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Dambros M[1], van Koeveringe G[1], de Jongh R[1], Bast A[1], van Kerrebroeck P[1]
1. University Hospital Maastricht

A LOW CONCENTRATION OF THE FLAVONOID AVOIDS THE PROGRESSIVE DECREASE OF BLADDER SMOOTH MUSCLE CONTRACTILITY INDUCED BY REPETITIVE FIELD STIMULATION.

Hypothesis / aims of study

There is increasing evidence that the generation of free radicals plays a role in the development of bladder dysfunction [1]. Studies have demonstrated that repetitive field stimulation (RFS) of smooth muscle strips isolated from the urinary bladder can be used to exhaust synaptic stores of acetylcholine and to produce direct neuronal damage [2]. Furthermore, RFS results in a significant increase in the accumulation of products of peroxidation within the smooth muscle membrane components which could be another cause of bladder dysfunction [2]. Flavonoids are a group of polyphenolic compounds and have recently gained tremendous interests, due to their broad pharmacological activity. The pharmacological effect can be explained by their inhibition of certain enzymes and their antioxidant activity. To the best of our knowledge, this is the first time, the protective effects of the flavonoid Galangin, during RFS, on the progressive decrease of bladder smooth muscle contratile responses, are demonstrated.

Study design, materials and methods

Male pig detrusor strips were mounted between stainless steel hooks in 20ml organ baths containing Krebs-buffer solution which was aerated continuously with 5% CO_2 – 95% O_2, at 37^0C. Mechanical responses were recorded using an isometric force transducer. Measurements were started after an equilibration period of 60 min with an initial tension of 2g. After an initial equilibration time, all strips were stimulated at 32Hz, and subjected to a metacholine concentration curve (MCC), then the incubation medium was changed to one with the following additions for each of 6 experimental groups: all tissues in group 1 were incubated with normal krebs; those in group 2 with Galangin (0,1µM); group 3 with Galangin (0,1µM) + Propranolol (1µM); group 4 with Galangin (0,1µM) + Verapamil (0,1µM); group 5 with Galangin (0,1µM) + Phentolamine (1µM) and those in group 6 with Galangin (0,1µM) + Atropine (1µM). All strips were equilibrated in these buffers for 30 minutes then stimulated with Electrical field stimulation (EFS) at 32Hz and a MCC was performed. Six strips from each group were stimulated for 1,5 hours at 32Hz for 15 seconds every 5 minutes. At the end of the period of RFS, all strips were stimulated at 32Hz and a MCC was perfomed. After that, all strips were washed 4 times with fresh buffer (containing the group-specific additions described above) and all groups were subjected to 1,5 hours of incubation (recovery period); responses to field stimulation at 32Hz and the MCC were re-assessed. Student's t-test and one factor ANOVA were used to determine the statistical significance to 0.05 levels.

Results

One and half hours of repetitive stimulation caused a progressive decrease in maximal contractile response to EFS and to a MCC (34% and 46% decrease, respectively – $p < 0,05$ versus initial response). Galangin avoided the progressive decrease in contractile response (the decrease of maximal contractile response was less than 3% - $p > 0,05$ versus initial response). Blockade of cholinergic and adrenergic nerves with atropine, phentolamine and propranolol did not modify the protective effect of galangin ($p > 0,05$). However, verapamil significantly reduced the effect of Galangin (the maximal contractile response decreased 36% - $p < 0,05$ versus group 2). Following the 1,5 hour period of recovery, the contractile responses to EFS and MCC in all groups showed no increase ($p > 0.05$ versus contractile responses after RFS).

Interpretation of results

We have demonstred that direct incubation of bladder smooth muscle in the presence of a low concentration of Galangin excerted a protective effect on bladder smooth muscle contractility by an action on L-type calcium channels and by reducing reactive oxygen species which are generated by repetitive field stimulation.

Concluding message

In the current experiments, Galangin excerted a protective effect from repetitive stimulation-induced fatigue. If the data are confirmed in-vivo, exogenenously administered Galangin might have a possible role in treating bladder dysfunction.

References

1. Protective effect of vitamin E on the response of the rabbit bladder to partial outlet obstruction. J Urol 166: 341-346, 2001.

2. Effect of repetitive stimulation on the contractile response of rabbit urinary bladder subjected to *in vitro* hypoxia or *in vitro* ischemia followed by reoxygenation. Pharmacology 57: 139 - 147, 1998.