L-acetylcarnitine causes antidepressant-like effect mediated by an enhanced acetylation in the transcription machinery: a new epigenetic path to the treatment of major depression

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INTRODUCTION
Stressful events occurring during critical periods of brain development trigger an enduring epigenetic program that is ultimately responsible for the low resilience to stress and the low threshold to associated with major depression. The term "epigenetics" refers to long-lasting changes in chromatin structure underlying modifications in gene expression that are not due to alterations in DNA sequence. Studies on models of chronic stress and depression have shown that L-acetylcarnitine (LAC) causes analgesia by acetylating p65 (a member of the NFκB family of transcription factors), thereby enhancing the expression of type-2 metabotropic glutamate receptors (mGlu2/3) in the dorsal horn of the spinal cord. This mechanism is shared by a number of HDAC inhibitors, including MS275 and SAHA. We examined the effect of LAC on depression-like behaviour using two relevant animal models. Here, we show the antidepressant effect of LAC that was observed 3 days after, and became more robust after two and three weeks of treatment. This temporal profile differed from that exhibited by the classical antidepressant, fluoxetine, which exhibited antidepressant effects only after 3 weeks of treatment.

MATERIALS AND METHODS
Materials: L-ac was synthesized and provided by Sigma Tau Laboratories (Milan, Italy). LAC (100mg/ml PBS) was purchased from Acros (Avonmouth, Bristol, UK)

Animal models: spontaneously depressed FSL rats and mice exposed to chronic unpredictable stress (CUS) were used. Both models of depression were performed by 24 h/day stress for 6 hours per day. Treatments: different groups of animals were treated intraperitoneally (i.p.) with or without LAC (100mg/ml PBS) once daily for 24 days and LY341495 (mGlu2/3 receptor antagonist) was administered i.p. 30 min prior to each LAC treatment.

Control animals were treated with saliné.

Forced swim test: we used the Porsolt's test to measure the duration of immobility, a behavioural correlate of negative mood. Animals were placed individually in a vertical glass cylinder (60 cm in height, 30 cm in diameter) filled with 30-cm-deep water (23–24°C). After 15 min (habitation session), animals were dried and returned to their home cages. The animals were replaced in the cylinder 24 h later, and the total duration of immobility in a 5-min observation period was recorded (test session).

Sucrose preference test: for 24 h, rats and mice were given a free choice between two bottles, one with 2% sucrose solution and the other with water. To prevent possible effects of side preference in drinking behavior, the position of the bottles was switched after 12 h. No previous water deprivation was applied before the test. The consumption of water and sucrose solution was estimated simultaneously in control and experimental groups by weighing the bottles.

cDNA synthesis and real-time PCR: RT-PCR analysis was performed by using the following primers: mGlu2: forward, ACCACCATGTCCTGTTAGGA; reverse, ACCCGGCTCTTGACCTGCA; mGlu3: forward, GGACCTGTGTTGCTGACCA; reverse, AACAACCCCTGTGTAAC3'-actin: forward, AGACCTGTGTTGAGTACG; reverse, TCCGGAAGACCTGTGCA.

Western blot analysis: brain tissues were homogenized at 4°C in ice-cold 0.1% SDS/lipid buffer containing protease inhibitors (1 mM PMSF, 1µg/ml aprotinin and 1 µg/ml leupeptin) and phosphate inhibitors (1 mM NaF, 1 mM NaVO₄ and 1 mM glycerol-2-phosphate) with a motor-driven Teflon–glass homogenizer (1700 rpm). Homogenates were collected at 13000 g for 4°C for 30 min and the supernatants were used for protein determinations. Samples containing 30 µg protein were resuspended in SDS–bromophenol blue reducing buffer with 40 mM dithiothreitol to limit the formation of receptor aggregates. The biochemical analysis was carried out using SDS-polyacrylamide gels and selective antibodies.

Release experiments on synaptosomes: after biochemical process, identical portions of the synaptosomal suspensions were superfused at 1 ml/min with standard physiological solution saturated with 95% O₂ and 5% CO₂ at 37°C containing 10 mM α-ketoglutaric acid to prevent GABA metabolism. Synaptosomes were first equilibrated during 36 min of superfusion, and then the consecutive 3 min fractions were collected. Synaptosomes were exposed transiently (90 s) at 1 min of superfusion to the depolarizing stimulus (120mM K⁺) and then resuperfused with the standard solution to control the concentration of K⁺. Superfusate samples were collected, and the endogenous glutamate and GABA content in each superfusate fraction was monitored by fluorometric analysis using a fluorescent N-(2-phenylphényl)carbodiimide and HPLC separation as described above.

Chromatin immunoprecipitation: analysis of mGlu2 and BDNF gene promoters was assessed by chromatin immunoprecipitation. Cross-link between proteins and DNA was obtained with 1% formaldehyde and chromatin immunoprecipitation was performed by a commercial kit (Upstate Cell assay kit) and a chromatin immunoprecipitation anti-acetylated H3 antibody (1:500). For real-time PCR analysis of immunoprecipitation chromatin we used specific primers for the mGlu2 and BDNF gene promoters.

BDNF incorporation and immunostaining: mice were injected with BDNF (2 µg) i.p. and sacrificed 24 h later. Brains were removed, post-fixed overnight in 4% PFA, and then transferred in 30% sucrose for cryoprotection. Serial 30 µm coronal sections were obtained from the frontal cortex of the hippocampus using a cryostat and stained for BDNF immunostaining using the following antibodies: mouse monoclonal anti-BDNF (1:20) and biotinylated anti-mouse antibodies (1:200). Immunoreactivity was detected using ABC Elite Kit (Vector Laboratories). Control staining was performed without the primary antibodies.

CONCLUSIONS
1. These data show for the first time that LAC, a drug marketed for the treatment of neuropathic pain, behaves as a putative antidepressant in a rat genetic model of depression and in a mouse environmental model of depression.
2. The action of LAC is mediated in part by an up-regulation of mGlu2 receptors and, perhaps, BDNF levels
3. LAC has an impact on the epigenetic regulation of the mGlu2 and BDNF gene promoters
4. Our data support the idea that enhancing hippocampal neuronal proliferation can serve to boost or augment the antidepressant response
5. Our data are supporting ongoing clinical studies which suggest the use of LAC as a novel add-on drug in the treatment of unipolar depression and dysthymia

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Values are mean ± S.E.M. of 8 animals per group. Statistical analysis was performed with One-Way ANOVA + Dunnett test.