THE SEVERITY OF MORPHINE ABSTINENCE CORRELATES WITH BRAIN FADD PHOSPHORYLATION: INTERACTION WITH α₂-ADRENOCEPTORS

A. Ramos-Miguel, A. Miralles, J.A. García-Sevilla
Laboratorio de Neurofarmacología – IUNICS, Universitat de les Illes Balears, and RETICS – Red de Transtornos Adictivos, Palma de Mallorca, Spain

INTRODUCTION
The phosphorylation status of Fas-associated death domain (FADD) protein has recently been implicated in various forms of neuroplasticity in brains of chronic morphine-treated rats [1,2] and postmortem brains of long-term opiate abusers [3]. To further explore the role of multifunctional FADD in the molecular mechanisms of morphine-induced physical dependence, the regulation of cortical phosphorylated (p-) FADD and its interaction with inhibitory α₂-adrenoceptors were assessed during the induction of spontaneous opiate withdrawal (SW) in morphine-dependent rats.

RESULTS
In morphine-dependent rats, SW induced a severe behavioral syndrome that peaked around 24 h and declined gradually up to 96 h (figure 2A). In the cerebral cortex of morphine-withdrawn rats, the content of oligomeric p-FADD, but not total FADD, was upregulated during the time course of SW (33-45% at SW 12-24 h) (figure 2C). Notably, there was a striking positive correlation between abstinence severity (behavioral scores) and cortical oligomeric p-FADD content (Spearman correlation coefficient: 0.59, n = 39, p < 0.0001) (figure 2C). It is well known that the activation of inhibitory α₂-adrenoceptors suppresses morphine abstinence symptoms in rodents. Accordingly, the selective inactivation of brain α₂-adrenoceptors (EEDQ) at SW 12 h markedly enhanced the intensity of morphine abstinence at SW 24 h (52% over vehicle-treated rats, p < 0.01) (figure 3B). Concomitantly with the potentiation of morphine abstinence, α₂-adrenoceptor inactivation further increased cortical oligomeric p-FADD content (34% over vehicle-treated rats, p < 0.05) (figure 3B).

METHODS
Groups of rats (adult male Sprague-Dawley) were chronically treated with escalating doses of morphine (10-100 mg/kg, i.p.), three times daily, for 6 days. Then, some rats (groups of n = 4-12) were left undisturbed for 2, 12, 24, 48, 72 and/or 96 h (SW), and others (n = 8) were treated with the α₂-adrenoceptor alkylating agent EEDQ (low dose of 1.6 mg/kg, i.p.) at SW 12 h, and target proteins were assessed at SW 24 h (see figure 1). Control rats received saline or vehicle solutions in parallel (n = 13-8). In chronic morphine-withdrawn rats (SW 2-96 h) some classical behavioral signs were checked (hostility, lacrimation, diarrhea) or counted (wet-dog shakes, teeth chattering, exploring behaviors) for a 10-min period, to evaluate the intensity of morphine SW. After the behavioral assays, the rats were killed by decapitation and the brains immediately removed to dissect the cerebral cortices. The contents of oligomeric and monomeric p-ser194 FADD in the cortical samples were assessed by Western blot analyses with specific antibodies. Results are expressed as means ± SEM of n rats per group. Statistical analyses of data from the behavioral (Kruskal-Wallis) and neurochemical (one-way ANOVA) assays were followed by Dunn’s or Bonferroni’s post hoc test respectively. In all figures, *p < 0.05; **p < 0.01; ***p < 0.001 when compared with the control group.

CONCLUSIONS
• These data suggest that the non-apoptotic/neuroplastic actions of p-FADD in brain are involved in the final molecular mechanisms leading to the expression of physical dependence on morphine in rats. Thus, cortical oligomeric p-FADD may play a functional role in the behavioral expression of morphine abstinence.
• α₂-Adrenoceptors had a crucial role in regulating simultaneously the intensity of morphine abstinence and the content of p-FADD in the cerebral cortex.

REFERENCES

DISCLOSURE
The authors state no conflict of interest.

Supported by SAF2008-01311 and RETICS RD06/001/003 (MICINN-FEDER, Spain).