Effect of intraperitoneal acetyl-\textit{l}-carnitine (ALCAR) on anxiety-like behaviours in rats

Joseph Levine\textsuperscript{1}, Zeev Kaplan\textsuperscript{1}, Jay W. Pettegrew\textsuperscript{2,3}, Richard J. McClure\textsuperscript{2,3}, Samuel Gershon\textsuperscript{3}, Igor Buriakovsky\textsuperscript{1} and Hagit Cohen\textsuperscript{1}

\textsuperscript{1} Ministry of Health Mental Health Center, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel
\textsuperscript{2} Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
\textsuperscript{3} Neurophysics Laboratory, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Abstract

Acetyl-\textit{l}-carnitine (ALCAR) is an acetyl derivative of carnitine, an endogenous molecule synthesized in vivo and supplemented by diet (mainly via meat and dairy products). Several parallel, double-blind, placebo-controlled studies have demonstrated that ALCAR treatment produces beneficial effects in geriatric depression. Since most antidepressants also have anti-anxiety effects we examined whether ALCAR shows anti-anxiety effects in a rat model of anxiety. Compared to a saline-injected control group, chronic administration of ALCAR at doses of 10 and 100 mg/kg (tested 24 h after the last dose administration) showed no effects, whereas doses of 50 and 75 mg/kg significantly reduced anxiety-like behaviours in the elevated plus-maze. Acute ALCAR (100 mg/kg), on the other hand (tested 6 h after administration), demonstrated anxiogenic effects. Our data suggest that chronic ALCAR administration may produce an inverted U-shaped curve of dose-dependent changes in anxiety-like behaviour. The precise mechanism by which ALCAR decreases anxiety-like behaviour after peripheral administration remains to be determined.

Received 4 March 2004; Reviewed 8 April 2004; Revised 22 April 2004; Accepted 2 May 2004

Key words: Acetyl carnitine, animal, anxiety disorders, models.

Introduction

\textit{Neurobiological properties of ALCAR}

Acetyl-\textit{l}-carnitine (ALCAR) is an acetyl derivative of carnitine, an endogenous molecule synthesized in vivo and supplemented by diet (mainly via meat and dairy products). Both carnitine and acetyl moieties have important neurobiological properties. The acetyl moiety of ALCAR can be used to maintain acetyl-CoA levels and carnitine is important in the \( \beta \)-oxidation of fatty acids. Both ALCAR and carnitine were reported to have beneficial effects on brain energy metabolism and to demonstrate neuroprotective properties (see Pettegrew et al., 2000 for extensive review of the literature; Beal, 2003).

\textit{ALCAR has shown efficacy in clinical depression and may also have anxiolytic effects}

Seven parallel, double-blind, placebo-controlled studies have examined ALCAR efficacy in various forms of geriatric depression (Bella et al., 1990; Fulgente et al., 1990; Garzya et al., 1990; Gecele et al., 1991; Nasca et al., 1989; Tempesta et al., 1987; Villardita et al., 1983). These papers were evaluated in a recent review of ALCAR by Pettegrew et al. (2000) and were reported to demonstrate favourable results for ALCAR treatment in a variety of depressive syndromes in humans. Five of these studies (Garzya et al., 1990; Gecele et al., 1991; Nasca et al., 1989; Tempesta et al., 1987; Villardita et al., 1983) were relatively small, each containing 20–28 subjects, and the maximal ALCAR dose administered was 2 g. Bella et al. (1990) and Fulgente et al. (1990) each studied 60 patients (30 on ALCAR and 30 on placebo) where 3 g/d ALCAR was administered, a dose reported to induce significant changes in high-energy phosphorous metabolism (Pettegrew et al., 1994, 1995, 2002;
Thal et al., 1996). The anxiety item of the Sandoz Geriatric Assessment Clinical Scale as measured in these last two studies showed no difference between ALCAR and placebo groups at baseline, whereas a statistically significant reduction (by Mann–Whitney test) was noticed for the ALCAR group compared to placebo after 60 d treatment ($p < 0.0001$ for both studies). These preliminary results, together with the findings that most antidepressants may also share anti-anxiety effects (Levine et al., 2001), led us to examine whether ALCAR can demonstrate anti-anxiety effects in an animal model.

Material and methods

Animals

A total of 133 adult male Sprague–Dawley rats weighing 150–200 g were used. The animals were habituated to the housing conditions for at least 10 d. During that time the rats were handled once daily and picked up with a gloved hand. The animals were housed four per cage in an animal room with stable temperature ($21^\circ C$) and reversed 12-h light/dark cycle (light cycle started at 21:00 hours) with ad libitum food and water. All behavioural testing was performed during the dark phase using a dim light (10:00–14:00 hours). The rats were weighed each day throughout the experimental period.

All treatment and testing procedures were approved by the Animal Care Committee of Ben-Gurion University of the Negev and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Behavioural testing and measurement

The elevated plus-maze (EPM)

Rationale for using the EPM model to explore anxiolytic effects of ALCAR

There are a variety of animal models to measure anxiety-like behaviour. The EPM model is frequently used as a first screening tool for the discovery of potential anti-anxiety agents. The model has several advantages: it is quick and easy to use, there is no prior training of animals or food and water deprivation required, and natural stimuli are used. Measurements of risk assessments have been recently added which increase the model’s ability to predict the anti-anxiety effects of drugs (Dawson and Tricklebank, 1995; Hogg, 1996; Wall and Messier, 2001). On the other hand, the model has several disadvantages: the rats cannot be reused (as exploratory behaviour of rats may habituate) and, thus, large numbers of rats are needed. In the face of restricted resources, this may limit the possibility of adding additional comparison groups to the placebo condition (i.e. positive control groups or a no-treatment group), especially in experiments where dose–response curves are examined, as in this study. Also, not all anxiolytic drugs show effects in this model. For example, 5-HT1A receptor agonists do not show a consistent effect in this model (both anxiolytic and anxiogenic effects have been reported). Chlordiazepoxide does seem to exert reliable and reproducible effects, and CCK-β receptor antagonists lacking robust effects in other anxiety models also demonstrate effects in this model. Interestingly other anxiolytic drugs such as myo-inositol and epi-inositol (Cohen et al., 1997) also seem to demonstrate anxiolytic effects in this model. While not all anxiolytic drugs show effects in this model this is also true for other models of anxiety, as summarized above regarding CCK-β antagonists (Dawson and Tricklebank, 1995; Hogg, 1996; Wall and Messier, 2001).

Description of the EPM apparatus

The maze employed was a black opaque Perspex platform elevated 50 cm above ground level, consisting of four arms as described by File (1993). Two opposing arms are enclosed by Perspex walls (40 cm high) on both the inner and outer edges of the platform and are referred to as ‘closed,’ while the two remaining opposing arms (referred to as ‘open’) are surrounded only by a Perspex ‘lip’ (1 cm high) which serves as a tactile guide to animals in the open areas. The apparatus was illuminated by dim red lighting arranged in such a manner as to provide similar lux levels in both the open and closed arms (40–60 lux). Individual rats were placed in the central platform, facing towards one of the four different arms in randomized order. Each 5-min session was recorded using an overhead video camera connected to a monitor/recorder in an adjacent observation room. Ratings were made by review of the video recordings using an Etho-Vision program (Noldus, The Netherlands) by observers blind to the original treatment conditions.

The maze was cleaned with a 5% ethanol/water solution and dried thoroughly between test sessions.

Procedure

Six measures of behaviour were assessed in the plus-maze: (1) time spent in the open arms; (2) time
spent in the closed arms; (3) the number of entries into the open arms; (4) the number of entries into the closed arms; (5) frequency of stretched-attend posture from closed to open arms, determined when the rat, located at the open end of the closed arms, exhibited an elongated body posture stretched forward with at least the snout passing over the open/closed divide; and (6) time of stretched-attend posture. Animals were scored as being in an open or closed arm only when all four paws passed over the open/closed divide.

Exploration activity was defined as the number of entries into any arm of the maze (total arm entries). Entry was defined as a rat having all four paws within one arm of the maze. In order to control for locomotor activity, number of entries into open arms was divided by the number of total arm entries.

‘Ratio time’ denotes the time spent in the open arms of the maze divided by the time spent in the closed arms of the maze. ‘Ratio entry’ denotes the number of entries into the open arms of the maze divided by the number of entries into the closed arms. A smaller score for ratio time or ratio entry means a higher manifestation of anxiety for the rat.

‘Risk assessment’ was defined when a rat poked its head, and possibly forepaws, into the open arms of the maze while its hindquarters remained in one of the closed arms of the maze (Adamec and Shallow, 1993). ‘Risk assessments’ [for both time (‘time risk’) and frequency (‘frequency risk’)] were calculated as a ratio of the total time spent in the closed arms of the maze (rats could only perform these behaviours from within the closed arms). Decreased scores for risk assessment express increased manifestation of anxiety-like behaviour.

Drugs

ALCAR (acetyl-carnitine, 10% solution diluted in 0.9% saline) vs. saline (sodium chloride, 0.9%) both injected intraperitoneally (i.p.).

**Experiment I – acute phase**

Forty adult Sprague–Dawley rats were used in these experiments. ALCAR or saline at doses of 100 and 500 mg/kg was injected i.p. (administered in a volume of 0.1 ml/100 g body weight). Behaviour in the EPM was measured 6 h after i.p. ALCAR administration. There were 10 rats in each group on each experimental day. For each dose level of the experiment, rats were randomly assigned into two groups: (1) ALCAR or (2) control.

**Experiment II – sub-chronic phase**

Twenty adult Sprague–Dawley rats were used in this experiment. ALCAR (10% solution diluted in 0.9% saline) was injected i.p. (administered in a volume of 0.1 ml/100 g body weight) at doses of 100 mg/kg, once daily (10:00 hours) for 6 d. Behaviour in the EPM was measured 24 h after the last i.p. ALCAR administration.

**Experiment III – chronic phase: finding a dose-response curve for chronic ALCAR administration**

Doses as low as 10 mg/kg ALCAR have been reported to exert biological effects in animal models (McKay Hart et al., 2002; Tolu et al., 2002). These authors reported that even a reduced dose of 10 mg/kg.d ALCAR therapy was significantly effective in preventing neuronal death at 2 wk in an axotomy model; however, in higher doses of ALCAR, morphological changes were more evident, thus demonstrating a dose–response effect on neuronal survival. Others have used higher doses of up to 75 mg/kg.d (Fernandez et al., 1990, 1992; Ghirardi et al., 1994; Markowska et al., 1990; Ramacci et al., 1988). We therefore decided to study chronic ALCAR dose–response curve effects in the plus-maze utilizing a range of doses from 10 to 100 mg/kg.d.

Seventy-three adult Sprague–Dawley rats were used in this experiment. ALCAR (10% solution diluted in 0.9% saline) was injected i.p. (administered in a volume of 0.1 ml/100 g body weight) at doses of 10, 50, 75 and 100 mg/kg, once daily (10:00 hours) for 14 d. Behaviour in the EPM was measured 24 h after the last dose of i.p. ALCAR.

**Statistical analyses**

One-way analysis of variance (ANOVA) was performed for between-group differences on the parameters described above.

**Results**

The effect of acute i.p. ALCAR (100 mg/kg) vs. placebo on rat anxiety-like behaviour

As shown in Table 1, i.p. administration of 100 mg/kg ALCAR enhanced anxiety-like behaviour as measured by time spent in closed vs. open arms, and by open-arm entries. ALCAR-treated rats spent significantly more time exploring the closed arms of the EPM ($F=5.8, p<0.03$), and less time in the open arms ($F=5.8, p<0.03$) than did the rats in the control group.
Table 1. Effect of acute, sub-chronic, and chronic ALCAR treatment on anxiety-like behaviour of Sprague–Dawley rats

<table>
<thead>
<tr>
<th>ALCAR treatment (n=number of animals)</th>
<th>Behavioural parameters</th>
<th>Time open arms (min)</th>
<th>Time closed arms (min)</th>
<th>Ratio time (open/closed)</th>
<th>No. of closed-arm entries</th>
<th>No. of open-arm entries</th>
<th>Total arm entries</th>
<th>Ratio [no. of entries (closed/open)]</th>
<th>Time risk</th>
<th>Frequency risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCAR (100 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>0.8 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>0.2 ± 0.07</td>
<td>4.8 ± 0.8</td>
<td>3.6 ± 0.8</td>
<td>8.4 ± 1.0</td>
<td>1.4 ± 0.6</td>
<td>2.9 ± 1.0</td>
<td>0.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>ALCAR (n=10)</td>
<td>0.25 ± 0.13</td>
<td>4.75 ± 0.13</td>
<td>0.06 ± 0.03</td>
<td>4.1 ± 1.0</td>
<td>0.7 ± 0.3</td>
<td>4.8 ± 1.2</td>
<td>0.15 ± 0.07</td>
<td>0.5 ± 0.2</td>
<td>0.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Statistics</td>
<td>F=5.8</td>
<td>F=5.8</td>
<td>F=4.9</td>
<td>ns</td>
<td>F=10.4</td>
<td>F=5.2</td>
<td>F=4.6</td>
<td>F=4.8</td>
<td>F=6.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.005</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.02</td>
<td></td>
</tr>
<tr>
<td><strong>Acute phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCAR (500 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>0.6 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>0.14 ± 0.06</td>
<td>6.4 ± 1.2</td>
<td>4.6 ± 1.3</td>
<td>11.0 ± 1.9</td>
<td>1.2 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>ALCAR (n=10)</td>
<td>0.08 ± 0.03</td>
<td>4.9 ± 0.03</td>
<td>0.015 ± 0.006</td>
<td>1.7 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>2.1 ± 0.4</td>
<td>0.3 ± 0.15</td>
<td>0.2 ± 0.2</td>
<td>0.04 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Statistics</td>
<td>F=6.3</td>
<td>F=6.3</td>
<td>F=5.1</td>
<td>F=13.8</td>
<td>F=10.2</td>
<td>F=21.4</td>
<td>ns</td>
<td>ns</td>
<td>F=5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.02</td>
<td>p &lt; 0.02</td>
<td>p &lt; 0.035</td>
<td>p &lt; 0.002</td>
<td>p &lt; 0.005</td>
<td>p &lt; 0.0002</td>
<td>ns</td>
<td>ns</td>
<td>p &lt; 0.035</td>
<td></td>
</tr>
<tr>
<td><strong>Sub-chronic phase (6 d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCAR (100 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.6 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>0.12 ± 0.05</td>
<td>5.6 ± 1.0</td>
<td>0.7 ± 0.3</td>
<td>6.3 ± 1.2</td>
<td>0.12 ± 0.5</td>
<td>2.2 ± 0.9</td>
<td>0.2 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>ALCAR</td>
<td>0.8 ± 0.2</td>
<td>4.2 ± 0.25</td>
<td>0.16 ± 0.07</td>
<td>5.8 ± 1.3</td>
<td>1.6 ± 0.45</td>
<td>7.4 ± 1.7</td>
<td>0.2 ± 0.06</td>
<td>4.4 ± 1.9</td>
<td>0.4 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Statistics</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic phase (14 d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCAR (10 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>0.3 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>0.06 ± 0.03</td>
<td>11.4 ± 1.5</td>
<td>0.3 ± 0.2</td>
<td>11.8 ± 1.6</td>
<td>0.02 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>0.15 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>ALCAR (n=10)</td>
<td>0.5 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>0.13 ± 0.05</td>
<td>8.6 ± 1.3</td>
<td>0.9 ± 0.5</td>
<td>9.5 ± 1.7</td>
<td>0.08 ± 0.04</td>
<td>0.03 ± 0.02</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Statistics</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic phase (14 d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCAR (50 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>0.4 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>0.09 ± 0.04</td>
<td>9.9 ± 2.6</td>
<td>0.4 ± 0.2</td>
<td>10.2 ± 2.7</td>
<td>0.04 ± 0.02</td>
<td>0.03 ± 0.02</td>
<td>0.1 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>ALCAR (n=6)</td>
<td>1.5 ± 0.4</td>
<td>3.5 ± 0.4</td>
<td>0.6 ± 0.3</td>
<td>11.2 ± 2.1</td>
<td>2.7 ± 0.8</td>
<td>13.9 ± 1.7</td>
<td>0.4 ± 0.3</td>
<td>0.1 ± 0.06</td>
<td>0.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Statistics</td>
<td>F=6.2</td>
<td>F=6.2</td>
<td>ns</td>
<td>ns</td>
<td>F=9.3</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>F=8.7</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.03</td>
<td>ns</td>
<td>ns</td>
<td>p &lt; 0.01</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>p &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>
Total entries into the open and closed arms (total arm entries) were reduced in rats treated with ALCAR, however, it was less than 50% of the total entries for the control group. We then calculated open-arm entries divided by total arm entries (to control for locomotor activity) finding significantly higher values for the control group compared to the ALCAR group ($F=8.7, p<0.009$; see Figure 1).

Administration of ALCAR also decreased the calculated parameters such as ratio time (open/closed), time risk, and the frequency risk compared to controls, reflecting an increase in anxiety-like behaviour.

The effect of acute i.p. ALCAR (500 mg/kg) vs. placebo on rat anxiety-like behaviour

As shown in Table 1, i.p. administration of 500 mg/kg ALCAR seemed to enhance anxiety-like behaviour as measured by time spent in closed vs. open arms, and by open-arm entries. ALCAR-treated rats (500 mg/kg) spent significantly more time exploring the closed arms of the EPM ($F=6.3, p<0.02$), and less time in the open arms ($F=6.3, p<0.02$) than did the control group rats (Table 1). Total entries into the open and closed arms (total arm entries) were significantly reduced in rats treated with ALCAR compared to controls ($F=21.4, p<0.0002$; see Table 1).

Administration of ALCAR decreased the calculated parameters as such ratio time ($F=5.1, p<0.04$) and the frequency risk ($F=5.2, p<0.04$), compared to controls, and this seems to reflect an increase in anxiety-like behaviour. However, the ALCAR-treated group of rats had significantly lower total arm entries compared to controls (less than 20% of that of the control group, probably reflecting much lower locomotor activity), and since locomotor activity may influence behaviour in the EPM – although parameters such as frequency risk suggest an increase in anxiety-like behaviour – it seems that a conclusion cannot be reached as to the anxiolytic effect of acute 500 mg/kg ALCAR in the present experiment.

The effect of sub-chronic i.p. ALCAR (100 mg/kg) vs. placebo on rat anxiety-like behaviour

As shown in Table 1, there were no significant differences between the groups in the anxiety-like behaviour parameters.

The effect of chronic i.p. ALCAR (10 mg/kg) vs. placebo on rat anxiety-like behaviour

As shown in Table 1, there were no significant differences between the groups in the anxiety-like behaviour parameters (see also Figure 1).
The effect of chronic i.p. ALCAR (50 mg/kg) vs. placebo on rat anxiety-like behaviour

As shown in Table 1, administration of 50 mg/kg ALCAR for 14 d significantly decreased anxiety-like behaviour as measured in the EPM. Time spent in the open arms \( (F = 6.2, p < 0.03) \) and open-arm entries \( (F = 9.3, p < 0.01) \) were significantly increased after administration of 50 mg/kg ALCAR for 14 d, compared to controls. There were no significant differences between the groups in the total arm entries parameter. Open-arm entries divided by total arm entries were higher for the ALCAR group compared to the control group \( (F = 5.3, p < 0.04; \text{Figure 1}) \).

Administration of 50 mg/kg ALCAR also increased the frequency risk compared to control rats, reflecting a decrease in anxiety-like behaviour.

The effect of chronic i.p. ALCAR (75 mg/kg) vs. placebo on rat anxiety-like behaviour

As shown in Table 1, administration of 75 mg/kg ALCAR for 14 d significantly decreased anxiety-like behaviour as measured in the EPM. Time spent in the open arms \( (F = 9.2, p < 0.008) \) and open-arm entries \( (F = 11.4, p < 0.004) \) were significantly increased compared to controls (see Table 1). There were no significant differences between the groups in the total arm entries parameter.

Open-arm entries divided by total arm entries were higher for the ALCAR group compared to the control group \( (F = 4.9, p < 0.04; \text{Figure 1}) \).

Administration of 75 mg/kg ALCAR increased the calculated parameters such as ratio time, and ratio entry, compared to control rats, reflecting a decrease in anxiety-like behaviour.

The effect of chronic i.p. ALCAR (100 mg/kg) vs. placebo on rat anxiety-like behaviour

As shown in Table 1, there were no significant differences between the groups in the anxiety-like behaviour parameters.

As shown in Figure 2, rats receiving daily injections of saline gained weight steadily from 179.3 ± 2.05 mg (mean ± S.E.M.) on day 1 to 298.3 ± 3.8 mg on day 14. In contrast, rats receiving daily injections of 100 mg/kg ALCAR gained less weight, from 186.9 ± 2.9 g on day 1 to 251.1 ± 4.1 g on day 14. The two-way ANOVA repeated-measures test indicated that a significant difference in body weight between saline-treated and ALCAR-treated rats was seen beginning at day 9 of injections, with ALCAR-treated rats gaining less weight than the control group. — – – , ALCAR; – – – – , control.

The effect of chronic i.p. saline on rat anxiety-like behaviour

Rats chronically injected with saline (all control groups pooled together) compared to rats treated acutely with saline (two control groups pooled together) showed increased anxiety-like behaviour as measured in the EPM. Time spent in the open arms \( (F = 5.9, p < 0.02) \) and open-arm entries \( (F = 34.1, p < 0.001) \) were significantly decreased after administration of chronic saline for 14 d.

Open-arm entries divided by total arm entries were higher for the acutely treated rats compared to the chronically treated group \( (F = 49.9, p < 0.0001) \).
Chronic as opposed to acute administration of saline decreased the calculated parameters such as ratio time and ratio entry, reflecting an increase in anxiety-like behaviour.

Discussion

**ALCAR is anxiolytic in chronic use**

Rats were injected with ALCAR either acutely (100, 500 mg/kg), sub-chronically (100 mg/kg) or chronically (10, 50, 75, 100 mg/kg). Acute administration was studied in the EPM after 6 h whereas sub-chronic and chronic administrations were tested 24 h after the last injected dose. Saline served as a control. Compared to saline injection, acute i.p. ALCAR administration at doses of 100 mg/kg significantly increased anxiety-like behaviour in rats. Chronic administration of ALCAR at doses of 50 and 75 mg/kg – but not 10 and 100 mg/kg – significantly reduced anxiety-like behaviours, demonstrating an inverted U-shaped dose–response curve. These data suggest that ALCAR, an antidepressant, may have also anti-anxiety effects in humans, as has been reported previously for a variety of antidepressants (Levine et al., 2001).

**ALCAR may be anxiogenic in acute use**

ALCAR administrated acutely (100 mg/kg) showed an anxiogenic effect. Similar results seem to have been obtained at 500 mg/kg; however, due to the substantial decrease in locomotor activity in the 500 mg/kg group, the results obtained may be taken as suggestive only. It is of note that due to limited resources we have not examined ALCAR effects in acute administration of lower doses (i.e. 10, 50, 75 mg/kg) and such doses may be found in future studies to carry anxiogenic effects, no effect or even anxiolytic effects. Also, since acute dose effects were examined after 6 h and were not followed at later time-points, it may be that the anxiogenic effects are time dependent, and future studies may address this point.

In humans, clomipramine has anti-panic efficacy after chronic treatment but may cause anxiety when administered acutely (Ramos et al., 1993). Humble and Wistedt (1992) reported that the antidepressant citalopram demonstrated beneficial effects in panic disorder in chronic treatment but caused a transient increase in panic-related symptoms in the first week of treatment. Thus, the adaptation of the behavioural effects of acute administration of ALCAR, which is seen in chronic administration of this drug is found also with other anxiolytic agents and may be due to receptor down-regulation or other processes. It is also possible that acute i.p. ALCAR is not anxiolytic due to lack of central nervous system activity. This line of thought is supported by the fact that acute ALCAR administration seems to penetrate the blood–brain barrier poorly and only small amounts of $[^3H]$acetyl-carnitine were found in the brain after pulse i.v. administration (Farrell et al., 1986). It also seems that the acetyl moiety and not the carnitine moiety penetrates the brain when the drug is given acutely (Kuratsune et al., 1997). On the other hand, when the drug is given chronically, significant elevation of ALCAR levels are found in the CSF suggesting that chronic administration raises brain ALCAR levels (Parnetti et al., 1992). One may thus speculate that the anxiogenic effect of ALCAR may be induced either by some peripheral mechanism or by the increase of the brain acetyl moiety, whereas the chronic effects are induced by the central nervous system effect of ALCAR.

**Methodological issues**

Three issues pertaining to the methods used require clarification. First, the time-period between the last administered dose of ALCAR in the various study groups and the time-point when the EPM test was done; second, the locomotor activity of the rats and its possible influence on the results obtained; and third, the effects of single vs. repeated (chronic) saline injections on anxiety-like behaviours of rats.

**Time-period between the last administered dose of ALCAR in the various study groups and the timing of the EPM test**

The fact that rats were tested for acute ALCAR effects 6 h after its administration was based on pharmacokinetic considerations. There are data demonstrating that following i.v. administration of 500 mg ALCAR to humans, plasma ALCAR levels decline gradually to baseline within 12 h whereas the ALCAR metabolite carnitine plasma levels decline gradually to baseline within 24 h. We thus decided to study ALCAR effects 6 h after its acute administration.

On the other hand rats administered the drug chronically were tested 24 h after the last administered dose. This is based on the assumption that the last injection administered may carry acute as well as chronic effects – which are not necessarily identical. In order to separate between the two and to measure chronic effects only, we tested the rats on the plus-maze 24 h after the last dose of ALCAR, a time-point when acute effects are not expected.
Finally, one may suggest testing the rats – administered chronic ALCAR – in the plus-maze at both 6 and 24 h following the last administered dose. Such a procedure may enable comparison between acute and chronic ALCAR administration. However, this is not recommended since habituation could develop to repeated testing in the plus-maze.

Controlling for locomotor activity

It is, in general, agreed that locomotor activity may influence results obtained in the EPM and that in order to interpret results obtained in this model as suggesting anxiogenic or anxiolytic effects of a given drug, one must first control for the level of locomotor activity induced by that drug (Wall and Messier, 2001). In general, the measurement of total arm entries can be taken as an indicative measurement of the locomotor activity of the study animals, although some authors suggest that closed-arm entries or a combined measure of both closed and total arm entries may be used (Wall and Messier, 2001). Presented here are data in which open-arm entries are divided by total arm entries in order to control for locomotor activity. We suggest that the results should be regarded with caution when a substantial decrease in total arm entries (serving as an indicative measure of locomotor activity) is induced by the active drug, even if a parameter of ‘open arm entries divided by total arm entries’ is taken into consideration, thus controlling for locomotor activity. Rats treated acutely with 500 mg/kg ALCAR had much lower total arm entries compared to their control group as well as compared to other study groups (mean of 2.1 for 500 mg/kg ALCAR vs. 5–12 in other groups). This suggests that ALCAR administered acutely in a dose of 500 mg/kg may have exerted some depressing effects on the locomotor activity of the rats, and that although the ‘open arm entries divided by total arm entries’ showed a statistically significant decreased ratio for the ALCAR group vs. the control group, this result should not be considered conclusive, but only indicative of anxiogenic effects of ALCAR.

Repeated administration of saline injections carries anxiogenic effects in the plus-maze

Chronic saline injections (compared to a single injection of saline) have anxiogenic effects. Future studies may make use of pumps in order to prevent the chronic stress associated with repeated injections. Interestingly, it was reported that a variety of attempts to increase baseline anxiety levels of rats prior to the plus-maze test – in order to heighten the test’s sensitivity to anxiolytic or anxiogenic effects – failed to shift preference for the closed arms. Such manipulations included immobilization or exposure to footshock (Dawson and Tricklebank, 1995). On the other hand, several environmental manipulations, such as daily brief handling or exposure to building noises, did change performance in the maze (Fernandes and File, 1993). We now report that repeated injections of saline are themselves anxiogenic. However, since both ALCAR and saline control groups were treated with the same number of injections administered in a similar fashion, we controlled for the effect of repeated injections on the rats’ performance in the plus-maze.

Two possible mechanisms of ALCAR anxiolytic activity

Two possible mechanisms of anxiolytic ALCAR activity may be offered: GABAergic activity and agonistic activity at 5-HT1A receptors. Fariello et al. (1988) reported a significant dose-dependent increase of nigral GABA following ALCAR treatment, an effect also demonstrated with the ALCAR metabolite carnitine. GABAergic agents have also shown consistent anxiolytic effects in the EPM and GABAergic agents are effective anxiolytic agents in humans. Therefore, there is a possibility that ALCAR exerts its anxiolytic effect in the plus-maze via enhancing GABAergic activity. However since GABAergic agents tend also to show acute anxiolytic effects – not demonstrated in the above study – other possible modes of action should be considered.

ALCAR administered to rats at a dose of 20 mg/d for 7 d was reported by Tolu et al. (2002) to increase dopamine and serotonin output in the nucleus accumbens shell and to prevent the development of escape behaviour to unavoidable stress. These authors suggested that such a behavioural effect may be mediated by the activation of 5-HT1A receptors, since administration of WAY-100635 (a 5-HT1A receptor antagonist) reversed the escape deficit, whereas antagonists of D1 receptors, β-adrenergic receptors and M1 and M2 muscarinic receptors had no effect.

Also, 5-HT1A agonists such as buspirone, gepirone and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) were reported by some (Dunn et al., 1989), but not all (Dawson and Tricklebank, 1995), to share anxiolytic effects in the EPM, while buspirone administered chronically was reported to demonstrate efficacy in human studies of anxiety disorders (Cohn and Rickels, 1989). It is thus suggested that ALCAR activity may be mediated via 5-HT1A receptors and
future studies may examine whether 5-HT1A antagonists may reverse chronic ALCAR anxiolytic effects in this model.

Conclusions
Chronic administration of ALCAR at doses of 50 and 75 mg/kg compared to placebo control significantly reduced anxiety-like behaviours in the plus-maze, while acute ALCAR (100 mg/kg) demonstrated anxiety-inducing effects. Our data as well as other data suggest that ALCAR administration may produce dose-dependent changes in anxiety-like behaviour. The precise mechanism by which ALCAR decreases anxiety-like behaviour after peripheral administration remains to be determined.

Acknowledgements
The authors thank Dr Yamima Osher for her valuable help.

Statement of Interest
None.

References


