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A possible effect of sulfhydryl reagents on the contractile activity of the rat detrusor muscle

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Abstract

We aimed to investigate the effect of sulfhydryl (SH) inactivating agents, ethacrynic acid and *N*-ethylmaleimide, on the contractile activity of rat detrusor muscle. Wistar Kyoto rats weighing 150–250 g were anaesthetized with ketamine and bled to death. The urinary bladders were surgically removed and detrusor strips were mounted under 0.5 g tension in organ baths. The responses were recorded with isotonic transducers on polygraph paper. After an equilibrium period, the tissues were contracted by electrical field stimulation, acetylcholine, ethacrynic acid or *N*-ethylmaleimide and the effects of L-cysteine, glutathione, verapamil, Ca^{2+}-free solution, sodium nitroprusside or atropine were then examined on these contractions. Verapamil, Ca^{2+}-free solution or atropine significantly reduced the contractions elicited by electrical field stimulation and acetylcholine whereas L-cysteine, glutathione or sodium nitroprusside had no effect on the contractions in response to these stimuli. L-Cysteine, glutathione, verapamil or Ca^{2+}-free solution significantly inhibited the contractions induced by ethacrynic acid or *N*-ethylmaleimide. Sodium nitroprusside slightly inhibited only the contraction induced by ethacrynic acid but not that with *N*-ethylmaleimide. Atropine has no action on the contractions in response to these SH reagents. These findings suggest that SH reagents may play a role in the contractile activity of rat detrusor muscle and this action seems to be related to the gating of Ca^{2+} channels. Further experiments are needed to determine the cellular mechanism(s) of action by which these SH reagents act on the detrusor smooth muscle.

Keywords: Sulfhydryl group; Detrusor muscle; Etachrynic acid; *N*-ethylmaleimide; Ca^{2+} channel

1. Introduction

A role of *N*-ethylmaleimide and ethacrynic acid, sulfhydryl (SH) alkylating agents (Rapoport and Murad, 1988; Lacampagne et al., 1995) in contraction of some tissues, such as guinea pig ileum (Salimi et al., 1979), rabbit ear arteries (Neering and Glover, 1979) and rat diaphragm muscle (Roed, 1989) has been demonstrated. Recent studies suggested a role of free SH groups in gating of the Ca^{2+} channels in some tissues, such as cardiac myocytes (Lacampagne et al., 1995; Suzuki et al., 1998), vascular endothelial cells (Elliott and Koliwad, 1997; Az-ma et al., 1999), rabbit heart sarcolemma and skeletal muscle (Murphy et al., 1990). SH reagents have been used to establish the role of SH groups in the function of Ca^{2+} channels and it has been shown that SH alkylating agents induced a Ca^{2+} current leading to an increase of tension in skeletal muscle fibers of a crustacean (Zuazaga and Del Castillo, 1985; Lizardi et al., 1992). It was also shown that *N*-ethylmaleimide increased the Ca^{2+} current in heart cells (Nakajima et al., 1990). It has been reported from a recent study that SH reagents exerted a stimulatory effect on the L-type Ca^{2+} current on frog ventricular myocytes and it was concluded that there are sites on the Ca^{2+} channels that are subject to direct modification by SH reagents (Yamaoka et al., 2000).

In the urinary bladder, the Ca^{2+} channels seem to be mainly of the L type (Anderson, 1993). Several experimental studies of animal detrusor tissue demonstrated the importance of extracellular Ca^{2+} entry through L-type Ca^{2+} channels and mobilisation of intracellular Ca^{2+} for tonic activity (Mostwin, 1986; Huddart and Butler, 1986; Batra et al., 1987; Kishii et al., 1992). Extracellular Ca^{2+} removal and L-type Ca^{2+} channel blockers reduce contractile activity (Andersson and Forman, 1986).

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Table 1
The effects of Ca^{2+}-free medium (Ca-F), 1 μM verapamil (VER) or 0.2 μM atropine (ATR) on the contractile responses of rat detrusor muscle to electrical field stimulation (EFS; 5 Hz) or acetylcholine (1 μM)

	Control	Ca-F	VER	ATR
EFS	106 ± 8.0	0*	51.0 ± 11.2*	56.0 ± 5.9*
Acetylcholine	120 ± 16	60.1 ± 9.2*	22.0 ± 5.10*	2.0 ± 1.3*

The values are presented as percents of control contraction (first series).
* Indicates significant differences from control ($P<0.05$) ($n=6$ for each group).

These results prompted us to investigate whether there is an actual contribution of SH reagents to the contractile activity of rat detrusor muscle or not. We thus compared the effect of *N*-ethylmaleimide and ethacrynic acid on the basal tonus of the isolated rat detrusor muscle with the neurogenic contractions induced by electrical field stimulation and myogenic contractions induced by acetylcholine and then examined the actions of L-cysteine, glutathione, Ca^{2+}-free medium, verapamil, sodium nitroprusside or atropine on the responses of the tissue to these stimuli.

2. Materials and methods

2.1. In vitro experiments

Wistar Kyoto rats weighing 150–250 g were anaesthetized with ketamine (25 mg/kg, intraperitoneally) and bled to death. The urinary bladders were surgically removed and were then placed in a Petri dish containing Krebs solution (composition in mM: NaCl, 119; KCl, 4.6; $CaCl_2$, 1.5; $NaHCO_3$, 15; NaH_2PO_4, 1.2; glucose, 11). Detrusor strips ($2 \times 2 \times 10$ mm) were prepared. The preparations were mounted under 0.5-g tension in 5-ml organ baths maintained at 37 °C and containing Krebs solution, which was aerated with 95% O_2 and 5% CO_2. Tissues were allowed to equilibrate for 1 h. The responses were recorded with isotonic transducers (Ugo Basile, 7006) on polygraph paper (Ugo Basile, Gemini 7070). After an equilibrium period, the tissues were contracted by electrical field stimulation (2, 5 and 10 Hz; 30 V, 0.1 ms), delivered as square waves by a Grass S88 stimulator via two parallel platinum electrodes embedded in Perspex. Stimulation was applied to the tissue for 30 s at 2-min intervals. In some experiments, acetylcholine (1 μM), ethacrynic acid (10, 30, 50 and 100 μM) or *N*-ethylmaleimide (5, 10, 15 and 25 μM) was added to the bathing medium to elicited contractions. After the responses to electrical field stimulation and the chemicals were recorded, the tissue was washed with fresh Krebs solution and thus relaxed back to the baseline. Thus, the first series of responses was obtained. After the tissue was left to rest for 30 min, the second series of responses was recorded as control in the same manner. In some trials, after the first series of responses was recorded, the preparation was replaced into medium with L-cysteine (10 or 100 μM), glutathione (100 or 200 μM), sodium nitroprusside (100 μM), verapamil (1 μM), atropine (0.2 μM) or Ca^{2+}-free solution and the second series of contractions due to chemicals or electrical field stimulation was examined.

2.2. Drugs and solutions

Stock solutions of acetylcholine, L-cysteine, glutathione, verapamil, sodium nitroprusside, atropine sulphate and *N*-ethylmaleimide were prepared in distilled water. Ethacrynic acid was dissolved in dimethyl sulfoxide (0.1% v v^{-1}). All drugs were purchased from Sigma.

2.3. Statistical considerations

The contractions were calculated. The mean values (± S.E.) for the first and the second series were calculated separately. In some groups, the values from the second series were expressed as percentages of mean values (± S.E.) for the first series. All data were evaluated with

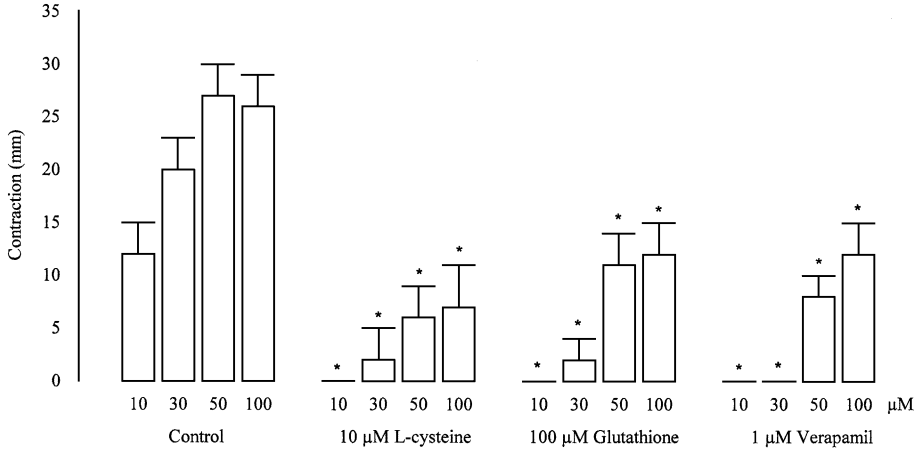

Fig. 1. The effects of 10 μM L-cysteine, 100 μM glutathione or 1 μM verapamil on the contractile responses of rat detrusor muscle to ethacrynic acid. Each column represents the mean contractile response. * Indicates significant differences from control ($P<0.05$) ($n=6$ for each group).

Table 2
The effects of Ca^{2+}-free medium (Ca-F), 100 μM sodium nitroprusside (SNP) or 0.2 μM atropine (ATR) on the contractile responses of rat detrusor muscle to 100 μM ethacrynic acid or 25 μM *N*-ethylmaleimide

	Control	Ca-F	SNP	ATR
Ethacrynic acid	93.0 ± 9.0	0*	62.0 ± 4.6*	88.0 ± 8.1
N-ethylmaleimide	120 ± 16	14 ± 6.2*	94.0 ± 15	92.0 ± 13

The results are presented as percents of control contraction (first series).
* Indicates significant differences from control ($P<0.05$) ($n=6$ for each group).

the Bonferroni-corrected *t*-test that was used in the one-way analysis of variance (ANOVA). *P* values of less than 0.05 were considered to be significant.

3. Results

3.1. Contractions induced by electrical field stimulation

Electrical field stimulation-induced contractions at all frequencies (2, 5 and 10 Hz) were significantly inhibited by Ca^{2+}-free solution, 1 μM verapamil or 0.2 μM atropine (Table 1) whereas these contractions were not affected by 100 μM L-cysteine, 200 μM glutathione or 100 μM sodium nitroprusside (not shown).

3.2. Contractions induced by acetylcholine

Atropine of 0.2 μM completely abolished the contraction induced by 1 μM acetylcholine (Table 1). The contractions in response to this agent were also significantly decreased by Ca^{2+}-free solution or 1 μM verapamil (Table 1) whereas 100 μM L-cysteine, 200 μM glutathione or 100 μM sodium nitroprusside had no effect on these contractions (not shown).

3.3. Contractions induced by ethacrynic acid

Ethacrynic acid (10, 30, 50 and 100 μM) caused dose-dependent reproducible contractions throughout the experiments. The contractions induced by ethacrynic acid at all concentrations were significantly inhibited by L-cysteine (10 or 100 μM), glutathione (100 or 200 μM) or verapamil (1 or 10 μM) (Fig. 1). Ca^{2+}-free solution or 100 μM sodium nitroprusside also abolished the contractions induced by 100 μM ethacrynic acid (Table 2). Atropine, 0.2 μM, had no significant action on the contraction induced by ethacrynic acid (Table 1).

3.4. Contractions induced by N-ethylmaleimide

N-ethylmaleimide (5, 10, 15 and 25 μM) also elicited a dose-dependent stable contraction that remained throughout the experiments similar to ethacrynic acid. L-Cysteine (10 or 100 μM), glutathione (100 or 200 μM) or verapamil (1 or 10 μM) completely abolished the contractions induced by *N*-ethylmaleimide at all concentrations in a dose-dependent manner (Fig. 2). These inhibitions could be surmounted by increasing the concentration of *N*-ethylmaleimide (Fig. 2). Ca^{2+}-free solution also caused a significant inhibition of the contractions induced by 25 μM *N*-ethylmaleimide whereas 100 μM sodium nitroprusside or 0.2 μM atropine did not affect these contractions (Table 2).

4. Discussion

In the present study, we demonstrated that SH alkylating agents, *N*-ethylmaleimide and ethacrynic acid, induced reproducible and reversible contractions that were inhibited by L-cysteine, glutathione, verapamil and Ca^{2+}-free medium in isolated rat detrusor muscle. These results suggest that SH reagents may play a role in the contractile activity of rat

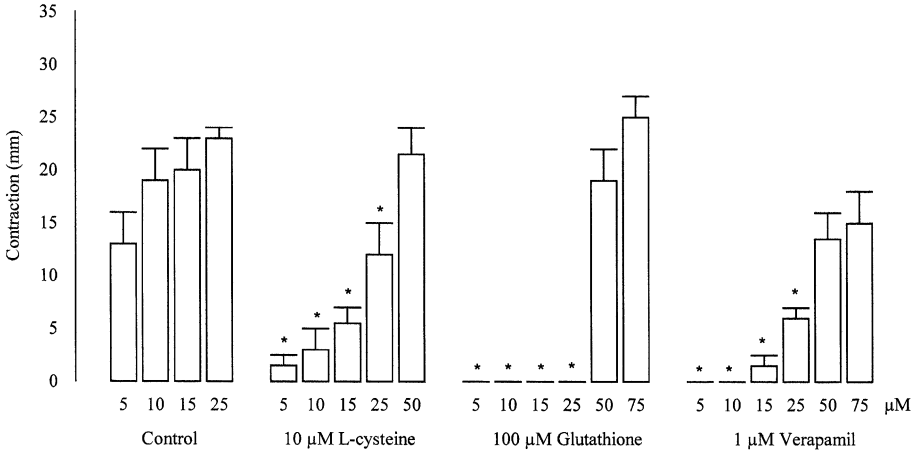

Fig. 2. The effects of 10 μM L-cysteine, 100 μM glutathione or 1 μM verapamil on the contractile responses of rat detrusor muscle to *N*-ethylmaleimide. Each column represents the mean contractile response. * Indicates significant differences from control ($P<0.05$) ($n=6$ for each group).

detrusor muscle, possibly related with the gating of L-type Ca^{2+} channels.

Electrical field stimulation, acetylcholine, *N*-ethylmaleimide and ethacrynic acid produced contractions in a frequency- or concentration-dependent manner. It is well known that in some animal species, bladder contraction is mediated by both cholinergic and nonadrenergic–noncholinergic mechanisms (Ambache and Zar, 1970; Taira, 1972; Anderson, 1993). An interesting finding of the present study was that the SH reagents elicited reproducible contractions. Although previous studies showed that these SH reagents caused an increase in the tension of some tissues such as guinea pig ileum, rabbit ear arteries and rat diaphragm muscle (Salimi et al., 1979; Neering and Glover, 1979; Roed, 1989), the contractile mechanism was unclear. In the present study, SH groups, L-cysteine and glutathione reduced the contractions induced by *N*-ethylmaleimide and ethacrynic acid markedly, but not those in response to electrical field stimulation or acetylcholine, indicating that there may be a specific interaction between SH reagents and endogenous SH groups in the tissue (Di Simplicio et al., 1985; Li et al., 1994; Garcia-Pascual et al., 2000). The fact that L-cysteine and glutathione abolished the contractile effects of *N*-ethylmaleimide and ethacrynic acid could be interpreted as prevention of the interaction of these SH reagents with thiols. Inhibition induced by SH groups on contractile responses of the tissue to *N*-ethylmaleimide could be surmounted by increasing the concentration of this agent. This finding may strengthen the possibility of an interaction between SH groups and *N*-ethylmaleimide. It was suggested that, in skeletal muscle fibers of a crustacean, Ca^{2+} channels were made functional by the SH-specific reagents and that the increase in tension was probably mediated by an increase in Ca^{2+} influx through the chemically induced Ca^{2+} channels (Lizardi et al., 1992). Also, similar evidence suggesting the role of free SH groups in gating of Ca^{2+} channels was obtained from studies on various tissues (Murphy et al., 1990; Aoki et al., 1993; Champbell et al., 1996; Donoso et al., 2000; Poteser et al., 2001). That there are sites on the Ca^{2+} channels that are subject to direct modification by SH reagents were shown recently for frog ventricular myocytes in a study using the whole-cell patch clamp technique (Yamaoka et al., 2000). In the present study, the contractions induced by *N*-ethylmaleimide and ethacyrnic acid may have resulted from the alkylating action of these SH reagents on SH groups in Ca^{2+} channel protein, leading to an increase in Ca^{2+} current (Zuazaga et al., 1985; Nakajima et al., 1990; Lizardi et al., 1992). However, further evaluation, using the patch clamp technique, is required to clarify the possible role of SH groups in gating of Ca^{2+} channels in the rat detrusor muscle.

A Ca^{2+} channel blocker, verapamil, and Ca^{2+}-free medium abolished the contractions induced by electrical field stimulation and acetylcholine. These findings are consistent with the results of the previous studies. The importance of extracellular Ca^{2+} in the contractile activity of detrusor muscle is well established (Mostwin, 1986; Huddart and Butler, 1986; Batra et al., 1987; Kishii et al., 1992). It was shown that Ca^{2+} channel antagonists have potent inhibitory effects on contraction of isolated detrusor muscle from various animal species (Sjögren and Andersson, 1979; Batra et al., 1987; Acevedo and Contreras, 1989; Damaser et al., 1997). It was also shown that Ca^{2+} channel antagonists abolish the NANC-mediated contractile component (Andersson et al., 1986; Bo and Burnstock, 1990) or atropine-resistant contractile component (Sjögren et al., 1982). Another interesting finding of the present study was that verapamil and Ca^{2+}-free medium also abolished the contractions induced by *N*-etylmaleimide and ethacrynic acid in rat detrusor muscle. Elevation of the concentration of *N*-etylmaleimide could also surmount the inhibition due to verapamil. These findings may indicate an important contribution of extracellular Ca^{2+} to the contractions induced by these SH reagents (Zuazaga et al., 1985; Nakajima et al., 1990; Lizardi et al., 1992; Gonzalez et al., 1993).

A nitric oxide donor, sodium nitroprusside, did not affect the contractions except the ethacyrinic acid-evoked responses. The finding that the contraction in response to ethacrynic acid was inhibited by sodium nitroprusside may indicate that nitric oxide has a modulator action on SH reagent-sensitive Ca^{2+} channel activity (Koivisto and Nedergaard, 1995; Andriambeloson et al., 1999). In a recent study, it was demonstrated that sodium nitroprusside inhibited L-type Ca^{2+} current in glomus cells of the rabbit carotid body (Summers et al., 1999). In this latter study, it is suggested that sodium nitroprusside is acting via a modification of SH groups on Ca^{2+} channel proteins. However, it is difficult to explain why the same agent did not reduce the response to *N*-ethylmaleimide. On the other hand, atropine did not exhibit any significant action on the contractions elicited by *N*-ethylmaleimide and ethacrynic acid. However, the same substance abolished the contractions in response to electrical field stimulation and acetylcholine. These results are consistent with those of the other previous studies demonstrating that the bladder contraction is mediated by both cholinergic and nonadrenergic–noncholinergic mechanisms (Ambache and Zar, 1970; Taira, 1972; Anderson, 1993). Our present findings may indicate that a cholinergic mechanism may not be involved in these SH reagent-evoked contractions in the rat detrusor muscle.

In conclusion, the results of the present study suggest that SH alkylating agents may play a role in the contractile activity of rat detrusor muscle and this action seems to be related to gating of Ca^{2+} channels.

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