Ang-(3-4), Generated from Ang II through an ACE-dependent Pathway, Suppresses Inhibition of Renal Plasma Membrane Ca^{2+}-ATPase by Ang II

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We previously demonstrated that 10^{-10} M angiotensin II (Ang II) inhibits the Ca^{2+}-ATPase resident in the basolateral membranes of kidney proximal tubule cells (BLM) in an AT_1R and AT_2R dependent-pathway, whereas high Ang II concentration (5×10^{-7} M) led to the recovery of the Ca^{2+} pump with simultaneous formation of two Tyr-containing metabolites. Therefore our objectives were identify these metabolites, the angiotensin receptor(s) and the transdution cascade implicated in this reactivation process, as well as the peptidases involved in the metabolites generation. The HPLC analysis reveal that Tyr and angiotensin-(3-4) [Ang-(3-4)] retention times matches with those of angiotensin-derivatives and that the dipeptide blocks the Ca^{2+}-ATPase inhibition by Ang II ($pA_{1/2}$ ~15.5), an effect that was abolished by an AT_2R antagonist. Moreover, Ang-(3-4) seems to impair the inhibitory effect of Ang II through dissociation of constitutive AT_1R/AT_2R heterodimers, which are also preserved with 10^{-10} M Ang II. Since Ang-(1-7) was formed after 2 min of BLM incubation with 5×10^{-7} M Ang II, the first enzymatic route studied is the Ang II$\rightarrow$Ang-(1-7) conversion where a carboxypeptidase Plummer's-sensitive (CP-P's sensitive) appears to be the key enzyme. Following the sequence, it is demonstrated that the Ang-(1-7)$\rightarrow$Ang-(1-5) conversion depends on ACE activity and Ang-(1-5)$\rightarrow$Ang-(1-4)$\rightarrow$Ang-(3-4) depends on another CP-P's sensitive. The metabolization of Ang II also requires aminopeptidases and neprilysin activities. In summary, it can be concluded that BLM have a complete peptidases machinery able to form Ang-(3-4) – from Ang II – in the vicinity of the Ca^{2+}-ATPase that may act as a physiological regulator of active Ca^{2+} fluxes by modulating angiotensin receptors interactions and by activating an AT_2R-linked signaling cascade.

Key words: Ang-(3-4), angiotensin metabolism, basolateral membranes of kidney proximal tubules, plasma membrane calcium ATPase, peptidases
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