Effects of hypoxia and glucose-removal condition on muscle contraction of the smooth muscles of porcine urinary bladder

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ABSTRACT. To elucidate the dependence of aerobic energy metabolism and utilization of glucose in contraction of urinary bladder smooth muscle, we investigated the changes in the reduced pyridine nucleotide (PNred) fluorescence, representing glycolysis activity, and determined the phosphocreatine (PCr) and ATP contents of the porcine urinary bladder during contractions induced by high K+ or carbachol (CCh) and with and without hypoxia (achieved by bubbling N2 instead of O2) or in a glucose-free condition. Hyperosmotic addition of 65 mM KCl (H-65K+) and 1 μM CCh induced a phasic contraction followed by a tonic contraction. A glucose-free physiological salt solution (PSS) did not change the subsequent contractile responses to H-65K+ and CCh. However, hypoxia significantly attenuated H-65K+ and CCh-induced contraction. H-65K+ and CCh induced a sustained increase in PNred fluorescence, representing glycolysis activity. Hypoxia enhanced H-65K+ and CCh-induced increases in PNred fluorescence, whereas glucose-free PSS decreased these increases, significantly. In the presence of H-65K+, hypoxia decreased the PCr and ATP contents; however, the glucose-free PSS did not change the PCr contents. In conclusion, we demonstrated that high K+- and CCh-induced contractions depend on aerobic metabolism and that an endogenous substrate may be utilized to maintain muscle contraction in a glucose-free PSS in the porcine urinary bladder.

KEYWORDS: hypoxia, PCr, PNred, porcine urinary bladder

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On the basis of electrophysiological and mechanical behaviors, smooth muscles are classified into phasic and tonic muscles [9, 13, 25]. Phasic smooth muscles generate action potentials and depolarization with high K+, which induces an initial phasic contraction [8]. Phasic muscles include the ileum, urinary bladder, uterus and vas deferens. In contrast, the excitatory electrical response of tonic smooth muscles is graded depolarization without action potentials. High K+-induced depolarization typically evokes a slowly developing sustained contraction in tonic muscles, which include the aorta and trachea. The contractile diversities among different smooth muscles appear to be attributed to variations depending on oxidative metabolism [4, 10], as well as cellular protein expression [2, 7, 14, 26] and electrophysiological responses [3, 28].

Knell et al. showed that hypoxia inhibited high K+-induced contraction in the guinea pig taenia coli, which included phasic muscles [15]. They suggested that high K+-induced contraction could not be maintained in the guinea pig taenia coli under the aerobic condition. On the other hand, some studies have shown that hypoxia has little effect on the contraction of vascular smooth muscle (tonic muscle), such as the rabbit aorta [4] and carotid artery [17]. These findings are considered to demonstrate that the dependence of muscle contraction on aerobic metabolism differs between the phasic type and the tonic type.

In guinea pig urinary bladder, cyanide inhibited high K+-induced contraction with decrease in high-energy phosphate compound (adenosine triphosphate, ATP and phosphocreatine, PCr) contents [12]. These results suggest that muscle contraction of guinea pig urinary bladder smooth muscle is highly dependent on aerobic metabolism. However, hypoxia decreased oxygen consumption and ATP contents in the rat urinary bladder, but only marginally inhibited carbachol (CCh)-induced contraction [29]. These data indicate that the relationship between contractions and aerobic metabolism in the urinary bladder remains unclear. Because it is very difficult to obtain human bladder tissue, porcine tissue has been used as a substitute, representing a large-animal model, to study the physiology and pathophysiology of the lower urinary tract as its anatomy and function are similar to those of the human urinary bladder [19]. To clarify the species difference in the effects of hypoxia, we focused on a larger animal porcine whose bladder has some structural similarity with that of human.

Glucose-free physiological salt solution (PSS) decreased high K+-induced contraction in the guinea pig taenia coli [1, 15, 27]. In contrast, glucose-free PSS did not influence the maintenance of contraction in the porcine carotid artery [17] and rabbit aorta [20]. In bovine trachea smooth muscle (tonic muscle), glucose-free PSS did not affect the maintenance of high K+-induced contraction [11]. Glucose-free PSS decreased contraction of the tonic component to musca-
rinic agonists and high K⁺ rather than the phasic component in the urinary bladder of the rabbit [16] and guinea pig [23]. The aforementioned studies imply that the effect of glucose removal differs among smooth muscle types.

However, the effect of the removal of glucose on contraction in the porcine urinary bladder is still unclear. Therefore, in the present experiment, we attempted to clarify the role of aerobic metabolism and the effects of removal of glucose on high K⁺- and CCh-induced contraction in the porcine urinary bladder by measuring muscle tension, reduced pyridine nucleotide (PNred) fluorescence and PCr and ATP contents.

MATERIALS AND METHODS

Muscle preparations and tension measurement: Urinary bladders from adult pigs of either sex were obtained from a local abattoir. The adjacent connective and fatty tissues were carefully removed, and strips were dissected out from the bladder dome. The muscle strips (4.5 mm wide and about 15 mm long) were incubated with PSS containing 136.8 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 11.9 mM NaHCO₃ and 5.6 mM glucose. The PSS was aerated with 95% O₂ and 5% CO₂ at 37°C to adjust the pH to 7.2. In some experiments, glucose was removed from the PSS. Hypoxia was induced by aerating the PSS with N₂ instead of O₂. Muscle tension was recorded isometrically.

RESULTS

Effects of hypoxia and glucose removal on high K⁺- and CCh-induced contraction: In the porcine urinary bladder, addition of H-65K⁺ or CCh induced an initial transient contraction followed by sustained contraction. Removal of glucose from the PSS (glucose-free PSS) did not change the H-65K⁺-induced contraction (Fig. 1A and 1C). However, hypoxia (bubbling with N₂ instead of O₂) decreased contraction (Fig. 1B and 1C). Figure 1D summarizes the effects of glucose-free PSS and hypoxia on contraction induced by 1 µM CCh. Glucose-free PSS did not affect CCh-induced contraction, whereas hypoxia decreased contraction.

Changes in PNred fluorescence in the high K⁺- and CCh-treated muscles: The application of H-65K⁺ and CCh induced a sustained increase in PNred fluorescence. Glucose-free PSS decreased the H-65K⁺-induced increases of PNred fluorescence (Fig. 2A and 2C). However, hypoxia significantly enhanced the H-65K⁺-induced increases of PNred fluorescence (Fig. 2B and 2C). Figure 2D summarizes the effects of glucose-free PSS and hypoxia on increases in PNred fluorescence induced by 1 µM CCh. Glucose-free PSS decreased the CCh-induced increases in PNred fluorescence, whereas hypoxia significantly enhanced the CCh-induced increases in PNred fluorescence.

Changes in PCr and ATP contents in high K⁺-treated muscles: To evaluate the changes in total energy for muscle contraction, we measured the PCr and ATP contents. In the presence of H-65K⁺, glucose-free PSS did not affect the PCr contents for 60 min, but significantly decreased the ATP extracts were once again spun, and 20 µl supernatant was applied to the HPLC. The HPLC system (Shimadzu Corp., Kyoto, Japan) consisted of a pump (LC-10AT), a system controller (SCL-10A), an auto injector (SIL-10AF), a column oven (CTO-10A) and a wave length-selectable detector (SPD-10Ai) set at 216 nm. Chromatography was performed by µRPC C2/C18 ST column (4.6 nm internal diameter and 100 nm length, GE Healthcare, UK., Ltd., Little Chalfont, Buckinghamshire, U.K.) using mobile phases of 50 mM KH₂PO₄ and 5 mM tetrabutylammonium hydrogen sulfate (TBAHS) (pH 6.0, buffer A), and 50 mM KH₂PO₄, 5 mM TBAHS and 40% methanol (pH 6.0, buffer B). The flow rate was 1.0 ml/min, and elution was initiated with 65% buffer A. During the first 14 min, buffer B was increased by 2.5%/min. This was followed by elution with 70% buffer B for 20 min and then 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 absorbance units full scale, and the oven temperature was usually set at 40°C. The PCr and ATP contents were expressed as µmol/g wet weight.

Statistics: Values were expressed as mean ±SEM. Statistical analyses were performed by Student’s t-test using two groups and a two-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test for more than three groups. P<0.05 or P<0.01 was considered significant.

Chemicals: Carbamylcholine chloride (carbachol) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).
Hypoxia significantly decreased the PCr and ATP contents for 60 min (Fig. 3).

**DISCUSSION**

In the present study, we investigated the influence of hypoxia and removal of glucose on high K⁺- and CCh-induced contraction to clarify the relationship between muscle contraction and aerobic metabolism, or the availability of exogenous glucose on the porcine urinary bladder. From the data obtained regarding PNred fluorescence and the high-energy phosphate compound contents on muscle contraction in the porcine urinary bladder, we found that the influence of hypoxia is similar to that in other phasic type muscles, but that of glucose removal is similar to that in tonic type muscles.

It has been shown that the dependence of muscle contractile tension on aerobic metabolism differs for the smooth muscle types. For example, hypoxia caused by bubbling N₂ instead of O₂ almost completely inhibited high K⁺-induced contraction in the guinea pig taenia coli [15] and the rabbit uterus [18]. These reports suggest that aerobic metabolism-dependent muscle contraction is high in the phasic type smooth muscle, such as the taenia coli and uterus. In contrast, hypoxia slightly inhibited high K⁺-induced contraction in the bovine trachea [11]. Moreover, the hypoxia slightly inhibited high K⁺-induced contraction in the rabbit aorta [4]. These findings suggest that aerobic metabolism-dependent muscle tension is low in the tonic type muscles, such as vascular and tracheal smooth muscles. The urinary bladder is classified as a phasic muscle. Sodium cyanide strongly inhibited high K⁺-induced contraction in the guinea pig urinary bladder [12, 24]. Moreover, papaverine, a nonselective relaxant, inhibited high K⁺- and CCh-induced contraction to the same degree via inhibition of aerobic metabolism [24].

![Fig. 1. Effects of glucose-free PSS and hypoxia (bubbling with N₂ instead of O₂) on 65.4 mM KCl (H-65K⁺) and carbachol (CCh)-induced contractions. Typical traces of the effect of glucose-free PSS (A) and hypoxia (B). The time-tension curves for the effects of glucose-free PSS and hypoxia on H-65K⁺(C)- and CCh (D)-induced contractions. The precontractions induced by H-65K⁺ solution at 10 min were taken as 100%. Each point represents the mean of 8–10 preparations. Vertical bars indicate the SEM. ** Significant difference from the control with P<0.01.](image1)

![Fig. 2. Effects of glucose-free PSS and hypoxia on H-65K⁺- or CCh-induced increases in reduced pyridine nucleotide (PNred) fluorescence. Typical traces of effects on glucose removal (A) and hypoxia (N₂ bubbling, B) on the H-65K⁺-induced increases in PNred fluorescence. The time-response curves for the effects of glucose-free PSS and hypoxia on H-65K⁺(C)- or CCh (D)-induced increases of PNred fluorescence. The pre-increases in PNred fluorescence induced by H-65K⁺ solution at 10 min were taken as 100%. Each point represents the mean of 8–10 preparations. Vertical bars indicate the SEM. *, ** Significant difference from the control with P<0.05 or P<0.01, respectively.](image2)
fluorescence and that hypoxia further increased high K\(^+\)-induced contraction by approximately 60% in the rat urinary bladder [29]. In the present study, hypoxia inhibited the CCh-induced contraction in the rat urinary bladder. However, the hypoxic condition slightly inhibited CCh-induced contraction in the bovine trachea [11]. Similarly, hypoxia did not affect tissue PCr and ATP contents in the porcine carotid artery [22]. In the present study, hypoxia inhibited high K\(^+\)-induced contraction and decreased the PCr and ATP contents in the porcine urinary bladder. Furthermore, high K\(^+\)-induced contraction is suggested to be highly dependent on aerobic metabolism in the porcine urinary bladder. The dependence of the rat urinary bladder [29] is different from that of the porcine urinary bladder because of the difference in experimental conditions (e.g., temperature, species and rearing environment, e.g., free feeding without much exercise) [5]. The pigs are fattened to be used as food; thus, a large quantity of energy substrates may be accumulated in the muscle layer of the urinary bladder. Therefore, we examined the change in high K\(^+\)- and CCh-induced contractions under glucose-free PSS conditions.

These data suggest that high K\(^+\)- and CCh-induced contraction is highly dependent on the aerobic metabolism in the urinary bladder. However, the hypoxic condition slightly inhibited CCh-induced contraction in the rat urinary bladder [29]. In the present study, hypoxia inhibited the high K\(^+\)- and CCh-induced contraction by approximately 60% in the porcine urinary bladder, suggesting that the high K\(^+\)- and CCh-induced contractions are highly dependent on aerobic metabolism in the porcine urinary bladder, which is similar to the guinea pig but not the rat.

Ozaki et al. [21] reported that high K\(^+\)-induced contraction was accompanied by an increase in oxidized flavoproteins (FPox) or PNred fluorescence in the guinea pig taenia coli. They suggested that the change in FPox fluorescence represented mitochondrial respiration activity and that PNred fluorescence represented glycolysis activity. In addition, they demonstrated that hypoxia further increased high K\(^+\)-induced PNred fluorescence in the guinea pig taenia coli. Our previous experiments showed that high K\(^+\) increased PNred fluorescence and that hypoxia further increased high K\(^+\)-induced PNred fluorescence in the guinea pig taenia coli [10]. These results suggest that hypoxia further increases glycolysis activity in the guinea pig taenia coli and the bovine trachea [11]. In the present study, high K\(^+\) and CCh sustainably increased PNred fluorescence in the porcine urinary bladder. In addition, hypoxia further increased high K\(^+\)- and CCh-induced PNred fluorescence in the porcine urinary bladder. These results suggest that hypoxia increases glycolysis activity even in the porcine urinary bladder. The PCr/creatine kinase system is considered to play 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 muscles. In the presence of high K\(^+\), hypoxia decreased the PCr and ATP contents in the guinea pig taenia coli [10]. Hypoxia slightly decreased high K\(^+\)-induced contraction, whereas it did not affect the PCr and ATP contents in the porcine urinary bladder. These data suggest that the effects of glucose-free PSS on muscle contraction differ according to smooth muscle types. Glucose-free PSS inhibited contractions, particularly those of tonic components, mediated by field stimulation, high K\(^+\) and muscarinic agonists in the rabbit [16] and guinea pig [23] urinary bladders. However, in the current study, glucose-free PSS did not affect the high K\(^+\)- and CCh-induced contraction in the porcine urinary bladder. These data show that the effect of glucose-free PSS on muscle contraction in the porcine urinary bladder is similar to that of the tonic but not phasic type of smooth muscle. In the porcine urinary bladder, glucose-free PSS inhibited high K\(^+\)-induced PNred fluorescence by approximately 40%. In contrast, glucose-free PSS did not affect high K\(^+\)-induced contraction or the PCr and ATP contents. These results raise the possibility that endogenous substrates, such as fatty acids or ketones, not involved in glycolysis are related to the maintenance of contraction of urinary bladder smooth muscle in glucose-free PSS. A further study is required to determine the differences in urinary bladders among species related to rearing environment or experimental conditions.

In conclusion, our results show that muscle contraction depends on aerobic metabolism in the porcine urinary bladder, which is similar to other phasic type muscles. However, endogenous substrates are probably used to maintain muscle
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contraction in glucose-free PSS in the porcine urinary bladder, which is similar to tonic type muscles. Further studies are required to determine what kinds of endogenous substrates are necessary to maintain muscle contraction in glucose-free PSS.

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