

THE IDENTIFICATION OF T-TYPE CALCIUM CHANNELS ON THE INTERSTITIAL CELLS OF CAJAL IN RAT BLADDER

Hypothesis / aims of study

To confirm the expression and location of T-type calcium channel subtypes on the interstitial cells of Cajal (ICCs) in rat bladder and to investigate the role of T-type calcium channels in mediating spontaneous activity of isolated bladder strips and isolated ICCs from rats bladder.

Study design, materials and methods

Bladders were removed from Sprague-Dawley (SD) rats. To confirm the expression and location of T-type calcium channel subtypes ($\alpha 1G$, $\alpha 1H$, $\alpha 1I$) on c-kit-positive ICCs in the rat bladder, whole mount preparations of rat bladders were fixed in 4% paraformaldehyde for immunohistochemical analysis and double labelled using antibodies directed to the ICC marker c-kit and $\alpha 1G$, $\alpha 1H$, $\alpha 1I$. And, to detect expression of $\alpha 1G$, $\alpha 1H$, $\alpha 1I$ and the c-kit tyrosine kinase receptor (c-kit), total cellular RNA was extracted from rat bladders and reverse transcribed to obtain complementary DNA (cDNA). Reverse transcription-polymerase chain reaction (RT-PCR) was then performed using primers specific to T-type calcium channel $\alpha 1G$, $\alpha 1H$, $\alpha 1I$ and the c-kit sequence and amplified products separated by agarose gel electrophoresis. Direct sequencing and comparing with the known sequences confirmed identity of amplified PCR products.

To investigate the functional role of T-type calcium channel on spontaneous action potential of ICCs, the changes of intracellular calcium ($[Ca^{2+}]_i$) were detected after isolated ICCs loading fluo-3 AM, using mibefradil (T-type calcium channel elective blocker) and glivec mesylate (Glivec, a c-kit tyrosine kinase inhibitor) to treat the cells. To investigate the role of T-type calcium channel on ICCs in mediating phasic contractions, bladder strips were isolated from adult rats and mounted in tissue baths. Strips were stimulated with low concentration of the muscarinic receptor agonist carbachol (CCH; $1\mu M$) to upgrade phasic contractions and the effect of mibefradil and glivec both at a certain concentration ($1\mu M$) were then investigated.

Results

Expression of c-kit and $\alpha 1G$, $\alpha 1H$, $\alpha 1I$ protein were detected co-localization in a network of same cells on the boundary of the SM bundles, orientated parallel to the axis of the bundles, by positive immunoreactivity to c-kit and $\alpha 1G$, $\alpha 1H$, $\alpha 1I$ specific antibodies. For molecular studies, we confirm the expression of $\alpha 1G$, $\alpha 1H$, $\alpha 1I$ and c-kit mRNA in rat detrusor.

In functional studies, ICCs were loaded with the intracellular Ca^{2+} indicator fluo-3AM and imaged using a confocal microscope. The ICCs spontaneously produced a Ca^{2+} wave that appeared to start at a focus in the cell and spread axially in 2 directions and along the branches. Mibefradil ($1\mu M$) significantly inhibited $[Ca^{2+}]_i$ ($p < 0.01$). Then glivec ($1\mu M$) was added to act on the same ICCs. Glivec decreased the $[Ca^{2+}]_i$ of ICCs further ($p < 0.01$). ($n = 50$ from ten animals).

What's more, bladder strips appeared phasic contractions and CCH could upgrade them. Mibefradil ($1\mu M$) significantly inhibited the amplitude of CCH-upgraded phasic activity of the detrusor strips but not the frequency ($p < 0.05$). Glivec ($1\mu M$) significantly inhibited the amplitude ($p < 0.05$) of phasic activity in tissue incubated with CCH and mibefradil but not the frequency. Another group of bladder strips, glivec was used firstly. Glivec significantly inhibited the amplitude ($p < 0.05$) of CCH-upgraded phasic activity of the detrusor strips but still not the frequency. However, mibefradil did not significantly inhibit the amplitude and frequency of phasic activity in tissue incubated with CCH and glivec.

Concluding message

These data confirm the presence of T-type calcium channels on c-kit positive ICCs in rat urinary bladder and their importance in mediating spontaneous phasic contractions of bladder strips and spontaneous activity of isolated ICCs.

Disclosures

Funding: This study was supported by the National Natural Science Foundation of China (Charity no. 81070606) **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** the Animal Research Ethics Committee of the Third Military Medical University