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Endothelin-1–induced contraction in cerebral vessels mediated by phospholipase C/protein kinase C cascade

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Endothelin-1–induced contraction in cerebral vessels mediated by phospholipase C/protein kinase C cascade. Endothelin (ET) vasoconstricts cerebral vessels potently, an effect mediated by ET_A receptors on the smooth muscle, although the subsequent signaling cascade is unclear. We tested whether the action of ET-1 is mediated by the phospholipase C (PLC)/protein kinase C (PKC) cascade. Isometric force was measured *in vitro* in ring segments of rat basilar (BA) and middle cerebral (MCA) arteries and expressed as a percentage of the contraction to 124 mM K^+. Concentration-effect curves for the constrictor effect of ET-1 (1 pM = 0.3 μM) in control segments or after 25 minutes preincubation with an inhibitor of PLC (neomycin 100 μM) or PKC (H7 10 μM) were constructed under resting tone. In untreated BA, 100 nM ET-1 induced a contraction of 119 ± 5.3% that fell significantly to 97 ± 2.8% and 98 ± 6.7% after neomycin or H7 pretreatment, respectively. In MCA, 100 nM ET-1 induced a contraction of 105 ± 3.2% that fell significantly to 93 ± 6.3% and 64 ± 8.1% after neomycin or H7, respectively. There was no significant shift of the ET-1 EC_{50} after PKC inhibition in either vessel or PLC inhibition in BA. In summary, the amplitude of ET-1–induced contraction in cerebral vessels is reduced significantly, whereas the sensitivity to the agonist is unchanged, after blocking PLC with neomycin or PKC with H7. This indicates noncompetitive inhibition. ET-1–induced contraction in cerebral vessels thus depends on activation of the PLC/PKC cascade.

Endothelins (ETs) dilate and constrict potently in the cerebral circulation. ET-1 is predominantly a vasoconstrictor [1]. In the isolated rat basilar artery (BA), dilation becomes evident only after precontraction and additional blockade of the ET_A receptor [2]. *In vivo*, minor dilation of pial arteries has been observed on exposure to 100 pM ET-1 [3, 4]. In the rat BA, ET-3 dilates at low concentrations (100 pM–10 nM) but constricts at higher concentrations if vessels are precontracted [5]. These opposite effects have been attributed to two different types of ET receptors: ET_A and ET_B. The ET_A receptor has a higher affinity for ET-1 and ET-2 than for ET-3, whereas the ET_B receptor is nonselective in this respect. The vascular ET_A receptor mediating vasoconstriction is located on the smooth muscle cell [6, 7], whereas the vascular ET_B receptor mediating relaxation is on the endothelial cell and is coupled to endothelial production of nitric oxide [5]. There is also evidence for a contraction-mediating ET_{B2} receptor subtype [8]. This study examines whether the ET-1–induced constriction of cerebral vessels via the ET_A receptor is mediated by activation of phospholipase C (PLC) and protein kinase C (PKC).

Key words: basilar artery, middle cerebral artery, endothelin, concentration-effect curves, neomycin.

METHODS

Male Wistar-Kyoto rats (280 to 320 g) were anesthetized with 6.9 mg/kg xylazine (Bayer, Leverkusen, Germany) and 30 mg/kg ketamine (Parke Davis, Berlin, Germany). Ring segments of middle cerebral artery (MCA) and BA were prepared for measurement of isometric force as described by Feger et al [1]. Segments were transferred into 5-ml organ baths containing modified Krebs-Högestätt solution (in mM): NaCl, 119; KCl, 4.6; NaH_2PO_4, 1.2; $CaCl_2$, 1.5; $MgCl_2$, 1.2; $NaHCO_3$, 15; glucose, 10. The bath solution was bubbled continuously with humidified 90% O_2/10% CO_2 to give a pH of approximately 7.30 at 37°C. The ring segments were mounted on two L-shaped wires (70 μm diameter stainless-steel for BA; 50 æm diameter tungsten for MCA), one of which was connected rigidly to the bath and the second to a force transducer. Concentration-effect curves were constructed under resting tone for the constrictor effect of ET-1 (1 pM = 0.3 μM) in untreated control segments and after 25 minutes preincubation with a PLC inhibitor (neomycin, 100 μM) or with a PKC inhibitor (H7, 10 μM). Contractions are expressed as a percentage (mean ± SEM) of the contraction induced by 124 mM K^+. The pD_2 ($-\log EC_{50}$) was calculated individually for each segment. Statistical analysis was performed by Duncan's test. $P < 0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Endothelin-1 vasoconstricted BA and MCA concentration dependently with maxima of 119% ± 5.3 and 105% ±

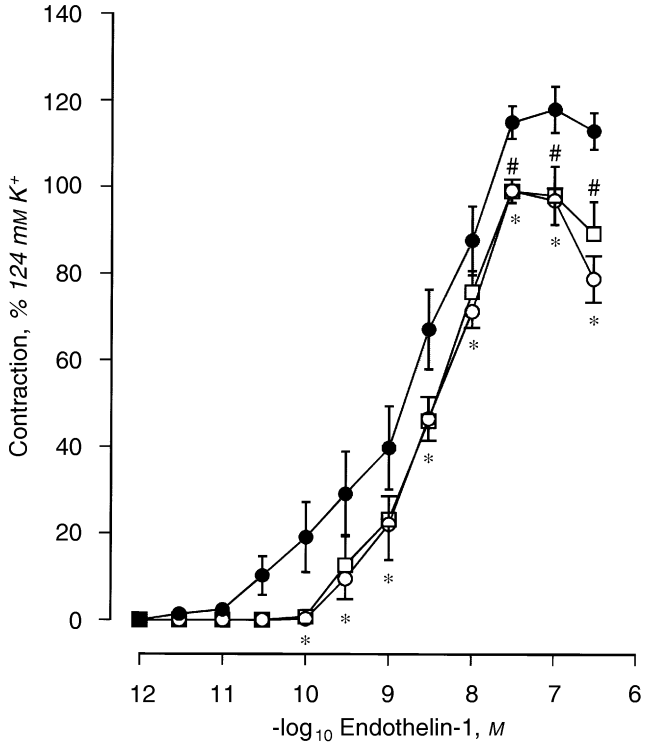

Fig. 1. Concentration-effect curves for the vasoconstriction of rat basilar artery rings elicited by endothelin-1 (ET-1) in the absence (control) or presence of H7 or neomycin. *, $^{\#}P < 0.05$ versus control for neomycin and H7, respectively. Symbols are: (●) control; (□) H7 preincubation; (○) neomycin preincubation.

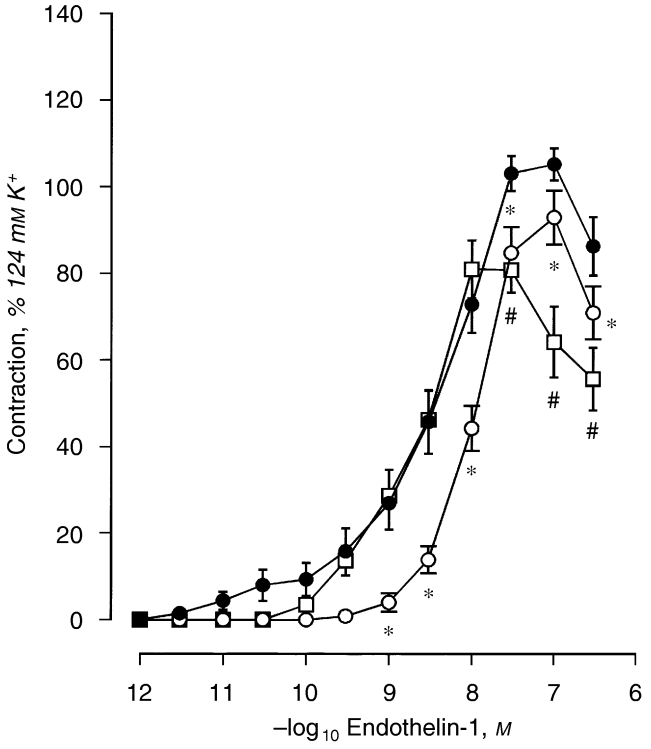

Fig. 2. Concentration-effect curves for the vasoconstriction of rat middle cerebral artery rings elicited by endothelin-1 (ET-1) in the absence (control) or presence of H7 or neomycin. *, $^{\#}P < 0.05$ versus control for neomycin and H7, respectively. Symbols are: (●) control; (□) H7 preincubation; (○) neomycin preincubation.

3.2% at 100 nM and pD_2 values of 8.76 ± 0.25 and 8.36 ± 0.09, respectively. Preincubation with the ET_A-receptor antagonist RO-61-1790 shifted the concentration-effect curves for ET-1 in both vessels to the right without attenuating the maximal response, indicating competitive antagonism. This confirms that in rat cerebral vessels, the ET_A receptor mediates endothelin-dependent vasoconstriction [1]. Preincubation of BA or MCA with neomycin or H7 weakened the ET-1–elicited contraction over the whole concentration range. In BA, the maximum response fell significantly to 97 ± 2.8% and 98 ± 6.7% with pD_2 values of 8.47 ± 0.01 and 8.51 ± 0.17 after neomycin or H7 pretreatment, respectively. In MCA, the maximum fell to 93 ± 6.3% (pD_2 7.80 ± 0.07) after neomycin and to 64 ± 8.1% (pD_2 8.56 ± 0.11) after H7. Because the maximum amplitudes, but not the pD_2, for both vessels fell, H7 blocks the ET-1–mediated constriction noncompetitively. Neomycin inhibits ET-1 in BA noncompetitively, but in the MCA, both pD_2 and the maximum response decreased. ET-1–induced contraction of BA and MCA thus depends on ET_A receptor-mediated activation of the PLC/PKC cascade.

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