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BRIEF COMMUNICATION

Differential Effect of Acetyl-l-Carnitine on Open Field Behavior in Young and Old Rats

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BLOKLAND, A., W. RAAIJMAKERS, F. J. VAN DER STAAY AND J. JOLLES. *Differential effect of acetyl-l-carnitine on open field behavior in young and old rats.* PHYSIOL BEHAV **47**(4) 783–785, 1990.—Acetyl-l-carnitine (ALCAR) exerts antiaging effects with regard to various physiological and neurochemical measures in rats. In this study we evaluated the effect of a chronic treatment with ALCAR (6 weeks, 50 mg/kg/day) on the open field behavior of 5-month-old and 26-month-old male Wistar rats. Old rats defecated more, made fewer crossings, and spent more time in the corner squares. However, it is difficult to interpret these findings in terms of an increase in emotional reactivity with age because locomotor performance generally has been found to be impaired in old rats. Consequently, treatment effects were analysed within age groups. ALCAR had no effect in young rats, but the old ALCAR-treated rats ambulated less and spent more time in the corner squares than the old control rats. We have interpreted this in terms of an enhanced emotional reactivity of old ALCAR-treated rats.

Acetyl-l-carnitine Open field Emotional reactivity Age differences

VARIOUS nootropic substances have been developed in the search for a potential pharmacological treatment of cognitive disturbances in elderly subjects. One of these substances is acetyl-l-carnitine (ALCAR). ALCAR is the acetyl derivate of carnitine, a naturally occurring substance which plays an important role in the energy and lipid metabolism of cells (4,5). As ALCAR can cross the blood-brain barrier, it could affect brain cells directly [see (1)].

Chronic ALCAR treatment is reported to exert antiaging effects as it prevents several age-associated neuroanatomical and neurochemical changes, particularly in the hippocampus (2,4). Another study has shown that the control of adrenocortical responsiveness is improved in aged ALCAR-treated rats (1). This finding is consistent with the anatomical and functional preservation of hippocampal glucocorticoid receptors found in old rats chronically treated with ALCAR (1). Furthermore, chronic ALCAR treatment has been shown to improve discrimination learning in 19-month-old rats compared to untreated controls (8).

ALCAR treatment increases the levels of carnitine and ALCAR in the brain. It has been suggested that basal forebrain cholinergic neurons are activated by the increase in high-affinity choline uptake activity in cortical and hippocampal but not striatal synaptosomes (4).

The effects of ALCAR on basal aspects of behavior are not clear. Drago and coworkers (5) found that young rats showed more ambulation and reared more frequently in an open field (OF) after acute injections of ALCAR (1 mg/kg, IP) than young untreated rats. Increased ambulation is commonly interpreted as reflecting a lower level of emotionality (3,16). We decided to study the effects of chronic ALCAR treatment on open field behavior. Besides increased ambulation (number of squares crossed) (10), fewer boli (9) and a decreased occupancy of corner squares (16) were taken to indicate lower "emotional reactivity." An increased "emotional reactivity," on the other hand, should express itself in changes in these measures in the opposite direction.

Both young and old rats were used in the present study. In view of the presumed nootropic activity of ALCAR, it is of interest to study the effects of chronic ALCAR on OF behavior in old rats. An age-associated decrease in locomotor activity (18) may interfere with the interpretation of the expected lower ambulation of old rats in the OF. Schuurman and Traber (15) reported a decrease in ambulation of about 30 percent between young (3–4 months) and

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aged (21–25 months) rats of the same strain used in this study. Since there are no relevant validation studies, it is not certain whether an age-related decline of OF ambulation represents a fall in emotional reactivity, in arousal (15), or in motor skills (7). We assume, however, that the OF measures are valid to detect ALCAR-induced emotional changes *within* age groups, which is the main objective of the present study. Furthermore, we hypothesized that aged rats would be less active than young rats as reported by others (7, 15, 18). We compared the untreated age groups to test this hypothesis.

METHOD

Animals

We used male random-bred Wistar rats [Bor:WISW(SPFCpb), Winkelmann, Borchen, West Germany] from two shipments, each consisting of eight young (2-month-old) and eight old (23-month-old) animals. Rats from each shipment were matched for body weight and assigned to one of four groups: young untreated (n=4), young ALCAR-treated (n=4), old untreated (n=4), and old ALCAR-treated (n=4). All animals were housed individually in standard Makrolon cages and had free access to food and water. The rats were kept under a reversed light-dark cycle (lights on between 2100 and 0900).

Treatment

Six weeks after the animals had been matched, the ALCAR-treated groups were given 50 mg/kg/day ALCAR, which was dissolved in a 0.1% solution of saccharine. The control groups received a comparable amount of saccharine solution (10 ml 0.1% per day). Four weeks after the treatment started, the rats were handled extensively on four successive days. Two weeks later the animals were tested in the OF.

Behavioral testing was carried out between 1000 and 1300. The drinking bottles containing the solutions were offered two hours before the OF test was performed. All animals consumed the total amount of the sweetened solutions within a few minutes. A drinking bottle containing tap water was always present.

Behavioral Procedure

The OF consisted of a square base (100 × 100 cm) subdivided into 36 equal squares by black lines; the walls were 30 cm high. The floor was made of white Trespa® and the four walls were made of transparent Plexiglas®. Two red fluorescent tubes provided very dim illumination (about two lux) on the floor of the apparatus.

Immediately after a rat was placed in the centre of the OF the movements of the rat were scored. A crossing was scored as soon as the rat crossed a line with both hind legs. After each session the number of boli was counted. The number of lines crossed and the total time spent in the corner squares were recorded manually with an AIM 64-microcomputer. The experimenter sat in front of the OF. Testing was carried out on four consecutive days in five-minute sessions.

Statistical Analysis

In order to enhance reliability, the data from the four sessions were aggregated as proposed by Ossenkopp and Mazmanian (13). Three measures of OF behavior were subjected to statistical analysis: mean number of boli per day (the mean number of boli were transformed to rank scores because of the frequent occurrence of zero scores, predominantly in young rats); the mean daily number of crossing of all squares; the mean time (t) in seconds per session spent in the corner squares in seconds transformed to the natural logarithm

$$\mathrm{Ln}[(t_1 + t_2 + t_3 + t_4)/4]$$

(subscripts refer to day 1 through 4 of testing) in order to remove inhomogeneity.

Shipment effects were analysed in an age by treatment by shipment analysis of variance (ANOVA). The behavioral measures of animals of both shipments were the same (all F's involving main effect and interactions with shipment were not significant). Thus, the second shipment can be considered as a replication of the first one. The data for both shipments were therefore pooled.

As mentioned in the Introduction, age-related changes were analysed by comparing the untreated groups with *t*-tests. The effects of the chronic ALCAR treatment were assessed within age groups by using *t*-tests. Age differences and treatment effects on the number of boli were analysed by Kruskal-Wallis one-way analysis of variance by ranks (χ^2-approximation).

RESULTS AND DISCUSSION

The treatment and control groups weighed the same after the data for both shipments had been pooled. The mean body weight (grams ±SEM) was 288.1 (±3.6) for the untreated young rats, 290.5 (±4.5) for the ALCAR-treated young rats, 499.4 (±11.1) for the untreated old rats, and 515.7 (±9.8) for the ALCAR-treated aged rats. At testing, 11 weeks after matching, the body weights were 379.6 (±10.4), 375.9 (±9.1), 507.1 (±12.3), and 515.0 (±17.5) grams, respectively. Thus, the young rats had gained weight; there was no indication that the ALCAR treatment affected the body weight. The weights of the aged rats were stable over the entire period from matching to testing.

The results of OF behavior are summarized in Table 1. Compared with the young untreated rats, the old untreated rats made fewer crossings, $t(14)=4.1$, $p<0.01$, spent more time in the corner squares, $t(14)=-2.7$, $p<0.05$, and defecated more, $\chi^2(1)=9.2$, $p<0.01$. Although it is tempting to conclude that aged rats react more emotionally than young rats when confronted with a novel environment, no attempt was made to interpret age differences in OF behavior because of the lack of data on the validity of these measures across ages. The decrease in ambulation in the old rats is quite similar to the value (ca. 30%) reported by others for rats of the same strain (15).

Chronic treatment with ALCAR had no effect on the OF behavior of young rats. This result contrasts with the findings reported by Drago and coworkers (5). The results of the two studies, however, cannot be compared directly because of procedural differences. Drago *et al.* used acute injections (1 mg/kg, IP), while we used a chronic oral administration of ALCAR. Another procedural difference is that Drago and coworkers tested the rats using the OF procedure described by Weijnen and Slangen (17). In this procedure rats are tested for one day in a brightly lit circular arena. In our study rats were tested in a dimly lit square OF on four consecutive days, in order to enhance the reliability of the OF measures (13,16).

Old ALCAR-treated rats made fewer crossings, $t(14)=2.30$, $p<0.05$, and spent more time in the corner squares, $t(14)=-2.71$, $p<0.05$, than the old untreated animals. This suggests that the treatment increased the emotional reactivity of the old rats when they were exposed to a novel environment. No treatment effect, however, was found for the number of boli. An alternative explanation in terms of a change in peripheral motor function cannot be excluded, but is highly unlikely. If any, the expected effect of the ALCAR treatment would be to increase ambulation in

TABLE 1

MEAN NUMBER (±SEM) OF BOLI (BETWEEN PARENTHESES: MEDIAN), MEAN NUMBER OF CROSSINGS OF ALL SQUARES AND THE AVERAGE TIME SPENT IN CORNER SQUARES (IN SEC AND TRANSFORMED TO THE NATURAL LOGARITHM) OF UNTREATED AND ALCAR-TREATED YOUNG (5 MONTHS) AND AGED (26 MONTHS) MALE WISTAR RATS (n=8 PER GROUP)

Group	Number of Boli	Number of Squares Crossed	Time in Corner Squares
		5 month old	
Untreated	0.06 ± 0.06 (0.0)	55.93 ± 2.81	4.75 ± 0.05
ALCAR-treated	0.28 ± 0.20 (0.0)	54.55 ± 3.92	4.75 ± 0.07
	$\chi^2 = -1.06$	$t = 0.76$	$t = -0.03$
		26 month old	
Untreated	2.81 ± 0.63 (3.0)	39.01 ± 2.97	4.93 ± 0.05
ALCAR-treated	3.19 ± 1.03 (2.9)	29.33 ± 2.99	5.10 ± 0.04
	$\chi^2 = -0.31$	$t = 2.30$*	$t = -2.71$*

*Treatment effect; $p<0.05$.

the old rats as a result of the stimulatory effects of ALCAR on the energy and lipid metabolism of muscle cells [see (1) and (4)]. A similar argument holds with regard to a possible increase in arousal or excitability. According to Onofrj *et al.* (12), ALCAR-treated rats show heightened arousal. An increase in ambulation would then be expected rather than the decrease observed.

In a recent study we assessed the validity of the number of crossings and time spent in the corner squares of an OF as measures of emotional reactivity (16). Young-adult rats of seven inbred strains were observed in the OF and a light-dark preference box. Both tests are supposed to measure emotional reactivity. Analysis by standard cross-validation techniques and by genetic correlations in a multitrait-multimethod-like design revealed that the above-mentioned OF measures are valid measures of emotionality in the rat. This suggests that chronic treatment with ALCAR enhances the level of emotionality in aged rats. Such a notion does not readily fit into the supposed action of ALCAR on the responsiveness of the corticosterone system.

There are few studies on the influence of aging on emotionality (6). Old rats are less capable of terminating an adrenocortical stress response (14), but this has not been related to OF behavior as far as we know.

The possibility that emotionality is expressed differently in young and old rats is supported by the finding that diazepam does not have the usual anticonflict effect in the Geller-Seifter test when applied to old rats (11). Such findings underline the necessity to assess the validity of the measures of emotionality in senescent rats, a study which we are currently carrying out.

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