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Short communication

Endothelin ET_{B1} receptor-mediated relaxation of rabbit basilar artery

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Abstract

This study tests whether endothelin receptor agonist-induced relaxation of the cerebral vasculature is mediated via endothelin ET_{B1} receptor activation. Sarafotoxin S6c, an endothelin ET_B receptor agonist, relaxed rabbit basilar artery constricted with serotonin in situ. BQ788 (*N-cis*-2,6-dimethylpiperidinocarbonyl L-γ-MeLeu-D-Trp ($COOCH_3$)-Nle), and RES-701-1 (Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp), endothelin $ET_{B1/B2}$ and endothelin ET_{B1} receptor antagonists, respectively, prevented sarafotoxin S6c-induced relaxation. RES-701-1 was selective for the ET_{B1} receptor, as the endothelin-1 constriction elicited in the presence of BQ610 (homopiperidenyl-CO-Leu-D-Trp (CHO)-D-Trp-OH), an endothelin ET_A receptor antagonist, was enhanced by RES-701-1, and relaxed by BQ788. These results represent the first demonstration of the presence of endothelin ET_{B1} receptors in the cerebral vasculature.

Keywords: Endothelin-1; Endothelin receptor; Relaxation; Basilar artery, rabbit; Endothelium; In situ

1. Introduction

While it is well established that endothelin ET_B receptors mediate endothelium-dependent relaxation of the vasculature, there is some controversy as to whether these endothelin ET_B receptors (endothelin ET_{B1} receptors) are pharmacologically distinct from endothelin ET_B receptors that mediate constriction and are located on the smooth muscle (endothelin ET_{B2} receptors). Evidence in support of distinct endothelin ET_B receptors includes observations in peripheral blood vessels that antagonists of the endothelin ET_B receptor, such as the endothelin $ET_{A/B}$ and endothelin ET_B receptor antagonists, PD142893 (Ac-D-Dip-Leu-Asp-Ile-Ile-Trp) and BQ788 (*N-cis*-2,6-dimethylpiperidinocarbonyl L-γ-MeLeu-D-Trp ($COOCH_3$)-Nle), respectively, block endothelin ET_B receptor-mediated relaxation, but not endothelin ET_B receptor-mediated constriction (Warner et al., 1993a,b; Douglas et al., 1995; Mizuguchi et al., 1997). However, others have suggested that this relative lack of blockade of endothelin ET_B receptor-mediated constriction is, in fact, the result of cross-talk between the endothelin ET_A and endothelin ET_B receptors (Clozel and Gray, 1995).

Additional evidence in support of the suggestion that distinct endothelin ET_B receptors mediate relaxation and constriction is based upon the ability of RES-701-1 (Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp), a purported selective endothelin ET_{B1} receptor antagonist (Tanaka et al., 1995), to block endothelin ET_B receptor-mediated relaxation of the rat aorta and rabbit saphenous vein, but not endothelin ET_B receptor-mediated constriction of the rabbit saphenous vein (Karaki et al., 1994; Sudjarwo et al., 1994; Douglas et al., 1995). However, these reports are also controversial, as others were unable to demonstrate that RES-701-1 selectively blocked the endothelin ET_{B1} receptor (He et al., 1995; Russell and Davenport, 1996).

In addition to the controversy surrounding distinct endothelin ET_B receptor types, the possibility that these

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receptor types may exist in the cerebral vasculature has not been investigated. The purpose of this study, therefore, was to further test whether distinct endothelin ET_B receptors mediate relaxation and constriction and, additionally, whether these distinct endothelin ET_B receptors are present in the cerebral vasculature. As we previously demonstrated in the rabbit basilar artery that the endothelin-1 constriction, elicited in the presence of an endothelin ET_A receptor-antagonist, is endothelin ET_B receptor mediated (Zuccarello et al., 1998a), this study tested whether RES-701-1 selectively blocked endothelin ET_B receptor-mediated relaxation in this vessel. Some of these results have appeared in abstract form (Zuccarello et al., 1998b).

2. Materials and methods

2.1. Animal preparation

2.1.1. General

New Zealand White male rabbits (weight, 3–4 kg) were anesthetized with ketamine HCl (30 mg/kg, i.m.) and xylazine (6 mg/kg, i.m.), intubated, and mechanically ventilated with room air supplemented with O_2. The respiratory rate and volume were adjusted to maintain expiratory P_{CO_2} between 35 and 37 mmHg. Heart rate and systemic pressure were measured with the use of a femoral artery catheter. Arterial P_{O_2} and P_{CO_2} were monitored and

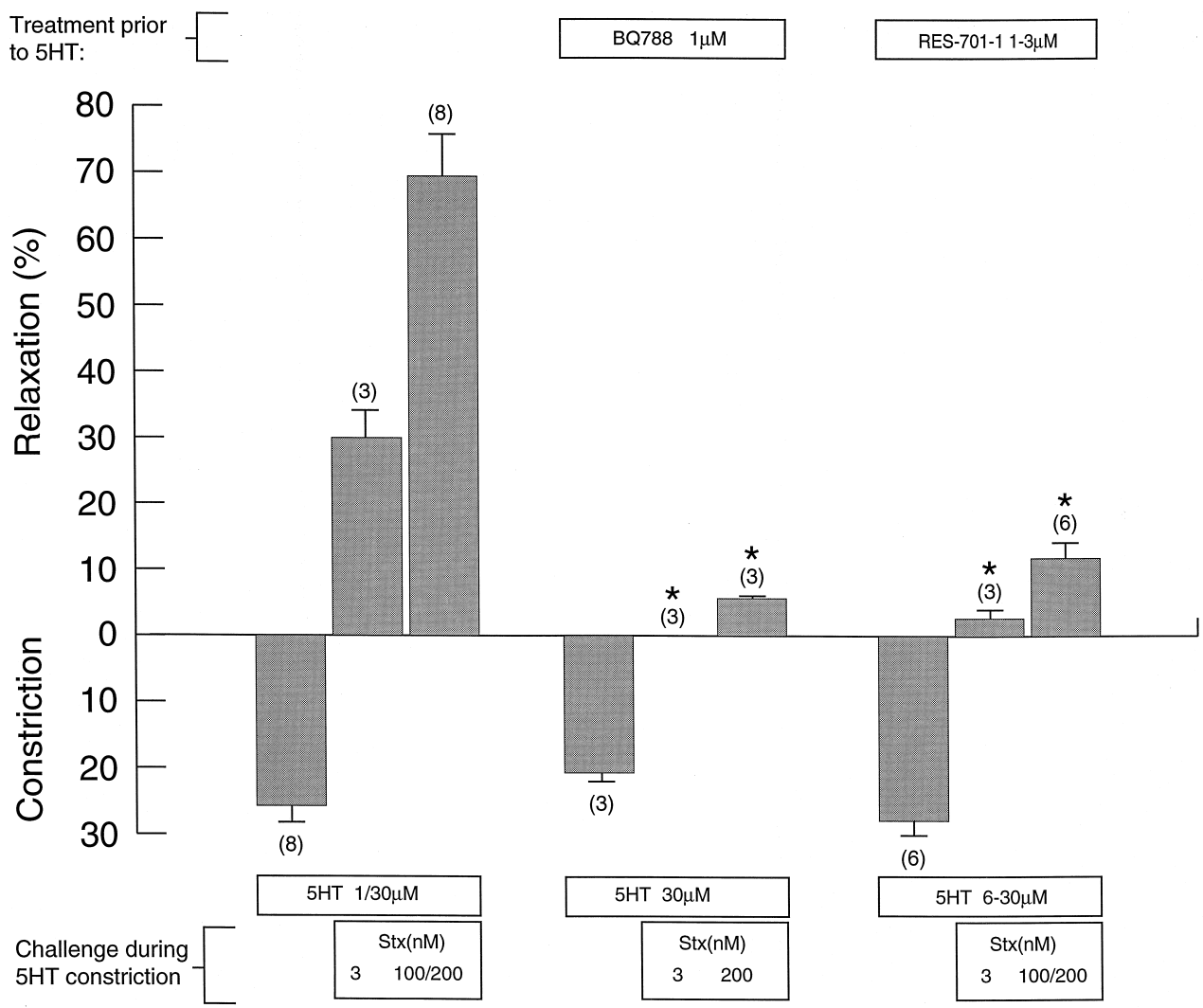

Fig. 1. Effects of BQ788 and RES-701-1 on sarafotoxin S6c-induced relaxation of serotonin-constricted rabbit basilar artery. Basilar artery was treated with 1 μM BQ610 (control), 1 μM BQ610 plus 1 μM BQ788, or to 1 μM BQ610 plus 1 or 3 μM RES-701-1 (synthetic and natural product). After 15 min, vessels were challenged with 1 or 30 μM serotonin (5-HT), which elicited similar magnitudes of constriction (22.6 ± 1.9 (5) and 30.7 ± 4.8 (3)%, respectively; means $\pm$ S.E. (*n*)). Serotonin-constricted vessels were then challenged with sarafotoxin S6c (Stx). Sarafotoxin S6c (0.2 μM)-induced relaxation of 1 μM serotonin constricted vessels, and 0.1 μM sarafotoxin S6c-induced relaxation of 30 μM serotonin constricted vessels were not significantly different (78.6 ± 9.8 (5) and 54.3 ± 4.1 (3)%, respectively; means $\pm$ S.E. (*n*)) and the results combined. Relaxation and constriction were calculated as percent of the serotonin constriction and of baseline diameter, respectively. Shown are means S.E. '*n*' (indicated in parentheses) represents the number of rabbits. * Significantly less than corresponding values from control vessels.

maintained within normal levels by adjusting the respiratory rate and/or tidal volume. Supplemental anesthesia and fluids were administered through a cannulated femoral vein. Core body temperature was monitored rectally and maintained at 37°C with a heating pad.

2.1.2. Basilar artery cranial window

Rabbits were anesthetized, placed in a head holder in the supine position, the clivus exposed by blunt dissection between the carotid sheath and trachea, and the trachea and esophagus retracted laterally. Compression of the carotid arteries and the descending vagus nerves was avoided. The muscle covering the basioccipital bone was removed by electrocautery. A rectangular osteotomy (4–5 mm wide) was then made at the base of the skull between the tympanic bullae with the use of a microdrill and microrongeur under an operating microscope. After a perfect hemostasis was achieved, the dura was opened and excised with microscissors, and the basilar artery exposed.

2.2. Contractility studies

The surgical field was illuminated with a 100-W halogen lamp, which was fitted with a heat filter to avoid warming the cranial window, and was visualized through a trinocular microscope. Basilar artery diameter was measured with a personal computer image analysis system (Image Analyzer, Magiscan) with the use of a video camera mounted on the phototube of the microscope. Head temperature was monitored with a needle inserted in the residual longus colli muscle and was maintained at 37–38°C.

The cranial window was suffused (1 ml/min) with artificial cerebrospinal fluid (mM: NaCl 121.8, KCl 3.2, $CaCl_2$ 2.5, $MgCl_2$ 1.26, $NaHCO_3$ 25.0, D-glucose 3.7; urea 6.0), maintained at 37°C, and gassed with 7% O_2/6% CO_2/87% N_2. Vessel diameter, blood pressure, heart rate, and arterial P_{O_2} and P_{CO_2} stabilized within 45 min after cerebrospinal fluid suffusion, and agents were then suf-

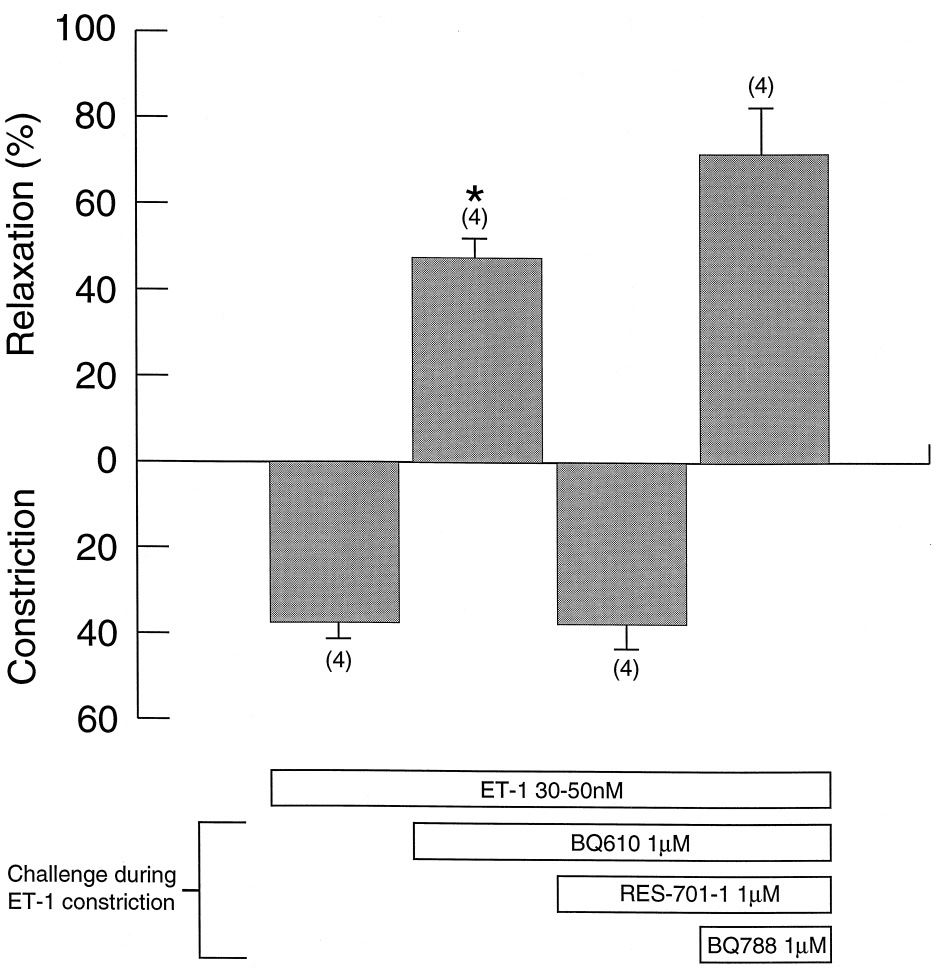

Fig. 2. Effects of RES-701-1 and BQ788 on BQ610-induced relaxation of endothelin-1-constricted rabbit basilar artery. Basilar artery was constricted with endothelin-1 (ET-1), and was then challenged with 1 μM BQ610. The vessel was then exposed to 1 μM RES-701-1 (synthetic and natural product), followed by 1 μM BQ788. Relaxation and constriction were calculated as percent of the endothelin-1 constriction and of baseline diameter, respectively. Shown are means ± S.E. '*n*' (indicated in parentheses) represents the number of rabbits. * Significantly less than BQ610 + RES-701-1 + BQ788 (Student's paired *t*-test).

fused over the craniotomy. Vessel diameter was recorded at the time of the plateau response to each agent. Each value of vessel diameter was the mean of 13 consecutive measurements taken at approximately 10-s intervals.

2.3. Statistical methods

Statistical significance between two means and multiple means was determined using Student's unpaired *t*-test and the Bonferroni procedure, respectively. Significance was accepted at the 0.05 level of probability. The magnitude of constriction was expressed as a percentage of basal diameter, measured in micrometers. The magnitude of relaxation was expressed as a percentage of the constriction, the latter measured as the difference in micrometers between basal and agonist-induced tone. Values are expressed as means ± S.E. '*n*' represents the number of animals.

2.4. Materials

Reagent sources were as follows: American Peptide for endothelin-1, RES-701-1, and sarafotoxin S6c, and Peptides International for BQ610 and BQ788. RES-701-1 was also obtained as a gift from Kyowa Hakko Kogyo.

3. Results

3.1. Endothelin ET_B receptor-mediated relaxation

To test whether endothelin ET_B receptor-mediated relaxation was present in the rabbit basilar artery, vessels were constricted with serotonin, and then challenged with sarafotoxin S6c, an endothelin ET_B receptor agonist (Williams et al., 1991). To prevent possible endothelin ET_A receptor-mediated constriction at greater sarafotoxin S6c concentrations, vessels were also exposed to 1 μM BQ610, an endothelin ET_A receptor antagonist (Ishikawa et al., 1992). Sarafotoxin S6c concentration-dependently relaxed the serotonin constriction (Fig. 1). BQ788 (1 μM), an endothelin $ET_{B1/B2}$ receptor antagonist (Ishikawa et al., 1994), prevented sarafotoxin S6c-induced relaxation (Fig. 1).

3.2. Endothelin ET_B receptor type

To determine the endothelin ET_B receptor type mediating sarafotoxin S6c-induced-relaxation, we tested whether RES-701-1, a purported selective endothelin ET_{B1} receptor antagonist (Tanaka et al., 1995), prevented sarafotoxin S6c-induced relaxation. RES-701-1 (1 and 3 μM) inhibited sarafotoxin S6c-induced relaxation (Fig. 1). The magnitudes of inhibition due to 1 and 3 μM RES-701-1 were similar and the results combined.

We then tested whether RES-701-1 selectively blocked endothelin ET_B receptor-mediated relaxation and not, in addition, endothelin ET_B receptor-mediated constriction. As we previously demonstrated that the endothelin-1 constriction elicited in rabbit basilar artery treated with BQ610 is endothelin ET_B receptor-mediated, i.e., relaxed by BQ788 (Zuccarello et al., 1998a), we tested whether endothelin-1 constricted vessels subsequently treated with 1 μM BQ610 were relaxed by RES-701-1. Endothelin-1 (30–50 nM) constricted vessels by 40%, and 1 μM BQ610 relaxed the constriction by nearly 50% (Fig. 2). However, 1 μM RES-701-1 did not relax the remaining endothelin-1 constriction and, in fact, reconstricted the vessel (Fig. 2). Subsequent addition of 1 μM BQ788 completely relaxed the reconstricted vessel (Fig. 2).

4. Discussion

The present study represents the first demonstration of endothelin ET_{B1} receptors in a cerebral vessel. This demonstration is supported by the observations that the purported selective endothelin ET_{B1} receptor antagonist, RES-701-1 (Tanaka et al., 1995) (1) abolished endothelin ET_B receptor-mediated relaxation, and (2) did not block endothelin ET_B receptor-mediated constriction in the rabbit basilar artery. In fact, RES-701-1 enhanced the endothelin-1 constriction that remained in the presence of BQ610, presumably due to inhibition of endothelin-1-induced endothelin ET_{B1} receptor-mediated relaxation.

While there is some controversy surrounding the presence of distinct endothelin ET_B receptor types, including that cross-talk between the endothelin ET_A and endothelin ET_B receptors underlies the apparent ability of endothelin $ET_{A/B}$ and endothelin ET_B receptor antagonists to block endothelin ET_B receptor-mediated relaxation and not endothelin ET_B receptor-mediated constriction (Clozel and Gray, 1995), the present results cannot be explained by possible cross-talk. That is, RES-701-1 actually enhanced the endothelin-1 constriction elicited in the presence of BQ610, an endothelin ET_A receptor antagonist (Ishikawa et al., 1992). Furthermore, the resulting constriction was relaxed by BQ788, a non-selective endothelin ET_B ($ET_{B1/B2}$) receptor antagonist (Ishikawa et al., 1994).

An additional controversy related to the possibility that distinct endothelin ET_B receptor types mediate relaxation and constriction are reports that RES-701-1 does not selectively block endothelin ET_{B1} receptors (He et al., 1995; Russell and Davenport, 1996). However, the inability to demonstrate RES-701-1 selectivity at endothelin ET_{B1} receptors (He et al., 1995; Russell and Davenport, 1996) may have resulted from the use of synthetic RES-701-1 and not the isolated natural RES-701-1, as the tertiary structure of the synthetic and natural products are different (Katahira et al., 1995). In this regard, the present studies were performed with both natural and synthetic RES-701-1 (see Materials), and similar results were obtained. The apparent endothelin ET_{B1} receptor selectivity of the cur-

rently used synthetic RES-701-1, as compared to the synthetic RES-701-1 used by other investigators, may relate to differences in synthetic procedures (Dr. Shawn Lee, American Peptide, personal communication; although see He et al., 1995).

In summary, the present results demonstrate that distinct endothelin ET_B receptors mediate endothelium-dependent relaxation and constriction in the cerebral vasculature. These results are consistent with the endothelin ET_{B1} and endothelin ET_{B2} receptor-mediation of these responses, respectively, as reported in the peripheral vasculature (Karaki et al., 1994; Sudjarwo et al., 1994; Douglas et al., 1995).

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