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THE EFFECT OF INTRA CELLULAR CALCIUM STORE INHIBITORS ON THE RATE OF FORCE DEVELOPMENT IN SMOOTH MUSCLE OF THE URINARY BLADDER.

Aims of Study

Previous research suggested a role of intra cellular calcium stores in the development of instable detrusor contractions. In order to determine the relevance of pathways in force development, in which intracellular stores are involved, the role of the calcium store inhibitors: Xestospongine C, Ryanodine and Thapsigargine was investigated.

Methods

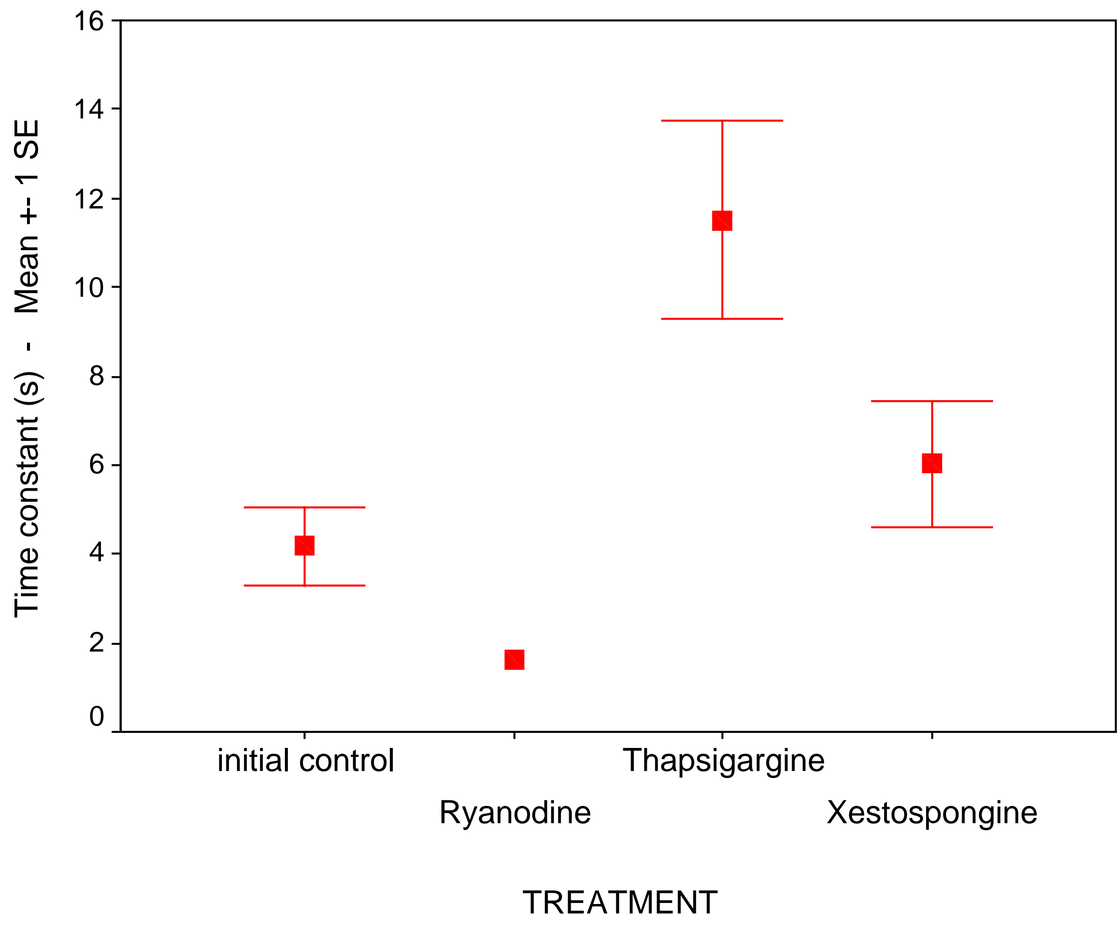
Urinary bladder muscle strips from pigs were mounted in a custom made organ bath and incubated for 15 minutes in Krebs solution. Before and after a treatment with Xestospongine C (4µM), Ryanodine (80µM) and Thapsigargine (50µM) respectively the strips were triggered with electric field stimulation (EFS) or acetylcholine (Ach40µM). Xestospongine C inhibits the IP3 (Inositol-tri-phosphate) mediated calcium release. Ryanodine blocks the ryanodine channel at the level of the sarcoplasmic reticulum membrane, the receptor responsible for the "calcium induced calcium release". Thapsigargine inhibits the endoplasmic reticular Ca^{2+}-ATPase and the IP3 mediated pathway. Three strips were incubated with Xestospongin C, three with Ryanodine and three with Thapsigargine. Two control strips were incubated with Krebs and underwent the same stimulation protocol. We calculated the maximum developed force (Fiso) and the rate of force development (time constant C). All calculations were processed in Matlab® 12.1. Analysis of variance and a paired t-test were used for statistical analysis.

Results

Force development was reduced in every muscle strip after each treatment with an inhibitor. EFS led to 35% of the initial force development in Xestospongine C, 4.7% in Ryanodine and 4.7% in Thapsigargine. After stimulation with Ach a similar effect was seen. Xestospongine C showed a reduction of force development to 17.6% of the initial force. Ryanodine to 2.8% and Thapsigargine to 12.38%. Analysis of variance showed that treatment was a significant source of variance in isometric force Fiso but also in timeconstant C. The force development rate during EFS became slower: in Xestospongine C treatment the rate reduced to 36% and in Thapsigargine to a significant 67% (p=0.016) of the initial rate. (see Fig 1) . When the responses in Xestospongine C and Thapsigargine are grouped together for both EFS and Ach stimulation a significant decrease in the mean rate of force development was seen (p=0.001 and p=0.02 respectively). In Ryanodine treatment only one strip was analysable. In this strip there was no change in the rate of force development which is in accordance with previous research.

Conclusions

Inhibition of the IP3 mediated intracellular pathway with Xestospongine C and Thapsigargine led to a decreased amplitude and rate of force development. When combining these findings with previous findings where Ryanodine was used, stimulation via the IP3 pathway appears to induce a faster force development than when the "Calcium induced calcium release" pathway is followed. Analysis of the rate of force development appears to be a useful tool for analysis of intracellular excitatory pathways. The understanding of the intracellular excitatory mechanisms and their time course might generate new ideas on the treatment of detrusor muscle overactivity.



Time constant (s) - Mean +- 1 SE
16
14
12
10
8
6
4
2
0
initial control
Ryanodine
Thapsigargine
Xestospongine
TREATMENT