

## NORADRENERGIC REGULATION OF MOTONEURONS INNERVATING THE URETHRAL SPHINCTER

### Hypothesis / aims of study

Duloxetine and reboxetine are two drugs that inhibit reuptake of norepinephrine and increase norepinephrine levels in the central nervous system. Both drugs have shown clinical efficacy in the treatment of stress urinary incontinence (1). It is still unclear, however, how increases in norepinephrine can modulate urethral sphincter activity. Spinal motoneurons of Onuf's nucleus innervate the urethral sphincter through the pudendal nerve. Because the cell somata of these motoneurons are densely innervated by noradrenergic terminals, Onuf's nucleus represents a putative site of action for reuptake inhibitors (2). Therefore, we hypothesized that elevated levels of norepinephrine due to reuptake inhibition may result in excitation of the motoneurons in Onuf's nucleus. To test this hypothesis, we studied the direct effects of norepinephrine on the motoneurons using an isolated spinal cord slice preparation.

### Study design, materials and methods

Visualized whole-cell patch-clamp electrophysiological recordings were made in acute spinal slices prepared from 6-14 day old female rats. Two days prior to recording, the fluorescent dye *Fast Dil* was injected into the ischioanal fossa of rats. Retrograde uptake of dye into pudendal nerve fibers traversing the ischioanal fossa accumulated in axons and cell somata of motoneurons in the spinal dorsolateral nucleus (DLN), the rat equivalent of human Onuf's nucleus. After at least two days of dye transport, 300 micron thick transverse lumbar spinal cord slices containing DLN were prepared from these rats. Slices were transferred to a recording chamber and perfused with an oxygenated artificial cerebrospinal fluid at room temperature. Conventional current-clamp recordings were made from *Fast Dil*-labelled DLN motoneurons identified using fluorescence optics. Norepinephrine was bath applied to the neurons at a concentration of 20 micromolar.

### Results

Bath application of norepinephrine significantly depolarized DLN motoneurons (approximately 9 mV), and in some cases evoked action potentials. To study the norepinephrine-induced changes in motoneuron intrinsic membrane properties, synaptic transmission was pharmacologically blocked and cells were maintained at -60 mV during recording. Under these conditions, norepinephrine significantly elevated input resistance by approximately 30 % and reduced rheobase by approximately 50 %. These changes were reversed with norepinephrine washout and were largely blocked by prazosin, an alpha-1 adrenergic receptor antagonist. In addition, norepinephrine significantly increased action potential frequency and reduced the amplitude of the afterhyperpolarization observed following action potentials by approximately 50 %. Both the increase in action potential frequency and the reduction in afterhyperpolarization were occluded by apamin, a small conductance calcium-activated potassium (SK) channel blocker.

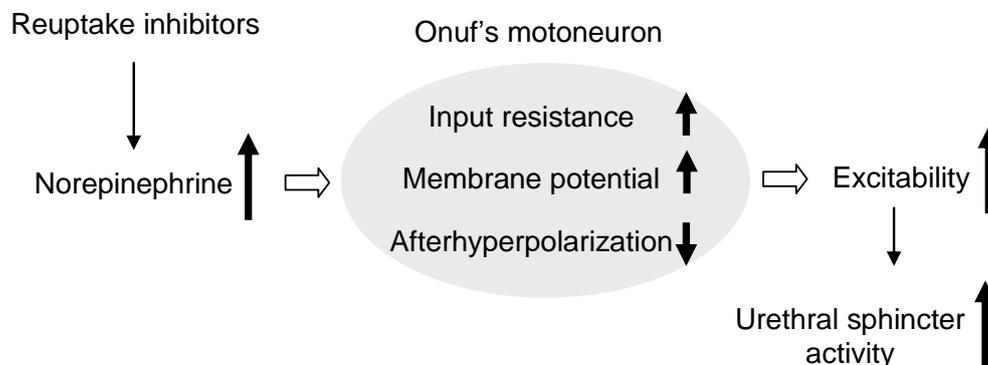


Figure 1. A model for the mechanism of norepinephrine-induced excitation of Onuf's motoneurons that mediates norepinephrine reuptake inhibition-enabled urinary continence.

### Interpretation of results

Norepinephrine produces multiple effects on DLN motoneurons. First, norepinephrine depolarizes DLN motoneurons through an alpha-1 adrenergic receptor-dependent mechanism. Second, norepinephrine increases input resistance also through an alpha-1 adrenergic receptor-dependent mechanism. These two modifications reduce the amount of current needed to excite DLN motoneurons. Consistent with this idea, prazosin has been shown to attenuate both basal and duloxetine-induced urethral sphincter activity by acting on the central nervous system in cats (3). Third, norepinephrine reduces the amplitude of afterhyperpolarization by attenuating a SK channel-mediated potassium conductance. This change results in an increase in frequency of repetitive action potentials. A receptor subtype responsible for the SK conductance attenuation is yet to be determined. In conclusion, norepinephrine effectively increases excitability of DLN motoneurons through multiple mechanisms. The norepinephrine-induced increase in excitability of DLN motoneurons could be a key mechanism for reuptake inhibitor-induced enhancement of pudendal nerve activity and increased urethral sphincter contraction.

### Concluding message

These results provide the first direct evidence for the excitatory effects of norepinephrine on DLN motoneurons. Furthermore, these results suggest that a norepinephrine-induced increase in urethral sphincter motoneuron activity may underlie the mechanism of action of monoamine reuptake inhibitors for treatment of stress urinary incontinence (see Figure 1).

## References

1. Int J Gynecol Obstet (2004) 86 Suppl 1; S38-52
2. J Clin Pract (2007) 61(8); 1349-55
3. J Urol (1984) 132(2); 403-9

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<b><i>What were the subjects in the study?</i></b>	<b>ANIMAL</b>
<b><i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i></b>	<b>Yes</b>
<b><i>Name of ethics committee</i></b>	<b>UROGENIX INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE</b>