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G-Proteins in alpha$_1$-adrenoceptor mediated prostatic smooth muscle contraction

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Abstract The role of signal transducing guanine-nucleotide binding proteins (G-proteins) in α_1-receptor mediated smooth muscle contractions was investigated in human hyperplastic prostatic tissue. The selective α_1-receptor agonist phenylephrine (PE) evoked dose dependent contractions antagonized by the α_1-receptor blockers prazosin (EC_{50} 10 nM) and YM 617 (EC_{50} 3 nM). Application of nifedipine (1-10,000 nM), a blocker of voltage-dependent L-type Ca^{2+}-channels (VDCC), inhibited the PE evoked contraction up to 65.4%. Pretreating the tissue strips with pertussis toxin (PTX, exotoxin from Bordetella pertussis; 5-25 µg/ml), inactivating a subpopulation of G-proteins, inhibited the PE induced contractions up to 73.9%. PTX pretreatment had no effect on contractions elicited by 125 mM K^+. Application of nifedipine to PTX pretreated tissue led to an additional inhibition of 13.7%. Our findings demonstrate the involvement of PTX-sensitive G-proteins in the signal transduction pathway of α_1-receptor induced contractions of prostatic smooth muscle. The remaining contractility of PTX pretreated tissue suggests additional participation of PTX insensitive mechanisms in α_1-receptor mediated prostatic smooth muscle contractions.

Key words BPH · α_1-adrenoceptor · pertussis toxin · G-proteins

Intravesical obstruction in symptomatic benign prostatic hypertrophy (BPH) is composed of two different factors [5]. A static component due to mechanical obstruction of the enlarged prostatic gland and a dynamic component related to α_1-adrenoceptor (α_1-receptor) mediated increased smooth muscle tone [9]. This has been confirmed by in vitro isometric contraction studies [9] and by radioligand binding studies identifying α_1-receptors [8] which were recently further classified by detecting mRNA transcripts of the α_{1C}-receptor subtype [18]. Signal transduction and intracellular mechanisms of the α_1-adrenergic mediated smooth muscle contraction are still unclear. Smooth muscle contractility is generally regulated by changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) composing of Ca^{2+} influx through VDCC and the release of Ca^{2+} from intracellular stores [3]. First messengers (hormones, neurotransmitters, biogenic mediators) modulate the activity of VDCC and intracellular Ca^{2+} stores in different tissue by increasing second messenger concentrations such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP) or inositol 1,4,5-trisphosphate (IP_3) [12]. Enzymes generating these second messengers are under the control of signal transducing G-proteins [1]. G-proteins are heterotrimeric (α-, β-, γ-subunits), membrane associated protein complexes and couple plasma membrane receptors to intracellular effector systems. The binding of guanine triphosphate (GTP) to the α-subunit of a G-protein is a major stimulus for the complex dissociation critical for the biological activity of the G-protein [4]. Pertussis toxin (PTX, islet activating protein), a bacterial exotoxin from Bordetella pertussis catalyzes the transfer of the adenosine 5′-diphosphate (ADP) ribose moiety of nicotinamide adenine dinucleotide (NAD) to the α-subunit of G-proteins of the G_i, G_0 or G_t family. This ADP-ribosylation uncouples the G-protein from its membrane receptor irreversibly and thus inactivates biological responses dependent on that receptor mechanism [22]. Thus PTX has been demonstrated to be a useful tool in identifying the role of G-proteins in receptor mediated cellular mechanisms.

The purpose of the present study was to investigate the involvement of PTX-sensitive G-proteins in the signal transduction of α_1-receptor mediated contractions in human prostatic tissue.

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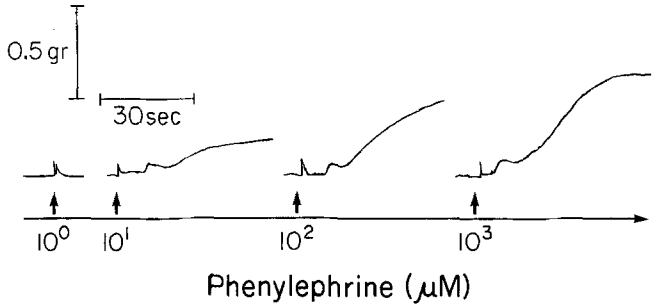

Fig. 1 Original recordings of contractions in human prostatic muscle strips induced by increasing PE concentrations. Arrows indicate the administration of PE

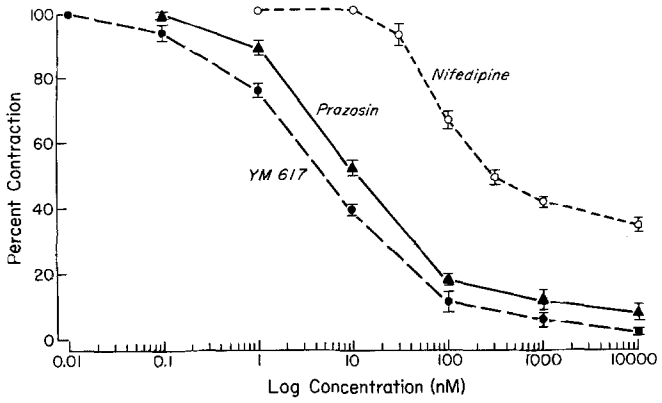

Fig. 2 Inhibitory effects of YM 617, prazosin and nifedipine on contractions of hyperplastic prostate tissue evoked by 100 μM PE. Logarithm of drug concentration (nM) is plotted versus percentage of control contraction. Points indicate means, vertical bars ± SEM

Materials and methods

Prostatic tissue was obtained from patients undergoing transurethral resection of the prostate (TURP) for symptomatic BPH. The tissue was removed as a large chip from the bladder neck down to the veru montanum at the 6 o'clock position in the central zone [16]. It was placed immediately in Reznikoff solution (Ham's F-12 nutrient mix with *L*-glutamine, $NaHCO_3$ 25 mM, penicillin/streptomycin 100 units/ml, 4 °C) and dissected free of surrounding tissue. Tissue strips (10 × 2 × 2 mm) were prepared and transferred to a 10 ml perfusion bath containing Krebs solution (in mM): NaCl 118, KCl 4.6, $CaCl_2$, $NaHCO_3$ 24, $MgSO_4$ 1.2, KH_2PO_4 1.6, glucose 11 (aerated with 5% CO_2, 95% O_2 37 °C, pH 7.4) and mounted individually under a resting tension of 0.5 g. High-potassium solution consisted of (in mM): KCL 123, $CaCl_2$ 1.2, $NaHCO_3$ 24, $MgSO_4$ 1.2, KH_2PO_4 1.6, glucose 11. Equilibration was allowed for 1 h, with the resting tension being maintained. In all experiments isometric contraciton of the tissue was registered on a Grass FTO3C force displacement transducer and recorded on a Grass 7*D* polygraph. Concentration response to agonist curves were obtained non-cumulatively, e.g. the agonist was washed out immediately after the maximum contraction was obtained. Several washes were continued over 30 min before increasing the agonist dose. The maximum contraction in the individual strip obtained was considered to be 100%. For studying the α_1-antagonists (α-blockers) and nifedipine the tissue was incubated in the organ bath with increasing concentrations for 15 min whereas tissue was exposed for 120 min at 37 °C in Krebs solution containing 5 or μg/ml PTX followed by exposure to 100 μg/ml PE. The contractile response was calculated as the percentage of the control contraction (100 μM PE).

PTX (SIGMA CO.), L-phenylephrine hydrochloride (SIGMA CO.), prazosin (SIGMA CO.), YM 617 (YAMANOUCHI PHARMACEUTICAL CO.) and nifedipine (SIGMA CO.) were prepared as stock solutions and diluted in the perfusion chambers in the respective concentrations. Data were expressed as mean ± SEM. Paired student's *t*-test was used when comparing the means of two groups ($P < 0.05$).

Results

Effect of PE and potassium

Figure 1 shows original tracings of PE induced prostatic tissue contractions. Application of agonist evoked smooth muscle contraction in a dose dependent manner whereas the maximum contraction was considered as 100% in each individual tissue strip. Submicromolar concentrations did not elicit any response ($n = 12$), whereas maximum contractions were obtained with millimolar PE concentrations ($n = 6$). The mean threshold concentration for PE was 3 μM (2.5 ± 0.7%, $n = 17$), the EC_{50} value was 30 μM (54.2 ± 3.9%, $n = 16$) and with 1 mM PE a mean contraction of 96.4 ± 1.5% ($n = 17$) was obtained. Exposure to 123 mM K^+ containing solution led to a sustained, reversible contraction ($n = 6$) which was 88.7 ± 4.3% ($n = 12$) of the contraction obtained with maximum PE concentrations (not shown).

Effect of α_1-blocker

Prazosin, a selective α-blocker reversibly inhibited the PE induced contractions in a dose dependent fashion (1-10,000 nM). 10 μM prazosin reduced PE elicited contraction to 5.2 ± 2.7% ($n = 7$), 50% reduction was obtained with 10 nM (52 ± 2.3%, $n = 9$). Application of 0.1 nM prazosin had no effect on PE induced smooth muscle contraction. The potent α-blocker YM 617 fully blocked α_1-mediated contraction at a concentration of 10 μM (2 ± 1%, $n = 7$). The EC_{50} value was in the range of 3 nM (51.7 ± 2.5%, $n = 8$). Fig. 2 summarizes the α_1-receptor blocking effects of prazosin and YM 617 in dose response curves.

Nifedipine effect

Nifedipine (0.01–10 μM), a blocker of VDCC, was used to study the contribution of gated Ca^{2+}-entry through VDCC to contractions induced by PE (100 μM). 10 nM nifedipine had no effect on PE induced contraction, whereas 0.3 μM inhibited the PE response by 49.2 ± 2.1% ($n = 7$). A saturating concentration of 10 μM nifedipine [7] inhibited the contractions by 65.4 ± 2.1% ($n = 5$).

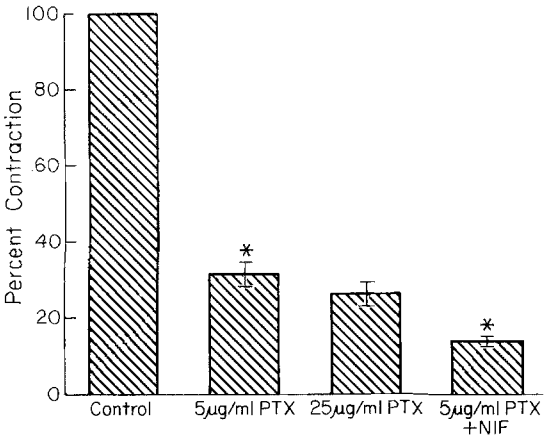

Fig. 3 Effect of pertussis toxin (*PTX*) in different concentrations on contractions evoked by 100 µM PE (*control*). Application of 10 µM nifedipine (*NIF*) further decreased the contraction. Vertical bars represent ± SEM; * $P < 0.05$

Higher nifedipine concentrations did not further inhibit the contractions. Fig. 2 shows the inhibition of PE induced contractions as a function of logarithmic nifedipine concentrations.

Effect of PTX

The inhibitory effect of PTX is summarized in Fig. 3. Incubating prostatic tissue with PTX (5 µg/ml) for 120 min reduced the PE induced contraction by 68.7 ± 3.2% ($n = 12$). Five times higher concentrations of PTX showed no significant further inhibition ($P > 0.1$, 71.8 ± 3.1%, $n = 8$). After a period of 60 min washout the initial PE effect could not be obtained. There was no inhibitory effect on the control strips pretreated in a PTX-free solution (98 ± 1.8%, $n = 7$). Nifedipine (10 µM) inhibited the remaining response after PTX pretreatment to 17.6 ± 1.2% ($n = 8$). Contractions induced by high concentrations of K^+, were unaffected in PTX treated strips (95.7 ± 3.3%, $n = 4$, of the control contraction).

Discussion

Our results provide evidence for the involvement of PTX-sensitive G-proteins in α_1-receptor mediated prostatic smooth muscle contraction. The inhibitory effect of PTX suggests that PTX-sensitve G-proteins transduce α_1-receptor mediated excitation. A nonspecific effect of PTX on Ca^{2+}-influx through VDCC or on the contractile apparatus was excluded by the fact that receptor independent contractions elicited with potassium were not affected by the toxin. Furthermore, PTX insensitive mechanisms seem to be involved in PE induced contraction since PTX blocked only 73% of the receptor mediated contraction.

PE, a selective α_1-receptor agonist elicited dose dependent contractions in hyperplastic prostatic tissue. This is in accordance with other reports identifying a substantial density and emphasizing the importance of α_1-receptors in the human prostate adenoma [6, 10, 15]. The functional role of this receptor in neurotransmission could be demonstrated by in vitro contraction experiments [9, 11] and radioligand receptor binding studies [8, 15]. To define the specificity of the prostatic receptor we applied prazosin and YM 617 as highly selective postsynaptic α_1-receptor blockers showing dose dependent reduction of PE induced responses (Fig. 2). This is in agreement with clinical studies demonstrating beneficial effects of α-blockers in symptomatic BPH [14].

G-proteins could be transducers in α_1-receptor induced elevation of $[Ca^{2+}]_i$ and subsequent contraction [1]. PTX is known to irreversibly inactivate G-proteins by ADP-ribosylation and was used in different tissue models [17, 22]. The consequence of this ADP-ribosylation is a general attenuation of the physiological reaction mediated by the G-protein and its associated receptor. We examined the effects of PTX in human hyperplastic prostatic tissue. PTX-pretreatment inhibited α_1-receptor induced contraction by 73%. A concentration of 5 µg/ml PTX which demonstrated good tissue penetration in other reports [2, 13] seemed to be sufficient to block G-proteins modulating α_1-receptor mediated contraction (68.7%) in our study. A concentration up to 25 µg/ml had no significant inhibitory effect (73.9%, Fig. 3).

VDCC blockage by nifedipine diminished α_1-receptor induced responses less than the toxin treated tissue. Thus PTX pretreatment may not only prevent Ca^{2+}-influx through VDCC but also may influence intracellular Ca^{2+} release mechanisms. This is supported by the fact that application of nifedipine to PTX pretreated strips led to an additional inhibition of 13.7%. These findings suggest a contribution of intracellular Ca^{2+} stores for α_1-receptor mediated contraction in the prostate and indicate that PTX-sensitive G-proteins regulate both the open probability of VDCC and intracellular Ca^{2+} release mechanisms. However, other reports suggest α_1-receptors coupling to two different G-proteins, one PTX-insensitive G-protein regulating the release of intracellular Ca^{2+} and another PTX-sensitive G-protein influencing VDCC function [21].

Application of high potassium containing solution depolarizes the smooth muscle cells by opening of VDCC. The resulting Ca^{2+} influx triggers the contraction itself. This effect is highly dependent on extracellular Ca^{2+} [19] and can be abolished by VDCC blockers. This is in accordance with our previous data (not demonstrated) which show that KCl induced contractions are fully inhibited by 10 µM nifedipine. Application of high potassium solution of PTX-treated tissue still elicited a contraction which was 95.7% of the contraction in PTX-untreated strips, indicating that PTX selectively inhibits G-proteins and leaves functional VDCC and contractile properties intact.

At least 15 different G-proteins are identified biochemically [1, 20]. G-proteins of the G_i-, G_o, and G_t-family are PTX-sensitive [22] and are suggested to couple membrane receptors to intracellular effector systems by directly interacting with VDCC or via second messengers [4]. However, in the present study no differentiation of G-protein subtypes on the basis of PTX treatment could be performed.

Our study demonstrates that PTX-sensitive signal coupling G-Proteins regulate α_1-receptor mediated contraction in the human hyperplastic prostate. Further studies are needed to elucidate the participation of non-PTX-sensitive G-proteins and second messengers involved in this tissue.

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