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L-GLUTAMINE DOES NOT INHIBIT THE RELEASE OF ENDOTHELIUM- DERIVED RELAXING FACTOR FROM FRESHLY ISOLATED RAT PERIPHERAL ARTERIES AND BOVINE ABDOMINAL AORTA

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In freshly isolated rat large peripheral arteries and bovine abdominal aorta rings, the effect of L-glutamine on the endothelium-dependent relaxations meditiated by the release of endothelium-derived relaxing factor (EDRF) was studied. The incubation of rat aorta, common carotid and superior mesenteric artery rings with 10^{-3} mol/l of L-glutamine (contact time 45 min) produced no changes in their relaxant responses to acetylcholine and histamine. The exposure of bovine abdominal aorta rings to the action of $3x10^{-4}$ mol/l of L-glutamine also did not alter their relaxant response to acetylcholine. Higher concentrations of L-glutamine than those used, reduced in parallel the control papaverine-induced and acetylcholine- or histamine induced relaxations. It was concluded that L-glutamine does not inhibit the endothelium-dependent relaxations mediated by the release of EDRF in freshly isolated rat large peripheral arteries and bovine abdominal aorta. The present experiments provide evidence that in these arteries the inhibitory effect of L-glutamine on the release of EDRF cannot be exerted when the endothelium is sufficient in L-arginine and when it does not synthesize L-arginine de novo.

Key words: L-glutamine, EDRF, L-arginine

INTRODUCTION

Endothelium-derived relaxing factor (EDRF) is authentic nitric oxide (Palmer et al., 1987; Hutchinson et al., 1987) or nitric oxide incorporated into a nitrosothiol compound such as S-nitrosocysteine (Myers et al., 1989., 1990). It has been proved that nitric oxide is derived from the guanidino group of L-arginine (Palmer et al., 1988; Schmidt et al., 1988), which can be generated by endothelial cells from an intracellular source (Hecker et al., 1990a). L-glutamine inhibits the generation of L-arginine and the release of EDRF from bovine aorta cultured endothelial cells (Hecker et al., 1990b), but not the release of EDRF

from freshly isolated rabbit aorta strips (Swierkosz et al., 1990). As the heterogeneity of many of the features of vascular smooth muscle and endothelium depends on animal species and anatomical location of blood vessels (Vanhoutte et al., 1986; Busse et al., 1985; Angus and Cooks, 1989; Marin and Sàchez-Ferrer, 1990), it was of interest to examine the effect of L-glutamine on the release of EDRF from freshly isolated rat peripheral arteries and bovine abdominal aorta.

MATERIAL AND METHODS

The experiments were performed on rings of rat thoracic aorta, common carotid and superior mesenteric artery and bovine abdominal aorta. Rats of either sex (250-300 g) were killed by a blow on the head. The arteries were carefully removed from the animal and placed in Krebs-Ringer-bicarbonate solution (mmol/l: NaCl, 118,3; KCl, 4.7; $CaCl_2$, 2,5; $MgSo_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; CaEDTA, 0.026; glucose, 11.1) at room temperature. A piece of bovine abdominal aorta between the last thoracic and the second lumbar vertebra was dissected immediately after the animal was killed in the abattoir and placed in Tyrode solution (mmol/1: NaCl, 137; KCl, 2,6; $CaCl_2$, 1.8; $MgCl_2$, 0.1; NaH_2PO_4, 0.42, $NaHCO_3$, 11,9; CaEDTA, 0.026; glucose, 11.1) at room temperature. This was usually performed about 2 to 3 hours before the beginning of the experiment.

After the removal of adjacent connective and adipose tissue, the arteries were cut into rings of 4 mm (rat arteries) or 5 mm (bovine aorta) in width. Particular attention was paid to avoid any damage to the endothelial cells. In some rings of bovine abdominal aorta, the endothelium was mechanically removed by a brief, gentle rubbing of the intimal surface (De May and Vanhoutte, 1981). Rings were mounted in an organ chamber of 60 ml capacity with both ends fastened; the lower end of the rings was tied to a glass-stick, and the upper end was attached to an isometric transducer (IMP-electronic, Tuzla, Yugoslavia). The organ chamber was filled with Krebs-Ringer-bicarbonate solution (37^0C) oxygenated with 95%O_2 – 5%CO_2 and with Tyrode solution (37^0C) oxygenated with 97%O_2 – 3%CO_2, respectively. Isometric tension was continuously recorded.

Each ring was gradually stretched to the optimal point (2 g for rat arteries and 5 g for bovine aorta) on its length-tension curve, and allowed to equilibrate for 40 min. The presence of the relaxing effect of acetylcholine in rings precontracted with phenylephrine was taken as evidence of the integrity of the vascular endothelium.

Concentration-response curves to acetylcholine and histamine were obtained in a cumulative fashion. The relaxations caused by acetylcholine and

histamine were expressed as a percentage o the maximal relaxations induced with papaverine (10^{-4} mol/l).

In bovine aorta the control response to acetylcholine and that a ter exposure to the action o L-glutamine were recorded in separate rings taken rom the same piece o aorta. In rat arteries the responses to acetylcholine and histamine, respectively, be ore and a ter the treatment with L-glutamine were recorded in the same ring. Ring segments were washed at least 3 times with 60 ml o physiological saline solution and allowed to equilibrate or 30 min a ter each exposure to acetylcholine and histamine, respectively, and remained in contact with it during the cumulative increase in concentration.

The ollowing drugs were used: acetylcholine iodide (Serva), histamine dihydrochloride (Serva), L-phenylephrine (Sigma), L-glutamine (Sigma) and papaverine hydrochloride (Merck). The drugs were dissolved in distilled water such that volumes o less than 0.2 ml were added to the organ chambers. Stock solutions o the drugs were reshly prepared every day. Concentrations o the drugs are expressed as the inal molar concentration in bath solution.

The EC_{50} and EC_{30} values were calculated or each preparation by linear interpolation between the two concentrations evoking responses just above and just below the 50% and 30%, respectively, o the maximum. For each series o experiments the geometric mean and s.e.m. o the values thus obtained are reported. The data are expressed as meano $\pm$ s.e.m. Statistical evaluation o the data was per ormed by Student's t-test or paired observations.

RESULTS

As in the previous experiments (Van de Voorde and Leusen. 1983; Rapoport and Murad, 1983; Katušić and Krstić, 1987a, Krstić et al., 1988 a, b), acetylcholine (10^{-9}-10^{-5} mol/l) caused concentration-dependent relaxations o the reshly isolated rat aorta, common carotid and superior mesenteric artery rings made to contract by phenylephrine (10^{-6}-10^{-7} mol/l) which gave 50% o their maximal tone. In all these arteries, concentration-response curves to the irst and second application o acetylcholine (7 experiments or each artery), and histamine (7 experiments or each artery), respectively, were comparable.The incubation o reshly isolated rat aorta, common carotid and superior mesenteric arteries with L-glutamine (10^{-3} mol/l; contact time 45 min) did not inhibit their relaxant responses to acetylcholine and histamine (Figures 1 and 2). The EC_{50} values or the relaxant responses to acetylcholine and histamine be ore and a ter the treatment by L-glutamine are given in Table 1. The exposure o the arteries to the action o $3x10^{-2}$ mol/l o L-glutamine reduced the relaxant responses to papaverine (10^{-4} mol/l; 4 experiments or each artery) and acetylcholine (10^{-9} - 10^{-5} mol/l) or histamine (10^{-8} - 10^{-4} mol/l) in parallel.

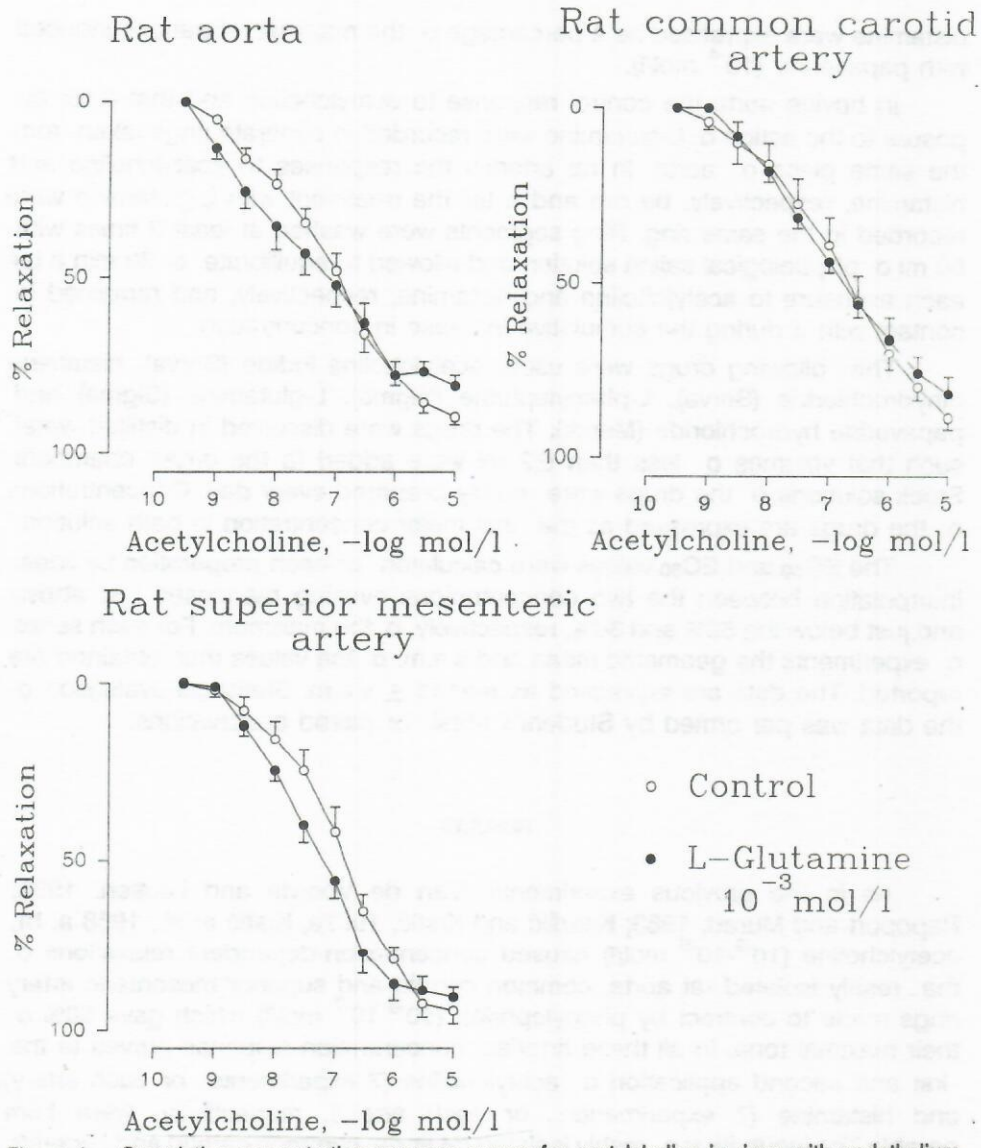

Figure 1. Cumulative concentration-response curves for acetylcholine in rings with endothelium alone and rings with endothelium in the presence of L-glutamine (rat thoracic aorta, common carotid and superior mesenteric artery). The relaxations were obtained during contractions induced by EC_{50} of phenylephrine (10^{-8} - 10^{-7} mol/l). The data are shown as mean ± s. e. m. (n=7, n=5 and n=5 for aorta, common carotid and mesenteric artery, respectively), and expressed as a percentage of the maximal relaxations induced by papaverine (10^{-4} mol/l; 100%=0.61±0.09 and 0.52±0.05 g in aorta, 100%=0.42±0.03 and 0.33±0.05 g in common carotid, and 100% = 046±0.06 and 0.39±0.04 g in mesenteric artery for rings with endothelium alone and with endothelium treated by L-glutamine, respectively).

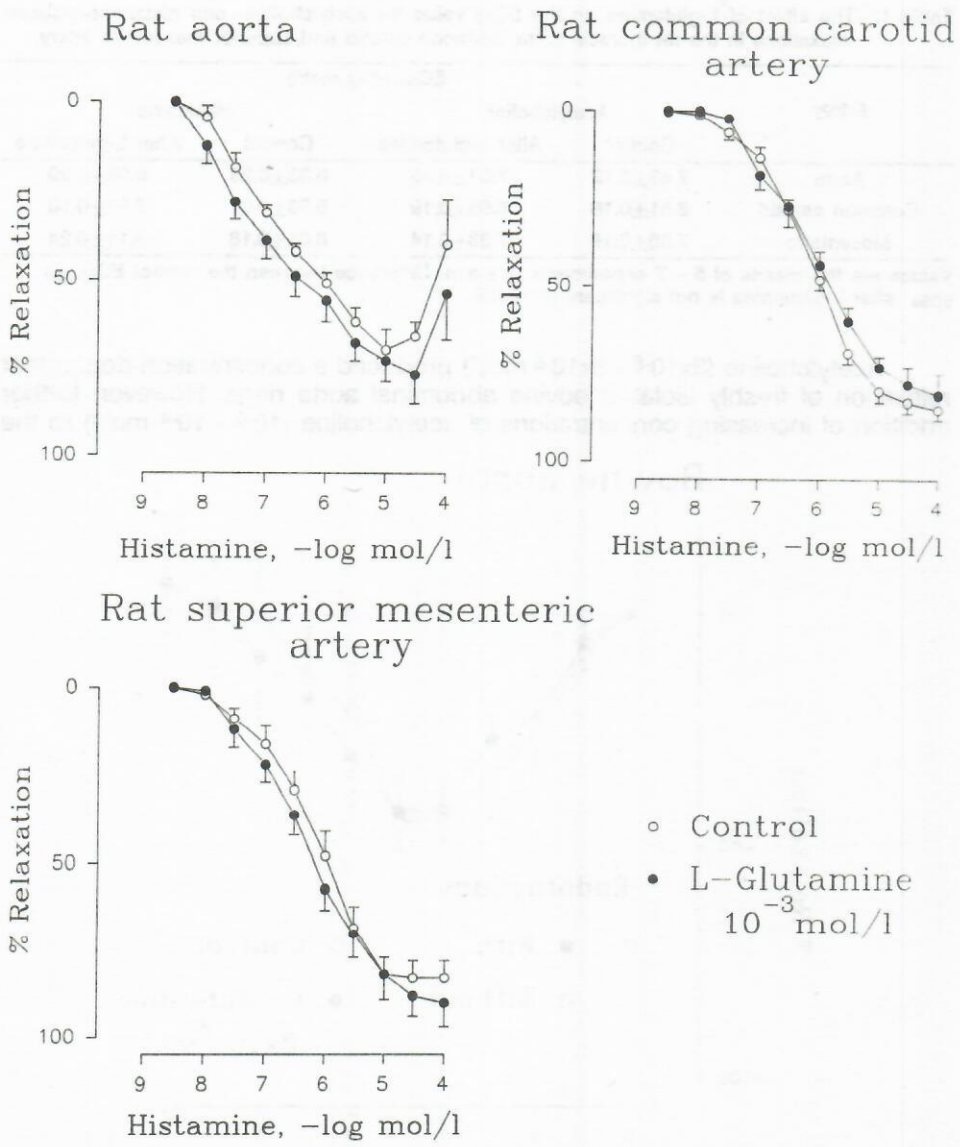

Figure 2. Cumulative concentration-response curves for histamine in rings with endothelium alone and rings with endothelium in the presence of L- glutamine (rat thoracic aorta, common carotid and superior mesenteric artery). The relaxations were obtained during contractions induced by EC_{50} of phenylephrine (10^{-8} - 10^{-7} mol/l). The data are shown as mean ± s.e.m. (n=6) and expressed as a percentage of the maximal relaxations induced by papaverine (10^{-4} mol/l; 100%=0.61±0.13 and 0.58±0.1 g in aorta, 100%=0.34±0.1 and 0.32±0.04 g in common carotid, and 100%=0.44±0.06 and 0.41±0.04 g in mesenteric artery for rings with endothelium alone and with endothelium treated by L-glutamine, respectively).

Table 1. The effect of L-glutamine on the EC_{50} value for acetylcholine- and histamine-induced relaxations in the rat thoracic aorta, common carotid and superior mesenteric artery.

Artery	EC_{50} (-log mol/l)			
	Acetylcholine		Histamine	
	Control	After L-glutamine	Control	After L-glutamine
Aorta	7.48±0.12	7.28±0.25	6.33±0.23	6.36±0.29
Common carotid	6.51±0.16	6.66±0.19	5.73±0.07	5.83±0.10
Mesenteric	7.35±0.18	7.23±0.14	6.01±0.18	6.11±0.24

Values are the means of 5 – 7 experiments ± s.e.m. Difference between the control EC_{50} and EC_{50} after L-glutamine is not significant ($p>0.05$).

Acetylcholine ($3x10^{-8}$ - $3x10^{-6}$ mol/l) produced a concentration-dependent relaxation of freshly isolated bovine abdominal aorta rings. However, further addition of increasing concentrations of acetylcholine (10^{-6} - 10^{-4} mol/l) to the

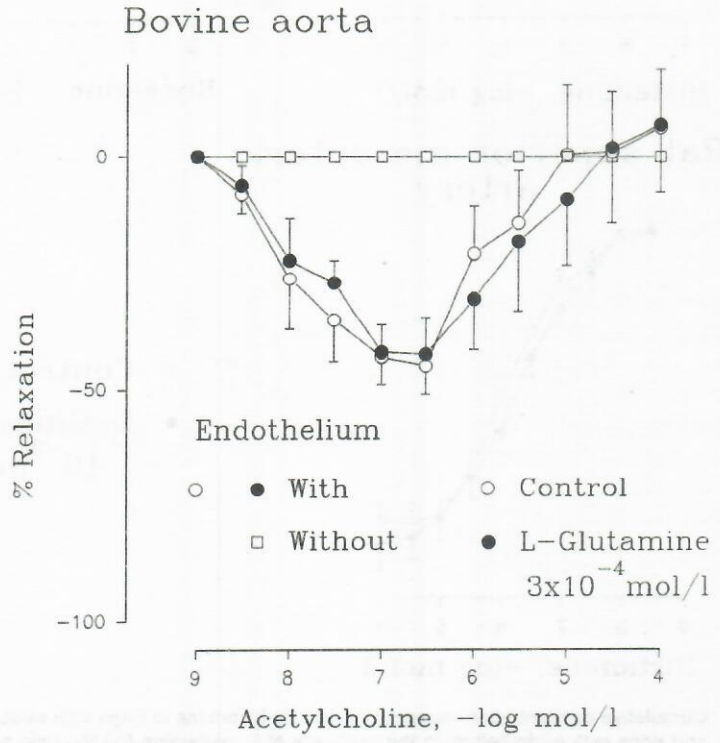

Figure 3. Cumulative concentration-response curves for acetylcholine in rings of bovine abdominal aorta with and without endothelium and with endothelium in the presence of L-glutamine. The relaxations were obtained during contractions induced by ED_{50} of phenylephrine (10^{-7} - $3x10^{-7}$ mol/l). The data are shown as mean ± s.e.m. (n=6), and expressed as a percentage of the maximal relaxations induced by papaverine (10^{-4} mol/l; 100%= 3.40±0.21, 6,88±0.85 and 3.46±0.92 g for rings with, without and with endothelium treated by L-glutamine, respectively).

bathing fluid elicited a concentration-dependent increase in tone of the preparation (Figure 3). Removal of the endothelium abolished the relaxant effect of acetylcholine (Figure 3). The incubation of freshly isolated abdominal aorta rings with $3x10^{-4}$ mol/l of L-glutamine (contact time 45 min) produced no changes in the relaxant effect of acetylcholine (Figure 3). The control EC_{30} value and EC_{50} value obtained in the presence of L-glutamine were 7.38 ± 0.33 and 7.33 ± 0.23 (-log mol/l; n=6). Higher concentrations of L-glutamine (10^{-4} and $3x10^{-3}$ mol/l) reduced or abolished both the relaxant responses to acetylcholine (10^{-8} -$3x10^{-6}$) and papaverine (10^{-4} mol/l; 4 experiments for each concentration of L-glutamine).

DISCUSSION

It is well-known that acetylcholine and histamine cause endothelium-dependent relaxations of freshly isolated rat aorta, common carotid and superior mesenteric artery mediated by the release of EDRF (Busse et al., 1985; Angus and Cocks, 1989; Krstić et al., 1990). In the present experiments, after removal of the vascular endothelium the acetylcholine-induced relaxations of the freshly isolated bovine abdominal aorta rings were abolished, which is in full agreement with the finding that these relaxations are mediated via the release of EDRF (Jezdimirović, 1989). L-glutamine inhibits EDRF release from cultured bovine endothelial cells by preventing the generation of L-arginine (Hecker et al., 1990b). Accordingly, it may be expected that L-glutamine suppresses the endothelium-dependent relaxations of blood vessels mediated by the release of EDRF. However, the acetylcholine-induced relaxations of the freshly isolated rabbit aorta strips mediated via the release of EDRF are not inhibited by L-glutamine (Swierkosz et al., 1990). L-glutamine did not affect the acetylholine- and histamine-induced relaxations of reshly isolated rat aorta, common carotid and superior mesenteric artery, indicating that the failure of L-glutamine to inhibit the acetylcholine-induced relaxations in the freshly isolated rabbit aortic strips is not due to species difference or anatomical location of the blood vessels.

L-arginine is the endogenous substrate of EDRF biosynthesis and its availability is rate-limiting for EDRF release (Hecker et al., 1990b, Mitchel et al., 1990). L-glutamine inhibits the release of EDRF from perfused rabbit aortae that were repeatedly challenged with acetylcholine, under which circumstances the endothelium is deficient in L-arginine and forced to generate L-arginine from an intracellular source (Swierkosz et al., 1990). The present experiments provide evidence that in rat large peripheral arteries and bovine abdominal aorta, like in the rabbit aorta, the inhibitory effect of L-glutamine on the release of EDRF cannot be exerted when the endothelium is sufficient in L-arginine and when it does not synthesize L-arginine de novo.

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L-GLUTAMIN NE INHIBIŠE OSLOBAĐANJE ENDOTELNOG RELAKSANTNOG FAKTORA IZ SVEŽE IZOLOVANIH PERIFERNIH ARTERIJA PACOVA I ABDOMINALNE AORTE GOVEČETA

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SADRŽAJ

Na sveže izolovanim prstenovima velikih perifernih arterija pacova i abdominalne aorte govečeta proučavano je dejstvo L-glutamina na relaksacije zavisne od endotela koje se prenose preko oslobođenog endotelnog relaksantnog faktora (EDFR). Inkubacija prstenova aorte, zajedničke karotidne i gornje mezenterične arterije pacova u 10^{-3} mol/l L-glutamina (vreme kontakta 45 min) nije izazvala promene u njihovoj relaksantnoj reakciji na acetilholin i histamin. Izlaganje prstenova abdominalne aorte govečeta dejstvu 3 x 10^{-4} mol/l L-glutamina, takođe, nije promenilo njihovu relaksantnu reakciju na acetilholin. Više koncentracije L-glutamina od onih koje su korišćene paralelno su redukovale kontrolne relaksacije izazvane papaverinom i acetilholinom ili histaminom. Zaključeno je da u sveže izolovanim velikim perifernim arterijama pacova i abdominalnoj aorti govečeta L-glutamin ne deluje na relaksacije zavisne od endotela koje se prenose preko oslobođenog EDRF-a. Dobijeni rezultati pružaju dokaz da se u ovim arterijama ne može ispoljiti inhibitorno delovanje l-glutamina na oslobađanje EDRF-a kad u endotelu ima dovoljno L-arginina i kad endotel ne sintetiše novi L-arginin.