Transmural Field Stimulation-induced Relaxation in the Rat Common Hepatic Artery

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Abstract

Hepatic arteries are reportedly innervated by vasoconstrictor and vasodilator nerves. Experiments were carried out to investigate the possible involvement of calcitonin gene related peptide (CGRP) and nitric oxide as neurotransmitters during the relaxation of the rat common hepatic artery produced by transmural electrical field stimulation (ES). Common hepatic arteries were excised under ether-anesthesia from 6 weeks-old female rats, and isometric tensions recorded from endothelium-damaged ring preparations. In the presence of atropine and guanethidine, ES relaxed arteries which had been previously contracted with vasopressin. The relaxation response to ES was attenuated by either tetrodotoxin or capsaicin-pretreatment. CGRP induced a concentration-dependent relaxation, which was inhibited by the CGRP antagonist CGRP₈₋₃₇. The ES-induced relaxation was attenuated either slightly by the nitric oxide synthesis inhibitor L-nitroarginine (L-NNA) or markedly by CGRP₈₋₃₇. The relaxation response was nearly abolished in the presence of both CGRP₈₋₃₇ and L-NNA. These results may indicate that the nerve stimulation-induced vasodilatation of the rat common hepatic artery is mediated mainly by CGRP and partly by nitric oxide.

Key words: rat common hepatic artery, vasodilator nerve, capsaicin, CGRP, nitric oxide

Introduction

Since the discovery of endothelium-derived relaxing factor (EDRF) by Furchgot and Zawadzki (1980), the importance of the endothelium in vasodilatation has been documented for many arteries (Ignarro, 1989), including the rat hepatic artery (Zygmunt *et al.*, 1994). In

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addition to endothelial regulation, hepatic circulation changes greatly in response to metabolic requirements of the body, under the control of both vasoconstrictor and vasodilator nerves. The importance of the sympathetic nervous system in the control of hepatic arterial blood flow through α -adrenergic receptor-mediated vasoconstriction has been well documented (see Lauth, 1980). In regard to the vasodilator regulation, recent studies on dog hepatic arteries reveal an involvement of β -adrenergic (Shiraishi *et al.*, 1997) and calcitonin gene-related peptide (CGRP)-mediated mechanisms (Shiraishi *et al.*, 1998). Electrical stimulation (ES) of the rat hepatic artery is shown to induce a tetrodotoxin-sensitive relaxation which is resistant to guanethidine treatment (Braitveit and Helle, 1991; Braitveit *et al.*, 1991). In the rat mesenteric artery, the vasodilatation produced by ES is mediated by CGRP (Kawasaki *et al.*, 1988). Histochemical examination shows that arteries in the rat hepatic region are rich in CGRP-containing nerves (Sasaki *et al.*, 1986). Nitric oxide (NO) has a strong vasodilator activity (Ignarro, 1989) and a rich distribution of the enzyme NO synthase has been demonstrated in the rat dorsal root ganglion (Aimi *et al.*, 1991).

Our previous work suggests that CGRP is involved in the nerve-stimulation induced relaxation of the rat common hepatic artery (Shimamura *et al.*, 1999). Recently, a possible involvement of CGRP in vasodilator responses of the rat common hepatic artery has been suggested from evidence that the acetylcholine-induced relaxation is sensitive to CGRP₈₋₃₇ (Hogestatt *et al.*, 2000). However, there is no direct evidence that CGRP is indeed involved in the ES-induced relaxation in this artery. In the present study, we have clarified the contribution of CGRP and NO in the rat common hepatic arterial neural vasodilatation using an antagonist of the CGRP receptor and an inhibitor of NO synthesis.

Materials and methods

Six weeks old female Wistar rats were anesthetized with diethyl ether and exsanguinated. All animals were treated ethically according to the rules of the Animal Use and Care Committee settled in The Kinki University Medical School. The common hepatic artery (outer diameter 0.4 mm) was dissected and placed in Tyrode's solution at room temperature. The lumen of the artery was perfused with 0.75% deoxycholate in distilled water or rubbed by a thin tungsten wire to damage the endothelium. A 1.5 mm long ring segment of the artery was prepared from each rat. This preparation was suspended horizontally by two tungsten wires (diameter 50 μ m) in a chamber containing 5 ml Tyrode's solution at 37°C. Isometric tension was measured using a transducer (Minebea, Load cell 10 GM, Karuizawa Japan), an amplifier (NEC-San-ei, Biophysigraph, Tokyo Japan) and recorded on a thermal pen recorder (NEC-San-ei, RECTI-HORI). The composition of the Tyrode's solution was (in mM): NaCl 137, KCl 5.4, NaHCO₃ 11.9, NaH₂PO₄ 0.4, CaCl₂ 2.0, MgCl₂ 1.0 and glucose 5.6, and the solution was aerated with 95% O₂ and 5% CO₂ (pH of the solution was 7.3 at 37°C). After a 2 hr incubation in Tyrode's solution under minimum passive tension, a passive tension of 2 mN was applied. When stable basal tension was achieved, arteries were allowed to produce tonic contraction in the presence of arginine vasopressin 10 nM (this allowed submaximal stable tonic contraction in the presence of guanethidine 10 μ M and atropine 1 μ M). A pair of platinum electrodes was

set close to the preparation and rectangular pulses of 0.5 msec duration applied for 6 sec at selected frequencies (1 Hz, 2 Hz, 5 Hz, 10 Hz, 20 Hz). At the end of experiments, the preparation was allowed to relax using solutions of papaverine 100 μ M and verapamil 10 μ M in Tyrode's solution. The amplitude of vasopressin-induced contraction was determined by subtracting this relaxed level. Peak amplitude of the relaxation produced by ES was expressed as a % of the vasopressin-induced contraction.

In seven urethane-chloralose (500 and 50 mg/kg i.p.) anesthetized rats of four weeks old of age, the left cervical vagus nerves were cut and the Th₈₋₁₀ dorsal root ganglia removed bilaterally after laminectomy. Bilateral vagotomy was not available because of the high incidence of death following surgery. The left cervical vagus nerve was cut preferentially because the hepatic vagal branch projected predominantly to the left side of the medulla (Adachi, 1981). Two weeks after surgery, the common hepatic artery was isolated and ES-induced responses examined.

Drugs used were: arginine vasopressin, tetrodotoxin, α -calcitonin gene-related peptide (CGRP), α -CGRP₈₋₃₇, capsaicin, acetylcholine chloride (all from Sigma, St. Louis, Mo, USA). Capsaicin was dissolved in ethyl alcohol at 10⁻² M. The final concentration of ethyl alcohol in the Tyrode's solution was 0.1%, and this concentration of alcohol did not affect contraction responses.

Data obtained were presented as mean $\pm$ S.E.M., with the number of animals used in parenthesis. Statistical analysis was performed by using the Student's *t*-test and a two-way ANOVA (Prizm 3.0, GraphPad, San Diego, USA). The P values of less than 0.05 were considered to be significant.

Results

When ES with a frequency of higher than 5 Hz was applied in the absence of any drug, transient (shorter than 30 sec) phasic contractions were elicited in the common hepatic artery. The amplitudes of contraction increased in a frequency-dependent manner, and with 20 Hz ES the amplitude of contraction reached $8.7 \pm 3.0\%$ ($n=7$) of the vasopressin-induced contraction. The ES-induced contraction was abolished by tetrodotoxin 1 μ M or prazosin 1 μ M.

Vasopressin 10 nM induced a contraction in the common hepatic artery with a sustained amplitude for up to 2 hrs. When ES was applied during the tonic contraction, a transient relaxation was elicited (Fig. 1). The amplitude and duration of the ES-induced relaxation increased as the stimulation frequency was increased. The relaxation was inhibited by tetrodotoxin 1 μ M. Application of capsaicin 10 μ M also produced a transient 10–15 min relaxation in arteries contracted with vasopressin. This transient relaxation was followed by a restoration of tension comparable to that observed before application of capsaicin. However, no ES-induced relaxation was elicited in arteries in the presence of capsaicin (Fig. 1).

In arteries contracted with 10 nM vasopressin, cumulative application of CGRP (0.1 to 10 nM) produced relaxation in a concentration-dependent manner. The relaxation induced by CGRP was abolished in the presence of CGRP₈₋₃₇ 1 μ M (Fig. 2), but was not significantly altered in the presence of L-nitroarginine (L-NNA) 100 μ M (data not shown).

Fig. 1. Electrical field stimulation (ES)-induced relaxation in an endothelium-damaged ring preparation of the rat common hepatic artery which was contracted by vasopressin 10 nM. The relaxation was inhibited by tetrodotoxine (TTX) 1 μ M. Capsaicin 10 μ M induced a similarly transient but longer relaxation than ES. After capsaicin treatment, ES at 10 Hz failed to induce relaxation.

Fig. 2. Calcitonine gene-related peptide (CGRP)-induced relaxation of the vasopressin-induced contraction and the effect of CGRP antagonist CGRP₈₋₃₇ on this relaxation. The CGRP inhibition of the vasopressin-induced contraction was concentration dependent. CGRP₈₋₃₇ 1 μ M inhibited this relaxation, while L-nitroarginine (NNA) 100 μ M had no effect on the relaxation. Five to nine experiments were performed for each data point.

The effects of CGRP₈₋₃₇ 1 μ M on the ES-induced relaxation were examined in common hepatic arteries contracted with vasopressin. In the presence of CGRP₈₋₃₇ 1 μ M, the amplitude of ES-induced relaxation was attenuated markedly at all frequencies tested (1–20 Hz) (Fig. 3). The effects of L-NNA 100 μ M on the ES-induced relaxation were also examined in vasopressin-contracted common hepatic arteries. The relaxation was again attenuated by L-NNA, but the attenuation was less significant compared to that seen in the presence of CGRP₈₋₃₇ (Fig. 4).

Attempts were made to observe the effects of CGRP₈₋₃₇ 1 μ M on the ES-induced relaxation

Fig. 3. Effect of CGRP₈₋₃₇ on the ES-induced relaxation of the vasopressin-induced contraction. The amplitude of relaxation was dependent on the ES frequency and the relaxation was inhibited by CGRP₈₋₃₇. Five to twelve experiments were performed for each data point.

Fig. 4. Effect of L-nitroarginine (NNA) on the ES-induced relaxation of the vasopressin-induced contraction. The amplitude of relaxation was dependent on the ES frequency and the relaxation was inhibited by NNA. Six to thirteen experiments were performed for each data point. Control data as for Figure 3.

Fig. 5. Effect of CGRP₈₋₃₇ on the ES-induced relaxation of the vasopressin-induced contraction in the presence of L-NNA. The amplitude of relaxation was inhibited by CGRP₈₋₃₇.

in the common hepatic arteries pre-treated with L-NNA 100 μ M. The relaxation evoked in the presence of L-NNA 100 μ M was markedly attenuated by additional application of CGRP₈₋₃₇ 1 μ M (Fig. 5). The amplitude of the ES-induced relaxation elicited in the presence of both L-NNA and CGRP₈₋₃₇ was $4.0 \pm 1.6\%$ ($n=5$), $10.2 \pm 0.8\%$ ($n=11$), $10.4 \pm 3.1\%$ ($n=5$) and $16.4 \pm 3.2\%$ ($n=5$) of the vasopressin-induced contraction at 2 Hz, 5 Hz, 10 Hz and 20 Hz, respectively.

In preparations from common hepatic arteries isolated from rats that had been operated to cut the left vagal nerve at the cervical level and to bilaterally remove the Th₈₋₁₀ dorsal root ganglia, similar ES experiments were performed to determine whether there was an altered relaxation response. The amplitude of relaxation was $59 \pm 5.8\%$ ($n=4$) of the contraction induced by vasopressin 10 nM, which was slightly smaller but not significantly different to that observed in intact rats ($69.5 \pm 8.8\%$, $n=11$).

Discussion

Vasoconstrictor innervation of rat mesenteric artery was reported to be mediated by α_{1A} , P_{2x} and NPY₁ receptors (Phillips *et al.*, 1998). In the present study, ES induced transient contraction of the common hepatic artery was abolished by prazosin, indicating that the contraction was mediated mainly through activation of α_1 adrenergic receptors.

Capsaicin has been used to release CGRP from peripheral nerves (Wharton *et al.*, 1986; Holzer, 1988), through vallinoid receptor (Lundberg, 1996). These capsaicin-sensitive nerve fibers are mostly involved in sensory pathways. Excitation of capsaicin-sensitive fibers induces hepatic arterial dilatation in the rat (Bradveit and Helle, 1991). CGRP has been shown to induce relaxation of the hepatic artery in an endothelium-independent manner in the rat (Zygmunt *et al.*, 1994, 1995). It has also been demonstrated that acetylcholine-induced relaxation of the hepatic artery is abolished by CGRP₈₋₃₇ (Hogstatt *et al.*, 2000), an antagonist of CGRP-1 receptors (Bell and McDermott 1996). Similar observations have also been reported in the rat mesenteric artery (Takenaga *et al.*, 1995). While these results clearly show the physiological importance of CGRP in rat hepatic arterial dilatation, no direct evidence has been reported to suggest the involvement of CGRP in the ES-induced relaxation of the rat hepatic artery. In the present study of the rat common hepatic artery, we have examined that capsaicin induces a relaxation and that following this, ES induced-relaxation is abolished. Our results which demonstrate the inhibition of the ES-induced relaxation by CGRP₈₋₃₇ indicate that the ES-induced relaxation is indeed produced by CGRP which is released in response to nerve excitation. These results are compatible with those of the previous studies.

The CGRP-induced relaxation of the rat common hepatic artery observed in the present experiments is not due to endothelium-derived relaxing factors, since endothelium-damaged arteries have been used. Similar observations of an endothelium-independent relaxation caused by CGRP have also been reported in the rabbit hepatic artery (Brizzolara and Burnstock, 1991).

It has been shown that the liver receives projections bilaterally from vagus nerves and the Th₇₋₁₀ sympathetic nerves (Lautt, 1996). Our results do not support the previous study by Sasaki *et al.* (1986) which suggests that CGRP neurons in hepatic blood vessels are derived from both vagus and splanchnic nerves bilaterally. This discrepancy may be partly due to the persistence of the right vagus and the remaining dorsal ganglia. However, the pathways by which CGRP neurons reach the common hepatic arteries need to be re-examined.

In the reversely perfused rat liver, an involvement of NO in flow regulation has been shown (Mittal *et al.*, 1994; Zhang *et al.*, 1997). Also in portal hypertensive rats, a possible involve-

ment of NO in a nerve-mediated relaxation is reported (Moll-Kaufmann *et al.*, 1998). Furthermore, NO has been shown to mediate bradykinin-induced activation of sensory nerves (Yu *et al.*, 1993). However, the present study has shown that the inhibition of NO synthesis induced only a slight decrease in the amplitude of ES-induced relaxation. Thus, the contribution of NO to the ES-induced relaxation is not a major feature in the common hepatic artery, although some traces of NO component are detectable.

In conclusion, we have confirmed that in the rat common hepatic artery, CGRP is indeed the main transmitter of the vasodilator sensory nerves. NO may also contribute to this relaxation, but to a limited extent.

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