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Comparison of Vascular Reactivity to Vasoactive Agents in Coronary Arteries Isolated from Cattle, Pigs and Horses

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Introduction

Responsiveness of coronary arteries isolated from dogs, pigs, monkeys and human to endogenous vasoactive agents has been investigated in vitro[1,3,9,14,18]. These reports show that there are remarkable species differences in vascular reactivity to endogenous vasoactive agents. In the case of histamine, for example, monkey and dog coronary arteries respond with a relaxation[8,16], but human and pig coronary arteries do with a contraction[5,15]. Little information, however, is available concerning cattle and horse coronary arterial responsiveness to endogenous vasoactive agents.

The present study was undertaken to compare the vascular reactivities of cattle, pig and horse coronary arteries to endogenous vasoactive agents, such as histamine, serotonin, acetylcholine and norepinephrine. A preliminary report of the results has been presented previously[10].

Materials and Methods

Tissue preparation

Freshly slaughtered cattle, pig and horse coronary arteries were obtained from local slaughterhouses and transferred to our laboratory, immersed in ice-cold buffered salt solution (NaCl, 119mM; KCl, 4.7mM; $CaCl_2$, 1.6mM; $MgCl_2$, 1.2mM; $NaHCO_3$, 25mM; KH_2PO_4, 1.2mM; glucose, 10mM) aerated with a mixture of 95% O_2 and 5% CO_2. The middle part of descending coronary arteries was dissected free and cleaned of adhering tissue, and cut into vascular rings approximately 3mm long to be used for tension measurements.

Isometric tension

To record isometric tension, each vascular ring was placed horizontally in organ chamber filled with 15ml of the above-mentioned salt solution aerated with 95% O_2 and 5% CO_2 (37℃, pH 7.4). The preparations were attached to a strain gauge (Nihon Kohden Kogyo Co., Japan) and isometric tension was recorded. The optimal resting tensions were applied to each ring; 1.0g in the pig coronaries and 2.0g in the cattle and horse coronaries, respectively. All preparations were allowed to be equilibrated for 120 min in the bathing media, during which time the solutions were replaced every 15 to 20 min. Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Nihon Kohden Kogyo Co., Japan). The contractile response to 60mM K^+

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was first obtained, and the coronary rings were washed repeatedly with fresh media and equilibrated. Concentration-response curves for the endogenous vasoactive agents were obtained by adding each agent directly to the bathing media in cumulative concentrations. Contractions induced by each agent were presented as relative values to contractions caused by 60mM K^+.

Drugs

Drugs used were histamine dihydrochloride (Nakarai), serotonin creatinine sulfate (Sigma), acetylcholine chloride (Daiichi) and *dl*-norepinephrine hydrochloride (Daiichi).

Statistical analysis

The results shown in text, table and figures were expressed as mean values (±S.E.M.). Statistical analyses were made using the Student's *t*-test. The significance was established when the probability level was equal to, or less than, 5%.

Results

Response to histamine

Fig. 1 shows the concentration-response curves to histamine in coronary arteries isolated from cattle, pigs and horses. In cattle and pig coronary arteries, the cumulative additions of histamine produced a concentration-dependent contraction. In horse coronary arteries, 4 out of 11 arteries showed a weak, but significant relaxation at 10^{-7} and 10^{-6} M histamine.

The contractions induced by 10^{-4} M histamine relative to those by 60mM K^+ were 148.6(±8.6)% in the pig coronary arteries, 103.9(±9.9)% in the horse coronary arteries and 87.7(±6.9)%

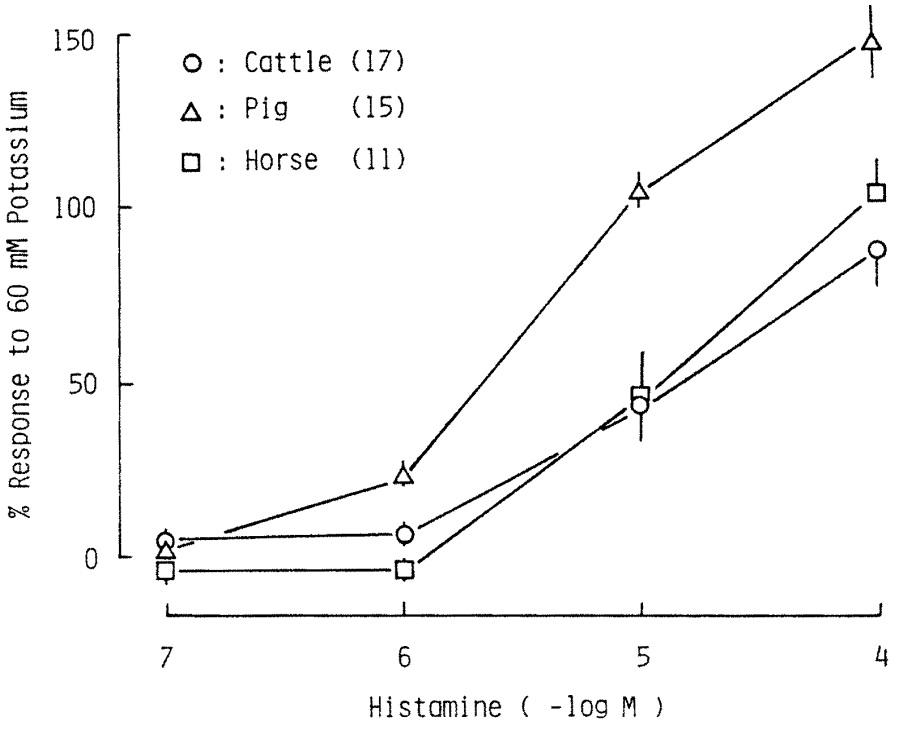

Fig. 1. Concentration-response curves to histamine in coronary arteries isolated from cattle, pigs and horses. Values are means ±S.E.M. Numbers in parentheses are the number of animals utilized.

in the cattle coronary arteries. Mean values of the apparent median effective concentration (EC_{50}) of histamine were 14.90 (±1.60) $\times 10^{-6}$ M in the horse coronary arteries, 14.70 (±1.62) $\times 10^{-6}$ M in the cattle coronary arteries and 5.01 (±0.93) $\times 10^{-6}$ M in the pig coronary arteries, respectively (Table 1).

Table 1. Mean EC_{50} values of histamine, serotonin and acetylcholine in coronary arteries isolated from cattle, pigs and horses

Species	Histamine	Serotonin	Acetylcholine
	$\times 10^{-6}$ M	$\times 10^{-7}$ M	$\times 10^{-7}$ M
Cattle	14.70±1.67 (17)	3.86±0.75*2 (15)	33.84±12.34*2 (12)
Pig	5.01±0.93*1 (15)	11.70±0.25 (15)	3.77±0.64 (12)
Horse	14.90±1.60 (11)	Non contraction (12)	2.52±0.74 (5)

Values are means ±S.E.M. Numbers in parentheses are the number of animals utilized.
*1 : Significantly different from cattle and horse ($P<0.05$).
*2 : Significantly different from pig and horse ($P<0.05$).

Response to serotonin

Fig. 2 shows the concentration-response curves to serotonin in coronary arteries isolated from cattle, pigs and horses. In cattle and pig coronary arteries, the cumulative additions of serotonin produced a concentration-dependent contraction. However, the horse coronary arteries showed no response to serotonin in a dose-range from 10^{-8} M to 10^{-5} M.

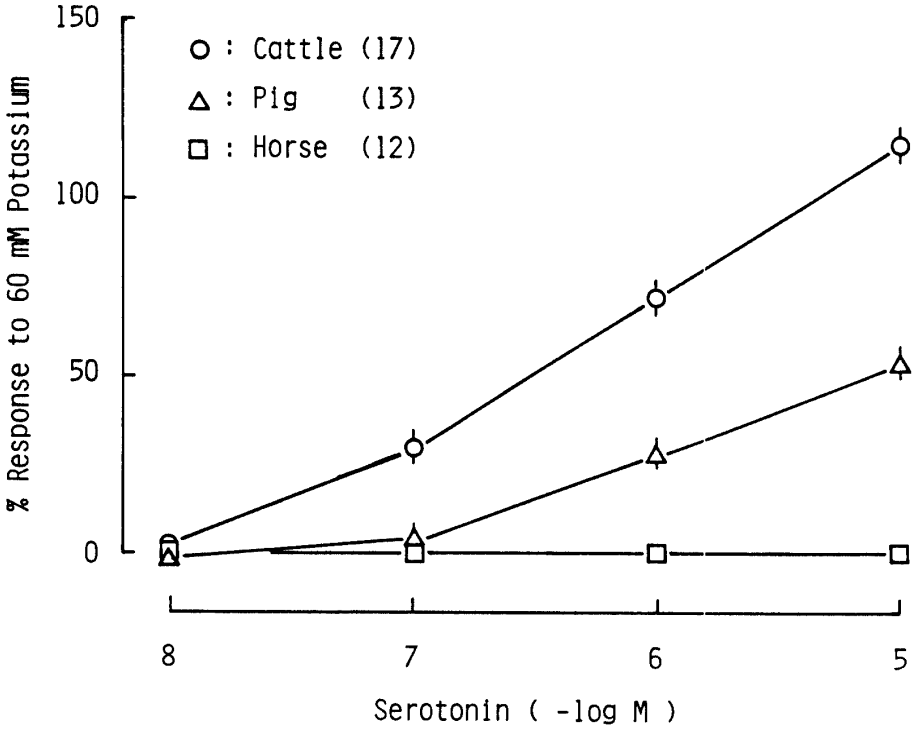

Fig. 2. Concentration-response curves to serotonin in coronary arteries isolated from cattle, pigs and horses. Values are means ±S.E.M. Numbers in parentheses are the number of animals utilized.

The contractions induced by 10^{-5} M serotonin relative to those by 60mM K^+ were 115.0(±5.2)% in the cattle coronary arteries and 53.1(±4.4)% in the pig coronary arteries. Mean EC_{50} values were 3.86(±0.75)$\times10^{-7}$ M in the cattle coronary arteries and 11.70(±0.25)$\times10^{-7}$ M in the pig coronary arteries, respectively (Table 1).

Response to acetylcholine

Fig. 3 shows the concentration-response curves to acetylcholine in coronary arteries from cattle, pigs and horses. The horse coronary arteries responded to acetylcholine with a contraction, dose-dependently. In cattle coronary arteries, 4×10^{-8} M acetylcholine caused a weak relaxation in 9 out of 15 coronary arteries, concentrations larger than 4×10^{-7} M caused the concentration-dependent contractions. In pig coronary arteries, 4×10^{-8} M to 4×10^{-6} M acetylcholine caused a concentration-dependent contraction; with higher concentrations (4×10^{-5} and 4×10^{-4} M) a decline occurred in the responses.

The contractions induced by 4×10^{-4} M acetylcholine relative to those by 60mM K^+ were 269.4(±22.5)% in the horse coronary arteries and 52.8(±9.8)% in the cattle coronary arteries, and the contraction induced by 4×10^{-6} M acetylcholine was 64.6(±10.2)% in the pig coronary arteries. Mean EC_{50} values were 2.52(±0.74)$\times10^{-7}$ M in the horse coronary arteries, 3.77(±0.64)$\times10^{-7}$ M in the pig coronary arteries and 33.84(±12.34)$\times10^{-7}$ M in the cattle coronary arteries, respectively (Table 1).

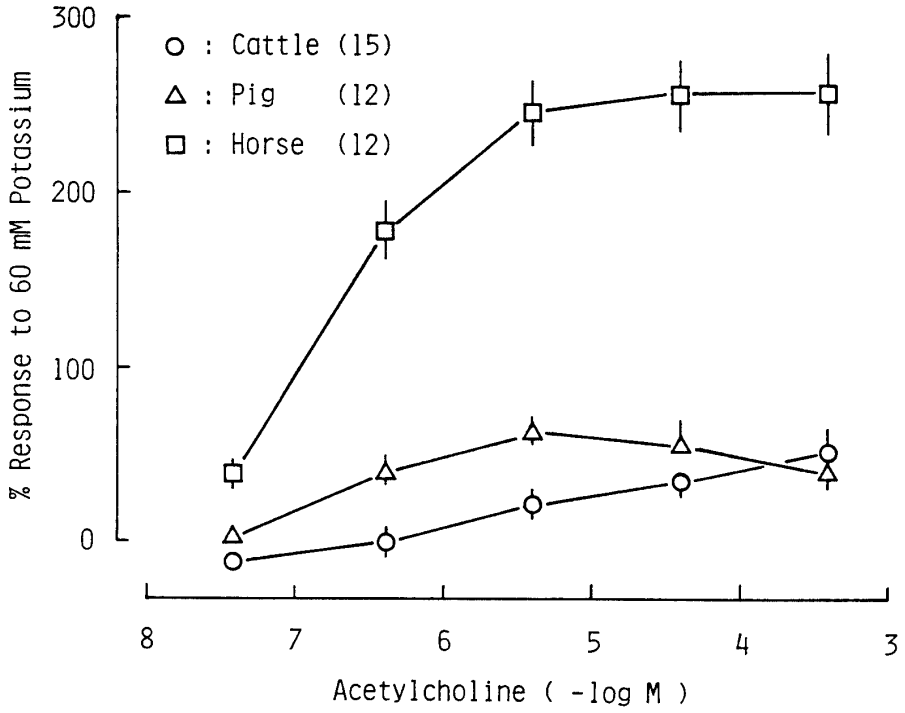

Fig. 3. Concentration-response curves to acetylcholine in coronary arteries isolated from cattle, pigs and horses. Values are means ±S.E.M. Numbers in parentheses are the number of animals utilized.

Response to norepinephrine

Fig. 4 shows the concentration-response curves to norepinephrine in coronary arteries isolated from cattle, pigs and horses. The cumulative additions of norepinephrine produced a concentration-dependent contraction in the horse coronary arteries. The contraction induced by 6×10^{-6} M norepinephrine relative to those by 60mM K^+ was 134.4 ($\pm$19.1)%, and the mean EC_{50} value was 1.22 ($\pm$0.24) $\times10^{-7}$ M in the horse coronary arteries. However, in the cattle and pig coronary arteries, norepinephrine produced a weak relaxation at relatively high concentration (6×10^{-6} M). The relaxations induced by 6×10^{-6} M norepinephrine relative to those by 60mM K^+ were -8.0 ($\pm$2.2)% in the cattle coronary arteries and -4.0 ($\pm$1.9)% in the pig coronary arteries, respectively.

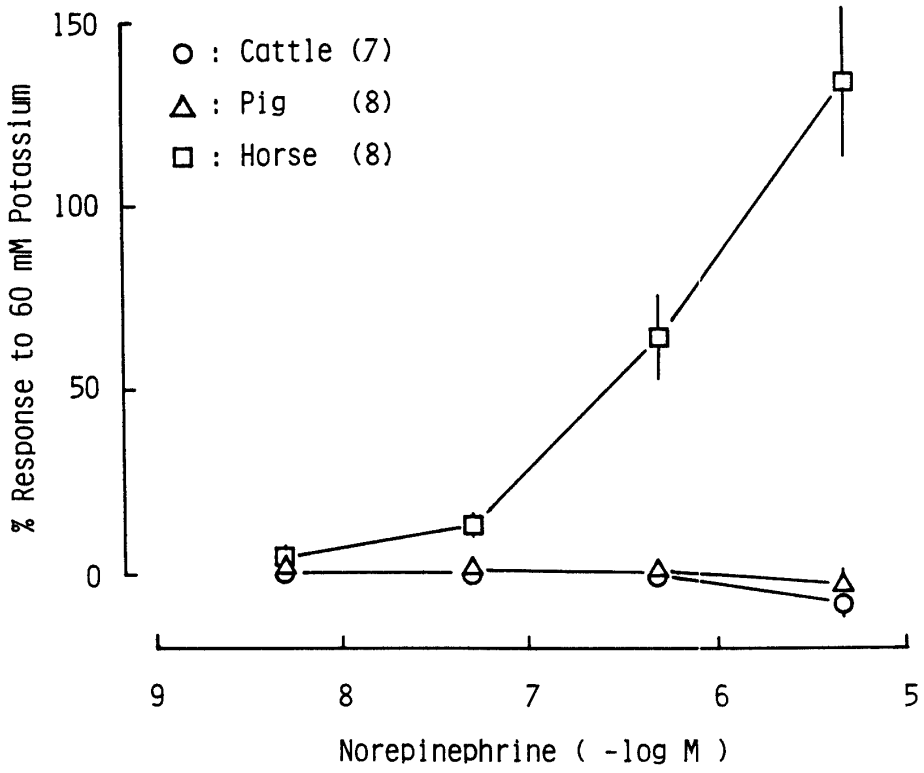

Fig. 4. Concentration-response curves to norepinephrine in coronary arteries isolated from cattle, pigs and horses. Values are means $\pm$ S.E.M. Numbers in parentheses are the number of animals utilized.

Discussion

There are many reports about the pharmacological properties of coronary arteries isolated from the experimental animals, however, there are few reports about the domestic animals. Present results, using the isolated coronary arteries from cattle, pigs and horses, have demonstrated the different reactivities to endogenous vasoactive agents in these species.

Histamine has been reported to contract the cattle and pig coronary arteries[6,11,18], but no information about horse coronary arteries is available, yet. As shown in Fig. 1, the isolated horse coronary arteries are contracted by histamine in the similar degree in cattle coronary arteries, and some horse coronary arteries (4 out of 11) show a weak relaxation at low concentrations of

histamine. This weak relaxation is similar to the results reported by Toda in human coronary arteries[17]. He has shown that the relaxation is abolished or reversed to contraction in the absence of endothelium. It is possible to assume that a weak relaxation by histamine in horse coronary arteries depends on an endothelium-dependent relaxation factor.

The action of serotonin observed here is of great interest (Fig. 2). Pig and cattle coronary arteries respond with a contraction to serotonin, as reported previously[6,11]. However, horse coronary arteries show no contractile response to serotonin. At present, there is no other available information to this phenomenon. It seems to be important to clarify a characteristic of horse coronary artery.

Constrictor effects of acetylcholine in the isolated pig and cattle coronary arteries have been previously reported[6,11]. The findings here for pig and cattle coronary arteries are similar to those in the above reports. Horse coronary arteries constrict to acetylcholine more strongly than pig and cattle coronary arteries do (Fig. 3). Dog coronary arteries contract[2] or relax[12] to acetylcholine, and both responses are blocked by atropine, so it is needful to examine whether the strong contraction observed in horse coronary arteries depends on the muscarinic receptors.

Norepinephrine has been reported to relax the pig and cattle coronary arteries[6,11], the relaxation being inhibited and reversed to contraction by propranolol, a β-adrenergic antagonist. No fixed information is obtained about horse coronary arteries. As shown in Fig. 4, horse coronary arteries respond to norepinephrine with a contraction as similar as isolated human coronary arteries[7,13]. It is generally known in resistant vessels that constrictor effects of norepinephrine are sensitive to α-adrenergic antagonists and relaxation to β-adrenergic antagonists. The results obtained in this study suggest the physiological importance of β-adrenergic receptors in the pig and cattle coronary arteries and α-adrenergic receptors in the horse coronary arteries, respectively.

In conclusion, there are many differences among cattle, pigs and horses in the direct coronary vasoconstrictor effects of histamine, serotonin, acetylcholine and norepinephrine. Further studies will be necessary to determine whether these species differences depend on the nature or number of receptors to each vasoactive agent in each coronary artery.

Summary

We compared the vascular reactivities to histamine, serotonin, acetylcholine and norepinephrine in the coronary arteries isolated from cattle, pigs and horses. Rings of descending coronary arteries were used. Coronary arteries were exposed to cumulative concentrations of each agent. The contractile reactivity to histamine was in the following order: pig$>$cattle$\fallingdotseq$horse. Some of the horse coronary arteries (4 out of 11) showed a relaxation at the concentrations of 10^{-7} and 10^{-6} M histamine. The contractile reactivity to serotonin was greater in cattle than in pig. Horse coronary arteries showed no contractile response to serotonin (10^{-8}-10^{-5} M), but responded to acetylcholine more strongly than cattle and pigs. Acetylcholine caused a weak relaxation at low concentration (4×10^{-8} M) in 9 out of 15 cattle used, and in pigs it showed concentration-dependent contractions at relatively low concentrations (4×10^{-8}-10^{-6} M), and relaxations at higher concentrations (4×10^{-5} and 4×10^{-4} M), respectively. Coronary arteries from cattle and pigs responded to norepinephrine with a relaxation, but those from horses responded to it with a contraction. These data indicate that there are many differences in the vascular reactivities to vasoactive agents among the cattle, pig and horse coronary arteries.

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