Noradrenaline-Induced Smooth Muscle Relaxation in the Specific Region of Canine Facial Vein: Implications for Facial and Cranial Circulation

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Abstract: This study was performed to investigate the heterogeneity of physiological and pharmacological properties in segments of the facial veins with special reference to selective brain cooling. Canine facial veins were isolated and the isometric tension of each segment was measured using the organ bath technique. Vessels in the segments of the facial veins that run opposite to the buccal cavity automatically produced myogenic tone and tended to show spontaneous contractions, but vessels in other segments did not. When no contractile agent was used for precontraction, noradrenaline and adrenaline produced dose-dependent relaxations in the former venous segments, but contractions in the latter ones. A Schild plot analysis for metoprolol against denopamine and for IC118,551 against salbutamol

showed that the venous segments running opposite the buccal cavity contained both β_1 - and β_2 -adrenoceptors, but the other venous segments contained only β_2 -adrenoceptors. Electrical field stimulation-induced tetrodotoxin-sensitive relaxations in the former venous segments were diminished by pretreatment with metoprolol, but not with IC118,551, indicating that the electrical stimulation-induced relaxation may be related to the activation of β_1 -adrenoceptors in the venous smooth muscles. In conclusion, the heterogeneity of the functional properties, especially in the distribution of β -adrenoceptors, in different segments of canine facial veins was observed in the present study, and autoregulatory mechanisms, humoral mechanisms, and neural mechanisms were suggested to affect cranial venous drainage.

Key words: venous smooth muscle, spontaneous rhythmic contraction, β -adrenoceptors, sympathetic innervation, cranial circulation.

The branches of facial veins form a complicated network that communicates with the cavernous sinus through the ophthalmic vein and orbital venous plexus [1]. This network is known to deliver cold venous blood from the nasal mucosa to the cavernous sinus, which is located at the bottom of the brain [2]. In some species, including humans, dogs [1], and reindeer [2], the carotid artery runs within the cavernous sinus, in which heat may be exchanged with warm blood ascending in the carotid artery. This mechanism has been directly demonstrated to mediate a selective cooling of the brain in reindeer [2].

Venous blood from nasal mucosa passes through not only the cavernous sinus, but also the buccal part of the facial vein. The distribution of blood flow in this venous network will be determined by the relative resistances in each segment of the network. Marked heterogeneity in functional and morphological properties has been demonstrated in different segments of the veins [3]. For example, the buccal segment of the facial vein shows unique characteristics, such as stretch- and flow-dependent temperature-sensitive intrinsic myogenic tone [4, 5], noradrenaline-induced vasorelaxation, electric field stimulation-induced vasorelaxation, a thick vascular wall accompany-

ing the relatively wide lumen, and a dense innervation of adrenergic and peptidergic fibers extending throughout the thickness of the vessel wall [3, 6]. Under heat stress, augmented nasal mucosal venous drainage, increased temperature of the buccal segment of the facial vein, and a suppression of the activity of sympathetic nerves together may cause constriction of this segment of the vein. Thus the constriction of this segment, which acts as a “facial venous sphincter,” may affect the distribution of blood flow through the venous network and contribute to the enhancement of selective brain cooling during heat stress. The physiological and pharmacological functional properties of each segment of facial veins, however, have not been well elucidated.

In this study, we first attempted to evaluate the mechanical properties of the veins, such as myogenic tone and spontaneous contractions; second, we attempted to evaluate the effects of catecholamines and other vasoactive substances on the venous smooth muscles; and third, we attempted to pharmacologically classify β -adrenoceptor subtypes in the segments of facial veins. Moreover, the current study was designed to characterize the mode of action of autonomic innervation in the isolated venous seg-

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ments. The results of this study may provide further understanding regarding the regulation of cranial venous blood drainage with special reference to selective brain cooling.

MATERIALS AND METHODS

Tissue preparation. Forty-two mongrel dogs of both sexes, weighing 8.2 ± 2.7 kg (mean $\pm$ SD), were anesthetized with sodium pentobarbital (25 mg/kg i.v.) and exsanguinated via the femoral veins. Facial veins were rapidly dissected out and cleaned of surrounding tissues. Preparations were dissected out from each segment of the veins, i.e., the mandibular facial vein (MFV), the proximal part of the buccal facial vein (pBFV), the central part of the buccal facial vein (cBFV), the proximal part of the deep facial vein (pDFV), the distal part of the deep facial vein (dDFV), the distal part of the buccal facial vein (dBV), the dorsal nasal vein (DNV), and the angularis oculi vein (AOV) (total 194 preparations) (Fig. 1). Ring preparations 4 mm wide were cut from the segments. All animals were treated in accordance with the guiding principles of the Physiological Society of Japan.

Measurement of mechanical activity. Two tungsten wires (100 μ m in diameter) were run through the lumen of the venous segment. The upper end of the segment was connected to the lever of a force-displacement transducer (Shinko Tsushin UL10, Tokyo, Japan), and the lower end was fixed to the bottom of the bath. The isometric tension detected by the transducer was amplified and recorded on a direct-writing oscillograph (Sanei Sokki, 8K, Tokyo, Japan). Each segment was suspended in a 10 ml organ bath and perfused at a constant rate of 4 ml/min with Krebs-bicarbonate solution, which was maintained at $37.0 \pm 0.5^\circ\text{C}$ and aerated with 95% O_2 and 5% CO_2 to give pH 7.4. The composition of the Krebs solution was as follows (in mM): NaCl, 120.0; KCl, 5.9; NaHCO_3 , 25.0; NaH_2PO_4 , 1.2; CaCl_2 , 2.5; MgCl_2 , 1.2; and glucose, 5.5. The resting tension of each segment was set at 0.5 g, which was optimal for inducing maximal contraction developed by a high potassium (80 mM) Krebs-bicarbonate solution. A high potassium Krebs-bicarbonate solution was prepared by replacing part of the NaCl with an equimolar amount of KCl.

All venous segments were allowed to equilibrate for 60 min in the oxygenated bathing medium before the start of the experiment. After this equilibration period, the strength and frequency of the spontaneous contractions were measured. The following protocols were then carried out.

Experimental protocols

Protocol 1: Contractile and relaxant responses of facial venous smooth muscles. After 80 mM K^+ Krebs-bicarbonate solution-induced contraction had been obtained, cumulative concentration-response curves for noradrena-

line (NA), adrenaline (Adr), prostaglandin $\text{F}_{2\alpha}$ ($\text{PG F}_{2\alpha}$), 5-hydroxytryptamine (5-HT), and histamine (His) were constructed in a randomized order in each preparation, using serial tenfold dilutions of each agent, as soon as a steady response to the previous administration had been achieved. Cumulative concentration-response curves for isoprenaline (ISP), acetylcholine (ACh), sodium nitroprusside (SNP), His, adenosine (ADO), adenosine triphosphate (ATP), and adenosine diphosphate (ADP) were similarly constructed in the venous rings, which had been contracted with 3×10^{-6} – 10^{-5} M 5-HT. The precontraction in each venous ring was $\sim 60\%$ of the contraction induced by myogenic tone plus 80 mM K^+ Krebs-bicarbonate solution.

As shown in Fig. 2, passive tension was determined as the maximal relaxation level of each ring preparation, which had been achieved at rest or during drug-induced relaxant responses. Myogenic tone was measured just before applying 80 mM K^+ Krebs-bicarbonate solution, and precontraction was defined as myogenic tone plus 5-HT-induced contraction. The degree of contraction induced by each drug was expressed as a percentage of the 80 mM K^+ Krebs-bicarbonate solution-induced contraction, and the degree of relaxation was expressed as a percentage of the precontraction.

Protocol 2: Classification of β -adrenoceptor subtypes in pBFV, cBFV, pDFV, and AOV. In this protocol, pBFV, cBFV, and pDFV were used with no discrimination because no significant difference in contractile and relaxant responses was observed among these veins in protocol 1. Venous ring preparations of pBFV, cBFV, pDFV, and AOV were precontracted by perfusion of the Krebs-bicarbonate solution containing 3×10^{-6} – 10^{-5} M 5-HT, which resulted in $\sim 60\%$ of the contraction induced by the 80 mM K^+ Krebs-bicarbonate solution. After the precontraction reached a plateau, cumulative concentration-response curves for β -adrenoceptor agonists, denopamine, a selective β_1 -adrenoceptor agonist, and salbutamol, a selective β_2 -adrenoceptor agonist, were obtained in each venous segment. The extent of relaxation was expressed as a percentage of the precontraction. In some experiments, selective β -antagonists, metoprolol (10^{-8} M, 10^{-7} M, 10^{-6} M, β_1) and ICI118,551 (10^{-8} M, 10^{-7} M, 10^{-6} M, β_2) were dissolved in the oxygenated Krebs-bicarbonate solution and perfused at least 20 min before the concentration-response curves for denopamine or salbutamol were determined.

A linear relationship was obtained when the molar concentration of the β -antagonists was plotted on the abscissa on a logarithmic scale against the dose ratio of the corresponding β -agonist minus unity on the ordinate on a logarithmic scale. The slope and pA_2 values for the β -antagonists, i.e., the antagonist concentration at which the dose ratio is equal to 2, were calculated as described by Arunlakshana and Schild (1959) to compare the blocking potency of the antagonists.

Protocol 3: Effects of electrical field stimulation on mechanical activity in AOV and cBFV. Electrical field stimulation was delivered in the AOV and cBFV via platinum electrodes at the top and bottom of the organ bath from a Square One modular stimulator (SEN 710, Isolator SS102J, Nihon Kodens, Tokyo, Japan) without touching preparations. The adjustment of electrical stimulation was monitored with an oscilloscope (VC4065, Hitachi, Tokyo, Japan). The segments were stimulated with 15 s trains of pulses at a nominal voltage of 25 V and pulse width of 0.1 ms.

After 80 mM K^+ Krebs-bicarbonate solution-induced contraction had been obtained, the isometric tension was measured. The frequency of the stimulation was shifted in steps from 0.5 to 1, 2, 4, 8, 16, and 32 Hz with an interval of 2 min. Then the bath solution was changed to Krebs-bicarbonate solution containing tetrodotoxin (TTX) (10^{-7} M), and the response to the same stimulation was again assessed after an equilibration period of 30 min.

In other cBFV segments, 5 repeated stimulations with an interval of 2 min were performed. Then phentolamine (10^{-6} M), phentolamine (10^{-6} M) + ICI118,551 (10^{-8} M), and phentolamine (10^{-6} M) + ICI118,551 (10^{-8} M) + metoprolol (10^{-7} M) were dissolved in the Krebs-bicarbonate solution and perfused at least 30 min before the stimulation-induced responses were observed in the order described at the stimulation frequency of 4 Hz. Phentolamine (10^{-6} M), phentolamine (10^{-6} M) + metoprolol (10^{-7} M), and phentolamine (10^{-6} M) + metoprolol (10^{-7} M) + ICI118,551 (10^{-8} M) were also administered at a frequency of 8 Hz. The degree of the responses was expressed as a percentage of the electrical stimulation-induced relax-

ation without drugs in each preparation.

Drugs. We used the following drugs: adenosine, adenosine 5'-diphosphate sodium salt, adenosine 5'-triphosphate disodium salt, l-adrenaline bitartrate, l-noradrenaline bitartrate, 5-hydroxytryptamine creatine sulfate, salbutamol hemisulfate, tetrodotoxin (Sigma, St. Louis, MO); prostaglandin $F_{2\alpha}$ (Ono Yakuhin, Osaka, Japan); denopamine (Tanabe, Tokyo, Japan); acetylcholine chloride, l-isoprenaline hydrochloride (Nikken Kagaku, Tokyo, Japan); metoprolol tartrate, phentolamine mesylate (Ciba-Geigy, Basel, Switzerland); and ICI118,551 (erythro-dl-1-(7-methylindan-4-yloxy)-3-(isopropylaminobutan-2-ol hydrochloride) (Imperial Chemical Industries, Wilmslow, Cheshire, UK).

Statistics. All results are expressed as the mean $\pm$ SEM unless otherwise indicated. The pD_2 was calculated as $-\log ED_{50}$ in which ED_{50} is the concentration of agonist causing 50% of maximum response. A one-way analysis of variance (ANOVA) followed by the Fisher's protected least significant difference (PLSD) test was used to test for statistical significance between groups. The differences between groups were considered significant at $p < 0.05$.

RESULTS

Spontaneous activity of the venous smooth muscle cells in the segments of canine facial veins

Spontaneous rhythmic contractions were observed in all pBFV and cBFV preparations, and in 54% of pDFV preparations (Fig. 1, B, C, and D and Table 1). On the other hand, spontaneous rhythmic contractions were not observed in the dBFV, DNV, or AOV and were observed in

Fig. 1. Diagrammatic anatomy of canine facial venous network and related veins (center) and representative recordings of the effects of noradrenaline (NA) in each segment of the vessels (A to H). EJV, external jugular vein. IMV, internal maxillary vein. EMV, external maxillary vein. OVP, orbital venous plexus.

Table 1. Spontaneous contraction of the isolated canine facial veins in each region.

Region	<i>n</i>	Number and percentage of spontaneously contractile vessels		Strength (g)	Frequency (min ⁻¹)	80 mM K ⁺ -induced contraction (g)
MFV	8	1	13%	0.25	6.0	2.7 ± 0.6
pBFV	20	20	100%	0.34 ± 0.05	6.1 ± 1.0	1.4 ± 0.2
cBFV	9	9	100%	0.23 ± 0.04	5.7 ± 0.9	1.3 ± 0.2
pDFV	13	7	54%	0.21 ± 0.09	5.0 ± 0.9	1.4 ± 0.1
dDFV	13	2	15%	0.25 ± 0.05	3.1 ± 0.1	2.2 ± 0.3
dBV	9	0	0%	no spontaneous contraction was observed		0.4 ± 0.1
DNV	15	0	0%	no spontaneous contraction was observed		2.9 ± 0.2
AOV	21	0	0%	no spontaneous contraction was observed		2.7 ± 0.2

Values are mean ± SE. *n*, number of experiments. Each region of facial vein is described in MATERIALS AND METHODS.

Table 2. 80 mM K⁺-induced contraction, myogenic tone, and $E_{\max}$ and pD_2 values of noradrenaline (NA) in the segments of the isolated canine facial veins.

Region	<i>n</i>	80 mM K ⁺ -induced contraction (g)	Myogenic tone (g)	NA-induced response	$E_{\max}$ (%)	pD_2
MFV	4	1.9 ± 0.4	0	contraction	>70 ± 8 ^a	NC
pBFV	6	1.2 ± 0.2	0.8 ± 0.2	relaxation	92 ± 2 ^b	6.2 ± 0.3
cBFV	4	1.3 ± 0.3	0.9 ± 0.2	relaxation	90 ± 4 ^b	5.7 ± 0.2
pDFV	5	1.2 ± 0.2	1.2 ± 0.3	relaxation	94 ± 3 ^b	5.8 ± 0.2
dDFV	4	1.9 ± 0.2	0	contraction	>36 ± 8 ^a	NC
dBV	6	0.4 ± 0.1	0	contraction	>141 ± 7 ^a	NC
DNV	5	2.8 ± 0.3	0	contraction	>51 ± 13 ^a	NC
AOV	6	2.5 ± 0.3	0	contraction	>78 ± 11 ^a	NC

Values are mean ± SE. *n*, number of experiments. a, maximum contractions expressed as a percentage of 80 mM K⁺-induced contractions. b, maximum relaxations expressed as a percentage of myogenic tone. pD_2 , $-\log [ED_{50}]$ in which ED_{50} is the concentration of NA causing a 50% of each maximum response. NC, not calculated. Each region of facial vein is described in MATERIALS AND METHODS.

Fig. 2. *In vitro* recording of a canine facial vein (proximal part of a deep facial vein). SNP, sodium nitroprusside. 5-HT, 5-hydroxytryptamine. a, passive tone. b, intrinsic myogenic tone. c, 80 mM K⁺-induced contraction. d, 5-HT-induced contraction. e, precontraction.

only 13% and 15% of MFV and dDFV preparations, respectively (Table 1).

Myogenic tone was observed in all pBFV, cBFV, and pDFV preparations (Table 2). No significant differences were observed in the value of developed myogenic tone among these 3 groups. No myogenic tone was observed in the MFV, dDFV, dBV, DNV, and AOV.

Adrenoceptor agonist-induced contractions and relaxations in the segments of canine facial veins

Figure 1 shows typical responses of the MFV, pBFV, cBFV, pDFV, dDFV, dBV, DNV, and AOV to NA. The addition of NA (10^{-8} to 10^{-4} M) caused concentration-dependent relaxations in the pBFV ($n = 6$), cBFV ($n = 4$), and pDFV ($n = 5$) without precontraction other than myogenic tone (Fig. 3, A and B). No significant differences were observed in the $E_{\max}$ and pD_2 of NA-induced relax-

ations among these 3 groups (Table 2). On the other hand, NA (10^{-8} to 10^{-4} M) caused concentration-dependent contractions in the MFV ($n = 4$), dDFV ($n = 4$), dBV ($n = 6$), DNV ($n = 5$), and AOV ($n = 6$) (Fig. 3, A and B). The pD_2 values of NA in the MFV, dDFV, dBV, DNV, and AOV were not calculated because up to 10^{-4} M of NA produced no maximum contractions (Fig. 1 and Table 2).

Similar relaxations were induced by ADR (10^{-8} to 10^{-4} M) in pBFV ($n = 6$, $E_{\max} = 83 \pm 9\%$, $100\% = 1.2 \pm 0.2$ g, $pD_2 = 6.0 \pm 0.2$) and pDFV ($n = 5$, $E_{\max} = 73 \pm 12\%$, $100\% = 1.5 \pm 0.4$ g, $pD_2 = 6.2 \pm 0.3$) without precontraction (Fig. 3C). Concentration-dependent contractions were produced by ADR in the MFV ($n = 5$), dDFV ($n = 6$), dBV ($n = 4$), DNV ($n = 5$), and AOV ($n = 5$) (Fig. 3C). ADR did not produce maximum contractions in these venous segments up to 10^{-5} M.

Figure 3F shows the concentration-response curves of ISP (10^{-8} to 10^{-5} or 10^{-4} M) in the pBFV ($n = 6$), cBFV ($n = 5$), pDFV ($n = 5$), dDFV ($n = 4$), DNV ($n = 4$), and AOV ($n = 6$) precontracted with 3×10^{-6} or 10^{-5} M 5-HT. In these venous segments, ISP produced marked relaxations in a dose-dependent manner ($E_{\max} = 80\text{--}94\%$, not significant among the groups). The decreasing order of potency for ISP in the relaxant response among the venous segments was as follows (pD_2): pDFV (6.9 ± 0.2) = cBFV (6.7 ± 0.4) = pBFV (6.7 ± 0.2) > AOV (6.4 ± 0.3) = dDFV (6.3 ± 0.2) > DNV (5.6 ± 0.2). The pD_2 of ISP in the pBFV, cBFV, and pDFV were significantly larger than that in the DNV.

Fig. 3. Cumulative dose-response curves to noradrenaline (NA), adrenaline (Adr), 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$), isoprenaline (ISP), acetylcholine (ACh), sodium nitroprusside (SNP), histamine (His), adenosine (ADO), adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP) in mandibular facial vein (MFV, solid circles), proximal part of buccal facial vein (pBFV, open circles), central part of buccal facial vein (cBFV, open triangles), proximal part of deep facial vein (pDFV, open squares), distal part of deep facial vein (dDFV, solid triangles), distal part of buccal facial vein (dBFV, solid squares), dorsal nasal vein (DNV, solid diamonds), and angularis oculi vein (AOV, inverted solid triangles) pretreated with 3×10^{-6} – 10^{-5} M 5-HT (FGHJKLM) or without any contractile agents (ABCDEI). Preparation numbers in each experiment were described in the text. Each value is presented as a mean $\pm$ SEM (vertical bars). The ordinate shows the isometric tension expressed in gram (A) or expressed as a percent contraction of 80 mM K^+ -induced contraction and/or percent relaxation of pre-contraction (B to M). The abscissa denotes the molar concentration of agonists on a logarithmic scale.

Agonist-induced contractile and relaxant responses in the segments of canine facial veins

5-HT (10^{-8} to 10^{-4} M) and PG $F_{2\alpha}$ (10^{-8} to 10^{-4} M) caused dose-dependent contractions in the pBFV ($n = 6$ and 6), cBFV ($n = 4$, 5-HT), pDFV ($n = 5$ and 5), dDFV ($n = 5$ and 4), dBFV ($n = 6$ and 5), DNV ($n = 4$ and 4), and AOV ($n = 5$ and 5). Cumulative concentration-response curves of these agents are summarized in Fig. 3, D and E. None of these agonists showed a plateau of the response at concentrations ranging from 10^{-8} to 10^{-4} M.

ACh (10^{-8} to 10^{-4} M) and SNP (10^{-8} to 10^{-4} M) produced dose-dependent relaxations in venous segments of pBFV ($n = 6$ and 6), cBFV ($n = 5$ and 6), pDFV ($n = 4$ and 5), dDFV ($n = 4$ and 4), DNV ($n = 4$ and 4), and AOV ($n = 6$ and 6) precontracted with 3×10^{-6} or 10^{-5} M 5-HT (Fig.

Table 3. $E_{\max}$ and pD_2 values of isoprenaline (ISP) in the segments of the isolated canine facial veins.

Region	<i>n</i>	80 mM K ⁺ -induced contraction (g)	Precontraction (g)	$E_{\max}$ (%)	pD_2
pBFV	5	1.4 ± 0.3	1.1 ± 0.1	86 ± 6	6.7 ± 0.2 ^a
cBFV	5	1.3 ± 0.2	0.8 ± 0.1	93 ± 3	6.7 ± 0.4 ^a
pDFV	8	1.4 ± 0.2	1.2 ± 0.1	82 ± 15	6.9 ± 0.2 ^b
dDFV	6	2.3 ± 0.7	1.6 ± 0.4	80 ± 11	6.3 ± 0.2
DNV	5	2.6 ± 0.3	1.3 ± 0.4	94 ± 4	5.6 ± 0.2 ^{a,b}
AOV	5	2.6 ± 0.5	1.3 ± 0.3	83 ± 8	6.4 ± 0.3

Values are mean ± SE. *n*, number of experiments. $E_{\max}$, maximum relaxations expressed as a percentage of precontraction, myogenic tone plus 5-HT (3×10^{-6} – 10^{-5} M)-induced contraction. pD_2 , $-\log [ED_{50}]$ in which ED_{50} is the concentration of ISP causing 50% of each maximum response. a, $p < 0.05$ pBFV vs. DNV and/or cBFV vs. DNV. b, $p < 0.01$ pDFV vs. DNV. Each region of facial vein is described in MATERIALS AND METHODS.

Fig. 4. Effects of metoprolol on relaxation induced by denopamine in pBFV (proximal part of buccal facial vein), cBFV (central part of buccal facial vein), pDFV (proximal part of deep facial vein) (**A**, $n = 6$), and AOV (angularis oculi vein) (**C**, $n = 6$). Effects of ICI118,551 on relaxation induced by salbutamol in pBFV, cBFV, and pDFV (**B**, $n = 4$), and AOV (**D**, $n = 4$). Open circles, control (**A**, 100% = 1.9 ± 0.3 g; **B**, 100% = 1.6 ± 0.3 g; **C**, 100% = 1.9 ± 0.2 g; **D**, 100% = 1.5 ± 0.3 g). Solid circles, 10^{-8} M antagonist. Solid triangles, 10^{-7} M antagonist. Solid squares, 10^{-6} M antagonist. Each value is presented as a mean ± SEM (vertical bars). In the ordinate, precontraction was taken as 100%. The abscissa denotes the molar concentration of agonists on a logarithmic scale.

3, G and H). The pD_2 of ACh and SNP in these venous segments was 5.2–5.9 and 5.6–6.1, respectively, and no significant differences were observed among the groups.

His (10^{-8} to 10^{-4} M)-induced responses were evaluated both in venous segments without precontraction and precontracted with 3×10^{-6} or 10^{-5} M 5-HT (Fig. 3, I and J). In venous segments without precontraction, the pBFV ($n = 6$) showed dose-dependent relaxation ($86 \pm 7\%$ at 10^{-4} M, 100% = 0.9 ± 0.2 g). On the other hand, the dDFV ($n = 4$), dBFV ($n = 6$), DNV ($n = 4$), and AOV ($n = 5$) demonstrated no contractions or only slight ones at high concentrations of His ($\sim 19\%$ at 10^{-4} M) in these segments. When the venous segments of the pBFV ($n = 4$), dDFV ($n = 3$), DNV ($n = 3$), and AOV ($n = 3$) were precontracted with 3×10^{-6} or 10^{-5} M 5-HT, similar dose-relaxant response curves were obtained with His among these segments.

Figure 3 KLM shows dose-response curves of ADO (10^{-8} to 10^{-4} M), ATP (10^{-8} to 10^{-4} M), and ADP (10^{-8} to 10^{-4} M) in the venous segments precontracted with 3×10^{-6} or 10^{-5} M 5-HT. ADO, ATP, and ADP produced no or only slight relaxation at a high concentration ($\sim 18\%$ at 10^{-4} M) in venous segments of the pBFV ($n = 6$), cBFV ($n = 5$), pDFV ($n = 4$), dDFV ($n = 4$), and AOV ($n = 5$) (ADO); pBFV ($n = 6$), cBFV ($n = 6$), pDFV ($n = 4$), dDFV ($n = 4$),

and AOV ($n = 5$) (ATP); and pBFV ($n = 4$), cBFV ($n = 5$), and AOV ($n = 4$) (ADP).

β -adrenoceptors in pBFV, cBFV, pDFV, and AOV

The addition of denopamine (10^{-9} M to 10^{-5} M), a selective β_1 -adrenoceptor agonist, caused dose-dependent relaxation in venous segments of the pBFV, cBFV, and pDFV ($n = 6$), which had been contracted with 10^{-5} M 5-HT, but not in the precontracted segment of the AOV ($n = 6$) (Fig. 4, A and C). The relaxant responses of the pBFV, cBFV, and pDFV to denopamine were suppressed by metoprolol (10^{-8} M to 10^{-6} M), a selective β_1 -adrenoceptor antagonist, in a dose-dependent manner ($n = 6$, Fig. 4, A and C). The slope and pA_2 for this antagonism in the Schild plot analysis are shown in Table 4.

Salbutamol (10^{-9} M to 10^{-5} M), a selective β_2 -adrenoceptor agonist, produced dose-dependent relaxations in all of the venous segments ($n = 4$ for pBFV, cBFV, and pDFV; $n = 4$ for AOV) precontracted with 10^{-5} M 5-HT (Fig. 4, B and D). ICI118,551 (10^{-8} M, 10^{-7} M), a selective β_2 -adrenoceptor antagonist, caused a parallel shift to the right of the concentration-response curve for salbutamol in the pBFV, cBFV, pDFV, and AOV (Fig. 4, B and D). The slope and pA_2 for this antagonism in the Schild plot

Table 4. Evaluation of the interaction of β -adrenoceptor agonists and antagonists in the segments of the isolated canine facial veins.

Region	Agonist	Antagonist	<i>n</i>	Slope	pA_2
pBFV, cBFV, pDFV	denopamine	metoprolol	6	1.23 ± 0.13 NS	7.53 ± 0.20
	salbutamol	ICI 118,551	4	0.90 ± 0.19 NS	8.86 ± 0.54
AOV	denopamine	metoprolol	6	no relaxation was observed	
	salbutamol	ICI 118,551	4	0.84 ± 0.05 NS	9.21 ± 0.17

Values are mean $\pm$ SE. *n*, number of animals. Each region of facial vein is described in MATERIALS AND METHODS. NS, not significantly different from unity.

Fig. 5. A: Representative recordings of 80 mM K⁺-induced contraction and frequency-dependent contractions of AOV (angularis oculi vein) induced by 15 s trains of electrical field stimulation at 25 V and 0.1 ms in pulse width. Pretreatment with 10⁻⁷ M tetrodotoxin abolished the contractions. **B:** Representative recordings of 80 mM K⁺-induced contraction and frequency-dependent relaxations of cBFV (central part of buccal facial vein) induced by the similar electrical field stimulation. Pretreatment with 10⁻⁷ M tetrodotoxin abolished the relaxations. **C:** Representative recordings of the effects of 10⁻⁶ M phentolamine, 10⁻⁷ M metoprolol, and 10⁻⁸ M ICI118,551 on the electrical field stimulation-induced relaxations in cBFV at a frequency of 8 Hz. **D:** Effects of 10⁻⁶ M phentolamine, 10⁻⁷ M metoprolol, and 10⁻⁸ M ICI118,551 on relaxant responses in pBFV (proximal part of buccal facial vein), cBFV, and pDFV (proximal part of deep facial vein) induced by 15 s trains of electrical field stimulation at 4 or 8 Hz, 25 V, and 0.1 ms in pulse width. The ordinate shows the isometric tension expressed as a percentage of control that was obtained on the absence of the drugs (100% = 0.60 $\pm$ 0.12 g at 4 Hz, *n* = 4; 100% = 0.81 $\pm$ 0.09 g at 8 Hz, *n* = 4). Each value is presented as a mean $\pm$ SEM (vertical bars). **p* < 0.05, ***p* < 0.01 vs. control. ††*p* < 0.01 vs. 10⁻⁶ M phentolamine. ‡‡*p* < 0.01 vs. 10⁻⁶ M phentolamine and 10⁻⁸ M ICI118,551.

analysis are shown in Table 4. In the presence of 10⁻⁶ M ICI118,551, salbutamol (up to 10⁻⁵ M) produced relaxation that was too small to be used in the Schild plot analysis.

Electrical field stimulation-induced mechanical responses of cBFV and AOV

Frequency-response relationship. When the frequency of electrical field stimulation was increased from 0.5 to 32 Hz, frequency-dependent contractile and relaxant responses could be obtained in the venous segments of the AOV (*n* = 4) and cBFV (*n* = 4), respectively (Fig. 5, A and

B). After 30 min of treatment with 10⁻⁷ M TTX, a sodium channel blocker, the electrical stimulation-induced contractions and relaxations were completely abolished (Fig. 5, A and B).

Effects of phentolamine, ICI118,551, and metoprolol on electrical field stimulation-induced relaxations of cBFV. Figure 5C demonstrates typical traces that show the effects of phentolamine, an α_1 - and α_2 -adrenoceptor antagonist, ICI118,551, and metoprolol on the electrical field stimulation-induced relaxations at a frequency of 4 Hz in a segment of canine facial vein. Pretreatment with 10⁻⁶ M phentolamine caused a significant increase in the electri-

cal field stimulation-induced relaxation at a frequency of 4 Hz in cBFV ($n = 4$) (Fig. 5D). Additional treatment with 10^{-8} M ICI118,551 caused no significant effect on the relaxation. Pretreatment together with 10^{-6} M phentolamine, 10^{-8} M ICI118,551, and 10^{-7} M metoprolol caused a significant decrease in the relaxation of the venous segments. At a frequency of 8 Hz, 10^{-6} M phentolamine significantly augmented the relaxant response of the venous segments, and additional treatment with 10^{-7} M metoprolol significantly decreased the relaxation ($n = 4$) (Fig. 5D). No further change was observed by additional treatment with 10^{-8} M ICI118,551.

DISCUSSION

Selective brain cooling

Venous blood from the nasal mucosa enters both the dBFV and AOV in the canine cranial drainage system [1]. The dBFV connects with the external jugular vein via the cBFV, pBFV, MFV, and external maxillar vein. On the other hand, the AOV connects with the cavernous sinus via the ophthalmic vein and orbital plexus. The proportion of blood flowing in each direction will be determined by the relative resistance in these pathways. Cooled blood delivered from the nasal mucosa to the cavernous sinus has been directly demonstrated to contribute to thermoregulation of the brain in *in vivo* experiments in reindeer [2]. When cooled blood flows into the orbital plexuses and the cavernous sinus at the base of the brain, heat might be exchanged with the cerebrospinal fluid. Furthermore, in the cavernous sinus heat would be exchanged with warm blood ascending in the carotid artery. In the canine cranial circulation, similar structures have been observed [1], and these thermoregulatory mechanisms may have a role in brain cooling during thermal stress.

A similar network of branches of the facial veins also exists in human [7] compared with that in dogs [1]. Moreover, Muller-Schweinitzer [8] also reported that the order of potencies of α -adrenoceptor agonists, adrenaline and noradrenaline, was similar in human and canine veins. Venous smooth muscle cells of the facial veins in human also produce physiological and pharmacological characteristics similar to those in dogs (as described below), such as intrinsic tone and β -adrenoceptor-induced relaxation caused by transmural nerve stimulation and NA [9]. Thus our study with canine facial veins may be applicable for understanding human cranial venous circulation.

In general, the smooth muscle layer in veins is thin, and contraction of the smooth muscles is known to produce few or no changes of the resistance of the vein. There are, however, marked regional differences in morphological and mechanical properties in the venous system. A distensibility of venous walls is related to each architecture of collagen fiber, elastic fiber, and smooth muscle cells in the walls [10]. Previous morphological studies demonstrated

that a specific area in the buccal region of facial veins has highly thickened media with prominent bands of circulatory smooth muscles in reindeer [2] and desert camel [11]. Further, functional venous occlusion was shown at a segment of the buccal facial veins by cineangiography in reindeer when body temperature was increased [2]. These findings strongly suggest that the contraction of a specific area of the facial veins acts as a sphincter and then produces the redistribution of venous flow in the cranial circulation.

Physiological and pharmacological properties of facial veins

All canine facial venous segments in buccal part, such as pBFV, cBFV, and pDFV, which run opposite to the buccal cavity, showed myogenic tone *in vitro*. Winquist and Bevan (1979) [12] demonstrated that the isolated buccal segment of the rabbit facial vein dramatically developed myogenic tone in response to stretch. This tone was exquisitely sensitive to small changes in temperature in the range of 33.5°C to 44.5°C [4]. The temperature-induced myogenic tone did not develop at 33.5°C, but approximately 80% of the maximum drug-induced tension was achieved at 44.5°C. Similar temperature-induced myogenic tone has also been observed in desert camels [13]. Thus thermal stress may induce an autoregulation of the cranial circulation, in which a contraction of the facial vein occurs at the buccal segment, and then a larger amount of cooled blood may be delivered from the nasal mucosa to the base of the brain rather than to the external jugular vein. Venous stretch induced by mouth opening may also affect the myogenic tone in the buccal segment of the facial vein.

Besides myogenic tone, spontaneous rhythmic contractions were observed mainly in the buccal part (pBFV, cBFV, pDFV) of the canine facial venous network and related veins. In the DNV of the desert camel, spontaneous contractions were also demonstrated accompanied by myogenic tone [13]. The canine posterior vena cava between the diaphragm and branches of renal veins [14] and the portal vein in the mouse, rat, guinea pig [15], and rabbit [16] are known to make spontaneous rhythmic contractions. These spontaneous contractions of smooth muscle cells in bovine mesenteric lymphatics have been demonstrated to propel lymph fluid to the adjacent lymphangion [17]. The physiological roles of the spontaneous contractions in these segments of the venous system, however, remain to be clarified.

In this study, NA and Adr induced dose-dependent relaxation in the buccal part (pBFV, cBFV, pDFV) only, but contractions in all other segments. In some parts of facial veins, NA-induced relaxation has also been demonstrated in rabbit [3], monkey [18], and man [9]. 5-HT and PG $F_{2\alpha}$ caused dose-dependent contractions in all segments. On the other hand, ACh and His caused dose-dependent re-

laxations in all segments precontracted with 5-HT. In an earlier study, however, 5-HT caused dose-dependent direct relaxation in rabbit facial veins [19], ACh caused dose-dependent muscarinic M_3 -receptor-mediated contraction in simian facial veins [20], and His caused dose-dependent contraction in rabbit facial veins [3], indicating that there are species differences in the responses to vasoactive agents. ADO, ATP, and ADP mediated no responses or only small ones in the segments. The present results suggested that humoral factors, such as NA, Adr, 5-HT, PG $F_{2\alpha}$, ACh, and His have some roles in the distribution of venous flow in the canine cranial venous drainage, but ADO, ATP, and ADP have relatively small roles in the drainage. Especially, NA and Adr reduce venous delivery from the nasal mucosa to the base of the brain via constriction of the MFV and AOV together with dilation of the pBFV and cBFV.

β -adrenoceptors

SNP induced dose-dependent relaxations with similar pD_2 in all of the venous segments precontracted with 5-HT. The pD_2 of ISP in the buccal part (pBFV, cBFV, pDFV) tended to be larger than in other portions. These findings indicate that the relaxant ability of the smooth muscle cells does not vary significantly among the vessels of these venous segments, but β -adrenoceptor-induced responses were more potent in the buccal part (pBFV, cBFV, pDFV) than in other segments.

In the buccal part (pBFV, cBFV, pDFV), both denopamine and salbutamol produced dose-dependent relaxations. Metoprolol and ICI118,551 caused a parallel shift to the right of the dose-response curves for denopamine and salbutamol, respectively. The pA_2 for metoprolol (7.53) in the buccal part (pBFV, cBFV, pDFV) was similar to that reported for β_1 -adrenoceptors in dog coronary arteries (7.48) [21] and bovine mesenteric lymphatics (7.59) [22]. Regarding β_2 -adrenoceptor-mediated responses, the pA_2 of 8.86 calculated for ICI118,551 in the same segments was similar to that for β_2 -adrenoceptors in the guinea pig trachea (8.81) [23] and in canine bronchi (8.91) [24]. These results suggest that venous dilations in these segments are produced via the activation of both β_1 - and β_2 -adrenoceptors.

In contrast with the findings for the buccal part (pBFV, cBFV, pDFV), dose-dependent relaxation was caused only by salbutamol in the AOV, not by denopamine. The pA_2 for ICI118,551 against salbutamol was 9.21. Thus only β_2 -adrenoceptors exist in the AOV and produce venous dilation.

Electrical field stimulation

In the present experiments, electrical stimulation produced frequency-related contractions in the AOV. On the other hand, the same stimulation induced frequency-related relaxation in the cBFV. These contractions and relax-

ations were totally abolished by tetrodotoxin, implying that the responses were neurogenic and not caused by a direct effect on the smooth muscles. Phentolamine significantly augmented the electrical stimulation-induced relaxation in the cBFV. Additional treatment with metoprolol, but not with ICI118,551, significantly reduced the relaxation. The concentration of phentolamine, metoprolol, and ICI118,551 selectively reduce α -, β_1 -, and β_2 -adrenoceptor-mediated responses, respectively [22, 25]. These present findings are consistent with the earlier observations that sympathetic nerve stimulation led to vasoconstriction in the AOV through α -adrenoceptor stimulation, and the relaxant response to sympathetic nerve stimulation was β_1 -adrenoceptor mediated in the buccal part of the rabbit facial vein [26].

Conclusions

In conclusion, the heterogeneity of the functional properties in different segments of canine facial veins was observed in the present study, and autoregulatory mechanisms that were induced by myogenic activities, humoral mechanisms mediated by vasoactive substances, and neural mechanisms related to the nervous activity of the vessels were suggested to affect cranial venous drainage. Especially, the buccal part (pBFV, cBFV, pDFV), which are located in the wall of the cheek, showed unique properties compared with other segments. These segments showed myogenic tone, spontaneous contractions, NA-induced relaxation, and electrical field stimulation-induced relaxation. Both β_1 - and β_2 -adrenoceptors were demonstrated in these segments, and the electrical field stimulation-induced relaxation was suggested to be mediated mainly via β_1 -adrenoceptors. These physiological characteristics in the veins may contribute to selective brain cooling during heat stress.

In the future, temperature-dependent changes in the physiological and pharmacological responsiveness in each segment of canine facial veins should be analyzed in detail. The mechanisms of brain cooling during exercise should be also examined because the increase of sympathetic activity may facilitate venous drainage through the buccal part (pBFV, cBFV, pDFV) and decrease venous flow from the nasal mucosa to the cavernous sinus.

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