A Novel Neurogenic Vasodilator Mechanism in Bovine Mesenteric Artery

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The presence of a neurogenic vasodilator mechanism was investigated in isolated bovine mesenteric arteries (BMAs) that were precontracted with phenylephrine. Electrical field stimulation induced tetrodotoxin-sensitive relaxations in guanethidine-pretreated BMAs. The relaxation occurred after a delay of about 5–8 seconds and amounted to 25–35% in different sets of experiments. The relaxation was not affected by classical receptor antagonists such as atropine (1 μM), cimetidine (3.9 μM), clemastine (2.8 μM), naloxone (1.2 μM), 8-phenyltheophylline (1 μM), propranolol (3.4 μM), ritanserin (5 μM), or droperidol (13 μM).

The nicotinic acetylcholine-receptor stimulant 1,1-dimethyl-4-phenyl-piperazinium iodide (10 μM) was without effect on the relaxation, and removal of the endothelium of the arteries also had no effect. The bee venom component apamin (1 μM), which has been shown to block the nonadrenergic, noncholinergic relaxation in intestinal and vascular smooth muscle from other species, was also found to be without effect on the relaxation induced by electrical field stimulation in BMAs. Pretreatment of the arteries with capsaicin (1 μM) had no effect per se and did not affect the relaxation induced by a subsequent stimulation. Capsaicin has been suggested to release neurotransmitter and eventually deplete neurons containing substance P and calcitonin gene-related peptide. Furthermore, exogenously applied calcitonin gene-related peptide (1–100 nM), substance P (10 nM–1 μM), and vasoactive intestinal peptide (0.3–30 nM) gave relaxations amounting to less than 10%. It is postulated that electrical field stimulation induces a neurogenic relaxation of a nonadrenergic, noncholinergic nature in BMAs. The relaxation is not dependent on an intact endothelium and seems not to be mediated by any of the known vasodilatory neuropeptides.

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Neuronal control of blood vessel tonus is well established and seems to be widespread within the cardiovascular system of most species. Neuronal control of vascular smooth muscle mediated by adrenergic nerves is well known, although evidence for the existence of a cholinergic innervation in certain vascular regions has been presented. During recent years several peptides have been suggested to play an important role in neurogenic vasodilation in different vascular regions. These peptides include vasoactive intestinal polypeptide (VIP) in various arteries, calcitonin gene-related peptide (CGRP) in peripheral and pial arteries in man, guinea pig, rat, and cat.


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electrodes to allow electrical field stimulation of the tissue. The isometric tension of the circular muscle layer was recorded with an isometric strain gauge transducer (model FT.03, Grass Instruments, Quincy, Massachusetts) and a Grass polygraph. To avoid release of noradrenaline during field stimulation, the arteries were pretreated for 10 minutes with 5 μM guanethidine; then 3.4 μM propranolol and 2.5 μM phenylephrine were added. The contraction was allowed to stabilize, and the arteries were stimulated with trains (70-second duration) of biphasic rectangular pulses (1 msec, 10 Hz, 100 V nominal output; representing supramaximal stimulation) with a Grass SD9 stimulator. In some preparations the endothelium was removed by gentle rubbing of the arteries with a scalpel. This procedure caused complete inhibition of acetylcholine-induced relaxation in BMAs precontracted with phenylephrine; this occurrence indicated successful removal of the endothelium.

Statistical Methods

Values are presented as mean±SEM. The level of statistical significance has been tested with Student’s t test using unpaired observations.

Drugs

Guanethidine was a gift from CIBA-GEIGY, Västra Frölunda, Sweden, and ritanserin was a gift from Janssen Pharmaceutica, Beerse, Belgium. Droperidol was from Janssen Pharmaceutica. Phenylephrine, atropine, acetylcholine, capsaicin, tetrodotoxin (TTX), scorpion toxin (from Androctonus australis) and 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) were from Sigma, St. Louis, Missouri. 8-phenyltheophylline was purchased from Calbiochem, San Diego, California. VIP was from Serva, Heidelberg, FRG, and CGRP I (human) was bought from Novabiochem, Läufelfingen, Switzerland. All peptides were dissolved in water and stored in aliquots in polyethylene vials at -80°C.

Results

Electrical field stimulation of guanethidine-treated and phenylephrine-contracted BMAs induced a relaxation after a delay of about 5–8 seconds. Guanethidine had to be used to deplete the arteries of noradrenaline, which otherwise was released during electrical field stimulation and induced a large contraction that masked the relaxatory responses. The relaxation initially developed rapidly, whereupon the rate of relaxation decreased. On cessation of stimulation, there was a slow regain in tension although in most cases the tension did not reach the prestimulation level (Figure 1). Repeated stimulations resulted in reproducible relaxations, and tachyphylaxis could not be induced even when the electrical stimulations were extended over 30 minutes (data not shown). TTX (3 μM) was tested on eight different arterial preparations and was found to completely block the relaxatory responses in all instances, which indicates that the relaxation induced by electrical field stimulation is of nervous origin (Figure 2). This assumption is further substantiated by the finding that scorpion toxin (from Androctonus australis) also induced relaxations in precontracted BMAs (Figure 3). This toxin belongs to the α-scorpion toxins, which are suggested to prevent inactivation of fast Na⁺ channels and thus maintain a depolarized state in the nerve. TTX (3 μM), which is known to prevent activation of fast Na⁺ channels, completely prevented the relaxation induced by scorpion toxin (data not shown).
To further characterize the relaxant response induced by field stimulation the dependency of pulse duration, field strength, and frequency was determined. The threshold level for induction of relaxation with regard to pulse duration was 0.4 msec. With pulse durations longer than 4–8 msec, a contraction was seen that lasted during the entire stimulation period. This contraction was not sensitive to TTX (2 μM), and on cessation of stimulation, a rebound relaxation was elicited. The relaxation was dependent on stimulation strength, with maximal relaxation occurring at a nominal output of 60–80 V. The relaxant response also showed a frequency-dependent pattern in the range 2–16 Hz. Higher frequencies did not further increase the relaxation.

To verify that guanethidine pretreatment did not have any adverse effects on the arterial tissue, similar experiments were performed on BMAs that had been contracted with histamine (5 μM) in the presence of the α-adrenoceptor antagonist phentolamine (5 μM), but without prior treatment with guanethidine. Electrical field stimulation of these vessels gave relaxations with the same characteristics as in the phenylephrine-contracted preparations.

The nicotinic acetylcholine-receptor stimulant DMPP did not induce any effect itself and also did not affect the relaxant response to a subsequent stimulation (Figure 3).

Removal of the endothelium did not affect the response to electrical field stimulation significantly; this finding indicated that the relaxation was not mediated through liberation of an endothelium-derived relaxing factor. The relaxatory response amounted to 28.9±2.6% (n=7) and 35.0±5.7% (n=8) (p=0.360) in the absence and presence of an intact endothelium, respectively (Figure 4).

Several "classical" receptor antagonists were used to further analyze the relaxatory response in BMAs. The muscarinic acetylcholine-receptor antagonist atropine (1 μM) had no significant effect on the relaxant response (Figure 4). The histamine (H₂)-receptor antagonist cimetidine (3.9 μM), the histamine (H₂)-receptor antagonist clemastine (2.8 μM), the morphine antagonist naloxone (1.2 μM), the purinergic (P₁)-receptor antagonist 8-phenyltheophylline (1 μM), the serotonin (5-HT₂)-receptor antagonist ritanserin (5 μM), and the dopamine receptor antagonist droperidol (13 μM) were found to be completely without effect on the relaxant response in the concentrations used (data not shown). Furthermore, exogenously applied dopamine in the concentration range 10 nM–1 mM had no relaxatory effect; on the contrary, a concentration-dependent increase in tension was seen at concentrations exceeding 10 μM (data not shown).

**Figure 3.** Top: Representative original recording showing effect of scorpion toxin (Androctonus australis) on tonus in bovine mesenteric artery precontracted with phenylephrine (PHE). Bottom: Representative original recording showing effect of 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) on relaxation induced by electrical field stimulation in bovine mesenteric artery precontracted with PHE. Horizontal bar indicates electrical field stimulation.

**Figure 4.** Bar graph showing effect of removal of endothelium or atropine pretreatment on the relaxation of bovine mesenteric artery induced by electrical field stimulation. Mean±SEM (n=5–8).
Treatment of the vessels with capsaicin (1 μM) did not affect the relaxatory response (Figure 5). Capsaicin has been shown to cause a very rapid depletion of CGRP-containing neurons and substance P-containing neurons in vitro. Furthermore, exogenously added CGRP showed very low relaxing potency in phenylephrine-contracted BMAs (Figure 6). Similarly, substance P and VIP, which have been shown to possess relaxing properties in vascular tissue from other species, were also nearly without relaxing effect on precontracted BMAs (Figure 6). The relaxation was also not affected by pretreatment with 1 μM apamin (Figure 5). The relaxation amounted to 20.7±4.2% and 26.4±7.2% (n=4) in control and apamin-treated arteries, respectively. Apamin has been shown to inhibit the non-adrenergic, non-cholinergic inhibitory system in guinea pig taenia coli and vascular smooth muscle from cat intestine.

Discussion

The present study shows the existence of a vasodilator system in BMAs, which can be activated by electrical field stimulation. This response seems to be mediated through activation of "fast" Na+ channels (i.e., neuronal type), since the response was totally blocked by TTX and since the relaxatory response could be mimicked by the α-scorpion toxin from Androctonus australis. This relaxation could be antagonized by TTX, a finding that supports a neurogenic action of the scorpion toxin. The inhibitory effect of TTX on the relaxation in BMAs is in marked contrast to the relaxatory response elicited in isolated canine coronary arteries by electrical stimulation, which was found to be unaffected by procedures known to block nerve conduction, such as TTX treatment or cold storage.

The response evoked in BMAs by electrical field stimulation was clearly of a nonadrenergic, non-cholinergic type, since guanethidine pretreatment, DMPP, and atropine did not have any effect on the relaxations. The lack of effect of DMPP also seems to speak against the existence of a ganglion situated in the vascular tissue. Furthermore, the relaxations seemed to be independent of an intact endothelium and could not be mimicked by exogenous application of VIP. These features clearly distinguish the relaxatory response in BMAs from the neurogenic nonadrenergic, noncholinergic vasodilation seen in cephalic vessels. The response elicited in these vessels during electrical field stimulation has been described as muscarinic and partially endothelium dependent in feline posterior auricular arteries or as an atropine-resistant relaxation, probably mediated by VIP, in cat middle cerebral arteries. The lack of effect of exogenously applied VIP also extends to substance P and CGRP. Substance P has been described as an endothelium-dependent vasodilator, whereas the vasorelaxant effect of CGRP seems to be dependent on an intact endothelium in some arteries but not in others. Furthermore, capsai-
cin, which has been suggested to cause release of neuronal CGRP and substance P, did not induce any effect by itself and did not block the relaxant effect of a subsequent stimulation. This is in marked contrast to the effect of capsaicin in pig coronary arteries and in perfused rat mesenteric bed. Previous studies in other vascular regions have shown that there is a correlation between peptidergic innervation and responsiveness to exogenously applied neuropeptides. These findings could suggest that the low effect of exogenously added VIP, CGRP, and substance P as found in the present study also indicates a very sparse innervation with nerves containing these peptides.

Since nonbovine neuropeptides were used in the present study, it could be argued that the very low potency of the exogenously added peptides is explained by species incompatibility. However, this seems unlikely since considerable interspecies activity of neuropeptides has been found in other studies. The BMAs used in the present study represent large arteries; their use could explain the very low potency of CGRP since this peptide has previously been shown to preferentially dilate small resistance arterioles. Evidence has previously been presented indicating that dopamine could act as a peripheral vasodilator released during nerve stimulation. However, dopamine seems unlikely to be of importance for the neurogenic vasodilation described in the present paper since exogenously applied dopamine did not induce relaxation in precontracted BMAs and the dopamine receptor antagonist droperidol did not inhibit the relaxation elicited by electrical field stimulation. The relaxatory response elicited by electrical field stimulation in BMAs shows marked similarity with the nonadrenergic, noncholinergic inhibitory system in guinea pig trachea. The shape of the relaxatory response is very similar, and both are insensitive to apamin treatment, which has been shown to block the nonadrenergic, noncholinergic relaxations in guinea pig taenia coli and in vascular smooth muscle of the intestine of the cat.

As is the case with the nonadrenergic, noncholinergic system in guinea pig trachea, the identity of the transmitter responsible for the relaxatory response in BMAs remains elusive, and further studies are obviously required to establish the nature of the mechanisms involved.

References


**KEY WORDS**

- bovine mesenteric artery
- neurogenic vasodilation
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