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Differential Desensitization of Ca^{2+} Mobilization and Vasoconstriction by ET_A Receptors in the Gerbil Spiral Modiolar Artery

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Abstract. Endothelins are known to be among the most potent endogenous vasoconstrictors. Vasoconstriction of the spiral modiolar artery, which supplies the cochlea, may be implicated in hearing loss and tinnitus. The purpose of the present study was to determine whether the spiral modiolar artery responds to endothelin, whether a change in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) mediates the response and which endothelin receptors are present. The vascular diameter and $[Ca^{2+}]_i$ were measured simultaneously by videomicroscopy and microfluorometry in the isolated spiral modiolar artery from the gerbil. ET-1 induced a transient $[Ca^{2+}]_i$ increase and a strong and long-lasting vasoconstriction. The transient $[Ca^{2+}]_i$ increase underwent rapid desensitization, was independent of extracellular Ca^{2+} and inhibited by the IP_3-receptor blocker (75 μM) 2-aminoethoxydiphenyl borate (2-APB) and by depletion of Ca^{2+} stores with 10^{-6} M thapsigargin. In contrast, the vasoconstriction displayed no comparable desensitization. The initial vasoconstriction was independent of extracellular Ca^{2+} but maintenance of the constriction depended on the presence of extracellular Ca^{2+}. The half-maximal concentration values (EC_{50}) for the agonists ET-1, ET-3 and sarafotoxin S6c were 0.8 nM, >10 nM and >100 nM, respectively. Affinity constants for the antagonists BQ-123 and BQ-788 were 24 nM and 77 nM, respectively. These observations demonstrate that ET-1 mediates a vasoconstriction of the gerbil spiral modiolar artery via ET_A receptors and an IP_3 receptor-mediated release of Ca^{2+} from thapsigargin-sensitive Ca^{2+} stores. The marked difference in desensitization between Ca^{2+} mobilization and vasoconstriction suggests that Ca^{2+} mobilization is not solely responsible for the vasoconstriction and that other signaling mechanisms must be present.

Key words: ET-1 — ET-3 — Sarafotoxin S6c — BQ-123 — BQ-788 — Cochlear blood flow

Introduction

The spiral modiolar artery originates via the anterior inferior cerebellar artery from the basilar artery and provides the main blood supply to the cochlea. Blood flow regulation in the spiral modiolar artery is of great interest since alterations of blood flow along this arteriole are thought to be involved in the pathogenesis of hearing loss and tinnitus. In general, blood flow is controlled by the vascular diameter, which depends on the contractile state of the vascular smooth muscle cells. The contractile state of the smooth muscle cells is controlled by the presence of vasoconstrictors and vasodilators.

Among the most potent vasoconstrictors are the endothelins (Yanagisawa et al., 1988). Endothelins are oligopeptides that occur in three isoforms, ET-1, ET-2 and ET-3 (Inoue et al., 1989). ET-1 is the most abundant isoform in the vasculature. ET-1 is produced mainly by endothelial cells and in small amounts by smooth muscle cells and perivascular nerves. Endothelial cells release as much as 75–90% of the secreted ET-1 toward the abluminal space (Yoshimoto et al., 1991; Unoki, Fan & Watanabe, 1999) where ET-1 interacts with endothelin receptors located on smooth muscle cells, pericytes and on the endothelial cells themselves. This release of endothelins allows vessels to control the vascular tone and thus blood flow.

There are two endothelin receptor subtypes that mediate vasoconstriction: ET_A- and ET_B-receptors (Naka-

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muta et al., 1991; Adachi et al., 1991; Cyr et al., 1991). ET_A receptors are most abundant on vascular smooth muscle cells. ET_B receptors are expressed in the vasculature predominantly by endothelial cells where they induce NO synthesis and cause a vasodilation (Tsukahara et al., 1994). Alternatively, some vessels express ET_B receptors in the smooth muscle cells where they mediate a vasoconstriction (LaDouceur et al., 1993; Haynes, Strachan & Webb, 1995; Pang et al., 1998). ET_A receptors are characterized by an agonist potency order of ET-1 > ET-3 >> sarafotoxin S6c (Watanabe et al., 1989; Vigne et al., 1990a). In contrast, ET_B receptors are characterized by an agonist potency order of sarafotoxin S6c = ET-1 = ET-3 (Watanabe et al., 1989; Williams, Jr. et al., 1991). Thus, a comparison of the potency of these agonists provides a means to distinguish between ET_A and ET_B receptors (Rubanyi & Polokoff, 1994). It is also possible to distinguish between ET_A and ET_B receptors using selective ET_A- and ET_B-receptor antagonists such as BQ-123 and BQ-788 (Ihara et al., 1992; Ishikawa et al., 1994). Further, ET_A receptors signal in many tissues mainly via $G_{q/11}$, phospholipase C and a mobilization of Ca^{2+}, whereas ET_B receptors signal mainly via a stimulation of nitric oxide production and an activation of guanylyl cyclase (Miasiro & Paiva, 1990; Vigne et al., 1990b; Rubanyi & Polokoff, 1994; Yang et al., 1994).

Endothelins have been shown to cause a vasoconstriction and a reduction in blood flow in a variety of organs including the cochlea. Endothelins have been demonstrated to be present in the cochlea and have been shown to cause a reduction in cochlear blood flow (Quirk et al., 1992; Jinnouchi et al., 1997). This reduction of cochlear blood flow may in part be due to an ET_A receptor-mediated vasoconstriction of capillaries in the spiral ligament of the lateral cochlear wall (Sadanaga, Liu & Wangemann, 1997). Whether endothelin receptors are present in the spiral modiolar artery, which provides the main blood supply to the cochlea, is currently unclear. The purpose of the present study was to determine whether the spiral modiolar artery responds to endothelin, whether a change in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) mediates the response and which endothelin receptors are present.

Parts of the present study have been presented in abstract form at recent meetings (Scherer, Wonneberger & Wangemann, 2000; Scherer & Wangemann, 2001a; Scherer & Wangemann, 2001b).

Materials and Methods

Preparation

Experiments were conducted on tissues isolated from gerbils. Gerbils were anesthetized with sodium pentobarbital (100 mg/kg i.p.) and decapitated under a protocol approved by the Institutional Animal Care and Use Committee at Kansas State University. The spiral modiolar artery was isolated from the cochlea by micro-dissection as described previously (Wangemann & Gruber, 1998). Briefly, the temporal bones were removed from the head, opened and placed into a micro-dissection chamber containing control solution (Solutions, *see below*) maintained at 4°C. The otic capsule enclosing the cochlea was opened and the spiral modiolar artery was visualized through the very thin bone surrounding the modiolus. The bone surrounding the modiolus was then carefully removed and the spiral modiolar artery, which is only loosely attached to the eighth cranial nerve, was isolated. Care was taken to not excessively stretch the artery.

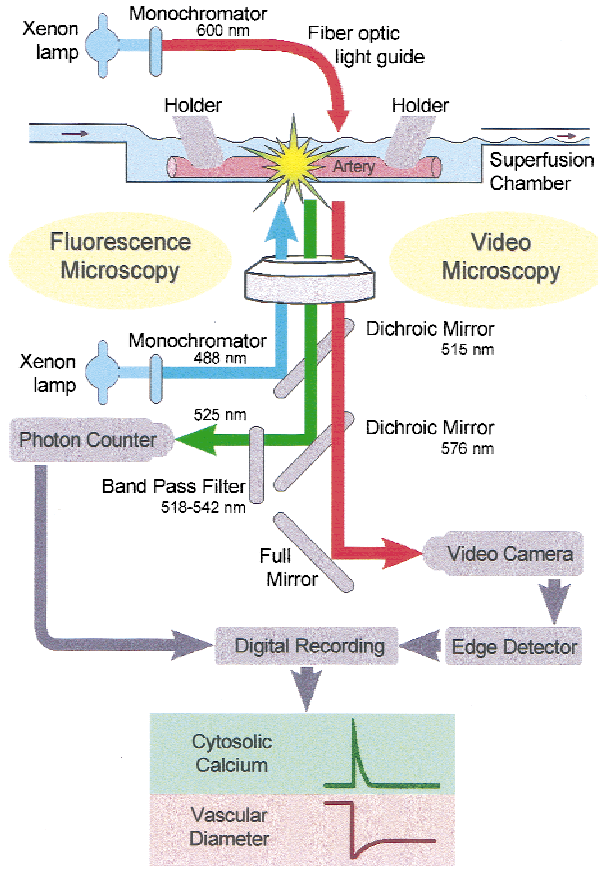

Fig. 1. Schematic of the method for simultaneous measurements of changes in the cytosolic Ca^{2+} concentration and changes in the vascular diameter with fluo-4 microfluorometry and video microscopy.

Simultaneous Measurement of Vascular Diameter and Intracellular Ca^{2+} ($[Ca^{2+}]_i$)

Video microscopy and microfluorometry were used simultaneously to measure the vascular diameter and to monitor $[Ca^{2+}]_i$ (Fig. 1). A 250 to 350 μm segment from the isolated spiral modiolar artery was loaded for 35 min in control solution containing 5 μM fluo-4-AM (Molecular Probes, Eugene, OR) at 37°C and then transferred into a bath chamber mounted on the stage of an inverted microscope (Diaphot, Nikon, Japan). In the bath chamber, the vessel was held in place with two blunted glass needles mounted on micromanipulators (Narashige, Japan). The mounted vessel was superfused at a rate of 2.5 times the bath

chamber volume per second. All experiments were performed at 37°C. The preparation was alternately illuminated with light of 600 and 488 nm (Deltascan, Photon Technology, South Brunswick, NJ). Epi-illumination was chosen for the 488 nm light path and transillumination for the 600 nm light path. The 488 nm light was reflected by a 515 nm dichroic mirror (Omega Optical, Brattleboro, VT) to reach the preparation. The fluorescence (500–550 nm) emitted in response to 488 nm excitation was transmitted through the 515 nm dichroic mirror, reflected by a 576 nm dichroic mirror (Chroma Technology, Brattleboro, VT) and limited with a band-pass filter to a wavelength between 518 and 542 nm (Chroma Technology). The fluorescence signal was detected by a photon-counter and recorded at a rate of 10 Hz (Photon Technology). Changes in the emission intensity were taken as changes in $[Ca^{2+}]_i$. The 600 nm wavelength light illuminated the preparation via a fiber-optic light guide. The image passed the 515 and 576 nm dichroic mirrors before it was detected by a chilled CCD video camera at a rate of 1.5–2.5 Hz (C5985, Hamamatsu, Japan). Images were mixed with a time signal (Time Code Generator, Fast Forward Video, Irvine, CA) and displayed on a monitor (PVM-137, Sony), as well as recorded on videotape (AG-1960, Panasonic). The outer diameter of the spiral modiolar artery was monitored by two video-edge detectors (Crescent Electronics, East Sandy, UT). The calibrated distance between the two tracked edges was monitored on-line with a chart-recorder (Kipp & Zonen, The Netherlands) as well as digitized at a frequency of 10 Hz (Photon Technology). Data were stored in ASCII format for later analysis (Origin 6.0, Microcal Software, Northampton, MA).

Solutions

The control solution contained (in mM) 150 NaCl, 5.0 HEPES, 3.6 KCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$ and 5.0 glucose, pH 7.4. In some experiments the extracellular Ca^{2+} concentration was raised to 10 mM by addition of $CaCl_2$ to the control solution. A maximal vasodilation was induced by the removal of extracellular Ca^{2+}. This nominally Ca^{2+}-free solution contained (in mM) 150 NaCl, 5.0 HEPES, 3.6 KCl, 1.0 $MgCl_2$, 1.0 EGTA and 5.0 glucose, pH = 7.4. BQ-123 and sarafotoxin S6c were obtained from Calbiochem (San Diego, CA). Unless otherwise indicated, all other chemicals were obtained from Sigma (St. Louis, MO).

Pharmacological Analysis

Dose-response curves were obtained using either a cumulative or a pulsatile protocol. The cumulative protocol entailed a stepwise increase of the agonist concentration. Several agonist concentrations could thus be tested in one vessel segment. The pulsatile protocol entailed that only one concentration was tested once in one vessel segment. Each concentration of agonist was applied for two minutes in both protocols. The agonist-induced increase in $[Ca^{2+}]_i$ was determined as difference at the peak. The agonist-induced vasoconstriction was determined by averaging the vascular diameter over the second minute of agonist application. Data were normalized to the maximal increase in $[Ca^{2+}]_i$ and the maximal vasoconstriction induced by an elevation of the extracellular Ca^{2+} concentration from 1 to 10 mM Ca^{2+} (Wonneberger, Scofield & Wangemann, 2000). Normalized data were fitted with the equation

$$E = E_{max} \cdot C^h/({EC_{50}}^h + C^h) \quad (1)$$

where E is the relative constriction (%), EC_{50} is the agonist concentration that induced a half-maximal effect, E_{max} is the maximal constriction, C is the concentration of agonist and h defines the slope (Limbird, 1986). For the analysis of pulsatile experiments, all data were taken together and fitted to equation *1* to determine E_{max}, EC_{50} and *h*. For analysis of cumulative experiments, all data were first taken together and fitted to equation *1* to determine E_{max} and *h*. E_{max} and *h* were then taken to obtain EC_{50} values from data obtained from individual vessel segments.

Affinity constants for antagonists (K_{DB}) were obtained from the Schild-equation

$$p(K_{DB}) = \log(B) - \log(DR - 1) \quad (2)$$

where B is the concentration of the antagonist and DR is the dose-ratio. The dose-ratio was obtained according to

$$DR = EC_{50\ Antagonist}/EC_{50\ ET-1} \quad (3)$$

where $EC_{50\ Antagonist}$ and $EC_{50\ ET-1}$ are EC_{50} values for ET-1 that were obtained in cumulative experiments in the presence and absence of antagonist. $EC_{50\ Antagonist}$ was obtained according to equation (*1*), in which E_{max} and *h* were fixed to the values obtained in the absence of antagonists.

Statistics

Data are presented as mean ± SEM. The number of experiments (n) represents the number of vessel segments. Statistical analyses involved Students paired *t*-tests and analyses of variance.

Results

The effects of ET-1 were compared to the effects induced by an elevation of the extracellular Ca^{2+} concentration from 1 to 10 mM that were set to 100%. ET-1 induced a transient increase in $[Ca^{2+}]_i$ to 137 ± 8% (n = 12), a strong and long-lasting vasoconstriction to 130 ± 14% (n = 12) and a robust increase in the vasomotion of the gerbil spiral modiolar artery (Fig. 2). The $[Ca^{2+}]_i$ returned to almost resting levels after the transient increase, while the constriction was maintained. These data are consistent with the view that ET-1 increased the Ca^{2+} sensitivity of the myofilaments such that a lesser $[Ca^{2+}]_i$ was sufficient for the maintenance of the constriction. The ET-1-induced vasoconstriction was not readily reversible upon removal of ET-1 from the perfusate. The constriction and the increased vasomotion were observed without a significant change for at least 20 minutes after removal of ET-1 from the superfusate (*data not shown*).

Dose-response curves, in general, can be obtained using a cumulative or a pulsatile experimental protocol. Both protocols should yield similar results as long as the effect of one dose does not affect the effect of another dose. Differences between cumulative and pulsatile dose-response curves may be an indication for a potentiation or a desensitization of the receptor. Thus, dose-response curves for ET-1 were obtained in the spiral modiolar artery in cumulative and in pulsatile fashion. Examples of original data are shown in Figs. 2 and 3, dose-response curves are shown in Fig. 4 and data are

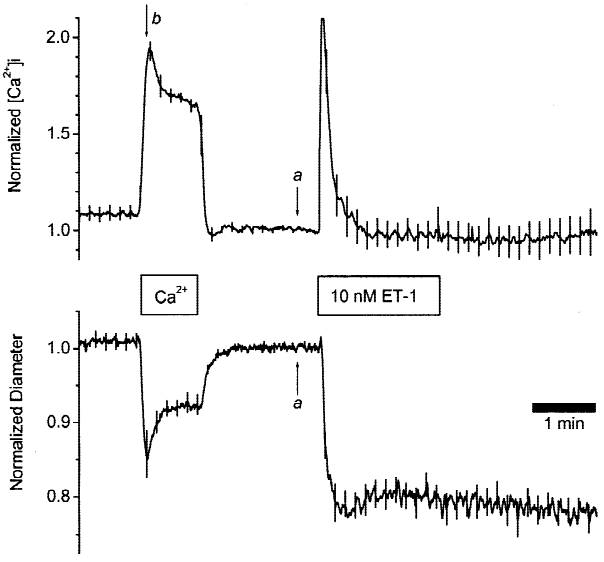

Fig. 2. Effect of endothelin (ET-1) on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the vascular diameter of the spiral modiolar artery: averages and SEM values of 5 original traces from pulsatile experiments. Note that ET-1 caused a transient increase in $[Ca^{2+}]_i$ and a sustained vasoconstriction and increase in vasomotion. An increase of the extracellular Ca^{2+} concentration from 1 to 10 mM (Ca) served as a control experiment. Measurements of $[Ca^{2+}]_i$ were normalized to the value obtained prior to the admission of ET-1 (value at time '*a*' was set to 1) and to the maximal increase in $[Ca^{2+}]_i$ elicited by the increase in the extracellular Ca^{2+} concentration (value at time '*b*' was set to 2).

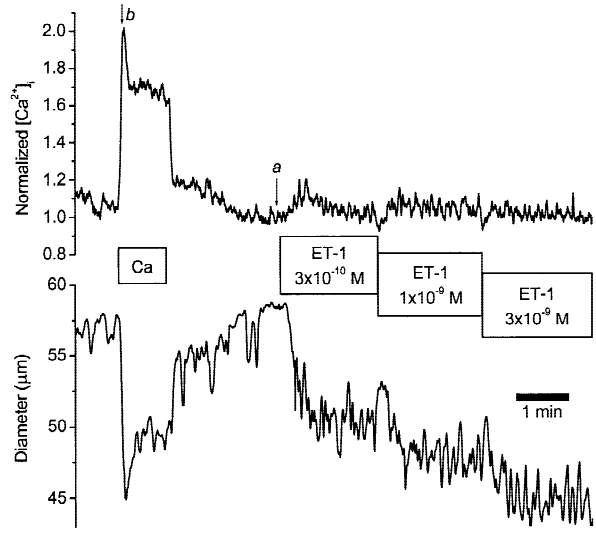

Fig. 3. Effect of endothelin (ET-1) on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the vascular diameter of the spiral modiolar artery: original traces from cumulative experiments. Note that cumulative doses of ET-1 have little effect on $[Ca^{2+}]_i$ but cause a stepwise increase in the sustained vasoconstriction. An increase of the extracellular Ca^{2+} concentration from 1 to 10 mM (Ca) served as a control experiment. Measurements of $[Ca^{2+}]_i$ were normalized to the value obtained prior to the admission of ET-1 (value at time '*a*' was set to 1) and to the maximal increase in $[Ca^{2+}]_i$ elicited by the increase in the extracellular Ca^{2+} concentration (value at time '*b*' was set to 2).

summarized in the Table. Cumulative and pulsatile dose-response curves for the ET-1-induced vasoconstriction were not significantly different. However, a significant difference was observed between cumulative and pulsatile dose-response curves for the increase in $[Ca^{2+}]_i$ (Fig. 4*a*). These observations document a pronounced desensitization of the ET-1-induced increase in $[Ca^{2+}]_i$. Intriguingly, this desensitization was not observed in the simultaneously measured vasoconstriction (Fig. 4*b*).

The observation that ET-1 induced in pulsatile experiments a transient increase in $[Ca^{2+}]_i$ raised the question whether this increase in $[Ca^{2+}]_i$ was due to an inflow of extracellular Ca^{2+} or due to a release of Ca^{2+} from intracellular stores. ET-1 was therefore applied in the absence of extracellular Ca^{2+} to distinguish between these two possibilities. Removal of the extracellular Ca^{2+} concentration caused a rapid decrease in $[Ca^{2+}]_i$ that was paralleled by a vasodilation (Fig. 5). In the absence of extracellular Ca^{2+}, ET-1 induced a transient increase in $[Ca^{2+}]_i$ to 122 ± 13% ($n = 8$) and a transient vasoconstriction. The $[Ca^{2+}]_i$ returned to near resting values while the constriction was in part maintained in the absence of extracellular Ca^{2+}. The vasoconstriction was restored when the extracellular Ca^{2+} concentration was reinstated. These observations suggest that ET-1 induced a release of Ca^{2+} from intracellular stores and that extracellular Ca^{2+} is required for the maintenance of the ET-1-induced vasoconstriction.

If the transient $[Ca^{2+}]_i$ increase observed in pulsatile experiments originated from intracellular Ca^{2+} stores via an IP_3-mediated process, it would be expected that the $[Ca^{2+}]_i$ increase would be reduced by blocking IP_3-receptors with 2-aminoethoxydiphenyl borate (2-APB) (Ma et al., 2000) and by depleting intracellular Ca^{2+} stores with thapsigargin. Indeed, the ET-1-induced transient $[Ca^{2+}]_i$ increase was reduced from 122 ± 13% ($n = 8$) to 29 ± 6% ($n = 8$) in presence of 75 µM 2-APB (Fig. 6) and from 137 ± 8% ($n = 12$) to 6 ± 1% ($n = 8$) after depletion of intracellular Ca^{2+} stores with 1 µM thapsigargin (Fig. 7). The simultaneously measured vasoconstrictions were reduced, too. These observations are consistent with the concept that ET-1 initiated the vasoconstriction via an IP_3-mediated release of Ca^{2+} from thapsigargin-sensitive Ca^{2+} stores.

The observation that ET-1 caused a long-lasting vasoconstriction raises the question which receptor is mediating this response. ET_A and ET_B receptors can be distinguished by the potency order of the agonist ET-1, ET-3 and sarafotoxin S6c. Thus, cumulative dose-response curves were obtained. ET-3 was a far less potent vasoconstrictor than ET-1 and the agonist sarafotoxin S6c had no significant effect up to a concentration of 10^{-7} M (Fig. 8). These observations suggest that the

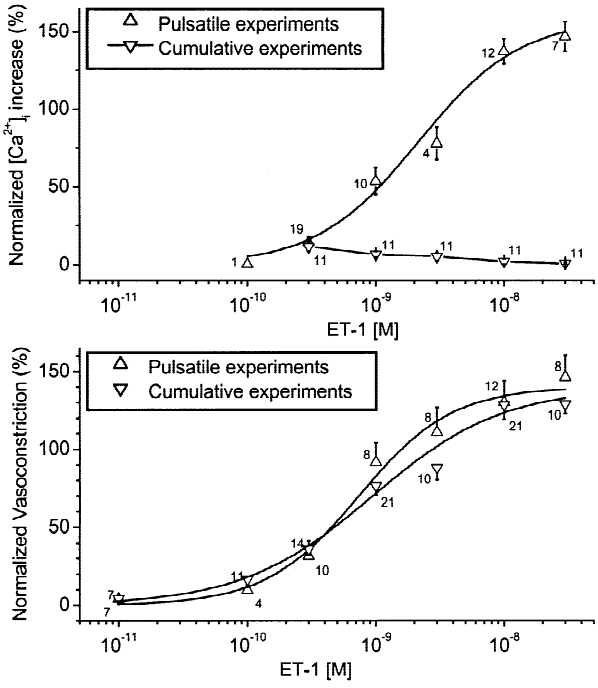

Fig. 4. Effect of endothelin (ET-1) on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the vascular diameter of the spiral modiolar artery: dose response curves from pulsatile and cumulative experiments. Data were normalized to the initial increase in $[Ca^{2+}]_i$ and to the vasoconstriction induced by an elevation of the extracellular Ca^{2+} concentration from 1 to 10 mM (set to 100%). Note that the increase in $[Ca^{2+}]_i$ was dose-dependent in pulsatile experiments but not in cumulative experiments although the simultaneously measured vasoconstrictions were dose-dependent in both protocols. This discrepancy demonstrates a rapid desensitization of the Ca^{2+} mobilization that is not present in the vasoconstriction. Numbers next to the symbols depict the number of vessel segments. E_{max}, EC_{50} and h values are summarized in the Table.

ET-1 induced vasoconstriction is mediated via ET_A receptors.

ET_A and ET_B receptors can be distinguished not only by the potency order but also by the affinity to the antagonists BQ-123 and BQ-788. Cumulative dose-response curves for the ET-1-induced vasoconstriction were obtained in the presence of 0.1 and 1 μM BQ-123 and 0.1 μM BQ-788 and compared to data obtained in the absence of antagonists (Figs. 9 and 10). Affinity constants for BQ-123 and BQ-788 were 24 nM (pK_{DB} = 7.61 ± 0.11; n = 15) and 77 nM (pK_{DB} = 7.12 ± 0.38; n = 5). These observations strengthen the conclusion that the ET-1-induced vasoconstriction of the spiral modiolar artery is mediated via ET_A receptors.

Discussion

Two lines of evidence support the conclusion that ET_A receptors mediate the ET-1 induced vasoconstriction of the spiral modiolar artery. First, endothelin agonists caused a vasoconstriction of the spiral modiolar artery with a potency order of ET-1 >> ET-3 = sarafotoxin S6c, which is characteristic for ET_A receptors (Watanabe et al., 1989; Vigne et al., 1990a; Rubanyi & Polokoff, 1994). Second, comparison of the K_{DB} values for BQ-123 and BQ-788 obtained in tissues known to express ET_A or ET_B receptors reveal patterns that are characteristic for these receptors (Fig. 11). The K_{DB} values obtained in the spiral modiolar artery clearly follow the pattern characteristic of ET_A receptors. These observations demonstrate that ET-1 mediates the vasoconstriction of the spiral modiolar artery via ET_A receptors. The presence of a significant population of ET_B receptors participating in the ET-1-induced vasoconstriction is unlikely since the selective ET_B receptor agonist sarafotoxin S6c up to a concentration of 100 μM had no significant effect. Taken together, these findings suggest that the ET-1-induced vasoconstriction is mediated solely via ET_A receptors.

ET_A receptors signal in many tissues mainly via $G_{q/11}$, phospholipase C, an IP_3-receptor-mediated mobilization of Ca^{2+} from intracellular Ca^{2+} stores and an influx of Ca^{2+} from the extracellular compartment (Miasiro & Paiva, 1990; Vigne et al., 1990b; Rubanyi & Polokoff, 1994; Yang et al., 1994). A similar signaling path is likely to be present in the spiral modiolar artery. Evidence for a mobilization of Ca^{2+} from stores comes from the observation that the transient increase in $[Ca^{2+}]_i$ was similar in magnitude in the presence and absence of extracellular Ca^{2+} (Figs. 2 and 5). Further evidence for a mobilization of Ca^{2+} from stores comes from the observation that the ET-1-induced transient increase in $[Ca^{2+}]_i$ was sensitive to depletion of the stores by thapsigargin (Fig. 7). Evidence of an involvement of IP_3-receptors comes from the finding that the transient $[Ca^{2+}]_i$ increase was inhibited by 2-APB (Fig. 6). The observation that the vasoconstriction was more transient in nature in the absence of extracellular Ca^{2+} and restored when the normal extracellular Ca^{2+} concentration was reinstated (Fig. 5) suggests that the maintenance of the vasoconstriction depended on an influx of Ca^{2+} from the extracellular compartment.

Additional signal transduction mechanisms are likely to be present in the spiral modiolar artery. The observation that the ET-1-induced vasoconstriction was maintained while the $[Ca^{2+}]_i$ returned to near resting levels suggests that ET-1 induced a change in the Ca^{2+} requirement of the contractile elements as observed in other preparations (Sudjarwo & Karaki, 1995). Apparently ET-1 caused a Ca^{2+} sensitization such that a lower $[Ca^{2+}]_i$ was sufficient to maintain the vasoconstriction. It is conceivable that this apparent Ca^{2+} sensitization was due to an increase in the Ca^{2+} sensitivity of the myofilaments (Somlyo & Somlyo, 2000).

Table. EC_{50}, E_{max} and h values for the ET-1-induced vasoconstriction obtained in pulsatile and cumulative experiments

	Protocol	EC_{50}	pEC_{50}	E_{max}	h	n
Vasoconstriction	Pulsatile	$(7.4 \pm 2.1) \times 10^{-10}$	9.13	140 ± 11	1.20 ± 0.35	57
	Cumulative	9.9×10^{-10}	9.00 ± 0.09	140 ± 11	0.85 ± 0.17	21
$[Ca^{2+}]_i$ increase	Pulsatile	$(2.2 \pm 0.6) \times 10^{-9}$	8.66	159 ± 13	1.09 ± 0.19	53
	Cumulative	—	—	—	—	11

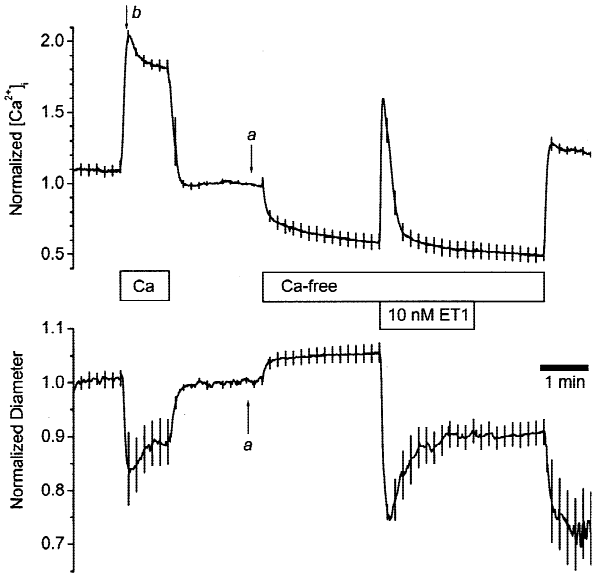

Fig. 5. Effect of endothelin (ET-1) on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the vascular diameter in the absence of extracellular Ca^{2+}: averages and SEM values of 6 original traces. Note that ET-1 in the absence of extracellular Ca^{2+} caused a transient increase in $[Ca^{2+}]_i$ and a transient vasoconstriction and that the vasoconstriction was restored when the extracellular Ca^{2+} concentration was reinstated. An increase of the extracellular Ca^{2+} concentration from 1 to 10 mM (Ca) served as a control experiment. Measurements of $[Ca^{2+}]_i$ were normalized to the value obtained prior to the removal of extracellular Ca^{2+} (value at time '*a*' was set to 1) and to the value obtained during the initial increase in the extracellular Ca^{2+} concentration (value at time '*b*' was set to 2). Measurements of the vascular diameter were normalized to the value obtained prior to the removal of extracellular Ca^{2+} (value at time '*a*' was set to 1). For clarity, SEM values are shown only in 10 sec intervals.

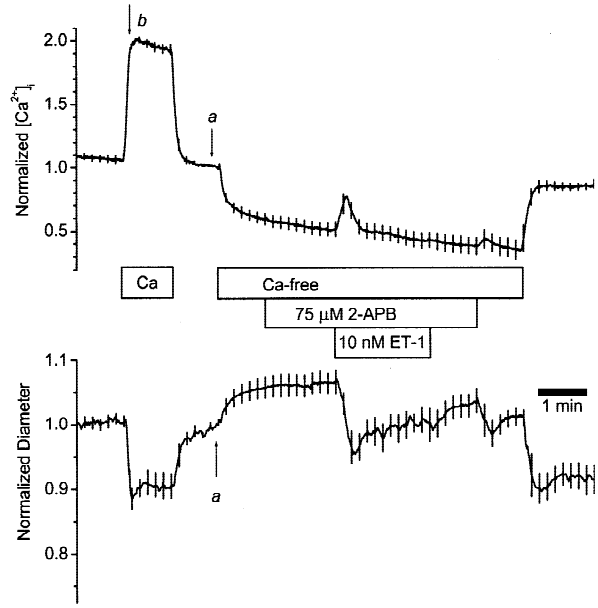

Fig. 6. Effect of endothelin (ET-1) on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the vascular diameter in the absence of extracellular Ca^{2+} and presence of 2-aminoethoxy-diphenyl borate (2-APB): averages and SEM values of 7 original traces. Note that ET-1 caused only a small transient increase in $[Ca^{2+}]_i$ and a small transient vasoconstriction. An increase of the extracellular Ca^{2+} concentration from 1 to 10 mM (Ca) served as a control experiment. Measurements of $[Ca^{2+}]_i$ were normalized to the value obtained prior to the removal of extracellular Ca^{2+} (value at time '*a*' was set to 1) and to the value obtained during the initial increase in the extracellular Ca^{2+} concentration (value at time '*b*' was set to 2). Measurements of the vascular diameter were normalized to the value obtained prior to the removal of extracellular Ca^{2+} (value at time '*a*' was set to 1). For clarity, SEM values are shown only in 10-sec intervals.

The vasoconstriction was maintained even longer than ET-1 was present in the superfusate. Such a resistance to washout has been observed in other preparations (Joshua, 1990; Kobayashi et al., 1990) and is not intrinsic to the chemical nature of the peptide agonists or the experimental setup since the peptide antagonists BQ-123 at a 100-fold higher concentration was easily washed out (Fig. 9). It is conceivable that ET-1 formed an essentially irreversible bond to the ET_A receptor and that the agonist-receptor complex continued signaling (Chun et al., 1995).

The observation that the mobilization of Ca^{2+} underwent rapid desensitization is consistent with observations in other preparations (Fu et al., 1989; Oles et al., 1997; Meyer, Oles & Pott, 2000). The finding, however, that this desensitization was not apparent in the simultaneously measured vasoconstrictions suggests that additional signaling mechanisms are present in the spiral modiolar artery. It is conceivable that these other signaling mechanisms are relatively independent of the Ca^{2+} mo-

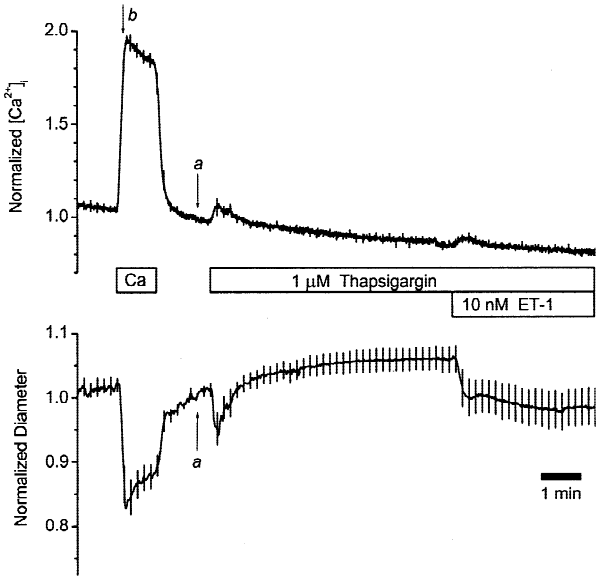

Fig. 7. Effect of endothelin (ET-1) on the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the vascular diameter in the presence of thapsigargin: averages and SEM values of 9 original traces. Note that ET-1 caused nearly no transient increase in $[Ca^{2+}]_i$ and a small vasoconstriction. An increase of the extracellular Ca^{2+} concentration from 1 to 10 mM (Ca) served as a control experiment. Measurements of $[Ca^{2+}]_i$ were normalized to the value obtained prior to the admission of thapsigargin (value at time '*a*' was set to 1) and to the value obtained during the initial increase in the extracellular Ca^{2+} concentration (value at time '*b*' was set to 2). Measurements of the vascular diameter were normalized to the value obtained prior to the admission of thapsigargin (value at time '*a*' was set to 1). For clarity, SEM values are shown only in 10 sec intervals.

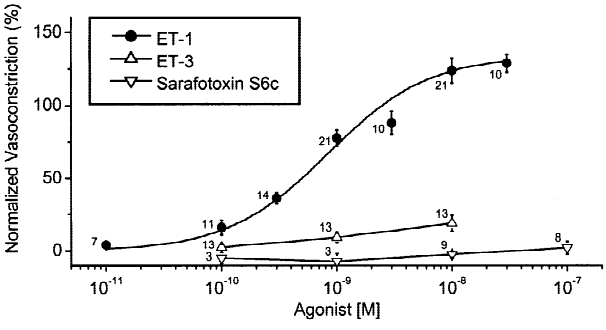

Fig. 8. Effect of endothelins (ET-1 and ET-3) and sarafotoxin S6c on the vascular diameter of the spiral modiolar artery: dose response curves from cumulative experiments. Data were normalized to the initial vasoconstriction induced by an elevation of the extracellular Ca^{2+} concentration from 1 to 10 mM (set to 100%). Note that the ET-3 and sarafotoxin S6c had hardly any effect up to concentrations of 10 and 100 nM.

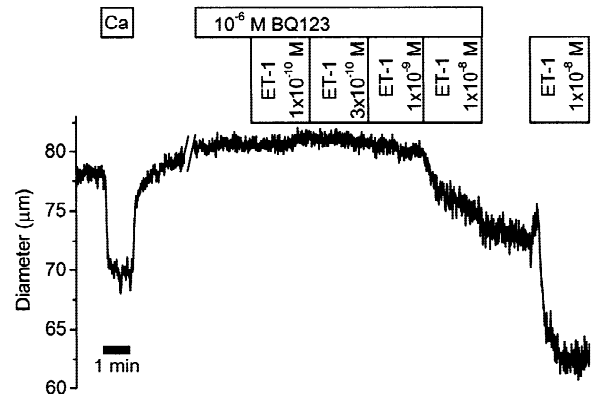

Fig. 9. Effect of endothelin (ET-1) on the vascular diameter in the presence and absence of the antagonist BQ-123: original trace. Note that cumulative doses of ET-1 had little effect in the presence of BQ-123 and that the highest dose (10 nM) elicited a marked vasoconstriction after washout of BQ-123. An increase of the extracellular Ca^{2+} concentration from 1 to 10 mM (Ca) served as a control experiment.

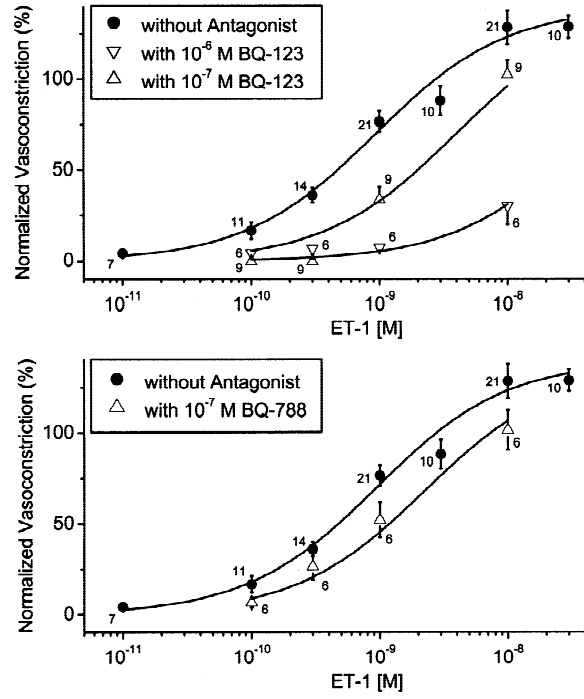

Fig. 10. Effect of endothelin (ET-1) on the vascular diameter in the absence and presence of the antagonists BQ-123 and BQ-788: dose response curves from cumulative experiments. Data were normalized to the initial vasoconstriction induced by an elevation of the extracellular Ca^{2+} concentration from 1 to 10 mM (set to 100%). Note that BQ-123 and BQ-788 shifted the dose-response curve for ET-1 to the right. The resulting affinity constants are given in the text.

bilization and mainly responsible for the Ca^{2+} sensitization and the maintained vasoconstriction.

Although the signaling mechanisms coupled to the ET_A receptors in the spiral modiolar artery are not yet fully understood, it is worthwhile speculating about the physiological and pathophysiological relevance of these receptors. ET-1 and ET_A receptors may be involved in a paracrine path controlling the vascular diameter. ET-1

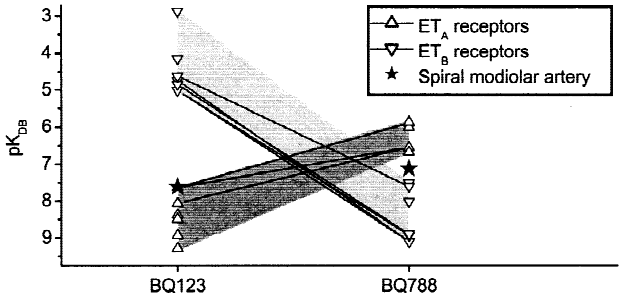

Fig. 11. Comparison of affinity constants (pK_{DB}) of the endothelin antagonists BQ-123 and BQ-788 for the endothelin receptors ET_A and ETB. Affinity constants obtained in the spiral modiolar artery (*stars*) are plotted together with affinity constants from preparations in which the endothelin receptor is known (Ishikawa et al., 1994; Ozaki et al., 1994; Ihara et al., 1995; Wu-Wong et al., 1996; Russell & Davenport, 1996; Tayag et al., 1996; Peter & Davenport, 1996). Note that a receptor subtype-specific pattern emerges and that the pK_{DB} values obtained in the spiral modiolar artery match the pattern characteristic for ET_A receptors.

concentrations at the ET_A receptors are likely to reach effective concentrations given that as much as 90% of the ET-1 secreted by endothelial cells is released toward the abluminal side (Yoshimoto et al., 1991; Unoki et al., 1999) and considering that the interstitial spaces in the spiral modiolar artery are very small. Alternatively, an endocrine path controlling the vascular diameter of the spiral modiolar artery is unlikely since plasma concentrations of ET-1 in humans are as low as 5×10^{-13} M (Lam et al., 1991), which is almost three decades below the threshold concentration for ET-1-induced constrictions. Even in pathophysiological situations where plasma ET-1 levels rise significantly, plasma concentrations remain at least one decade below the threshold concentration for ET-1-induced vasoconstriction of the spiral modiolar artery. Thus, ET-1 and ET_A receptors are most likely part of a paracrine pathway controlling the vascular diameter of the spiral modiolar artery.

In conclusion, endothelins cause a strong and long-lasting vasoconstriction of the spiral modiolar artery, which is mediated via ET_A receptors. The signaling path involves IP_3-receptor-mediated mobilization of Ca^{2+} from intracellular Ca^{2+}-stores and an apparent Ca^{2+} sensitization of the contractile elements. The observation that Ca^{2+} mobilization underwent desensitization and that the vasoconstriction did not desensitize suggest that other signaling pathways must be present. The observation that the ET-1-induced vasoconstriction was long-lasting suggests that ET-1 may play a major role in the pathogenesis of syndromes such as sudden hearing loss and tinnitus.

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