

The role of ATP-sensitive potassium channel on acute urinary retention and subsequent catheterization in the rat

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ABSTRACT

We investigated the role of K_{ATP} channel on acute urinary retention (AUR) induced bladder dysfunction. Eight-week-old female Sprague-Dawley rats were divided into seven groups: a sham-operated control group, an AUR group, and five AUR groups treated with: two different K_{ATP} channel openers namely nicorandil (3 or 10mg/kg), or cromakalim (100 or 300 μ g/kg), or one K_{ATP} channel inhibitor namely glibenclamide (5mg/kg). The drugs were administered 30min before induction of AUR. After the urethra was obstructed with a clip, AUR was induced by intravesical infusion of 2.5mL of saline via cystostomy. Following a 30min obstruction the bladder was allowed to drain with a catheter in place for 60min with real-time monitoring of intravesical pressure and blood flow. After the experimental period, the bladder function was assessed, using organ bath techniques (carbachol and 100mM KCl). AUR increased the intravesical pressure and decreased the blood flow. The subsequent catheterization decreased the intravesical pressure and increased the blood flow. AUR group reduced significantly the contractile responses to both carbachol and KCl compared with the control group. Nicorandil and cromakalim but not glibenclamide prevented the bladder dysfunction after AUR suggesting that K_{ATP} channel openers may prevent the bladder dysfunction caused by AUR and subsequent catheterization.

Keywords: acute urinary retention, nicorandil, cromakalim, glibenclamide, pharmacological preconditioning

1. Introduction

Clinically, acute urinary retention (AUR) is often observed in patients with benign prostate hyperplasia (Meigs et al, 1999), diabetic autonomic neuropathy (Ewing and Clarke, 1982) or as a drug-related side effect (Verhamme et al, 2008). AUR is a significant public health issue with significant consequences to the affected individual (Choong and Emberton, 2000). Traditionally, prolonged bladder overdistension has been thought to simply produce stretch damage of the detrusor muscle and other bladder microstructures, resulting in reduced contractility of the detrusor muscle. Several reports have indicated that the biochemical and metabolic status of the bladder is altered after prolonged overdistension (Tammela et al, 1993; Gabella et al, 1999; Saito et al, 2010). Previous studies from this and other laboratories have demonstrated that ischemia/reperfusion injury might be at least partly responsible for the prolonged bladder dysfunction occurring after overdistension (Lin et al, 2000; Saito and Miyagawa, 2001). Both in humans and animals, acute bladder overdistension induces a reduction in blood flow to bladder tissue resulting in partial ischemia and hypoxia; after subsequent catheterization, the blood supply recovers, allowing reperfusion (Lin et al, 2000; Saito and Miyagawa, 2001). As with ischemia/reperfusion-injury of other organs, ischemia followed by subsequent reperfusion in the bladder leads to oxidative stress, inflammation, and neutrophil infiltration, which might contribute to the delayed recovery of the bladder function. Recently, we reported that treatment with edaravone, a radical scavenger or sivelestat, a neutrophil elastase inhibitor are able to prevent the bladder dysfunction induced by rat ischemia/reperfusion or AUR models (Kono et al, 2008; Shimizu et al, 2009).

Noma (1983) originally described the ATP-sensitive potassium channel (K_{ATP}

channel) in guinea pig cardiac membrane patches (Noma, 1983). Since then, K_{ATP} channels have been detected in several kinds of tissues and they are localized in the sarcolemmal, mitochondrial and nuclear membranes (sarco K_{ATP} channels, mito K_{ATP} channels, and nuclear K_{ATP} channels, respectively) (Zhuo et al, 2005). K_{ATP} channels play an important role in ischemia/reperfusion-injury in many tissues. It was found that ischemia leads to cardiac cell apoptosis, which contributes to the injury while preconditioning prevents apoptosis in ischemic hearts. Opening of K_{ATP} channels can protect cardiac myocytes against ischemia/reperfusion -injury, called pharmacological preconditioning (Zhuo et al, 2005). There are some reports indicating that nicorandil is a selective mito K_{ATP} channel opener, and cromakalim is non-selective K_{ATP} channel opener whereas glibenclamide is a non-selective K_{ATP} channel inhibitor (Date et al, 2005).

Therefore, we hypothesized that K_{ATP} channel openers might prevent the bladder dysfunction caused by the AUR and subsequent catheterization. In the current international literature there is no information available regarding the effects of K_{ATP} channel openers or inhibitors on bladder dysfunction following AUR and subsequent catheterization. Thus the aim of the present study was to investigate the effects of K_{ATP} channel openers, nicorandil and cromakalim, and K_{ATP} channel inhibitor, glibenclamide in the bladder dysfunction after AUR and subsequent catheterization.

2. Materials and methods

2.1. Animals and experimental design

All animal experiments were conducted in accordance with the guidelines set by the Tottori University Committee for Animal Experimentation. The rat experimental model was created as described in our previous reports (Saito and Miyagawa, 2001; Shimizu et al, 2009). Eight-week-old female Sprague-Dawley rats (160-200g; SLC, Shizuoka, Japan) were randomly divided into seven groups (n=5-6 in each group). Group A consisted of sham-operated control rats, and groups B, C, D, E, F and G undergone 30 min of overdistension followed by 60 min of drainage; group B was non-drug treated; groups C and D were treated with nicorandil at a dose of 3 or 10 mg/kg body weight, respectively; groups E and F were treated with cromakalim at a dose of 100 or 300 µg/kg body weight, respectively; and group G was treated with glibenclamide 5mg/kg body weight. Each drug was administered intra-abdominally 30min before inducing AUR. Each rat was anaesthetized with sodium pentobarbital (50mg/kg, intra-abdominally). The rat distal urethra was then clamped with a small clip; subsequently a cystostomy was made and 2.5 ml (0.5ml/min) of saline was infused with an infusion pump (TOP-5200; TOP, Tokyo, Japan) to induce AUR. Thirty minutes after inducing urinary retention, the cystostomy tube was opened and the bladder was allowed to empty for another 60 min. The rats were euthanized with an overdose of pentobarbital (60mg/kg, intra-abdominally).

2.2. Simultaneous measurements of intravesical pressure and blood flow in the bladder

A cystometry catheter was connected to an external pressure transducer (P2310;

Gould, Eastlake, OH) for the measurement of the intravesical pressure. Simultaneously, the blood flow in the rat bladder was measured in vivo with a laser Doppler flow meter (BRL-100; Bioresearch Co., Nagoya, Japan) according to the method we described in our previous reports (Saito and Miyagawa, 2001; Shimizu et al, 2009).

2.3. Tissue preparation and measurement of contractile force of the bladder

Functional studies were conducted according to the methods described in our previous reports (Saito et al, 2008, 2010; Shimizu et al, 2009). Briefly, longitudinal strips of the posterior wall of the bladder dome were mounted in organ baths (25 ml) containing Krebs-Henseleit solution, and bubbled with 5% CO₂ and 95% O₂ (37°C). One hook was suspended from a transducer (type 45196 A; San-ei Instruments, Tokyo, Japan), and the lower hook was fixed to a plastic support leg attached to a micrometer (Mitutoyo, Tokyo, Japan). Each strip was equilibrated unstretched for 30 min. A load of 1.0 g was applied to each strip by micrometer adjustment, and the load was readjusted to this level 30 min later. Changes in the tone of the strips were measured isometrically using force transducers, and the data were recorded on a personal computer (Macintosh G3; Apple Computer, Cupertino, CA) using the Chart v3.6.9 software and the PowerLab/16sp data acquisition system (AD Instruments, Castle Hill, Australia). Cumulative concentration response curves to carbachol were constructed, and contractile forces to 100 mM of KCl were also measured.

2.4. Data analysis

The EC₅₀ and E_{max} values to carbachol were determined using a Macintosh computer (G3) loaded with the Chart v3.6.9 software and the Power Lab/16sp data

acquisition system. Contractile data were calculated as grams of active force per cross-sectional area in square millimeters. The cross-sectional area was calculated by using the following equation: cross-sectional area = weight/ (length × 1.05), where 1.05 is the assumed density of the smooth muscle (Saito et al, 2008; Shimizu et al, 2009). A statistical comparison of differences between groups was performed with the use of analysis of variance and Fisher's multiple comparison tests. P values less than 0.05 were considered to be statistically significant.

2.5. Drugs and chemicals

Nicorandil was kindly supplied by Chugai Pharmaceutical Co. Ltd (Tokyo, Japan). Cromakalim and glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals are available commercially.

3. Results

3.1. Measurement of intravesical pressure and blood flow in the rat bladder during urinary retention and subsequent catheterization

Figure 1 shows the result of the simultaneous measurement of intravesical pressure and blood flow in the rat bladder during urinary retention and subsequent catheterization. In all the experimental groups, urinary retention increased the intravesical pressure and decreased blood flow, and subsequent catheterization decreased intravesical pressure and increased blood flow. Treatment with nicorandil or cromakalim tended to increase the blood flow during the catheterization phase in a dose dependent fashion, and treatment with glibenclamide decreased the blood flow during the catheterization phase compared to that in group B. At the end of the experiments (95 minutes since induction of AUR), p values of group B vs groups C, D, E, F and G in the blood flow were 0.308, 0.028, 0.812, 0.395 and 0.180, respectively. On the other hand, there were no significant differences in the intravesical pressure of the bladder between any of the other groups during the experimental period. These data suggest that the treatment with K_{ATP} channel openers or K_{ATP} channel inhibitor did not alter the intravesical pressure during AUR and subsequent catheterization, and that the treatment with K_{ATP} channel openers significantly increased the blood flow during the catheterization phase.

3.2. Measurement of contractile response to carbachol and 100mM KCl

The E_{max} and EC_{50} values for the contractile response of bladder strips to carbachol and the response to KCl (100 mM) were determined, and the results are shown in Table 1. The E_{max} values of carbachol in the AUR rats (group B) were markedly smaller than those of the control rats (group A). Treatment with nicorandil and cromakalim K_{ATP} channel

openers (group C-F) significantly prevented this injury. On the other hand, treatment with the K_{ATP} channels inhibitor glibenclamide failed to prevent the bladder smooth muscle dysfunction (group G). The EC_{50} values of the group B were significantly greater than those of the control group (group A). However, apart from the significant difference between groups A and B, there were no statistically significant differences between any of the other groups (Table 1). Contractile responses to 100 mM KCl showed a similar pattern to that for the E_{max} values of carbachol in each group.

4. Discussion

In the present study, we clearly demonstrated that during the AUR phase the urinary bladder was under ischemia and that during and after drainage the bladder was under reperfusion. Interestingly, although in the bladder blood flow there were no significant differences between any of the groups during AUR period, treatment with nicorandil or cromakalim increased the blood flow during the catheterization phase in a dose-dependent manner compared to that in the non-drug treated AUR animals. On the other hand, treatment with glibenclamide decreased the blood flow during the catheterization phase compared to that in the non-drug treated AUR animals. These phenomena may be explained by the action of the K_{ATP} channel openers. In fact, it is possible that the activation of the K_{ATP} channels by the K_{ATP} channel openers might have resulted in dilated vessels so that blood flow increased during the drainage phase. In addition, as nicorandil is reported to have properties as both K_{ATP} channel opener and nitric oxide (NO) donor (Kamiyama et al, 2007), it is possible that NO directly dilated the vessels in the bladder. Another possible mechanism could be that K_{ATP} channel openers directly relax the bladder smooth muscle and subsequently the blood flow increase during the drainage phase.

Gopalakrishnan and associates (1999) provided evidence for the molecular identity of K_{ATP} channels expressed in guinea pig bladder smooth muscle cells. Employing pharmacological and molecular analysis, they demonstrated that the K_{ATP} channels in the bladder smooth muscle cells are composed of SUR2B associated with a single inward rectifier, $K_{IR} 6.2$. (Gopalakrishnan et al, 1999). Fabiyi and co-workers (2003) reported that, K_{ATP} channel openers inhibit both neurogenic and myogenic bladder contraction (Fabiyi et al 2003). Since then some investigators have reported the inhibitory effects of

K_{ATP} channel openers on bladder contractility for the purpose of clinical use in patients with overactive bladder (Kamiyama et al, 2007; Yunoki et al, 2008; Akino et al, 2008). However, there is no information available concerning the role of K_{ATP} channels on AUR associated bladder dysfunction as a result of ischemia/reperfusion-injury.

The role of K_{ATP} channels in cardiovascular system has been studied adequately and their most prominent function is that the opening of these channels can protect cardiac myocytes against ischemic injury. More specifically sarco K_{ATP} channels are reported to regulate the cell energy metabolism in two ways: a) one is that opening of sarco K_{ATP} channel accelerates phase 3 repolarization and slows down depolarization, which will shorten action potential duration and prevent the reversal of Na^+/Ca^{2+} exchanger resulting in inhibition of Ca^{2+} entry in the cell and b) the second way is induction of membrane potential hyperpolarization (Zhuo et al, 2005). On the other hand in case of energy crisis, such as in an ischemia/reperfusion-injury the mitochondrial production of ATP is impaired by alternation of mitochondrial membrane potential, imbalanced trans-membrane ions transport, or over-production of free radicals. The opening of mito K_{ATP} channels prevent the mitochondria from these disturbances by controlling the mitochondrial Ca^{2+} ion concentration and matrix swelling (Zhuo et al, 2005).

Previously we clearly demonstrated that mechanical preconditioning prevents ischemia/reperfusion induced bladder dysfunction in the rat (Hisadme et al, 2007). In order to investigate the effect of pharmacological preconditioning on AUR-induced bladder dysfunction, we used three drugs namely nicorandil, cromakalim and glibenclamide. Nicorandil is reported to be a selective mito K_{ATP} channel opener, and cromakalim is reported to be a non-selective K_{ATP} channel opener whereas glibenclamide

is reported to be a non-selective K_{ATP} channel inhibitor (Date et al, 2005). Present functional studies showed that nicorandil and cromakalim but not glibenclamide prevented the bladder dysfunction after AUR. Our data indicate that pharmacological preconditioning protects the bladder smooth muscle against ischemic (and reperfusion) insult, and that this effect is mediated via the opening of the mito K_{ATP} channels. However, there are still controversies about the existence of the mito K_{ATP} channels in the mitochondria (Zhuo et al, 2005). Further investigation is required to clarify the underlying molecular mechanisms of K_{ATP} channels in preventing AUR-induced bladder dysfunction.

In conclusion, K_{ATP} channel openers prevented the bladder dysfunction caused by AUR and subsequent catheterization.

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Figure legends

Intravesical pressure and blood flow measured simultaneously during experimental duration.

Nic 3: AUR rats treated with nicorandil at 3 mg/kg body weight, Nic10: AUR rats treated with nicorandil at 10 mg/kg body weight, Cro 100: AUR rats treated with cromakalim at 100µg /kg body weight, Cro 300: AUR rats treated with cromakalim at 300µg /kg body weight, and Glib 5: AUR rats treated with glibenclamide 5 mg/kg body weight.

Table 1. Functional studies in the experimental rats

Group	E_{max} (g/mm ²)	EC_{50} (10 ⁻⁶ M)	KCl (g/mm ²)
A (control)	4.43 ± 0.15 ^{a,c}	0.70 ± 0.19 ^a	2.61 ± 0.17 ^{a,b,c}
B (AUR)	2.53 ± 0.19	1.13 ± 0.19	1.39 ± 0.14
C (nicorandil at 3 mg/kg)	3.63 ± 0.33 ^{a,c}	0.77 ± 0.10	2.01 ± 0.20 ^a
D (nicorandil at 10 mg/kg)	3.97 ± 0.25 ^{a,c}	0.81 ± 0.08	2.40 ± 0.23 ^{a,c}
E (cromakalim at 100µg /kg)	4.14 ± 0.22 ^{a,c}	0.97 ± 0.37	2.36 ± 0.18 ^{a,c}
F (cromakalim at 300µg /kg)	4.26 ± 0.38 ^{a,c}	0.74 ± 0.11	2.46 ± 0.25 ^{a,c}
G (glibenclamide 5 mg/kg)	2.92 ± 0.16	1.10 ± 0.24	1.72 ± 0.12

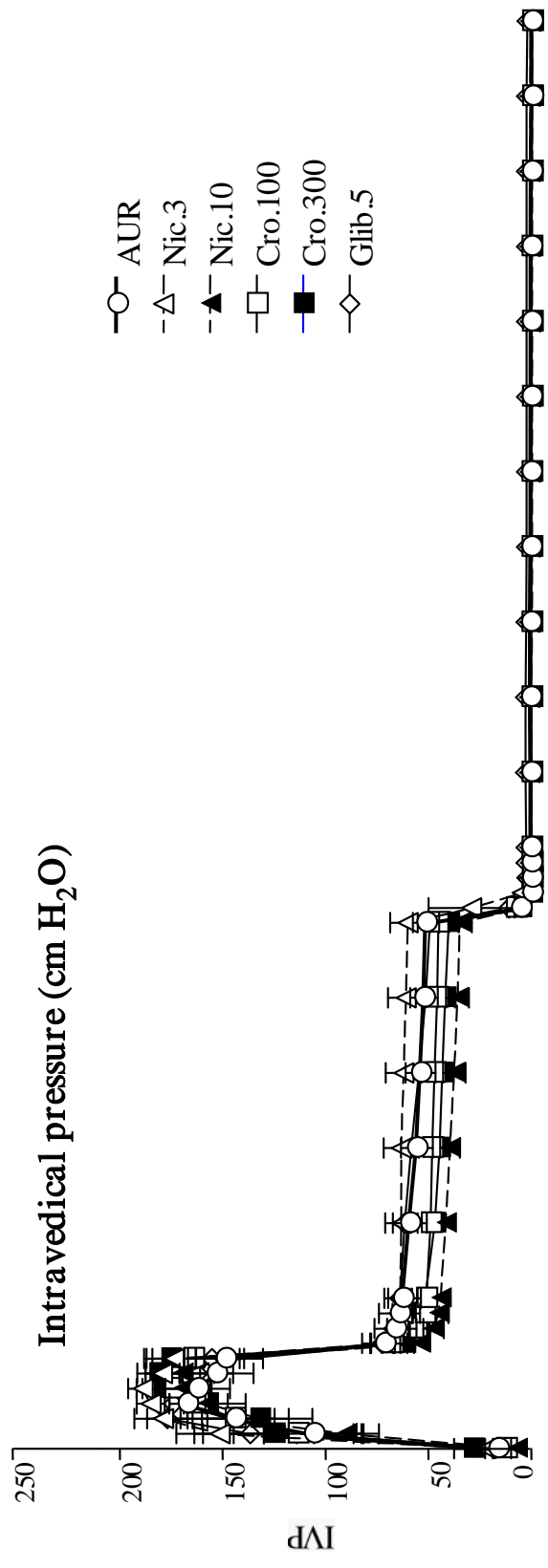
Data are shown as mean ± SEM of five to six separate determinations in each group. E_{max} and EC_{50} values are for carbachol effect in experimental rat bladder strips. KCl mean contractile force to 100 mM KCl. A: control rats, B: AUR rats, C: AUR rats treated with nicorandil at 3 mg/kg body weight, D: AUR rats treated with nicorandil at 10 mg/kg body weight, E: AUR rats treated with cromakalim at 100µg /kg body weight, F: AUR rats treated with cromakalim at 300µg /kg body weight, G: AUR rats treated with glibenclamide 5 mg/kg body weight. EC_{50} : effect concentration 50.

^a significantly different from group B. (p<0.05)

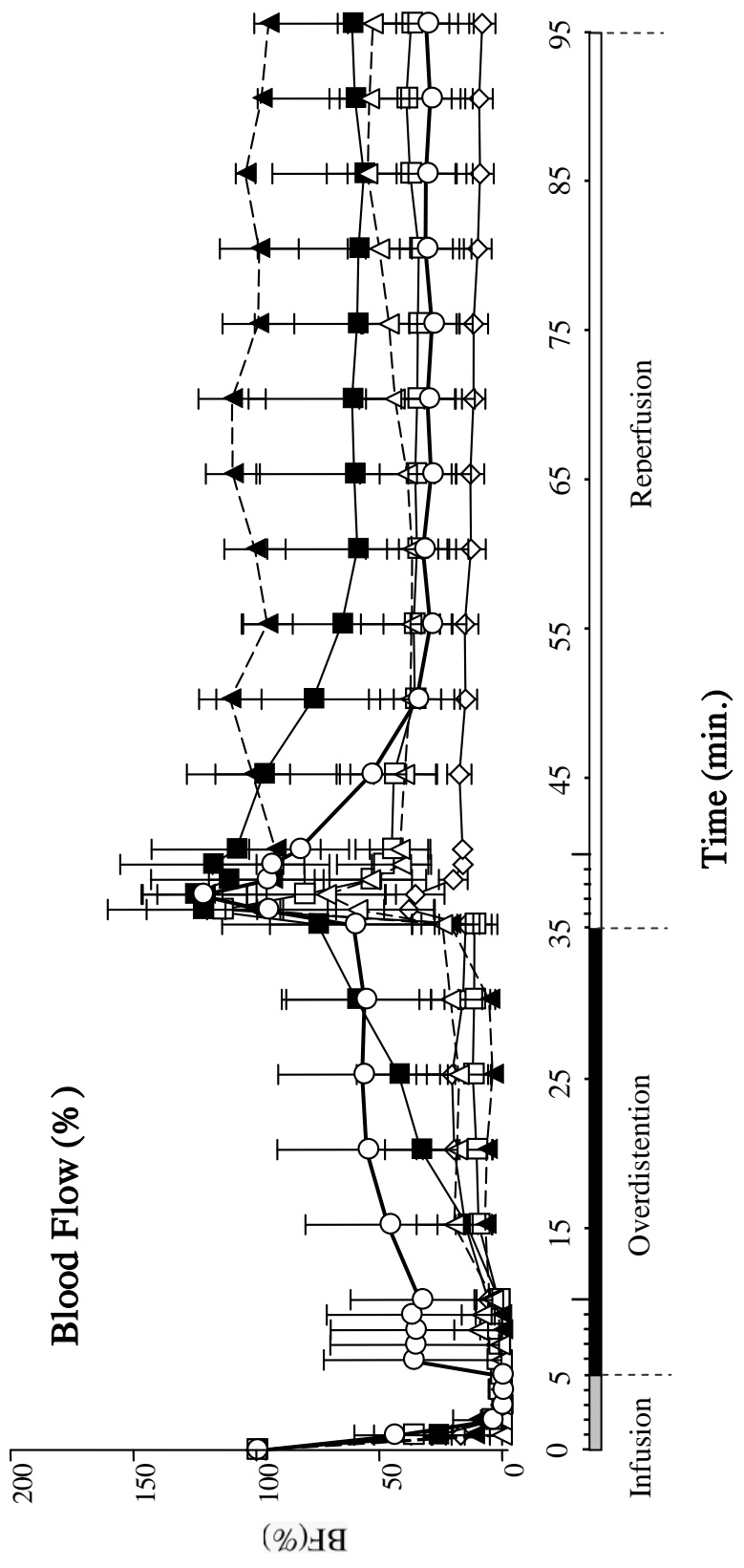
^b significantly different from group C. (p<0.05)

^c significantly different from group G. (p<0.05)

Intravascular pressure (cm H₂O)



Blood Flow (%)



Reperfusion

Overdistention

Infusion

Time (min.)