F) Fluorescence spectroscopy. Another method of studying structural alterations in proteins is the examination of the intrinsic fluorescence. Compared with native LDH, the maximum signal intensity at 340 nm of the LDH_{tr} is about 30% reduced.

Reactions with antibodies. Another mechanism of reactivation was observed in the presence of antibodies directed against native LDH. Antibodies react with surface determinants of a protein, which are elements of the tertiary structure. Antibodies against native LDH also react with the dimers, and this results in a reactivation of up to 70% (fig. 8, 2). Reactivation of the dimers was only detected in the supernatant solution at low concentrations of antibodies. At higher concentrations of antibodies a precipitation of antigen-antibody-complex starts, whose course is similar to that observed for the native enzyme. This result implies that the reactive surface determinants of LDH_{tr} and the native enzyme have extensive similarities. In the complex formed with low concentration of antibodies the LDH_{tr} is reactivated but not precipitated (soluble complex). This increase of activity is a result of a rearrangement of structural deviations and reassociation directed by the antibodies. Only the reactivated form of the LDH_{tr} is able to precipitate with higher concentrations of antibodies such as the native LDH. On the other hand, the ternary complex (LDH_{tr}-NAD-SO_3) and the native enzyme show similar precipitation profiles at the beginning of the reaction with antibodies, which indicated that both have the same surface determinants (fig. 8, 3 and 8, 1). This corroborates with the result that structural properties are rearranged when the ternary result that structural properties are rearranged when the ternary complex is formed or the active site is rearranged.

Abbreviations. CD, circular dichroism; DEAE, diethylaminoethyl; fru-1,6-P_2, fructose-1,6-bisphosphate; IgG, immunoglobulin G; LDH_{tr}, tryptic digested lactate dehydrogenase (isoenzyme from porcine heart) without any ligands); LDH, lactate dehydrogenase (isoenzyme from porcine heart); TPCK, N-tosyl-L-phenylalanyl-chloromethane; Tris/HCl, tris(hydroxymethyl)-aminomethane hydrochloride.

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High sensitivity of porcine cerebral arteries to endothelin

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Summary. The threshold concentration of endothelin to induce contractions in porcine cerebral arteries (anterior cerebral, Willis ring and basilar artery) was lower than those for coronary and renal arteries. The median effective concentrations (ED_{50}) of endothelin in cerebral arteries were also significantly lower than those for coronary and renal arteries. There was no significant difference in the sensitivity to endothelin among cerebral arteries, or between coronary and renal arteries. The maximal percentage of contractions induced by endothelin, as compared to that induced by 10^{-1} M potassium chloride, was not significantly different between the arteries.
Key words. Endothelin; porcine; cerebral artery; coronary artery; renal artery.

Large cerebral arteries play a major role in the regulation of blood flow in the brain [1]. With regard to the effects of vasoactive substances on cerebral and peripheral arterial tonus, there are larger differences between cerebral and peripheral arteries in the sensitivity to agonists [2, 3]. This heterogeneity of response to various agonists exists even among the cerebral arteries [4]. Endothelin, which has recently been isolated from endothelial cells, plays an im-

portant functional role in the regulation of local blood flow[4] and may be a candidate as a causative agent of cerebral vasospasms[5]. It has been reported that large cerebral arteries extending from Willis rings are responsible for cerebral vasospasm after subarachnoid hemorrhage[6]. However, there have been no reports concerning differences in the susceptibility to endothelin among cerebral arteries or between cerebral and peripheral arteries. We therefore examined the contractile responses to endothelin in cerebral (basilar, anterior cerebral and Willis rings), coronary and renal arteries to evaluate the significance of endothelin in cerebral circulation.

Materials and methods

Porcine brain, heart and kidney were obtained immediately after slaughter and immersed in cold modified Krebs solution. Ring segments of anterior cerebral, Willis ring and basilar arteries, and distal portions of the left-descending coronary and the interlobular renal arteries (diameter 0.6–1.0 mm, length 3 mm) were isolated. The preparations were fixed vertically between hooks under a resting tension of 1.5 g in 20-ml siliconized glass tissue baths containing modified Krebs solution. The composition of modified Krebs solution was as follows (mM): Na^+ 143.0; K^+ 5.9; Ca^{2+}; Mg^{2+} 1.2; Cl^- 153.9; HCO_3^- 25.0; SO_4^{2-} 1.2; $H_2PO_4^-$ 1.2; dextrose 10.0. 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.) and the changes in isometric tension were measured.

Following a 1-h equilibration period, contraction was obtained with 10^{-1} M potassium chloride. After washing with fresh Krebs solution and stabilizing the resting tension of these arteries, endothelin (Peptide Institute Inc. Osaka, Japan) was added to the baths cumulatively in concentrations ranging from 10^{-11} to 10^{-7} M. Contraction induced by endothelin was expressed as a percentage of contraction induced by 10^{-1} M potassium chloride. The values presented in the text and figures are expressed as means ± standard errors of means (SEM). Doses of endothelin required to produce 50% maximal contractions (ED_{50}) were calculated by probit transformation[7]. Statistical analyses were performed using one-way analysis of variance followed by least significant difference (LSD) test for multiple comparisons. Significance level was set at $p < 0.05$.

Results

Typical recordings of the contractile responses to endothelin in basilar, coronary and renal arteries are shown in figure 1. The threshold concentrations of endothelin to induce contraction were 10^{-10} to 3×10^{-10} M in cerebral arteries and 10^{-9} to 3×10^{-9} M in coronary and renal arteries. The values of median effective concentrations (ED_{50}; $\times 10^{-9}$ M) for endothelin-induced contrac-

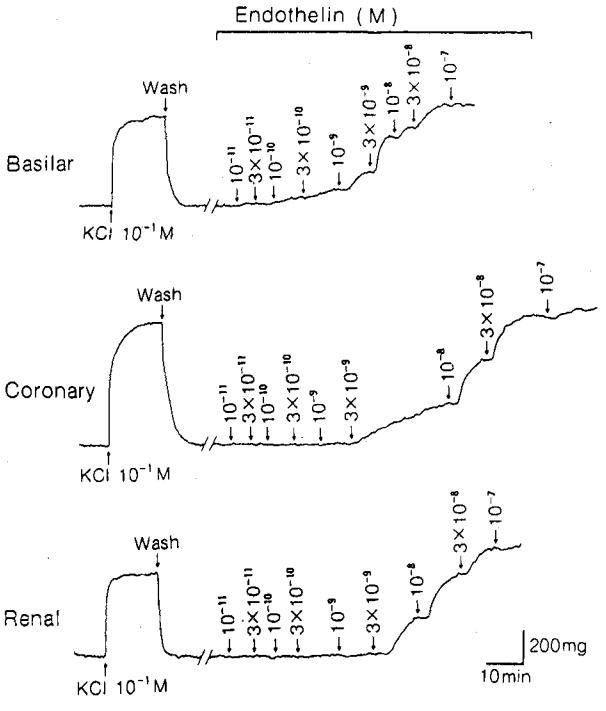

Figure 1. Typical tracings of the responses to endothelin in basilar, coronary and renal arteries. Endothelin induced contractions at 10^{-10} M in basilar arteries and at 3×10^{-9} M in coronary and renal arteries.

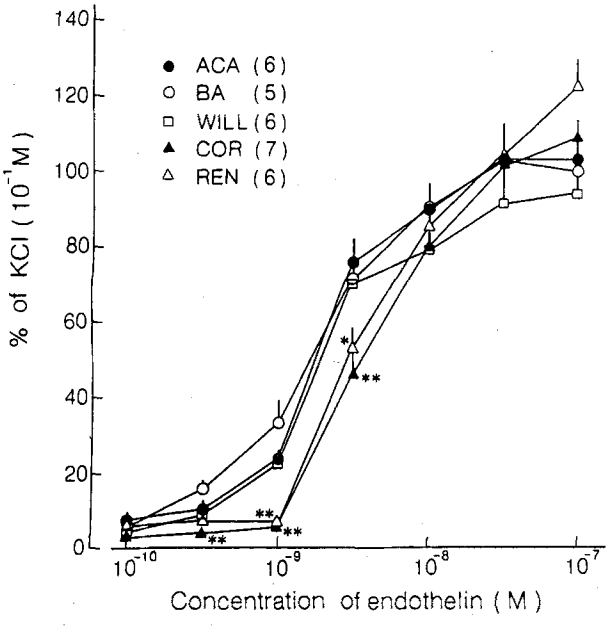

Figure 2. The responses to endothelin in anterior cerebral (ACA), basilar (BA) and Willis ring (WILL), coronary (COR) and renal (REN) arteries. The number of preparations is indicated in parentheses. The responses to endothelin in cerebral arteries were more sensitive than those in coronary and renal arteries. Symbols ($^*p < 0.05$; $^{**}p < 0.01$) indicate significant differences from the values in cerebral arteries at each endothelin concentration.

tile responses were 1.9 ± 0.2 (n = 8) in anterior cerebral arteries, 1.9 ± 0.4 (n = 6) in Willis ring and 1.7 ± 0.8 (n = 5) in basilar arteries, 4.2 ± 1.9 (n = 7) in coronary arteries and 4.6 ± 1.4 (n = 6) in renal arteries. The ED_{50} values for cerebral arteries were significantly ($p < 0.01$) less than those of coronary and renal arteries. There were no significant differences in either ED_{50} or the contractions at each endothelin concentration among cerebral arteries or between coronary and renal arteries. The maximal contractions following endothelin, expressed as a percent of those induced by 10^{-1} M potassium chloride, were 109.3 ± 10.4 (n = 8) in anterior cerebral arteries, 93.9 ± 12.4 (n = 6) in Willis ring, 102.6 ± 18.1 (n = 5) in basilar arteries, 108.5 ± 4.6 (n = 7) in coronary arteries and 123.3 ± 7.0 (n = 6) in renal arteries, respectively, with no significant differences among the arteries. The increased tone induced by endothelin did not return to a resting level within 60 min, even after repeated washing, in the arteries tested.

Discussion

The present study indicated that cerebral arteries were more sensitive to endothelin than coronary and renal arteries, since the threshold concentration of endothelin to induce contraction and the ED_{50} values of the endothelin-induced contractile responses in the cerebral arteries were lower than those in coronary and renal arteries. In a previous report by Yanagisawa et al.[4], no variations in the effect of endothelin were observed in various large isolated arteries in vitro. However, they did not compare the effect of endothelin on various arteries in the same species. In contrast, in vivo experiments have demonstrated heterogeneity of sensitivity to endothelin in various vascular beds: Pernow et al.[8] reported markedly more potent selective vasoconstrictive effects of endothelin on the renal circulation compared to the coronary, femoral or bronchial circulation. Faraci[9] showed that low concentrations of endothelin caused some dilation of pial arterioles of rat brain, whereas high concentrations of endothelin resulted in constriction. In contrast, in the basilar artery endothelin induced only constriction, not dilation.

Although we cannot elucidate how these different responses to endothelin in various vascular beds may occur, our study showed that the large cerebral arteries were more sensitive to endothelin than the large coronary and renal arteries. It has been shown that large arteries have a greater resistance in the cerebral circulation than in other vascular beds[1]. Thus, our findings appear to indicate that endothelin-induced contractions of large cerebral arteries may play a more significant role in the cerebral circulation than do the endothelin-induced contractions of the large coronary and renal arteries in the coronary or renal circulation.

Saito et al.[10] reported that ED_{50} values of endothelin in cat cerebral arteries are 2.6×10^{-10} M, which are almost one-tenth of the values obtained in the present study. Our data are fairly consistent with the data for human cerebral arteries reported by Martin de Aguilera et al.[11]. The discrepancies may be due to species differences in the sensitivity to endothelin in each set of cerebral arteries. In addition to the species differences, there are regional differences in the sensitivity to various agonists within cerebral arteries. Toda et al.[12] reported heterogeneity in the response of isolated dog proximal and distal middle cerebral arteries to vasoconstrictors. McCulloch and Edvinsson[3] reported that the sensitivity to norepinephrine differs among feline cerebral arteries. In contrast, serotonin, which is released from platelets during subarachnoid hemorrhage, had similar effects on large cerebral arteries in the cat and dog[3,13]. Up to now there have been no reports comparing the endothelin sensitivity of the large cerebral arteries in one species. In the present study, the sensitivity to endothelin did not differ among three porcine cerebral arteries (anterior cerebral, basilar and Willis ring).

Endothelin produced long-lasting cerebral vasospasm in dogs[5]. In our study, the increased tone of cerebral arteries induced by endothelin was found to be resistant to repeated washing, reflecting the long-lasting effect of endothelin in cerebral arteries. The findings also indicate that endothelin might be a candidate for the generation of cerebral vasospasms.

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