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Effects of Thiopental, Pentobarbital, and Ketamine on Endothelin-Induced Constriction of Porcine Cerebral Arteries

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Contractile mechanisms of endothelin, a newly isolated vasoactive substance from endothelium, were evaluated in anterior cerebral arteries (ACA). Furthermore, the effects of thiopental, pentobarbital, ketamine, and diltiazem on the endothelin-induced cerebral vasoconstriction were also studied. Endothelin induced cerebral arterial contractions in concentrations above 3×10^{-10} M. The median effective concentration (ED_{50}: $\times 10^{-9}$ M) of endothelin was 2.1 ± 0.7 (n = 6). Endothelin did not elicit contractions in preparations soaked in Ca^{2+}-free solution, but addition of 2.5 mM Ca^{2+} to the baths induced marked contractions. Thiopental and pentobarbital attenuated endothelin-induced contractions at concentrations above 3×10^{-4} M, while ketamine was effective above 10^{-3} M. In contrast, diltiazem decreased endothelin-induced vasoconstriction at 10^{-6} M. The findings suggest that endothelin may cause contractions of porcine cerebral arteries by influx of Ca^{2+} through Ca^{2+} channels. The cerebral vasomotion induced by endothelin, however, does not seem to be influenced by clinical doses of barbiturates and ketamine. (Key words: Anesthetics: intravenous; ketamine; pentobarbital; thiopental. Artery: cerebral. Pharmacology, calcium channel blocking drug: diltiazem. Species: swine. Vasoconstriction: endothelin.)

ENDOTHELIN, an endogenous potent vasoconstrictor of the 21 aminopeptide type, has recently been isolated from endothelial cells.[1] This substance is thought to be important in the control of local blood flow[1] because it is released by several stimuli from endothelial cells.[2,3] Disturbances in the control of endothelin production may contribute to the pathogenesis of vascular spasm.[1] It has been reported that barbiturates and ketamine, which may greatly alter cerebral hemodynamics, influence transmembrane calcium movements in cerebral arteries.[4–6] Recent studies have suggested that endothelin may cause contraction through activating influx of calcium.[1,7] Because knowledge of the interactions between endothelin and the anesthetics in cerebral arteries may provide better understanding of cerebral hemodynamics under anesthesia, we have investigated the effects of barbiturates (thiopental and pentobarbital), ketamine, and diltiazem on the endothelin-induced contractions in porcine cerebral arteries.

Materials and Methods

Porcine brains were obtained immediately after slaughter and immersed in cold modified Krebs solution. The ring segments of anterior cerebral arteries (ACA) were isolated (diameter: 0.5–1 mm; length: 3 mm). The preparations were fixed vertically between hooks under a resting tension of 1.5 g in 20-ml siliconized glass tissue baths containing a modified Krebs solution. The composition of the modified Krebs solution was as follows (mM): Na^+ 143; K^+ 5.9; Ca^{2+} 2.5; Mg^{2+} 1.2; Cl^- 153.9; HCO_3^- 25; SO_4^{2-} 1.2; $H_2PO_4^-$ 1.2; dextrose 10. The solution was maintained at 37° C and aerated with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). The specimens were connected to a transducer (model TB-612T, Nihon Koden Kogyo Co, Tokyo, Japan) and the changes in isometric tension were measured. After a 1-hour equilibration period, the contractions with 10^{-1} M potassium chloride were obtained. After washing with fresh Krebs solution and stabilizing the resting tension of the arteries, endothelin was added to the baths in cumulative fashion ranging from 10^{-11}–10^{-7} M. The contraction by endothelin was expressed as percent contractions of that by 10^{-1} M potassium chloride. To evaluate the relation between Ca^{2+} and endothelin-induced cerebral vasoconstriction, the contractions with 10^{-1} M potassium chloride were obtained first. After washing, the preparations were soaked in a Ca^{2+}-free Krebs solution for 30 min, during which the bathing solution was replaced every 10 min. Then, 10^{-11}–10^{-7} M endothelin was applied to the bath. After the responses to 10^{-7} M endothelin were obtained, 2.5 mM Ca^{2+} was added to the bath.

In the second part of this study, the effects of thiopental, pentobarbital, ketamine, or diltiazem on endothelin-induced contractions were studied in porcine anterior cerebral arteries. After contractions with 10^{-9} M endothelin were stabilized, 10^{-6}–10^{-3} M thiopental, pentobarbital, ketamine, or 10^{-7}–10^{-4} M diltiazem were applied to the baths in cumulative concentrations. At the end of the experiments 10^{-4} M papaverine was added. Relaxation pro-

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duced by thiopental, pentobarbital, ketamine, or diltiazem was expressed as the percent relaxation of that by 10^{-4} M papaverine.

The values presented in the text and figures are expressed as mean ± standard error of mean (SEM). Doses of endothelin required to produce 50% maximal contractions (ED_{50}) were calculated by probit transformation.[8] The percent relaxations by barbiturates, ketamine, and diltiazem were analyzed statistically by one-way analysis of variance followed by least significance differences (LSD) test for multiple comparisons. $P < 0.05$ was considered to be statistically significant. Endothelin was purchased from Peptide Institute Inc., Osaka, Japan.

Results

Endothelin elicited contractions of anterior cerebral arteries in concentrations greater than 3×10^{-10} M (fig. 1, upper panel). The values of median effective concentrations (ED_{50}; $\times 10^{-9}$ M) of endothelin-induced contractile responses of anterior cerebral artery were 2.1 ± 0.7 (n = 6). The maximum percentage of contraction following endothelin as compared with that following 10^{-1} M potassium chloride was 104 ± 10.1 (n = 6). In Ca^{2+}-free solutions, contractions induced by 10^{-11}–10^{-7} M endothelin were not observed, while addition of 2.5 mM Ca^{2+} to the baths caused marked contractions (fig. 1, lower panel). The contractions induced by restoration of 2.5 mM Ca^{2+} were 125.0 ± 11.6% (n = 5) of those by 10^{-1} M potassium chloride.

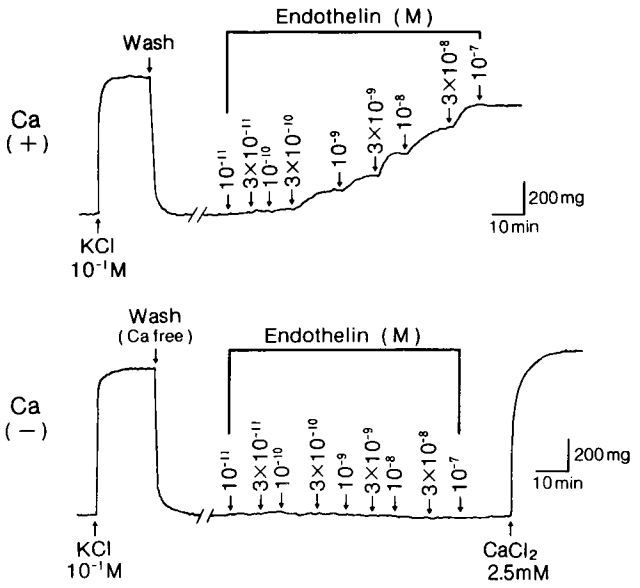

FIG. 1. *Upper panel:* Typical tracings of the contractile responses to endothelin in anterior cerebral artery in the presence of Ca^{2+}. Endothelin-induced contraction at 3×10^{-10} M. *Lower panel:* Typical tracings of endothelin-induced responses in anterior cerebral arteries soaked with Ca^{2+}-free solutions. Endothelin, 10^{-11}–10^{-7} M, did not induce contraction, while addition of 2.5 mM Ca^{2+} to the baths caused marked contraction.

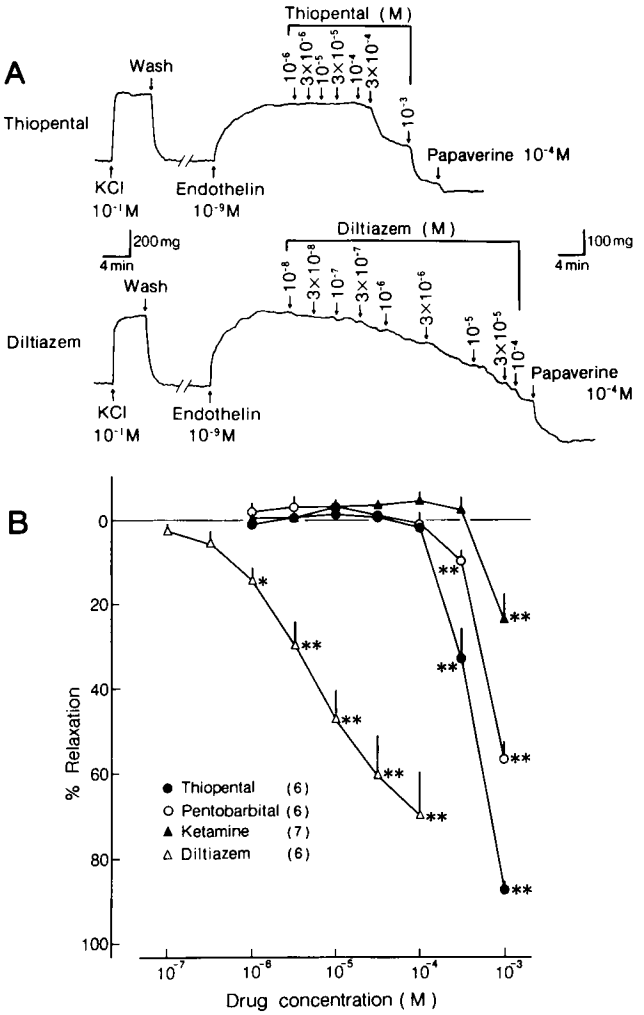

FIG. 2. *A* Typical tracings of the relaxant responses to thiopental and diltiazem in anterior cerebral arteries previously contracted with 10^{-9} M endothelin. Thiopental attenuated the endothelin-induced contraction at high concentrations. *B* The attenuation by barbiturates (thiopental: ● and pentobarbital: ○), ketamine (▲), and diltiazem (△) of the endothelin-induced cerebral vasoconstriction. The number of preparations is indicated in parentheses. $*P < 0.05$, $**P < 0.01$, significantly decreased the endothelin-induced contraction. High concentrations of thiopental, pentobarbital, and ketamine attenuated the endothelin-induced contraction, whereas diltiazem attenuated it at lower concentrations.

Thiopental and pentobarbital at concentrations above 3×10^{-4} M attenuated the endothelin-induced contractions. Ketamine at 10^{-3} M attenuated the endothelin-induced contractions. In contrast, diltiazem at 10^{-6} M antagonized the endothelin-induced contractions (fig. 2). The contractions induced by 10^{-9} M endothelin were 69.0 ± 4.8% (n = 25) of those induced by 10^{-1} M potassium chloride.

Discussion

Endothelin, a newly isolated substance from endothelial cells, has a potent, strong, and characteristically long-lasting vasoconstrictor activity.[1] With regard to the contractile mechanism of endothelin in cerebral arteries, Saito *et al.*[7] reported that endothelin caused contraction of cat cerebral arteries through activating the influx of Ca^{2+}. These findings are in agreement with our present study, because the endothelin-induced cerebral vasocontraction was shown to be highly susceptible to the calcium channel blocking drug diltiazem, and addition of Ca^{2+} to the preparations pretreated with Ca^{2+}-free solution and endothelin caused marked contraction. These findings suggest that endothelin may contract porcine cerebral arteries by an influx of Ca^{2+} through Ca^{2+} channels.

Yanagisawa *et al.*[1] reported that endothelin may be important in the control of systemic blood pressure and/or local blood flow. Recent work has showed that endothelin production in endothelial cells is enhanced by shear stress[2] or thrombin generated during the clotting of blood.[1,3] Disturbances in the control of endothelin production would lead to hypertension or vasospasm.[1] Thus, endothelin is thought to be a possible mediator-controlling local blood flow as well as vasospasm after hemorrhage. It has been reported that cerebral vasospasm may occur especially in large cerebral vessels.[9] Recent work showed that intracisternal injection of lowest dose of endothelin produced prolonged cerebral vasospasm.[10] Studying the effects of anesthetics on cerebral vascular tone induced by endothelin, a possible mediator for cerebral circulation and/or for cerebral vasospasm, may give us important knowledge for control of cerebral circulation during anesthesia. In the previous *in vitro* studies,[5,6] barbiturates or ketamine attenuated agonist-induced cerebral vasocontraction through a blocking of Ca^{2+} influx. In the present study, high concentrations of barbiturates (3×10^{-4} M) and ketamine (10^{-3} M) also attenuated the endothelin-induced vasocontraction. However, clinical concentrations of barbiturates (10^{-5}–2×10^{-5} M)[11] and ketamine (10^{-4} M)[12] neither enhanced nor attenuated the endothelin-induced cerebral vasocontraction. Thus, the cerebral vasomotion induced by endothelin does not seem to be influenced by clinical doses of barbiturates or ketamine. A calcium channel-blocking drug might be one of drugs of choice to relieve cerebral vasospasm during anesthesia because low concentrations of diltiazem attenuated the cerebral vasoconstriction induced by endothelin, a possible mediator of cerebral vasospasm.

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