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THE ROLE OF CHLORIDE CHANNELS ON THE REGULATION OF BLADDER SMOOTH MUSCLE TONE IN RATS.

Hypothesis / aims of study
Ion channels have been proved to be of functional importance in the regulation of bladder smooth muscle (SM) tone. The role of chloride channels on the bladder SM tissue has not been elucidated. We investigated the physiological roles of CLC-3 chloride channel and calcium-activated chloride channel (CaCC) on the maintenance of bladder SM tone in isolated rat bladder tissues.

Study design, materials and methods
Bladder smooth muscle tissue strips (2 x 2 x 10 mm) were suspended in tissue bath chambers for isometric tension experiments. Contractions elicited by KCl were examined in the condition of changing concentration of extracellular chloride (ECl) from 138 mM to 8 mM and substitution of extracellular Cl- to Br- or I-. Contractions elicited by norepinephrine (NE) were examined in the presence of chloride transport inhibitors: bumetanide (BUM), 4-(2-hydroxyethyl)-1-1- piperazine ethanesulphonic acid (HEPES) without bicarbonate, ethacrynic acid (ETH), and chloride channel blockers: 4,4'-diisothiocyano-2,2'-stilbene-disulfonic acid (DIDS), anthracene-9-carboxylic acid (A9C) and niflumic acid (NFA) (All concentration: 10^-8M~1M).

Results
In bladder SM strips the KCl induced contractility decreased significantly as the concentration of ECl changed from 138 to 8 mM ($p<0.01$, figure 1). The KCl elicited contractile response also decreased significantly as the extracellular Cl- was substituted by Br- or I- ($p<0.01$, figure 2). In addition, pretreatment with BUM, HEPES or ETH could significantly suppress the NE induced contractility in a concentration dependent manner (all $p<0.01$, figure 3). Pretreatment with DIDS, A9C or NFA could also significantly reduce the NE elicited contractile response in a concentration dependent manner (all $p<0.01$, figure 4).

Interpretation of results
This study demonstrates that alteration of ECl concentration or substitution of extracellular Cl- by Br- or I- can inhibit the contractility of rat bladder SM induced by KCl. These results have revealed that bladder SM strips possess the nature similar to volume-activated chloride currents described in other cells [1], suggesting the possibly important role of CLC-3 chloride channel on rat bladder SM. On the other hand, interference with either the distribution of chloride across the membrane or the ability of chloride channels to open markedly suppresses contractile responses of rat bladder SM to NE, showing the typical characteristics of CaCC on the tissue.

Concluding message
Our results imply that both CLC-3 chloride channel and CaCC are of functional importance in the regulation of bladder smooth muscle tone.

Figure 1. Alteration of ECl (n=6).

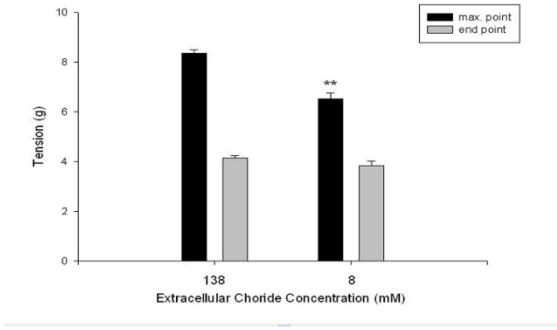


Figure 2. Substitution of Cl- by Br-, I- (n=6).

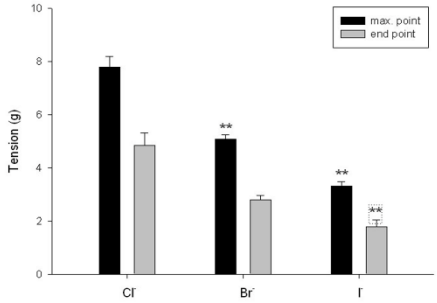


Figure 3. Inhibitory dose response curve of chloride transport inhibitors (n=6 in each experiment).

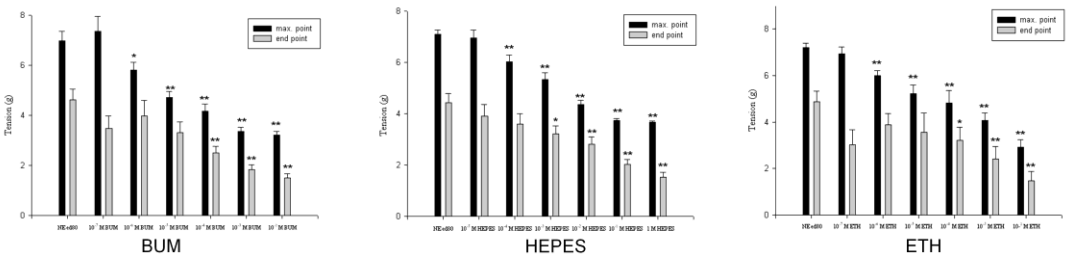
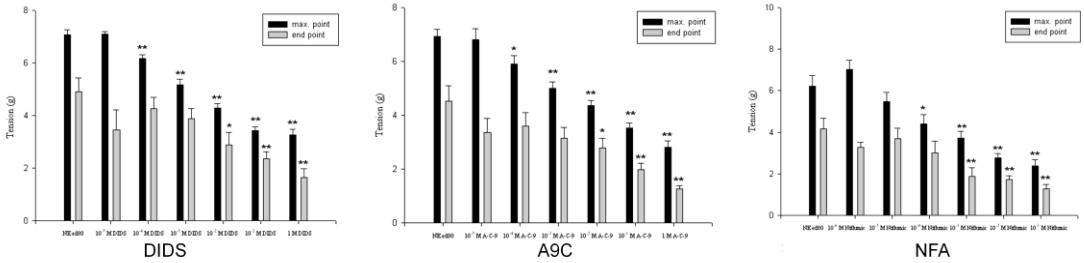


Figure 4. Inhibitory dose response curve of chloride channel blockers (n=6 in each experiment).



References

1. von Weikersthal SF, Barrand MA, Hladky SB: Functional and molecular characterization of a volume-sensitive chloride current in rat brain endothelial cells. J Physiol 1999; 516 (Pt 1): 75.

Disclosures

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Institutional Animal Care and Use Committee (IACUC)