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Behavioral Effects of Acetyl-l-carnitine in the Male Rat

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DRAGO, F., G. CONTINELLA, G. PENNISI, M. C. ALLORO, M. CALVANI AND U. SCAPAGNINI. *Behavioral effects of acetyl-l-carnitine in the male rat.* PHARMACOL BIOCHEM BEHAV 24(5) 1393–1396, 1986.—The behavioral activity of carnitine acetylate derivative, acetyl-l-carnitine has been studied in the male rat. Intraperitoneal (IP) injection of acetyl-l-carnitine was followed by an increase in ambulation and rearing items in the open field behavior. Both the number of conditioned avoidance response (CARs) and the percentage of learners in the acquisition of shuttle-box active avoidance behavior appeared to be increased by IP or intracerebroventricular (ICV) injection of the drug at different doses. Subchronic administration of the drug mimicked the effects found after acute injection. The number of CARs in the extinction of shuttle-box active avoidance behavior appeared to be increased after acute IP or ICV injection, and after subchronic administration of acetyl-l-carnitine. The retention of passive avoidance behavior was facilitated by IP injection of the substance. The behavioral effects of acetyl-l-carnitine may involve central mechanisms, e.g., cholinergic neurotransmission in the brain.

Acetyl-l-carnitine Open field Active avoidance behavior Passive avoidance behavior Acetylcholine

ACETYL-L-CARNITINE is the acetylate derivative of carnitine, naturally occurring constituent of many biological systems [6,10]. There is evidence that changes in the tissue levels of l-carnitine or acetyl-l-carnitine can be related to pathological states associated with disorders of lipid metabolism [12]. Recent data show that acetyl-l-carnitine influences excitability of retinal neurons in man [8]. Furthermore, myocardial excitable tissue benefits by anti-fatigue and inotropic effects of acetyl-l-carnitine. In fact, in the isolated rabbit heart, this substance increases significantly the contractile force depressed by anoxia [9]. Some authors have recently suggested that acetyl-l-carnitine might represent a store of active acetyl groups [16]. Furthermore, recent data show that this substance is widely distributed in the tissues, including the brain [7]. Thus, acetyl-l-carnitine may exert neurotropic functions.

Recently, a significant increase in rearing has been described in rats injected systemically with acetyl-l-carnitine [13]. This effect has been related to a central cholinergic activity of this substance. In fact, cortical application of the drug exerts an excitatory action on acetylcholine-sensitive neurons and changes the components of the visual evoked potentials in a manner consistent with cholinergic activation [13]. Furthermore, evidence has been presented that acetyl-l-carnitine can influence serotonergic and noradrenergic transmission in the brain [15].

The above findings prompted us to examine the behavioral effects of acetyl-l-carnitine in the male rat. The present study includes experiments where tests for conditioned and unconditioned behaviors have been used.

METHOD

Animals

Male rats of Wistar strain (purchased from Morini, S.Polo d'Enza, Italy), weighing 140–150 g were used. The animals were housed 5 to a cage and kept at room temperature (20°C). All animals had free access to commercial food and water, under a constant light-dark cycle (lights on between 8.00 and 20.00). Seven days prior to beginning the experimental session a number of animals were implanted with permanent plastic cannula into their lateral ventricle (foramen interventriculare, König and Klippel, A6360). Only animals that recovered from the surgery and were in good health were used. Individual animals were tested in only a single dose condition of a single behavioral experiment.

Drugs and Treatment

Acetyl-l-carnitine (Sigma-Tau, Pomezia, Italy) was dissolved in saline and injected intraperitoneally (IP) at the doses of 0.1, 0.5 and 1 mg/kg, or intracerebroventricularly (ICV) at the dose of 1 μg/1 μl/rat. Control animals received injections of the saline only. IP and ICV injections were performed 60 and 30 min before the behavioral testing, respectively. In a different experiment, subchronic treatment was

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TABLE 1

EFFECTS OF INTRAPERITONEAL INJECTION OF ACETYL-L-CARNITINE (1 mg/kg) ON AMBULATION AND REARING ITEMS OF OPEN FIELD BEHAVIOR OF THE MALE RAT

Treatment Groups	Ambulation	Rearing
Saline (8)	80.5 ± 6.0	12.3 ± 1.8
Acetyl-l-carnitine (8)	109.4 ± 10.1*	17.8 ± 1.9*

Values are mean ± SEM.

Acetyl-l-carnitine or saline were injected IP 60 min prior to beginning the test.

In the parentheses the number of animals per each group.

*Significantly different as compared to controls ($p<0.05$, Student's t-test, two-tailed).

TABLE 2

EFFECTS OF INTRAPERITONEAL OR INTRACEREBROVENTRICULAR (ACUTE OR SUBCHRONIC) INJECTION OF ACETYL-L-CARNITINE ON THE ACQUISITION OF SHUTTLE-BOX ACTIVE AVOIDANCE BEHAVIOR OF THE MALE RAT

Treatment Groups	CARs (mean ± SEM)	Learners (%)
Intraperitoneal (acute)		
Saline (10)	10.2 ± 1.6	20
Acetyl-l-carnitine		
0.1 mg/kg (8)	15.0 ± 1.6*	25
0.5 mg/kg (8)	18.9 ± 1.6*	50
1 mg/kg (10)	22.2 ± 1.9*	50
Intracerebroventricular (acute)		
Saline (8)	9.4 ± 2.3	25
Acetyl-l-carnitine		
1 μg/1 μl (9)	19.6 ± 2.6†	66‡
Intraperitoneal (subchronic)		
Saline (10)	10.1 ± 0.9	20
Acetyl-l-carnitine		
1 mg/kg/die (10)	22.4 ± 1.2†	70‡

Acetyl-l-carnitine or saline were injected IP 60 min and ICV 30 min prior to beginning the test. Subchronic treatment was made for 7 days.

In parentheses the number of animals per each group.

*Significantly different as compared to controls ($p<0.05$, Dunnett's test for multiple comparisons).

†Significantly different as compared to controls ($p<0.05$, Student's t-test, two-tailed).

‡Significantly different as compared to controls ($p<0.05$, Fischer's exact t-test).

performed injecting IP acetyl-l-carnitine at the dose of 1 mg/kg/day for 7 days, the last injection being made 1 hr before the behavioral test.

Behavioral Procedures

Open field behavior was studied in a circular arena as described by Weijnen and Slangen [17]. Ambulation (number of floor units entered), rearing, grooming (number of bouts) and defecation (number of boli) scores were recorded during a 5-min observation session.

Active avoidance behavior was studied in a shuttle-box task. Acquisition of shuttle-box active avoidance behavior was studied as described elsewhere [3]. Briefly, animals were trained to avoid the unconditioned stimulus (US) of a scrambled electrical footshock (0.20 mA, AC) delivered through the grid floor of a box divided into two sections by a barrier. The conditioned stimulus (CS) was a buzzer presented for 5 sec before the US. If no escape occurred within 20 sec of CS/US presentation, the shock was terminated. A maximum of thirty conditioning trials were given in a single session with a variable intertrial interval averaging 60 sec. The learning criterion was 5 consecutive conditioned avoidance responses (CARs). For those animals that reached the criterion in less than 30 trials, the remaining trials until 30 were considered as CARs. Indexes of avoidance behavior were the total number of CARs and the percentage of learners. Two hr after the acquisition session, the animals were re-tested in the same behavioral apparatus for extinction of active avoidance behavior. Thirty non-reinforced trials were presented, in which the CS was terminated immediately after the rat had crossed the barrier within 5 sec or after 5 sec in the absence of avoidance. For those animals that showed 5 negative consecutive responses, the remaining trials until 30 were considered negative.

Passive avoidance behavior was studied in a step-through type of passive avoidance behavior [1]. Briefly, the rats were adapted to the apparatus consisting of a large dark compartment equipped with a grid floor and a mesh-covered elevated runway attached to the front center of the dark chamber. Adaptation training was followed by a single trial in which the rats were placed on the elevated platform and allowed to enter the dark box. Three such trials were given on the next day with an intertrial interval of 5 min. After the third trial the rats received a single 2-sec unavoidable scrambled footshock (0.25 mA, ac) immediately after entering the dark compartment. Retention of the response was tested 24 hr after the learning trial. The rats were placed on the elevated runway and the latency to reenter the shock compartment was recorded up to a maximum of 300 sec.

All behavioral experiments were performed between 10.00 and 17.00 at natural sun light. The number of animals tested in each group is given in Tables 1–4.

Animals bearing ICV cannulas were killed at the end of the behavioral procedures. Localization of cannulas was checked by injecting Evans blue and macroscopical inspection of the colouring of the walls of the ventricular system in formaline fixed brains.

Experimental Design

Experiment 1. The influence of acetyl-l-carnitine on open field behavior was studied in rats injected IP with 1 mg/kg of the substance. Control animals received IP injection of saline.

Experiment 2. The influence of acetyl-l-carnitine on acquisition and extinction of shuttle-box active avoidance behavior was studied in rats injected IP with 0.1, 0.5 or 1 mg/kg of the substance. Acetyl-l-carnitine was also injected ICV (1 μg/1 μl/rat) to another group of animals. Subchronic treat-

TABLE 3

EFFECTS OF INTRAPERITONEAL OR INTRACEREBROVENTRICULAR (ACUTE OR SUBCHRONIC) INJECTION OF ACETYL-L-CARNITINE ON THE EXTINCTION OF SHUTTLE-BOX ACTIVE AVOIDANCE BEHAVIOR OF THE MALE RAT

Treatment Groups	CARs
Intraperitoneal (acute)	
Saline (10)	4.1 ± 0.2
Acetyl-l-carnitine	
0.1 mg/kg (8)	8.0 ± 1.1*
0.5 mg/kg (8)	11.5 ± 1.8*
1 mg/kg (10)	13.8 ± 1.8*
Intracerebroventricular (acute)	
Saline (8)	2.4 ± 0.1
Acetyl-l-carnitine	
1 μg/1 μl (9)	12.6 ± 1.9†
Intraperitoneal (subchronic)	
Saline (10)	2.8 ± 0.9
Acetyl-l-carnitine	
1 mg/kg/die (10)	12.6 ± 1.8†

Values are mean ± SEM.

Acetyl-l-carnitine or saline were injected IP 60 min and ICV 30 min prior to beginning the extinction session of shuttle-box active avoidance task. Subchronic treatment was made for 7 days.

In parentheses the number of animals per each group.

*Significantly different as compared to controls ($p<0.05$, Dunnett's test for multiple comparisons).

†Significantly different as compared to controls ($p<0.05$, Student's t-test, two tailed.

ment (1 mg/kg/day for 7 days) was performed in a different group of rats. All control animals were injected IP or ICV with saline.

Experiment 3. The effect of acetyl-l-carnitine on passive avoidance behavior was studied in rats injected IP with 0.5 or 1 mg/kg of the drug 60 min prior to retention trial. Controls received saline alone.

Statistical Analysis

The statistical differences were analysed using the Student t-test, two tailed and the Dunnett's test for multiple comparisons for parametric data. The Steel's test was used for non-parametric data, and the Fischer's exact t-test for frequencies. A probability level of 0.05 or less was accepted as significant difference.

RESULTS

Experiment 1

Rats injected IP with acetyl-l-carnitine showed a significant increase in ambulation and rearing, as compared to saline-injected control animals (Table 1). No change was found in grooming and defecation between animals treated with the drug and control rats (data are not shown).

Experiment 2

IP injection of acetyl-l-carnitine facilitated the acquisition of shuttle-box active avoidance behavior in a dose-dependent manner (Table 2). The total number of CARs was higher in rats treated with the drug, and a higher percentage of these animals reached the learning criterion as compared to saline-injected controls. Similar effects were found in animals injected ICV with acetyl-l-carnitine. These animals showed a higher number of CARs and a higher percentage of learners (Table 2) as compared to control rats. Subchronic treatment with acetyl-l-carnitine also increased significantly the number of CARs and the percentage of learners (Table 2). No change was found in the non-discriminated barrier crossing in the active avoidance training after drug treatment (data not shown).

The effects of IP injection of acetyl-l-carnitine on extinction of shuttle-box active avoidance behavior are shown in Table 3. The number of CARs performed by animals treated with the drug was significantly higher than that shown by controls. The effect of the substance seemed to be dose-dependent. Also ICV injection of acetyl-l-carnitine was followed by a significant increase in the number of CARs performed during the extinction session (Table 3). Similar effects were found in animals injected for 7 days with acetyl-l-carnitine. These animals performed a higher number of CARs than that shown by saline-injected control animals (Table 3).

TABLE 4

EFFECTS OF INTRAPERITONEAL INJECTION OF ACETYL-L-CARNITINE ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR OF THE MALE RAT

Treatment Groups	Latency (median in sec)
Saline (8)	32
Acetyl-l-carnitine	
0.5 mg/kg (8)	121*
1 mg/kg (9)	206*

Acetyl-l-carnitine or saline were injected IP 60 min prior to beginning the test.

In parentheses the number of animals per each group.

*Significantly different as compared to controls ($p<0.05$, Steel's test for multiple comparisons).

Experiment 3

IP injection of acetyl-l-carnitine facilitated the retention of passive avoidance response, as indicated by the median latency to reenter the shock compartment (Table 4). In fact, the latency of rats injected with acetyl-l-carnitine was significantly higher than that shown by saline-injected controls. A graded effect of the drug was found depending on the dose.

DISCUSSION

The present experiments provide further evidence that acetyl-l-carnitine possesses central effects. In fact, it can influence both conditioned and unconditioned behaviors in the male rats. The acquisition of active avoidance behavior was facilitated and the extinction was inhibited by both acute and subchronic administration of the drug. Furthermore, these effects were mimicked by ICV injection of acetyl-l-carnitine, suggesting that they were mediated by central rather than peripheral mechanisms.

Ambulation and rearing items of open field behavior appeared to be increased after peripheral injection of acetyl-l-

carnitine. The hypothesis cannot be ruled out that these effects involved a peripheral component, due to the action of acetyl-l-carnitine on the energetic metabolism of the muscles [4]. However, as ambulation and rearing behaviors involve motivation factors, it is likely that the effect of acetyl-l-carnitine on these items also concerns central mechanisms.

Furthermore, the increased ambulation found in animals treated with acetyl-l-carnitine may be responsible for a facilitated acquisition and an inhibited extinction of active avoidance performance. However, also the retention of passive avoidance behavior was facilitated by acetyl-l-carnitine. As reduced rather than increased locomotor activity may eventually favour the response in this test, it is tempting to conclude that the facilitation of learning and memory processes found in rats treated with acetyl-l-carnitine does not depend on motor performance of the animals. Another possibility is that acetyl-l-carnitine may have increased the arousal of animals. In fact, the increased open field activity and the facilitated active avoidance acquisition observed after the drug treatment are consistent with an increase in arousal. Also, an increase in arousal could have facilitated the retention of passive avoidance behavior.

It is not yet known by which mechanism of action acetyl-l-carnitine exerts behavioral effects. Direct application of acetyl-l-carnitine in rat cortex is followed by excitation of acetylcholine-sensitive neurons and changes of components of the visual evoked potentials in a manner consistent with cholinergic activation [13]. Other authors reported increased visual evoked potentials in the lateral geniculate nucleus of rats after local application of carnitine esters [11]. Furthermore, modifications of behavioral patterns found in peripherally-treated animals reflect cholinergic activation. In fact, these animals show hyperexcitability [13], in a sense of "fear related" behavior [18]. This altered behavior could be attributed to the high colinergic activity of hippocampal pathways [2]. Thus, it is possible that the effects found in the present experiments involve changes in central cholinergic neurotransmission induced by IP or ICV administration of acetyl-l-carnitine. Indeed, the structural resemblance of acetyl-l-carnitine to acetylcholine was recently studied and the conformational analysis predicted cholinergic activity of the substance [14]. In addition, acetyl-l-carnitine has been considered a store of active acetyl groups [16] which may be used for the synthesis of acetylcholine [7].

Recent data showed that acetyl-l-carnitine can influence serotonine and noradrenaline neurotransmission in the brain [15]. Thus, the involvement of these monoamines in the behavioral effects of the drug is also possible.

As an alternative, acetyl-l-carnitine may act by influencing directly the excitability of neurons in behaviorally-competent brain areas. In fact, the substance can affect the excitability of retinal neurons in man [8] and of myocardial cells in rabbits [9]. In this case, acetyl-l-carnitine may improve lipid metabolism of the excitable cells and, hence, favour conduction of nerve impulses at the membrane level. In fact, carnitine and its derivatives are involved in the oxidation of fatty acids in many biological systems [5].

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