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Role of intracellular Ca^{2+} stores in smooth muscle of human penile erectile tissue

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Abstract *Objective*: In human erectile tissue smooth muscle contraction and detumescence are highly dependent on an increase in cytosolic [Ca^{2+}]. The Ca^{2+} influx can be derived from the extracellular space or from intracellular sarcoplasmic stores. The role of both pathways was evaluated in an organ bath study on human cavernosal strips. *Patients and methods*: The tissue was obtained from 12 patients with chronic erectile dysfunction. The effects of Ca^{2+}-free solution, ryanodine, caffeine and of nifedipine on electrically and adrenergically induced contractions were evaluated. *Results*: Following an incubation period of 10 min in Ca^{2+}-free solution the electrically induced contraction was reduced to 20%, whereas the contraction induced by phenylephrine (PE) was only reduced to 64 ± 6% (mean ± SEM). Ryanodine inhibited the PE-contraction to 30 ± 6% and the additional application of caffeine or nifedipine further reduced the contraction to 11% and 8%. *Conclusion*: The results give evidence for a role of intracellular Ca^{2+}-stores in human cavernosal tissue. Whether the more marked effect of ryanodine in tissue from patients with erectile failure in comparison with similar experiments in rabbit cavernosal tissue might be a sign of an increased cavernosal contractility in these patients remains to be shown in future experiments with normal erectile tissue.

Key words Penile erection · Human corpus cavernosum smooth muscle · Calcium channel blocker · Intracellular calcium

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Introduction

Elevated smooth muscle tonus in human corpus cavernosum tissue is considered to be a possible cause for chronic erectile failure [10]. The presynaptic release of norepinephrine leads to a rise of free cytoplasmic Ca^{2+}, thus inducing smooth muscle contraction and detumenescence [12]. It has been shown that contraction in human erectile tissue is highly dependent on the concentration of extracellular Ca^{2+} [6, 17]. However, electrically and norepinephrine-induced contractions could not be abolished completely by blockade of the extracellular influx using the Ca^{2+}-channel blocker nifedipine in vitro [6, 8]. These observations suggest a role of other Ca^{2+} sources in human cavernosal tissue. On one hand, the plasmalemma with voltage-gated and chemically gated receptor operated Ca^{2+} channels is responsible for the regulation of intracellular Ca^{2+} concentration. The sarcoplasmic reticulum, on the other hand, has been identified as the main intracellular Ca^{2+} store in vascular smooth muscle [2, 4]. Moreover, two seperate types of Ca^{2+} channels exist in the sarcolemmal membrane: Ca^{2+}-activated channels and inositol 1,4,5-triphosphate-activated channels [1, 14]. The exact role of these intracellular Ca^{2+}-stores and the mechanisms of their regulation in the human cavernosal tissue are not yet clear.

Therefore it seemed of interest to investigate the influence of changes of Ca^{2+} availability in human cavernosal tissue. The second aim of the study was to elucidate the role of intracellular Ca^{2+} stores for smooth muscle contraction in human erectile tissue.

Materials and methods

Tissue preparation

Human cavernosal tissue was taken at the time of implantation of a penile prosthesis from 12 patients with chronic erectile failure. The etiological background for erectile dysfunction in these patients was as follows: general arteriosclerosis ($n = 2$), diabetes mellitus

($n = 3$), severe Peyronie's disease ($n = 3$), radical prostatectomy ($n = 2$), transurethral resection of the prostate ($n = 1$), multiple surgery for bladder exstrophy ($n = 1$). Since these patients represent a very heterogenous etiological pattern with small numbers for each group no subgroups were considered for further evaluation of the experimental results.

A total of 55 tissue strips were prepared, with a mean of 4.6 strips per patient. The tissue was cut into pieces of $1 \times 2 \times 5$ mm, armed with a silk tie and mounted between two metal hooks in a Schuler organ bath chamber containing 10 ml of a Krebs solution.

Tension measurements

Tension was maintained by an adjustable connection to a Grass force-displacement transducer FT03 (Grass Instruments, Quincy, Mass.) for measuring isometric tension. The impulses were amplified by means of a pre-amplifier (Grass Low Level DC Pre-Amplifier Model 7PI) and an amplifier (Grass DC Driver Amplifier Model 7DA) and transferred to the recording unit. A Grass 7D polygraph was used for recording. Tension of the preparations was adjusted to 5 mN for the erectile tissue and equilibration was allowed for 1 h. After the equilibration period a contraction of each tissue strip was provoked by adding high K^+ solution (124 mmol/l) in order to control the contractile capacity. One strip from each cavernous tissue always served as control.

Drugs and solutions

Krebs solution was composed as follows: NaCl 118 mmol/l, $NaHCO_3$ 24 mmol/l, KCl 4.6 mmol/l, KH_2PO_4 1.6 mmol/l, $CaCl_2$ 1.2 mmol/l, $MgSO_4$ 1.2 mmol/l and glucose 11 mmol/l. The solution was aerated with 5% CO_2 and 95% O_2, maintaining pH at 7.4. A thermoregulated water circuit maintained the temperature at 37°C. Isotonic high-K^+ solution (124 mmol/l) was prepared by replacing the NaCl in the normal Krebs solution by equimolar amounts of KCl. Ca^{2+}-free solution contained 0.1 mml/l ethylene glycol-bis (β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA, Sigma, St louis, Mo.) and $CaCl_2$ was omitted.

The following drugs were used: phenylephrine (Sigma), nifedipine (Sigma), caffeine (Sigma) and ryanodine (ICN, Montreal, Canada). Stock solutions and subsequent dilutions in Krebs solution were prepared daily for phenylephrine (PE) and caffeine. A stock solution of nifedipine and ryanodine (10^{-2} mol/l) was prepared with ethanol and further dilutions were performed in distilled water. In separate experiments as well as in the permanent controls ethanol showed no effects on the tissue. Direct exposure of nifedipine to light was avoided.

Phenylephrine stimulation

Initially a PE dose relation to K^+ (124 mmol/l)-induced contraction was provided. PE was added cumulatively (10^{-3}–10^{-4} mol/l) to the tissue after each level of contraction had stabilized. For all subsequent experiments a PE concentration of 10^{-4} mol/l was applied and the results were compared with the pretreatment PE response. Incubations with nifedipine, ryanodine and caffeine were carried out 15–20 min before application of PE.

Transmural electrical stimulation

For transmural electrical stimulation the tissue strips were mounted between two parallel platinum electrodes (6 mm apart). A Grass S44 stimulator and a current amplifier were used. Square waves (20 V, duration 0.8 ms, stimulation interval 120 s) were provided in trains of 5 s at 20 Hz. The amplitude of electrically induced contractions following treatment with Ca^{2+}-free solution was compared with the average amplitude of three pretreatment contractions, which was considered as 100%.

Statistical analysis

Results are expressed as mean value ± standard error of the mean. Analysis of variance was applied to compare responses and $P < 0.05$ was considered significant.

Results

Effects of transmural electrical stimulation

Transmural electrical stimulation of tissue strips at baseline tensions produced a frequency-dependent contractile response. Between 10 and 40 Hz a strong increase in the contractile force was found, beyond 80 Hz no further increase could be achieved ($n = 7$). At 20 Hz reproducible contractions of 60% ± 8% of the maximum response were seen (Fig. 1).

Ca^{2+}-free solution

Incubation of the tissue strips in Ca^{2+}-free solution reduced the electrically induced contractions depending on time ($n = 5$). However, after 15 min a contraction of 20% of the preincubation value could still be elicited (Fig. 2).

PE (10^{-4} mol/l)-induced contractions were also significantly inhibited after incubation in Ca^{2+}-free solution ($n = 5$). After an incubation period of 10 min, however, a remaining contraction of 64% ± 6% was still found (Fig. 3).

Ryanodine, caffeine, nifedipine

Preincubation for 20 min with 10^{-4} mol/l ryanodine reduced the following PE-induced contraction to 30% ± 6% of the preincubation value ($n = 4$). This means a statistically significant difference also in comparison to the inhibition caused by Ca^{2+}-free solution ($P < 0.05$ variance analysis). Preincubation with

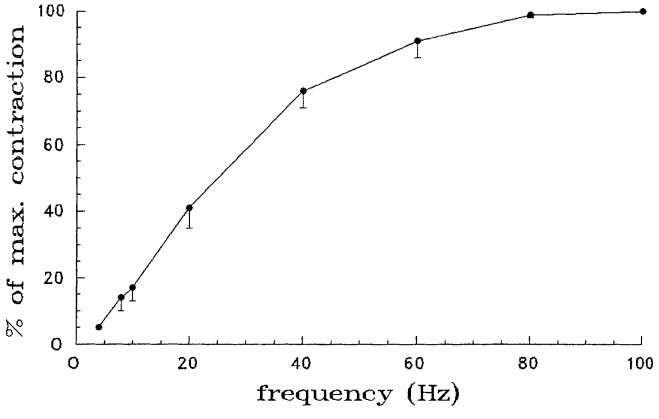

Fig. 1. Frequency-dependent change of contraction force in human erectile tissue

ryanodine and additional administration of caffeine (10^{-3} mol/l) further reduced the PE-induced contraction to 11% ± 0.3% ($n = 3$) (Fig. 3). In three strips the same experiment was repeated in the presence of nifedipine (10^{-5} mol/l), but only a slight further reduction to 8% ± 1% was achieved ($n = 3$, Fig. 3). Incubation of the tissue with ryanodine and nifedipine (10^{-5} mol/l, $n = 3$), however, lead to a marked reduction in contraction force to 10% ± 3% of the pretreatment value (Figs. 3 and 4).

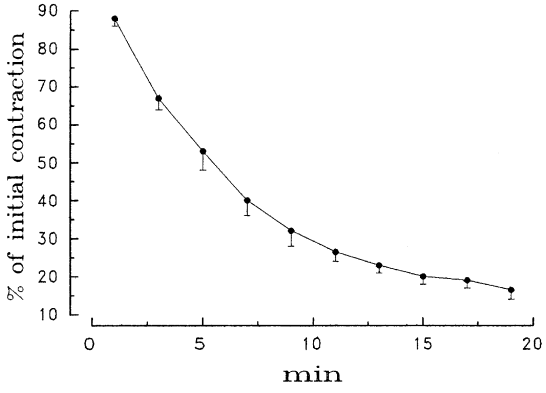

Fig. 2. Time-dependent reduction of electrically induced contraction force of human corpus cavernosal tissue after incubation in Ca^{2+}-free solution

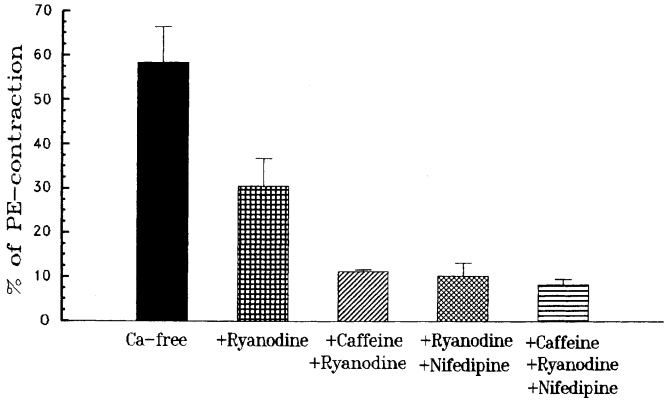

Fig. 3. Reduction of phenylephrine (PE) (10^{-4} mol/l)-induced contraction after incubation with Ca^{2+}-free solution, Ryanodine (10^{-4} mol/l), ryanodine and caffeine (10^{-3} mol/l), ryanodine and nifedipine (10^{-5} mol/l) and ryanodine, caffeine and nifedipine

Discussion

The important role of the sarcoplasmic reticulum as an intracellular Ca^{2+}-store has been identified in vascular smooth muscle cells [4]. However, in human penile erectile tissue the significance of these stores for the erectile mechanisms remains to be clarified.

As a limitating factor of the results presented here, it must be considered that tissue was taken from patients with chronic erectile failure. Cellular Ca^{2-} regulation of these cavernosal smooth muscle cells may possibly be altered due to the underlying disease. Thus all results can only be regarded as specific for patients with chronic erectile failure and cannot simply be transferred to normal cavernosal tissue.

At the cellular level an increase of free cytoplasmic Ca^{2+}, which depends partly on Ca^{2+} influx from the extracellular space, is responsible for cavernosal smooth muscle contraction [6, 8, 17]. However, different studies, give evidence that intracellular Ca^{2+} stores may also play an important role in smooth muscle contraction of human erectile tissue since blockade of the extracellular Ca^{2+} influx by nifedipine could not abolish electrically or PE-induced contractions [6, 8]. Furthermore, following incubation in Ca^{2+}-free solution smooth muscle contractions of human cavernosal tissue could still be induced [6, 17]. In an extensive study with various Ca^{2+} channel blockers such as verapamil and nifedipine, Kerfoot et al. [8] could demonstrate that all of these substances were effective in inhibiting electrically induced contractions and relaxed norepinephrine-induced contractions. Their results show clearly that Ca^{2+} channel blockers are capable of inhibiting the neuronal signals which lead to the cavernosal smooth muscle tone, and furthermore can suppress the adrenergically induced tone. The resulting cavernosal relaxation may possibly lead to penile tumenescence and erection.

The results of the present study confirm these previous findings. After incubation in nominally Ca^{2+}-free solution both electrical and α_1-adrenergic receptor stimulation with the α_1-adrenoceptor agonist PE still provoked contractions of human cavernosal smooth muscle tissue strips which decreased with time. PE stimulation elicited markedly stronger contractions after 10 min than did electrical stimulation. A possible explanation may be that the application of repeated

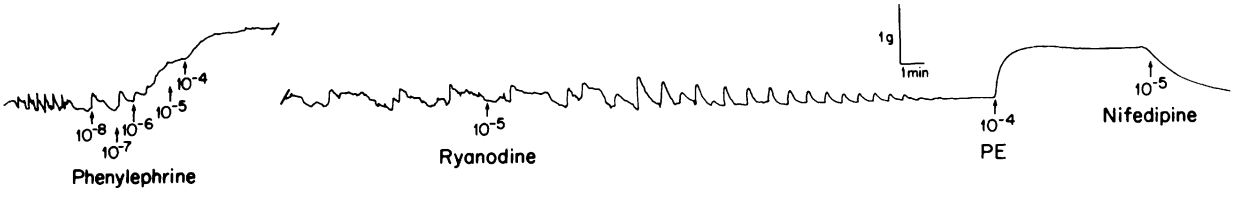

Fig. 4. Original drawing, showing a dose response curve with PE. Following wash-out the tissue is incubated with ryanodine, which abolishes the spontaneous contractions and diminishes the following PE response. Further addition of nifedipine relaxes the tissue close to the baseline

electrical stimuli leads to a quicker cytoplasmic Ca^{2+} depletion. Comparable results could be achieved in cavernosal tissue of New Zealand white rabbits in previous experiments [15].

To investigate intracellular Ca^{2+}-stores, ryanodine, a natural plant alkaloid, is a useful tool [3, 13, 16]. Ryanodine selectively depletes Ca^{2+} from the sarcoplasmic reticulum without affecting plasma membrane Ca^{2+} channels or the contractile apparatus [13]. The effect of ryanodine can be described as stimulation of Ca^{2+} leakage from the intracellular stores so that the drug acts as a functional blocker of intracellular Ca^{2+} stores due to Ca^{2+} depletion [7].

In the present investigation preincubation with ryanodine reduced the subsequent PE-induced contraction to a third of the preincubation value, which is also a statistically significant difference in comparison with the effects of Ca^{2+}-free solution. This gives further evidence for the importance of ryanodine–sensitive intracellular Ca^{2+} stores in human erectile tissue smooth muscle cells.

In a previous study the effect of ryanodine on cavernosal tissue of New Zealand white rabbits was investigated [15]. The inhibiton of the contraction there was markedly less than in human tissue since in the rabbit studies the PE–induced contraction was only reduced to 70% of the pretreatment value. A higher activity or sensitivity of the intracellular ryanodine-sensitive Ca^{2+} store in tissue of patients with chronic erectile failure may therefore be possible. In vascular smooth muscle of patients with arteriosclerosis an increased expression of the inositol 1,4,5-triphosphate receptor has already been found [11], which might lead to an increased sensitivity of intracellular Ca^{2+} stores, thus elevating vascular muscle tone. However, direct comparison of cavernosal tissue from erectile-healthy patients and patients with chronic erectile failure remains to be done.

Further evidence for a role of intracellular Ca^{2+} stores in human cavernosal smooth muscle derives from the experiments with caffeine and nifedipine. Pretreatment of tissue strips with caffeine and ryanodine further significantly reduced PE-induced human cavernosal smooth muscle contractions. Caffeine acts directly on the sarcoplasmic reticulum, increasing the Ca^{2+}-induced Ca^{2+} leakage without releasing Ca^{2+} from any other cell organelle [4]. Thus both substances, ryanodine and caffeine, act in the same direction in depleting Ca^{2+} from intracellular Ca^{2+} stores.

Similar to the results achieved with the combination of ryanodine and caffeine, the combination of ryanodine with nifedipine leads to a significant reduction of PE-induced contractions. Nifidepine, a specific L-type blocker of plasmalemmal voltage-gated Ca^{2+} channels, inhibits Ca^{2+} influx from the extracellular space in the concentration used in this experiment [9]. The potency of nifedipine and other Ca^{2+} channel blockers to inhibit electrically and adrenergically induced contractions has already been shown [8]. These observations and our results re-emphasize the significance of extracellular and intracellular Ca^{2+} stores in human cavernosal smooth muscle contraction.

Taking all results into consideration one should expect an almost complete abolition of PE-induced contraction if all of the three agents – ryanodine, caffeine and nifedipine – are combined. Previous studies on New Zealand white rabbits, in which this combination of drugs was applied, showed an almost complete inhibition of PE-induced contractions, which is consistent with this expectation [15]. In this investigation, however, a small contractile response to PE was still seen.

An intact erection depends strongly on the fine balance between contractile and relaxing influences on cavernosal smooth muscles. A slight increase in contractility may lead to a disproportionally larger reduction in relaxation [15]. Therefore, minimal changes in intracellular Ca^{2+} stores might be responsible for the development of erectile failure. Evidence for a role of intracellular ryanodine-sensitive Ca^{2+} store in patients with chronic erectile failure is provided by the markedly increased inhibition of smooth muscle contraction by ryanodine in these patients.

These data confirm the well-known importance of extracellular Ca^{2+} stores for penile smooth muscle contraction and give further evidence for the presence and a significance of intracellular Ca^{2+} stores. However, comparative studies of intracellular Ca^{2+} stores in patients with and without chronic erectile failure have yet to be performed.

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