Effects of Glucose Deprivation on the Contractile Response of the Rabbit Bladder to Repetitive Stimulation

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The urinary bladder requires an adequate energy supply to maintain contractile function. The primary metabolic fuel is glucose. Through glycolysis and oxidative phosphorylation, high energy phosphates are generated, which in turn supply the metabolic energy for the contractile activities of the urinary bladder. The aim of this study was to determine the effects of glucose deprivation and recovery from glucose deprivation on the phasic and tonic components of the contractile responses of rabbit bladder strips to field stimulation, bethanechol, and KCl. The results can be summarized as follows: In response to glucose deprivation, (1) the tonic responses to field stimulation, bethanechol, and KCl all decreased at a significantly greater rate than the phasic responses; (2) the phasic and tonic responses to field stimulation were both reduced to less than 10% of control within 70 minutes of initiating glucose deprivation; (3) the tonic responses to bethanechol and KCl were reduced to approximately 10% of control within 180 minutes whereas the phasic responses remained stable at 40 and 30%, respectively; and (4) glucose replacement stimulated a rapid and nearly complete recovery of the phasic and tonic components of the responses to field stimulation, bethanechol, and KCl. These results indicate that the tonic responses to all forms of stimulation are more sensitive to glucose deprivation than the phasic responses.

Key words: glucose deprivation, bladder, stimulation

INTRODUCTION

Bladder contraction is biphasic in nature. Stimulation induces a rapid rise in intravesical pressure followed by a sustained period of increased pressure. Bladder emptying occurs primarily during the sustained increase in intravesical pressure [Levin et al., 1987; Wein et al., 1991; Andersson, 1993]. In general, contractile function is dependent upon the availability of metabolic energy. Functional compartmentation refers to the observation that specific cellular functions appear to utilize energy derived from selective sources such as glycolysis, oxidative phosphorylation,
or cytosolic ATP hydrolysis (i.e., specific compartments) [Paul, 1989; Casteels and Wuytack, 1975; Campbell et al., 1988; Ishida and Paul, 1989; Lynch and Paul, 1989]. In this regard, we have evidence that the phasic and tonic components of the response to receptor mediated contraction derives metabolic energy from different sources [Van Arsdalen et al., 1983; Zhao et al., 1992; Bilgen et al., 1992]. Specifically, the phasic response is supported by anaerobic metabolic energy, whereas the tonic component is related to oxidative energy. A prior study demonstrated that incubation of isolated strips in the absence of glucose inhibited the tonic response to field stimulation to a significantly greater degree than inhibition of the phasic response, indicating that the tonic response to field stimulation is more dependent upon active metabolic processes than the phasic response [Hypolite et al., 1991]. The specific aim of this current study was to expand these studies and directly compare the effect of repetitive field stimulation (neurohumoral transmission) in the presence and absence of glucose, to the responses to bethanechol (receptor-mediated contraction) and KCl (depolarization-mediated contraction).

MATERIALS AND METHODS

Tissue Preparation

Mature male New Zealand White rabbits weighing 2–3 kg were sedated with an i.m. injection (0.7 ml/kg) of a ketamine/xylazine mixture (29.2 mg/kg ketamine, 8.3 mg/kg xylazine). Surgical anesthesia was maintained with 1 ml of 50 mg/ml pentobarbital given over the course of surgery. After a midline incision, the ureters were ligated and the urinary bladder was rapidly removed as an intact organ. The urinary bladder body was separated from the base above the level of the ureteral orifices, and placed in Tyrode’s solution containing glucose in 37°C and equilibrated with 95% O₂ and 5% CO₂.

Isolated Muscle Strip Methodology

Six longitudinal muscle strips of equal size were obtained from each bladder body and were placed in individual baths with 40 ml of oxygenated normal Tyrode’s buffer (NaCl 124.9 mM, KCl 2.5 mM, NaHCO₃ 23.8 mM, MgCl₂ · 6H₂O 0.5 mM, NaH₂PO₄ · H₂O 0.4 mM, CaCl₂ 1.8 mM, glucose 5.6 mM) at 37°C. One end of each strip was connected to a force displacement transducer, and changes in muscle tension were measured and recorded on a Grass Model 7D Polygraph. Basal tension of 2 g was applied to each strip. Each strip was allowed to incubate for a 1 hour period at the start of the experiment. At the end of this time, the bath solution in three baths was replaced by glucose-free Tyrode’s solution.

The schedule for field stimulation and drug administration was designed to produce a maximal level of fatigue. The time between stimulation was based on the minimum period for recovery of basal tension after drug washout. Bethanechol (250 µM; Sigma Chemical Co., St. Louis, MO) was left in contact with the strips for 4 minutes. The strips were then washed 3 times with Tyrode’s solution. The interval between bethanechol stimulations was 30 minutes. KCl (120 mM) was left in contact with the strips for 4 minutes. The strips were then washed 3 times with Tyrode’s solution. The interval between KCl stimulations was 10 minutes. Field stimulation (32 Hz, 80 V, 1 ms) was maintained for 2 minutes. The interval between field
stimulations was 10 minutes. At the end of each incubation in glucose-free medium (100 minutes for FS, 180 minutes for KCl and Bethanechol), the medium was changed to Tyrode’s containing glucose (1 mg/ml) and stimulations continued for an additional 90 minutes.

Each strip was used for only one form of stimulation. The effects of glucose-free incubation on the contractile response were calculated based on the response of each strip to stimulation in the presence of glucose, and are presented as percent of control response.

Statistics

Statistical analysis was performed using analysis of variance followed by Newman Keuls multiple comparison tests. A probability of \( P < 0.05 \) was accepted as statistically significant.

RESULTS

Responses in the Presence of Glucose

The initial phasic and tonic responses to Bethanechol, KCl, and FS before glucose removal are presented in Table I. The phasic responses to KCl and FS were significantly greater than the tonic response. The phasic and tonic responses to FS, Bethanechol, and KCl in the presence of glucose progressively decreased by approximately 40% during repetitive stimulation (Fig. 1). In individual experiments continued to 270 minutes, there were no further reductions in the responses to FS, Bethanechol, or KCl compared to 150 minutes.

Responses in the Absence of Glucose

The data presented for the effect of glucose deprivation are calculated as the percent of response in the presence of glucose for the same incubation period. The tonic response to field stimulation began to decrease immediately, falling to 25% of control by 30 minutes (Fig. 2). Phasic tension in the absence of glucose showed the same rate of decline as phasic tension in the presence of glucose over the first 60 minutes (thus showing near 100% of control tension). After 60 minutes, there was a rapid decrease in phasic tension over the next 15 minutes. Both the phasic and tonic responses decreased to less than 20% of control responses by 70 minutes of stimulation in glucose-free medium. The addition of glucose at 100 minutes mediated a rapid, simultaneous, and complete recovery of both phasic and tonic responses.

The tonic responses to Bethanechol (Fig. 3) and KCl (Fig. 4) decreased progressively to approximately 10% of control by 180 minutes. The phasic response to KCl progressively decreased at the same rate as the tonic response for 50 minutes to approximately 50% of control, and then levelled off while the tonic response continued to decrease. The phasic response to Bethanechol decreased at the same rate as the tonic response for 30 minutes (1 stimulation) and then levelled off at approximately 40% of control. In both cases, the tonic response fell to a significantly lower level than the phasic response. The addition of glucose to the medium resulted in a rapid and full recovery of both the tonic and phasic responses to control levels.
Fig. 1. The effects of repetitive stimulation in the presence of glucose on the phasic and tonic responses of rabbit bladder strips to FS (32 Hz, 1 ms, 80 V), bethanechol (250 μM), and KCl (120 mM). Each point represents the mean ± SEM of strips from 6 or 8 individual animals.

### TABLE I. Initial Response of Control Bladder to Field Stimulation, Bethanechol, and KCl

<table>
<thead>
<tr>
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<th>Phasic response (g tension)</th>
<th>Tonic response (g tension)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field stimulation (32 Hz)</td>
<td>14.4 ± 0.75</td>
<td>3.5 ± 0.08**</td>
</tr>
<tr>
<td>Bethanechol (250 μM)</td>
<td>6.32 ± 0.34</td>
<td>5.92 ± 0.55</td>
</tr>
<tr>
<td>KCl (120 mM)</td>
<td>11 ± 0.50</td>
<td>6.7 ± 0.3**</td>
</tr>
</tbody>
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*The initial (first) contractile response of rabbit bladder strips to bethanechol (250 μM), KCl (120 mM), and FS (32 Hz, 1 ms, 80 V) in the presence of glucose. Each bar represents the mean ± SEM of strips from 6 or 8 individual animals.

**Significantly different from phasic tension, \( P < 0.05 \).

### DISCUSSION

The response of the isolated urinary bladder to nerve stimulation is biphasic in nature, consisting of a rapid phasic contraction mediated by the release of acetylcholine and ATP with subsequent activation of muscarinic and purinergic post-synaptic receptors [Levin et al., 1986, 1987; Wein et al., 1991; Andersson, 1993]. This phasic response is followed by a sustained tonic increase in tension which is mediated entirely by muscarinic receptor stimulation [Levin et al., 1986]. Although it is generally accepted that smooth muscle function is dependent on the metabolism of
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Field Stimulation

Fig. 2. The effects of repetitive stimulation in the absence of glucose on the phasic and tonic responses of rabbit bladder strips to FS. Each point represents the mean ± SEM of strips from 6 or 8 individual animals. *Significantly different from phasic tension \( (P < 0.05) \). At the arrow, the medium was changed to Tyrodes containing glucose (1 mg/ml).

Bethanechol

Fig. 3. The effects of repetitive stimulation in the absence of glucose on the phasic and tonic responses of rabbit bladder strips to bethanechol. Each point represents the mean ± SEM of strips from 6 or 8 individual animals. *Significantly different from phasic tension \( (P < 0.05) \). At the arrow, the medium was changed to Tyrode's containing glucose (1 mg/ml).

substrates, there is little detailed information on the relationships between substrate utilization and contractile function [Haugaard et al., 1987; Hellstrand and Vogel, 1985; Wendt, 1987, 1989; Levin et al., 1988, 1989; Arner et al., 1993; Polyanska et al., 1993; Scott and Coburn, 1989]. Bladder emptying occurs primarily during the tonic phase of the contractile response [Levin et al., 1986, 1987; Wein et al., 1991; Andersson, 1993].

Similar to our previous study [Hypolite et al., 1991], the results of this study
Fig. 4. The effects of repetitive stimulation in the absence of glucose on the phasic and tonic responses of rabbit bladder strips to KCl. Each point represents the mean ± SEM of strips from 6 or 8 individual animals. *Significantly different from phasic tension ($P < 0.05$). At the arrow, the medium was changed to Tyrodes containing glucose (1 mg/ml).

indicate that the tonic response to field stimulation was significantly more sensitive to glucose deprivation than was the phasic response. The current study utilized 10 minute intervals between field stimulations whereas the previous study utilized 20 minute intervals. The shorter time period probably increases the onset of fatigue. This may be the reason that the phasic response to repetitive stimulation in the presence of glucose in the current study decreased to a greater extent than in the previous study.

Interestingly, the rate of decrease of the phasic and tonic components of the responses to bethanechol and KCl were similar after removal of glucose, although the tonic response fell to a significantly lower level than the phasic response. Comparatively, by 30 minutes, the tonic response to FS was reduced by 80%, whereas the tonic response to KCl or bethanechol was reduced by only 40%. It took 90 minutes for the tonic responses to bethanechol and KCl to fall below 80%. These differences in the rate of decline in response to repetitive stimulations among FS, bethanechol, and KCl may be due to the differences in the interval between stimulations.

As mentioned in the introduction, functional compartmentation refers to the concept that specific cellular functions utilize energy derived from selective sources [Ishida et al., 1994; Ishida and Paul, 1989; Lynch and Paul, 1989]. Three major sources of energy have been identified: cytosolic ATP, glycolytic-generated ATP (via membrane-bound glycolytic enzymes), and oxidative generation of ATP (mitochondrial oxidative phosphorylation) [Ishida et al., 1994; Ishida and Paul, 1989; Lynch and Paul, 1989; Hardin et al., 1992, 1993]. Recent evidence demonstrates that whereas the initial generation of tension in smooth muscle is linked to cytosolic ATP and anerobic generation of ATP, the maintenance of tension is supported by oxidative energy generation [Lynch and Paul, 1989; Levin et al., 1994, 1995]. On a cellular level, intracellular calcium uptake in smooth muscle plasmalemmal vesicles preferentially utilize membrane-bound glycolytic ATP generation (in comparison to cytosolic ATP), whereas, Na-K ATP'ase [Hardin et al., 1992, 1993] utilizes oxidative
energy. Our data showing that the tonic responses to all forms of stimulation are significantly more sensitive to glucose deprivation than are the phasic responses are consistent with the concept that phasic tension generation is supported by ATP whereas tonic tension maintainance is supported by oxidative energy generation.

Hypolite demonstrated that although both carbohydrates and fat were important substrates for the production of energy in the urinary bladder, bethanechol stimulation increased the metabolism of glucose, but not palmitic acid [Hypolite et al., 1989]. Further studies demonstrated that bethanechol stimulation results in a significantly greater breakdown of ATP than does stimulation by KCl, indicating that receptor mediated contraction requires a significantly greater expenditure of metabolic energy than depolarization-mediated contraction [Levin et al., 1991]. This observation is consistent with studies in the literature in which receptor-mediated stimulation resulted in a greater utilization of metabolic energy than KCl stimulation [Peterson, 1982; Davidheiser, 1986; Davidheiser et al., 1984].

Physiologically, all bladder contraction is mediated through receptor-mediated stimulation (via the release of neurohumoral transmitters) [Wein et al., 1991; Andersson, 1993]. In addition, emptying is a function of the tonic phase of the response to stimulation [Levin et al., 1986]. The current studies support the hypothesis that the primary defect induced by glucose deprivation is a decrease in the tonic response to stimulation, and that the urinary consequences would be a specific reduction in the ability to sustain increased pressure during a micturition contraction and a selective decrease in the ability of the bladder to empty.

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REFERENCES


