The role of extracellular Ca\(^{2+}\) in carbachol-induced tonic contraction of the pig detrusor smooth muscle

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Received: 16 March 1994/Accepted: 25 May 1994

Abstract. The role of extracellular Ca\(^{2+}\) in the tonic-contractile response to muscarinic receptor stimulation was investigated in isolated detrusor smooth muscle from the pig urinary bladder.

Carbachol (10\(^{-8}\)–10\(^{-5}\) M) produced a concentration-dependent contractile response in isolated pig detrusor smooth muscle strips consisting of an initial phasic component followed by a tonic component. During the plateau of the tonic contractions induced by carbachol at the submaximal concentration of 10\(^{-6}\) M, the inhibiting effects of atropine, EGTA, nifedipine (a voltage-dependent calcium channel antagonist), H-7 (a protein kinase C (PKC) inhibitor) and YM934 (a potassium channel opener) on the contractions were evaluated. Atropine (10\(^{-10}\)–3×10\(^{-8}\) M) concentration-dependently inhibited the tonic contractions induced by carbachol. In the same experimental conditions, EGTA (4 mM) and nifedipine (10\(^{-9}\)–3×10\(^{-7}\) M) depressed the tonic contractions in a concentration-dependent manner as did H-7 (10\(^{-5}\)–3×10\(^{-5}\) M) and YM934 (10\(^{-8}\)–10\(^{-6}\) M). However, H-7 (10\(^{-5}\)–3×10\(^{-5}\) M) and YM934 (10\(^{-6}\) M) were very weak in inhibiting the contractions induced by KCl (50 mM) in isolated pig detrusor smooth muscle strips.

These results suggest that the tonic-contractile response induced by carbachol in pig detrusor smooth muscle strips is dependent mainly on depolarization of the cell membranes and an influx of extracellular Ca\(^{2+}\), and also suggest that this depolarizing response may be due to inactivation of ATP-sensitive potassium channels through muscarinic activation of PKC.

Key words: Tonic contraction – Extracellular Ca\(^{2+}\) – Protein kinase C – Depolarization – ATP-sensitive potassium channel – Carbachol – Detrusor smooth muscle

Introduction

In the urinary bladder, muscarinic receptor stimulation causes a biphasic contractile response consisting of an initial phasic component followed by a tonic component (Bhat et al. 1989; Yoshida et al. 1992). Smooth muscle contraction is produced by a rise in the concentration of Ca\(^{2+}\) within the cytoplasm, the origin of which as intracellular or extracellular has yet to be determined (Bolton 1979; Somlyo 1985). Several reports demonstrate that in urinary bladder preparations the contractile response, especially the initial phasic contraction, induced by muscarinic receptor stimulation are not completely abolished by treatment with calcium antagonists or in extracellular Ca\(^{2+}\)-free conditions (Maggi et al. 1989; Lowe and Noronha-Bloq 1991; Elliott et al. 1992), suggesting that this phasic contraction is dependent mainly on intracellular Ca\(^{2+}\). Mostwin (1985) also showed that muscarinic receptor stimulation releases Ca\(^{2+}\) from an intracellular Ca\(^{2+}\)-free store in guinea-pig detrusor smooth muscle cells. On the other hand, little information is available concerning the origin of Ca\(^{2+}\) in the tonic-contractile response induced by muscarinic receptor stimulation in urinary bladder preparations. Recently, Rasmussen and his colleagues (1987) proposed a two-phase model of smooth muscle contraction. In this model, these two phases of contraction are regulated by different Ca\(^{2+}\) dependent pathways: the initial phasic phase is mediated by activation of calmodulin-dependent myosin light-chain kinase (MLCK) and phosphorylation of myosin light chains, and the second tonic phase is linked to activation of protein kinase C (PKC). Furthermore, it is reported that phorbol esters, which are activators of PKC, produce tonic contractions in smooth muscle preparations (Rasmussen et al. 1984; Park and Rasmussen 1985; Yoshida et al. 1992), suggesting that PKC plays an important role in tonic contraction of smooth muscle. The aim of the present study is thus to determine the dependence on extracellular Ca\(^{2+}\) of the tonic-contractile response of pig detrusor smooth muscle induced by muscarinic receptor stimulation, and to investigate...
whether this tonic-contractile response is mediated by activation of PKC.

Methods

Tissue preparations. Urinary bladders were obtained from pigs of either sex which were killed at an abattoir. The urinary bladder was opened by a ventral incision from the urethra to the dome, and the mucosa was removed from the bladder smooth muscle in ice-cold Krebs-Henseleit (K-H) solution (pH 7.3) bubbled with a 95% O2 and 5% CO2 gas mixture. For mechanical recordings, strips of detrusor smooth muscle measuring about 10x2x1 mm were cut from the bladder dome.

Contractile response study. Fine silk ligatures were tied to each end of the detrusor smooth muscle strips, and each strip was then transferred to a 30 ml organ bath containing K-H solution bubbled with a 95% O2 and 5% CO2 gas mixture at 37°C. One end of the strip was attached to a fixed hook in the organ bath and the other end to a force displacement transducer (SB-1T, Nihon Kohden, Tokyo, Japan). The strips were allowed to equilibrate for at least 2 h, during which a resting tension of 0.5 g was applied, and changes in the force of contraction were measured isometrically with the force transducer.

To determine a suitable dose of carbachol, a concentration response curve of carbachol was constructed in a stepwise manner after the response to the previous concentration had reached a plateau. The interval between each concentration varied from 1 to 10 min, depending on the carbachol concentration. A suitable response was obtained with carbachol at 10^-6 M. Strips were then contracted at this concentration; after the plateau was reached, cumulative concentration response curves to the test drugs were constructed in the continuous presence of carbachol.

In separate experiments, contractions were repeatedly elicited with KCl at the high concentration of 50 mM every 30 min until responses were reproducible. The contact period for KCl was 10 min. To evaluate the effects of the PKC inhibitor H-7 and the potassium channel opener YM934 on the KCl-induced contractions, H-7 (10^-5 and 3x10^-5 M) or YM934 (3x10^-2 M) was dissolved in distilled water containing 20% dimethyl sulfoxide and 20% ethanol. Nifedipine (3x10^-5 M) was dissolved in distilled water containing 20% dimethyl sulfoxide and ethanol in the tissue bath was always <0.01%, which in our study had no effect on the carbachol- and KCl-induced contractions. Other drugs were dissolved in distilled water.

A Krebs-Henseleit solution of the following composition was used (mM): NaCl, 121.4; KCl, 5.9; CaCl2, 2.5; MgCl2, 1.2; NaHCO3, 10.0 and glucose, 11.1.

Statistical analysis. Data in the text are expressed as mean±SEM. Statistical analysis was performed by Student’s t-test. A P-value less than 0.05 was considered statistically significant.

Results

Contractile response study

Carbachol (10^-8 – 10^-5 M) produced a concentration-dependent contractile response in isolated pig detrusor smooth muscle strips consisting of an initial phasic component followed by a slow tonic component. The mean tension of the phasic and tonic components recorded with carbachol at the submaximal dose of 10^-6 M was 6.47±0.85 g and 2.61±0.54 g, respectively. The tonic contraction was maintained over 2 h after the tonic contraction had reached a plateau (Fig. 1a). Inhibitory effects

Drugs and drug solutions. The following drugs were used: carbachol chloride (carbachol) and nifedipine (Sigma Chemical Co., St Louis, Mo., USA), atropine sulphate (Tanabe Pharmaceutical Co., Ltd., Tokyo, Japan), YM934: 2-(3,4-dihydro-2,2-dimethyl-6-nitro-2H-1,4-benzoxazin-4-yl)pyridine N-oxide (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan), H-7: 1-(5-isoquinolinesulfonyl)-2-methylpipеразин дидроксид (Segusakuy Co., Tokyo, Japan) and EGTA: ethyleneglycol bis (β-aminoethyl ether)-N,N'-tetraacetic acid (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). YM934 (3x10^-2 M) was dissolved in distilled water containing 20% dimethyl sulfoxide and 20% ethanol. Nifedipine (3x10^-5 M) was dissolved in distilled water containing 20% ethanol. The final concentration of dimethyl sulfoxide and ethanol in the tissue bath was always <0.01%, which in our study had no effect on the carbachol- and KCl-induced contractions. Other drugs were dissolved in distilled water.

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