Documenta Ophthalmologica 70: 89–96 (1988)

Effects of intravenous L-acetylcarnitine on retinal oscillatory potentials

WALTER G. SANNITA,[1,2] LUISA LOPEZ,[1] LINO MAGGI[1] & GUIDO ROSADINI[1]

[1]Center for Neuropsychoactive Drugs, Institute of Neurophysiopathology, University; Center for Cerebral Neurophysiology, National Council of Research; Genova, Italy; [2]Department of Psychiatry and Behavioral Sciences, State University of New York, Stony Brook, N.Y., USA

Key words: drug plasma concentration, flash full-field stimulation, L-acetylcarnitine, retinal oscillatory potentials

Abstract. L-acetylcarnitine is a compound with cholinergic properties and putative action on the visual system and the glucose metabolism. Ten healthy, emmertropic volunteers (age range: 21 to 28 years) were studied. Each subject was administered 5, 10, and 30 mg/kg acute intravenous doses of L-acetylcarnitine and matching placebo. Retinal oscillatory potentials to full-field flash stimulation were recorded before and 30, 60, and 120 min after administration. A systematic reduction of the implicit time of the P2 and N2 oscillatory potential components was observed after administration of the 10 and 30 mg/kg doses: significant changes were not evident at the 5 mg dose or after placebo. The latency reduction was significantly correlated with the postdrug increment of the L-acetylcarnitine plasma concentration. No other systematic modification in latency of amplitude was observed.

Introduction

L-acetylcarnitine is produced in vivo in normal liver and brain, has a cholinergic effect on peripheral receptors, and increases activity of adenosine 5′-triphosphate; there is some evidence for a role in central nervous system neurotransmission and regulatory functions in fatty acid oxidation [1,2,3,4]. The administration of L-acetylcarnitine or its precursor carnitine to insulin-dependent diabetic patients allows the control of glucose blood levels with diet only or with insulin intake significantly lower than in untreated patients, and carnitine plasma concentration is itself reduced in diabetic patients [5,6,7].

Therapeutic potentialities in diabetes are conceivable; and diabetic com-

The results were presented in part at the XXV I.S.C.E.V. Symposium, Sarasota (Florida), April 26–30, 1987.

plications of the visual system are a possible indication, as L-acetylcarnitine is reported to be active on the visual system [8] and to counterbalance retinal modifications induced by aging [9]. An electrophysiological study was performed in healthy volunteers to verify the effect of L-acetylcarnitine on retinal oscillatory potentials (OPs), which display a peculiar sensitivity in the diagnosis of diabetic retinopathies [10,11,12,13,14,15,16], and to provide guidelines for further studies on patients.

Methods

Ten young, healthy male volunteers, ranging 21 to 28 years (mean: 24.3 +/ − 1.8 yrs) in age and 63 to 80 kg (mean: 67 +/− 3.9 kg) in weight, were recruited among a student population, after ruling out any evidence or history of relevant systemic or ocular diseases, drug allergy, or drug dependence. The screening electroretinogram (ERG) and OPs were in all cases normal; visual acuity was not worse than 9/10 and ametropia did not exceed 0.5 diopters. All subjects had been previously included in other neuropharmacological studies and were fully acquainted with laboratory setting, experimental design, recording procedures, and investigators. They abstained from taking any psychoactive compound in the 72-hour period preceding each experimental session and in the 24 hours following it. Four sessions were run at weekly intervals beginning at 8.30 a.m. after a normal night sleep and at least 90 min after a light standardized meal. After baseline determinations each volunteer was administered a single 5, 10, or 30 mg/kg dose of l-acetylcarnitine or a matching placebo. Administration was by intravenous infusion (3 min) and double blind according to a balanced order design. Postdrug recordings were at 30, 60, and 120 min after drug or placebo administration. Volunteers were informed about the characteristics of the compound they were administered and signed written consent.

Flash-evoked retinal OPs were recorded by Ag/AgCl dermal electrodes positioned symmetrically on lower eyelids (reference positioned on linked mastoids; ground electrode at the vertex) and maintained on location during each experimental session. Full-field flash stimuli (photostimulator Grass PS22; light intensity: 140 apostilb at the eye level) were presented to eyes open and with pupils undilated. Eye movements were monitored online with a TV infrared-sensitive camera, and 15 artefact-free epochs were averaged. Amplifiers were set at 160–1000 Hz to minimize contamination from lower frequency potentials (e.g. electroencephalographic or muscle activity). Analog to digital conversion was at 50 KHz, on 100 msec epochs, with final

resolution at 5.12 sample/msec (OTE-Biomedica Neuroaverager 1239 system). Methods are described in detail elsewhere [17].

Blood samples were collected in baseline conditions and after drug or placebo administration, after each OP recording. Plasma was extracted by centrifugation, and L-acetylcarnitine was essayed by radioimmunoassay methods [18].

Amplitude (peak-to-peak) and implicit time values were defined for eight OP wavelets, hereafter defined P(ositive)1, N(egative)1, P2, N2, P3, N3, P4, and N4 depending on polarity and sequence of appearance [17] (Fig. 1). Postdrug amplitude and implicit time measures were expressed as percentage variations from baseline values in order to minimize individual variability, and the hypothesis of no difference between predrug and postdrug measurements was verified by paired t test for placebo and each L-acetylcarnitine dose. The existence of significant correlation between each OP variable and L-acetylcarnitine plasma concentration was verified by computing the Kendall's coefficient of correlation [19].

Results

The protocol requirements concerning sleep, drug assumption, and the like were satisfied by all volunteers, and adequate OP recordings were possible in all cases.

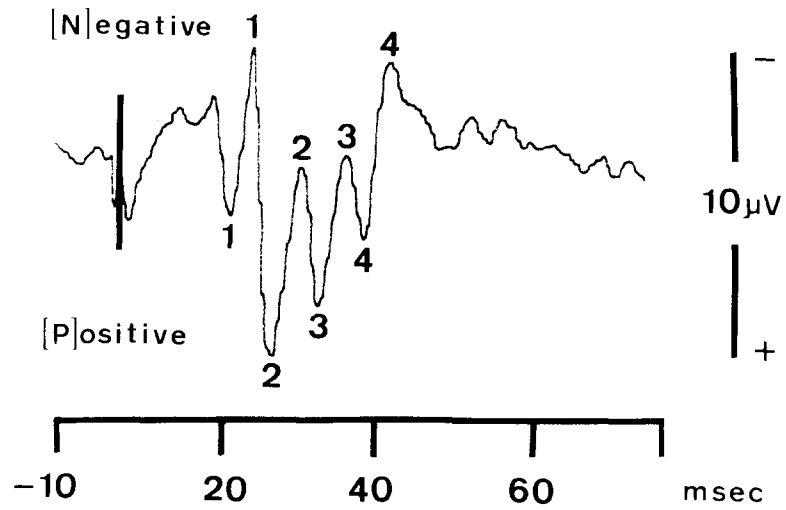

Fig. 1. Specimen of baseline oscillatory potential showing positive and negative components recorded in the study. Average on 15 epochs; right eye.

The plasma concentration of L-acetylcarnitine did not differ significantly across sessions in baseline condition, although there were slightly different average values, and was significantly increased by the administration of l-acetylcarnitine at either dose, with a significant correlation between dose and drug plasma concentration (Kendall's coefficient of correlation: 3.12, $p < 0.001$)(Fig. 2).

A systematic and significant reduction of the implicit time of the P2 component followed administration of L-acetylcarnitine at the 10 or 30 mg/kg dose; a comparable effect on the N2 latency, although restricted to the 30 mg/kg dose, was observed as well (Figs 3,4). This reduction was bilateral and consistent across subjects (with one exception at the 10 mg/kg dose)(Fig. 3); it was maximum on average at the 30-min control and transient at the 10 mg/kg dose, but still evident 120 min after administration of the 30 mg/kg dose (Fig. 4). No systematic or statistically significant (baseline versus postdrug *t* test) modification was observed after administration of placebo

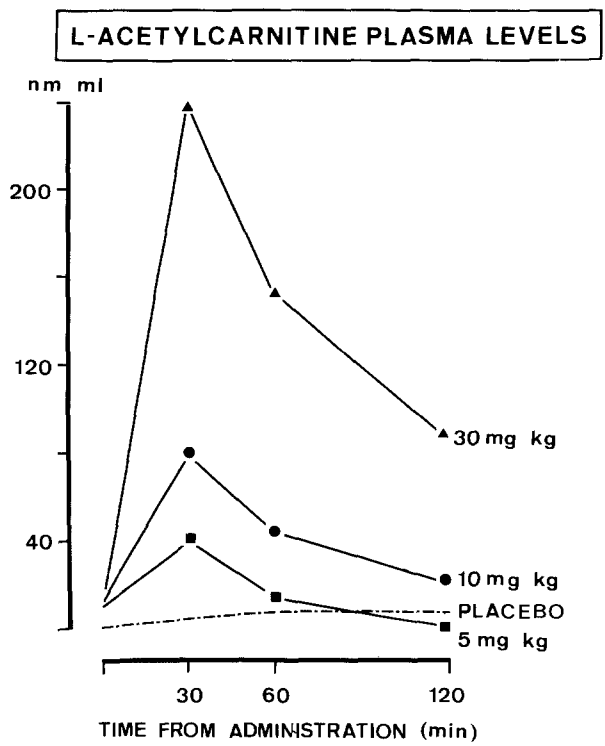

Fig. 2. Averaged L-acetylcarnitine plasma concentration values in baseline condition prior to and after acute intravenous administration of placebo or L-acetylcarnitine, 5, 10, and 30 mg/kg.

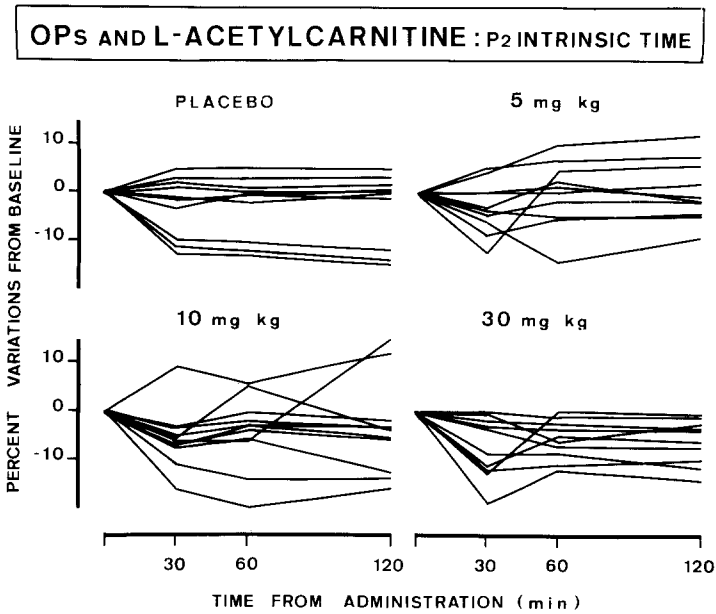

Fig. 3. Individual modifications (expressed as percent variation from baseline) of the P2 component implicit time after acute intravenous administration of placebo or L-acetylcarnitine, 5, 10, and 30 mg/kg. Right eye.

or the 5 mg/kg dose (Figs 3,4); systematic changes in the latency of any other OP component or in amplitude were never detected.

No correlation was found in baseline conditions between the amplitude or implicit time of any OP component and the L-acetylcarnitine plasma concentration (Kendall's coefficient of correlation; P2: $z = 0.87$; NS; Nz: $z = 0.79$). The average modifications of the P2 and N2 implicit time matched the drug plasma levels for apparent peak, time ongoing, and duration. The variations from baseline of both P2 and N2 implicit time were significantly correlated with the postdrug increment of L-acetylcarnitine plasma concentration (Kendall's coefficient of correlation; P2: 2.43, $p < 0.008$; N2: 2.94, $p < 0.002$).

Discussion

Consistency across subjects, latency from administration, time ongoing, and relation to dose of the implicit time modifications following after drug administration conceivably allow statistical accidents to be ruled out; the attribution of the latency modifications to drug action appears therefore

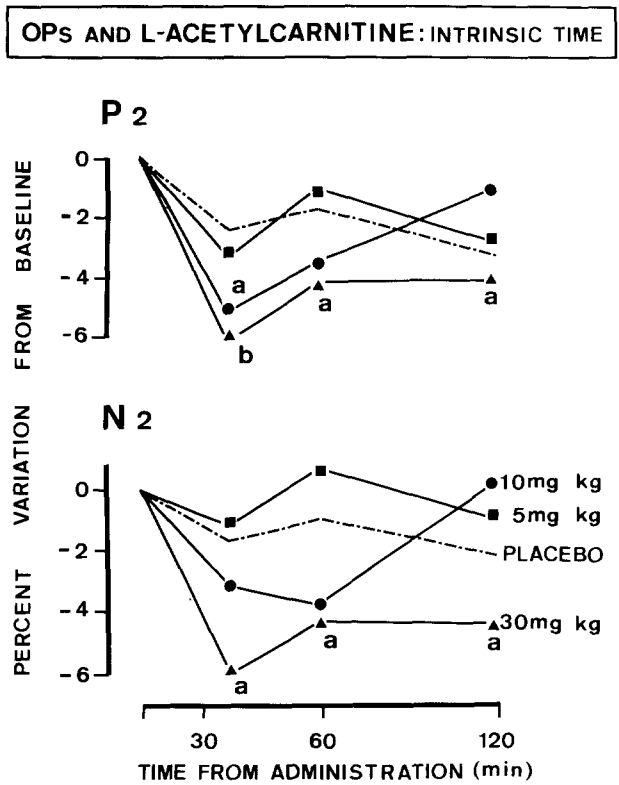

Fig. 4. Average modifications, expressed as percent variation from baseline, of the implicit time of P2 and N2 components of the oscillatory potentials after placebo or 5, 10, and 30 mg/kg L-acetylcarnitine, acute intravenous administration. Right eye; ten subjects. Across-subject statistical significance at the predrug versus postdrug comparison (paired *t* test) is indicated as follows: a = $p < 0.05$; b = $p < 0.01$.

justified despite the relatively limited amount of change (on average 6% after both the 10 and 30 mg/kg doses) and despite the restriction to two OP components. In systematic studies on healthy volunteers the quantitative electroencephalographic effects of single therapeutic doses of neuroactive drugs are known to be often as selective and usually within physiological variability [20,21,22,23,24,25,26], and this may conceivably apply as well to other electrophysiological phenomena, including retinal potenials. A relevant variability of the amplitude measurements was conversely observed that is likely to depend on the recording method and to account for the absence of systematic and drug-attributable modifications.

The effects on OPs parallelled the drug plasma concentration, and the existence of a statistical correlations between the postdrug changes of these two variables adds to the specificity of the OP modifications with respect to

L-acetylcarnitine action. A correlation between electrophysiological effects and drug plasma concentration is uncommon in human neuropharmacology [27], mostly due to structural and functional peculiarities of the blood-brain barrier [28,29].

The mammalian retina shares most of these barrier characteristics, and a correlation between retinal effects and drug plasma concentration can be expressive of selective drug transport and/or activity. The cholinergic mechanisms of L-acetylcarnitine action are still to be confirmed [8,30]. Oscillatory potentials are known to be sensitive to pharmacological interferences within the gamma-amenobytyric acid- or glycine-mediated retinal systems [31,32,33,34]; and an effect of L-acetylcarnitine on this(these system(s) is also conceivable. Regardless of possible mechanisms of action, the results substantiate the sensitivity of retinal OPs to neurotropic drugs also when administered systematically.

Acknowledgement

The authors wish to thank Miss Rossella Mancini for technical assistance, and SIGMA-TAU Farmaceutici, Roma, Italy, for supplying the compound administered and for the quantitative assessment of plasma concentration levels.

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