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THE INFLUENCE OF NITRIC COMPOUNDS ON THE REACTIVITY OF THE ISOLATED BOVINE ABDOMINAL AORTA IN THE PRESENCE OF LIPOPOLYSACCHARIDE (LPS)

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Nitric compounds, such as glyceryl trinitrate (GTN) and sodium azide, are well known vasodilators. Their vasodilative effect is accomplished via nitric oxide (NO), released during the biotransformation of these compounds in vascular smooth muscle and endothelium. Lipopolysaccharides (LPS) are endotoxins derived from gram-negative bacteria. In the vascular system, LPS cause severe functional disorders, inducing excessive synthesis and release of NO from the vascular endothelium and smooth muscles. The fact that the effect of both nitrovasodilatators and LPS on blood vessels is mediated by nitric oxide prompted us to study their possible synergistic vasodilative interaction, i.e. we wanted to investigate if there is a functional relationship between the activity of nitric compounds and LPS on the bovine abdominal aorta.

The experiments were conducted on isolated bovine abdominal aorta, incubated for 4 hours in an organ bath with Tyrode solution. Glyceryl trinitrate and sodium azide bring about a dose-dependent relaxation of isolated bovine abdominal aorta previously contracted with noradrenaline. With an EC_{50} of $1.531 \times 10^{-9}M \pm 0.13$, glyceryl trinitrate is a more potent relaxant than sodium azide whose EC_{50} is $2.322 \times 10^{-9}M \pm 0.02$. In the presence of LPS, the maximal relaxant effect of GTN was intensified in contrast to the control relaxant effect of GTN on isolated bovine abdominal aorta. The relaxant effect of sodium azide in the presence of LPS was significantly intensified in contrast to the control relaxant effect of this nitric compound. The applied lipopolysaccharide definitely induced the production of NO, which, together with NO derived from nitric compounds (glyceryl trinitrate and sodium azide), caused greater maximal relaxation of the isolated blood vessel, i.e. pretreatment with LPS enhances the biotransformation of both nitric compounds; to a higher degree of sodium azide.

Key words: Lipopolysaccharide, glyceryl trinitrate, sodium azide, bovine abdominal aorta.

INTRODUCTION

The vasodilative effect of nitric compounds has been known for a long time. Thus, organic nitrates and inorganic nitrites such as: glyceryl trinitrate, isosorbitol dinitrate, sodium nitroprusside, sodium azide, sodium nitrite and sodium oxide, cause passing, dose-dependent relaxation of previously contracted isolated coronary, pulmonary, mesenteric artery and bovine abdominal aorta (Ignarro et al., 1988; Jezdimirović et al., 1991), rat aorta and mesenteric artery (Shirasaky et al., 1988) and human coronary artery (Forstermann et al., 1988).

It has been shown that the mechanism of action of nitric compounds proceeds to a great extent via nitric oxide (NO). After application of organic nitrates and nitrites, biotransformation occurs in vascular smooth muscles, with the formation of NO. (Bennett et al., 1994; Oates, 1995; Kearney et al., 1998). This free, unstable radical, then chemically reacts with the SH-group of cysteine or some other thiol, producing S-nitrosocysteine, which activates soluble guanylate cyclase (sGC). The activated guanylate cyclase increases the production of cGMP in smooth muscle cells, which decreases the Ca^{2+} ion content in the cytosol. All this has as a conseqence the relaxation of vascular smooth muscle and vasodilatation (Moncada et al., 1991; Gyton, 1991; Schulz and Triggle, 1994).

Lipopolysaccharides (LPS) are endotoxins derived from gram-negative bacteria which, induce the expression of inducible NO-synthase (iNOS), in vascular tissue, The activation of this enzyme in vascular endothelial and smooth muscle cells results in an increased synthesis and release of NO. This NO, causes a marked vasodilatation, hyporeactivity of blood vessels to vasoconstrictors, as well as an increase in capillary permeability, namely, the main symptoms of endotoxic shock (Villamor et al., 1995; Thiemermann, 1995; Parrat, 1995).

Under the influence of the constitutive NO-synthase (cNOS) a small amount of NO is synthesized and released in the endothelium, smooth muscles of blood vessels and myocytes of the cardiovascular system. This biologically active compound, as an endogenous vasodilative mediator, takes part in the physiological regulation of the vascular tonus (Adams, 1995; Rees, 1996).

Having in mind that nitric oxide (NO) is a compound with an important role in the relaxant activity of nitrovasodilatators, as well as the fact that this free radical is an endogenous vasodilatative mediator and, under pathophysiological conditions, such as septic shock, it can be considered responsible for the development of cardiovascular insufficiency, we thought it necessary to undertake a study of the functional relationship between the activities of nitrovasodilatators and LPS on an isolated blood vessel.

MATERIALS AND METHODS

The experiments were performed on the isolated abdominal aorta of male Simmenthal cattle, aged 12 to 14 months and of body weight about 500 kg. A part of the abdominal aorta was always dissected from the same anatomical region, i.e. between the last thoracic and second lumbar vertebra. The isolated blood

vessels were cut into rings, 3-5 mm wide, and immersed in organ baths, containing 25 ml of nutritive medium (Tyrode solution) of the following composition: NaCl 137, KCl 2.6, $CaCl_2$ 1.8, $MgCl_2$ 0.1, NaH_2PO_4 0.42, $NaHCO_3$ 11.9 and glucose 11.1 (in mM). The medium was aerated with a mixture of oxygen (95%) and carbon dioxide (5%), at a constant temperature of 37°C and pH 7.2.

The isometric tension of the isolated aorta was recorded by an isometric transducer and preamplifier (Ugo Basile 7080) on the Gemini 2-channel recorder (Ugo Basile 7070), with an initial preloading of the preparation of 2.5g.

For each experiment two segments of the same blood vessel were used. One of them was treated with nitrovasodilatators (glyceryl trinitrate or sodium azide) only, and the second segment with LPS from E.coli 055:B5 (1μg/ml organ bath) and nitrovasodilatators. The segments were first incubated in the organ bath for 4 hours, with or without LPS, and subsequently precontracted with noradrenaline ($1.53x10^{-7}$M) and relaxed with glyceryl trinitrate (from $4.21x10^{-11}$M to $1.63x10^{-7}$M) or sodium azide (from $7.68x10^{-11}$M to $5.96x10^{-8}$M). Every registration of those effects was repeated six times, i.e. 6 isolated segments of blood vessels taken from 6 animals. The following substances were used: LPS from E.coli 055:B5 (Difco Laboratories), glyceryl trinitrate (Nirmin^R - Zorka Šabac Farmacija), sodium azide (Fluka) and noradrenaline hydrochloride (Serva).

The obtained results are expressed as the mean (±s.e.m.) in percentages (%) of the greatest relaxant effect observed (100%). Statistical evaluation was done by the paired and unpaired Student's test for the dependent measurements and the Test of Parallelism for the probability levels 0.05 and 0.01.

RESULTS

Glyceryl trinitrate ($4.21x10^{-11}$M to $1.63x10^{-7}$M) and sodium azide ($7.68x10^{-11}$M to $5.96x10^{-8}$M) both produced maximum cumulative-concentration dependent relaxation of isolated bovine abdominal aorta, that had previously been incubated for 4 hours and precontracted with noradrenaline ($1.53x10^{-7}$M) by 63.63% (Figures 1. and 2.). Glyceryl trinitrate was a more potent relaxant than sodium azide, ($EC_{50}=1.531 \times 10^{-9}$M± 0.13 for GTN and $EC_{50}= 2.322 \times 10^{-9}$M ±0.02 for sodium azide).

After an incubation period of 4 hours, lipopolysaccharide from E. coli (1μg/ml), intensified, but not significantly, the relaxant effect of glyceryl trinitrate on the isolated bovine abdominal aorta, compared to the control relaxant effect of this organic nitrate. In the presence of LPS, the maximal relaxant effect of glyceryl trinitrate was obtained with the concentration of $1.63x10^{-8}$M, while the same relaxant effect was obtained with a 10 times higher glyceryl trinitrate concentration in the control experiment, i.e. with $1.63x10^{-7}$M. Consequently, the control cumulative-concentration dependent curve for glyceryl trinitrate and the curve for both glyceryl trinitrate and LPS crossed (Figures 3 and 4).

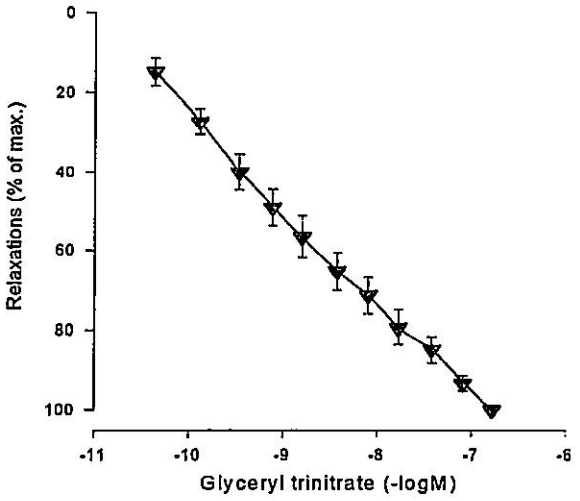

Figure 1. The dose-response curve indicating the relaxant effect of increasing concentrations of glyceryl trinitrate on the isolated bovine abdominal aorta, incubated for 4 hours in Tyrode solution and precontracted with noradrenaline ($1.53x10^{-7}$M) by 63.63%. The isometric relaxation increase of the maximal achievable relaxant effect is shown on the ordinate. Data are expressed as mean ± s.e.m. of 6 observations.

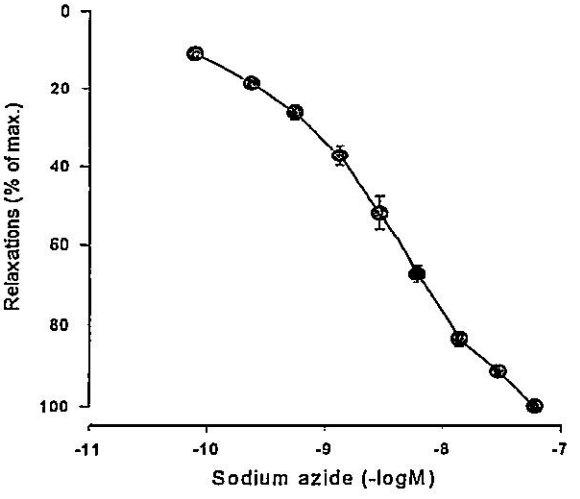

Figure 2. The dose-response curve indicating the relaxant effect of increasing concentrations of sodium-azide on the isolated bovine abdominal aorta, incubated for 4 hours in Tyrode solution and precontracted with noradrenaline ($1.53x10^{-7}$M) by 63.63%. The isometric relaxation increase of the maximal achievable relaxant effect is shown on the ordinate. Data are expressed as mean ±s.e.m. of 6 observations.

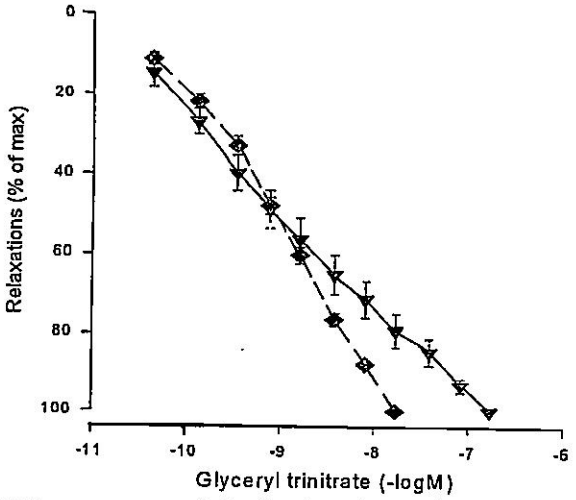

Figure 3. The dose-respones curves indicating the relaxant effect of increasing concentrations of glyceryl trinitrate on the isolated bovine abdominal aorta, to the presence of LPS. The line marked with inverted triangles (♦) is the dose-response curve showing the relaxant effect of increasing concentrations of glyceryl trinitrate in preparations incubated only in Tyrode solution for 4h. The line marked with rhombuses (♦), shows the dose-response relationship for glyceryl trinitrate in preparations incubated in the presence of LPS (1mg/ml) for 4h. Before every test with glyceryl trinitrate, the isolated bovine abdominal aorta was precontracted with noradrenaline ($1.53x10^{-7}$M) of 63.63%.

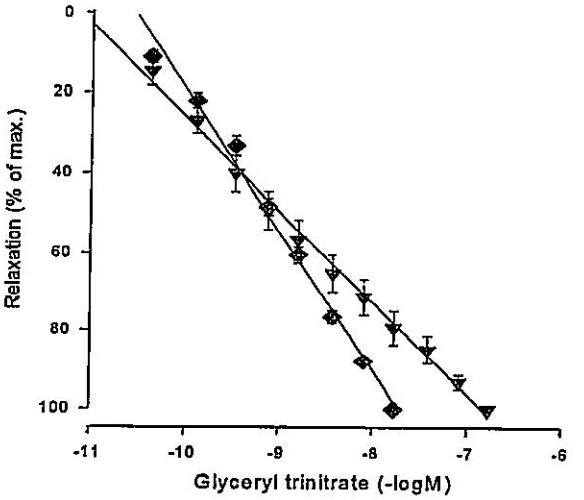

Figure 4. The regression lines showing the dose-response relationship for glyceryl trinitrate on the isolated bovine abdominal aorta incubated in the presence of LPS. The line marked with inverted triangles (♦ y=260.21+23.38x, r=0.998) shows the dose-response relationship for glyceryl trinitrate in preparations incubated for 4h only in Tyrode solution. The line marked with rhombuses (♦ y=375.62+35.64x, r=0.995), shows the dose-response relationship for glyceryl trinitrate in preparations which were incubated in LPS (1μg/ml) for 4h before addition of increasing concentrations of glyceryl trinitrate to the medium. The differences in relaxant effects of glyceryl trinitrate with and without LPS were not significant.

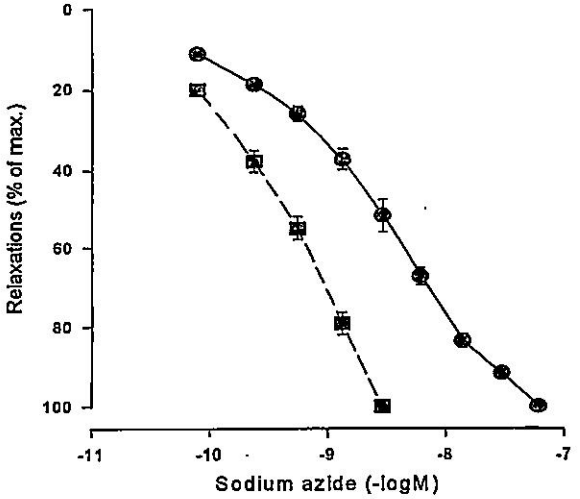

Figure 5. The dose-respones curves indicating the relaxant effect of increasing concentrations of sodium-azide on the isolated bovine abdominal aorta, to the presence of LPS. The line marked with circles (●) is the dose-response curve showing the relaxant effect of increasing concentrations of sodium azide in preparations incubated only in Tyrode solution for 4h. The line marked with squares (■), shows the dose-response relationship for sodium azide in preparations incubated in the presence of LPS (1μg/ml) for 4h. Before every test with sodium azide, the isolated bovine abdominal aorta was precontracted with noradrenaline ($1.53x10^{-7}$M) by 63.63%.

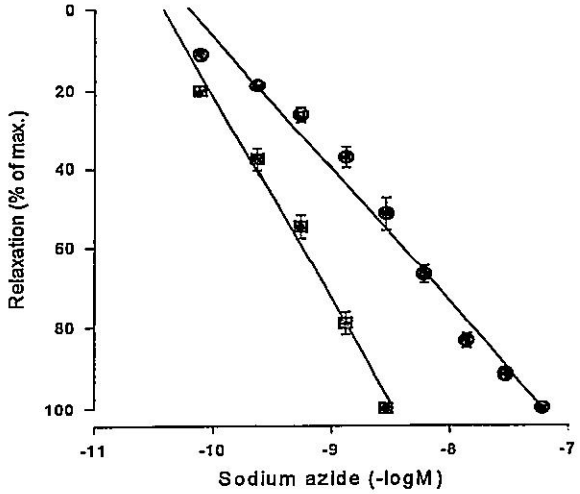

Figure 6. The regression lines showing the dose-response relationship for sodium azide on the isolated bovine abdominal aorta incubated in the presence of LPS. The line marked with circles (●y=341.64+33.5x, r=0.989) shows the dose-response relationship for sodum-azide in preparations incubated for 4h only in Tyrode solution. The line marked with squares (■) y=535.45+51.38x, r=0.993), shows the dose-response relationship for sodium azide in preparations which were incubated in LPS (1μg/ml) for 4h before addition of increasing concentrations of sodium azide to the medium. The significant difference was pronounced particularly in the region of higher concentrations of sodium azide.

In the presence of LPS, the relaxant effect of sodium azide on the isolated bovine abdominal aorta was more significantly intensified ($P<0.01$) than the control relaxant effect of this nitric compound. Therefore, the cumulative-concentration dependent curve for sodium azide in the presence of LPS was shifted to the left in relation to the control cumulative-concentration dependent curve for this nitric compound. The maximal relaxant effect of sodium azide alone, in the control experiment, was achieved with 5.96×10^{-8}M; whereas, identical relaxation of isolated bovine abdominal aorta in the presence of LPS was achieved at a 21 times lower sodium azide concentration (2.84×10^{-9}M) (Figures 5 and 6.).

DISCUSSION

Nitric compounds, such as organic nitrates and inorganic nitrites, are donors of nitric oxide and produce relaxation of vascular smooth muscles. Their relaxant effect on blood vessels relies, to a great extent, on their ability to metabolize to NO in vascular endothelial and smooth muscle cells. The released radical then activates guanylate cyclase and enhances the cGMP level, thus causing vasodilatation (Bennett et al., 1994; Oates, 1995; Kearney et al., 1998).

In our experiments GTN and sodium azide produced dose-dependent relaxation of bovine abdominal aorta precontracted with noradrenaline. In the presence of LPS this relaxant effect of GTN was significantly intensified; so that the maximal relaxant effect was achieved with a markedly lower concentration of this nitric compound than in the control experiment.

Similar results were obtained by Salvemini and others (1992). They showed that in rats, LPS intensifies GTN hypotensive responses and that methylene blue, as an inhibitor of soluble guanylate cyclase, blocks the intensified hypotensive effect of endotoxins. The same authors demonstrated, *in vitro*, in cultured bovine aortic endothelial and smooth muscle cells that GTN and LPS induce an increase in the production of cGMP, as its level was higher than in the culture of cells treated exclusively with glyceryl trinitrate.

In the experiments performed on isolated carotid, mesenteric, cranial and coronary artery of pigs, LPS also intensified the relaxant effect of GTN. The authors advanced the hypothesis that LPS brings about a rapid decrease in basal NO release from the endothelium, resulting in complete harnessing of the exogenous source of this free radical as well as in increased reactivity of vascular smooth muscles to glyceryl trinitrate (Arden et al., 1994).

Other authors obtained different results from similar experiments. Namely, they showed that LPS from E.coli reduces relaxation induced by sodium nitroprusside on rat isolated endothelium-denuded aorta (Tsuchida et al., 1994; Tsuchida et al., 1995). Hasegawa and others (1999) noticed, decreased reactivity of rat isolated endothelium-denuded aorta to GTN in the presence of LPS. The authors explained such findings by the hypothesis that excessive release of NO, induced by LPS, reduces the activity of guanylate cyclase, an enzyme that functions as a nitrovasodilatator receptor in vascular smooth muscles.

These findings are definitely supported by our results for an intact isolated blood vessel obtained with glyceryl trinitrate and sodium azide in the presence of LPS. It is evident that the reduction in the sodium nitroprusside relaxant effect, on the endothelium-denuded blood vessel, under the influence of endotoxins, is the result of NO formation in the endothelium, i.e. the triggering of its relaxant activity. Our results, as well as those of other authors (Salvemini et al., 1992; Arden et al., 1994) indicate that the enhancend relaxation of the isolated intact bovine abdominal aorta, caused by GTN and sodium azide in the presence of LPS, most probably is the result of NO synergistic activity, this NO being induced by the biotransformation of exogenously applied nitric compounds in vascular smooth muscles, as well as of that NO produced by activation of iNOS in endothelial and smooth muscle cells of the same blood vessel.

Not less interesting is the assumption that LPS reduces the physiological formation of NO from endothelial cells, this being compensated for by exogenous sources, such as nitric compounds (Arden et al., 1994). Moreover, it can be supposed that this heightened sodium azide relaxant effect, on the isolated bovine abdominal aorta, is the consequence of certain differences in the mechanism of relaxation. Namely Jezdimirovć and others (1991) showed that intact isolated bovine abdominal aorta is more intensely relaxed under the influence of sodium azide than abdominal aorta without the endothelium. However, the presence of the endothelium did not affect the reactivity of the blood vessel, when treated with glyceryl trinitrate.

The diametrically opposite differences in the relaxant effect of the nitric compounds on the isolated blood vessel, in the presence of endotoxins (LPS), existing between our findings and those of Tsuchida and others (1994, 1995) and Hasegawa and others (1999), could be probably explained by the significant role the endothelium plays in relaxation. Namely those authors conducted their experiments on endothelium-denuded blood vessels, while we used an intact isolated blood vessel in our experiments.

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UTICAJ NITRO-JEDINJENJA NA REAKTIVNOST IZOLOVANE ABDOMINALNE AORTE GOVEČETA U PRISUSTVU LIPOPOLISAHARIDA (LPS)

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Nitro-jedinjenja, kao što su gliceril trinitrat (GTN) i natrijum azid su poznati vazodilatatori. Mehanizam njihovog vazodilatatornog delovanja odvija se preko azotnog oksida (NO), koji se oslobađa u toku biotransformacije ovih jedinjenja u glatkim mišićima i endotelu krvnih sudova. Lipopolisaharidi (LPS) su endotoksini poreklom iz gram-negativnih bakterija koji u vaskularnom sistemu, prouzrokuju

teške funkcionalne poremećaje, tako što indukuju prekomernu sintezu i oslobađanje NO-a iz endotela i glatkih mišića krvnih sudova. Imajući u vidu da se delovanje nitrovazodilatatora, kao i LPS-a na krvne sudove, odigrava posredstvom azotnog oksida, želeli smo da ispitamo da li između njih postoji sinergističko vazodilatatorno dejstvo, odnosno da utvrdimo funkcionalnu povezanost u mehanizmu delovanja nitro-jedinjenja i LPS-a na abdominalnoj aorti govečeta.

Eksperimenti su izvedeni na izolovanoj abdominalnoj aorti govečeta, koja je inkubirana 4 časa u vodenom kupatilu za izolovane organe sa Tyrode-ovim rastvorom. Gliceril trinitrat i natrijum azid prouzrokuju dozno-zavisnu relaksaciju prethodno noradrenalinom kontrahovane izolovane abdominalne aorte govečeta. Gliceril trinitrat je potentniji i prouzrokuje jače relaksantno delovanje jer mu je $EC_{50}=1.531 \times 10^{-9}M \pm 0.13$ u odnosu na natrijum azid, čija je $EC_{50}=2.322 \times 10^{-9}M \pm 0.02$. U prisustvu LPS-a maksimalan relaksantni efekat GTN-a je potenciran u odnosu na kontrolni relaksantni efekat GTN-a, na izolovanoj abdominalnoj aorti govečeta. Relaksantni efekat natrijum azida u prisustvu LPS-a je izrazito potenciran, u odnosu na kontrolni relaksantni efekat ovog nitro-jedinjenja. Primenjeni lipopolisaharid je indukovao produkciju NO-a, koji zajedno sa NO-om poreklom iz nitro-jedinjenja (gliceril trinitrata i natrijum azida) prouzrokuje jaču maksimalnu relaksaciju izolovanog krvnog suda, odnosno pretretman sa LPS-om povećava biotransformaciju oba nitro-jedinjenja, ali više natrijum azida.