Pharmacological Detection of an Integrity of Endothelium in Pig, Cattle and Horse Basilar Arteries in Vitro

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Pharmacological Detection of an Integrity of Endothelium in Pig, Cattle and Horse Basilar Arteries *in Vitro*

Atsushi MIYAMOTO, Mitsuharu MATSUMOTO* and Akira NISHIO
(*Laboratories of Veterinary Pharmacology and* *Veterinary Anatomy)
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Introduction

Since the report of Furchgott and Zawadzki[6], it has been shown that the endothelium plays an important role in relaxation and contraction caused by a variety of vasoactive substances, such as acetylcholine (ACh), bradykinin, substance P and calcium ionophore A23187(A23187)[12]. It is well known, however, that there are species and regional differences in the capacity of these vasoactive substances to evoke the endothelium-dependent response[13].

In basilar arteries, Kanamura *et al*[7] have shown that human basilar arteries are relaxed by ACh and A23187, and the relaxations induced by both the substances are significantly attenuated after denudation of the endothelium. Similar results have been reported in the rabbit and dog basilar arteries[3,4]. Recently we have found that ACh did not evoked endothelium-dependent relaxation in pig basilar artery. To our knowledge, there have been few available data about the domestic animals, such as cattle and horses. Therefore, the present study was designed for pharmacological detection of an integrity of endothelial cells in pig, cattle and horse basilar arteries *in vitro*.

Materials and Methods

Basilar arteries from freshly slaughtered pigs, cattle and horses were obtained at two abattoirs and transferred to our laboratory immersed in ice-cold physiological salt solution (119 mM NaCl, 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) and were aerated with a mixture of 95% O_2 and 5% CO_2. The basilar artery was dissected free and cleaned of adhering tissues, and two rings (outer diameter: 0.5-0.8 mm in pigs, 1.0-1.4 mm in cattle and 1.0-1.5 mm in horses) about 4 mm long were cut off. One ring was mounted vertically between two L-shaped stainless steel holders, with the upper part fixed to an isometric force transducer (TB-611T, Nihon Kohden, Japan) and suspended in a 5-ml water-jacketed organ bath filled with oxygenated physiological salt solution at 37℃ (pH 7.4). The other ring was denuded mechanically by gentle rubbing of the intimal surface with a stainless steel rod having a diameter equivalent to the lumen size of each artery, then was mounted as above. The arterial ring mounted in the organ bath was left to equilibrate for at least 120 min under a resting tension of 7.5 mN for pig preparations or 10 mN for cattle and horse preparations, either of which was most optimal for inducing maximal contraction.

The arterial ring with or without endothelium was precontracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (10^{-7} M for pig preparations and 10^{-6} M for cattle and horse preparations)

then was exposed to one of the vasoactive substances cumulatively. The maximal relaxations were expressed as the percentage to relaxation induced by 10^{-4} M papaverine. The presence or absence of the endothelial cells was determined morphologically by scanning electron microscopy (JEOL).

The drugs used were $PGF_{2\alpha}$ (Ono, Japan), acetylcholine hydrochloride (ACh; Daiichi, Japan), histamine dihydrochloride, papaverine hydrochloride, methylene blue, indomethacin and sodium fluoride (NaF) (Nacalai, Japan), bradykinin acetate, calcium ionophore A23187 (A23187) and Lys^8-vasopressin acetate (Sigma, USA), Arg^8-vasopressin (Wako, Japan), L-nitro-arginine (Aldrich, USA), and substance P (Peptide Institute Inc., Japan).

The results shown in the text are expressed as mean values ± S.E.M. Statistical analysis was carried out by Scheffé's test after one-way analysis of variance. Significance was established when the probability level was equal to, or less than 5%.

Results

Fig.1 shows the effect of ACh on tone in pig, cattle and horse arterial rings, with and without endothelium, precontracted with $PGF_{2\alpha}$. ACh (10^{-9}-10^{-6} M) produced a relaxation of the $PGF_{2\alpha}$-mediated tone in some arterial rings (9 out of 27 horses) with endothelium of

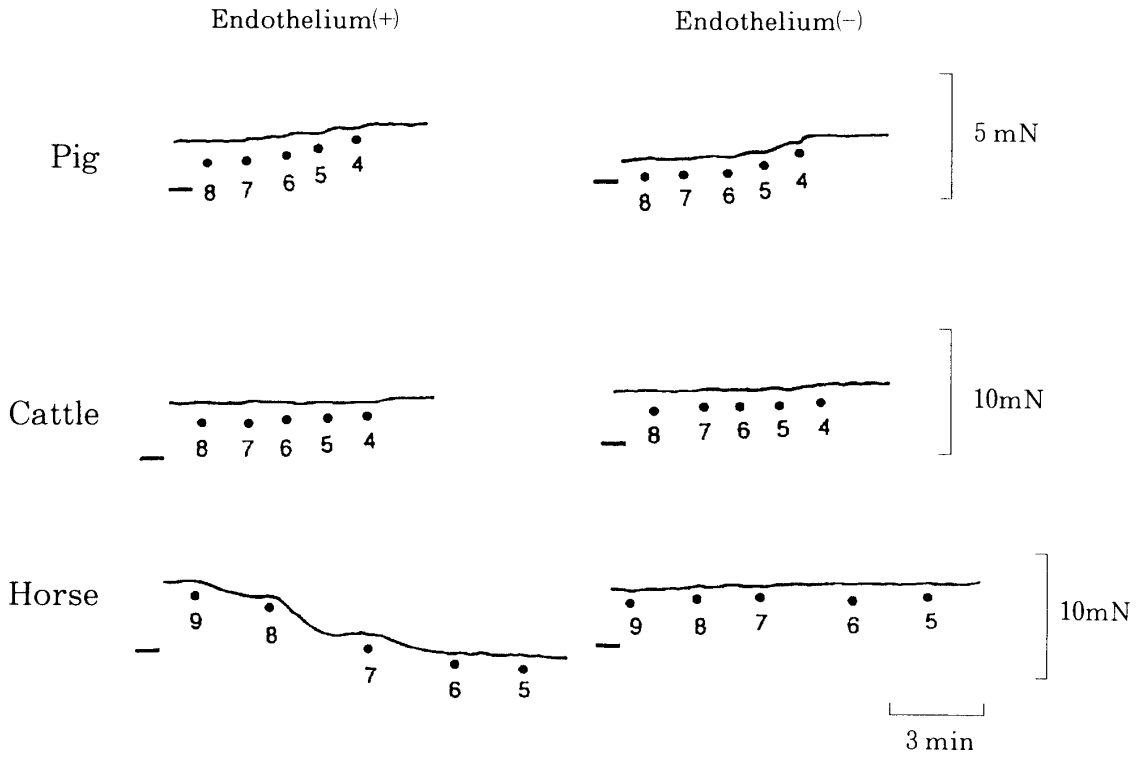

Fig.1. Typical responses to acetylcholine in pig, cattle and horse basilar arteries, with and without endothelium, precontracted by $PGF_{2\alpha}$ (10^{-7}-10^{-6}M). The concentration of $PGF_{2\alpha}$ was chosen to obtain a stable contraction corresponding approximately to 50% of the maximal response elicited by 60 mM KCl. The tension level before addition of $PGF_{2\alpha}$ is shown as a horizontal line on the left of each tracing. The concentration of acetylcholine is expressed as a negative logarithm (M).

Table 1. Effects of L-nitro-arginine, methylene blue and indomethacin on the maximal relaxation induced by acetylcholine in $PGF_{2\alpha}$-contracted horse basilar arteries with intact endothelium.

Treatment (Concentration)	N[a]	% Relaxation[b]
Control	4	−80.6 ± 8.1
L-nitro-arginine (10^{-4} M)	4	− 8.5 ± 6.4*
Methylene blue (10^{-5} M)	4	− 7.8 ± 7.8*
Indomethacin (10^{-5} M)	4	−77.7 ± 8.0

The values are expressed as the mean ± S.E.M.

[a]: Number of animals used.

[b]: Relaxation induced by 10^{-4} M papaverine was taken as 100%.

*: Significantly different from control ($P<0.05$).

Table 2. The presence (+) and absence (−) of the endothelium-dependent relaxation in pig, cattle and horse basilar arteries precontracted by $PGF_{2\alpha}$

Drug[a]	Concentration	Endothelium-dependent relaxation		
		Pig	Cattle	Horse
Acetylcholine	10^{-9}-10^{-6} M	− (6)	− (6)	+ (9/27)[b]
Histamine	10^{-8}-10^{-4} M	− (6)	− (6)	− (6)
Bradykinin	10^{-8}-10^{-6} M	+ (6)	− (6)	− (6)
Vasopressin	10^{-8}-10^{-6} M	− (6)	− (6)	− (6)
Substance P	10^{-9}-10^{-6} M	− (6)	− (6)	− (4)
A23187	10^{-8}-10^{-6} M	− (6)	− (6)	− (6)
NaF	10^{-2}-3×10^{-2} M	+ (6)	+ (6)	+ (6)

[a]: Each drug was applied cumulatively on the basilar arteries with endothelium precontracted by $PGF_{2\alpha}$ (10^{-7}-10^{-6}M).

[b]: Basilar arteries, 9 out of 27 horses, showed the endothelium-dependent relaxation. Numbers in parentheses indicate the number of animals used.

the horse. The relaxation was abolished completely after denudation of the endothelium. In the arterial rings of the pig and cattle, ACh produced no relaxation irrespective of the presence or absence of endothelium. The effects of L-nitro-arginine (10^{-4} M), methylene blue (10^{-5} M) and indomethacin (10^{-5} M) on the ACh-induced relaxation in horse basilar arteries with intact endothelium, are shown in Table 1. In response to ACh ($pEC_{50}=6.99\pm0.27$, $n=4$), L-nitro-arginine and methylene blue markedly inhibited the endothelium-dependent relaxation, whereas indomethacin had no significant effect on the relaxation. After the end of each experiment, scanning electron microscopic studies showed the presence of the intact endothelium in the pig and cattle basilar arteries and in some horse basilar arteries which had shown no endothelium-dependent relaxation.

Table 2 shows the effect of some other vasoactive substances on the endothelium-dependent responses in pig, cattle and horse basilar arteries. Histamine (10^{-8}-10^{-4} M) caused no endothelium-dependent relaxation in arteries precontracted with $PGF_{2\alpha}$. Bradykinin (10^{-8}-10^{-6} M) caused the endothelium-dependent relaxation in pig basilar arteries, but not in cattle and horse basilar arteries precontracted with $PGF_{2\alpha}$. The pEC_{50} value and the maximal relaxation in pig basilar arteries were 8.82 ± 0.16 and 89.7 ± 6.4% ($n=5$), respectively.

Lysine^8-vasopressin for pigs and arginine^8-vasopressin for cattle and horses, caused no endothelium-dependent responses in each basilar artery precontracted with $PGF_{2\alpha}$. Substance P (10^{-9}-10^{-6} M) and A23187 (10^{-8}-10^{-6} M) caused no endothelium-dependent response in basilar arteries from the three species. Sodium fluoride (NaF; 10^{-2}-3 x 10^{-2} M) caused the endothelium-dependent relaxation in all the basilar arteries with intact endothelium precontracted with $PGF_{2\alpha}$. The pEC_{50} values of NaF in the pig (1.66 ± 0.01; n=4) and cattle (1.63 ± 0.02; n=4) basilar arteries were significantly lower than that in horse basilar arteries (1.95 ± 0.05; n=5). The maximal relaxation values were 67.8 ± 3.8, 64.3 ± 6.0 and 79.0 ± 10.0% in the same order. These was no significant difference among the three mean values. After the denudation of endothelium the relaxing effect of NaF was abolished completely (data not shown).

Discussion

The present results indicate that NaF is one of the available pharmacological agents suitable for detecting an integrity of the endothelium in pig, cattle and horse basilar arteries *in vitro*, and bradykinin also is available in pig basilar artery for detecting an integrity of the endothelium *in vitro*. ACh is one of the most widely used vasoactive substances to elicit the endothelium-dependent relaxation in the various vessels isolated from various species[5,6]. In pig and cattle basilar arteries with intact endothelium, however, ACh caused no relaxation. Some horse basilar arteries with intact endothelium showed relaxation (Fig.1). Treatment with methylene blue or L-nitro-arginine markedly inhibited the endothelium-dependent relaxation; however, indomethacin had no effect (Table 1). From these results, it was assumed that ACh is depending on the formation and release of endothelium-derived relaxing factor(s) (EDRF) (presumably nitric oxide), and not on prostacyclin, derived from endothelial cells; however, ACh seems to be an unsuitable vasoactive substance to detect pharmacologically an integrity of the endothelium in pig, cattle and horse basilar arteries. In the present experiment, it is unclear whether the lack of the endothelium-dependent relaxation is dependent on the absence of receptors, or on the lack of mechanisms in the formation or release of EDRF, or on a delicate balance kept between endothelium-dependent relaxation and endothelium-dependent contraction. Present results, however, suggest that pig basilar arteries have no muscarinic receptors which liberate EDRF. Bradykinin showed the endothelium-dependent relaxation in pig basilar arteries (Table 2). This result agreed with the previous report in this species[9]. Whalley *et al*[15] have reported that bradykinin and ACh caused the endothelium-dependent relaxation in the human basilar arteries. These results support the previous suggestion that the pig basilar artery does not possess ACh receptors on the endothelial cells. It has been reported that the histamine-induced relaxation is mediated by endothelial H_1-receptors responsible for the release of EDRF in monkey[11] and rat[1] basilar arteries. In pig, cattle and horse basilar arteries, however, histamine showed no endothelium-dependent relaxation. It has also been reported that vasopressin[8,14], substance P[8] and A2318 7[7,8,10] cause the endothelium-dependent relaxation in dog, monkey and human basilar arteries *in vitro*. In pig, cattle and horse basilar arteries, vasopressin, substance P and A23187 caused no endothelium-dependent relaxation. Recently Cushing *et al*[2] have shown that NaF produces the endothelium-dependent relaxation in dog, pig, cattle and human coronary arteries. In this experiment, NaF produced the endothelium-dependent relaxation in pig, cattle and horse

basilar arteries. In the denuded basilar arteries of these species, NaF produced contraction (data not shown), as shown in the denuded coronary arteries[2].

Summary

Acetylcholine produced endothelium-dependent relaxation in some of the horse basilar arteries precontracted with prostaglandin $F_{2\alpha}$, but not in the pig and cattle basilar arteries with intact endothelium. The effect of acetylcholine in horses was completely abolished by endothelial denudation, and was markedly inhibited by L-nitro-arginine and methylene blue, but not by indomethacin. Bradykinin produced the endothelium-dependent relaxation in pigs but not in cattle and horses. Histamine, vasopressin, substance P and calcium ionophore A23187 produced no endothelium-dependent responses, however, NaF produced the endothelium-dependent responses in all the three species.

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