**LETTER TO THE EDITOR**

**Determination of Sex Differences in Activities of Angiotensin-Converting Enzyme 2 (ACE2) Requires an Activity Assay That Doesn’t Underestimate ACE2**

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**To the Editor:** We read with interest the recent article, “Sex Differences in Angiotensin-Converting Enzyme Modulation of Ang (1–7) Levels in Normotensive WKY Rats,” by Bhatia et al. in which they report their investigation of the enzymatic mechanisms whereby female rats have higher levels of angiotensin (1–7) (Ang (1–7)) than male rats. They measured the enzymatic activities of angiotensin-converting enzyme (ACE), ACE2, and nephrilysin in the kidney cortex and medulla from normotensive WKY rats. They hypothesized that female rats would have higher activities than male rats of nephrilysin or ACE2, which can generate Ang (1–7) from angiotensin I and angiotensin II, respectively. However, they found no statistically significant sex differences in nephrilysin or ACE2 activities. They did observe higher cortical ACE activity as well as higher cortical Ang (1–7) concentrations in female rats than male rats. These observations led to the conclusion that ACE has a role for renal cortical Ang (1–7) formation in female rats.

For measuring ACE2 activity, Bhatia et al. used the fluorogenic substrate 7Mca-YVADAPK(Dnp)-OH. Because the substrate can also be hydrolyzed by ACE, they included the ACE inhibitor captopril in the reaction mixture. They then determined the ACE2 activity as the hydrolysis rate of 7Mca-YVADAPK(Dnp)-OH that could be inhibited by 1 µM of the ACE2 inhibitor DX600. For the ACE2 activity assay, Bhatia et al. refer to an article describing the assay that our laboratory used in the past. However, we recently discovered that both rat ACE2 and mouse ACE2, in contrast with human angiotensin-converting enzyme 2, are poorly inhibited by 1 µM DX600.

We further estimated that only 19% of the ACE2 activity in mouse kidney was inhibited by 1 µM DX600. Similar incomplete inhibition of rodent ACE2 by 1 µM DX600 was observed by Ye et al. Using 10 µM of the fluorogenic substrate 7Mca-APK(Dnp)-OH, they observed around 50% inhibition of the hydrolysis rate from rat kidney cortex with 1 µM DX600. Unless Bhatia et al. optimized the ACE2 reaction conditions to allow effective inhibition of rat kidney ACE2 with 1 µM DX600, the reported renal ACE2 activities are likely substantially underestimated.

Because the means of the ACE2 activities determined by Bhatia et al. were higher in female rats than male rats, although not statistically significant, it is conceivable that the full renal ACE2 activities would show a significant sex difference. To determine whether there are sex differences in renal ACE2 activity, an ACE2 activity assay that doesn’t underestimate ACE2 should therefore be used. Such an assay can be carried out with 10 µM 7Mca-APK(Dnp)-OH at pH 6.5 in the absence and presence of 10 µM DX600.

**DISCLOSURE**

The authors declared no conflict of interest.

**REFERENCES**


