AGE-DEPENDENT EXPRESSION OF TRPM4 CHANNEL IN GUINEA PIG DETRUSOR SMOOTH MUSCLE IS ASSOCIATED WITH ALTERED CONTRACTILITY

Sarah E. Maxwell[1], M. Dennis Leo[1], John Malysz[1], Georgi V. Petkov[1,2,3]

[1]Department of Pharmaceutical Sciences, College of Pharmacy; [2]Department of Pharmacology, College of Medicine;
[3]Department of Urology, College of Medicine, University of Tennessee Health Science Center, Memphis, TN

NIH-NIDDK

CAIRIBU 2020 Annual Meeting

ABSTRACT

INTRODUCTION AND OBJECTIVE: During development, maturation, or aging, the expression and function of detrusor smooth muscle (DSM) ion channels can change, thus affecting micturition. Increasing evidence supports a novel role of transient receptor potential melastatin type 4 (TRPM4) channels in DSM physiology. However, it remains unknown whether the properties of TRPM4 channels in DSM fluctuate over different life stages. Here, we examined TRPM4 channel protein expression and the effects of TRPM4 channel inhibitors, 9-phenanthrol (9-Phen) and glibenclamide (Glib), on phasic and tonic contractions of DSM isolated strips from juvenile and adult guinea pigs.

METHODS: Male Hartley-Albino guinea pigs, juveniles (5-9 weeks old; N=23) and adults (6-18 months old; N=23), were used in this study. All studies were on DSM without mucosa. Western blot was employed for the determination of total TRPM4 expression as well as for the detection of intracellular and plasma membrane fractions using cell surface biotinylation labelling. Isometric tension recordings of DSM isolated strips were performed using increasing cumulative concentrations of 9-Phen or Glib. *P*<0.05 (two-tailed) was considered statistically significant for Student's t-test or two-way ANOVA followed by the post hoc Sidak multiple comparison test.

RESULTS: Compared to juveniles, adults displayed a 50-70% reduction (P<0.05) in total DSM TRPM4 protein expression, while the surface-to-intracellular expression ratio remained the same for the two age groups. In both adult and juvenile DSM, cell surface TRPM4 protein expression (~80%) predominated over its intracellular fraction (~20%), revealing optimized channel trafficking mechanisms toward the cell membrane. 9-Phen showed lower potencies and/or maximum efficacies in adults than juveniles for inhibiting amplitude (P<0.05) and muscle force (P<0.05) of spontaneous and 20 mM KCl-induced phasic DSM contractions. Compared to 9-Phen, Glib also attenuated both spontaneous and KCl-induced DSM contractions, but with less pronounced effects in adults.

CONCLUSIONS: We reveal that total, surface, and intracellular TRPM4 channel protein fractions were decreased in adult compared to juvenile DSM while channel trafficking remained intact. Further, 9-Phen exerted a reduced inhibitory effect on adults, both spontaneous and 20 mM KCl-induced phasic DSM contractions, where the expression of the total TRPM4 protein was reduced compared to juveniles. Collectively, our data show age-dependent expression of TRPM4 channel protein and altered function in DSM.

INTRODUCTION

Overactive bladder becomes more prevalent with age in both males and females [1]. There have also been age and maturation related changes in muscarinic receptor function observed in the DSM of various animal species [2]. TRPM4 channels, which are highly permeable to monovalent cations, were recently recognized as functional regulators of DSM excitability and contractility. Previously, TRPM4 channels have been identified in DSM of mice [2], rats [3, 4], guinea pigs [5], and human bladder [6]. However, the trafficking and subcellular expression profile of these channels has yet to be examined, specifically the surface/intracellular ratio within DSM. Two commonly utilized TRPM4 channel inhibitors are 9-phenanthrol and glibenclamide which have IC_{50} values of approximately 20-40 μM for recombinant TRPM4 channels expressed in HEK-293 cells [7, 8]. Glibenclamide has been shown in rats to promote urine storage increasing void volume and reducing voiding frequency using in vivo cystometry employing different urinary bladder disease-induced models [8].

OBJECTIVE

The present study aimed to determine whether TRPM4 channel expression, trafficking, and function in DSM changes during maturation.

METHODS

Cell surface biotinylation and Western blotting: Surface biotinylation of live tissue and total protein quantification were performed as previously described with slight modifications [9, 10]. Surface and intracellular TRPM4 channel bands were expressed as a percent of total protein. Western blotting for total protein was performed following standard protocol.

Isometric DSM tension recordings: Mucosa-free isolated DSM strips were mounted for isometric tension recordings as previously described [4, 5, 6]. The effects of inhibitors of TRPM4, 9-phenanthrol and glibenclamide were evaluated on DSM spontaneous phasic contractions and 20 mM KCl-induced contractions. TTX (1 μM) was present throughout the experiments.

REFERENCES

1. Andersson K, and Arner A (2004) *Physiol Rev* 84: 935-986.
2. Kolta MG *et al.* (1984) *Mech Ageing Dev* 27: 183-188.
3. Kullmann F *et al.* (2018) *Naunyn Schmiedebergs Arch Pharmacol* 391: 1191-1202.
4. Parajuli S *et al.* (2013) *Channels (Austin)* 7:537-540.
5. Smith AC *et al.* (2013) *Am J Physiol Renal Physiol* 304:F918-929.
6. Smith A *et al.* (2013) *Am J Physiol Cell Physiol* 304:C467-477.
7. Hristov KL *et al.* (2016) *Am J Physiol Cell Physiol* 310:C600-611.
8. Grand T *et al.* (2008) *Br J Pharmacol* 153: 1697-1705.
9. Ozhathil LC *et al.* (2018) *Br J Pharmacol* 175: 2504-2519.
10. Henkel A, and Bieger S (1994) *Anal Biochem* 223:329-331.
11. Leo M *et al.* (2015) *Am J Physiol Cell Physiol* 309: C392-402.

RESULTS

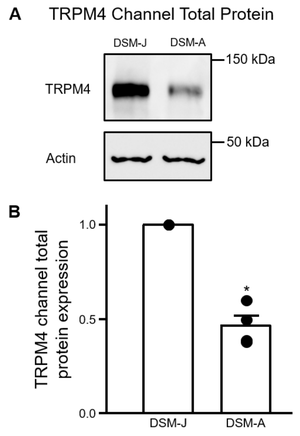
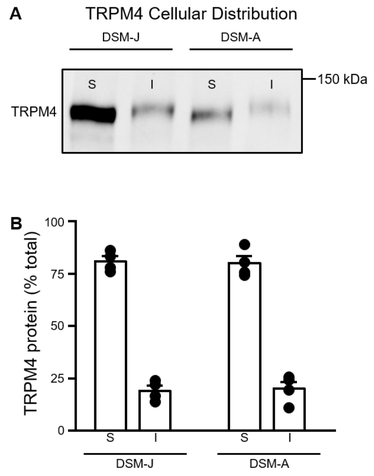


Figure 1. TRPM4 channel total protein is reduced in DSM-A compared to DSM-J. A) Representative Western blots show that the molecular weight of TRPM4 was 130 kDa in both DSM-J and DSM-A. B) TRPM4 channel total protein expression (shown as fold-change normalized to DSM-J and actin) was reduced by ~50% in DSM-A compared to DSM-J (*N*=4 for both groups, **P* <0.05).



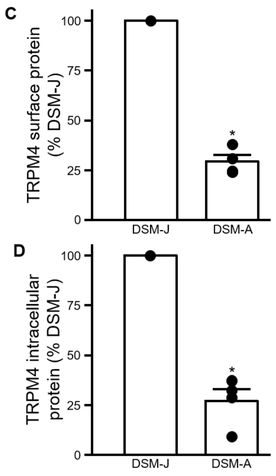
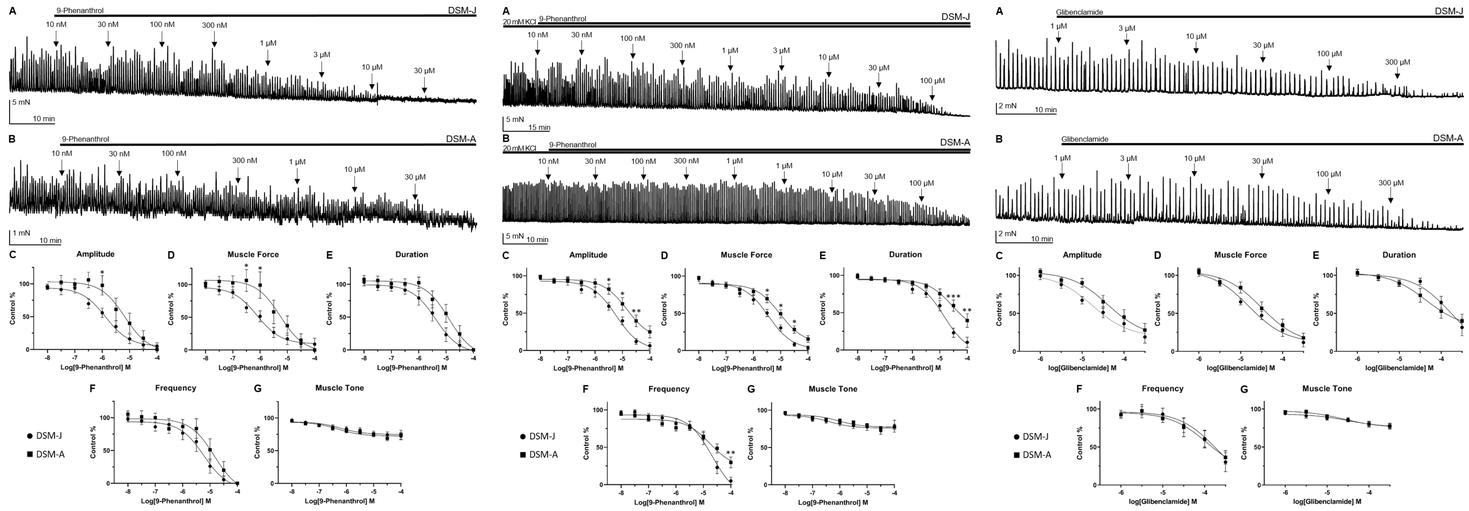


Figure 2. Cellular distribution of TRPM4 channel protein remains unchanged between DSM-J and DSM-A. A) Representative Western blot illustrates the cellular distribution of TRPM4 protein in DSM. B) Both DSM-J and DSM-A indicated 80% of TRPM4 protein was located in the cell surface fraction compared to the intracellular fraction. C) Surface TRPM4 protein expression was reduced by ~70% in DSM-A compared to DSM-J (*N*=4 both groups, **P* <0.05) D) DSM-A showed an ~70% reduction in intracellular TRPM4 protein compared to DSM-J. S, surface fraction; I, intracellular fraction (*N*=4 for both groups, **P* <0.05).



Phasic Contraction Parameter	DSM-J IC_{50}, Mean (95% CI) Max Inhibition, Mean ± SEM (n=6-13, N=4)	DSM-A IC_{50}, Mean (95% CI) Max Inhibition, Mean ± SEM (n=4-12, N=7-9)
Amplitude	1.3 (0.7 – 2.6) μM 94.8 ± 5.2%	6.7 (2.4 – 20.5) μM 100.0 ± 0.0%
Muscle Force	0.8 (0.4 – 1.6) μM 91.0 ± 9.1%	5.5 (1.9 – 17.8) μM 100.0 ± 0.0%
Duration	4.1 (2.1 – 8.2) μM 100.0 ± 0.0%	12.8 (4.9 – 3.5) μM 100.0 ± 0.0%
Frequency	5.9 (2.8 – 12.5) μM 100.0 ± 0.0%	14.4 (4.8 – 45.0) μM 100.0 ± 0.0%
Muscle Tone	0.6 (0.07 – 4.6) μM 27.8 ± 5.7%	0.5 (0.1 – 2.0) μM 25.6 ± 7.0%

Figure 3. 9-Phenanthrol inhibits spontaneous phasic contractions with lower potency in DSM-A compared to DSM-J. A) Representative isometric tension recording from a DSM-J isolated strip illustrating the effect of cumulative applications of 9-phenanthrol (10 nM–30 μM) on DSM spontaneous phasic contractions. B) Representative isometric tension recording from a DSM-A isolated strip illustrating the effect of cumulative applications of 9-phenanthrol (10 nM–30 μM) on DSM spontaneous phasic contractions. C-G) Cumulative concentration-response curves for the inhibitory effects of 9-phenanthrol (10 nM–100 μM) on DSM phasic contraction amplitude (C), muscle force (D), duration (E), frequency (F), and muscle tone (G), (DSM-J: *n*=6-13, *N*=4; DSM-A: *n*=4-12, *N*=7-9). The two-way ANOVA analysis revealed a statistically significant difference in phasic contraction amplitude and muscle force between DSM-J and DSM-A [DSM-J vs. DSM-A, Amplitude: F(1,19)=4.803, *P* <0.05; Muscle force: F(1,18)=4.746, *P* <0.05]; asterisks denote statistically significant differences for DSM-J vs. DSM-A (two-way ANOVA, post hoc Sidak multiple comparison test, **P* <0.05).

Phasic Contraction Parameter	DSM-J IC_{50}, Mean (95% CI) Max Inhibition, Mean ± SEM (n=10-23, N=8-10)	DSM-A IC_{50}, Mean (95% CI) Max Inhibition, Mean ± SEM (n=19-33, N=10-13)
Amplitude	5.9 (3.5 – 8.2) μM 93.6 ± 4.3%	14.8 (7.1 – 30.8) μM 75.1 ± 8.0%
Muscle Force	3.6 (2.2 – 5.2) μM 96.3 ± 2.5%	9.3 (5.5 – 15.9) μM 84.5 ± 4.6%
Duration	14.3 (8.1 – 24.8) μM 89.7 ± 7.0%	27.9 (1.1 – 83.6) μM 60.6 ± 9.1%
Frequency	20.8 (10.9 – 40.3) μM 95.0 ± 5.0%	17.4 (7.2 – 42.8) μM 70.3 ± 7.3%
Muscle Tone	0.4 (0.03 – 3.9) μM 21.5 ± 8.1%	0.9 (0.2 – 4.3) μM 22.5 ± 3.6%

Figure 4. 9-Phenanthrol inhibits 20 mM KCl-induced phasic contractions with lower potency and maximum efficacy in DSM-A compared to DSM-J. A) Representative isometric tension recording from a DSM-J isolated strip illustrating the effect of cumulative applications of 9-phenanthrol (10 nM–100 μM) on DSM 20 mM KCl-induced phasic contractions. B) Representative isometric tension recording from a DSM-A isolated strip illustrating the effect of cumulative applications of 9-phenanthrol (10 nM–100 μM) on DSM 20 mM KCl-induced phasic contractions. C-G) Cumulative concentration-response curves for the inhibitory effects of 9-phenanthrol (10 nM–100 μM) on DSM phasic contraction amplitude (C), muscle force (D), duration (E), frequency (F), and muscle tone (G), (DSM-J: *n*=10-23, *N*=8-10; DSM-A: *n*=19-33, *N*=10-13). The two-way ANOVA analysis revealed a statistically significant difference in phasic contraction amplitude, muscle force, and duration between DSM-J and DSM-A [DSM-J vs. DSM-A, amplitude: F(1,45)=6.054, *P* <0.05; muscle force: F(1,50)=4.802, *P* <0.05; duration: F(1,42)=4.768, *P* <0.05]; asterisks denote statistically significant differences for DSM-J vs. DSM-A (two-way ANOVA, post hoc Sidak multiple comparison test, **P* <0.05, ***P* <0.01, ****P* <0.001).

Phasic Contraction Parameter	DSM-J IC_{50}, Mean (95% CI) Max Inhibition, Mean ± SEM (n=9-10, N=5-6)	DSM-A IC_{50}, Mean (95% CI) Max Inhibition, Mean ± SEM (n=10, N=6)
Amplitude	17.6 (6.9 – 45.2) μM 81.1 ± 8.1%	40.6 (15.4 – 106.8) μM 72.0 ± 8.7%
Muscle Force	15.8 (8.2 – 30.6) μM 88.2 ± 7.4%	31.4 (14.2 – 69.1) μM 82.1 ± 6.1%
Duration	169.4 (45.7 – 627.9) μM 68.4 ± 11.0%	43.9 (13.7 – 140.6) μM 60.1 ± 9.1%
Frequency	187.3 (21.8 – 161.3) μM 69.8 ± 12.5%	113.9 (20.0 – 647.8) μM 63.6 ± 8.9%
Muscle Tone	32.0 (4.2 – 245.3) μM 22.6 ± 4.0%	25.4 (8.8 – 73.7) μM 22.8 ± 3.0%

Figure 5. Glibenclamide inhibits spontaneous phasic contractions with lower potency in DSM-A compared to DSM-J. A) Representative isometric tension recording from a DSM-J isolated strip illustrating the effect of cumulative applications of glibenclamide (1-300 μM) on DSM 20 mM KCl-induced phasic contractions. B) Representative isometric tension recording from a DSM-A isolated strip illustrating the effect of cumulative applications of glibenclamide (1-300 μM) on DSM 20 mM KCl-induced phasic contractions. C-G) Cumulative concentration-response curves for the inhibitory effects of glibenclamide (1–300 μM) on DSM phasic contraction amplitude (C), muscle force (D), duration (E), frequency (F), and muscle tone (G), (DSM-J: *n*=9-10, *N*=5-6; DSM-A: *n*=10, *N*=6).

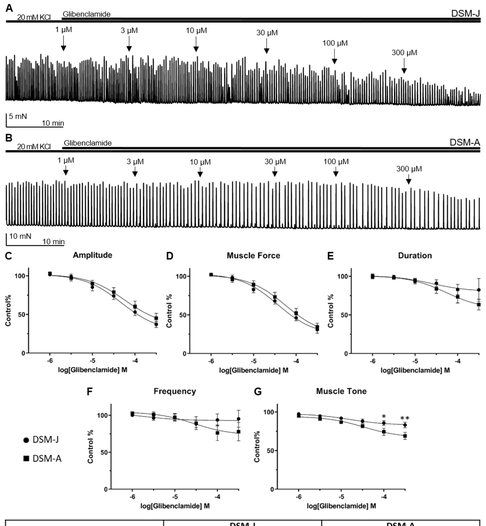


Figure 6. Glibenclamide inhibits 20 mM KCl-induced phasic contractions with lower potency in DSM-A compared to DSM-J. A) Representative isometric tension recording from a DSM-J isolated strip illustrating the effect of cumulative applications of glibenclamide (1-300 μM) on DSM 20 mM KCl-induced phasic contractions. B) Representative isometric tension recording from a DSM-A isolated strip illustrating the effect of cumulative applications of glibenclamide (1-300 μM) on DSM 20 mM KCl-induced phasic contractions. C-G) Cumulative concentration-response curves for the inhibitory effects of glibenclamide (1–300 μM) on DSM phasic contraction amplitude (C), muscle force (D), duration (E), frequency (F), and muscle tone (G), (DSM-J: *n*=27-29, *N*=10; DSM-A: *n*=15-21, *N*=7). The two-way ANOVA analysis revealed a statistically significant difference in phasic contraction muscle force between DSM-J and DSM-A [DSM-J vs. DSM-A, amplitude: F(1,45)=6.054, *P*<0.05; muscle force: F(1,47)=4.811, *P*<0.05]; asterisks denote statistically significant differences for DSM-J vs. DSM-A (two-way ANOVA, post hoc Sidak multiple comparison test, **P*<0.05, ***P*<0.01).

SUMMARY & CONCLUSIONS

- Total, surface, and intracellular TRPM4 channel protein were decreased in adult DSM compared to juvenile while maintaining the ratios of surface-to-intracellular in both groups.
- Cell surface TRPM4 protein expression (~80%) predominated over its intracellular fraction (~20%) showing conserved channel trafficking mechanisms toward the cell membrane.
- Preferential expression of TRPM4 channels within the plasma membrane would be expected for essential regulators of DSM cell function.
- The inhibitory effect of 9-phenanthrol on DSM-A was significantly reduced on spontaneous and 20 mM KCl-induced phasic DSM contractions compared to DSM-J.
- **Collectively, our data reveal that a reduction in the expression of TRPM4 channel protein with aging alters guinea pig DSM function.**
- **Future comparative studies on human DSM isolated from patients with or without overactive bladder from various age groups will provide insights as to if TRPM4 channel dysfunction is a contributing factor to OAB that increases with age.**

ACKNOWLEDGEMENT

Funding: P20 DK-123971

R01 DK-106964, and Van Vleet Endowment to **Georgi V. Petkov**. R01 HL-149662 to M. Dennis Leo