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THE ROLE OF CITRIC ACID CYCLE METABOLITES ON DETRUSOR CONTRACTION

Hypothesis / aims of study

The pathophysiology of overactive bladder syndrome remains to be clarified. Its pathogenesis is linked with several of the components of the metabolic syndrome. Increasing evidence has demonstrated the unique role of the citric acid metabolite succinate in the pathogenesis of metabolic syndrome. The first goal of our novel study was to determine the effect of succinate on detrusor contraction or relaxation by activating its specific G-protein coupled receptor GPR91. Then, we aimed to establish a reproducible and consistent experimental in vitro method to study factors impacting bladder contractility. Lastly, we assessed the impact of prolonged exposure to succinate.

Study design, materials and methods

Detrusor strips were isolated from female rats of a hypertensive strain (SS2BN) and used for in vitro organ bath functional studies, which included contraction to potassium chloride (KCl), carbachol and electric field stimulation (EFS). In the second part of the study, these strips were mounted on agarose culture dishes under minimal tension. These strips were maintained in DMEM/F12 culture media and were subjected to daily stimulation with KCl. The contractile responses of these strips were assessed by measuring their contraction to KCl and carbachol at days 0 (i.e. non-cultured), 3, 5, and 6. The last part of the study involved looking at the effect of organ culture in the presence of 20 mM of succinate in comparison to control media after 3 days.

Results

Muscle strips incubated in organ bath with Krebs-Ringer buffer displayed a potent contraction in response to KCl (60 mM), carbachol (3 nM to 100 µM), and EFS. Addition of increasing concentrations of succinate gradually inhibited contractions elicited by moderate concentrations of carbachol (1 µM) and KCl (15 mM) (Figure 1), but not with EFS. Interestingly, higher concentrations of succinate restored contraction.

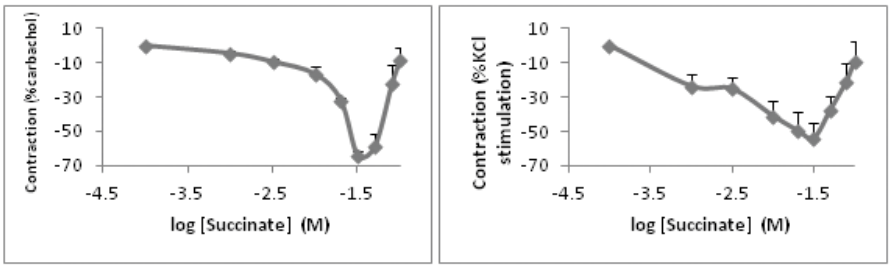

Figure 1. Succinate inhibits muscle contraction elicited by carbachol (1 µM) and KCl (15 mM), and restores contraction at higher concentrations.

Maleic acid, an agonist of GPR91 displayed the same curve as succinate. Similarly, malonate, an inhibitor of the conversion of succinate to fumarate, and dimethyl succinate, a metabolised permeable analog of succinate, both mimicked the effect of succinate (Figure 2).

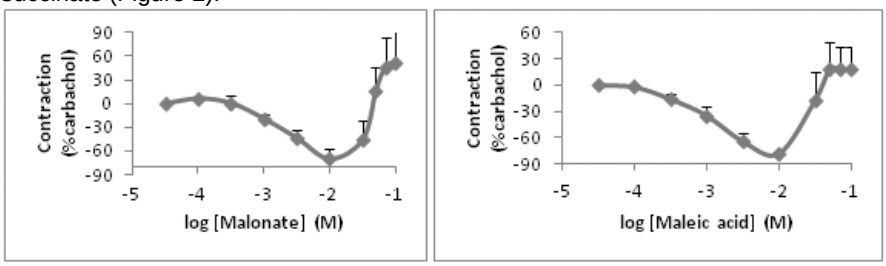

Figure 2. Malonate and maleic acid inhibits the contractile effect of carbachol (1 µM) on detrusor muscle strips), and restores contraction at higher concentrations.

Conversely, intermediates of glycolysis, glucose, fructose, glyceraldehyde-3P, glycerol and pyruvate, displayed no effects on carbachol or KCL contraction.

Bladder strips were then cultivated for up to 6 days in order to establish a reproducible method that would allow studying the effect of chronic exposure to different factors on the detrusor. The contractile response to KCl significantly decreased from day 0 (572.8 ± 99.2 g/g tissue, mean ± SE) to day 3 (183.1 ± 61.2 g/g tissue) ($P<0.05$, One-way ANOVA), but then remained stable at days 5 (226 ± 57.4 g/g tissue) and 6 (205.6 ± 50.1 g/g tissue). The EC_{50} of the carbachol showed no statistically significant difference between days 0, 3, 5, and 6. On the other hand, there was a statistically significance between the E_{max} values, but only between day 0 (866.2 ± 80.9 g/g tissue) and 6 (429.9 ± 33.2 g/g tissue) ($P<0.01$, One-way ANOVA).

Subsequently, to determine the effect of a longer exposure of succinate, bladder strip were cultivated for 72 hrs in a culture medium containing with or without succinate (20 mM). Detrusor strips cultivated for three days with succinate displayed a lower contractile activity to stimulation by KCl and carbachol (E_{max}) compared to control media (Figure 3).

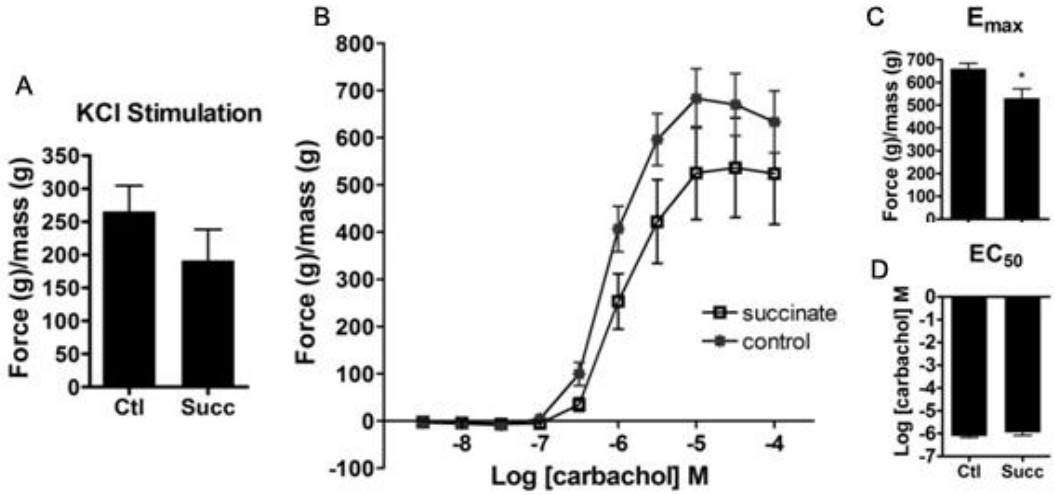

Figure 3. Detrusor strips cultured for 3 days in 20 mM succinate and control media. A: Stimulation of detrusor strips with 60 mM KCl. B: Gradual stimulation of detrusor strips with carbachol (from 3nM to 100 mM). C: Carbachol E_{max}. D: Carbachol EC_{50}. *P<0.05 Student's T-test.

Interpretation of results

Succinate and related metabolites impact bladder contraction in a biphasic fashion, with initial relaxation at moderate concentrations and restoration of contraction at high concentrations. This may result from direct activation of the succinate receptor GPR91 or by modulating energy metabolism in mitochondria. Lack of an effect of succinate on EFS suggests that it doesn't mediate neuronal contraction. The organ culture method applied to rat detrusor strips is a valid, replicable and reliable tool to study their contractile properties in vitro. The response to KCl stimulation was higher with non-cultured strips, but remained similar for days 3, 5, and 6. Moreover, carbachol stimulation remained constant up to day 5. Organ culture in the presence of succinate reduces the contractile response of detrusor strips to KCl and carbachol.

Concluding message

Organ culture is a reliable method to study the effect of chronic exposure of factors, such as hormones and metabolites, on bladder contractility. Succinate may relax bladder contraction acutely by counteracting the effect of carbachol or KCl, while chronic exposure may desensitize the bladder to contractile agents.

Disclosures

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