A new approach to the real time monitoring of exogenous ethanol in brain of freely moving animals: development and characterization of an implantable biosensor

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Ethyl alcohol, or ethanol, may be considered one of the most widespread central nervous system (CNS) depressants in western countries. Moderate amounts of this beverage could reduce stress and increase feelings of happiness. In particular red wine may have positive effects on the heart and a low regular consumption may help in early outcome after acute infarct and diminish cardiac death risk. Despite these positives properties, ethanol is also known to induce physical damages and addiction when chronically and high consumed (Guiraud et al. 2004).
Ethyl alcohol is one of the most diffused psychotropic agents (Mehta and Ticku, 1999). Its psychoactive effects are mainly associated with the interaction with the GABAergic and glutamatergic systems, while the positive reinforcing properties are related to activation of mesolimbic dopaminergic pathways, resulting in a release of dopamine in the nucleus accumbens (Theile et al. 2011). Because of its toxicological and neurobiological implications, the detection of ethanol in brain extracellular fluid (ECF) is of great importance.
In this study we describe the development and characterization of an implantable biosensor for the amperometric detection of brain ethanol in real time. Ten different designs were characterized in vitro in terms of Michaelis–Menten kinetics (V_{MAX} and K_M), sensitivity (linear region slope, limit of detection (LOD), and limit of quantification (LOQ), and electroactive interference blocking. Starting from a fixed enzyme solution (Alcohol Oxidase, 200 U ml^{-1}), different approaches were used in order to identify the best biosensor design for in vivo-experiments: enzyme stabilizers as polyethyleneimmine or glycerol (or combinations of both) were used and also OPD polymer or different nets for entrapping enzyme layers (Rocchitta et al 2012)
Thus, all parameters were monitored in selected designs up to 28 days after fabrication in order to quantify their stability. Finally, the best performing biosensor design was selected for implantation in the nucleus accumbens and coupled with a previously developed telemetric device for the real-time monitoring of ethanol in freely moving, untethered rats.
Ethanol (1 g kg^{-1} i.g.) was then administered systemically to animals, either alone or in combination with ranitidine (30 mg kg^{-1} i. p.), an alcohol dehydrogenase inhibitor, while the biosensor signal was continuously recorded. The implanted biosensor, integrated in the low-cost telemetry system, was demonstrated to be a reliable device for the short-time monitoring of exogenous ethanol in brain ECF and represents a new generation of analytical tools for studying ethanol toxicokinetics and the effect of drugs on brain ethanol levels
Afterwards, a further *in-vitro* characterization of the biosensor was performed. With the aim of enhancing ethanol biosensor performance, different enzyme loadings were investigated in terms of above-mentioned parameters. As well as in the previous study, the responses of biosensors were studied over a period of 28 days. At the same time, a study on the oxygen dependence was performed in order to identify the most suitable biosensor design for in vivo experiments. Besides, a further characterization about pH dependence was conducted, in order to complete its validation.
The overall findings confirm the original biosensor design to be the best of those investigated for *in vivo* applications up to one week after implantation.

References:

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