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Endothelin ET_B receptor-mediated constriction in the rabbit basilar artery

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Abstract

The present study tests whether endothelin ET_B receptor activation can mediate endothelin-1 constriction in the rabbit basilar artery in situ. Endothelin-1 (30 nM) induced 27% constriction of vessels pretreated with 1 μM BQ610 (homopiperidenyl-CO-Leu-D-Trp (CHO)-D-Trp-OH), an endothelin ET_A receptor antagonist, and the resulting constriction was completely relaxed by BQ788 (*N-cis*-2,6-dimethylpiperidinocarbonyl L-γ-MeLeu-D-Trp ($COOCH_3$)–Nle), an endothelin ET_B receptor antagonist. Similarly, 30 nM endothelin-1 induced 30% constriction of vessels pretreated with 1 μM BQ788, and the resulting constriction was completely relaxed by BQ610. In contrast, sarafotoxin S6c, an endothelin ET_B receptor agonist, did not induce constriction. This study suggests that in the basilar artery (1) endothelin ET_B receptor activation can result in constriction and (2) the ability to elicit constriction is in some way dependent upon the agonist that activates the endothelin ET_B receptor.

Keywords: Endothelin; Basilar artery; Receptor

Although few studies have investigated the relative contribution of endothelin ET_A vs. endothelin ET_B receptor activation in the endothelin-1 constriction of cerebral vessels, the generality that has emerged is that the endothelin-1 constriction is essentially endothelin ET_A receptor-mediated (Adner et al., 1993; Salom et al., 1993; Feger et al., 1994, 1997; Petersson et al., 1996; Nilsson et al., 1997). In possible contrast to the demonstrations that endothelin-1 constriction of cerebral vessels is largely endothelin ET_A receptor-mediated, we concluded that endothelin ET_A receptors mediate a significant component of the endothelin-1-dependent spasm of the rabbit basilar artery following subarachnoid hemorrhage (Zuccarello et al., 1994). While the apparent presence of functional endothelin ET_B receptors in the rabbit basilar artery smooth muscle following subarachnoid hemorrhage may have resulted from the induction of these receptors (Hino et al., 1996), alternatively it may be considered that endothelin-1 constriction of normal rabbit basilar artery also involves endothelin ET_B receptor activation. Therefore, this study tested whether endothelin ET_B receptor activation results in constriction of the rabbit basilar artery.

Rabbit basilar artery cranial window was prepared as previously described (Zuccarello et al., 1994). Drugs were introduced into the cranial window via superfusion in physiologic salt solution. Vessel diameter was measured with image analysis. 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. Statistical significance between means was determined using Student's unpaired *t*-test. Significance was accepted at the 0.05 level of probability. Values are expressed as means $\pm$ S.E. The variable *n* represents the number of animals.

To investigate whether endothelin ET_B receptor activation results in constriction, we tested whether endothelin-1 constricted vessels pretreated with BQ610 (homopiperidenyl-CO-Leu-D-Trp (CHO)-D-Trp-OH), an endothe-

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lin ET_A receptor antagonist. Endothelin-1 (30 nM) in the presence of 1 μM BQ610 induced 27% constriction (Fig. 1). Increasing the BQ610 concentration to 2 μM following attainment of the plateau constriction did not induce relaxation (Fig. 1), suggesting that the BQ610 pretreatment effectively blocked endothelin ET_A receptors.

We then tested whether the endothelin-1 constriction observed in the presence of BQ610 was endothelin ET_B receptor-mediated. Indeed, challenge with 1 and 10 μM BQ788 (*N-cis*-2,6-dimethylpiperidinocarbonyl L-γ-MeLeu-D-Trp ($COOCH_3$)–Nle), an endothelin ET_B receptor antagonist, partially and completely relaxed the endothelin-1 constriction, respectively (Fig. 1).

As a control to determine the ability of BQ610 to inhibit the endothelin-1 constriction, we tested whether BQ610 relaxed endothelin-1 constricted vessels pretreated with BQ788. BQ610 (1 μM) completely relaxed the 30 nM endothelin-1 constriction elicited in the presence of BQ788 (Fig. 1). The magnitude of endothelin-1 constriction elicited in the presence of BQ788 (30%) was similar to that elicited in the presence of BQ610 (27%; Fig. 1), suggesting that cross-talk may exist between the endothelin ET_A and endothelin ET_B receptors.

To further test whether endothelin ET_B receptor activation results in constriction, vessels were challenged with sarafotoxin S6c, a selective endothelin ET_B receptor agonist. However, sarafotoxin S6c concentrations as great as 0.2 μM, and in the presence or absence of 1 μM BQ610, elicited little constriction (data not shown).

The present study suggests that the ability to detect endothelin ET_B receptor-mediated constriction in the rabbit basilar artery is dependent upon the agonist. That is, endothelin-1 induced significant endothelin ET_B receptor-mediated constriction, as defined by BQ788-induced relaxation in the presence of BQ610, while sarafotoxin S6c induced little constriction. The inability of sarafotoxin S6c to induce constriction was unexpected, as the endothelin ET_B receptor-mediated contractile efficacies of sarafotoxin S6c and endothelin-1 are similar. Another explanation is that sarafotoxin S6c may induce greater release of a relaxant factor as a result of endothelial endothelin ET_B receptor activation, thereby preventing endothelin ET_B receptor-mediated constriction. However, the relaxant efficacies of sarafotoxin S6c and endothelin-1 may be similar, as maximal sarafotoxin S6c-induced relaxation of serotonin constricted rabbit basilar artery was approximately 70% (unpublished observation), and maximal endothelin-1-induced relaxation of prostaglandin $F_{2\alpha}$ constricted rat basilar artery in the presence of an endothelin ET_A receptor antagonist was approximately 50% (Feger et al., 1997).

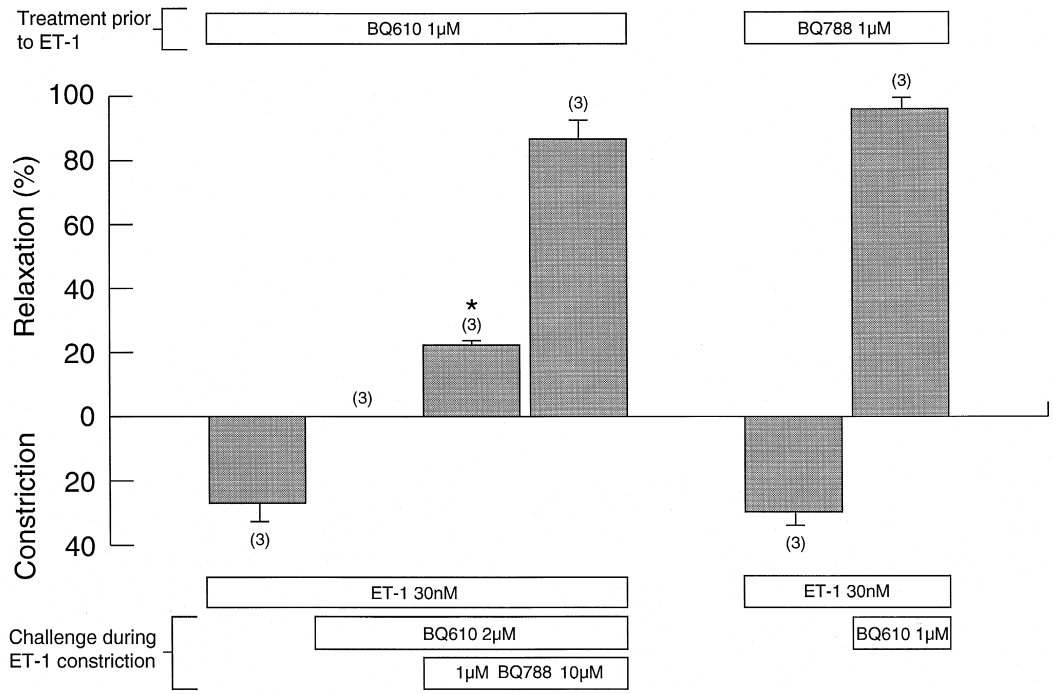

Fig. 1. Effects of BQ788 and BQ610 on the endothelin-1-induced plateau constriction in BQ610- and BQ788-pretreated rabbit basilar artery. Basilar artery was pretreated with 1 μM BQ610 for 15 min prior to 30 nM endothelin-1. Following attainment of a plateau constriction, an additional 1 μM BQ610 was added, followed by 1 and 10 μM BQ788. Other vessels were pretreated with 1 μM BQ788 for 15 min prior to 30 nM endothelin-1 and then, following attainment of a plateau constriction, 1 μM BQ610 added. Relaxation and constriction were calculated as percents of the endothelin-1 constriction and of baseline diameter, respectively. Shown are means $\pm$ S.E. *n*, indicated in parentheses, represents the number of rabbits. $*$ Significantly less than BQ610 plus BQ788.

In contrast to the present demonstration of endothelin ET_B receptor-mediated constriction in the rabbit basilar artery, Petersson et al. (1996) concluded that endothelin-1 constriction of this vessel in vitro was mainly endothelin ET_A receptor-mediated. However, Petersson et al. (1996) demonstrated that an endothelin ET_A receptor antagonist only partially prevented the constriction to 30 nM endothelin-1, and the constriction to 100 nM endothelin-1 remained uninhibited. Similarly, endothelin ET_A receptor antagonist in vitro also competitively shifted the endothelin-1 concentration–constriction curve to the right in cerebral vessels from other species (Adner et al., 1993; Salom et al., 1993; Feger et al., 1994; Nilsson et al., 1997). Thus, it would be of interest to test whether constrictions due to higher endothelin-1 concentrations elicited in the presence of endothelin ET_A receptor antagonist are sensitive to relaxation by endothelin ET_B receptor antagonist.

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References

Adner, M., You, J., Edvinsson, L., 1993. Characterization of ET-A receptors in the cerebral circulation. NeuroReport 4, 441–443.

Feger, G.I., Schilling, L., Ehrenreich, H., Wahl, M., 1994. ET-induced contraction and relaxation of rat isolated basilar artery: effect of BQ-123. J. Cereb. Blood Flow Metab. 14, 845–852.

Feger, G.I., Schilling, L., Ehrenreich, H., Wahl, M., 1997. Endothelium-dependent relaxation counteracting the contractile action of endothelin-1 is partly due to ET_B receptor activation. Res. Exp. Med. 196, 327–337.

Hino, A., Tokuyama, Y., Kobayashi, M., Yano, M., Weir, B., Takeda, J., Wang, X., Bell, G.I., Macdonald, R.L., 1996. Increased expression of endothelin B receptor mRNA following subarachnoid hemorrhage in monkeys. J. Cereb. Blood Flow Metab. 16, 688–697.

Nilsson, T., Cantera, L., Adner, M., Edvinsson, L., 1997. Presence of contractile endothelin-A and dilatory endothelin-B receptors in human cerebral arteries. Neurosurgery 40, 346–353.

Petersson, J., Hanson, G.C., Lindberg, B.F., Högestätt, E.D., 1996. Contractile effect of big ET-1 and its conversion to ET-1 in rabbit cerebral arteries. Naunyn-Schmiedeberg's Arch. Pharmacol. 354, 656–661.

Salom, J.B., Torregrosa, G., Barberá, M.D., Jover, T., Alborch, E., 1993. ET receptors mediating contraction in goat cerebral arteries. Br. J. Pharmacol. 109, 826–830.

Zuccarello, M., Lewis, A.I., Rapoport, R.M., 1994. Endothelin ET_A and ET_B receptors in subarachnoid hemorrhage-induced cerebral vasospasm. Eur. J. Pharmacol. 259, R1–R2.