REGULATION OF PHOSPHORYLATED FORMS OF FADD, MAPKs, AND PEA-15 IN THE PREFRONTAL CORTEX OF HUMAN OPIOID ABUSERS

A. Ramos-Miguel¹, M.J. García-Fuster¹, L.F. Callado², R. La Harpe³, J.J. Meana², J.A. García-Sevilla¹

¹Laboratorio de Neurofarmacología, IUNICS, Universidad de las Islas Baleares, and RETICS, Spain
²Departamento de Farmacología, Universidad del País Vasco, and CIBERSAM, Spain
³Centre Romand de Médicine Légale, Université de Genève, Switzerland

PURPOSE

The phosphorylation status of Fas-associated death domain (FADD) protein has been proposed as a molecular target in opioid-induced neural plasticity in rodent models [1]. The principal aim of this study was to evaluate the regulation of p-Ser194 FADD (p-FADD) in brains of opioid abusers who had died of an opiate overdose. In addition, alterations in various signaling pathways involved in neuroplasticity (MAPKs, mitogen-activated protein kinases; PEA-15, phosphoprotein-enriched in astrocytes; Akt, the kinase that mediates PEA phosphorylation) (see Fig 1) were investigated.

RESULTS

In the prefrontal cortex of opioid abusers, the immunodensity of monomeric p-FADD (23 kDa) was increased (all addicts: 51%, n=24; ST: 23%, n=10; LT: 76%, n=14) compared with that in sex-, age-, and PMD-matched controls (n=20), being this up-regulation more remarkable in LT addicts (LT vs ST: 43%) (Fig 3A). Oligomeric p-FADD (116 kDa) was also increased but to a lesser extent (all addicts: 27%; ST: 36%; LT: 20%) (Fig 3A). At the subcellular level (one selected LT addict) monomeric p-FADD was only localized in the nucleus and its content was increased, whereas oligomeric p-FADD showed increases in the cytosol and nucleus (Fig 3B). The contents of p-ERK1/2 and p-JNK MAPKs were decreased in LT abusers (54% and 43% respectively), and that of activated p38 MAPK in ST abusers (55%) (Fig 4). The phosphorylation (Ser116) of PEA-15, a protein linking ERK and FADD [3], was also reduced (all addicts: 34%; ST: 33%; LT: 36%), as well as the amount of p-Akt in LT abusers (30%) (Fig 4).

CONCLUSIONS

- The phosphorylation of monomeric FADD (and the induction of protein oligomerization) may participate in the mechanisms of synaptic plasticity involved in the development of opiate addiction.
- The observed alterations of the canonical MAPK pathways further demonstrated their relevant roles in opioid-induced neuroplasticity [2].
- Finally, p-Ser116 PEA-15, a protein involved in ERK-FADD interaction, could represent a novel molecular target in opiate addiction.

METHODS

Specimens of the right prefrontal cortex (BA 9) were collected from 24 well-defined opioid abusers (14M/10F; 36±2 yr; 20±2 PMD) and 20 healthy matched-controls (15M/5F; 36±2 yr; 20±2 PMD; with a negative blood toxicology). Postmortem delays (PMD) longer than 40 h did not allow the quantification of some phospho-proteins (Fig 2). All opioid abusers showed the presence of fatal concentrations of morphine (0.47±0.09 µg/ml) or methadone (0.72±0.18 µg/ml) in blood samples. The quantification of opiates and metabolites in hair samples was used as an indicator of short-term (ST, absence of opiates) or long-term (LT, presence of opiates) opiate abuse [2]. The target proteins were quantified by Western immunoblot analyses with specific antibodies.

REFERENCES