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 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 acute and chronic pain have shown that L-acetylcarnitine (LAC) causes analgesia by acetylating p65 (a member of the NFkB family of trascription factors), thereby enhancing the expression of type-2 metabotropic glutamate (mGlu2) receptors in the dorsal horns 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 visible after 3 days, and became more robust after two and three weeks of treatment. This temporal profile differed from that exhibited by the classical antidepressant, which showed antidepressant-like effects only after 3 weeks of treatment.

MATERIALS AND METHODS

Materials:L-ac was synthesized and provided by Sigma Tau Laboratories (Pomezia, Italy); LY341495 was purchased from Tocris Cookson (Avonmouth, Bristol, UK)

Animal models: spontaneously depressed FSL rats and mice exposed to chronic unpredictable stress (two times in a day, spaced out of 6 hours)

Treatments: different groups of animals were treated intraperitoneally (i.p.) once daily with LAC (100mg/kg) for 21 days and LY341495 (1mg/kg) alone and in combination with LAC on the 22nd day of the experiment. Control animals were treated with saline

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 into a vertical glass cylinder (60 cm in height, 30 cm in diameter) filled with 30-cm-deep water (23–24° C). After 15 min (habituation session),animals were dried and returned to their home cages. The animals were replaced in the cylinder 24 h later, and the total period 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 another with water. To prevent possible effects of side preference in drinking behavior, the position of the bottles was switched after 12 h. No previous food or 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 using the following primers: mGlu2: forward, AGCACCTTTGCTGGTTAGGA, reverse, ACCCGAGCTCTTCAGACTCA; mGlu3:forward,GCAGTTTGTCTTGGTCAGCA,reverse,AACACACCCTT GGTCAAAGC;β-actin: forward, AGCACTGTGTTGGCGTACAG, reverse, AGAGCTACGAGCTGCCTGAC.

Western blot analisys:brain tissues were homogenized at 4° C in ice-cold 0.1% SDS-lysis buffer contaning protease inhibitors (1 mM PMSF, 1µg/ml aprotinin and 1 µg/ml of leupeptin) and phosphatase inhibitors (1mM NaF, 1 mM Na_3VO_4 and 1mM glycerol-2-phosphate) with a motor-driven Teflon-glass homogenizer (1700 rpm). Homogenates were centrifuged at 13000 rpm, 4° C, for 20 min and the supernatant was used for protein determinations. Samples containing 30µg 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**.**

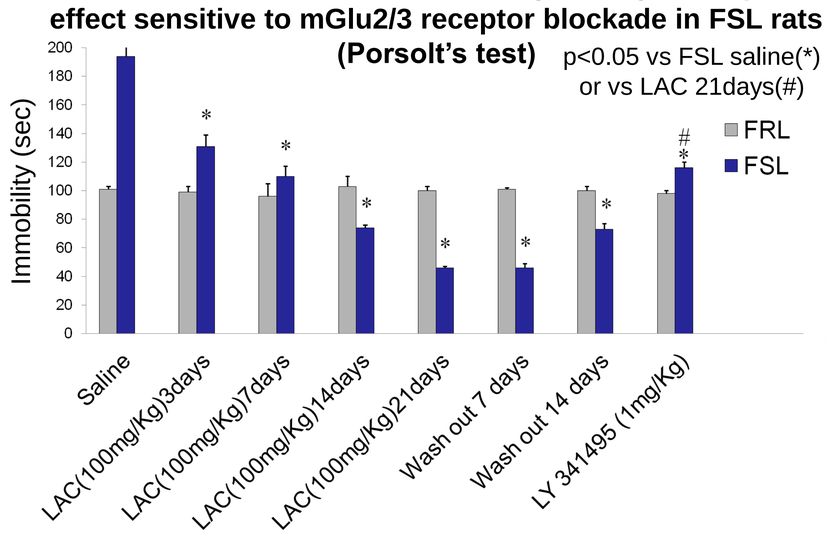
Release experiments on synaptosomes: after biochemical process, identical portions of the synaptosomal suspensions were superfused at 0.5 ml/min with standard physiological solution aerated with 95% O_2 and 5% CO_2, at 37° C containing 10 µm aminooxyacetic acid to prevent GABA metabolism. Synaptosomes were first equilibrated during 36 min of superfusion, and then four consecutive 3 min fractions were collected. Synaptosomes were exposed transiently (90 s) at *t* = 39 min of superfusion to the depolarizing stimuli (12mM K^+) and then resuperfused with standard medium. LY379268 was added concomitantly with K^+. Superfusate samples were collected, and the endogenous glutamate and GABA content in each superfusate fraction was monitored by fluorometric detection after *o*-phthalaldehyde derivatization 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 ChIP assay kit) and a chromatin immunoprecipitation anti-acetyl H3 antibody (1:500). For real-time PCR analysis of immunoprecipitated chromatin we used specific primers for the mGlu2 and BDNF gene promoters.

BrdU incorporation and immunostaining: mice were injected with BrdU (Sigma; 2 i.p. injections of 50 mg/kg, two times in a day for three days with 6 h of interval) and killed 24 h later. Mice were anesthetized with chloralium hydrate (320 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 8. 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 whole rostrocaudal extent of the hippocampus and processed for BrdU immunostaining using the following antibodies: mouse monoclonal anti-BrdU (1:20) and biotinylated anti-mouse antibodies (1:200). 3,3-Diaminobenzidine tetrachloride was used for detection (ABC Elite kit). Control staining was performed without the primary antibodies.

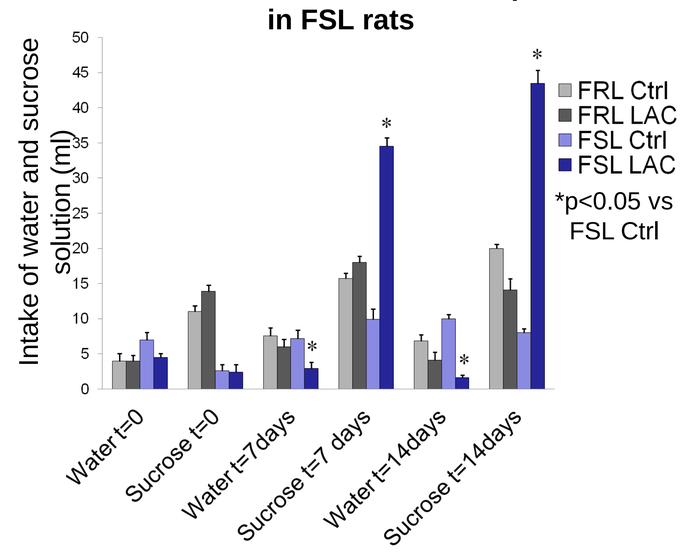
Values are mean + S.E.M. of 8 animals per group. Statistical analysis was performed by One-Way ANOVA + Dunnett test

1 LAC treatment causes a fast and long-lasting antidepressant effect sensitive to mGlu2/3 receptor blockade in FSL rats (Porsolt’s test)

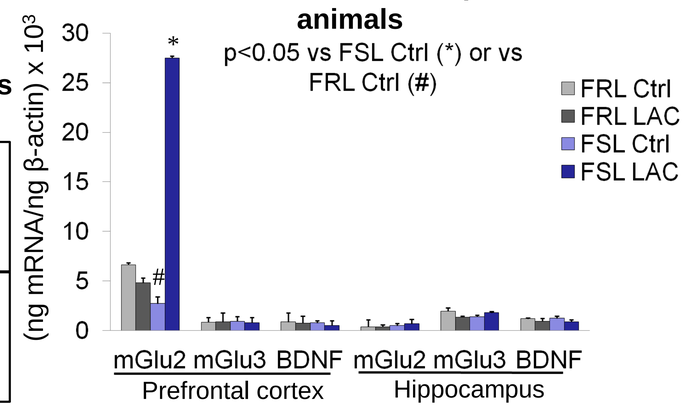


Systemic treatment with LAC (100 mg/kg, i.p., once a day for 21 days) significantly reduced the immobility time at the forced swim test after 1 week of treatment, when conventional antidepressants drugs are usually inactive. The antidepressant-like activity of LAC was maintained for at least 3 weeks. Its action was attenuated by the mGlu2/3 receptor antagonist, LY341495 (1 mg/kg, i.p.)

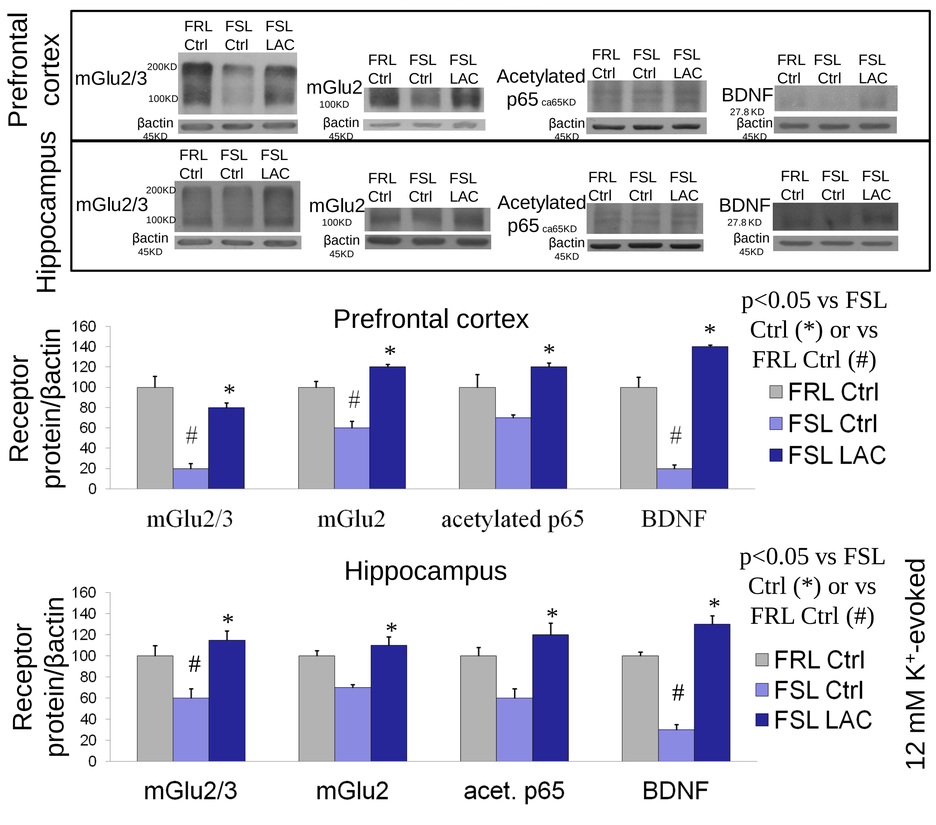
2 LAC treatment enhances sucrose preference in FSL rats



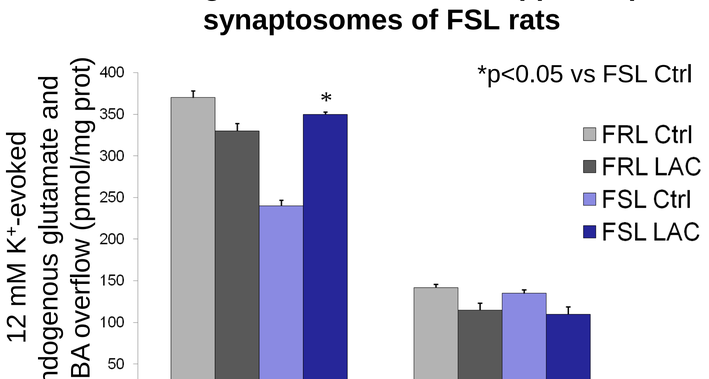
3 LAC treatment increases the levels of mGlu2 mRNA in the frontal cortex of depressed treated animals



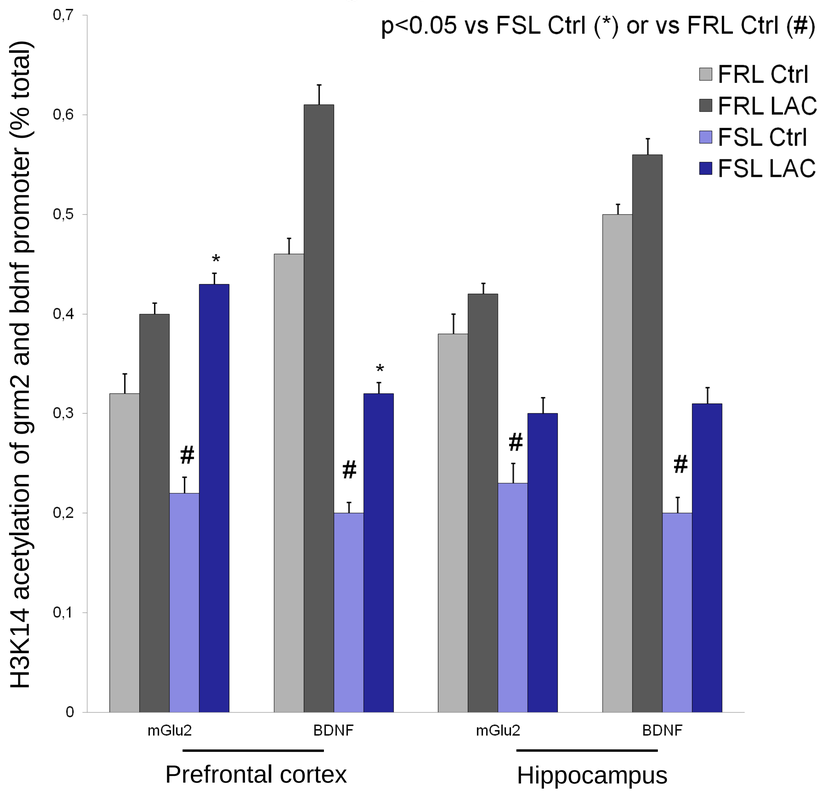
4 LAC treatment enhances the expression of mGlu2/3 receptors, acetylated p65, and BDNF in the prefrontal cortex and hippocampus of FSL rats



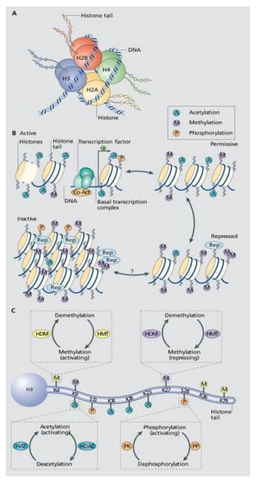
5 LAC treatment reverses the deficit in the evoked release of glutamate seen in hippocampal synaptosomes of FSL rats and no changes in GABA release hippocampal synaptosomes of FSL rats



6 LAC enhances H3K14 histone acetylation at the grm2 and bdnf gene promoters



Chromatin immunoprecipitation



Epigenetic changes are mediated by methylation of CpG islands of gene promoters and histones, which can either suppress or activate gene expression, and acetylation of histones or transcription factors, which always activates gene expression. A critical role for acetylation in the pathophysiology of depressive disorders is suggested by the evidence that antidepressant treatment enhances histone acetylation, and inhibitors of histone deacetylases (HDACs) relieve depressive symptoms in experimental animal models

7 In FSL rats, a 7-day treatment with LAC increases the number of proliferating neuroprogenitors in the hippocampus, as shown by BrdU immunostaining

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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No potential conflict of interest