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Alpha melanocyte stimulating hormone (α-MSH) does not modify pentylenetetrazol- and pilocarpine-induced seizures

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ABSTRACT

Aims: Alpha-melanocyte stimulating hormone (α-MSH) is a pro-opiomelanocortin (POMC)-derived peptide involved in different neurological functions that also exerts anti-inflammatory effects, including in the central nervous system (CNS). Although inflammation has been implicated in seizures and epilepsy, no study has systematically investigated whether α-MSH modifies seizures. Therefore, in the current study we determined whether α-MSH alters pentylenetetrazol (PTZ)- and pilocarpine-induced seizures.
Main methods: Adult male Swiss mice were injected with α-MSH (1.66, 5 or 15 μg/3 μL, intracerebroventricular (i.c.v.)) or systemic (0.1, 0.3 or 1 mg/kg, intraperitoneally (i.p.)). Five to sixty minutes after the injection of the peptide, animals were injected with PTZ (60 mg/kg, i.p.) or pilocarpine (370 mg/kg, i.p.). Latency to myoclonic jerks and tonic–clonic seizures, number of seizure episodes, total time spent seizing and seizure intensity, assessed by the Racine and Meurs scales were recorded. Interleukin 1 beta (IL-1β) levels in the hippocampus were measured by a commercial enzyme-linked immunoabsorbent assay (ELISA).
Key findings: Neither intracerebroventricular (1.66, 5 or 15 μg/3 μL, i.c.v.) nor systemic (0.1, 0.3 or 1 mg/kg, i.p.) administration of α-MSH altered PTZ- and pilocarpine-induced seizures. IL-1β levels in the hippocampi were not altered by α-MSH, PTZ or pilocarpine.
Significance: Although inflammation has been implicated in seizures and epilepsy and α-MSH is a potent anti-inflammatory peptide, our results do not support a role for α-MSH in seizure control.

Introduction

Neurotrauma, stroke, infection, and febrile seizures are all associated with acute seizures and increased risk of developing epilepsy (Dedeurwaerdere et al., 2012). Several lines of evidence support a relationship between inflammation and epilepsy (Cremer et al., 2009; Dedeurwaerdere et al., 2012; Perucca et al., 2007; Vezzani et al., 2011a). Inflammation is triggered by molecular patterns associated to pathogens (PAMPs) or molecular patterns associated to damage (DAMPS) (Maroso et al., 2010). These molecules activate the host defense system and also increase neural tissue excitability (Matsuo et al., 2006; Rodgers et al., 2009). PAMPs and DAMPs bind to toll-like receptors (TLR) and advanced glycation end product receptors (RAGE), which are expressed mainly on the cell surface of astrocytes and microglial cells in the central nervous system (Medzhitov, 2007). TLR have also been reported in neurons (Zhou et al., 2009), particularly in dysplastic neurons from epileptic patients (Zurolo et al., 2011). Interestingly, the epicortical application of lipopolyssacaride (LPS) produces spontaneous epileptiform discharges (Rodgers et al., 2009) and high-mobility group box-1 (HMGB1), a non-histone nuclear protein, facilitates kainate- and bicuculine-induced seizures (Maroso et al., 2010). Moreover, it is fairly known that activation of TLR increases microglial tumor necrosis factor (TNF)-α, interleukin 6 (IL-6), macrophage inflammatory protein-1α (MIP-1α) and interleukin 1 beta (IL-1β) expression (Allan et al., 2005; Dunne and O'Neill, 2003; Farooqui et al., 2007; Li et al., 2011). In this context, several studies have described that these pro-inflammatory cytokines increase seizure susceptibility (Galic et al., 2012; Li et al., 2011; Vezzani et al., 2012).

Alpha-melanocyte stimulating hormone (α-MSH) is a thirteen amino acid neuropeptide derived from the post-translational processing of pro-opiomelanocortin (POMC) (Brzoska et al., 2008; Butler, 2006; Catania et al., 2000) that binds to Gs protein-coupled melanocortin receptors (MC-Rs) (Gantz and Fong, 2003; Mountjoy

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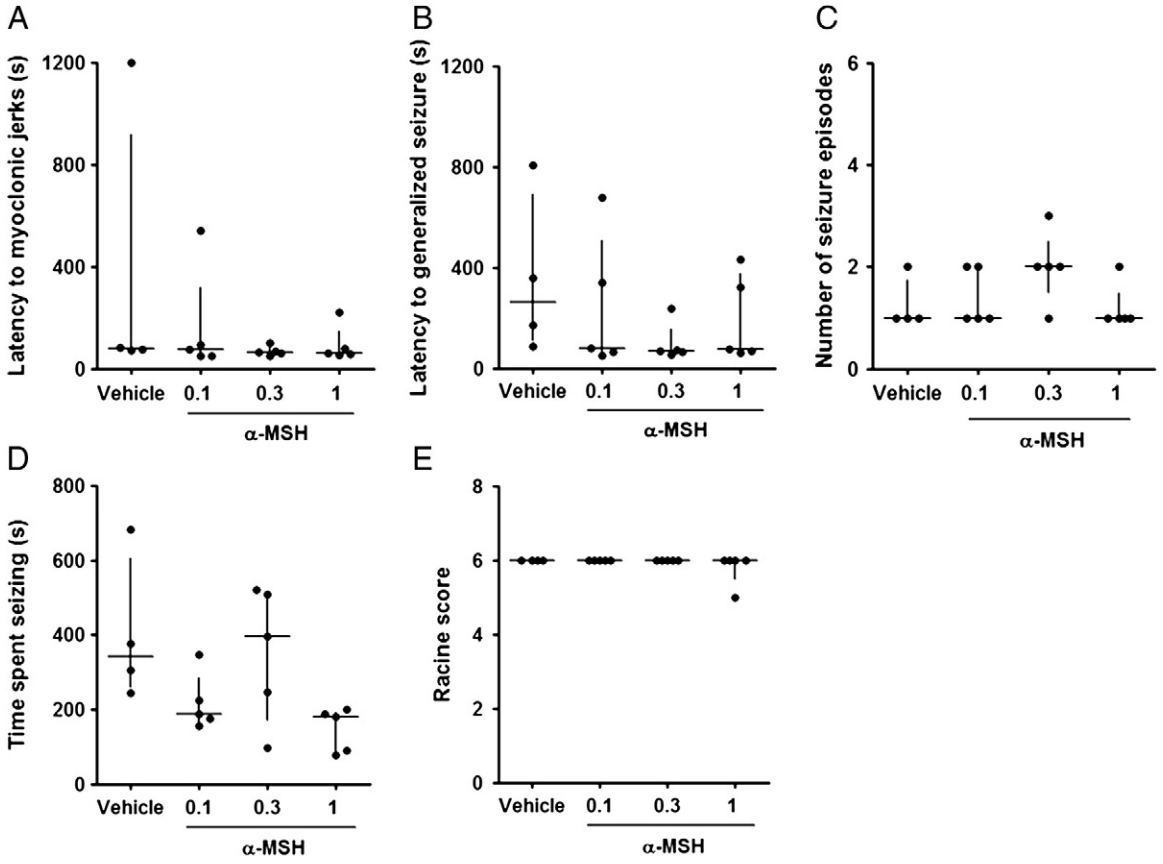

Fig. 1. Lack of effect of α-MSH (0.1, 0.3 or 1 mg/kg, i.p., 15 min before PTZ) on PTZ-induced seizures. Latency to myoclonic jerks (A); latency to generalized tonic–clonic seizures (B); number of seizure episodes (C); total time spent seizing (D) and Racine scale (E). Data are presented as median and interquartile range for n = 4–5 in each group.

et al., 1992). Three out five of MC-R subtypes described (MC1-R, MC3-R and MC4-R) have anti-inflammatory role (Caruso et al., 2004; Catania et al., 2004; Ichiyama et al., 1999; Maaser et al., 2006; Mountjoy et al., 2003). α-MSH binding to these MC-Rs downregulates various pro-inflammatory cytokines and increases the expression of the anti-inflammatory cytokine IL-10 (Bhardwaj et al., 1996; Redondo et al., 1998). α-MSH downregulates pro-inflammatory cytokines by inhibiting IkB degradation. As a result, nuclear factor kB (NFkB) is maintained complexed with IkB in the cytoplasm, and does not trigger the transcription of target genes that encode pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α (Brzoska et al., 2008; Catania, 2008; Catania et al., 2010; Hiscott et al., 1993; Muceniece and Dambrova, 2010).

A number of studies have suggested a role for inflammatory mediators in pentylenetetrazol (PTZ)- and pilocarpine-induced seizures (Funck et al., 2011; Maroso et al., 2010; Oliveira et al., 2008; Rambo et al., 2009; Vezzani et al., 2010, 2011a, 2011b), and that α-MSH triggers anti-inflammatory mechanisms in the central nervous system (CNS). However, no study has determined whether α-MSH modify PTZ- and pilocarpine-induced seizures. Therefore, in the current study we investigated whether α-MSH decreases the seizures elicited by these chemoconvulsants.

Material and methods

Animals

Adult male Swiss mice (28 ± 3 g; n = 184), housed ten to a cage, and maintained under controlled light and environment (12-h light/dark cycle, 24 ± 1 °C, 55% relative humidity) with free access to food (Guabi, Santa Maria, Brazil) and water, were used. All animals were obtained from the Animal House of the Federal University of Santa Maria.

Behavioral tests were conducted during the light phase of the cycle (from 9:00 a.m. to 5:00 p.m.). All experiments reported in this study were conducted in accordance with the policies of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised in 1996, and with the Institutional and National regulations for animal research. All efforts were made to reduce the number of animals used to a minimum, as well as to minimize their suffering (Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria authorization number: 008/2013).

Reagents

PTZ, pilocarpine and methylscopolamine bromide were purchased from Sigma (St. Louis, MO, USA). α-MSH was purchased from Bachem (CA, USA). Drugs were dissolved in sterile 0.9% NaCl or sterile phosphate-buffered saline (PBS), pH 7.4. The murine IL-1β kit was purchased from PreproTech (São Paulo, Brazil).

Surgical procedures

All animals were anesthetized with ketamine (100 mg/kg, intraperitoneally (i.p.)) and xylazine (10 mg/kg, i.p.), placed in a rodent stereotaxic apparatus, and were implanted with one 27-gauge guide cannula placed 1 mm above the right lateral ventricle, at the following coordinates

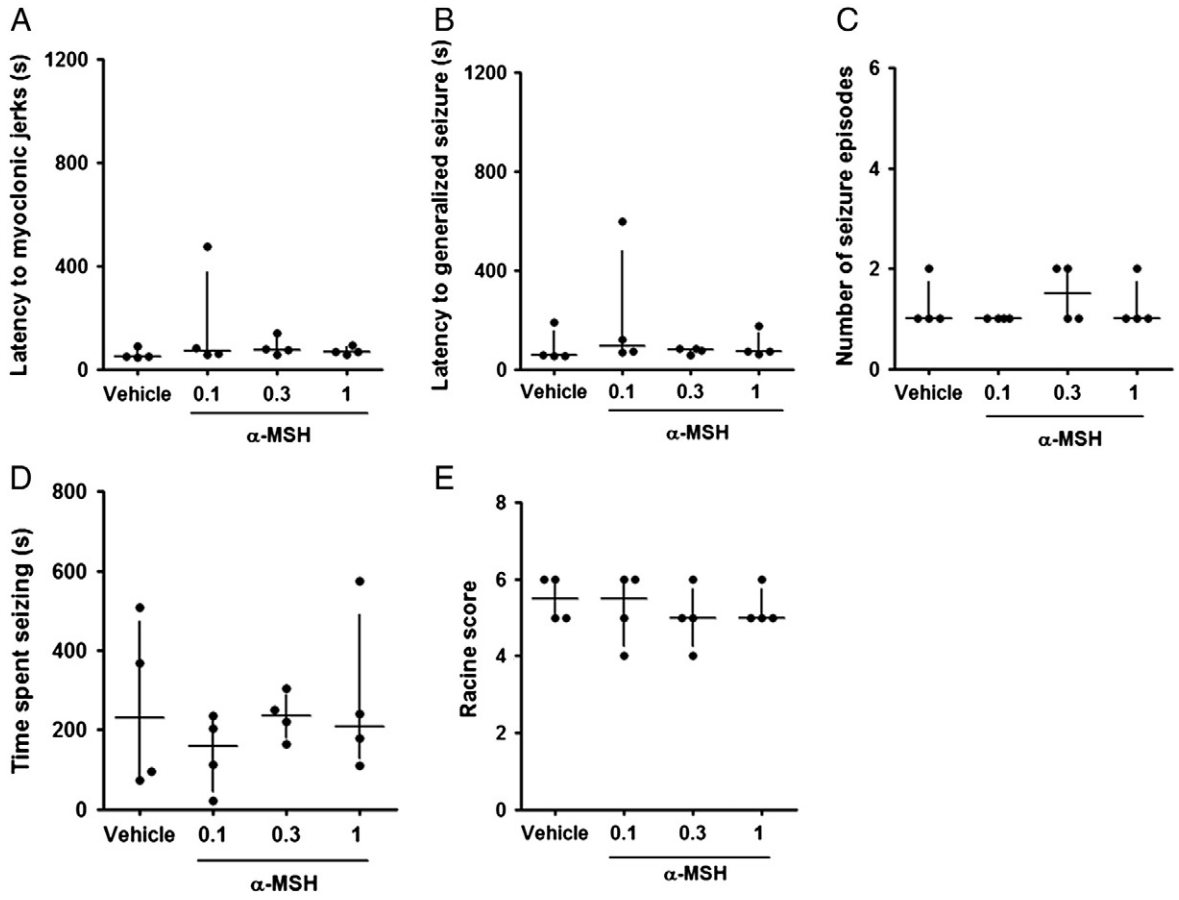

Fig. 2. Lack of effect of α-MSH (0.1, 0.3 or 1 mg/kg, i.p., 30 min before PTZ) on PTZ-induced seizures. Latency to myoclonic jerks (A); latency to generalized tonic–clonic seizures (B); number of seizure episodes (C); total time spent seizing (D) and Racine scale (E). Data are presented as median and interquartile range for n = 4 in each group.

relative to bregma: AP 0 mm, ML 0.9 mm, V 1.6 mm from the dura (Paxinos and Franklin, 2008). Chloramphenicol (200 mg/kg, i.p.) (Oliveira et al., 2008) was administrated immediately before the surgical procedure to prevent infection.

Injection procedures

The effect of α-MSH on PTZ- or pilocarpine-induced seizures was investigated by injecting the animals with α-MSH (0.1, 0.3 or 1 mg/kg, i.p., in those experiments in which the effect of the systemic administration of α-MSH was investigated or 1.66, 5 or 15 μg/3 μL, intracerebroventricular (i.c.v.), in those experiments in which the effect of the central administration of α-MSH was investigated) or respective vehicle (sterile 0.9% NaCl, 10 mL/kg, i.p. or PBS, i.c.v.). All intracerebroventricular injections were performed by using a Hamilton syringe and a 30-gauge needle fitted into the guide cannula. The tip of the infusion needle protruded 1.0 mm beyond that of the guide cannula into the right lateral ventricle. The needles were left in place for additional 60 s to minimize backflow.

Experiment 1: effect of systemic α-MSH on PTZ-induced seizures

The effect of systemic α-MSH on PTZ-induced seizures was investigated by administrating α-MSH at the doses 0.1, 0.3 or 1 mg/kg (i.p.), or sterile 0.9% NaCl (10 mL/kg, i.p.), 15, 30 or 60 min before the injection of PTZ (60 mg/kg, i.p.) (Funck et al., 2011). The animals were evaluated for the appearance of behavioral seizures, as described below. α-MSH doses and injection intervals were chosen based on a previous study (Ichiyama et al., 1999), which has shown an anti-inflammatory action of systemic α-MSH in the CNS.

Experiment 2: effect of i.c.v. α-MSH on PTZ-induced seizures

Since the systemic administration of α-MSH did not alter PTZ-induced seizures, we hypothesized that this effect could be due to its low blood brain barrier permeability (Wilson, 1988). Therefore, the effect of i.c.v.-administered α-MSH on PTZ-induced seizures was investigated. α-MSH (1.66, 5 or 15 μg/3 μL) or vehicle (3 μL of PBS, pH 7.4) were injected 15 min before PTZ (60 mg/kg, i.p.). Immediately after PTZ injection, the animals were evaluated for the appearance of behavioral seizures, as described below. Initial α-MSH doses and injection intervals were chosen based on a previous study (Izumi et al., 1973).

Experiment 3: effect of systemic α-MSH on pilocarpine-induced seizures

Considering that α-MSH did not alter PTZ-induced seizures, and that anticonvulsant activity depends on the chemical agent used to induce seizures (Oliveira et al., 2008), we tested whether α-MSH altered pilocarpine-induced seizures. Animals were injected with methylscopolamine bromide (1 mg/kg, i.p.) (Muller et al., 2009) to attenuate the peripheral cholinergic effects of pilocarpine. Fifteen minutes after the injection of methylscopolamine, animals were injected with saline or α-MSH (0.1, 0.3 or 1 mg/kg, i.p.). Pilocarpine (370 mg/kg, i.p.) (Costa et al., 2012) was administered 30 min thereafter.

Experiment 4: effect of i.c.v. α-MSH on pilocarpine-induced seizures

Since the systemic administration of α-MSH did not alter pilocarpine-induced seizures, we decided to test whether α-MSH, administered by the i.c.v. route, prevented seizures. Animals were injected with methylscopolamine bromide (1 mg/kg, i.p.) to prevent the peripheral cholinergic effects of pilocarpine. Fifteen minutes after the injection of methylscopolamine, animals were injected with α-MSH

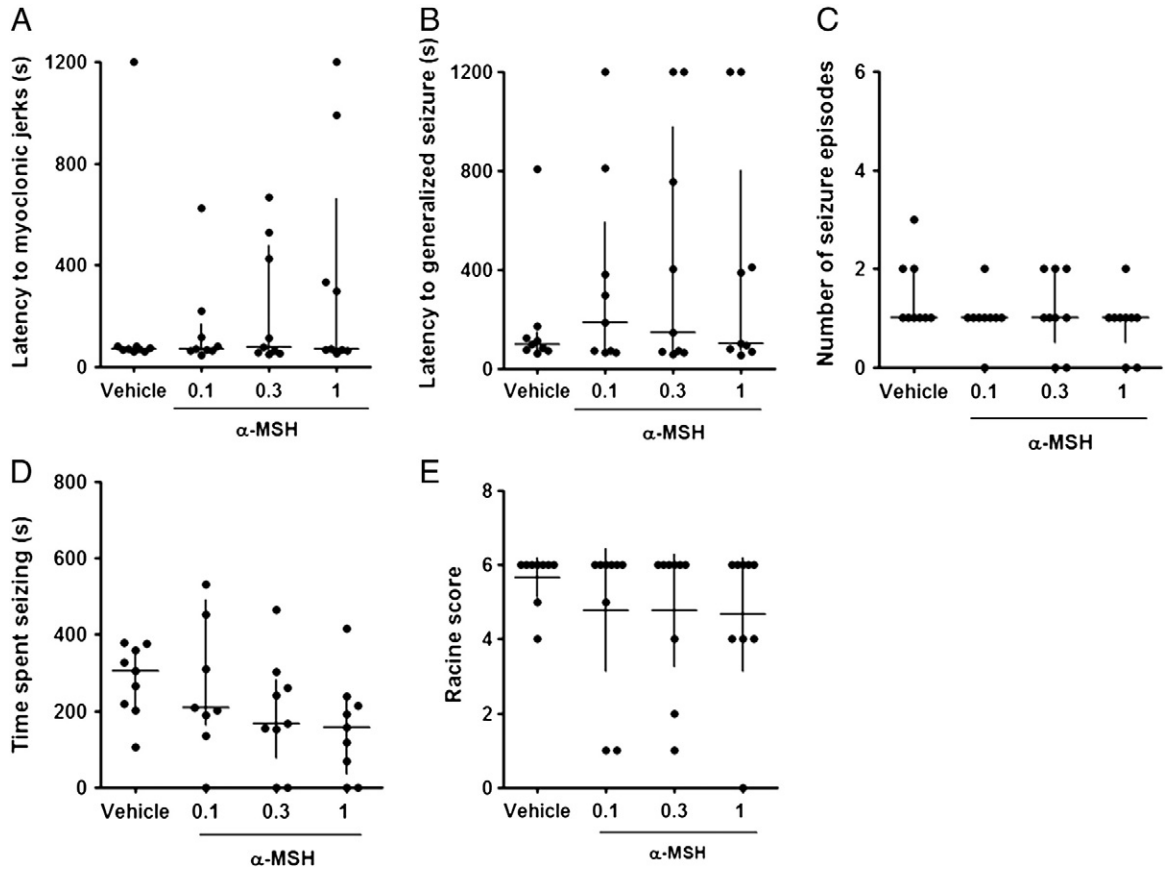

Fig. 3. Lack of effect of α-MSH (0.1, 0.3 or 1 mg/kg, i.p., 60 min before PTZ) on PTZ-induced seizures. Latency to myoclonic jerks (A); latency to generalized tonic–clonic seizures (B); number of seizure episodes (C); total time spent seizing (D) and Racine scale (E). Data are presented as median and interquartile range for n = 9 in each group.

(1.66, 5 or 15 μg/3 μL) or vehicle (3 μL of PBS, pH 7.4). Pilocarpine (370 mg/kg, i.p.) was administered 30 min thereafter.

Experiment 5: effect of early i.c.v. α-MSH on pilocarpine-induced seizures

Considering that the half-life of α-MSH is relatively short (Ahmed et al., 2013), we decided to decrease the time between the administration of the peptide and pilocarpine, in order to guarantee that α-MSH would be present in the brain when pilocarpine was injected. Animals were injected with methylscopolamine bromide (1 mg/kg, i.p.) to prevent the peripheral cholinergic effects of pilocarpine. Twenty minutes after the injection of methylscopolamine, animals were injected with α-MSH (15 μg/3 μL) or vehicle (3 μL of PBS, pH 7.4). Pilocarpine (370 mg/kg, i.p.) was administered 5 min thereafter.

Experiment 6: effect of early i.c.v. α-MSH and of PTZ and pilocarpine on IL-1β levels in the hippocampus

The effect of α-MSH, PTZ- or pilocarpine on IL-1β levels was investigated by injecting the animals with α-MSH (1.66 μg/3 μL, i.c.v.) or respective vehicle (sterile 0.9% NaCl, 10 mL/kg, i.p. or PBS, i.c.v.). α-MSH or vehicle (3 μL of PBS, pH 7.4) were injected 15 min before PTZ (60 mg/kg, i.p.). Those animals subjected to pilocarpine-induced seizures were first injected with methylscopolamine bromide (1 mg/

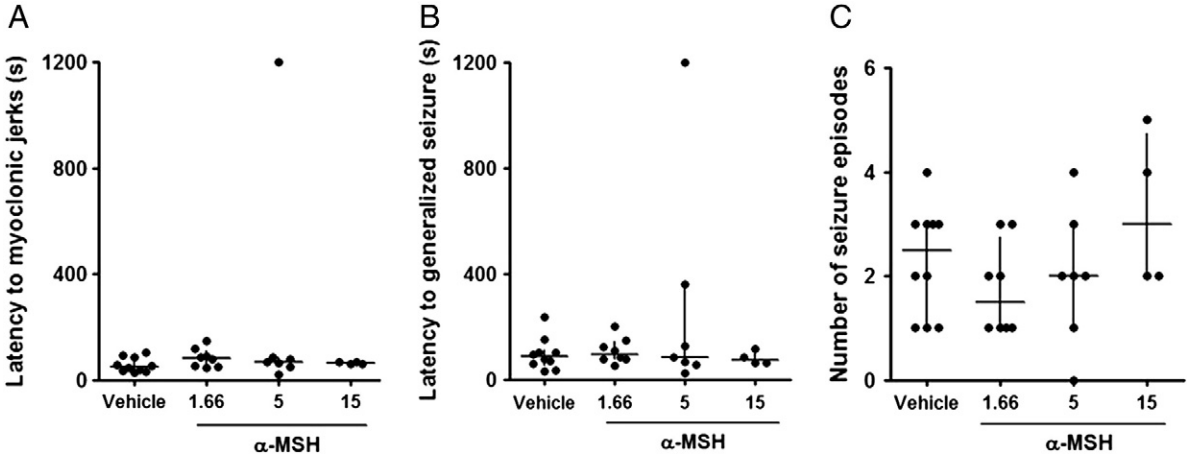

Fig. 4. Lack of effect of α-MSH (1.66 μg, 5 μg or 15 μg/3 μL, i.c.v., 15 min before PTZ) on PTZ-induced seizures. Latency to myoclonic jerks (A); latency to generalized tonic–clonic seizure (B); and number of seizure episodes (C). Data are presented as median and interquartile range for n = 4–10 in each group.

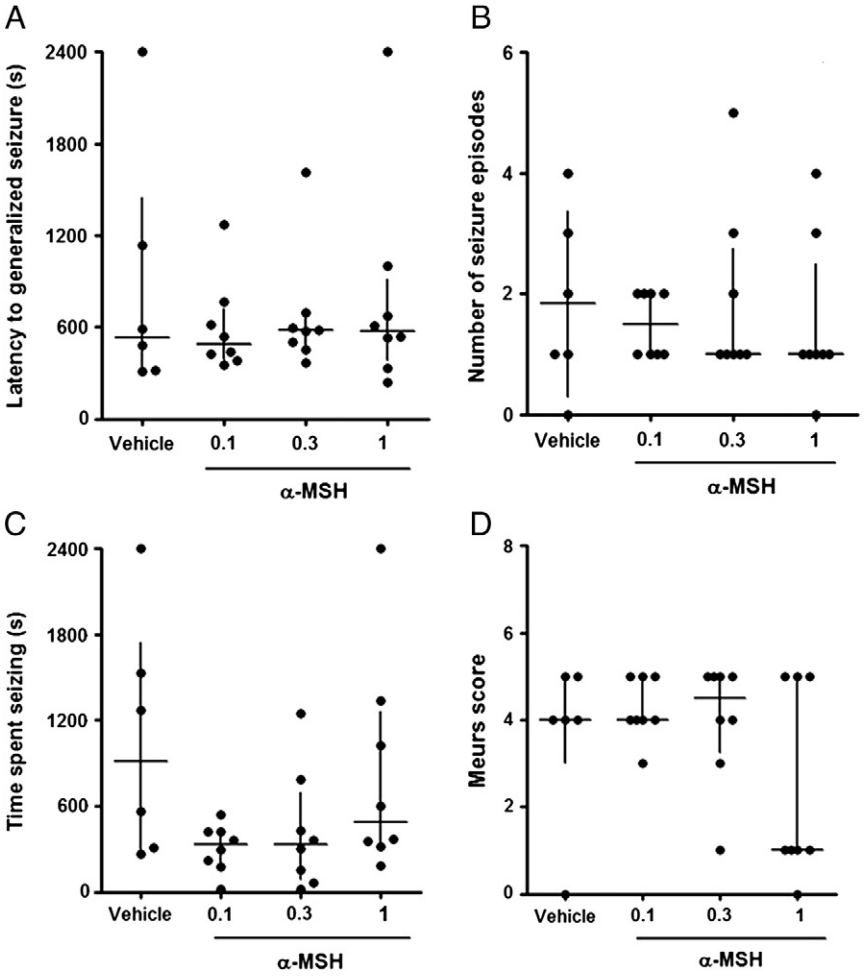

Fig. 5. Lack of effect of α-MSH (0.1, 0.3 or 1 mg/kg, i.p., 30 min before pilocarpine) on pilocarpine-induced seizures. Latency to generalized tonic–clonic seizure (A); and number of seizure episodes (B); and total time spent seizing (C) and Meurs scale (D). Data are presented as median and interquartile range for n = 6–8 in each group.

kg, i.p.) to prevent the peripheral cholinergic effects of pilocarpine and, 15 min thereafter, they were injected with α-MSH (1.66 μg/3 μL) or vehicle (3 μL of PBS, pH 7.4). Pilocarpine (370 mg/kg, i.p.) was administered 30 min after α-MSH. PTZ-injected animals were killed by decapitation 20 min after PTZ injection, and the hippocampi were dissected and homogenized in appropriated buffer (PBS containing 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and bovine serum albumin (BSA) 0.5%, pH 7.4). Samples were centrifuged at 25,000 *g* for 10 min and the supernatant was used to measure IL-1β levels, which were corrected for total protein content. Protein content was measured by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL).

Seizure evaluation

After the injection of PTZ or pilocarpine, the animals were videomonitored for 20 or 40 min, respectively, for the appearance of seizures and the following parameters were recorded: latency to myoclonic jerks (only for PTZ-injected animals), latency to generalized tonic–clonic seizures, number of seizure episodes and total time spent seizing, according to Ferraro et al. (1999). The severity of PTZ-induced seizures was scored by the modified Racine scale (Luttjohann et al., 2009), as follows: (1), sudden behavioral arrest and/or motionless staring; (2), facial jerking with muzzle or muzzle and eye; (3), neck jerks; (4), clonic seizure in a sitting position; (5), convulsion including clonic and/or tonic–clonic seizure while lying on the belly and/or pure tonic seizure; and (6), convulsion including clonic and/or tonic–clonic seizure while lying on the side and/or wild jumping. The severity of pilocarpine-induced seizures was evaluated by the Meurs scale (Meurs et al., 2008), as follows: (0) normal, non-epileptic activity; (1) mouth and facial movements, hyperactivity, grooming, sniffing, scratching, wet dog shakes; (2) head nodding, staring, tremor; (3) forelimb clonus, forelimb extension; (4) rearing, salivating, tonic–clonic activity; and (5) falling, status epilepticus. Mice were sacrificed 20 or 40 min after PTZ or pilocarpine injection, respectively.

Statistical analysis

Latency to myoclonic jerks and to generalized tonic–clonic seizures, number and total time spent seizing were analyzed by Kruskal–Wallis test followed by nonparametric Dunn's multiple comparison test because these variables did not meet ANOVA assumptions (normal distribution and homoscedasticity). These data are presented as median and interquartile range. IL1β levels were analyzed by a factorial 2 (saline or α-MSH) × 3 (saline, PTZ or pilocarpine) ANOVA, and are presented as mean and S.E.M. A probability of $p < 0.05$ was considered significant.

Results

Experiment 1

Figs. 1, 2 and 3 show that α-MSH (0.1, 0.3 or 1 mg/kg, i.p.) injected 15, 30 or 60 min before PTZ, respectively, did not alter the latency to PTZ-induced myoclonic jerks (A), tonic–clonic seizures (B), number of seizure episodes (C), total time spent seizing (D) and Racine scale (E).

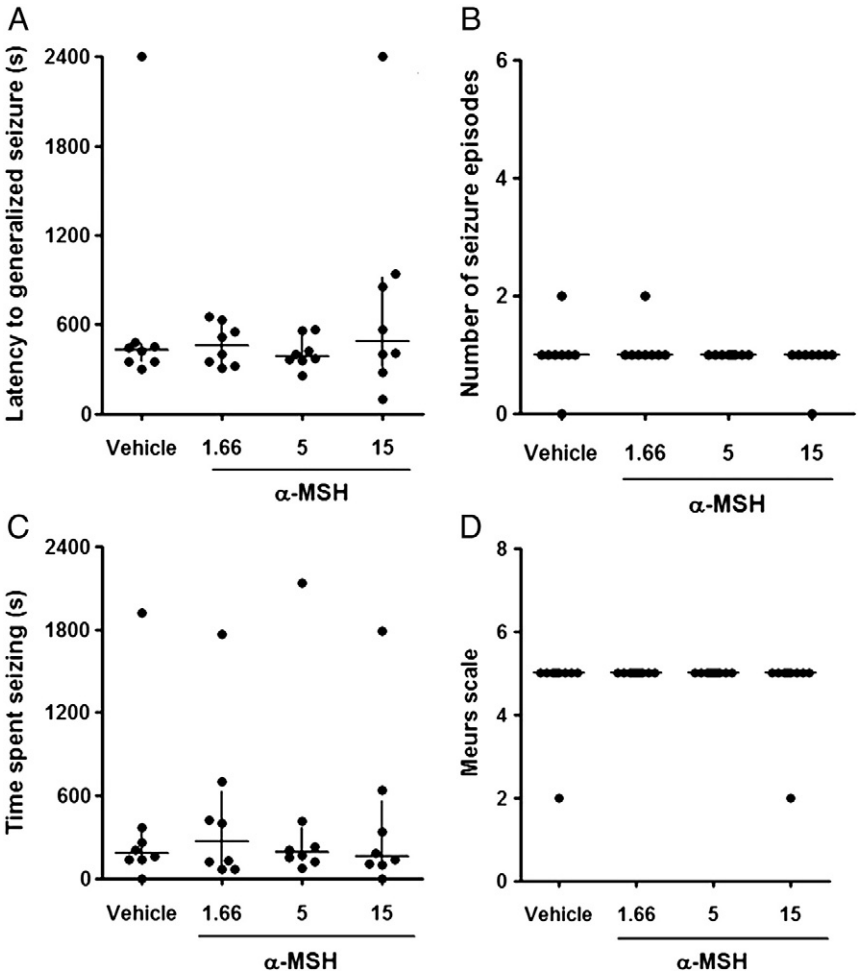

Fig. 6. Lack of effect of α-MSH (1.66, 5 or 15 μg/3 μL, i.c.v., 30 min before pilocarpine) on pilocarpine-induced seizures. Latency to generalized tonic–clonic seizure (A); number of seizure episodes (B); total time spent seizing (C) and Meurs scale (D). Data are presented as median and interquartile range for n = 8 in each group.

α-MSH also did not alter seizure severity, as assessed by the modified Racine score.

Experiment 2

Fig. 4 shows that α-MSH (1.66, 5 or 15 μg/3 μL, i.c.v.), injected 15 min before PTZ, did not alter the latency to PTZ-induced myoclonic jerks (A), tonic–clonic seizures (B), number of seizure episodes (C), total time spent seizing (D) and seizure severity, as assessed by the modified Racine scale (E).

Experiment 3

Fig. 5 shows that α-MSH (0.1, 0.3 or 1 mg/kg, i.p.), injected 30 min before pilocarpine, did not alter the latency to pilocarpine-induced tonic–clonic seizures (A), number of seizure episodes (B), total time spent seizing (C) and Meurs scale (D). α-MSH also did not alter seizure severity, as assessed by the modified Meurs score.

Experiment 4

Fig. 6 shows that α-MSH (1.66, 5 or 15 μg/3 μL, i.c.v.), injected 30 min before pilocarpine (370 mg/kg, i.p.), did not alter the latency to pilocarpine-induced tonic–clonic seizures (A), the number of seizure episodes (B), total time spent seizing (C) and Meurs score (D).

Experiment 5

Fig. 7 shows that α-MSH (15 μg/3 μL, i.c.v.), injected 5 min before pilocarpine (370 mg/kg, i.p.), did not alter the latency to pilocarpine-induced tonic–clonic seizures (A), the number of seizure episodes (B), total time spent seizing (C) and Meurs score (D).

Experiment 6

Fig. 8 shows that IL-1β levels in the hippocampi were not altered by α-MSH (1.66 μg/3 μL, i.c.v.), PTZ (60 mg/kg, i.p.) or pilocarpine (370 mg/kg, i.p.). Notwithstanding, a naïve group (control data, not included in the factorial ANOVA) presented hippocampal IL-1β levels approximately 45% lower than injected animals, indicating that the surgical procedure and cannula implantation, per se, increased the hippocampal IL-1β content. Whole data analysis (including the naïve group) by a single factor analysis revealed a significant effect $F(6,22) = 3.71$; $p = 0.011$. Accordingly, post hoc analysis (Student–Newman–Keuls multiple comparisons test) revealed that the mean of the naïve group differed from all other groups.

Discussion

The current study showed that neither systemic nor intracerebroventricular administration of α-MSH attenuated seizures, regardless of α-MSH dose, time between peptide and convulsant injection, or convulsant agent used.

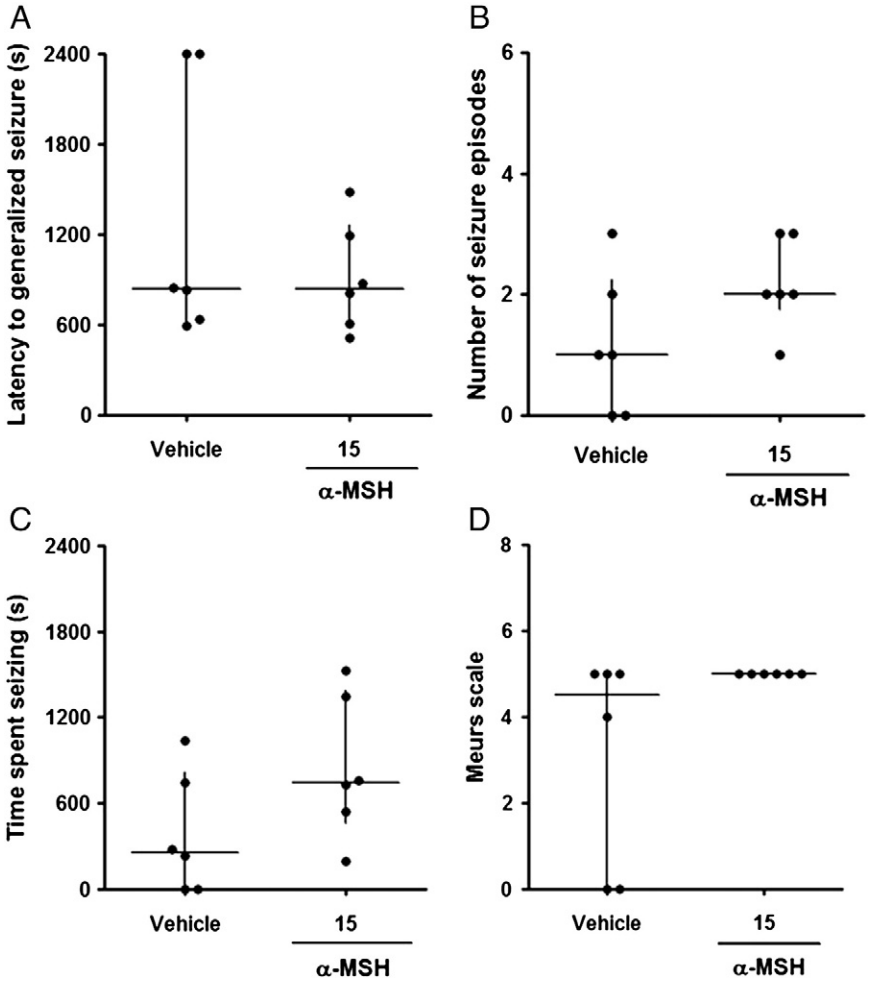

Fig. 7. Lack of effect of α-MSH (15 μg/3 μL, i.c.v., 5 min before pilocarpine) on pilocarpine-induced seizures. Latency to generalized tonic–clonic seizure (A); number of seizure episodes (B); total time spent seizing (C) and Meurs scale (D). Data are presented as median and interquartile range for n = 6 in each group.

Only a few studies have investigated the effect of α-MSH or its fragments on seizures. The i.c.v. administration of the peptide ORG-2766, a 4–9 fragment of adrenocorticotropic hormone (ACTH) and desacetyl α-MSH sequences, decreases seizures in electrically kindled mice (Goldman and Berman, 1984). Notwithstanding, ORG-2766 does not displace labeled α-MSH in cell preparations, even at concentrations within the millimolar range (Schioth et al., 1995), suggesting that it does not interact with α-MSH binding sites. Therefore, it seems unlikely that α-MSH receptors are involved in the anticonvulsant effect of ORG-2766 in mice, as initially thought. Moreover, ORG-2766 did not significantly alter seizure frequency of four children with intractable seizures (Pentella et al., 1982). Willig and Lagenstein (1980) studied the effect of the 4–10 ACTH fragment (which corresponds to the desacetyl 4–10 α-MSH fragment) on seizure frequency of seven epileptic children and, similar to Pentella et al. (1982), did not find any improvement in seizure frequency or in the EEG pattern of the studied patients. On the other hand, Croiset and De Wied (1992), have found that whereas ACTH(1–39), ACTH(1–24), ACTH(1–18), ACTH(1–16) and ACTH(18–39) were not active, subcutaneous pretreatment with smaller ACTH-like fragments, such as ACTH(4–9), ACTH(4–10), ACTH(4–10)(7D-Phe), ACTH(7–16), and ORG-2766, reduced the severity of pilocarpine (2.4 mg/2 μL, i.c.v.)-induced seizures. The authors have argued that anti-epileptic activity appeared to reside in the sequence 1–16 and, more specifically, in the sequences 4–7 and 7–16, of the ACTH molecule.

Plotnikoff and Kastin (1976), have suggested that α-MSH (0.01–1 mg/kg, i.p.) by itself decreases the percentage of mice that present

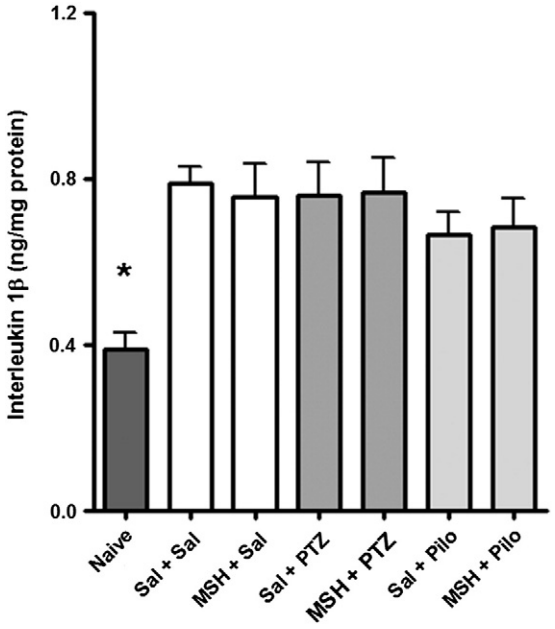

Fig. 8. Lack of effect of α-MSH (1.66 μg/3 μL, i.c.v.), PTZ (60 mg/kg, i.p.) or pilocarpine (370 mg/kg, i.p.) on IL-1β levels in the hippocampus. All groups differ from naive (SNK test) for a $p < 0.05$.

audiogenic-evoked seizures. Notwithstanding, the differences between groups reported in that study were not statistically significant. On the other hand, Izumi et al. (1973) have shown that α-MSH (10 μg, i.c.v.) decreases the latency to ouabain-elicited seizures in rats, suggesting that this peptide may facilitate seizures. However, the large injected volume (50 μL) into the left lateral ventricle may have added a confounding factor (increased cranial pressure and expanded extracellular volume, for instance), and contributed for the observed effects (Routtenberg, 1972).

Our first set of experiments demonstrated that systemically-administered α-MSH does not alter PTZ-induced seizures. Regarding this point, it is worth mentioning that, until the present moment, neither active nor facilitated transport has been reported for any α-MSH fragment or analog in the blood–brain barrier (BBB). Current evidence suggests that diffusion of the peptide across the BBB is poor in mice, similar to other water-soluble macromolecules, such as inulin (Wilson, 1988). Notwithstanding, systemic α-MSH suppresses fever induced LPS (25 μg/kg, i.p.) via central melanocortin receptors regardless of suppression of corticosterone and IL-6 release (Huang et al., 1998), suggesting that despite its low BBB permeability, low systemic doses of α-MSH alter central functions.

The limited penetration of the peptide into the CNS (Rapoport et al., 1980; Wilson, 1988) in mice motivated the experiment that investigated whether i.c.v. injected α-MSH decreased PTZ-induced seizures. However, α-MSH (1.66, 5 or 15 μg/3 μL, i.c.v.), did not prevent or attenuate PTZ-induced seizures measured as latency to myoclonic jerks and tonic–clonic seizures, and number of seizure episodes (Fig. 4A–C).

Although PTZ-induced seizures have been associated with variable degree of BBB disruption (Lorenzo et al., 1975), and inhibited by selected anti-inflammatory agents (Oliveira et al., 2008), pilocarpine-induced status epilepticus have been reported to highly depend on increased BBB permeability (Marchi et al., 2007) and on early peripheral (Marchi et al., 2009) and central inflammation (Fabene et al., 2010). Therefore, considering that an anti-inflammatory role for α-MSH has been demonstrated in both periphery (Hiltz and Lipton, 1989) and central nervous system (Lasaga et al., 2008; Muceniece and Dambrova, 2010), we hypothesized that α-MSH would probably be more effective in an experimental model of status epilepticus that depends on peripheral and central inflammation, the pilocarpine model. However, the systemic administration of α-MSH, at doses recognized to decrease peripheral and central inflammatory markers (Ichiyama et al., 1999; Rajora et al., 1997), did not attenuate pilocarpine-induced seizures, further suggesting a lack of anticonvulsant effect of α-MSH. In this respect, it is interesting that a single large dose of systemic α-MSH inhibits fever, but not other aspects of the LPS-induced acute phase response (Martin and Lipton, 1990). Therefore, based on these findings, one might also propose that the inhibitory effect of α-MSH on the inflammatory response may not quantitative or qualitatively comprehend those involved in the development of pilocarpine-induced seizures. Regarding this point it is particularly interesting that operated mice presented high levels of IL-1β compared with the naïve group, regardless of the pharmacological treatment (Fig. 8). Therefore, it is possible that α-MSH is not able to modify an already established inflammatory response, though α-MSH (i.c.v.) has been reported to decrease IL1β-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis (Cragnolini et al., 2004).

All mice injected i.c.v. with 1.66, 5 or 15 μg of α-MSH exhibited unusual behaviors, such as hindlimb and forelimb stretchings and yawning (supplementary content), whereas those mice injected with α-MSH by the i.p. route did not. Such a behavioral repertoire has been previously shown in animals subjected to intracerebral injection of α-MSH, but not after intravenous injections of the peptide (Bertolini et al., 1988; Ferrari et al., 1963). The observation that the i.c.v. injection of the peptide induces a known typical behavioral repertoire indicates that animals received biologically active doses of the peptide, and rule out the possibility that insufficient amounts of α-MSH or other factor other than the lack of anticonvulsant action of the neuropeptide underlie the currently reported lack of effect of α-MSH. At last, it is worth mentioning that control unpublished results show that both PTZ- and pilocarpine-induced seizures are attenuated by phenobarbital (20 mg/kg, per oral route (p.o.)), confirming the vast literature showing that PTZ- and pilocarpine-induced seizures are sensitive to known anticonvulsants, and the suitability of these animal models for investigating drugs with anticonvulsant activity (Moshé et al., 2006). Notwithstanding, we do not rule out an anticonvulsant effect of α-MSH in other models.

In summary, our data support that either systemic or intracerebroventricularly-administered α-MSH does not alter PTZ- or pilocarpine induced seizures.

Conclusion

The current results do not imply a protective role for α-MSH against PTZ- and pilocarpine-induced seizures.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2013.09.006.

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