The Use of "Sniffer Cells" in the Detection of Angiotensin II Release in Brain Slices

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The brain is sensitive to Angiotensin (Ang) II and has all the precursors and enzymes required for synthesis. However, it is not known if the neurons in the brain release Ang II as a neurotransmitter. To address this question, Sniffer cells (Chinese Hamster Ovary [CHO] cells modified to express the AT1a receptors and GCaMP) were developed to detect Ang II. Introducing Ang II to the cell, AT1a receptors bind the Ang II and then cause the intracellular calcium concentration to increase. This increase in calcium causes the GCaMP in the cells to increase fluorescence. The CHO cells were plated on a glass cover slip and imaged on an inverted microscope where they were continuously perfused with artificial cerebral spinal fluid. CHO cell fluorescence was measured in response to varying concentrations of Ang II and its derivatives (Ang III, Ang 1-7 and Bradykinin). Fluorescence was measured in response to other common neurotransmitters such as glutamate and GABA. In response to Ang II administration, Sniffer cell fluorescence increased and this increase in fluorescence is specific to AT1a receptor activation. The AT1a dependent increases in fluorescence were dose dependent for Ang II and Ang III only. These experiments demonstrate that Sniffer cells adhere to the brain slice and detect extracellular Ang II *in vitro.* Based on these findings, Sniffer cells are a viable method to detect Ang II release and changes in release in response to experimental manipulation.

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