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Role of angiotensin II receptor subtypes in porcine basilar artery: Functional, radioligand binding, and cell culture studies

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Abstract

We aimed to clarify responsiveness to angiotensin (Ang) II in the porcine basilar artery and the role of Ang II receptor subtypes by functional, radioligand binding, and cell culture studies. Ang II induced more potent contractions in the proximal part than in the distal part of isolated porcine basilar arteries. The contraction induced by Ang II was inhibited by the Ang II type 1 (AT_1) receptor antagonist losartan, but the Ang II type 2 (AT_2) receptor antagonist PD123319 enhanced it. After removal of the endothelium, the effect of losartan remained but the effect of PD123319 was abolished. The specific binding site of [^{3}H]Ang II on the smooth muscle membrane was inhibited by losartan, but not by PD123319. Stimulation of angiotensin II increased nitric oxide (NO) production in cultured basilar arterial endothelial cells. This production was inhibited by PD123319 and the NO synthase inhibitor L-N^G-nitroarginine. These results suggest that the contraction induced by Ang II might be mediated via the activation of AT_1 receptors on the basilar arterial smooth muscle cells and be modulated via the activation of AT_2 receptors on the endothelial cells, followed by NO production.

Keywords: Angiotensin II receptors; Cerebral arteries; Endothelium; Losartan; Nitric oxide; PD123319

Introduction

Angiotensin (Ang) receptor subtypes are generally classified into two main groups, Ang II type 1 (AT_1) and Ang II type 2 (AT_2) receptors (Widdop et al., 2003). The distributions of the two receptor subtypes are different (Timmermans et al., 1993). In general, AT_2 receptors are predominant in the fetus; however, after birth the expression of AT_2 receptors is restricted to some regions and AT_1 receptors become predominant (Matsubara, 1998; Horiuchi et al., 1999). The AT_1 receptor is concerned with vasoconstriction in various arteries, whereas the AT_2 receptor is concerned with vasorelaxation in the uterine (McMullen et al., 1999; Hannan et al., 2003), mesenteric (Matrougui et al., 1999; Touyz et al., 1999; Matrougui et al., 2000), and cerebral (Tsutsumi and Saavedra, 1991) arteries. However, the role of the AT_2 receptor remains unclear.

Ang II induces contraction in the basilar arteries of the monkey (Toda et al., 1990), rabbit (Zerrouk et al., 1996), and dog (Manabe et al., 1989; Yen et al., 1990). However, these reports have not demonstrated the Ang receptor subtypes responsible for contraction in these arteries. Similarly, there is no information about responsiveness of the porcine basilar artery to Ang II and its related Ang receptor subtypes. Therefore, we aimed to clarify the response of the porcine basilar artery to Ang II and determine the role of Ang II receptor subtypes in this response by functional, radioligand binding, and cell culture studies.

Material and methods

Tissue preparation

Basilar arteries were obtained from freshly slaughtered pigs (both sexes, about 6 or 7 months old, Landrace–Large White–Duroc crossbreed) at a local slaughterhouse and transferred to our laboratory in ice-cold physiological saline (119 mM NaCl,

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4.7 mM KCl, 1.6 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 10 mM glucose, pH 7.4) aerated with carbogen (95% (v/v) O_2, 5% (v/v) CO_2). Each artery was dissected free of adherent tissues.

Reagents

We used the following reagents: Ang II acetate salt, [Sar[1], Thr[8]]angiotensin II, bradykinin, PD123319 ditrifluoroacetate salt, uridine 5′-triphosphate sodium salt (UTP), Dulbecco's modified Eagle's medium (DMEM), nutrient mixture F-12 HAM, penicillin, streptomycin and amphotericin B (Sigma, Saint Louis, MO, USA), heat-inactivated horse serum (Invitrogen Corp., NY, USA), fluorescent acetylated low-density lipoprotein (Harbor Bio-Product, Norwood, MA, USA), Ang II (5-L-isoleucine), [tyrosyl-3.5-^{3}H(N)] (Perkin Elmer, Boston, MA, USA), NO_2/NO_3 assay kit (Wako, Osaka, Japan). Losartan potassium was a gift of Merck and Co., Inc. (Whitehouse Station, NJ, USA).

Functional study

Several (three or 4) rings approximately 4 mm long were cut from each artery. The rings were mounted vertically between two L-shaped stainless steel holders, with the upper part fixed to an isometric force transducer (TB-611T, Nihon Kohden Kogyo, Tokyo, Japan), and immersed in a 5-ml water-jacketed organ bath containing oxygenated salt solution at 37 °C (pH 7.4). Each suspended ring was left to equilibrate for at least 120 min under a resting tension of 7.5 mN. This tension was chosen because it allowed us to induce maximum contractions in the artery. KCl (60 mM) was applied every 30 min until the amplitude of the contraction reached a constant value. Changes in the KCl concentration of the physiological saline were compensated for by equimolar adjustment of the NaCl concentration. The isometric tension was displayed on an ink-writing recorder (WI-641G, Nihon Kohden Kogyo, Tokyo, Japan). The cumulative concentration–response curve of Ang II or the contraction with a single application was obtained by adding a solution of Ang II directly to the fluid in the bath. Antagonists were added to the bathing media 30 min before Ang II. There were no effects of antagonists on the resting tension.

The presence of endothelial cells was confirmed pharmacologically by testing the relaxant response to bradykinin under precontracted conditions with UTP (this response is abolished by endothelial denudation; Miyamoto et al., 1999), and morphologically by scanning and transmission electron microscopy after the experiments.

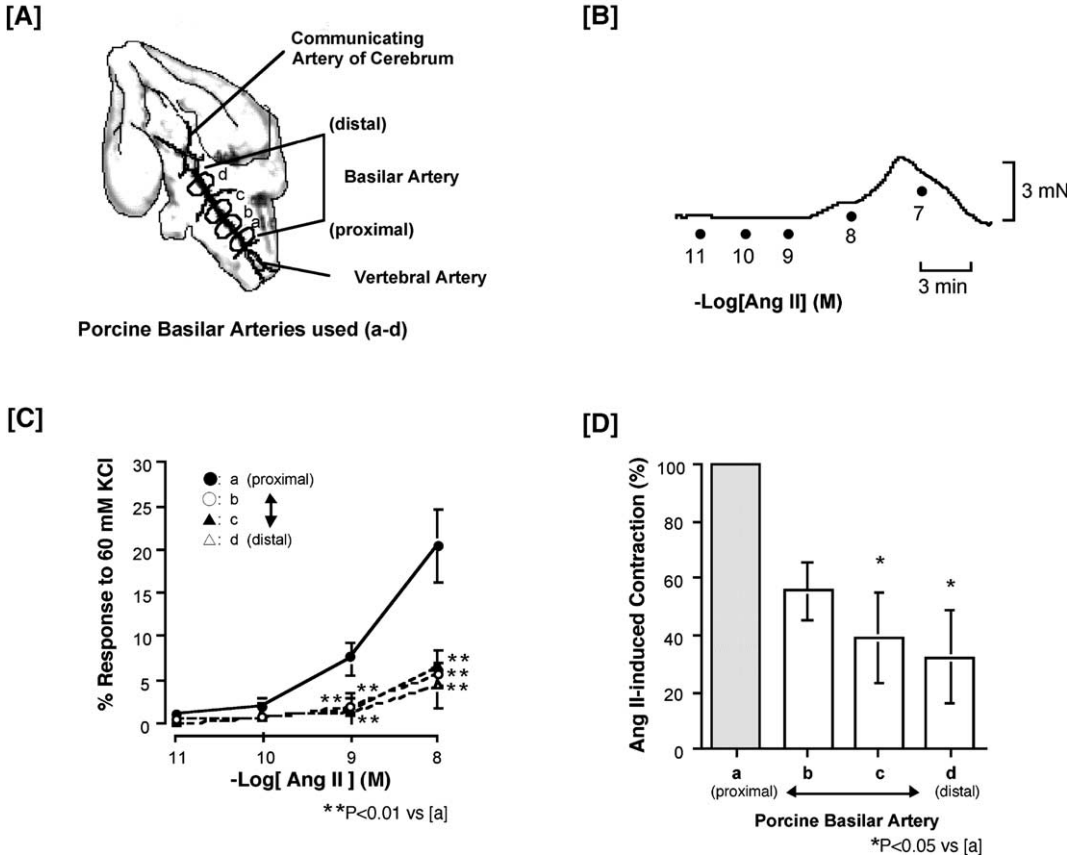

Fig. 1. Typical response to angiotensin (Ang) II [B] in isolated porcine basilar arterial rings obtained from region a [A]. Regional differences in responsiveness to Ang II are shown in [C]. After removal of the endothelium, the regional difference in response to Ang II (10 nM) remained [D]. The regions of arteries (a–d) used in [C] and [D] were shown in [A]. Each bar represents the mean±s.e.m. from 7 [C] or 5 [D] pigs.