Physiol. Res. 50: 439-442, 2001

SHORT COMMUNICATION

Carnitine Pretreatment Can Partially Change the Excitability of the Immature Nervous Tissue

D. MAREŠOVÁ, H. RAUCHOVÁ[1], K. JANDOVÁ, I. VALKOUNOVÁ, J. KOUDELOVÁ, S. TROJAN

Institute of Physiology, First Faculty of Medicine, Charles University and [1]Institute of Physiology, Academy of Sciences of the Czech Republic and Center for Experimental Cardiovascular Research, Prague, Czech Republic

Received November 9, 2000
Accepted January 3, 2001

Summary

The possible protective action of L-carnitine on neuronal excitability was studied in 21-day-old male Wistar rats with implanted electrodes. Administration of L-carnitine did not change the elicitation and duration of the epileptic seizures (cortical afterdischarges, ADs) in rats under normobaric oxygen atmosphere conditions. However, in animals exposed to 30 min hypobaric hypoxia the duration of the ADs was shortened after the second, fourth and sixth stimulation (in comparison with the first evoked ADs) while carnitine-treated rats retained their neuronal excitability and the duration of ADs was shortened only after the third stimulation.

Key words

Carnitine treatment • Epileptic seizures • Hypobaric hypoxia • Neuronal excitability • Rat

Endogenous L-carnitine, a betaine derivative of β-hydroxybutyrate, plays a key role in fatty acid metabolism (Schulz 1991). It is essential for the transport of activated long-chain fatty acids from the cytosolic compartment into the mitochondrial matrix where β-oxidation takes place (for reviews see Bieber 1988, Rebouche and Seim 1998, Zeyner and Harmeyer 1999).

During the past decade, numerous reports have demonstrated the protective effect of carnitine in various physiological and pathological processes (Ferrari *et al.* 1992). For instance, experimental data obtained in laboratory animals as well as accumulating clinical cases have suggested that carnitine (and some of its derivates) may be an important protective substance in acute and chronic states of myocardial ischemia and in some cardiovascular diseases the origin of which is not primarily of ischemic origin (Kolář 1994). Furthermore, it was found that carnitine is able to substantially decrease the negative effects of the anesthetic halothane on energy-linked processes in rat liver mitochondria

(Toninello *et al.* 1986) or to prevent acute ammonia toxicity (Matsuoka and Igisu 1993) and to enhance the efficacy of ammonia elimination as urea and glutamine (O'Connor and Costell 1990). L-carnitine could also protect the brain from ischemic insults (Matsuoka and Igisu 1992) and prevent or attenuate the level of lipid peroxidation in the brain (Koudelová *et al.* 1994, 1996, 1999) or in heart (Luo *et al.* 1999). Administration of L-carnitine had anticonvulsive effects in pentylenetetrazol-induced seizures in mice (Yu *et al.* 1997) and improved brain stem auditory evoked potentials in alloxan-diabetic rats (Yildiz *et al.* 1996).

The aim of the present study was to evaluate the influence of L-carnitine pretreatment on the excitability of cortical neurons in rats non-exposed and exposed to 30 min intensive hypobaric hypoxia under the same conditions when we observed significant protection from lipid peroxidation in the brain after carnitine administration (Koudelová *et al.* 1999).

The experimental animals were 21-day-old male Wistar rats of our own breed housed under standard temperature and light conditions and fed a complete laboratory diet and water *ad libitum*. We chose animals of this age because 21-day-old rats had previously shown very low resistance to the lack of oxygen (Koudelová and Mourek 1992). All performed experiments were in agreement with the guidelines of the Animal Protection Law of the Czech Republic, completely compatible with the European Convention on Animal Protection.

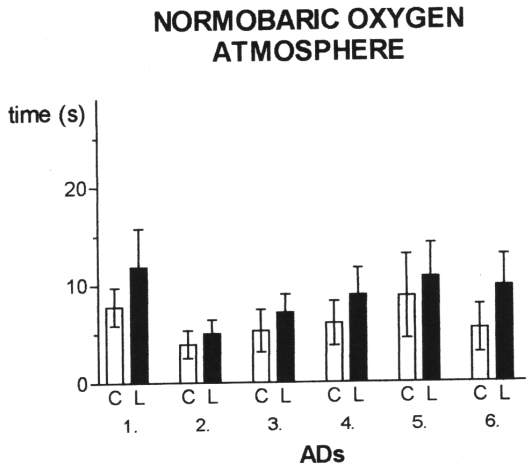


Fig. 1. *The duration (in seconds) of cortical afterdischarges (ADs) in control (C) and L-carnitine-treated (L) rats after repetitive stimulation of sensorimotor cortex. Values are given as means ± S.E.M. Abscissa: the first to sixth ADs of C and L rats.*

Under general ether anesthesia, the stimulation electrodes were placed on the right sensorimotor cortex and registration electrodes on the left sensorimotor cortex and at both visual areas. An indifferent electrode was placed on the nasal bone. Epileptic seizures (cortical afterdischarges, ADs) were evoked in freely moving animals with implanted silver electrodes by bipolar stimulation. Parameters of the stimulation were as described previously (Marešová *et al.* 2001): 8 Hz, pulse duration of 0.5 ms, the required intensity for the elicitation of epileptic seizure 3-5 mA. Stimulation of the sensorimotor cortex lasted 15 s and was repeated 5 times, always 1 min after the end of previous seizure activity. Unipolar and bipolar electrocorticographic activity was registered and its duration and shape of ADs were assessed. Experiments were performed after a recovery period (always more than one hour) tested in animals by placing and righting reflexes.

All animals were divided into four groups: the first two groups (control and carnitine-treated rats) were kept under a normobaric oxygen atmosphere. The other two groups (control and carnitine-treated rats) were exposed to 30 min intensive hypobaric hypoxia (simulating an altitude of 9000 m with pO_2 6.4 kPa). The control animals received no injections because it had been demonstrated in pilot experiments that an injection of physiological saline had no influence on ADs in these rats. Carnitine-treated animals received similarly an intraperitoneal injection of L-carnitine (generous gift of Sigma Tau, Italy) in the amount of 80 mg per 100 g body weight 30 min before electrical stimulation, as in our previous experiments (Koudelová *et al.* 1994, 1996, 1999). The animals exposed to hypobaric hypoxia received carnitine 15 min before exposition to hypoxia and stimulation began 15 min after the end of hypoxia exposure. All experimental groups consisted of at least 8 animals. The results were statistically evaluated by the t-test (using the GraphPadPrism program). Level of significance was set at 5 %.

The electrical stimulation of the sensorimotor cortex elicited ADs in all 21-day-old rats in a normobaric oxygen atmosphere (Fig. 1). The ADs were composed of spike-and-wave complexes with a frequency of 2-3 Hz. Synchronous movements with the stimulation of the head and/or forelimbs were registered during stimulation and evoked ADs – Racine's scale 2 and 3 (Racine 1972). The duration of the ADs did not significantly change with the repetition of the stimulation. The pretreatment with carnitine did not influence the elicitation and duration of

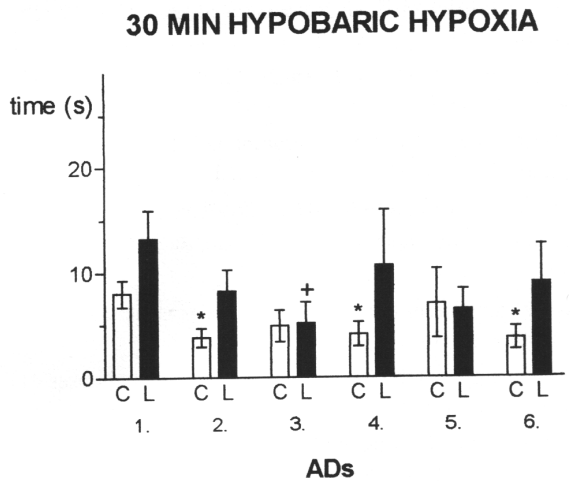


Fig. 2. *The duration (in seconds) of cortical afterdischarges (ADs) in control (C) and L-carnitine-treated (L) rats after repetitive stimulation of sensorimotor cortex. Values are given as means ± S.E.M., asterisks indicate significant differences p<0.05 from the first ADs in control rats, whereas the cross denotes such a significant difference in rats treated with L-carnitine. Abscissa: the first to sixth ADs of C and L rats.*

the ADs (Fig. 1). Intensive hypobaric hypoxia lasting 30 min shortened the duration of ADs after the second, fourth and sixth stimulation ($p<0.05$) in comparison with the first control ADs (Fig. 2). While the carnitine-treated animals in the normobaric oxygen atmosphere did not exhibit significant changes in the duration among six individual ADs (Fig. 1), the carnitine-treated animals exposed to hypobaric hypoxia only exhibited a shortened duration of the third ADs (Fig. 2). A similar influence of two other carnitine derivates (acetyl-L-carnitine or propionyl-L-carnitine) on the elicitation and duration of ADs was found in the same experimental arrangements (Marešová *et al.* 2000).

Chronic L-carnitine pretreatment (4 weeks, 20 mg/100 g body weight/day) had beneficial effects on diabetic central neuropathy in alloxan-diabetic rats as revealed by brain stem auditory evoked potentials (Yildiz *et al.* 1996). Single dose of L-carnitine used in our present study (80 mg/100 g body weight) exhibited a significant protective effect against increased lipid peroxidation in different parts of the rat brain (cortex, subcortical structures, cerebellum and medulla oblongata) in animals exposed to hypobaric hypoxia (Koudelová *et al.* 1994). L-carnitine pretreatment with a similar dose suppressed both clonic and tonic seizures induced by pentylenetetrazol, when the interval between L-carnitine and pentylenetetrazol administration was 15-30 min (Yu *et al.* 1997), whereas higher doses of L-carnitine (160 or 320 mg/100 g body weight) exhibited more pronounced effects.

Carnitine is found in all mammalian tissues including the brain. However, its physiological role in the brain is not quite clear because this tissue does not utilize long chain fatty acids as an energy source. Matsuoka and Igisu (1992) suggested that carnitine is capable of protecting the brain from ischemia due to its hitherto undefined function. In the present study, the carnitine pretreatment in immature (21-day-old) rats exposed to hypobaric hypoxia was partially able to restore the excitability of neurons involved in the generation of ADs.

Acknowledgements

This study was partially supported by research grant No. 303/98/0473 and 305/99/0976 from the Grant Agency of the Czech Republic, grant No. 241/1998 from Charles University (Prague) and 111100001 from MŠMT.

References

BIEBER LL**:** Carnitine. *Annu Rev Biochem* **57:** 261-283, 1988.

FERRARI R, DIMAURO S, SHERWOOD G (eds): *L-carnitine and its Role in Medicine: From Function to Therapy.* Academic Press, Harcourt Brace Jovanovich Publishers, London 1992, 433 p.

KOLÁŘ F: Carnitine and heart diseases (in Czech). *Cor Vasa* **36:** 190-198, 1994.

KOUDELOVÁ J, MOUREK J: Different degrees of lipid peroxidation in the CNS of young and adult rats exposed to short-term hypobaric hypoxia. *Physiol Res* **41:** 207-212, 1992.

KOUDELOVÁ J, MOUREK J, DRAHOTA Z, RAUCHOVÁ H: Protective effect of carnitine on lipoperoxide formation in rat brain. *Physiol Res* **43:** 387-389, 1994.

KOUDELOVÁ J, RAUCHOVÁ H, MOUREK J, TROJAN S: Hypoxia and hyperoxia induced lipid peroxidation and protective effect of carnitine and vitamin E in brain tissue. *J Neurochem* **66** (Suppl 2): S46, 1996.

KOUDELOVÁ J, RAUCHOVÁ H, ŠLAPETOVÁ V, MOUREK J, DRAHOTA Z: Hypoxia-induced lipid peroxidation in rat brain: protective effect of phosphocreatine and carnitine. *Physiol Res* **48** (Suppl 1): **S**111, 1999.

LUO X, REICHETZER B, TRINES J, BENSON LN, LEHOTAY DC: L-carnitine attenuates doxorubicidin-induced lipid peroxidation in rats. *Free Radic Biol Med* **26:** 1158-1165, 1999.

MAREŠOVÁ D, JANDOVÁ K, VALKOUNOVÁ I, TROJAN S, RAUCHOVÁ H: Can carnitine affect the excitability of the immature nervous tissue ? *Physiol Res* **49:** P22, 2000.

MAREŠOVÁ D, VALKOUNOVÁ I, JANDOVÁ K, BORTELOVÁ J, TROJAN S: Excitability changes of cortical neurons during the postnatal period in rats exposed to prenatal hypobaric hypoxia. *Physiol Res* **50:** 215-219, 2001.

MATSUOKA M, IGISU H: Preservation of energy metabolites by carnitine in the mouse brain under ischemia. *Brain Res* **590:** 334-336, 1992.

MATSUOKA M, IGISU H: Effects of L and D-carnitine on brain energy metabolites in mice given sublethal doses of ammonium acetate. *Pharmacol Toxicol* **72:** 145-147, 1993.

O'CONNOR JE, COSTELL M: New roles of carnitine metabolism in ammonia cytotoxicity. *Adv Exp Med Biol* **272:** 183-195, 1990.

RACINE RJ: Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* **32:** 281-294, 1972.

REBOUCHE CJ, SEIM H: Carnitine metabolism and its regulation in microorganisms and mammals. *Annu Rev Nutr* **18:** 39-61, 1998.

SCHULZ H: Beta oxidation of fatty acids. *Biochim Biophys Acta* **1081:** 109-120, 1991.

TONINELLO A, BRANCA D, SCUTARI G, SILIPRANDI N, VINCENTI E, GIRON G: L-carnitine effect on halothane-treated mitochondria. *Biochem Pharmacol* **35:** 3961-3964, 1986.

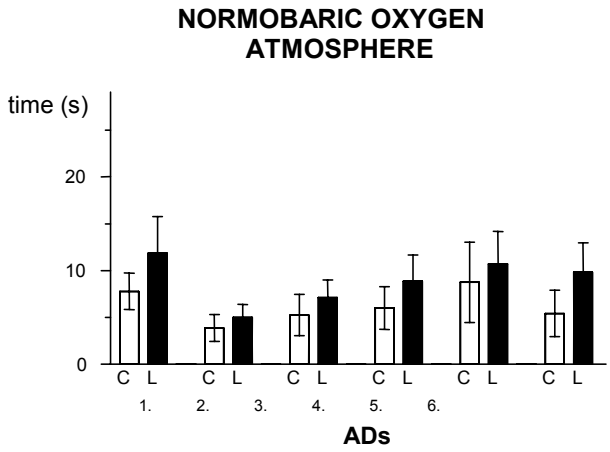
YILDIZ O, OZATA M, OZKARDES A, CORAKCI A, GUNDOGAN N, GUNDOGAN M: L-carnitine treatment improves brain stem auditory evoked potentials in diabetic rats. *Neuroreport* **25:** 2957-2959, 1996.

YU Z, IRYO Y, MATSUOKA M, IKEDA M: Suppression of pentylenetetrazol-induced seizures by carnitine in mice. *Naunyn-Schmiedebergs Arch Pharmacol* **355:** 545-549, 1997.

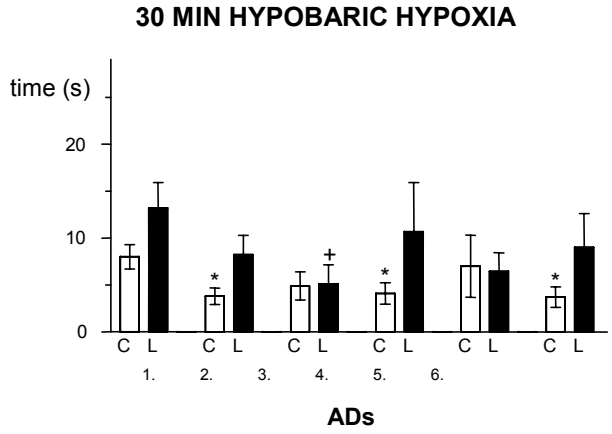
ZEYNER A, HARMEYER J: Metabolic functions of L-carnitine and its effects as feed additive in horses. A review. *Arch Anim Nutr* **52:** 115-138, 1999.

Reprint requests

H. Rauchová, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, e-mail: rauchova@biomed.cas.cz


NORMOBARIC OXYGEN
ATMOSPHERE
time (s)
20
10
0
C L C L C L C L C L C L
1. 2. 3. 4. 5. 6.
ADs

T


30 MIN HYPOBARIC HYPOXIA
time (s)
20
10
0
C L C L C L C L C L C L
1. 2. 3. 4. 5. 6.
ADs