The Potent Free Radical Scavenger α-Lipoic Acid Improves Cognition in Rodents

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INTRODUCTION

α-Lipoic acid or thioctic acid is a coenzyme for the pyruvate dehydrogenase complex in the mitochondrial matrix. Presently it is mainly in theraputic use against diabetic polyneuropathy.[1,2] At least part of its therapeutic implications can be explained by the free radical scavenging properties of α-lipoic acid[3] and particularly of its reduced form dihydrolipoic acid.[4,5] Free radical damage may be an important cause of aging and age-related disease,[6] including Alzheimer's disease[6] and Parkinson's disease.[7] The membrane hypothesis of aging[8] argues that chronic damage by free radicals leads to changes in cell membrane properties that cause a deterioration of cellular function. Based on the membrane hypothesis of aging, we investigated whether a treatment with α-lipoic acid could improve memory function in young and aged mice. Because of the potential therapeutic importance of the results, we performed a parallel experiment in young rats using α-lipoic acid and the known cognition enhancer piracetam[9,10] to see whether the results in mice can be generalized.

MATERIALS AND METHODS

Animals

Female NMRI mice had been purchased from Interfauna, Tuttlingen, Germany. Young-adult ones (3–6 months) had been transferred to our animal facilities at least two weeks before the beginning of the tests. Old female NMRI mice were received at the age of 12 months as retired breeders and kept in our animal facilities from then on until they were 20 to 23 months old. Young (2 months) male CrI: (Wi)BR rats had been purchased at Charles River, Bad Sulzfeld, Germany. They were transferred to the testing facilities 4 days before the test.

Treatment

Orally treated mice received 100 mg/kg body weight α-lipoic acid in Methocel (1%) administered once per day for 15 days for the test of habituation in the open field. Controls received Methocel alone. Mice treated by intraperitoneal injection (ip treatment) got 100 mg/kg body weight α-lipoic acid as its trometamol salt (Thioctacid T solution for injection from Asta Medica, Frankfurt, Germany) for 15 days during the test in the Morris water maze. Controls were treated with saline. On days of behavioral testing, the treatments were given after the tests. Rats received 1 and 10 mg/kg α-lipoic acid in 5% dimethylaminoacetamide (DMA) or 100 mg/kg and 300 mg/kg piracetam in saline intraperitoneally one hour before the tests. Controls received the respective vehicle alone.

Behavioral Testing

Habituation in the Open Field

This test has been described in detail in Stoll *et al.*[11] In short, mice were placed in an open field (50 × 50 × 20 cm) the floor of which was divided in squares. Horizontal activities were measured as the number of squares entered with the forepaws. The animals were placed in the open field for 3 minutes, repeating this exposure after 15 minutes and after 24 hours. The test started on day 14 of oral treatment.

Morris Water Maze

The maze consisted of a black round pool (diameter 56 cm) that was filled with water of 27°C. The pool was divided into 4 quadrants. A black platform (diameter 4 cm) was placed about 1 cm below the waterline in the center of a quadrant. One test block included 4 trials on the first day and 1 trial on the second day. If a mouse did not find the platform within 60 seconds, it was guided to it. After finding the platform, the animal had to stay there for 30 seconds. Starting positions varied from trial to trial, platform positions from block to block. Six test blocks were performed: the first one began on day 4 before the start of ip treatment, the second to sixth ones on days 1, 4, 8, 11, and 14 of ip treatment. Swimming distances were measured with a Video Path Analyzer.

Active Avoidance Learning

The rats were placed in the active avoidance learning box (31 cm × 35 cm × 50 cm) where a tone signal (1.6 kHz, 8 s) (the conditioned stimulus) was followed by a foot shock (40 V, 12 s) (the unconditioned stimulus). Animals could avoid the shock by jumping to a suspended pole. During training and the retention test (24 hours after the training), each animal got 20 randomly distributed stimulus combinations. The test criterion for a conditioned response (CR) was that a rat jumped onto the pole in three succeeding stimulus combinations during the tone signal. Further, the latency from the start of the tone up to jumping onto the pole was registered.

Statistics

Statistical analyses apart from the Mann-Whitney rank sum test were performed with the SAS system for personal computers, release 6.03. In the Morris water maze task, swimming speeds of mice finding the platform are significantly higher than those of animals who do not, possibly distorting the distribution of the distances swum. To adjust for this difference as individually as possible and, therefore, with as little influence on variance as possible, the distance swum by a mouse that did not find the platform in a particular trial was adjusted. It was set to the maximum of the measured swimming distance and of the distance computed from the average swimming speed of the animal in the case of finding the platform in the block. That is, the computed swimming distance was the product of the individual blockwise swimming speed and the trial time of 60 seconds.

RESULTS

Habituation in the Open Field

A highly significant repetition of trials effect indicating learning was found in young (y), 3 months old, and aged (a), 20–23 months old, mice (see TABLE 1 for statistics). *Post hoc* comparisons of horizontal activities using the paired *t*-test revealed habituation in the 15 minute trial for both age groups in treated (tr) and control (co) animals (y-co: $p < 0.0001$, $t = 7.78$, $n = 15$; y-tr: $p < 0.0001$, $t = 6.87$, $n = 10$; a-co: $p < 0.0001$, $t = 7.40$, $n = 19$; a-tr: $p < 0.0001$, $t = 10.85$, $n = 10$). They also revealed no significant loss of habituation in the 24 hours trial as compared to the 15-minute trial for young mice either treated or controls. Aged control mice showed significant forgetting ($p < 0.003$, $t = 3.43$, $n = 19$). However, no significant forgetting between the 15-minute trial and the 24-hour trial could be found in aged treated mice.

Morris Water Maze

The time-course of swimming distances is shown in FIGURE 1. Both young (co: $n = 17$, tr: $n = 16$) and aged mice (co: $r = 14$, tr: $n = 14$) showed significant effects of block (y: $p < 0.0001$, $F = 7.35$, 5 DF; a: $p < 0.0001$, $F = 15.06$, 5 DF) and trial (y: $p < 0.0001$, $F = 15.77$; 4 DF; a: $p < 0.0001$, $F = 8.89$, 4 DF). The interaction of block and trial was significant in young animals ($p < 0.0069$, $F = 1.98$, 20 DF). A significant effect of treatment was found only in aged mice ($p < 0.0444$, $F = 4.43$, 1

TABLE 1. Habituation in the Open Field in Young and Aged Mice under Treatment with 100 mg/kg α-Lipoic Acid[a]

	Q0	Q15′	Q24h	*n*
Young control	98.07 ± 2.72	64.33 ± 5.27	75.53 ± 4.54	15
Young treated	88.70 ± 5.88	57.30 ± 6.37	68.30 ± 6.94	10
Aged control	61.79 ± 5.81	77.00 ± 5.51	77.00 ± 5.51	19
Aged treated	111.80 ± 13.17	64.90 ± 9.59	66.30 ± 7.76	10

[a]Q0 is the initial horizontal activity, Q15′ the activity 15 minutes after the first trial, Q24h the activity 24 hours after the first trial. All means are given with SEMs. For statistics see text.

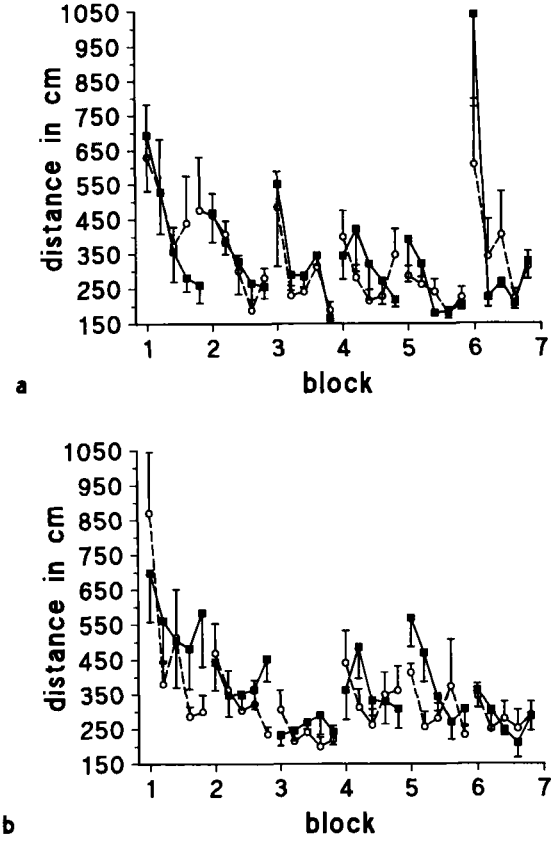

FIGURE 1. Learning in the Morris water maze in young (1a) and aged (1b) female NMRI mice under treatment with 100 mg/kg α-lipoic acid. Controls are indicated as open circles, treated animals as solid squares. A significant main effect of treatment could be found in aged mice. Values are means ± standard errors of the means (SEMs). For statistics see text.

DF). However, there were no significant interactions among treatment, block, and trial. For this reason, a separate analysis of the last three training blocks was performed. An interaction of treatment and trial close to significance could be found there ($p < 0.0611, F = 2.32, 4$ DF).

Active Avoidance Learning

Controls did not reach the criterion for a CR either during training or during the retention trial (FIGURE 2, FIGURE 3). However, both α-lipoic acid (FIGURE 2) and piracetam (FIGURE 3) improved performance during training and the retention trial in a dose-dependent way. Latencies decreased from training to the retention trial. This decrease was more pronounced in animals treated with either 1.0 or 10.0 mg/kg thioctic acid or 300 mg/kg piracetam (TABLE 2).

DISCUSSION

Age differences in habituation in the open field[11,12] and the Morris water maze task[13] have been described previously. The results presented in this paper give further support to the hypothesis that α-lipoic acid is able to improve cognitive function in rodents. The results of habituation in the open field and of the Morris water maze task support the notion that age-related deficits of cognition are attenuated: aged mice showed an improvement of memory in the habituation in the open field task as can be seen in the 24 hour activities (for a further discussion of this topic see Stoll *et al.*[11] A significant main effect of treatment for aged mice in the Morris water maze task hints at an age-specific effect of α-lipoic acid on learning or memory. Further, the interaction between treatment and trial during the last three water maze blocks that was close to significance in aged mice suggests a time-

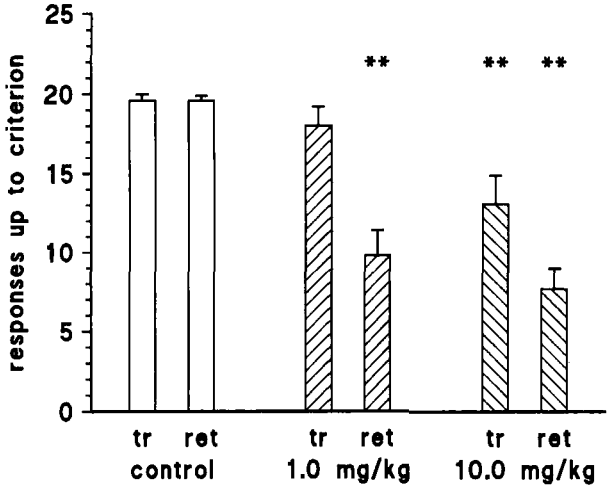

FIGURE 2. Active avoidance learning in young male rats under treatment with 1.0 and 10.0 mg/kg α-lipoic acid. Values are means ± SEMs. ** indicates $p < 0.01$ in the Mann-Whitney rank-sum test (1.0 mg, 24 hour retention: U = 3.5; 10.0 mg, training: U = 2.9; 10.0 mg, 24 hour retention: U = 3.5).

dependent effect of α-lipoic acid. This is in accordance with the hypothesis that increased oxidative stress with aging may be one cause of age-related cognitive decline. Since α-lipoic acid improves glucose utilization in a manner additive to insulin[14–17] and since glucose meabolism is impaired in patients with Alzheimer's disease,[18,19] a specific effect of α-lipoic acid may add to its radical scavenger function in aged individuals. This notion is supported by casuistic descriptions of a partial attenuation of behavioral deficits in Alzheimer's patients.[20] Improved active avoidance learning in young rats hint in a further direction: a nootropic component in the action of α-lipoic that is also found in piracetam cannot be excluded. Preliminary data for aged rats hint in the same direction.[21] Apart from improvements in cognition, α-lipoic acid may have additional effects on motor functions as may be suggested by the high initial activity of treated aged mice in the open field (TABLE 1) even though there was no significant difference to aged controls. Effects on motor

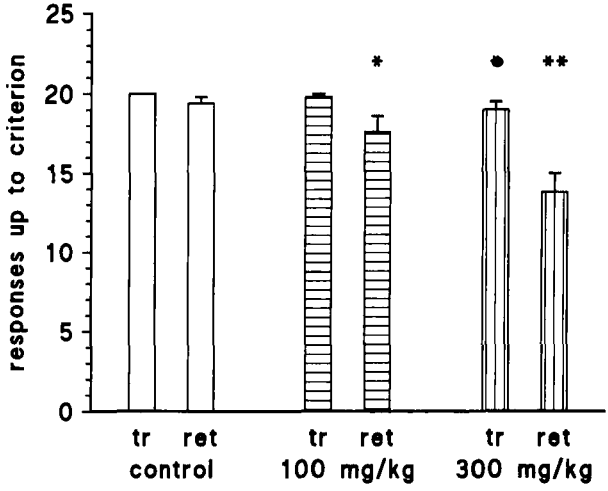

FIGURE 3. Active avoidance learning in young male rats under treatment with 100 and 300 mg/kg piracetam. Values are means ± SEMs. * indicates $p < 0.05$, ** indicates $p < 0.01$ in the Mann-Whitney rank-sum test (100 mg, 24 hour retention: U = 1.9; 300 mg, training: U = 2.2; 300 mg, 24 hour retention: U = 3.5).

functions may influence the results of the Morris water maze task and active avoidance learning as well. Our data hint at an action of α-lipoic acid on different areas of learning, as in the Morris water maze and active avoidance learning, and memory, as in habituation and the water maze. The effects seem to depend on species, model, and age. However, improved cognitive function is found in all models described here. Further experiments taking into account these possible differential effects of α-lipoic acid as well as possible effects on motor functions are necessary to elucidate the effects and the mechanisms of action of α-lipoic acid. The same is true regarding its biochemical mechanisms of action for the effects on learning and memory as suggested previously.[11] In summary, our data suggest that a treatment with free radical scavengers may be a useful treatment for age-related memory decline.

TABLE 2. Active Avoidance Learning in Young Male Rats under Treatment with α-Lipoic Acid or Piracetam[a]

Treatment	mg/kg	Training	U	Retention	U	*n*
Control	—	11.7 ± 0.3	—	8.0 ± 0.4	—	10
α-Lipoic acid	1.0	10.6 ± 0.3[c]	2.3	6.2 ± 0.2[c]	3.3	10
α-Lipoic acid	10.0	9.0 ± 0.3[c]	3.6	4.9 ± 0.5[c]	3.7	10
Control	—	10.8 ± 0.4	—	7.9 ± 0.3	—	10
Piracetam	100	10.8 ± 0.3	0.1	7.7 ± 0.3	0.6	10
Piracetam	300	9.9 ± 0.3[b]	1.9	7.0 ± 0.3[b]	2.3	10

[a]The mean latencies to jump to the pole after the tone signal started are given with SEMs. [b]Indicates $p < 0.05$, [c]$p < 0.01$ versus control in the Mann-Whitney rank sum test. U is the U-value of the Mann-Whitney Rank sum test.

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