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CONTRASTING EFFECTS OF GAP JUNCTION BLOCKERS ON CONTRACTILITY OF RAT URINARY BLADDER STRIPS

Hypothesis / aims of study

Gap junctions are present at the coupling of different cell types within the urinary bladder [1]. Detrusor, the smooth muscle of the bladder, displays contractile phasic activity (PA) during urine storage. This PA could be facilitated by cell-to-cell communication e.g. via gap junctions. Increased PA may result in pathologies. Bladders from young animals manifest phasic contractile activity, which changes with ageing and enables generation of insight into factors moderating such activity, which could be relevant in the clinical context e.g. for overactive bladder syndrome. Hence, we characterize the effects of known gap junction blockers: isomers 18α- and 18β-glycyrrhetinic acid (18α-,18β-GA) and carbenoxolone (CBX) [2] on intact (including mucosa) and mucosa-denuded detrusor bladder tissue.

Study design, materials and methods

Bladders were isolated from male Wistar rats (P19-24 days). Denuded and intact tissue strips (5-8mm long) were placed in superfusion tissue organ baths, maintained in oxygenated Krebs buffer at 37°C and tied to a tension transducer to a resting tension of 1g. In the absence of measureable spontaneous PA in intact strips and in all denuded strips, 1μM carbachol (CCh) or 1 μM physostigmine (PhS) were used to induce PA. Changes in the contractile force were measured (ADInstruments) throughout the control period, single dose drug exposure and a washout period, each 30min. 1,10 and 30μM 18β-GA; 30μM 18α-GA and 50μM CBX were used on CCh-stimulated tissue, and 30μM 18β-GA and 50μM CBX we used on basal and PhS-stimulated tissue. The effect of a drug or drug vehicle on PA was investigated by measuring the amplitude (g per mg tissue) and frequency of PA (number of contractions during 5 min). Data show mean percentage change ± SEM from at least 7 strips (n) from at least 6 animals (N). Two-tailed paired t-test at $p<0.05$ was considered significant.

Results

Amplitude of bladder tissue CCh-induced PA decreased with increasing concentrations of the 18β-GA. In intact tissue 10μM 18β-GA decreased PA amplitude by 15.6 ± 4.7% ($p < 0.05$). Amplitude decreased by 33.3 ± 4.9% ($p < 0.001$) and 32.7 ± 3.4% ($p < 0.01$) with 30μM 18β-GA and 18α-GA, respectively. PA frequency increased by 20.1 ± 5.8% ($p < 0.01$) at 30μM 18β-GA.
The effect of 18β-GA was stronger in denuded detrusor strips. 10μM 18β-GA decreased PA amplitude by 36.1 ± 2.0% ($p < 0.001$) and 30μM 18β-GA by 42.6 ± 9.1% ($p < 0.01$). At 30μM 18β-GA PA frequency increased by 51.2 ± 24.0% ($p < 0.05$).
Similar effects were observed upon stimulation of intact strips with 1μM Physostigmine (PhS): 30μM 18β-GA decreased amplitude by 28.3±6.7% ($p<0.05$) without altering frequency of PA. Basal spontaneous PA amplitude was decreased by 19.5±4.3% ($p<0.05$).
In contrast, 50μM CBX showed a trend of increasing PA amplitude in CCh stimulated intact and denuded. Simultaneously PA frequency decreased in intact and denuded strips by 23.6 ± 4.9% ($p < 0.001$) and 20.3±7.2% ($p<0.05$), respectively. PhS stimulated intact strips increased the amplitude of contractions by 48.7±18.1% ($p<0.05$) while decreasing the frequency by 30.9±0.6% ($p<0.001$). Basal PA was unaffected by 50μM CBX.

Interpretation of results

This data show two contrasting effects of gap junction blockers on stimulated rat bladder tissue strips. 18β-GA acts as a blocker in agreement with previously shown decrease in bladder pressure in whole organ experiments [1]. In contrast to 18β-GA and previous report on blocking muscle slow wave activity [3], we find CBX having a different effect. While 18β-GA significantly decreases PA amplitude while slightly increasing the frequency, CBX increases PA amplitude significantly decreasing the frequency. Both drugs have much less pronounced effects on unstimulated basal PA.

Concluding message

18β-GA increased frequency and decreased amplitude of bladder tissue strip PA. CBX decreased PA frequency - a different observation to 18β-GA – and showed a trend in increasing PA amplitude - opposite to 18β-GA effect. This is an unexpected difference & will be further characterized.

References

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2. (2) Palani D et al (2007). Auton Neurosci 137: 56-62
3. (3) Hashitani H et al (2004) J Physiol 559.2:567–581

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