Progress report

Project title: The renin-angiotensin system and the lower urinary tract in diabetes
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Our specific aims are as follows:

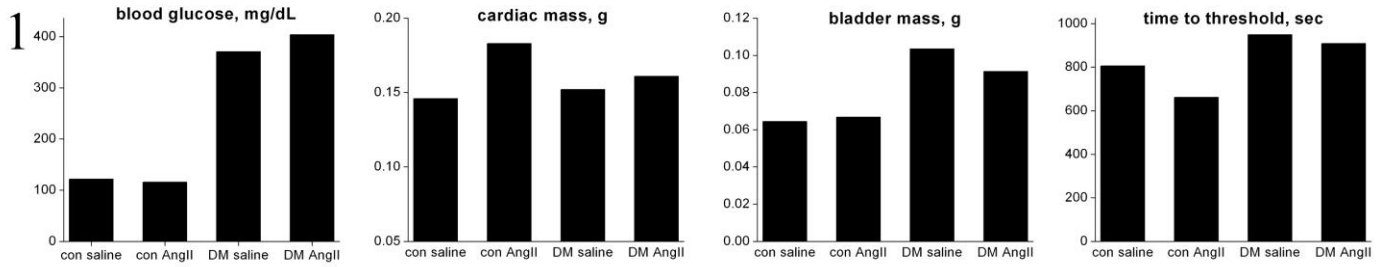
Urological complications develop in up to 80% of diabetics, the most common being lower urinary tract (LUT) dysfunction which chronically and seriously compromises quality of life in diabetics. Overactivity of the renin-angiotensin system (RAS) is an important component of diabetes. However, although the RAS is known to affect the function of the lower urinary tract (LUT), and although both RAS overactivity and LUT dysfunction are important complications of diabetes, virtually nothing is known about the effects of the RAS on the LUT in diabetes. Moreover, despite the signal importance of mouse models in the study of diabetes, little is known about the effects of RAS activity on the LUT in normal mice, let alone diabetic mice. Our **overarching hypothesis** is that RAS overactivity in the setting of diabetes affects LUT function in a time-dependent manner. To explore this hypothesis, we will study mice after 6 or 14 wk of hyperglycemia and with and without chronic angiotensin II treatment. Our associated specific aims are:

Specific aim 1. Using cystometry and leak-point pressure methodology, determine the effects of chronic angiotensin II treatment on the LUTs of 129/SvEv mice with and without Akita diabetes.

Specific aim 2. Using isolated preparations from the bladder and urethra *in vitro,* determine the effects of acute and chronic angiotensin II treatment on LUT contractility in mice with and without Akita diabetes and show the functional dependence on AT1 vs AT2 receptors.

Specific aim 3. Using fixed LUTs and histologic and immunohistochemical methods, determine the effects of diabetes and chronic angiotensin II treatment on the expression and distribution of angiotensin II and its AT1 and AT2 receptors and on the smooth muscle and collagen of the LUT.

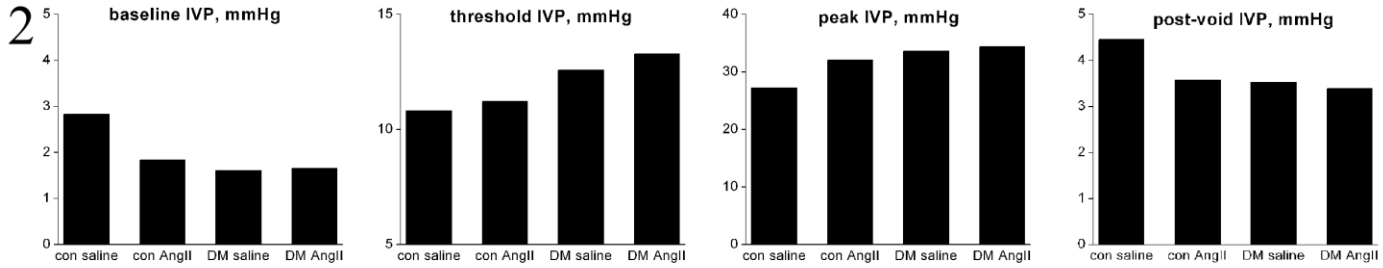
Because our contract was a long time getting finalized and there were impediments to beginning data acquisition, we are not very far along the road to completion of the study. Accordingly, the data presented below are very much partial, standard errors are not shown, and statistical comparisons are premature.



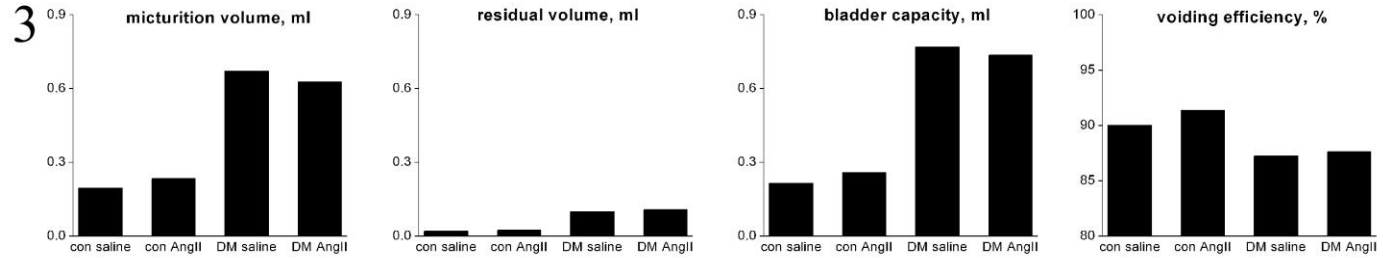
By definition, blood glucose levels are higher in the diabetic than the control mice (Figure 1, left panel). All diabetic mice had blood glucose values above 300 mg/dL. Cardiac mass was higher in control mice with chronic (4 weeks) 700 ng/kg/min angiotensin II than in control mice without angiotensin; there is a much smaller difference between diabetic mice with and without angiotensin II, but note that the numbers of mice involved are much smaller at this time. Bladder mass is conspicuously augmented in diabetic mice; no effect of angiotensin on bladder mass is convincingly apparent at this juncture.

When doing cystometry in animals whose bladders have very different functional capacities, it is necessary to choose whether to fill all bladders at the same rate regardless of their capacity, or at the same fractional rate with respect to capacity so that all fill in roughly the same amount of time. We chose to keep the fractional rate of filling about the same, which we accomplished by varying the rate of filling to keep the time between the start of infusion and intravesical pressure reaching threshold near 10 min. The final panel

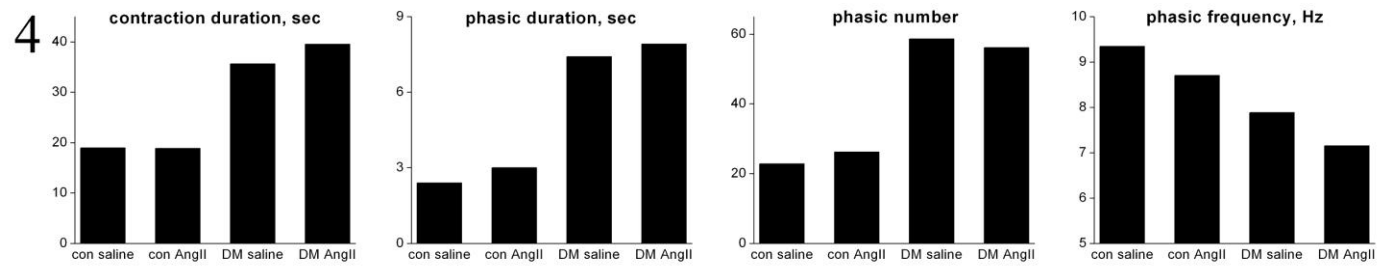
of Figure 1 above, "time to threshold," merely confirms that the fractional rate of filling is relatively constant among groups.



The pressure within the bladder at different functional times is shown for the four groups in Figure 2 above. Baseline intravesical pressure (IVP) – the pressure in the empty bladder before filling begins – is 1.6-2.8 mmHg in all groups, and post-voiding IVP is similar at 3.4-4.4 mmHg. Threshold IVP was lower in control than in diabetic rats, but even so ranged only from 10.7-13.3 mmHg. Finally, there is more spread in peak IVP, with values ranging from 27-34 mmHg, but no pattern of control vs. diabetic nor saline vs. angiotensin II is readily apparent. Regarding peak IVP, it is especially worth noting that the ability of the diabetic mice to generate forceful bladder contractions is not at all compromised.



The primary cystometric variables for the four groups are shown in Figure 3 above; the first 3 panels all have the same range for the *y*-axis. The micturition volume was much higher for diabetic than for control mice, with no obvious difference between mice treated with saline vs. angiotensin II for four weeks. Residual volume was also higher for diabetic than control mice, with no obvious effect of angiotensin II treatment. Accordingly, bladder capacity was much higher for diabetic than for control mice. It is very important to note that, despite the higher residual volume in diabetic mice than control mice, the diabetic mice had such large void volumes that voiding efficiency remained high. As shown in the final panel of Figure 3, voiding efficiency was indeed lower in diabetic than control mice, but was nonetheless very high: 87.2% and 87.6% for the two diabetic groups vs. 90.7% and 91.4% for the two control groups.



How do the diabetic mice succeed in voiding as effectively as they do, despite the relatively long course of their diabetic state (onset of diabetes at 3 wk, study at 20 wk)? As noted in regards to Figure 1, the diabetic mice do generate the same peak pressures during voiding as do control mice, and their bladder mass is conspicuously augmented. As shown in the first panel of Figure 4, the duration of the voiding contraction is also markedly augmented. Moreover, the duration of phasic activity – that period during which the external urethral sphincter is phasically relaxing and contracting, necessary for voiding – is markedly increased in the diabetic mice, with the number of "high frequency oscillations (HFOs)" of IVP (and thus the number of external urethral sphincter contractions) being similarly elevated. This suggests that maintenance of strong bladder contractions for as long as needed to void completely – perhaps requiring the observed increase in bladder mass – with maintenance of external urethral sphincter phasic activity as long as needed sums up the most important adjustments made by the 20-week Akita +/- diabetic mouse bladder.

The last panel of Figure 4 is interesting, though we must await study of additional mice to confirm what appears to be shown. First, the HFO frequency is higher in control mice than in diabetic mice; second, in a given state (eugylcemic or diabetic), HFO frequency is higher in untreated than in angiotensin II-treated mice. The latter would likely require an action of angiotensin II on central pattern generators in the lumbar spinal cord, and it is known that angiotensin II receptors are indeed present in many spinal cord neurons. Assuming that these differences still hold after more mice are studied, it remains to be shown whether the variation in HFO frequency is an important contributor to voiding efficiency.

STZ-treated diabetic mice studied by Daneshgari's group (Am J Physiol 290:R1728-R1735, 2005) had residual volumes considerably higher than ours at all study intervals (3-20 wk post-STZ), and showed clear signs of decompensation – decreased peak IVP, decreased micturition volume in the face of increasing residual volume, and increased resting pressure – with increasing time post-STZ. In regards to the absence of significant decompensation in the Akita diabetic mice, it must be noted that the streptozotocin-treated mice studied by Daneshgari's group had considerably higher blood glucose levels that ours, averaging 356 mg/dL at 3 wk post-STZ, similar to our mice, but 582, 548, and 591 mg/dL at 9, 12 and 20 wk post-STZ.

Further studies. The original plan was to study mice with pumps implanted at two different ages, 5 vs. 16 wk, with final study at 13 vs. 20 wk, to assess the relative effects of angiotensin II on compensated vs. decompensated bladders. However, the data above thus far suggest that the Akita +/- diabetic mouse bladder is not meaningfully decompensated at 20 wk, and full study of the younger mice may be terminated early if there is no sign that they are meaningfully different from the older diabetic mice.

We have studied a number of mice with and without Akita diabetes, and with and without overexpression of renin (using the RenTg transgenic mouse strain). The data are acquired but not yet fully analyzed.

Cystometric study of mice (including those above) often reveals the presence of multiple "premicturition contractions," not unlike those seen in normal cats (but not rats nor humans) and in all animals with neurogenic voiding. We have begun to study some mice with two chronically implanted balloons, one intravesically (as for the studies above) and one intraabdominally but away from the bladder. Limited data suggest that many bladder contractions are not accompanied by abdominal contractions, and that many which are initially accompanied by abdominal contractions outlive them; that is, those elevations of bladder pressure are not merely the manifestations of an abdominal contraction. On the other hand, it appears in many of the latter cases that the abdominal contraction is initiated immediately before the bladder contraction, suggesting the operation of a somatovesical reflex whereby abdominal contractions incite (longer-lasting) bladder contractions. (On still another hand, it appears that some abdominal contractions are preceded by bladder contractions, suggestive of a vesicosomatic reflex.) Much more study is required to reach any satisfactorily firm conclusions.

Studies of *in vitro* contractility have not begun.

Note that for Specific aim 3, the bladders of the mice studied in Specific aim 1 have all been fixed with the bladders inflated to the functional capacity determined in the conscious mice the same day, and will be morphologically studied as outlined for Specific aim 3.