Involvement of α- and β-Adrenergic Receptors in Skeletal Muscle Blood Flow Changes During Hyper-/Hypocapnia in Anesthetized Rabbits

Kyotaro Koshika, DDS, PhD;* Rumi Kaneko, DDS;† Mai Shionoya, DDS;† Kotaro Shimizu, DDS;† Yuka Sendai, DDS;† Nobutaka Matsuura, DDS;† Yui Akiike, DDS, PhD;‡ and Tatsuya Ichinohe, DDS, PhD§

*Associate Professor, Department of Dental Anesthesiology, Tokyo Dental College, Tokyo, Japan, †Postgraduate Student, Department of Dental Anesthesiology, Tokyo Dental College, Tokyo, Japan, ‡Senior Assistant Professor, Department of Dental Anesthesiology, Tokyo Dental College, Tokyo, Japan, §Professor and Chairman, Department of Dental Anesthesiology, Tokyo Dental College, Tokyo, Japan

Objective: This study investigated the involvement of α_1- and β_2-adrenergic receptors in skeletal muscle blood flow changes during variations in $ETCO_2$.

Methods: Forty Japanese White rabbits anesthetized with isoflurane were randomly allocated to 1 of 5 groups: phentolamine, metaproterenol, phenylephrine, butoxamine, and atropine. Heart rate (HR), systolic blood pressure (SBP), common carotid artery blood flow (CCBF), masseter muscle tissue blood flow (MBF), and quadriceps muscle tissue blood flow (QBF) were recorded and analyzed at 3 periods: (1) baseline, (2) during hypercapnia (phentolamine and metaproterenol groups) or hypocapnia (phenylephrine, butoxamine, and atropine groups), and (3) during or after receiving vasoactive agents.

Results: MBF and QBF decreased during hypercapnia. The decrease in MBF was smaller than that in QBF. SBP and CCBF increased, while HR decreased. Both MBF and QBF recovered to their baseline levels after phentolamine administration. MBF became greater than its baseline level, while QBF did not fully recover after metaproterenol administration. MBF and QBF increased during hypocapnia. The increase rate in MBF was larger than that in QBF. HR, SBP, and CCBF did not change. Both MBF and QBF decreased to ~90% to 95% of their baseline levels after phenylephrine or butoxamine administration. Atropine showed no effects on MBF and QBF.

Conclusion: These results suggest the skeletal muscle blood flow changes observed during hypercapnia and hypocapnia may mainly involve α_1-adrenergic but not β_2-adrenergic receptor activity.

Key Words: Adrenergic receptor; Hypercapnia; Hypocapnia; Skeletal muscle blood flow.

Skeletal muscle blood flow is regulated by various mechanisms.[1,2] In general, nitric oxide, carbon dioxide, lactate, adenosine, adenosine triphosphate, and serotonin have vasodilatory effects, while endothelin and serotonin have vasoconstrictive effects. Although carbon dioxide has direct vasodilatory effects, an elevation in $PaCO_2$ also enhances sympathetic nervous system (SNS) activity, which leads to vasoconstriction with a net result of reduced blood flow at the skeletal muscle tissue level.[1] In anesthetized rabbits, increases in end-tidal carbon dioxide ($ETCO_2$) tension reduced skeletal muscle blood flow while decreases in $ETCO_2$ resulted in increased skeletal muscle blood flow.[3–5] Because $ETCO_2$ values nearly equal $PaCO_2$ values,[6] it can be considered that decreases in skeletal muscle blood flow induced by $ETCO_2$ elevation are attributable to an enhancement of SNS activity rather than direct vasodilatory effects of carbon dioxide.

Constriction of skeletal muscle vessels induced by SNS activation is more profound in fast-twitch muscles than slow-twitch muscles.[7] Meanwhile, although the densities of vascular α1- and β-adrenergic receptors are similar in slow- and fast-twitch muscles, resistance

Received February 2, 2022; accepted for publication January 23, 2023.

Address correspondence to Dr Kyotaro Koshika, Department of Dental Anesthesiology, Tokyo Dental College, 2-9-18 Kanda-Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan; koshikakyotarou@tdc.ac.jp.

Anesth Prog 70:58–64 2023 | DOI 10.2344/anpr-70-02-02

arterioles are far more numerous in slow-twitch muscles.[8,9] Accordingly, it has been speculated that the differences in vascular regulation in fast- and slow-twitch muscles are attributable to the differences in vascular innervation or SNS activity and in the densities or functional reserves of vascular α-adrenergic receptors.[10]

If changes in skeletal muscle blood flow induced by variations in $ETCO_2$ arise primarily from altered SNS activity, then, as an example, the increase in skeletal muscle blood flow that occurs with decreased $ETCO_2$ may mainly involve the inhibition of α_1-adrenergic receptors rather than the activation of β_2-adrenergic receptors. Therefore, the purpose of this study was to investigate the activity of which adrenergic receptors, α_1 or β_2, is primarily involved in skeletal muscle blood flow changes during variations in $ETCO_2$. In this study, we targeted 2 skeletal muscles: the masseter muscle which predominantly has slow-twitch fibers, and the quadriceps muscle which predominantly has fast-twitch fibers.[11,12]

METHODS

Forty Japanese White rabbits (Sankyo Labo) weighing ~2.5 kg were used in this study. All animals received humane care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals and The Guidelines for the Treatment of Experimental Animals of Tokyo Dental College.[13] The animals were randomly allocated evenly (n = 8) into 1 of 5 groups: (1) phentolamine, (2) metaproterenol, (3) phenylephrine, (4) butoxamine, and (5) atropine groups.

Anesthesia and Experimental Preparation

General anesthesia was induced with isoflurane (4%) and oxygen delivered using a mask. Before skin incisions for each of the experimental procedures, 2% lidocaine (0.5 mL) was injected into the surgical field. A #20 Fr, noncuffed, pediatric tracheal tube 10 cm in length[6] was inserted into the trachea through a tracheostomy. The left auricular marginal vein and right femoral artery were cannulated with 22- and 20-gauge indwelling catheters, respectively. After intravenous (IV) acetated Ringer's solution was started at 10 mL/kg/h, the animals were paralyzed with a rocuronium infusion (14 μg/kg/min).[14]

Animals were ventilated with a tidal volume of 35 to 50 mL and a respiratory rate of 35 to 45 breaths per minute to maintain the $ETCO_2$ at 30 mm Hg. However, supplemental CO_2 was added to the inhaled oxygen to maintain the study's baseline $ETCO_2$ of 40 mm Hg. To produce hypercapnic conditions, additional CO_2 was mixed in to maintain $ETCO_2$ at 60 mm Hg. To produce hypocapnic conditions, supplemental CO_2 was stopped, and $ETCO_2$ was maintained at 30 mm Hg. Thus, hypercapnic and hypocapnic conditions were achieved without any changes in the ventilator settings. Exhaled gas was sampled at the connector between a tracheal tube and an anesthesia circuit. $ETCO_2$ and isoflurane concentrations were continuously monitored using an anesthetic gas monitor (Capnomac Ultima, Datex). Femoral artery blood pressure was continuously monitored with a pressure transducer (P231D, Gould).

After the skin incision along the left lower margins of the mandible without local anesthesia, the fascia of the left masseter muscle was detached to expose muscle tissue. A needle probe of the hydrogen clearance tissue blood flowmeter (UHE-100, Unique Medical) was inserted 3-mm deep into the anterior portion of the left masseter muscle to measure masseter muscle tissue blood flow (MBF). Then, after a skin incision was made along the left femoral region without local anesthesia, the left quadriceps muscle was exposed. A needle probe of the hydrogen clearance tissue blood flowmeter was inserted 5-mm deep into the center of the left quadriceps muscle to measure quadriceps muscle tissue blood flow (QBF).

After completion of experimental preparations, isoflurane was reduced to achieve an end-tidal concentration of 1.0% (0.5 minimum alveolar concentration in rabbits)[15] and maintained at that level for more than 60 minutes to stabilize hemodynamic and respiratory variables. A heating lamp was used to maintain body temperature at ~39.0 °C.

Measurements

Heart rate (HR) was recorded by a tachograph triggered by blood pressure wave. Common carotid artery blood flow (CCBF) was measured with an ultrasound flowmeter (T108, Transonic). A flow probe (type 3SB) was applied to the isolated left common carotid artery. HR, systolic blood pressure (SBP), and CCBF were continuously recorded using a tachometer (HRM-100, Unique Medical). MBF and QBF were analyzed using a data collection analysis system (UCO, Unique Medical).

Measurements were performed at 3 periods: (1) baseline, (2) hypercapnia/hypocapnia, and (3) during or after receiving vasoactive agents. After baseline, $ETCO_2$ was changed to 30 or 60 mm Hg and maintained

Table 1. Hemodynamic and Tissue Blood Flow Changes During Hypercapnia ± Phentolamine.*

	Baseline	*Hypercapnia*	*Phentolamine*
HR, bpm	300.6 ± 17.0	286.9 ± 16.0†	297.5 ± 18.5‡
SBP, mm Hg	131.9 ± 10.3	140.6 ± 11.5†	125.6 ± 8.6‡
CCBF, mL/min	31.9 ± 6.1	37.1 ± 7.0†	33.5 ± 6.1‡
MBF, mL/min/100 g	45.4 ± 9.0	33.2 ± 6.7†	46.9 ± 7.4‡
QBF, mL/min/100 g	62.2 ± 7.2	37.9 ± 8.2†	63.1 ± 8.5‡

* Mean ± SD. CCBF, common carotid artery blood flow; HR, heart rate; MBF, masseter muscle tissue blood flow; QBF, quadriceps muscle tissue blood flow; SBP, systolic blood pressure.
† $P < .05$ versus baseline.
‡ $P < .05$ versus hypercapnia.

at this level for 15 minutes, and measurements were repeated for hypercapnia/hypocapnia. Thereafter, rabbits received 1 of 5 vasoactive IV agents.

During hypercapnic conditions, rabbits received phentolamine (an α_1- and α_2-receptor antagonist) or metaproterenol (a β_2-receptor agonist). In the phentolamine group, the third measurement was performed 3 minutes after an IV bolus of phentolamine (100 μg/kg; Regitine, Novartis Pharma). In the metaproterenol group, the third measurement was performed 15 minutes after starting an IV infusion of metaproterenol (0.2 μg/kg/min; metaproterenol sulfate, FUJIFILM Wako Pure Chemical Corporation).

During hypocapnic conditions, rabbits received phenylephrine (an α_1-receptor agonist), butoxamine (a β_2-receptor antagonist), or atropine (a muscarinic receptor antagonist). In the phenylephrine group, the third measurement was performed 15 minutes after starting an IV infusion of phenylephrine (0.1 μg/kg/min; Neo-Synesin Kowa, Kowa) infusion. In the butoxamine group, the third measurement was performed 15 minutes after starting an IV infusion butoxamine (750 μg/kg/min; butoxamine hydrochloride, Sigma-Aldrich-Merck) infusion. In the atropine group, the third measurement was performed 3 minutes after an IV bolus of atropine (100 μg/kg; Atropine Sulfate, Nipro ES Pharma). Finally, after the metaproterenol, phenylephrine, or butoxamine infusions were stopped and the $ETCO_2$ levels recovered to 40 mm Hg, rabbits were kept at rest for more than 30 minutes. The experiments were concluded after the observed variables recovered to baseline levels.

Statistical Analysis

Sample size was determined by an a priori power analysis (α error = .05, β error = .20, effect size = 1.2) using two masseter muscle tissue blood flow measurements at $ETCO_2$ levels of 30 mm Hg (33.3 ± 2.8 mL/min/100 g) and 40 mm Hg (27.4 ± 4.0 mL/min/100 g) from our previous study.[3] A total of at least 8 rabbits per group (N = 40) were calculated as the required sample size.

All data were expressed as mean ± standard deviation. One-way repeated-measures analysis of variance (ANOVA) using a linear mixed models was applied to compare values at baseline, hyper-/hypocapnia, and after IV agent administration for HR, SBP, and CCBF. Two-way repeated-measures ANOVA using a linear mixed model with (1) MBF/QBF and (2) values at baseline, hypercapnia/hypocapnia, and after IV agent administration as 2 variable factors was performed. In the case of a significant interaction, 1-way ANOVA was performed considering all groups as independent,[16] and, if significant, multiple comparisons of all groups were performed using the Tukey method. SPSS version 28.0.0.0 (IBM Japan) was used. Statistical significance was determined using $P < .05$.

RESULTS

In all 5 groups, 2-way repeated-measures ANOVA showed a significant interaction between 2 muscles and 3 observed periods.

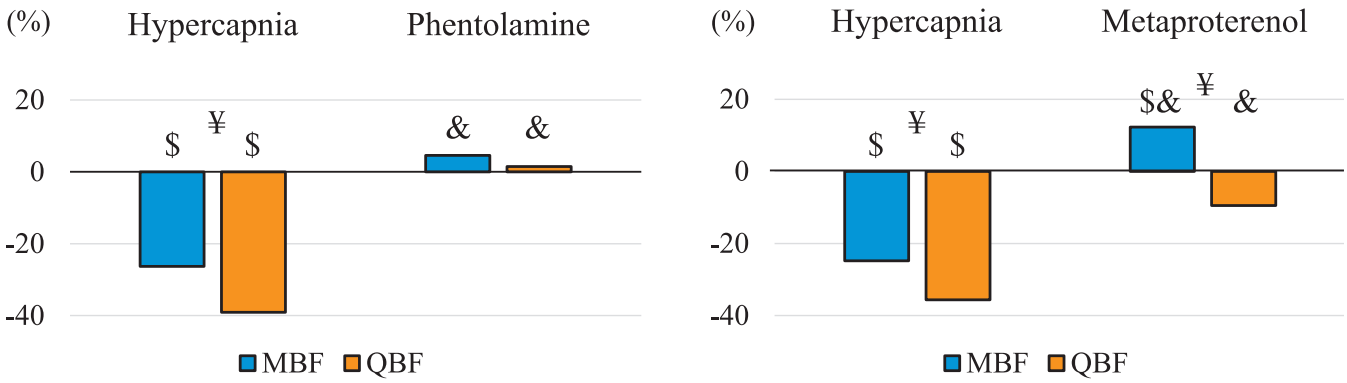
Hypercapnia Groups

Compared with baseline, both the phentolamine and metaproterenol groups had decreases in MBF and QBF during hypercapnia ($P < .05$). Decreases were larger in QBF than in MBF. SBP and CCBF increased, and HR decreased.

In the phentolamine group, all of these variables essentially recovered to baseline after phentolamine administration (Table 1, Figure 1).

In the metaproterenol group, both MBF and QBF increased relative to hypercapnia ($P < .05$), and although MBF increased beyond baseline ($P < .05$), QBF did not fully recover to baseline after metaproter-

Figure 1. Mean changes in muscle blood flow during hypercapnia and after phentolamine or metaproterenol administration.



MBF and QBF decreased during hypercapnia, while the decrease in MBF was smaller than that in QBF. Both MBF and QBF recovered to their baseline levels after phentolamine administration. In contrast, although MBF became greater than its baseline level, QBF did not fully recover to its baseline level after phentolamine administration. Data are expressed as the percentage change in respective baseline values. MBF, masseter muscle tissue blood flow; QBF, quadriceps muscle tissue blood flow. [a] $P < .05$ versus baseline; [b] $P < .05$ versus hypercapnia; [c] $P < .05$ between the 2 groups.

enol administration ($P < .05$; Table 2, Figure 1). HR remained decreased compared with baseline, SBP recovered to baseline, and CCBF increased as compared with baseline and hypercapnia ($P < .05$).

Hypocapnia Groups

As compared with baseline, the phenylephrine, butoxamine, and atropine groups had increases in MBF and QBF during hypocapnia ($P < .05$). Increases were greater in MBF than in QBF. On the other hand, there were no significant changes in HR, SBP, or CCBF.

In the phenylephrine group, MBF, QBF, and HR decreased after phenylephrine administration relative to baseline and hypocapnia ($P < .05$; Table 3, Figure 2).

Similar results were noted in the butoxamine group as both MBF and QBF decreased after butoxamine administration relative to baseline and hypocapnia ($P < .05$; Table 4, Figure 2).

In the atropine group, MBF and QBF showed no significant changes relative to hypocapnia but remained increased relative to baseline after atropine administration ($P < .05$; Table 5).

DISCUSSION

Results of this study demonstrated that MBF and QBF decreased during hypercapnia, while the decrease rate in MBF was smaller than that in QBF. During hypercarbic conditions, an increase in SNS tone occurs, which produces increased α- and β-adrenergic receptor activity; however, the overall decrease in MBF and QBF indicates constriction of the skeletal muscle blood vessels. General mechanisms of vasoconstriction include increased α_1-receptor activity or suppressed β_2-receptor activity. In fact, in the present study, the vasoconstriction that occurred during hypercarbia was corrected by suppressing α-receptor activity with phentolamine (an α blocker) or increasing β_2-receptor activity with meta-

Table 2. Hemodynamic and Tissue Blood Flow Changes During Hypercapnia ± Metaproterenol.*

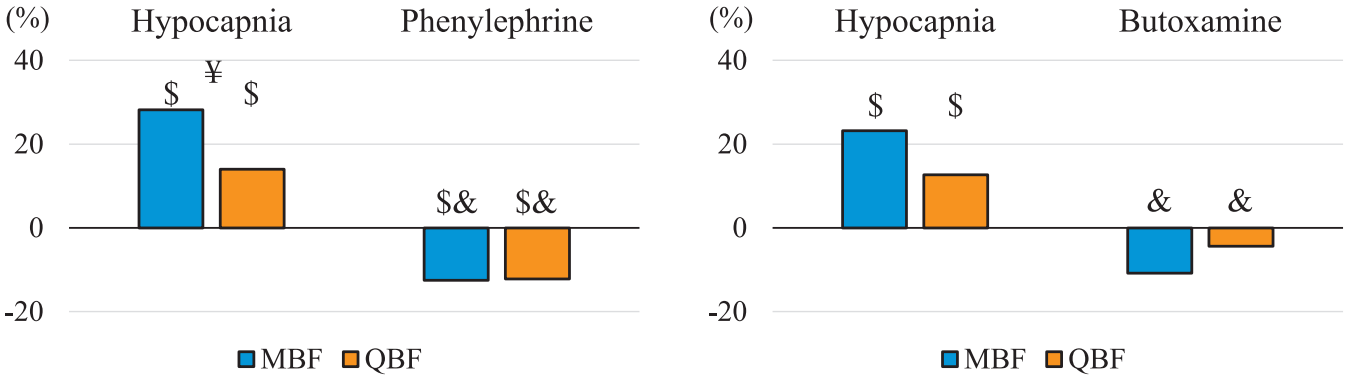
	Baseline	*Hypercapnia*	*Metaproterenol*
HR, bpm	301.3 ± 15.5	291.3 ± 15.8†	293.1 ± 15.6†
SBP, mm Hg	132.5 ± 17.9	141.3 ± 17.9†	135.6 ± 16.2‡
CCBF, mL/min	30.6 ± 7.0	37.1 ± 7.5†	42.0 ± 9.4†,‡
MBF, mL/min/100 g	42.0 ± 6.5	31.5 ± 5.7†	47.0 ± 6.9†,‡
QBF, mL/min/100 g	63.5 ± 8.4	41.1 ± 7.7†	57.4 ± 9.0†,‡

* Mean ± SD. CCBF, common carotid artery blood flow; HR, heart rate; MBF, masseter muscle tissue blood flow; QBF, quadriceps muscle tissue blood flow; SBP, systolic blood pressure.

†$P < .05$ versus baseline.

‡ $P < .05$ versus hypercapnia.

Figure 2. Mean changes in muscle blood flow during hypocapnia and after phenylephrine or butoxamine administration.



MBF and QBF increased during hypocapnia, while the increase in MBF was larger than that in QBF. Both MBF and QBF decreased to about 90% to 95% of their baseline levels after phenylephrine or butoxamine administration. Data are expressed as the percentage change in respective baseline values. MBF, masseter muscle tissue blood flow; QBF, quadriceps muscle tissue blood flow. [a] $P < .05$ versus baseline; [d] $P < .05$ versus hypocapnia; [c] $P < .05$ between the 2 groups.

Table 3. Hemodynamic and Tissue Blood Flow Changes During Hypocapnia ± Phenylephrine.*

	Baseline	*Hypocapnia*	*Phenylephrine*
HR, bpm	301.3 ± 16.4	295.0 ± 14.1	282.5 ± 12.8†,‡
SBP, mm Hg	125.6 ± 8.6	125.0 ± 8.0	128.4 ± 9.2
CCBF, mL/min	30.5 ± 8.8	32.1 ± 8.7	28.3 ± 7.1
MBF, mL/min/100 g	42.0 ± 7.7	53.2 ± 6.2 [a]	36.5 ± 7.0†,‡
QBF, mL/min/100 g	57.3 ± 4.5	65.3 ± 6.3 [a]	50.4 ± 7.5†,‡

* Mean ± SD. CCBF, common carotid artery blood flow; HR, heart rate; MBF, masseter muscle tissue blood flow; QBF, quadriceps muscle tissue blood flow; SBP, systolic blood pressure.
† $P < .05$ versus baseline.
‡ $P < .05$ versus hypocapnia.

Table 4. Hemodynamic and Tissue Blood Flow Changes During Hypocapnia ± Butoxamine.*

	Baseline	*Hypocapnia*	*Butoxamine*
HR, bpm	288.8 ± 26.4	286.9 ± 22.5	281.3 ± 19.6
SBP, mm Hg	131.3 ± 15.5	131.3 ± 15.5	130.0 ± 13.6
CCBF, mL/min	30.1 ± 6.7	29.1 ± 6.2	28.8 ± 4.9
MBF, mL/min/100 g	43.3 ± 5.2	53.4 ± 7.5†	39.0 ± 9.8†,‡
QBF, mL/min/100 g	60.9 ± 5.0	68.5 ± 4.7†	58.0 ± 3.7†,‡

* Mean ± SD. CCBF, common carotid artery blood flow; HR, heart rate; MBF, masseter muscle tissue blood flow; QBF, quadriceps muscle tissue blood flow; SBP, systolic blood pressure.
† $P < .05$ versus baseline.
‡ $P < .05$ versus hypocapnia.

Table 5. Hemodynamic and Tissue Blood Flow Changes During Hypocapnia ± Atropine.*

	Baseline	*Hypocapnia*	*Atropine*
HR, bpm	287.5 ± 23.8	285.6 ± 19.2	286.9 ± 23.1
SBP, mm Hg	128.8 ± 13.9	128.8 ± 14.6	128.8 ± 13.0
CCBF, mL/min	29.8 ± 7.0	29.1 ± 6.2	29.1 ± 5.8
MBF, mL/min/100 g	43.0 ± 5.5	53.0 ± 7.0†	52.3 ± 7.4†
QBF, mL/min/100 g	60.3 ± 6.0	68.6 ± 4.6†	68.0 ± 5.2†

* Mean ± SD. CCBF, common carotid artery blood flow; HR, heart rate; MBF, masseter muscle tissue blood flow; QBF, quadriceps muscle tissue blood flow; SBP, systolic blood pressure.
† $P < .05$ versus baseline.

proterenol (a β_2 agonist). However, since SNS tone increases during hypercarbia and both α- and β-receptor activity increases as a result, vasoconstriction by suppressing β_2-receptor activity is not possible. Thus, results of this study suggest that the decreased skeletal muscle blood flow during hypercarbia is mainly attributable to α_1-adrenergic receptor stimulation via increased SNS activity.

In addition, it has been reported that the vasoconstrictor effects of lumbar sympathetic stimulation on the resistance vessels of the soleus muscle, a slow-twitch muscle, were much smaller than seen in fast-twitch muscles.[17] It has also been reported that SNS-mediated vasoconstriction was greater in fast-twitch muscles than in slow-twitch muscles,[18] and the same results were obtained in this study. Both MBF and QBF recovered to baseline after administering phentolamine (an α blocker). In contrast, although MBF increased beyond baseline and CCBF also increased, QBF failed to fully recover to baseline after metaproterenol (a β_2 agonist) administration. When an α blocker was applied, the vasoconstrictive effects seemed to be reversed, suggesting that α-adrenergic receptor activity was primarily responsible for the vasoconstriction seen during hypercarbia. In addition, vasodilation by the β_2 agonist was shown to correct the decreased skeletal muscle blood flow due to increased α-adrenergic receptor activity. Since MBF increased beyond baseline level and QBF failed to fully recover to baseline after metaproterenol administration, there may be an increased distribution of β_2-adrenergic receptors in the masseter than in the quadriceps vasculature.

Contrary to hypercarbia, MBF and QBF increased during hypocarbia, indicating dilation of the skeletal muscle vessels. General mechanisms of vasodilation include suppressed α_1-adrenergic receptor activity or increased β_2-adrenergic receptor activity; however, SNS tone is decreased during hypocarbia, so vasodilation by increased β_2-adrenergic receptor activity is not possible. Therefore, the increase in skeletal muscle blood flow during hypocarbia is likely attributed to suppressed SNS tone, leading to decreased α_1-adrenergic receptor activity. The results of this study also indicate that both phenylephrine (an α_1-adrenoceptor agonist) and butoxamine (a β_2 blocker) inhibit the increase in skeletal muscle blood flow during hypocarbia by α_1-mediated vasoconstriction and by inhibiting β2-mediated vasodilation.

On the other hand, it has been reported that sympathetic denervation resulted in a 2.7-fold increase in blood flow to the soleus muscle (fast-twitch muscle) and an 8.7-fold increase in flow to the white portion of the gastrocnemius muscle (slow-twitch muscle).[18] In addition, α blockade by phentolamine caused increased blood flow in fast-twitch muscles, whereas muscles composed of greater than 20% slow-twitch fibers showed no effect.[19] Thus, the increases in skeletal muscle blood flow during hypocarbia observed in this study cannot be fully explained by only suppressed α_1-adrenergic receptor activity due to decreased SNS tone. One possible mechanism is the redistribution of tissue blood flow in oral and maxillofacial tissues. It is suggested that tissue blood flow in the mandibular bone marrow redistributes to the masseter muscle during hypocarbia and remifentanil infusion. This mechanism may explain the results obtained in this study.[3–5,20] In addition, the involvement of β_2-receptors in vasodilatation observed during low-dose epinephrine infusion (0.01 μg/kg/min)[21] and sympathetic cholinergic fibers is negligible.

In this study, the masseter muscle and the quadriceps muscle were utilized. Skeletal muscles consist of slow- and fast-twitch fibers, and 50% to 90% of the total muscle fibers in the anterior and deep portion of the masseter have slow-twitch properties.[11] In contrast, 90% of the total muscle fibers in the quadriceps muscle have fast-twitch properties.[12] Thus, we believe that the masseter and quadriceps muscles would be good indicators for the slow- and fast-twitch muscles, respectively. Phentolamine, a nonselective α-adrenergic receptor antagonist, was used in this study due to the lack of an available injectable, selective α1-adrenergic receptor antagonist in Japan. Therefore, the involvement of α_2-adrenergic receptors during vasoconstriction and vasodilatation could not be ruled out. In fact, it is reported that SNS control of blood flow in muscle appeared to be mediated through postsynaptic α_1- and α_2-adrenergic receptors located on the vascular smooth muscle.[18]

In this study, anesthesia was maintained with 1.0% isoflurane throughout the experiment. This concentration was equal to 0.5 minimum alveolar concentration (MAC) of isoflurane in the rabbit.[15] No major differences in tissue blood flow were observed during anesthesia with isoflurane, sevoflurane, or desflurane at a 0.5 MAC level.[22] Therefore, the effects of vasodilatation induced by isoflurane use in this study should be minimal.

CONCLUSION

The findings from this study suggest that α_1-receptor activity but not β_2-receptor activity may be mainly involved in the changes in skeletal muscle blood flow during hypercapnia and hypocapnia. Redistribution mechanisms in oral and maxillofacial tissues may also be associated with MBF changes.

DISCLOSURE

None of the authors have any relevant financial relationship(s) with a commercial interest.

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