Abstract—Stimulation of perivascular nerve terminals leads to a release of various neurotransmitters such as norepinephrine, epinephrine, acetylcholine, nitric oxide, and calcitonin gene-related peptide (CGRP). Because some of these substances have been shown to cause smooth muscle hyperpolarization by direct or endothelium-dependent mechanisms, we hypothesized that the liberation of 1 or more of these transmitters may lead to neurogenic hyperpolarization in arterial muscle cells. The present study was designed to determine the presence or absence of neurogenic hyperpolarization and, if present, its underlying mechanisms in isolated rat mesenteric resistance arteries, through the use of conventional microelectrode techniques. The experiments were performed under the combined blockade of α-adrenoceptors and purinoceptors with phentolamine and suramin to eliminate depolarizing responses to nerve stimulation. Under these conditions, perivascular nerve stimulation (5 Hz, 30 seconds) evoked smooth muscle hyperpolarization (−3.3±0.3 mV, n=15), which was abolished by tetrodotoxin, indicating the neurogenic origin of the response. This neurogenic hyperpolarization was resistant to atropine, nitro-L-arginine, or CGRP8-37, a CGRP antagonist, but was abolished by guanethidine and β-blocker propranolol. This hyperpolarization was also abolished by glibenclamide, an ATP-sensitive K⁺ channel (KATP) blocker, but was unaffected by apamin, a Ca²⁺-activated K⁺ channel blocker. In separate experiments, exogenous norepinephrine caused glibenclamide-sensitive hyperpolarization in the presence of phentolamine. On the other hand, norepinephrine-induced depolarization in the absence of phentolamine was enhanced by propranolol. These findings suggest that neurally released catecholamines cause membrane hyperpolarization through the activation of KATP by β-adrenoceptors. Such hyperpolarization may play an important role in the control of arterial membrane potential by opposing α-adrenergic depolarization. (Hypertension. 2000;35[part 2]:379-384.)

Key Words: receptors, adrenergic, beta nervous system, sympathetic potassium membranes hyperpolarization muscle, smooth, vascular rats

Vascular tone is under the control of sympathetic and parasympathetic nerves as well as nonadrenergic, noncholinergic nerves, including sensory afferent nerves that release peptides peripherally. Stimulation of these nerve terminals may lead to a release of various neurotransmitters such as norepinephrine, epinephrine, adenosine 5’-triphosphate (ATP), acetylcholine (ACh), nitric oxide (NO), vasoactive intestinal peptide, and calcitonin gene-related peptide (CGRP). Some of these substances have been shown to cause smooth muscle hyperpolarization by direct or indirect mechanisms when applied exogenously: For example, norepinephrine and CGRP may act directly on smooth muscle cells to elicit hyperpolarization. ACh may produce hyperpolarization either directly or indirectly through endothelium-derived hyperpolarizing factor.

In accord with these observations, neurogenic hyperpolarization has been demonstrated in certain vascular beds and the evaluation of such responses in resistance arteries has been hampered by depolarizing responses and contractions during repetitive nerve stimulation, which result in difficulty in maintaining microelectrode impalements. Furthermore, little is known regarding the ionic mechanisms of neurogenic hyperpolarization.

The mesenteric circulation is one of the largest vascular beds, and its vascular conductance is thought to be a major determinant of total peripheral resistance. The mesenteric artery is densely innervated by adrenergic and cholinergic nerves as well as sensory nerves containing peptides such as CGRP. Perivascular nerve stimulation in this vascular bed usually evokes excitatory junction potentials (EJPs) and slow depolarization, but such responses could be blocked by a combined application of the P2X-purinoceptor antagonist suramin and α-blocker phenolamine, enabling us to study neurogenic hyperpolarization in resistance arteries. The present study was designed to determine the presence or absence of neurogenic hyperpolarization in isolated rat
30 to 70 were stimulated by passing brief square stimulating pulses (duration To record neurogenic hyperpolarization, the perivascular nerves and a sharp return to zero potential on withdrawal of the electrode. smooth muscle cell; a stable membrane potential for voltage on impalement of the microelectrode into the vascular successful impalement included the following: an abrupt drop in (silver–silver chloride). An electrical stimulator (SEN-3201, Nihon recorder (RJG-4002, Nihon Koden). oscilloscope (VC-11, Nihon Koden), and recorded with a pen signals were amplified (MEZ-7200, Nihon Koden), monitored on an 36°C) bubbled with 95% O2 -5% CO2 (pH 7.3 to 7.4) at a rate of 3 Krebs solution of the following composition (in mmol/L): Na 137.4, K 5.9, Mg 2+ 1.2, Ca 2+ 2.5, HCO3 - 15.5, H2PO4 - 1.2, Cl - 134, and glucose 11.5. Recording of Membrane Potentials and Neurogenic Hyperpolarization The arteries were placed in an experimental chamber (capacity 2 mL). Tissues were carefully pinned to a rubber bed fixed at the bottom of the chamber and were superfused with Krebs solution (36°C) bubbled with 95% O2-5% CO2 (pH 7.3 to 7.4) at a rate of 3 mL/min. After an equilibration of at least 60 minutes, the membrane potentials of vascular smooth muscle cells were recorded with the use of conventional glass capillary microelectrodes filled with 3 mol/L KCl, with tip resistances of 50 to 80 MΩ. Criteria for successful impalement included the following: an abrupt drop in voltage on impalement of the microelectrode into the vascular smooth muscle cell; a stable membrane potential for ≥2 minutes; and a sharp return to zero potential on withdrawal of the electrode. To record neurogenic hyperpolarization, the perivascular nerves were stimulated by passing brief square stimulating pulses (duration 30 to 70 μs, intensity 30 to 60 V) through a suction electrode (silver–silver chloride). An electrical stimulator (SEN-3201, Nihon Koden) was used to supply a train of pulses. Signals were amplified through an amplifier (MEZ-7200, Nihon Koden), monitored on an oscilloscope (VC-11, Nihon Koden), and recorded with a pen recorder (RJG-4002, Nihon Koden).

**Methods**

**Preparation of Arteries**

Five- to 8-week-old male Wistar rats (Charles River, Atsugi, Japan) were used in the present study. The rats had free access to tap water and were fed a normal rat chow. The study protocol was approved by the Animal Experimentation Ethics Committee of Kyushu University. Rats were anesthetized with ether and exsanguinated. The third or fourth branch of the mesenteric artery was excised and bathed in cold Krebs solution to give the final chamber concentrations. High K+ solution was prepared by equimolar substitution of Na+ by K+ or vice versa.

**Statistical Analysis**

Data are expressed as mean±SEM; n refers to the number of animals examined. Statistical significance was determined with a paired Student’s t test. A level of P<0.05 was considered statistically significant.

**Results**

The resting membrane potential of the rat mesenteric resistance arteries was −68.1±0.8 mV (n=23). After confirming the presence of EJPs, tissues were incubated for >60 minutes with phenolamine (3×10−6 mol/L), an α-adrenoceptor antagonist, and suramin (10−4 mol/L), a selective P2-purinoceptor antagonist, which abolished EJPs and depolarizing responses to nerve stimulation. Application of these drugs did not alter the membrane potential (−68.1±0.8 mV and −67.8±0.9 mV in the absence and presence of 3×10−6 mol/L phenolamine together with 10−4 mol/L suramin; n=23; P=NS). Under these conditions, stimulation of perivascular nerves elicited membrane hyperpolarization.

Figure 1 shows actual recordings of the electrical responses of smooth muscle cells to perivascular nerve stimulation (5 or 10 Hz, 30 seconds) as well as the effects of various blockers on these responses. Glibenclamide (10−6 mol/L) and ampin
(10⁻⁶ mol/L) significantly depolarized the membrane by 2.3±0.6 mV (n=8) and 1.8±0.5 mV (n=4), respectively. Various other blockers used did not alter the membrane potential. The average amplitudes of hyperpolarization elicited by 10-second trains of stimuli delivered at 5 Hz and 30-second trains of stimuli delivered at 5 and 10 Hz were −1.5±0.2 mV (n=14), −3.3±0.3 mV (n=15), and −3.8±0.5 mV (n=15), respectively. In the following experiments, perivascular nerves were stimulated by 30-second trains of stimuli at 5 or 10 Hz.

These hyperpolarizations were abolished by TTX (10⁻⁶ mol/L), guanethidine (10⁻⁵ mol/L), and propranolol (3×10⁻⁶ mol/L), a β-adrenergic receptor antagonist, but were unaffected by atropine (3×10⁻⁶ mol/L), a muscarinic cholinergic receptor antagonist (Figure 1 and Table). L-NNA (10⁻⁴ mol/L), an NO synthase inhibitor, also did not alter the amplitude of hyperpolarization (Figure 1 and Table). Neither capsaicin (10⁻⁵ mol/L), which directly acts on sensory nerve terminals to release and ultimately deplete neuropeptides,²¹ nor CGRP8-37 (10⁻⁷ mol/L), a CGRP antagonist,¹³ altered the amplitude of the hyperpolarization (Figure 1 and Table).

Figure 2 shows the membrane hyperpolarizations elicited by perivascular nerve stimulation (5 Hz, 30 seconds) in 3 different [K⁺]₀ solutions (1, 5.9, and 20 mmol/L). The membrane potentials in 1 mmol/L, 5.9 mmol/L, and 20 mmol/L [K⁺]₀ were −65±2.6 mV, −66±2.1 mV, and −56.5±1.7 mV, respectively (n=4 for each). The amplitude of hyperpolarization was increased in low K⁺ solution (1 mmol/L) but decreased in high K⁺ solution (20 mmol/L) compared with values obtained in normal K⁺ solution (5.9 mmol/L) (Figure 2).

Stimulation-evoked hyperpolarization was nearly abolished by glibenclamide (10⁻⁶ mol/L), an inhibitor of ATP-sensitive K⁺ channels (KATP),²² but was unaffected by apamin (10⁻⁶ mol/L), a blocker of small-conductance Ca²⁺-activated K⁺-channels (KCa²⁺)²³ (Figure 3 and Table).

In the presence of phenolamine (3×10⁻⁶ mol/L), exogenously applied norepinephrine (10⁻⁶ mol/L) produced a membrane hyperpolarization that was abolished by propranolol (3×10⁻⁶ mol/L) (Figure 4) (norepinephrine 10⁻⁵ mol/L; −4.1±0.3 and −0.3±0.3 mV before and after treatment with 3×10⁻⁶ mol/L propranolol; n=5). Glibenclamide (10⁻⁶ mol/L), a KATP blocker, also abolished the norepinephrine-induced hyperpolarization (norepinephrine 10⁻⁵ mol/L; −5.1±0.6 and −0.3±0.3 mV before and after treatment with 10⁻⁶ mol/L glibenclamide; n=5). Conversely, in the absence of phenolamine, norepinephrine depolarized the membrane, and this depolarization was enhanced by propranolol (3×10⁻⁶ mol/L) by an amplitude of 6.3±1.2 mV (n=7).

Isoproterenol-induced hyperpolarization was unaltered by pretreatment with phentolamine together with suramin (isoproterenol 3×10⁻⁷ mol/L; −3.8±0.6 and −3.9±0.5 mV before and after treatment with 3×10⁻⁶ mol/L phenolamine and 10⁻⁷ mol/L suramin, respectively; n=5; P=NS).

**Discussion**

The present study demonstrated that neurally released catecholamines induce membrane hyperpolarization through the activation of KATP by β-adrenergic receptors in the rat mesenteric resistance arteries. Such β-adrenergic, neurogenic hyperpolarization may play an important role in the control of arterial membrane potential by opposing α-adrenergic depolarization.

**Resting Membrane Potential of the Rat Mesenteric Artery**

The resting membrane potential of the rat mesenteric artery observed in this study is somewhat more negative than those in previous studies.³ The difference may arise from several differences in experimental conditions. First,
we used peripheral resistance arteries, and the membrane might be more negative in peripheral, small arteries than in proximal, large arteries. Second, the preparations were not subject to transmural pressure in this study. Considering the experimental evidence of pressure-dependent depolarization of muscle cells,24 this might be associated with more negative membrane potential. Indeed, it has been shown that the membrane potential of arterial smooth muscle cells at low transmural pressure measured in vitro lies in the range of $-60$ to $-75$ mV.25,26

Transmitters Involved in Neurogenic Hyperpolarization

Perivascular nerve stimulation in the combined presence of phentolamine and suramin evoked slow hyperpolarization in the rat mesenteric resistance arteries. This hyperpolarization was abolished by TTX and guanethidine, which indicates that the hyperpolarization is mediated by transmitters released from sympathetic nerve terminals. Several previous studies, primarily conducted in venules or in arterioles, have demonstrated the presence of neurogenic hyperpolarization, and various transmitters have been proposed to account for this phenomenon.7–13

Hashitani et al13 have demonstrated that in guinea pig choroidal arterioles, perivascular nerve stimulation evokes biphasic hyperpolarization consisting of inhibitory junction potentials (IJPs) and slow hyperpolarization along with some depolarization and have suggested that IJPs may result from the activation of muscarinic receptors, whereas slow hyperpolarization appears to involve NO, derived either from nitrergic nerve terminals or from the endothelium. In guinea pig small intestinal arterioles11 and in rabbit lingual artery,7 nerve stimulation also elicits hyperpolarization that is blocked by muscarinic receptor blockers and is mimicked by exogenous ACh. ACh is thought to act on the vascular endothelium to release endothelium-derived hyperpolarizing factor5,6 or to exert a direct action on muscarinic receptors located on the vascular smooth muscle to induce hyperpolarization.7 In the present study, stimulation-evoked hyperpolarization was not affected by atropine or the NO synthase inhibitors, which suggests that neither cholinergic, muscarinic transmitters, nor NO is involved in neurogenic hyperpolarization in the rat mesenteric artery.

CGRP may be released from sensory nerve terminals on peripheral nerve stimulation and cause vasodilatation.17 Furthermore, in rabbit arteries, CGRP has been shown to activate $K_{ATP}$ and produce hyperpolarization.4 Capsaicin activates and subsequently depletes capsaicin-sensitive inhibitory transmitter stores.21 In the guinea pig mesenteric artery, IJPs have been shown to be mediated by capsaicin-sensitive afferent nerves.12 The rat mesenteric vascular bed is densely innervated by sensory nerve fibers containing various peptides.17 However, the lack of effect of capsaicin and CGRP8-37, a CGRP antagonist, on stimulation-evoked hyperpolarization in the rat mesenteric artery makes it unlikely that neurally released peptides from sensory nerves such as CGRP are involved in the hyperpolarization.
In rat mesenteric resistance artery, β-adrenoceptors exist on the smooth muscle membrane, and we have recently demonstrated that exogenously applied β-agonists such as isoproterenol cause membrane hyperpolarization in this preparation. In the present study, stimulation-evoked hyperpolarization was abolished by propranolol and guanethidine, and in the presence of phentolamine, exogenously applied norepinephrine elicited hyperpolarization that was also blocked by propranolol. Together with the lack of effect of various other blockers on the hyperpolarization as mentioned above, it may be reasonable to conclude that neurogenic hyperpolarization in rat mesenteric resistance arteries results from the activation of β-adrenoceptors by catecholamines released from sympathetic nerve terminals.

In rabbit facial vein and cat gastric submucosal venules, hyperpolarization evoked by perivascular nerve stimulation is inhibited by β-adrenergic blockers and is mimicked by exogenously applied catecholamines, which indicates that neurally released catecholamines elicit hyperpolarization by β-adrenoceptors. To the best of our knowledge, this is the first study to demonstrate β-adrenergic, neurogenic hyperpolarization in resistance arteries, which may play an important role in the control of total vascular resistance.

In this study, preparations were preincubated with suramin and phentolamine to eliminate EJPs and depolarizing responses by purinoceptors or α-adrenoceptors in response to nerve stimulation. The possibility that these drugs augment β-adrenergic, neurogenic hyperpolarization nonspecifically seems unlikely because neither the amplitude of isoproterenol-induced hyperpolarization nor the resting membrane potential was altered by these treatments. However, we cannot exclude the possibility that the amount of catecholamines released may have been augmented by suramin and phentolamine by eliminating prejunctional inhibition of transmitter release by α2-adrenoceptors and P2X-purinoceptors located on prejunctional nerve terminals.

Ionic Mechanisms of Neurogenic Hyperpolarization

The amplitude of β-adrenergic hyperpolarization produced by perivascular nerve stimulation in the rat mesenteric artery was increased in low K+ solution and decreased in high K+ solution, which suggests that hyperpolarization may be due to an increase in K+ conductance. It has been suggested that β-adrenoceptor–mediated hyperpolarization in vascular smooth muscle cells involves either KCa or KATP. We have previously demonstrated that β-agonist–induced hyperpolarization in rat mesenteric resistance arteries is mediated by KATP but not by KCa. In the present study, KATP blocker glibenclamide but not KCa blocker apamin inhibited nerve-mediated hyperpolarization, which is consistent with the view that neurally released catecholamines activate KATP channels, thereby leading to smooth muscle membrane hyperpolarization. Voltage-activated K+ channels, such as voltage-dependent K+ channels (Kv) and large-conductance KCa (BKCa), may be less available for activation in this preparation because of the very negative resting membrane potential.

Only limited information is available regarding the ionic mechanisms of neurogenic hyperpolarizations, including those involving β-adrenoceptors. In the study by Hashitani et al., on neurogenic hyperpolarization in guinea pig choroidal arteries, cholinergic IJPs appeared to result from the activation of KCa, whereas slow hyperpolarization, mediated by NO, appeared to result from activation of KATP channels. Although the activation of KATP by NO has been demonstrated in certain vascular beds, NO is unlikely to account for the neurogenic activation of KATP in the present study because the NO synthase inhibitor was without effect on hyperpolarization. Thus, although KATP may be involved in neurogenic hyperpolarization both in our study and in that by Hashitani et al., the transmitters responsible for the activation of KATP appear to differ.

Physiological Implications

In the present study, norepinephrine-induced depolarization in the absence of phentolamine was enhanced by the β-blocker propranolol, which suggests that norepinephrine stimulates both α-adrenoceptors and β-adrenoceptors simultaneously and that β-adrenoceptor–mediated hyperpolarization counteracts α-adrenoceptor–mediated depolarization to a certain extent. Furthermore, in the rabbit facial vein and in situ cremaster muscle vascular bed, a blockade of β-adrenoceptors with propranolol leads to a membrane depolarization, which suggests that the membrane potential is continuously modulated by β-adrenoceptor–mediated hyperpolarization under physiological conditions.

The present findings are not in themselves sufficient to elucidate whether neurogenic hyperpolarization actually affects the vascular tone. However, considering the importance of membrane potential as a determinant of smooth muscle tone, even a subtle change in membrane potential might be expected to influence muscle tone, especially in the presence of contractile agonists, and several studies have demonstrated that neurogenic vasodilatation is correlated with hyperpolarization. Furthermore, several recent studies including those on rat mesenteric artery have demonstrated the involvement of KATP channels in β-agonist–induced vasorelaxation. It is thus conceivable that the membrane hyperpolarization produced by neurally released catecholamines through activation of KATP may play some sort of modulatory role in the regulation of arterial tone in rat mesenteric circulation.

Sympathetic discharge rates occurring in vivo are not certain, but it has been suggested that the normal pattern of sympathetic discharge is irregular and the frequency of such activity may reach very high values, although the average discharge rate may rarely exceed 10 Hz. Nevertheless, caution should be exercised in extrapolating our findings to in vivo conditions because we used the supramaximal level of nerve stimulation.

In conclusion, neurally released catecholamines may cause membrane hyperpolarization by β-adrenergic receptors and through the activation of KATP in rat mesenteric resistance arteries. Such hyperpolarization may play an important role in the control of arterial membrane potential by opposing α-adrenergic depolarization.
References

Sympathetic Control of Arterial Membrane Potential by ATP-Sensitive K⁺-Channels
Kenichi Goto, Koji Fujii, Isao Abe and Masatoshi Fujishima

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