Effects of potassium channel modulators on myogenic spontaneous phasic contractile activity in human detrusor smooth muscle from neurogenic patients

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OBJECTIVES

▪ *Among the several mechanisms involved in the pathogenesis of detrusor overactivity, alteration of bladder smooth muscle properties plays a critical role. Such impairment may result in an increase in spontaneous phasic contractile activity as it has been observed in detrusor strips from patients with idiopathic OAB and in animal models of OAB caused by partial bladder outlet obstruction.*

▪ *We have previously demonstrated that adenosine triphosphate-sensitive potassium (K_{ATP}) and large conductance calcium-activated potassium (BK_{Ca}) channels play a role in the modulation of myogenic spontaneous phasic contractile activity developed by detrusor strips from patients without overactive bladder (control patients) (Darblade et al, 2006, Urology).*

The aims of this study were:

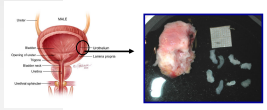
➢ to characterize spontaneous phasic contractile activity developed by detrusor smooth muscle from patients with neurogenic detrusor overactivity (NDO) and to compare it with spontaneous phasic contractile activity developed by detrusor from patients without OAB (control patients)

➢ to evaluate the role of K_{ATP} and BK_{Ca} channels on myogenic spontaneous phasic contractions of detrusor strips form patients with NDO.

MATERIALS & METHODS

Human detrusor strip preparation

Human bladder samples were obtained from 22 different patients with no known OAB undergoing cystectomy for bladder cancer and from 10 different neurogenic patients who underwent partial or total cystectomy. Detrusor strips with or without urothelium were mounted isometrically at a resting tension of 500 mg in a 5 ml organ bath filled with Krebs-HEPES buffer maintained at 37°C and bubbled with 95%O2-5%CO2. The strips were connected to force transducers for isometric tension recordings (Pioden Controls Ltd, UK). Following amplification, the tension changes were computerized via MacLab™/8 using Chart™ 5 software (AD Instruments Ltd).



In vitro contractile experiments

The strips were equilibrated for 90 minutes. In a first set of experiment, the strips were incubated for 30 min with a drug cocktail containing blockers for known transmitter receptors in the bladder wall, including the muscarinic antagonist atropine (10^{-6} M), alpha-adrenergic antagonist phentolamine (10^{-6} M), beta-adrenergic antagonist propranolol (10^{-6}M), purinergic antagonist suramin (10^{-5} M), and neuronal sodium channel blocker tetrodotoxin (10^{-6} M). In a second set of experiments, the strips were incubated with 10 µM pinacidil (K_{ATP} channel opener) or vehicle during 30 minutes and with 10 µM glibenclamide (K_{ATP} channel blocker) or vehicle for an additional 30 minute period. The strips were also incubated with 30 µM NS1619 (BK_{Ca} channel opener) or vehicle during 30 minutes and with 100 nM iberiotoxin (BK_{Ca} channel blocker) or vehicle for an additional 30 minute period.

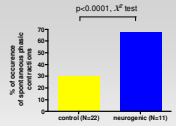
Data Analysis

Phasic contractile activity was quantified by calculating the area under the force-time curve (AUC, mg.min^{-1}) using the low points of the phasic contractions as the baseline, the maximal amplitude (mg), the frequency (contractions per minute), and the developed tension (mg). Analyses were performed with Elphy, version 3.0.0.45, software (CNRS-UNIC, France). For the evaluation of the effect of K channel modulators, the AUC, amplitude, frequency and developed tension are expressed as the percentage in the change of initial values measured before exposure to every tested pharmacologic agent. Data are expressed as the mean±SEM. Comparisons were performed using Student's *t* test. *P* values less than 0.05 were considered significant (GraphPad Prism, version 4.02).

RESULTS

Characterization of spontaneous phasic contractile activity of human detrusor from neurogenic patients- Comparison with control patients

▪ **Occurrence and characterization of spontaneous phasic contractile activity**



Characterization of spontaneous contractile activity after 90 min of equilibration

Urothelium	AUC (mg/min)		Amplitude (mg)		Frequency (contractions per min)		Developed tension (mg)	
	-	+	-	+	-	+	-	+
Control	398.7±107.1	573.6±82.2	488.5±81.1	523.3±81.1	1.8±0.3	2.9±0.6	786.3±74.9	946.8±75.5
Neurogenic	1071.0±178.3*	961.2±277.1	929.1±115.3*	666.9±200.8	2.7±0.3	2.5±0.2	1011.0±119.2	1163.0±150.5

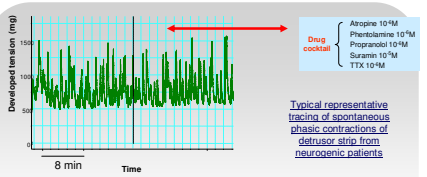
*p<0.05 versus control, student t-test

➢ Increase in occurrence of spontaneous phasic contractions in detrusor from neurogenic patients when compared to detrusor from control patients

➢ The AUC and the amplitude of phasic spontaneous contractions developed by detrusor strip from neurogenic patients are increased when compared to the strips from control patients

➢ The presence of urothelium does not modify amplitude, AUC nor frequency of phasic contractions in strips from neurogenic nor control patients

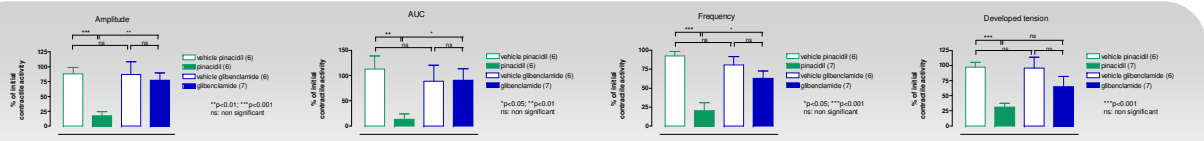
▪ **Myogenic origin of spontaneous contractile activity of detrusor from neurogenic patients**



Typical representative tracing of spontaneous phasic contractions of detrusor strip from neurogenic patients

➢ The incubation with the drug cocktail did not modify the AUC, amplitude, frequency or developed tension of phasic spontaneous activity of the strips with or without urothelium from neurogenic patients nor from control patients suggesting a myogenic origin for these contractions.

Effect of K_{ATP} channel modulators on spontaneous phasic contractile activity of human detrusor from neurogenic patients



➢ Pinacidil markedly inhibited spontaneous phasic contractile activity of detrusor strips. It reduced the amplitude, the AUC, the frequency and the developed tension of phasic contractile activity.

➢ This inhibitory effect was reversed by the addition of glibenclamide, more particularly on the amplitude, the AUC and the frequency of phasic contractile activity.

Effect of BK_{Ca} channel modulators on spontaneous phasic contractile activity of human detrusor from neurogenic patients

➢ The incubation of NS1619 followed by the incubation of iberiotoxin did not elicit any significant changes in phasic contractile activity

CONCLUSIONS

➢Myogenic spontaneous phasic contractions are increased in detrusor strips from patients with NDO. While neuroplasticity or alteration in nerve transmissions are largely described to be involved in NDO processes, such modification in detrusor smooth muscle properties has never been yet clearly described before in neurogenic patients.

Spontaneous phasic contractile activity observed in organ baths represents a promising *in vitro* modelling of neurogenic DO. The investigation of the effect of drugs at modulating these contractions could robustly support development of new pharmacological compounds for the treatment of OAB, particularly for neurogenic patients.

➢K_{ATP} channels are involved in the regulation of myogenic phasic contractile activity of detrusor strips from patients with NDO. These results are in line with the results we previously obtained in control patients. In contrast, BK_{Ca} channels do not participate in the regulation of these phasic contractions of detrusor from NDO patients while we previously demonstrated that, they played a role in the regulation of phasic contractions in control patients. These results might be very helpful for the development of K channels openers for the treatment of NDO.