Aerobic metabolism on muscle contraction in porcine iris sphincter

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ABSTRACT. Eyes are supplied O_2 through the cornea and vessels of the retina and iris, which are tissues characterized by aerobic metabolism. Meanwhile, there are no reports on the association between iris sphincter contraction and aerobic metabolism. In this paper, we studied the aforementioned association. Eyes from adult pigs of either sex were obtained from a local abattoir. A muscle strip was connected to a transducer to isometrically record the tension. O_2 consumption was measured using a Clark-type polarograph connected to a biological oxygen monitor. Creatine phosphate (PCr) and adenosine triphosphate (ATP) contents were measured in the muscle strips by high-performance liquid chromatography (HPLC). Iris sphincter muscles were measured in resting, contractile or hypoxic phases. Contraction was induced by hyperosmotic 65 mM KCl (H-65K$^+$) or carbachol (CCh), and hypoxia was induced by aeration with N_2 instead of O_2 or by addition of sodium cyanide (NaCN). H-65K$^+$- and CCh-induced muscle contraction, involved increasing O_2 consumption. Hypoxia and NaCN significantly decreased H-65K$^+$- and CCh-induced muscle contraction and/or O_2 consumption and PCr contents. Our results suggest that the contractile behavior in porcine iris sphincter highly depends on mitogen oxidative metabolism.
KEY WORDS: aerobic metabolism, hypoxia, iris sphincter, porcine

Hypoxia reduces the tension of smooth muscles, such as skeletal and cardiac muscles [2, 11]. However, the changes in the contraction of the smooth muscles during hypoxia differ between organs and tissues. Aeration with N_2 instead of O_2 abolishes contraction in the guinea pig taenia coli by high K$^+$-induced contraction [7]. Moreover, sodium cyanide (NaCN), an inhibitor of mitochondrial respiration, significantly inhibits high K$^+$-induced contraction in the guinea pig urinary bladder [9]. In contrast, hypoxia has little effects on the contraction of elastic vascular smooth muscles, such as those in the aorta and carotid arteries [1, 13]. Hypoxia also slightly reduces high K$^+$-induced contraction of smooth muscles of the bovine trachea [8].

O_2 can be supplied to ocular tissues and aqueous humor through the cornea and by the vessels of the retina and iris. The retina is one of the highest oxygen-demanding tissues. Abnormal oxygen distribution in the retina is an important cause of eye diseases, such as diabetic retinopathy [14, 18], glaucoma [10] and retinal vascular occlusion [3]. In contrast, tissues in the lenses of rabbits and humans have a low oxygen demand [12, 16]. Moreover, during hypoxic conditions, O_2 consumption falls to 50% in rabbit lens [15]. However, to

our knowledge, there are no reports on oxygen distribution and consumption in the iris. Moreover, the association of muscle contraction of the porcine iris sphincter and aerobic metabolism remains unclear.

It has been thought that creatine phosphate (PCr)/creatine kinase system plays a role in the transport of high-energy phosphates from the mitochondrial compartment to the sites of energy utilization, correlating with oxidative metabolism in mammalian smooth muscle. In guinea pig urinary bladder, cyanide inhibited high K$^+$-induced contraction with decrease in PCr and ATP [9]. This report suggests that the high energy phosphate compound plays an important role in maintaining muscle contraction in aerobic metabolism.

In the present study, we examined the aforementioned association, O_2 consumption, and contents of PCr and ATP after exposure to NaCN or bubbling N_2 of high K$^+$- and carbachol (CCh)-induced smooth muscle contraction in the porcine iris sphincter.

MATERIALS AND METHODS

Muscle preparations and tension measurement: Eyes from adult pigs of either sex were obtained from a local abattoir. Two strips of iris sphincter muscles were cut from each eye (with the ciliary margin removed). In the present study, we only used porcine eye from a local abattoir. Therefore, we didn't need every examine from Nippon Veterinary and Life Science University ethics committee. The muscle strips were incubated with physiological salt solution (PSS) containing 136.8 mM NaCl, 5.4 mM KCl, 2.5 mM CaCl$_2$, 1.0 mM MgCl$_2$, 11.9 mM NaHCO$_3$ and 5.6 mM glucose. PSS was

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aerated with 95% O_2 and 5% CO_2 for adjusting pH to 7.2 at 37°C. For inducing hypoxia, PSS was aerated with 95% N_2 instead of O_2.

Muscle tension was isometrically recorded. One end of each strip was bound to a glass holder, and the other end was connected by a silk thread to a strain gauge transducer (TB-611T; Nihon Kohden, Tokyo, Japan) in an organ bath containing PSS with a resting tension of 0.25 g. The muscle strips were equilibrated for 30 min for obtaining stable contracility induced by hyperosmotic 65 mM KCl (H-65K$^+$).

Oxygen consumption measurement: Two strips of iris sphincter muscles cut from each eye were incubated for 30 min with PSS. After the incubation, the muscle strips were put into the organ bath with PSS (3 ml) saturated with oxygen at 37°C. Furthermore, we measured O_2 consumption under each condition (control, addition of hyperosmotic 65 mM KCl (H-65K$^+$) or 0.3 μM CCh and 100 μM or 1 mM NaCN). Each condition was monitored for 15 min by a Clark-type polarograph electrode (YSI) connected with a biological oxygen monitor (YSI model 5300; YSI Japan Corp., Tokyo, Japan). O_2 consumption was expressed as μM/g/min.

Creatine phosphate and adenosine triphosphate assay: PCr and adenosine triphosphate (ATP) contents in the strips were measured by high-performance liquid chromatography (HPLC) as previously reported [7]. The strips were incubated with PSS for 30 min and contracted by H-65K$^+$ or 0.3 μM CCh. After the contraction, NaCN (100 μM or 1 mM) was added, or the strips were aerated with 95% N_2 for 20 min. Furthermore, the strips were rapidly frozen in liquid nitrogen and stored at −80°C until homogenization in 6% perchloric acid (0.9 ml). The homogenate was centrifuged at 15,000 ×g for 5 min, and the supernatant was neutralized with 0.2 ml of 2 M KHCO$_3$. The neutralized extracts were centrifuged again, and 20 μl of the supernatant was subjected to HPLC.

The HPLC system (Shimadzu Corp., Kyoto, Japan) comprised a pump (LC-10AT), a system controller (SCL-10AT), an autoinjector (SIL-10AF), a column oven (CTO-10A) and a wave length-selectable detector (SPD-10Ai) set at 216 nm.

Chromatography was performed using μRPC C2/C18 ST (internal diameter, 4.6 mm; and length, 100 mm; Amersham Biosciences, Piscataway, NJ. U.S.A.) using mobile phases of 50 mM KH$_2$PO$_4$ and 5 mM tetrabutylammoninum hydrogen sulphate (TBAHS) (pH 6.0, buffer A) and 50 mM TBAHS and 40% methanol (pH 6.0, buffer B). The flow rate was 1.0 ml/min, and the elution was initiated by 65% buffer A. In the first 14 min, buffer B increased at a rate of 2.5%/min. This step was followed by elution with 70% buffer B for 20 min and subsequently with 100% buffer A for 10 min. These procedures were programmed with the system controller. The sensitivity of the detector was usually set at 1.0 AUFS and the oven temperature at 40°C. PCr and ATP contents were expressed as μM/g wet weight (wt).

Chemicals: CCh (Sigma–Aldrich, St. Louis, MO, U.S.A.) and NaCN (Wako Pure Chemical, Osaka, Japan) were used.

Statistics: Values are expressed as means ± S.E.M. Statistical analyses were performed by Student's *t* test. A probability (*p*) value of <0.05 was considered statistically significant.

RESULTS

Effects of NaCN and hypoxia on high K$^+$- and CCh-induced tension in the porcine iris sphincter: H-65K$^+$ induced a large phasic contraction followed by a tonic one in the porcine iris sphincter (Fig. 1A). Similarly, CCh (0.3 μM) also induced a phasic contraction followed by a tonic one. Furthermore, the effects of aeration with N_2 instead of O_2 on H-65K$^+$- and CCh-induced contraction were investigated. As shown in Fig. 1B and C, hypoxia reduced H-65K$^+$- and CCh-induced contraction by 4.8% and 3.7%, respectively. Moreover, NaCN inhibited H-65K$^+$- and CCh-induced contraction in a concentration-dependent manner (Fig. 1D), and the values for 50% inhibition (IC$_{50}$) were 95.4 μM and 85.4 μM, respectively. These observations are consistent with the observation that aerobic metabolism inhibitors influence H-65K$^+$- and CCh-induced contraction.

Effects of NaCN on high K$^+$- and CCh-induced increases in O_2 consumption: In the porcine iris sphincter, the tension of H-65K$^+$- and 0.3 μM CCh-induced muscle contraction measured 10 min after application of the reagent was 9.23 ± 0.98 g/g wet wt and 10.19 ± 1.12 g/g wet wt, respectively (Table 1).

Table 1 shows that the change in O_2 consumption was 0.04 ± 0.01 μM/g/min in the resting phase. The rate of O_2 consumption significantly increased after adding H-65K$^+$ and CCh (0.3 μM), amounting to 0.05 ± 0.01 and 0.04 ± 0.01 μM/g/min, respectively. Furthermore, stimulation with 1 μM CCh increased the tension and O_2 consumption (Table 1).

H-65K$^+$- and CCh-induced increases in O_2 consumption were significantly inhibited in a concentration-dependent manner by NaCN (100 μM and 1 mM) (Table 1). Thus, the application of H-65K$^+$ and CCh appeared to contract the porcine iris sphincter through an aerobic metabolism-dependent muscle tension.

Effects of NaCN and hypoxia on high K$^+$- and CCh-induced increases in PCr and ATP contents in the porcine iris sphincter: As shown in Table 2, hypoxia significantly reduced H-65K$^+$- and 0.3 μM CCh -induced increases in PCr contents. Furthermore, NaCN reduced PCr contents in a concentration-dependent manner (Table 2). In contrast, NaCN and hypoxia did not affect ATP contents, significantly (Table 2).

DISCUSSION

The following results suggest that the contraction of porcine iris sphincter muscles is highly dependent on aerobic glycolysis in the following way: 1) high K$^+$- and CCh-induced contractions almost completely disappeared during hypoxia (achieved using N_2 instead of O_2) and NaCN treatment, 2) increases in high K$^+$- and CCh-induced oxygen consumption decreased with NaCN in a concentration-dependent manner and 3) in the presence of high K$^+$, and CCh, PCr contents significantly decreased during hypoxia and NaCN treatment.

The presence of high K$^+$ or CCh in porcine iris sphincter muscles led to a transient and early contraction and a sub-
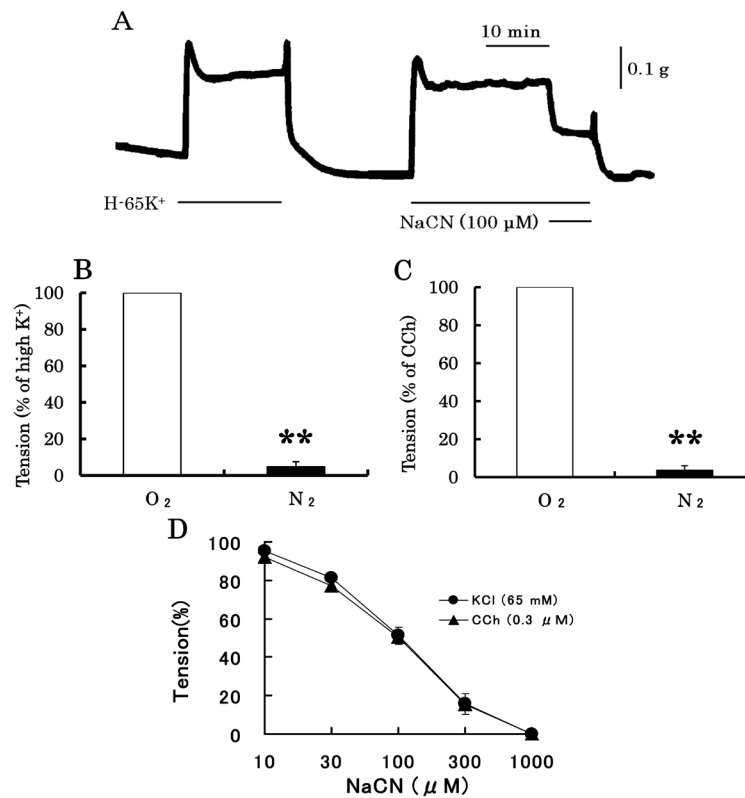
Fig. 1. Effects of hypoxia (bubbling with N_2 instead of O_2) or NaCN on H-65K$^+$-induced and CCh-induced contraction. Typical trace of the effect of NaCN in H-65K$^+$-induced contraction (A). Effects of hypoxia instead of O_2 on H-65K$^+$-induced (B) and CCh-induced (C) contraction. Values of aeration with N_2 instead of O_2 were obtained 10 min after changing the aeration from O_2 to N_2. Values at 10 min were assigned as 100%. Effects of NaCN on H-65K$^+$- or CCh-induced contraction (D). Each point represents the mean of 4–5 preparations. **Significantly different from the O_2 with $P<0.01$.

Table 1. Changes of the tension and the O_2 consumption in various conditions

Condition	Tension (g/g wet wt) (N)	O_2 consumption (μM/g/min) (N)
Resting phase	NA	0.04 ± 0.01 (23)
H-65K$^+$	9.23 ± 0.98 (5)	$0.05 \pm 0.01^*$ (9)
+NaCN (100 μM)	No data	$0.02 \pm 0.01^{**}$ (4)
+NaCN (1 mM)	No data	$0.01 \pm 0.01^{**}$ (5)
CCh (0.3 μM)	10.19 ± 1.12 (8)	$0.04 \pm 0.01^*$ (8)
+NaCN (100 μM)	No data	$0.02 \pm 0.01^{\dagger}$ (4)
+NaCN (1 mM)	No data	$0.01 \pm 0.01^{\dagger\dagger}$ (4)
CCh (1 μM)	13.29 ± 0.87 (8)	$0.05 \pm 0.01^*$ (4)

Tension was obtained 10 min after the application of H-65K$^+$ and CCh. NaCN was applied 10 min after application of H-65K$^+$ and CCh (0.3 μM). O_2 consumption rates were obtained 10 min after the application of reagents. *$P<0.05$, vs resting; **$P<0.01$, vs. H-65K$^+$; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, vs. CCh (0.3 μM). NA=not applicable.

Table 2. Effects NaCN and N_2 on phosphocreatine (PCr) and ATP contents

Condition (N)	PCr level (μM/g wet wt)	ATP level (μM/g wet wt)
H-65K$^+$ (9)	2.71 ± 0.35	0.21 ± 0.06
+NaCN (100 μM) (6)	2.16 ± 0.26	0.15 ± 0.02
+NaCN (1 mM) (6)	$1.26 \pm 0.17^{**}$	0.32 ± 0.12
+N_2 (6)	$0.81 \pm 0.06^{**}$	0.66 ± 0.17
CCh (0.3 μM) (5)	2.46 ± 0.49	0.62 ± 0.19
+NaCN (100 μM) (8)	1.78 ± 0.29	0.55 ± 0.11
+NaCN (1 mM) (4)	$1.45 \pm 0.12^{\dagger}$	0.86 ± 0.24
+N_2 (8)	$0.37 \pm 0.03^{\dagger\dagger}$	0.07 ± 0.02

All samples were treated with each reagent or aeration for 20 min. After the treatment, samples were frozen and measured. **$P<0.01$ vs. H-65K$^+$; $^{\dagger}P<0.05$; $^{\dagger\dagger}P<0.01$ vs. CCh (0.3 μM)

sequent sustained tonic phase, revealing that the sphincter muscles are phasic muscles similar to guinea-pig taenia coli. Oxygen consumption also increased, which is consistent with a report on guinea-pig taenia coli [19]. Our findings suggest that the aerobic glycolysis metabolic pathway is involved in the development of contractile tension in porcine iris sphincter muscles.

In porcine iris sphincter muscles, hypoxia and NaCN (1 mM) abolished the high K$^+$- and CCh-induced contractions. These results are consistent with a study by Ishida

and Paul (1990) [6] on taenia coli, suggesting that aerobic metabolism is involved in the contractile response of porcine iris sphincter muscles. Recently, we demonstrated that muscle contraction in porcine urinary bladder, a phasic muscle, depends on aerobic metabolism [9], but not in bovine trachea, a tonic muscle [8]. Our present data more suggest that contraction of phasic muscles depends on aerobic metabolism rather than that of tonic muscles.

In smooth muscle energy metabolism, ATP is generated from the glycolysis pathway, TCA cycle and electron transport chain, consequently phosphorylating creatine to produce PCr for energy storage. ATP required for smooth muscle contraction is supplied by the phosphorylation of ADP into ATP via creatine kinase dephosphorylation of stored PCr. With regard to smooth muscle energy metabolism, hypoxic conditions decrease muscle contraction, intracellular ATP and PCr contents, and increase intracellular ATP contents because of exogenous glucose uptake, regardless of changes in intracellular Ca^{2+}, and improve muscle contraction [5]. In the present study, we also found that intracellular PCr contents in contracted porcine iris sphincter muscles were significantly suppressed during hypoxia or NaCN treatment. This indicates that stored PCr is utilized because ATP from the glycolysis pathway is not supplied in time, owing to the inhibition of aerobic metabolism. Furthermore, PCr contents markedly diminished, because of the inhibition of aerobic metabolism, suggesting that ATP production from the TCA cycle and electron transport chain are important. However, hypoxia or NaCN did not affect ATP contents, significantly. It has been reported that there is compartmentation of ATP synthesis and utilization in smooth muscle [6]. Further studies will clarify the relationship between muscle contraction and changes of ATP contents.

Visual function impairment following hypoxia associated with military and civilian aviation activities is well known [4]. Changes in blinking rates and pupil size fluctuations have been observed under hypoxic conditions [17]. Hence, our present data may partly explain the mechanism of visual function impairment following hypoxia in pilots.

In summary, hypoxia and NaCN inhibited high K^+- and CCh-induced contractions by decreasing O_2 consumption and PCr contents in porcine iris sphincter muscles. These findings suggest that the contractile tension of these muscles highly depends on aerobic metabolism.

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