PRESYNAPTIC GLP-1 RECEPTORS ENHANCE GLUTAMATE AND GABA RELEASE FROM PURIFIED MOUSE CORTICAL AND HIPPOCAMPAL SYNAPTOSOMES

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Glucagon-like peptide 1 is a hormone belonging to the incretin family that has been extensively investigated for its peripheral effects on the regulation of glucose homeostasis (Cho et al., 2014). This hormone acts by stimulating GLP-1 receptors (GLP-1R) that belong to the family of G protein coupled receptors able to stimulate adenylyl cyclase activity. In recent years, the GLP-1/GLP-1R system in the central nervous system has attracted the attention of neuroscientists, especially for its pro-mnesic and neuroprotective effects that could be therapeutically exploited in different neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD; Holscher, 2014; Tramutola et al., 2017). On the basis of these results, the GLP-1R agonists exenatide and liraglutide have been trialled in small cohorts of AD and PD patients with promising results (Aviles-Olmos et al., 2013, 2014; Gejl et al., 2016).

Despite these clear-cut evidences, little is known on the physiological effects of GLP-1R on central neurotransmission, especially at the presynaptic level.

In this view, we have investigated the functional effects of GLP-1R activation on the release of glutamate and GABA, respectively the major excitatory and inhibitory neurotransmitters of the CNS.

To this purpose, we have used the technique of purified isolated nerve terminals (synaptosomes) in superfusion. Synaptosomes were obtained from homogenised mouse cerebral cortex or hippocampus and purified on Percoll® gradients essentially according to Nakamura et al. (1993). Purified synaptosomes have been used for release studies, using [3H]D-aspartate or [3H]GABA as markers of glutamatergic and GABAergic nerve terminals, and for western blot and immunofluorescence analyses to further confirm the presence of presynaptic GLP-1Rs.

Western blot analysis revealed the presence of GLP-1Rs in mouse cortical and hippocampal synaptosomal preparation.

During chemical depolarization with 15 mM KCl, exposure of cortical purified synaptosomes to increasing concentrations of the GLP-1R selective agonist exendin-4 (1-30 nM) caused a significant enhancement of [3H]D-asp release (maximal effect 36,81□4,52% at 30 nM). The effect of exendin-4 (10 nM) was completely prevented by the selective GLP-1R antagonist exendin-3 (10 nM) and by the adenylyl cyclase inhibitor 2',5'-dideoxyadenosine (10 □M). Under the same experimental conditions, also the KCl-induced release of [3H]D-asp in the hippocampus was increased by exendin-4 in a concentration- (35,22□2,95% at 10 nM, 43,64□13,70 at 30 nM) and exendin3-dependent manner.

Similarly, the KCl-induced release of [3H]GABA was enhanced both in purified cortical and hippocampal synaptosomes by exendin-4 and its effect was prevented by exendin-3 or the adenylyl cyclase inhibitor 2',5'-dideoxyadenosine.

Using anti-GLP1-R, anti-synaptophysin, anti-VGLUT1 and anti–VGAT antibodies, our confocal microscopy analysis demonstrated that GLP-1Rs are present on 40% of cortical and on 20% of hippocampal glutamatergic nerve terminals, whereas they are localized on 17% of cortical and on 34% of GABAergic nerve endings.

In conclusion, our data show for the first time that GLP-1 receptors are localized at the presynaptic level onto cortical and hippocampal glutamatergic and GABAergic synaptic boutons and their activation lead to the increase of the release of the two neurotransmitters. These presynaptic receptors could represent additional mechanisms through which the neurohormone GLP-1 exerts its effects in the CNS.

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