Neurourology and Urodynamics 6:109–118 (1987)

Surface Spectrofluorometry of the Rabbit Urinary Bladder

Robert M. Levin, Michael R. Ruggieri, Joseph A. Hypolite, S. Bruce Malkowicz, and Alan J. Wein

Division of Urology, University of Pennsylvania School of Medicine, and the Philadelphia Veterans Administration Medical Center, Philadelphia

Proper function of the urinary bladder is dependent on the delivery of a normal supply of blood, oxygen, and nutrients to the tissue. The smooth muscle elements of the bladder utilize metabolic energy in the form of ATP and other high-energy compounds to support bladder contraction. One of the first metabolic indications of contractile activity would be alterations in the oxidative state (redox) of the tissue, which is reflected by the NADH/NAD ratio. NADH is a metabolic intermediate synthesized during substrate metabolism and utilized during energy production and utilization. We have studied NADH metabolism in the in vitro bladder strip preparation utilizing a surface spectrofluorometer. This instrument monitors NADH fluorescence in tissue preparations with the use of an optical fiber probe placed on the serosal surface of the strip. The bladder strips displayed rhythmic contractile activity. This was correlated with alterations in NADH fluorescence. There was an excellent correlation (correlation coeficient of 0.986) between the frequency of the spontaneous activity of both contraction and fluorescence. An increase in tension was followed (in seconds) by a decrease in fluorescence. The basal NADH fluorescence was modulated by metabolic inhibitors as would be expected by their metabolic actions: Anoxia and sodium azide (metabolic inhibitors of oxidative phosphorylation) produced a marked increase in the redox state (NADH fluorescence), whereas DNP (a mitochondrial uncoupler) produced a marked decrease. Bethanechol stimulated an increase in tension and a reduction in fluorescence.

The above findings suggest that NADH surface spectrofluorometry reflects NADH metabolism in the detrusor smooth muscle and that fluctuations in such spectra correlate well with functional activity. NADH surface spectrofluorometry displays potential as a noninvasive method of monitoring one aspect of detrusor metabolism.

Key words: urinary bladder, metabolism, glycolysis, surface spectrometry

This work was supported in part by grants from the Veterans Administration, NIH grants RO-1-DK-2-6508-05 and RO-1-DK-33559-01, and the McCabe Fund.

Received for publication August 8, 1986; accepted January 22, 1987.

Address reprint requests to Dr. Robert Levin, Division of Urology, 3006 Ravdin Ctyd Building, Hospital of the University of Pennsylvania, 3400 Spruce St, Philadelphia, PA 19104.

INTRODUCTION

The urinary bladder is a smooth muscle organ whose function is to store and expel urine. The filling and storage phase of micturition consists of relatively passive enlargement of the bladder at a low intravesical pressure. The expulsion phase consists of a highly coordinated contraction that actively expels the bladder's content through an adaptively shaped low-resistance urethra. This active contraction utilizes intracellular energy, primarily in the form of adenosine triphosphate (ATP) and other metabolic high-energy compounds [Butler et al, 1977; Wrogeman and Stephens, 1977; Levin et al, 1982, 1983].

The urinary bladder metabolizes glucose to generate the energy required to function. Glucose (or glycogen, the stored form of carbohydrates) is metabolized to pyruvate and lactic acid via glycolysis and to CO_2 via the tricarboxylic cycle [Rohner et al, 1967; Lundholm et al, 1977; Levin et al, 1987]. The energy generated from the metabolism of glucose is stored and utilized in the form of intracellular ATP. An intermediate of ATP generation is NADH (the reduced form of nicotinamide-adenine dinucleotide). This compound is generated during active glycolysis and pyruvate metabolism by the tricarboxylic cycle. During oxidative phosphorylation within the mitochondria, NADH is oxidized by the cytochromes to NAD (the oxidized form of nicotinamide-adenine dinucleotide), which is linked metabolically to the generation of ATP (from ADP). The redox state of the tissue (the ratio NADH/NAD) is an indicator of metabolic state of the cell. One would predict that during relaxation, the ratio would be high, that during active contraction, the ratio would decrease, and that during anoxia, ischemia, or other forms of metabolic dysfunctions, the ratio would either increase or decrease depending on the site of the dysfunction. Thus, the redox state of the tissue can be used as an indicator of its metabolic state.

Utilizing a surface spectrofluorometer we have monitored the redox potential of isolated strips of rabbit urinary bladder muscle to determine if this methodology can be used as an indicator of the metabolic state of the bladder.

MATERIALS AND METHODS

Tissue Preparation

Mature male New Zealand white rabbits were sedated with an intramuscular (I.M.) injection (0.7 ml/kg) of a ketamine/xylazine mixture (29.2 mg/ml ketamine, 8.3 mg/ml xylazine). Surgical anesthesia was maintained with 1 ml of 50 mg/ml pentobarbital given over the course of surgery (15 minutes). The urinary bladder was rapidly removed and separated between body and base at the level of the ureteral orifices. The bladder dome was dissected free of all fat and connective tissue, the bladder was mounted with the serosal side up on a ring (40 mm diameter) and suspended as a circular strip within a 300-ml isolated muscle chamber containing Tyrodes solution at 37°C equilibrated with 95% O_2, 5% CO_2. The time between the surgical removal of the bladder and mounting the strip in the bath was 3–5 minutes.

Measurement of Spontaneous and Evoked Contractile Activity

A 5-0 silk suture is tied off center to the mounted tissue and connected to a Harvard isometric transducer connected to a strip-chart recorder. One gram of tension is placed on the tissue by raising the transducer; this basal tension was stable throughout the study.

Measurement of Oxygen Tension

The oxygen tension within the bath is continuously monitored using a Gilson oxygraph with the oxygen probe placed in the isolated muscle bath. Anoxia is produced by changing the aeration from the oxygen mixture to 95% nitrogen, 5% CO_2.

Measurement of Fluorescence

NADH fluorescence is continuously monitored using an MB2 surface spectrofluorometer produced by the University of Pennsylvania Biomedical Instrumentation Group. Complete details and diagrams of this device were published previously by Britton Chance and co-workers [Chance, 1976; Franke et al, 1976, 1980]. This group has utilized this instrument to evaluate the metabolic state of the kidney under a variety of conditions. We have modified its use for the study of urinary bladder smooth muscle. Briefly, the MB2 fluorometer generates light by using a mercury arc in a separate housing. An emission beam of 366 nm is generated by using a lens filter system. The emitted light is applied to the tissue via a trifurcated optical fiber probe. One set (45%) of fibers carries the emitted light to the tissue, and the second group of optical fibers (45%) carries the fluorescent light to a differential amplifier where the amplitude of the 450 nm fluorescence is monitored. The third group of optical fibers (10%) carries reflected light of 366 nm to the differential amplifier where an automatic correction is made in the fluorescence for this reflected light. Detailed studies on the metabolism of the kidney have demonstrated that this technique is capable of the continuous monitoring of the redox state of a tissue [Chance, 1976; Franke et al, 1976, 1980].

Quantitative Measurement of NADH and NAD

Isolated bladder strips were incubated in oxygenated Tyrodes for 30 minutes, at which time they were subjected to the following experimental protocols: 1) 10 minutes of anoxia, 2) 10 minutes in the presence of 200 μM DNP, 3) 5 minutes of 200 μM bethanechol, 4) an additional 10 minutes in oxygenated Tyrode's (as a control). At the end of the experimental period, the tissue was rapidly frozen and stored in liquid nitrogen. At the time of analysis, each tissue was divided under liquid nitrogen into two equal pieces; half was quantitatively analyzed for NADH and the other half was analyzed for NAD according to the enzymatic methods described by Bergemeyer [1963].

Pharmacological Manipulations

Anoxia was produced by changing the aeration from an oxygen mixture (95% O_2, 5% CO_2) to a nitrogen mixture (95% N, 5% CO_2). The metabolic inhibitors dinitrophenol (DNP) (200 μM) and sodium azide (200 μM) were added to the bath in single additions of 1.0 ml inhibitor, which was dissolved in Tyrode's solution. Dose-response curves to bethanechol (1—200 μM) and potassium chloride (12.5—200 mM) were generated through the cumulative addition of increasing concentrations of the agent utilizing 1-ml additions.

Statistical significance was determined by using analysis of variance and posthoc analysis.

RESULTS

Figure 1 displays the recording of the spontaneous activity of both fluorescence and contraction. The recorder has two pens for the simultaneous recording of both contraction and fluorescence. In order for the pens to pass each other, one is displaced 5 mm in front of the other. The recordings have been corrected for this displacement of the recorder pens so that one can observe the temporal relationship between the two functions. Close inspection of these curves reveals that an inverse relationship exists between contraction and fluorescence. As tension increases, fluorescence decreases, and as tension decreases, fluorescence increases. Peak tension is generally associated with the lowest fluorescence.

Figures 2 and 3 display the effect of bath temperature on the amplitude and frequency of spontaneous activity of both fluorescence and contraction. For these studies, the bath temperature was slowly cooled by decreasing the temperature of the circulating water bath while continuously recording contractile and fluorescence activity. Decreasing the temperature of the Tyrode's solution produced a gradual decrease in both the magnitude and frequency of spontaneous activity of both contraction and fluorescence. Both functions decreased to a similar extent. Below 20°C, spontaneous variation of fluorescence and contraction approached 0.

Figure 4 displays the effect of various metabolic inhibitors on the fluorescence of the bladder. "Oxygen" depicts the normal state of the in vitro bladder in the isolated bath. Changing the aeration from oxygen to nitrogen rapidly produces an anoxic condition. Within seconds, the fluorescence began to increase, reaching a maximum within approximately 10 minutes. A lesser increase in fluorescence was obtained by incubating the tissue in the presence of sodium azide (200 μM). Dinitro-

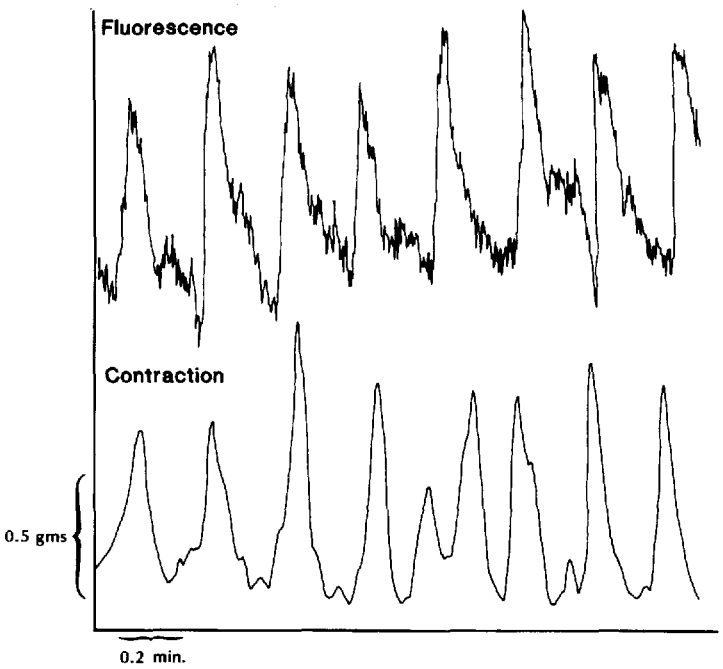

Fig. 1. Spontaneous activity of contraction and fluorescence. Representative tracing of the spontaneous activity of contraction and fluorescence.

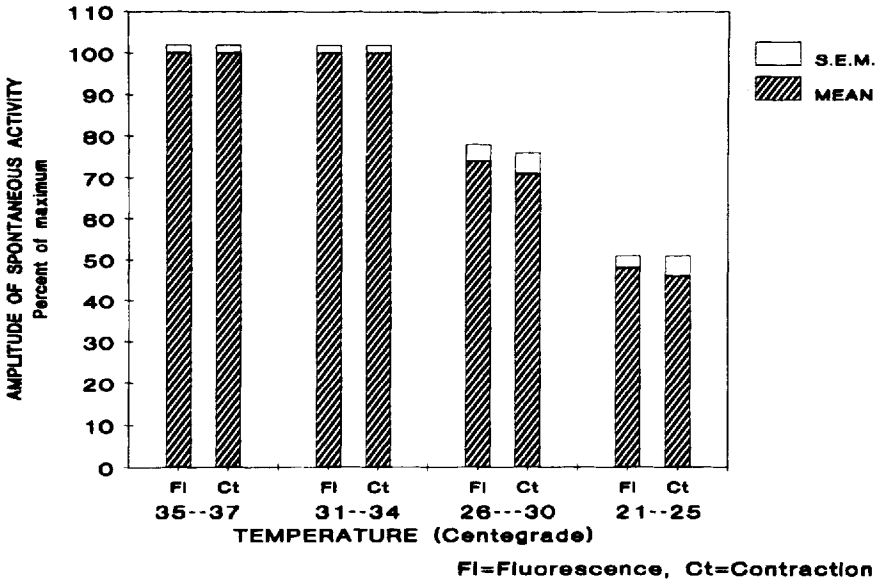

Fig. 2. Effect of temperature on the amplitude of spontaneous activity. The amplitude of spontaneous activity for both contraction and fluorescence was measured during the gradual reduction of isolated bath temperature. Each bar represents the mean +/− SE of between five and seven individual preparations.

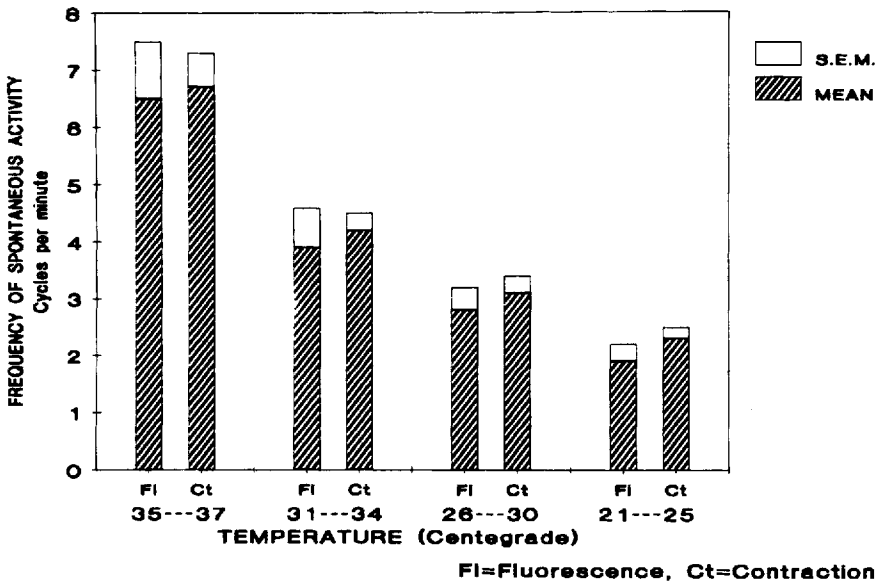

Fig. 3. Effect of temperature on the frequency of spontaneous activity. The frequency of spontaneous activity for both contraction and fluorescence was measured during the gradual reduction of isolated bath temperature. Each bar represents the mean +/− SE of between five and seven individual preparations.

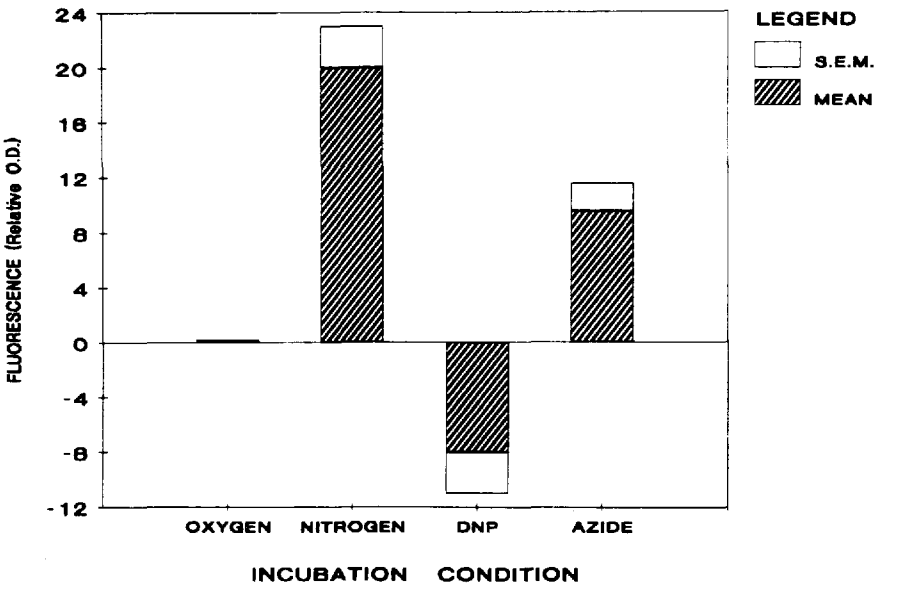

Fig. 4. The effect of metabolic inhibitors on fluorescence. The effect of a the following metabolic inhibitors on the in vitro fluorescence was determined: Control (95% oxygen 5% carbon dioxide), anoxia (95% nitrogen, 5% carbon dioxide), sodium azide (200 μM), and dinitrophenol (200 μM). Each bar represents the mean +/− SE of between five and seven individual preparations.

phenol (200 μM) produced a gradual decrease in fluorescence. All metabolic inhibitors produced gradual changes in fluorescence that reached maximum amplitude within approximately 10 minutes and remained constant throughout the exposure.

Figure 5 displays a representative tracing of the effect of bethanechol on fluorescence and contraction. Bethanechol (200 μM) produced a rapid increase in tension that was followed within 3–5 seconds by a prolonged decrease in fluorescence. Although not present in this particular tracing, in about 50% of the studies, there was a moderate increase in fluorescence immediately prior to the marked decrease. Figure 6 displays the bethanechol dose-response curves for fluorescence and contraction. The ED_{50}s for contraction and fluorescence were 6.4 μM and 3.5 μM, respectively. Prior administration of atropine (10 μM) completely inhibited both the contractile and fluorescence response to bethanechol (data not shown).

Figure 7 displays the KCl dose-response curves for fluorescence and contraction. The ED_{50}s for contraction and fluorescence are 37 and 16 mM, respectively. As with bethanechol, the change in fluorescence is more sensitive to KCl stimulation than is contraction.

To insure that changes in the redox potential observed with the spectrofluorometer actually reflected qualitative changes in the redox potential, NADH/NAD was quantitatively determined in tissue strips incubated under a variety of conditions. The ratio of NADH to NAD (as percentage NADH) in oxygenated tissue was 64%. Incubation for 10 minutes in the presence of anoxia increased the ratio to 83% NADH, whereas incubation in the presence of DNP reduced the percentage NADH to 21%. Incubation for 5 minutes in the presence of 200 μM bethanechol reduced the NADH percentage to 40% (from 64%). Mechanically increasing the tension on the bladder strip during recording did not significantly affect the fluorescent reading;

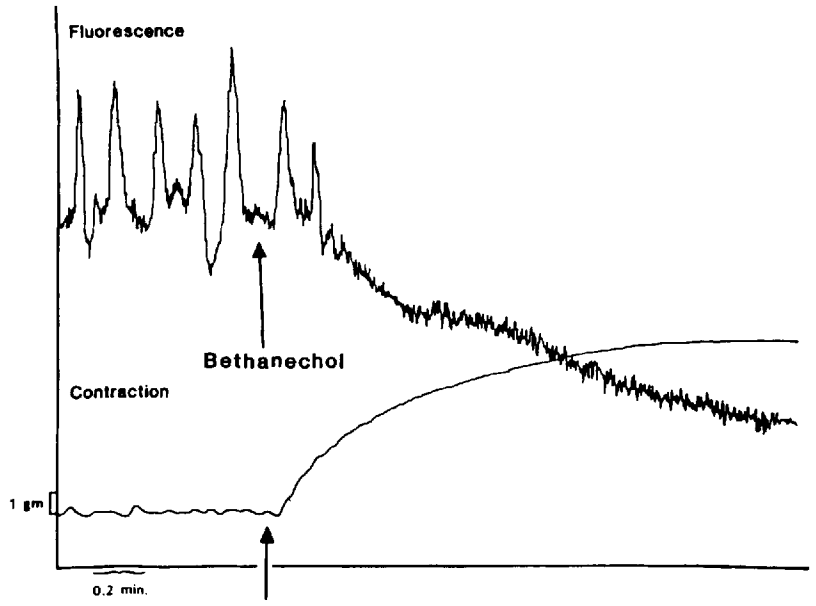

Fig. 5. Effect of bethanechol on contraction and fluorescence. A representative tracing of the effect of bethanechol (200 μM) on contraction and fluorescence.

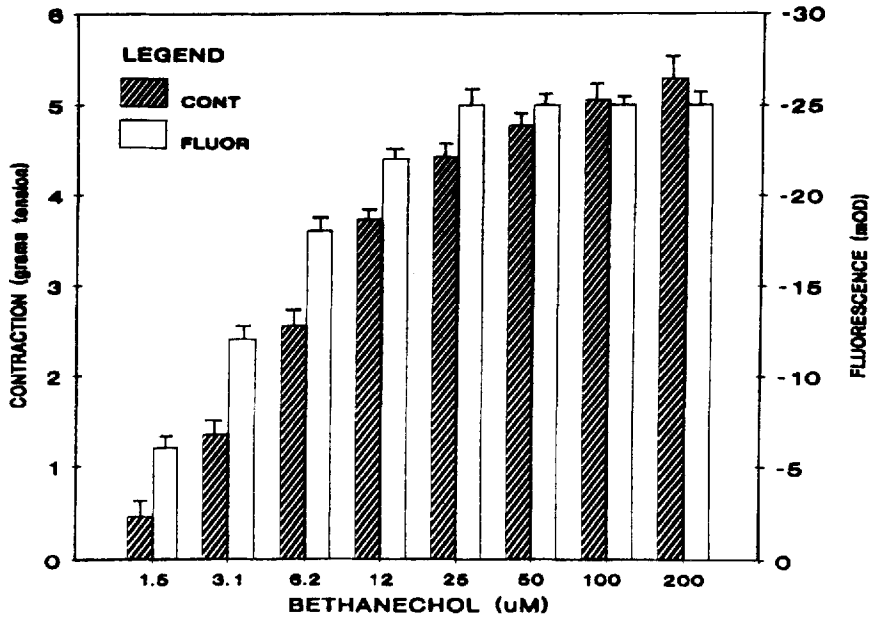

Fig. 6. Dose response of the bladder to bethanechol. The effect of bethanechol on bladder contraction and fluorescence is presented. Each bar represents the mean +/− SE of between five and seven individual preparations. The ED_{50}s for contraction and fluorescence is 6.4 and 3.5 μM respectively.

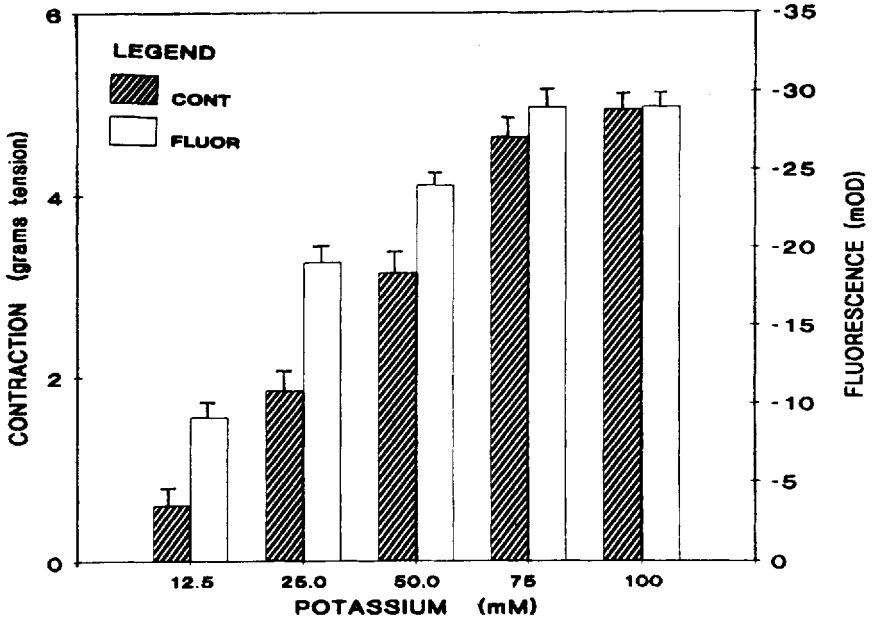

Fig. 7. Dose response of the bladder to KCl. The effect of KCl on bladder contraction and fluorescence is presented. Each bar represents the mean +/− SE of between five and seven individual preparations. The ED_{50}s for contraction and fluorescence are 37 and 16 μM respectively.

thus, the changes in fluorescence recorded during bethanechol and KCl stimulation were not secondary to the increase in tension, but were related to the pharmacological stimulation.

DISCUSSION

The urinary bladder, similar to other smooth muscle structures, metabolizes carbohydrates as its main source of metabolic energy [Levin et al, 1987; Lundholm et al, 1977; Rohner et al, 1967]. There are three phases of carbohydrate metabolism and energy production: 1) Glycolysis metabolizes glucose to lactic acid or pyruvate. Glycolysis can generate 2 ATP molecules per glucose molecule. 2) Pyruvate is metabolized to acetyl-Co A with the generation of CO_2 and the reduction of one molecule of NAD to NADH. 3) The Krebs cycle metabolizes the acetyl-Co A to CO_2 with the reduction of three molecules of NAD to NADH [Lehninger, 1965; Lundholm et al, 1977].

NADH is oxidized via the mitochondrial cytochrome system to NAD, with the generation of H_2O. The oxidation of NADH is directly linked to oxidative phosphorylation, which generates ATP (from ADP). One molecule of acetyl-Co A can generate 12 molecules of ATP [Lehninger, 1965].

The ratio of NADH to NAD is a sensitive indicator of the metabolic state of the bladder. During resting conditions, an intermediate ratio exists within the bladder smooth muscle. If one inhibits the electron transport chain (cytochromes), one would expect an increase in the concentration of NADH relative to NAD, and thus an increase in the redox state of the tissue. This is exactly what was demonstrated for

both anoxia and sodium azide. Both methods would prevent the generation of H_2O via cytochrome oxidase.

DNP uncouples respiration from oxidative phosphorylation, and as a result significantly increases the rate of the oxidation of NADH to NAD and generation of H_2O. Experimentally, a significant decrease in the redox state of the bladder was observed in the presence of DNP.

Increasing the use of metabolic energy markedly stimulates respiration and would be expected to significantly reduce the intracellular concentration of NADH. Experimentally, both bethanechol and KCl stimulated contractility and induced a reduction in NADH fluorescence. In fact, the reduction in NADH was a more sensitive indicator of bethanechol (or KCl) administration than was the measurement of contraction. Atropine administration completely inhibited both the contractile and fluorescence response to bethanechol but not to KCl.

There appears to be a direct correlation between contraction and fluorescence. "Spontaneous activity" is a commonly observed property of in vitro isolated strip preparations [Levin et al, 1986; Sibley, 1984; Van Duyl, 1985]. This activity is myogenic in nature and cannot be inhibited by agents such as atropine, tetrodotoxin, hexamethonium, and phentolamine [Levin et al, 1986]. Although the functional significance of spontaneous contractile activity is a controversial topic, it can be utilized to study the relationship between intracellular metabolism and myogenic contraction. An increase in contraction was directly related to a decrease in fluorescence. Decreasing the temperature of the isolated bath reduced both the amplitude and frequency of the spontaneous activity of both contraction and fluorescence. Thus, we feel that an increase in contractile activity (either spontaneous or evoked) directly induces a decrease in the redox state of the tissue.

The above studies have demonstrated that surface spectrofluorometry of the urinary bladder provides a sensitive method for monitoring the metabolic state of the tissue under a variety of in vitro conditions. Since the bladder depends on metabolic energy for contractile function, the ability to noninvasively monitor intracellular metabolism in vivo would provide us with important new information regarding the development of specific pathologies. Future studies will be directed at adapting this methodology for the noninvasive evaluation of the metabolic state of the in vivo bladder.

REFERENCES

Bergmeyer H (ed) (1963): "Methods of Enzymatic Analysis." New York: Academic Press, pp 2045–2060.

Butler RM, Siegman JJ, Mooers SU, Davies RE (1977): High energy phosphate utilization during force development and force maintenance in mammalian smooth muscle. In Casteels T, Godfraind T, Ruegg JC (eds): "Excitation Contraction Coupling in Smooth Muscle." Amsterdam: Elsevier, pp 463–469.

Chance B (1976): Pyridine nucleotide as an indicator of the oxygen requirements for energy linked functions of mitochondria. Circ Res 38:I33–I38.

Franke H, Barlow CH, Chance B (1976): Oxygen delivery in perfused kidney: NADH fluorescence and renal functional state. Am J Physiol 231:1082–1089.

Franke H, Barlow CH, Chance B (1980): Surface fluorescence of reduced pyridine nucleotide of the perfused rat kidney: Interrelation between metabolic and functional states. Contrib Nephrol 19:240–247.

Lehninger AL (1965): "Bioenergetics." New York: W.A. Benjamin, Inc., pp 51–113.

Levin RM, Brendler K, Van Arsdalen KN, Wein AJ (1983): Functional response of the rabbit urinary bladder to anoxia and ischemia. Neurourol Urodyn 2:233–243.

Levin RM, Haugaard N, Ruggieri MR, Wein AJ: (1987): Biochemical characterization of the rabbit urinary bladder base and body. Neurourol Urodyn 6:57–61.

Levin RM, High J, Wein AJ (1982): Metabolic and contractile effects of anoxia on the rabbit urinary bladder. J Urol 128:194–196.

Levin RM, Ruggieri MR, Velagapudi S, Gordon D, Altman B, Wein AJ (1986): Relevence of spontaneous activity to urinary bladder function. An in-vitro and in-vivo study. J Urol 136:517–521.

Lundholm L, Andersson RG, Armquist HJ, Lundholm EM (1977): Glycolysis and glycogenolysis in smooth muscle, In Stephens NL (ed): "Biochemistry of Smooth Muscle." Baltimore: University Park Press, pp 159–207.

Rohner TJ, Komins JI, Kirchner P, Adams R (1967): Utilization of glucose by normal, defunctionalized, and denervated bladder muscle. Invest Urol 5:12–18.

Sibley GNA (1984): A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J Physiol (Lond) 354:431.

Van Duyl WA (1985): Spontaneous contractions in urinary bladder smooth muscle: Preliminary results. Neurourol Urodyn 4:301.

Wrogemann K, Stephens NL (1977): Oxidative phosphorylation of smooth muscle. In Stephens NL (ed): "Biochemistry of Smooth Muscle." Baltimore: University Park Press, pp 41–50.