IMOP ANR 07-NEURO-016-01

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BIOLOGIE & SANTÉ 2011

In vivo cellular dynamics of opioid receptors

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Context

Drug addiction is a chronic relapsing disorder, resulting from a gradual adaptation of the brain to repeated drug exposure. The opioid system is a major player with mu and delta receptors contributing to very distinct aspects of addictive behaviors. Mu receptors represent the primary target for both therapeutic and adverse activities of morphine *in vivo* and mediate reinforcing properties of non-opioid drugs of abuse (cannabinoids, alcohol and nicotine). Delta receptors on the other hand are involved in many behaviors relevant to drug abuse, including the modulation of basal emotional states, and a possible implication in context-induced craving and relapse.

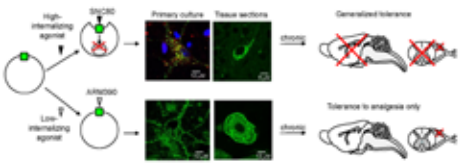
Objectives

Identify changes in receptor localization, dynamics and function to understand adaptations of the endogenous opioid system using novel knock-in mice models expressing a functional fluorescent delta (DOR-eGFP) or mu (MOR-mcherry) receptor.

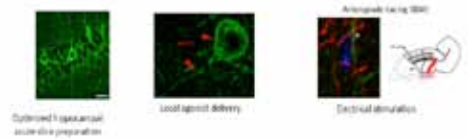
Results

I. In vivo physiological dynamics in context-induced withdrawal.
Using DOR-eGFP mice, endogenous activation and subsequent *in vivo* internalization of a G protein-coupled receptor were for the first time directly visualized under physiological conditions. Morphine was repeatedly administered in a given environment at a dose inducing physical dependence. This paradigm elicited context-induced withdrawal upon re-exposure of drug-free animals and led to activation of the hippocampus. Subsequent DOR-eGFP activation and internalization were detected in a small subset of CA1 neurons uncovering spatially and temporally controlled opioid peptide release. In addition, the internalization profile was distinct from the one previously described upon pharmacological stimulation.

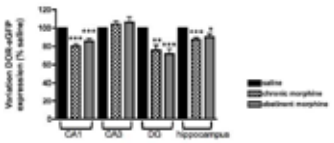
II. In vivo dynamics following pharmacological stimulation.
In our DOR-eGFP mice, the prototypic delta agonist SNC80, produced robust receptor internalization that ultimately resulted in a transient acute behavioral desensitization, which was directly correlated with receptor internalization and G-protein uncoupling. In contrast to SNC80, treatment with the non-internalizing delta agonist ARM390 did not produce behavioral desensitization, and delta receptors remained coupled on the cell surface. As was anticipated from cellular studies, chronic treatment with SNC80 also resulted in widespread receptor degradation, leading to generalized behavioral tolerance to agonist effects. Chronic treatment with ARM390 also produced tolerance despite any obvious changes to the receptor. In addition, ARM390-tolerant animals normally responded to other delta agonist-induced behavioral effects. These data illustrate biased agonism *in vivo*, a concept according to which distinct agonists acting at the same receptor can engage different active receptor conformations leading to agonist-dependent signaling or regulatory responses.



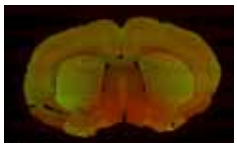
III. Ex vivo real-time dynamics in a neuronal integrated network.
To date, information about delta opioid receptor dynamics were almost exclusively collected in heterologous systems. DOR-eGFP mice enable real-time visualization with subcellular resolution of an endogenous receptor expressed at physiological level. We addressed real-time receptor dynamics in a context that maintains neuronal connectivity to determine how receptor activation and trafficking affect neuronal activity and plasticity. In optimized acute hippocampal slice preparation, we compared receptor trafficking initiated by local agonist delivery to dynamics induced by endogenous peptide release promoted by electrical stimulation that mimics physiological conditions. This will lead towards identification of the molecular determinants underlying receptor trafficking.



IV. Long-lasting effect of chronic morphine on delta receptors
Fine mapping of receptor distribution in DOR-eGFP mice showed for the first time that chronic morphine induces long-lasting changes in the number of hippocampal delta receptor-expressing cells. These modifications likely impact on hippocampal physiology, and may influence delta receptor-mediated regulation of drug-context association or drug seeking behavior.



V. 3D mapping of mu and delta fluorescent receptors in the CNS
Knock-in mice expressing mu receptors in fusion with a red fluorescent protein (MOR-mcherry) were generated and characterized. These mice were crossed with DOR-eGFP mice. The distribution of both receptors through the central nervous was studied in details to provide the first on -ine 3D reference atlas.



Conclusion and Perspectives

Our novel model of fluorescent mice has proven a unique tool to tackle opioid receptor trafficking after physiological or pharmacological stimulation. It has led to uncovering novel aspects that will bring better insights into the molecular mechanisms underlying opiate action.

Publications

- Pradhan A.A. *et al.* (2009) *In vivo delta opioid receptor internalization controls behavioral responses to agonist.* PLoSOne 4, 1-11
- Pradhan A.A. *et al.* (2010) *Ligand-directed trafficking of the delta opioid receptor in vivo:two paths towards analgesic tolerance. J. Neurosci. 30,16459-16468*
- Pradhan A.A. *et al.* (soumis) *The delta opioid receptor: an evolving target for the treatment of brain disorders.*
- Faget L. *et al.* (soumis) *Direct visualization of delta opioid receptor internalization under physiological conditions.*
- Erbs E. *et al.* (soumis) *Chronic morphine durably decreases delta opioid receptor in the mouse hippocampus.*
- Rezaï X. *et al.(* in preparation) *Delta opioid receptor real-time dynamics in brain slices.*
- *Erbs E. et al.* (in preparation) *Mu and delta opioid receptor 3D mapping in the mouse central nervous system.*

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