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Lloyd J C[1], Degoski D J[2], Brooks J M[2], Dolber P C[3], Fraser M O[4]
1. *Duke University Medical Center,* ***2.*** *Institute for Medical Research/Durham Veterans Affairs Medical Center,* ***3.*** *University of Texas Medical Branch at Galveston,* ***4.*** *Duke University and Durham Veterans Affairs Medical Centers*

DIFFERENTIAL EFFECTS OF STEPWISE PHARMACOLOGICAL AUTONOMIC DENERVATION OR DIRECT SMOOTH MUSCLE RELAXATION ON URODYNAMIC PARAMETERS IN CHRONIC SPINAL CORD INJURED RATS

Hypothesis / aims of study
Suprasacral spinal cord injury (SCI) often results in neurogenic detrusor overactivity (NDO), causing low compliance and unsafe bladder pressures. We undertook a series of experiments designed to determine the relative contributions of parasympathetic and sympathetic nervous system activities, and spontaneous myogenic activity on urodynamic indices in chronic SCI rats. Chronic SCI rats underwent cystometric evaluation during which time they either receive repeated vehicle, parasympathetic antagonists, sympathetic antagonists or smooth muscle relaxants (ion channel blockers and β-adrenergic agonists).

Study design, materials and methods
Female Sprague-Dawley rats (n=46, 250-275 g body weight) underwent complete spinal cord transection at vertebral level T9-10 under isoflurane anaesthesia. The bladders of these rats were expressed by manual crede twice daily until terminal experimentation. During the week preceding terminal experimentation, the animals were placed in Ballman restraint cages for 3 x 1 hour sessions over 3 days in order to acclimate them to the cages. On the day of terminal experimentation, the chronic SCI rats (≥4 weeks post-SCI) were fitted with unilateral jugular and transvesical bladder catheters for drug delivery and cystometric testing, respectively, and the animals were mounted in Ballman restraint cages with food and water provided *ad libitum*. The bladder catheters were connected to an infusion pump and pressure transducers via 3-way stopcocks.

Following a 1 hour recovery period from surgery, the animals underwent conscious cystometry with flow rates adjusted to achieve single fill cycles within 15-20 minutes. The bladders were emptied and a single fill cystometrogram was performed and continued filling followed to produce a period of continuous cystometry over a period of 1 hour. This pattern was repeated for all treatments. Following the control period, each animal received an intravenous vehicle control (normal saline). Control group animals received 3 additional vehicle doses. In the parasympathetic denervation group (Para), rats sequentially received atropine (pure antimuscarinic), NF−449 ($P2X_1$ purinergic antagonist) and hexamethonium (HEX, autonomic ganglion blocker). In the sympathetic denervation group (Symp) rats received phentolamine (P; α−adrenergic antagonist), propranolol + SR59230A (provides a complete β−adrenergic block) and HEX. The smooth muscle relaxant (SM) rats received verapamil (Ca^{2+} channel blocker), CL−316,243 (β_3−adrenergic agonist) and isoproterenol (β_{1-3}−adrenergic agonist). Table 1 illustrates the dosing regimen for each group.

Parameters analysed included true bladder capacity (TBC), functional bladder capacity (FBC), voiding efficiency (VE), maximum voiding bladder contraction amplitude (VC-MA), non-voiding bladder contraction count (NVC#) and maximum NVC amplitude (NVC-MA), compliance (C) and area under the curve of the filling pressure (AUC-FP). Data were analysed by 2−Way RM ANOVA and comparisons were made across dosing positions (e.g. Dose 2, Dose 3) to the repeated vehicle group, alpha = 0.05. It is here noted that the HEX group is a treatment “duplication”, albeit with different preceding treatments, across both autonomic groups, and therefore may be seen to serve as an internal control. In the case of HEX inducing overflow incontinence, some indices were measured up to the first point of leak, some were not measurable.

Results
For the Para group, Atropine produced increases in TBC and decreases in VC-MA, NF-449 additionally increased FBC, and HEX further increased TBC and additionally increased AUC-FP. Symp alone had no apparent effect, suggesting that tonic sympathetic tone may not be present in SCI rats. For the SM group, the β−adrenergic agonists increased TBC, decreased VC-MA, and increased compliance. See Table 2 for more details.

Table 1. Treatment Design, n=10-12/group

Group	**Treatment**			
	Dose 1	**Dose 2**	**Dose 3**	**Dose 4**
Vehicle	Vehicle	Vehicle	Vehicle	Vehicle
Para	Vehicle	Atropine	NF-449	HEX
Symp	Vehicle	Phentolamine	Propranolol + SR-59230A	HEX
SM	Vehicle	Verapamil	CL-316-243	Isoproterenol

Table 2. Statistically significant results relative to repeated vehicle alone

		Treatment						Treatment			
		Dose 1	Dose 2	Dose 3	Dose 4			Dose 1	Dose 2	Dose 3	Dose 4
True Bladder Capacity						Compliance					
	Para	—	60%↑**	77%↑****	130%↑****		Para	—	—	—	—
	Symp	—	—	—	128%↑****		Symp	—	—	—	—
	SM	—	—	42%↑*	41%↑*		SM	—	—	200%↑**	—
Functional Bladder Capacity						AUC of Filling Pressure					
	Para	—	—	69%↑*	NA		Para	—	—	—	172%↑****
	Symp	—	—	—	NA		Symp	—	—	—	197%↑****
	SM	—	—	—	—		SM	—	—	—	—
Voiding Efficiency						Non-Voiding Bladder Contraction Count					
	Para	—	—	—	NA		Para	—	—	—	—
	Symp	—	—	—	NA		Symp	—	—	—	—
	SM	—	—	—	—		SM	—	—	—	—
Max Voiding Bladder Contraction Amplitude						Max Non-Voiding Bladder Contraction Amplitude					
	Para	—	23%↓***	16%↓*	NA		Para	—	—	—	—
	Symp	—	—	—	NA		Symp	—	—	—	—
	SM	—	—	16%↓*	16%↓*		SM	—	—	—	—

Interpretation of results

The positive results attained were expected, and replicate previous results by ourselves and others. However, we have failed to replicate, in a statistically meaningful sense, other previous results, such as expected decreases in NVC-MA with both atropine and the β−adrenergic agonists, and the expected increase and decrease in NVC# with the same compounds, respectively. We are also surprised that an enhancement of TBC was not accompanied by an increased C following Para treatment. We suspect that these unexpected negative results may be due to an under-powering of the study, given the number of comparisons and the low number of animals/group. We have yet to analyse the data using a within groups approach rather than across all groups approach to determine whether this is a reasonable conclusion.

The lack of effect of sympathetic blockade suggests that ongoing sympathetic tone is not important in the generation of neurogenic bladder, at least in females. Indeed, the data support the notion that enhanced sympathetic tone, at least at the β−adrenergic receptor, can be therapeutic for this type of neurogenic bladder.

Concluding message

We hereby provide evidence in a single study that pharmacologic denervation of the parasympathetic nervous system and sympathomimetic application of β−adrenergic receptor agonists are both capable of ameliorating neurogenic bladder complications resulting from suprasacral SCI.

Disclosures

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