability of pulmonary endothelial monolayers and the endothelial layer of intimal explants. Am. J. Pathol. 122, 140–151.

Nádasy, G.L., Székács, B., Juhász, I., Monos, E., 1992. Pharmacological modulation of prostacyclin and thromboxane production of rat and cat venous tissue slices. Prostaglandins 44, 339–355.

Parillo, J.E., 1990. Septic shock in humans: advances in the understanding of pathogenesis, cardiovascular dysfunction and therapy. Ann. Intern. Med. 113, 227–242.

Reichlin, S., 1985. Neuroendocrinology. In William's Textbook of Endocrinology. Saunders, Philadelphia, pp. 492–511.

Robert, A., Kauffman, G.L., 1989. Stress ulcers, erosions, and gastric mucosal injury. In: Gastrointestinal Disease, Pathophysiology, Diagnosis, Management. Saunders, Philadelphia, pp. 772–792.

Robertson, G.L., 1987. Posterior pituitary. In: Baxter's Endocrinology and Metabolism. McGraw-Hill, New York, pp. 335–356.

Root, R.K., Jacobs, R., 1991. Septicemia and septic shock. Harrison's Principles of Internal Medicine. McGraw Hill, New York, pp. 507–516.

Sokol, H.W., Valtin, H., 1982. The Brattleboro rat. Ann. New York Acad. Sci. 394, 1–828.

Vanner, S., Jiang, M.-M., Brooks, V.L., Surprenant, A., 1990. Characterization of vasopressin actions in isolated submucosal arterioles of the intestinal microcirculation. Circ. Res. 67, 1017–1026.