Development of nex microfluidic-amperometric device for monitoring dopamine secretion from PC12 cells suspension using a nanostructured microsensor

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Parkinson's disease is a degenerative disorder of the central nervous system. The motor symptoms of Parkinson's disease result from the death of dopamine-generating cells in the substantia nigra. The PC12 cells are used as an *in vitro* model for the study of age-related diseases associated to an impairment of the movement consequent to a deficit of DA (Fornai et al., 2007). In this study we developed a microfluidic device for the detection of dopamine (DA) secreted by PC12 cells using microdialysis and constant potential amperometry (CPA). The proposed system combines microdialysis technique with electrochemical detection. A dual channel in vitro apparatus, derived from a previously described design (Migheli et al., 2008), was coupled with multiwall carbon nanotubes (MWCNT) based microsensors for the flow-through detection of dopamine (DA) secreted from PC12 cells. Nanostructured microsensors were manufactured by deposition of solubilized MWCNT on top of epoxycarbon-based sensors or directly incorporated in the resin itself. Then microsensors were calibrated with different concentrations of DA. The microdialysis device was loaded with a solution containing PC12 cells while a constant phosphate-buffered saline (PBS) medium perfusion was carried out using miniaturized peristaltic pump. In a "treatment–control' experimental design, after a first period of stabilization and DA baseline recording, nicotine (from 0.125 mM to 1 mM), nicotinic antagonistic (mecamylamine 10 μM) and KCl (75 mM), both in presence or in absence of Ca^{2+}, were added to the perfusion fluid to capillaries in order to detect drugs-evoked and/or calcium-dependent DA secretion. The electronic circuitry was derived from previously published schematics and optimized for dual sensor constant potential amperometry applications (Migheli et al., 2008). The microdialysis system was tested and validated *in vitro* under different experimental conditions, and DA secretion was confirmed by high-performance liquid chromatography coupled with electrochemical detection (HPLC–EC). PC12 cell viability was quantified before and after each experiments by trypan blue assay. Nicotine exposure induced a non linear fitting (hyperbolic) DA secretion, suggesting a saturation of nicotine receptors at nicotine concentration higher than 0.4 mM. Mecamylamine administration prevented nicotine-induced DA release. KCl exposure, in either the presence or absence of extracellular Ca^{2+}, induced proportional DA secretion to extracellular K^+ and Ca^{2+} but also PC12 cell number. The proposed apparatus is suitable as a reliable model for studying the effects of different drugs on DA secretion through the direct comparison of extracellular DA increase in treatment–control experiments performed on the same initial PC12 cell population. The DA sensor may be easily replaced with different microsensors or biosensors that allow the *in vitro* detection of other important molecules, such as nitric oxide, glutamate, glucose and lactate.

Fornai et al .(2007). *Brian Research.*1129:174-190
Migheli et al. (2008) *Anal. Biochem*. 380, 323–330