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Pharmacology

Vasomotor effects of 5-hydroxytryptamine, histamine, angiotensin II, acetylcholine, noradrenaline, and bradykinin on the cerebral artery of bottlenose dolphin (*Tursiops truncatus*)

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ABSTRACT. From an evolutionary aspect, dolphins share a very close phylogenetic relationship with pigs. Previously, we characterized porcine cerebral artery responsiveness to intrinsic vasoactive substances. Therefore, here, we investigated dolphin (*Tursiops truncatus*) cerebral artery responsiveness to 5-hydroxytryptamine (5-HT), histamine (His), angiotensin (Ang) II, acetylcholine (ACh), noradrenaline (NA), and bradykinin (BK) to characterize their related receptor subtypes. We also compared dolphin cerebral artery responsiveness with porcine cerebral artery responsiveness. We found that 5-HT and His induced concentration-dependent contraction of the dolphin cerebral artery. Ketanserin (a 5-HT_2 antagonist) and methiothepin (a 5-HT_1 and 5-HT_2 antagonist) shifted the concentration-response curve for 5-HT to the right. Although diphenhydramine (an H_1 antagonist) shifted the concentration-response curve for His to the right, cimetidine (an H_2 antagonist) had no such effect. Ang II and ACh did not produce any vasomotor actions. NA induced concentration-dependent relaxation. Propranolol (a β antagonist) shifted the concentration-response curve for NA to the right, whereas phentolamine (an α antagonist) had no significant effect. BK induced relaxation followed by contraction in pre-contracted arteries with intact endothelium. HOE140 (a B_2 antagonist) shifted the concentration-response curve for BK to the right, whereas des-Arg^9-[Leu^8]-BK (a B_1 antagonist) had no significant effect. These results suggest that 5-HT_1, 5-HT_2, and H_1 receptor subtypes are important in arterial contraction and that β and B_2 receptor subtypes modify these contractions to relaxations. The responsiveness of the dolphin cerebral artery is very similar to that of porcine cerebral artery, supporting their evolutionary linkage.

KEY WORDS: cerebral artery, dolphin, receptor, vasoconstrictor, vasodilator

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The responsiveness of cerebral arteries to intrinsic vasoactive substances is species specific, and some reactivities are unique and characteristic. As an example, although noradrenaline (NA) is a vasoconstrictor that induces the contraction of the cerebral artery in dogs [33] and guinea pigs [3], it induces relaxation of the cerebral artery in cattle [1] and pigs [18]. Moreover, the intensity of relaxation in pigs is much greater than that in cattle. Therefore, a large relaxation induced by NA is one of the distinctive characteristics of the porcine cerebral artery. In another example, bradykinin (BK), a vasorelaxant, induces relaxation in human cerebral arteries, but induces a very strong contraction in the equine cerebral artery [32]. The contraction induced by BK in the

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equine cerebral artery is greater than that induced in the equine cerebral artery by NA, histamine (His), or 5-hydroxytryptamine (5-HT). Therefore, a BK-induced contraction is a distinctive characteristic of the equine cerebral artery. To our knowledge, cerebral arterial responsiveness to these vasoactive substances in one animal species is not identical o that in other species. Therefore, characterization of cerebral artery reactivity in different species of animal may be useful to investigate evolutionary linkage among animals.

Although the dolphin is an aquatic mammal, it shares many characteristics with terrestrial mammals. Cetaceans evolved from ancient even-toed animals (Artiodactyla) at the end of the cretaceous period (approximately 55 million years ago) [6]. The ancestors of cetaceans first lived in a terrestrial environment and then adapted to an aquatic environment. Several anatomical, morphological, and physiological adaptations to living underwater have been well studied, including the streamlined body shape, the location of the blowhole, the higher basal metabolic rate, and the lower maximum rate of oxygen consumption to maintain thermoregulation [13, 36].

The vascular system of marine mammals plays a key role during the dive response, where high fluctuations in oxygen availability or consumption may be encountered. Potential vasodilator/ vasoconstrictor mechanisms of cerebrovascular control and increased cerebral blood flow during voluntary diving are consistent with the dynamics of cerebral blood flow in hypercapnia in terrestrial mammals [4]. Local vasodilator and neural-mediated vascular control mechanisms both ensure the brain can access the available blood oxygen [4]. Considering the evolutionary and adaptive changes in dolphin, a study of cerebral artery responsiveness to intrinsic vasoactive substances in dolphin would be of considerable interest. Cerebral artery responsiveness in dolphins could then be compared with that in terrestrial mammals, especially those with a close evolutionary relationship.

Because of their close phylogeny [5, 27], numerous comparative studies between dolphins and pigs have been conducted in other fields [15, 26, 28, 31]. There are, however, unanswered questions concerning the physiological changes that have occurred over the course of evolution in dolphins as a result of adaptation to the marine environment.

Although there has been extensive research on the vascular reactivity of different terrestrial and amphibious animals, information regarding vascular reactivity in aquatic animals is limited. We have previously characterized the cerebral artery responsiveness to intrinsic vasoactive substances in pig [16–18, 21–24], and have extensively researched pig cerebral artery function [10, 21, 22, 24]. In addition, it has been demonstrated a close phylogenetic relationship between pig and dolphin [5, 27]. Here, we report the responsiveness of isolated dolphin (*Tursiops truncatus*) cerebral arteries to 5-HT, His, Ang II, ACh, NA, and BK.

MATERIALS AND METHODS

Tissue preparation

We isolated cerebral arteries from the heads of dead bottlenose dolphin (*Tursiops truncates*) (both sexes, indeterminate age range, body weight 200 ± 26.7 kg), which had been captured in Taiji, Japan, during drive hunt fishing practices permitted by the Wakayama Prefecture government. Section of the cerebral arteries (proximal part of meningeal artery) were then gently isolated from the brain and transferred to ice-cold physiological saline (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, pH 7.4) aerated with carbogen (95% (v/v) O_2, 5% (v/v) CO_2) and transferred to our laboratory. The location of the sampled artery section apparently corresponds with that of basilar artery in terrestrial mammals. Each artery was immediately dissected free of adherent tissues under a stereomicroscope. All experiments were performed in accordance with the Kagoshima University Guidelines for Animal Experimentation.

Reagents

The following reagents were all obtained from Sigma-Aldrich (St. Louis, MO, USA) and used at the indicated final concentrations:

5-HT (10^{-9}–10^{-5} M); ketanserin tartrate (10^{-8}–10^{-7} M); methiothepin maleate (10^{-8}–10^{-7} M); His hydrochloride (10^{-6}–10^{-3} M); diphenhydramine hydrochloride (10^{-7}–10^{-4} M); cimetidine (10^{-5} M); Ang II acetate salt (10^{-9}–10^{-5} M); NA (10^{-9}–10^{-5} M); phentolamine mesilate (10^{-5} M); propranolol hydrochloride (10^{-8}–10^{-6} M); BK acetate salt (10^{-9}–10^{-6} M); des-Arg^9- [Leu^8]-BK (10^{-5} M); *Nω*-nitro-L-arginine (L-NNA; 10^{-4} M); and sodium nitroprusside (SNP, 10^{-4} M).

The following reagents were obtained and used at the indicated final concentrations: HOE140 (10^{-8}–10^{-6} M; Peptide Institute, Osaka, Japan); indomethacin (10^{-5} M; Nacalai tesque, Kyoto, Japan); ACh chloride (10^{-9}–10^{-5} M; Daiichi Sankyo, Tokyo, Japan). All drugs were dissolved in distilled water.

Functional studies

Three or four rings of approximately 4 mm length were cut from each artery. Each ring was mounted horizontally between two L-shaped stainless steel holders (outer diameter, 0.5 mm), with one part fixed to an isometric force transducer and immersed in a 4 m*l* water-jacketed a micro tissue organ bath (UMTB-1, Unique Medical Co., Ltd., Tokyo, Japan) containing oxygenated salt solution at 37°C (pH 7.4). Each suspended ring was allowed to equilibrate for at least 120 min under a resting tension of 0.50 g. This tension was chosen to allow induction of maximum contractions in the artery. KCl (60 mM) treatment was applied every 30 min until the amplitude of contractions reached a constant value. Changes in the KCl concentration of the physiological saline were compensated by equimolar adjustment of the NaCl concentration. The isometric tension was recorded using an amplifier (AP-621G, Nihon Kohden Kogyo, Tokyo, Japan), digitized with an analog-digital converter (PowerLab/8SP, ADInstruments Co., Castle Hill, NSW, Australia), and stored on the hard disk of a personal computer. The cumulative concentration-response curve of each

agonist was obtained by adding a solution of agonist directly to the fluid in the bath. Antagonists were added to the bathing media 30 min before adding the agonist. The antagonists had no effect on the resting vascular tone. The log concentration ratio of EC_{50} values (i.e., concentration producing half-maximum response) in the absence or presence of antagonists was calculated and plotted against the logarithm of antagonist concentration to obtain pA_2 values.

Statistical analyses

Results are expressed as means ± standard error of mean. Statistical analyses were performed by the Student's *t* test or Bonferroni test after one-way analysis of variance (Stat View J-4.5, Abacus Concepts Inc., Berkeley, CA, USA). Significance was established when the probability level was equal to or less than 5%.

RESULTS

Responsiveness to 5-HT, His, Ang II, ACh, NA, and BK

We first investigated the vascular responsiveness to 5-HT, His, Ang II, ACh, NA, and BK in resting tension. We then confirmed the relaxation in the presence of these agonists in pre-contraction with U46619 (a thromboxane A_2 analog; 10^{-8} M). Finally, we generated concentration-response curves for all the agonists in isolated dolphin cerebral arteries with endothelial cells (Fig. 1). 5-HT and His induced contraction in a concentration-dependent manner in resting tension, but no relaxation was observed for these agonists in pre-contraction. Ang II and ACh did not induce any changes under either condition. NA induced relaxations under both conditions, but the magnitude of the relaxation in pre-contraction was greater than that in resting tension. Endothelial removal had no effect on NA-induced relaxations. BK induced complicated and unstable response, including relaxation and contraction in resting tension. In pre-contraction, however, BK induced concentration-dependent relaxation (10^{-9}–10^{-7} M) followed by contraction (10^{-6} M). Table 1 shows the pEC_{50} values and maximal responses for the agonists examined. Although L-NNA (a NO synthase inhibitor, 10^{-4} M) induced contraction (8.15 ± 0.59% to 60 mM KCl) under resting tension, indomethacin (a cyclo-oxygenase inhibitor, 10^{-5} M) induced relaxation (2.2 ± 0.24% to 60 mM KCl) under contraction induced by L-NNA (data not shown). The magnitude of contraction induced by 60 mM KCl was 0.35 ± 0.03 g (n=9).

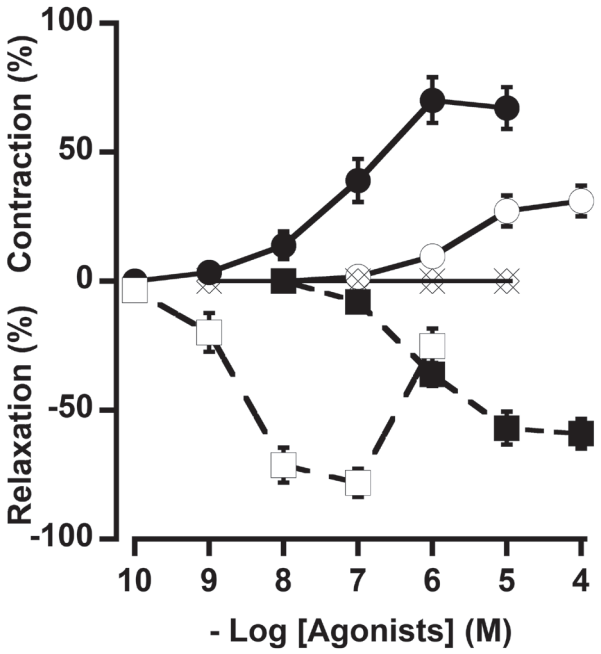

Fig. 1. Responsiveness of the isolated dolphin cerebral artery with intact endothelium to 5-hydroxytryptamine (5-HT: ●), histamine (His: ○), angiotensin II (Ang II: ×), acetylcholine (ACh: ◇), noradrenaline (NA: ■), and bradykinin (BK: □). Relaxation in response to NA and BK was investigated in pre-contraction with U46619 (a thromboxane A_2 analog; 10^{-8} M). The contraction induced by 60 mM KCl and relaxation induced by sodium nitroprusside (10^{-4} M) was taken as 100%. Each point represents the mean ± SEM for 6–8 dolphins.

Effects of ketanserin and methiothepin on 5-HT-induced contraction

We investigated the effects of ketanserin (a 5-HT_2 antagonist) and methiotheipin (a 5-HT_1 and 5-HT_2 antagonist) on the 5-HT-induced concentration-response curve in isolated dolphin cerebral arteries. Ketanserin (10^{-8}–10^{-7} M) shifted the concentration-response curve for 5-HT to the right (Fig. 2A). The calculated pA_2 value for ketanserin was 8.52 ± 0.09 and its slope was 0.87 ± 0.08 (Fig. 2B), which was not significantly different from unity. Methiothepin (10^{-8}–10^{-7} M) also shifted the concentration-response curve for 5-HT to the right (Fig. 3).

Effects of diphenhydramine and cimetidine on His-induced contraction

We investigated the effects of diphenhydramine (a H_1 antagonist) and cimetidine (a H_2 antagonist) on the concentration-response curve for His. Diphenhydramine (10^{-6}–10^{-4} M) shifted the concentration-response curve for His in parallel to the right (Fig. 4A). In contrast, cimetidine (10^{-5} M) had no significant effect on the concentration-response curve for His (Fig. 4A). The calculated pA_2 value for diphenhydramine was 7.26 ± 0.13 and its slope was 1.31 ± 0.16, which was not significantly different from unity (Fig. 4B).

Table 1. The pEC_{50} values and maximal responses for agonists

Agonists	pEC_{50}	Max (%)
5-HT	7.44 ± 0.08	70.12 ± 8.85[a)]
Histamine	5.82 ± 0.06	31.10 ± 5.93[a)]
ACh	–	No response
Ang II	–	No response
Noradrenaline	6.15 ± 0.12	−59.13 ± 5.75[b)]
Bradykinin	8.8 ± 0.14	−78.18 ± 5.50[b)]

a) Contraction induced by 60 mM KCl was taken as 100%. b) Relaxation induced by 10^{-4} M sodium nitroprusside was taken as 100%. Each point represents the mean ± SEM for 6−8 dolphins.

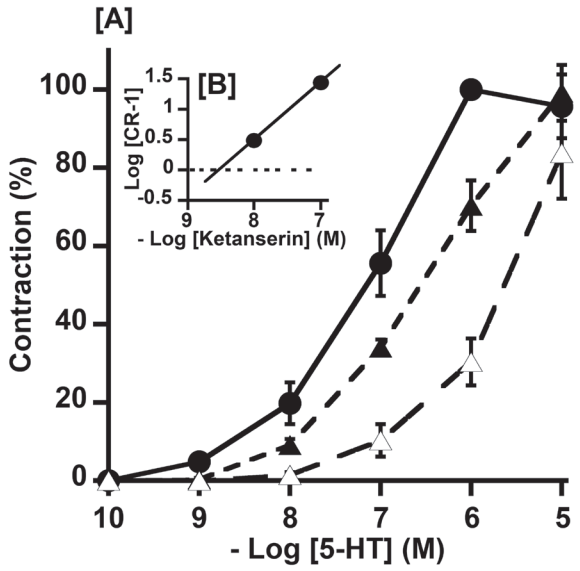

Fig. 2. Effect of the 5-HT_2-receptor antagonist ketanserin (▲: 10^{-8} M and △: 10^{-7} M) on 5-hydroxytryptamine (5-HT)-induced contraction (●) [A] and Schild plot of ketanserin [B] in isolated dolphin cerebral arteries with intact endothelium. The maximum contraction induced by 5-HT in the absence of ketanserin was taken as 100%. Each point represents the mean ± SEM for 6 dolphins. CR indicates the equieffective 5-HT concentration ratio [concentration producing 50% maximal concentration (EC_{50}) in the presence of ketanserin/EC_{50} in the absence of ketanserin].

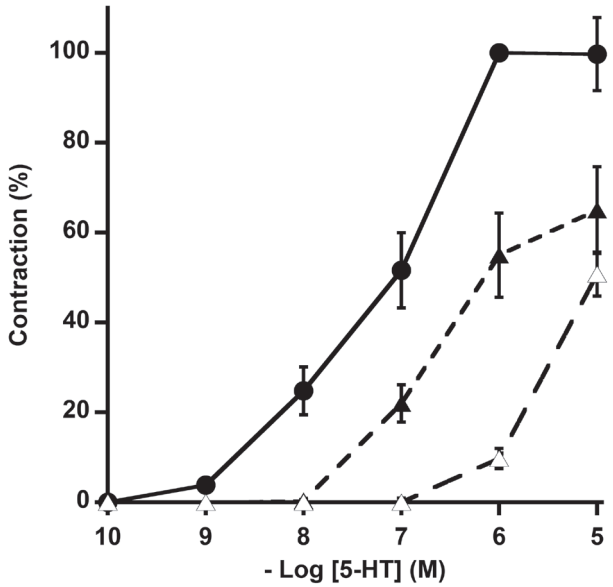

Fig. 3. Effect of the 5-HT_1 and 5-HT_2-receptor antagonist methiothepin (▲: 10^{-8} M and Δ: 10^{-7} M) on 5-hydroxytryptamine (5-HT)-induced contraction (●) in isolated dolphin cerebral arteries with intact endothelium. The maximum contraction induced by 5-HT in the absence of methiothepin was taken as 100%. Each point represents the mean ± SEM for 6 dolphins.

Effects of phentolamine and propranolol on NA-induced relaxation

We examined the effects of phentolamine and propranolol, non-selective α and β-adrenoceptor antagonists, respectively, on the concentration-response curve for NA. Phentolamine (10^{-5} M) showed no significant effect. Propranolol shifted the concentration-response curve for NA parallel to the right in a concentration-dependent manner (Fig. 5A). The calculated pA_2 value for propranolol was 8.01 ± 0.11 and its slope was 1.56 ± 0.13 (Fig. 5B), which was not significantly different from unity.

Effects of endothelial removal, L-NNA, and indomethacin on BK-induced relaxation

Endothelial denudation completely abolished both BK-induced relaxation and contraction. Pretreatment with L-NNA significantly inhibited BK-induced relaxation but enhanced contraction. Indomethacin had no significant effect on BK-induced relaxation but abolished BK-induced contraction (Fig. 6).

Effects of B_1 and B_2 receptor antagonists on BK-induced relaxation

To characterize the BK receptor subtypes, the arteries were pretreated with B_1 and B_2 receptor antagonists. Des-Arg^9-[Leu^8]-BK (a B_1 antagonist) had no significant effect on BK-induced response of the dolphin cerebral arteries. HOE140 (10^{-8}–10^{-6} M; a B_2 antagonist) shifted the BK-induced concentration-response curve to the right (Fig. 7A). The calculated pA_2 value for HOE140 was 8.30 ± 0.08 and its slope was 1.16 ± 0.04 (Fig. 7B), which was not significantly different from unity. The pA_2 value for HOE140 was calculated from the relaxation response part of the BK-induced responses in dolphin cerebral arteries.

DISCUSSION

To the best of our knowledge, this is the first study to demonstrate the responsiveness of the isolated dolphin cerebral artery to 5-HT, His, Ang II, ACh, NA, and BK as well as to investigate the receptor subtypes involved in this responsiveness.

Our results revealed that 5-HT-induced concentration-dependent contractions of the isolated dolphin cerebral artery. The pEC_{50} value (7.44 ± 0.08) of 5-HT in dolphin cerebral arteries was similar to that observed in pig cerebral arteries (7.70 ± 0.10), a response mediated via the activation of 5-HT_1 and 5-HT_2 receptors [23]. Ketanserin, a 5-HT_2-receptor antagonist, shifted the concentration-response curve of 5-HT to the right (Fig. 2A). The pA_2 value for ketanserin (8.52 ± 0.09) observed in this study is similar to that observed for human mesenteric arteries (8.40 ± 0.25) [7] and equine cerebral arteries (8.91) [25]; however, it is lower than that observed for the porcine cerebral artery (9.58 ± 0.13) [23]. Methiothepin, a 5-HT_1, and 5-HT_2-receptor antagonist,

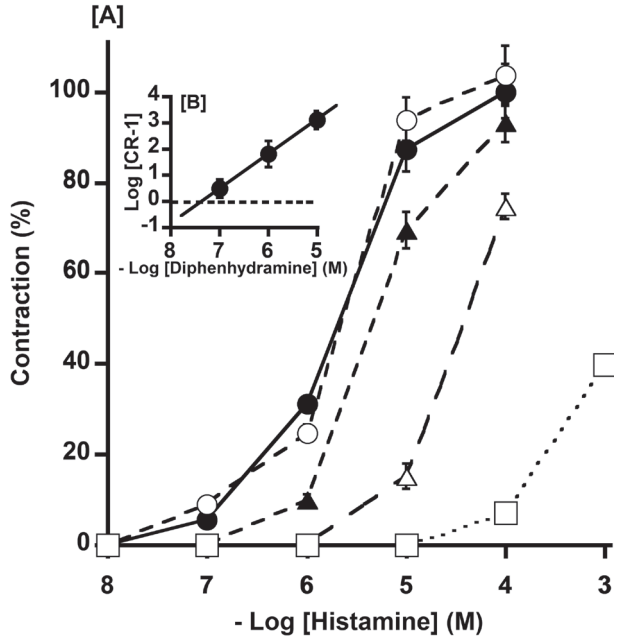

Fig. 4. Effects of the H_1 receptor antagonist diphenhydramine (▲: 10^{-7} M, △: 10^{-6} M, □: 10^{-5} M) and the H_2 receptor antagonist cimetidine (○: 10^{-5} M) on histamine (His)-induced contraction (●) [A] and Schild plot of diphenhydramine [B] in the isolated dolphin cerebral artery with intact endothelium. The contraction induced by His in the absence of antagonist was taken as 100%. Each point represents the mean ± SEM for 7 dolphins. CR: see Fig. 2.

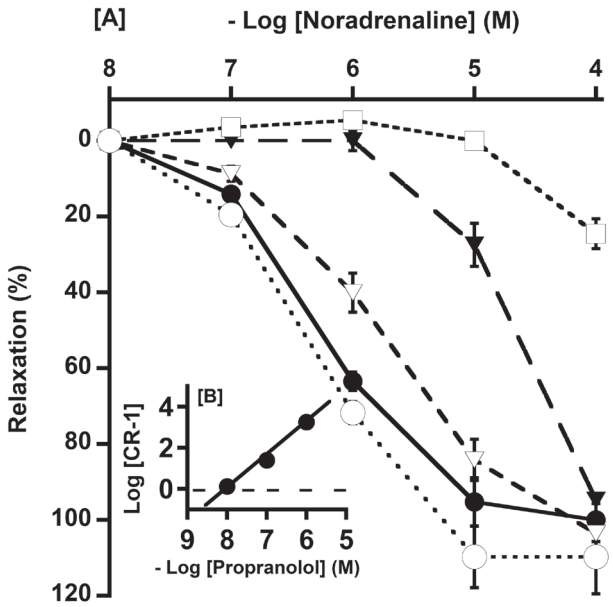

Fig. 5. Effects of the β-adrenoceptor antagonist propranolol (▽: 10^{-8} M, ▼: 10^{-7} M and □: 10^{-6} M) and the α-adrenoceptor antagonist phentolamine (○: 10^{-5} M) on noradrenaline (NA)-induced relaxation (●) [A] and Schild plot of propranolol [B] in the isolated dolphin cerebral artery with intact endothelium. The relaxation induced by NA in the absence of antagonist was taken as 100%. Each point represents the mean ± SEM for 6 dolphins. CR: see Fig. 2.

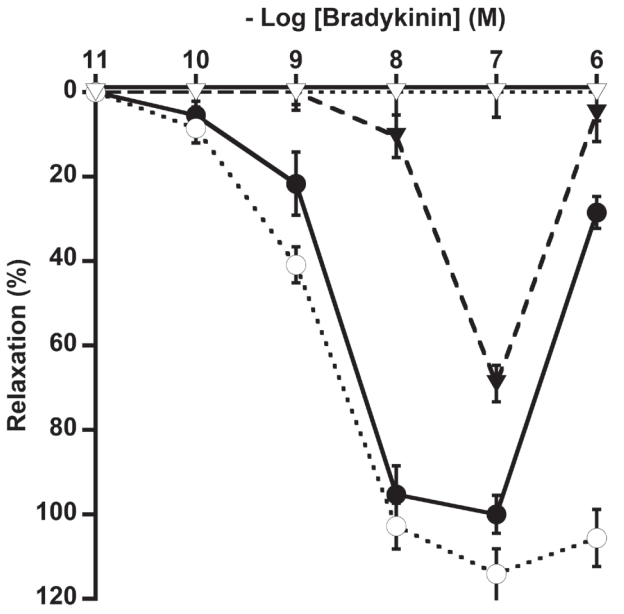

Fig. 6. Effects of *Nω*-nitro-L-arginine (▼: 10^{-4} M), indomethacin (○: 10^{-5} M), and endothelial removal (▽) on bradykinin (BK)-induced biphasic responses (●) in the isolated dolphin cerebral artery. The relaxation induced by BK in the absence of inhibitor was taken as 100%. Each point represents the mean ± SEM for 6 dolphins.

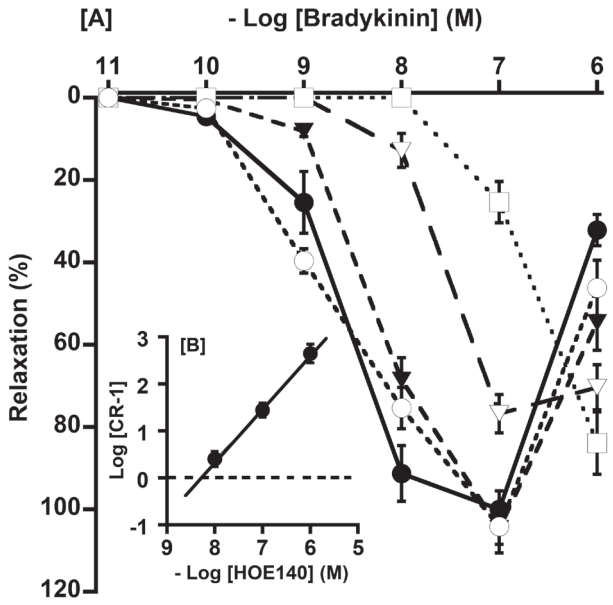

Fig. 7. Effects of the B_1 receptor antagonist des-Arg^9-[Leu^8]-bradykinin (○, 10^{-5} M) and the B_2 receptor antagonist HOE140 (▼: 10^{-8} M, ▽: 10^{-7} M and □: 10^{-6} M) on bradykinin (BK)-induced biphasic responses (●) [A] and Schild plot of HOE140 in the isolated dolphin cerebral artery [B]. The relaxation induced by BK in the absence of antagonist was taken as 100%. Each point represents the mean ± SEM for 6 dolphins. CR: see Fig. 2.

shifted the concentration-response curve of 5-HT to the right and downward (Fig. 3). Methiothepin is reported to have high affinity to the 5-HT_2 receptor (pK_B or pA_2=9.0) and low affinity to the 5-HT_1 receptor (pK_B or pA_2=7.7) [9]. Therefore, we consider that methiothepin may inhibit 5-HT_2-related contraction at a low concentration and both 5-HT_1 and 5-HT_2-related contraction at a high concentration. A similar phenomenon has been observed in the porcine cerebral artery which has 5-HT_1 and 5-HT_2 receptors [23]. Our data indicate that 5-HT-induced contractions in the dolphin cerebral artery involve both 5-HT_1 and 5-HT_2 subtypes. Similar findings have been reported for the equine cerebral artery [25].

His induced concentration-dependent contractions in the isolated dolphin cerebral artery. The pEC_{50} value (5.82 ± 0.06) of His in the dolphin cerebral artery was close to that in porcine cerebral artery (5.17 ± 0.16) [16]. The H_1 receptor antagonist diphenhydramine shifted the concentration-response curve of His to the right, whereas the H_2 receptor antagonist cimetidine had no significant effect. These results suggest that H_1 receptor activation induces the contraction of the dolphin cerebral artery. Contraction of the resting vascular tone in response to His has also been reported in pigs [16], cattle, horses [20], and guinea pigs [3]. The calculated pA_2 value for diphenhydramine was 7.26 ± 0.13, which is very close to the values reported for bovine (7.61) and porcine (7.77) cerebral arteries [16, 20].

Ang II and ACh did not induce any vasomotor action in the dolphin cerebral artery. In contrast, Ang II induced a very weak contraction in the porcine cerebral artery, with a variation in proximal to distal part responses and a variation in repeated application responses [24]. ACh did not induce any vasomotor action in the porcine cerebral artery either (unpublished data). Thus, muscarinic receptors may be absent or poor in the dolphin cerebral artery. Diphenhydramine is a potent muscarinic antagonist in addition to being an H_1-selective antihistamine [14]. However, ACh did not produce any vasomotor action in resting tension or pre-contraction in dolphin cerebral artery. Therefore, we consider that diphenhydramine may not affect muscarine receptors in this artery. Differences in the responsiveness to these substances may be due to the absence of their receptors on smooth muscle or endothelial cells.

NA induced relaxation in the dolphin cerebral artery in a concentration-dependent manner. The pEC_{50} value of NA (6.15 ± 0.12) in the dolphin cerebral artery was similar to that in the porcine cerebral artery [18]. A non-selective β-adrenoceptor antagonist, propranolol (10^{-8}–10^{-6} M), inhibited NA-induced relaxation in a concentration-dependent manner. Moreover, pretreatment with 10^{-5} M propranolol avoided this relaxation and induced slight contractions, which could be blocked by pretreatment with phentolamine, a non-selective α-adrenoceptor antagonist (data not shown). Together, these results suggest that the relaxation induced by NA is mediated through the stimulation of β-adrenoceptors and that few α-adrenoceptors modify NA-induced relaxations. These results were similar to those obtained for porcine cerebral [18] and coronary arteries [37].

BK-induced relaxation was abolished in arteries after endothelial denudation as shown in Fig. 6. Pretreatment with L-NNA shifted the concentration-response curve for BK to the right, and indomethacin abolished BK-induced contraction. These results suggest that endothelium-dependent responses to BK are primarily mediated via NO (relaxation event) and contractile prostaglandins (PGs). This result was also consistent with previous findings on the porcine cerebral artery [17]. In pigs, $PGF_{2\alpha}$ has been identified as contractile PG [10].

In the present study, the relaxing and contracting effects of BK were significantly inhibited by HOE140 but not by the B_1 receptor antagonist des-Arg^9-[Leu^8]-BK, as shown in Fig. 7. These data indicate that the dilating and contracting responses of BK in the dolphin cerebral artery are mediated by the B_2 receptor and not by the B_1 receptor. B_1 receptor-mediated responses are generally not observed under normal physiological conditions [34]. The calculated pA_2 value of HOE140 was 8.30 ± 0.08, which is similar to that reported for the guinea-pig ileum (8.42) [8] and human umbilical vein (8.52) [30]. Although relaxation induced by the activation of endothelial B_2 receptors has been reported in human [35] and mouse [11] cerebral arteries, contraction induced by the activation of endothelial B_2-receptors in cerebral arteries has only been reported in the porcine cerebral artery [17].

The coexistence of two different BK receptor subtypes (B_1 and B_2) in the same artery may cause a biphasic response to BK [29]. However, as observed with the porcine cerebral artery, dolphin cerebral artery demonstrated a biphasic response owing to only one type of BK receptor (B_2). It will be of interest to determine how the signal from the B_2 receptor regulates the pathways of both the cyclo-oxygenase and NO synthase systems in endothelial cells and to determine why the relaxant response was first evoked before the contractile response. It has been previously reported that the stimulation of B_2 receptors activates the NO synthase pathway [12] and the cyclo-oxygenase pathway [2] via the activation of heterotrimeric G-proteins of the Gi and Gq family [19]. Thus, further studies are needed to clarify this issue.

In summary, we investigated the responses of the dolphin cerebral artery to several pharmacological agents that are modulators of cerebrovascular circulation in both normal and pathophysiological states. We demonstrated that 5-HT and His induce contractions in the dolphin cerebral artery, NA and BK induce relaxation, and Ang II and ACh induce no response. Our results show that dolphins and pigs show a high degree of similarity in cerebral artery responsiveness to intrinsic vasoactive substances, thus strengthen the evidence of their close phylogenetic relationship.

CONFLICT OF INTEREST. The authors have no conflict of interest to declare.

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