Superoxide mediates excitatory actions of angiotensin II in the rostral ventrolateral medulla during acute stress

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Reactive oxygen species (ROS) are thought to be important intracellular mediators of angiotensin II (AII) actions in the brain (Zimmerman *et al*., 2002). We recently found that AII in the rostral ventrolateral medulla (RVLM) mediates the blood pressure (BP) response to emotional stress in rabbits (Mayorov & Head, 2003). In the current study, we examined the role of the superoxide radical ($\bullet O_2^-$) and nitric oxide (NO) in this action of AII in the RVLM.

We first evaluated the role of superoxide in the stress-induced neuronal excitation in the RVLM. We tested the cardiovascular response to airjet stress before and after injections of cell permeable superoxide dismutase (SOD) mimetics tempol, tiron or 3-carbamoyl proxyl (3-CP) into this region in conscious rabbits. Eight minute airjet stress evoked a sustained increase in BP (+12±2 mmHg). Bilateral microinjections of equimolar doses (20 nmol; n=7-9) of tempol, tiron or 3-CP into the RVLM did not alter resting BP. Tempol and tiron attenuated the pressor response to airjet by 57±12% and 52±8%, respectively. By contrast, 3-CP which is structurally similar to tempol but has a lower superoxide scavenging activity, did not alter the stress response. The SOD mimetics did not affect the renal sympathetic nerve activity (RSNA) baroreflex or the pressor response to local microinjection of glutamate.

In another series of experiments, we determined whether NO is important in mediating the circulatory stress reactions in the RVLM. Microinjections of NO donors, sodium nitroprusside or S-nitroso-N-acetylpenicillamine (1-20 nmol), dose-dependently increased BP, indicating that NO predominantly plays an excitatory role in the RVLM of the conscious rabbit. Microinjection of N(G)-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor (10 nmol), did not affect the pressor stress response, measured 10-20 min after injection. However, this response was diminished by 55±13% one hour later. Notably, L-NAME decreased the gain of the RSNA baroreflex by 38±12%, suggesting that NO is involved in modulating baroreflexes.

In further experiments, we tested whether the inhibitory action of tempol in the RVLM depends on local NO levels. Co-injections of L-NAME and tempol (n=4) did not affect resting BP, but attenuated the pressor stress response by 31±8%, indicating that the SOD mimetic acted, at least in part, via a NO-independent mechanism. Finally, we determined whether ROS in the RVLM mediate the pressor action of exogenously applied AII. Unilateral microinjections of AII (100 pmol) increased BP by 12±3 mmHg. Tiron and tempol attenuated by the pressor response to AII by 59-64%. By contrast, L-NAME tended to increase the pressor response to AII.

It is plausible that stress-induced activation of the AII – superoxide signalling pathway is not confined to the RVLM. We have found, in a pilot study, that microinjections of either AT_1-receptor antagonist candesartan (500 pmol) or the SOD mimetics into the dorsomedial hypothalamus also attenuated the pressor response to airjet by 30-46%.

Overall, these results suggest that the stress-induced neuronal excitation in the RVLM involves activating a redox sensitive signaling pathway in rabbits. Local superoxide, but not NO is critically important in mediating the acute pressor effects of emotional stress in rabbits. Together with our previous findings (Mayorov & Head, 2003), these results also indicate that superoxide is a key intracellular signaling molecule in the acute excitatory action of AII on the RVLM vasomotor neurons.

Mayorov & Head (2003). *Hypertension*, 41, 1168-1173.

Zimmerman, M.C., Lazartigues, C., Lang, J.A., Sinnayah, P., Ahmad, I.M., Spitz, D.R. & Davisson R.L. (2002). *Circulation Research*, 91, 1038-1045.