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Dihydroergocristine and Memory Alterations of Aged Male Rats

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DRAGO, F., C. VALERIO, B. SCALISI, V. D'AGATA AND U. SCAPAGNINI. *Dihydroergocristine and memory alterations of aged male rats*. PHARMACOL BIOCHEM BEHAV **30**(4) 961–965, 1988.—The ergot alkaloid derivative dihydroergocristine (DHECS) was injected acutely or subchronically to aged male rats of the Sprague-Dawley strain, 26 months old, at the dose of 0.05 or 0.1 mg/kg. Learning and memory ability of the animals were studied with tests of avoidance behavior. The acquisition of active avoidance behavior was studied with the shuttle-box and pole-jumping tasks. In the latter, the extinction of active avoidance behavior was also studied. A step-through type of passive avoidance task was used to examine the retention of passive avoidance responses. The acquisition of the active avoidance behavior and the retention of the passive avoidance response were reduced in aged animals as compared to those of young animals. Acute treatment of old rats with DHECS was followed by a facilitation of acquisition of active avoidance behavior in the shuttle box and of retention of passive avoidance responses in the dark box. The effect on the acquisition and extinction of pole-jumping behavior after a single injection of DHECS at the beginning of the acquisition session was restricted to the first acquisition trial. A more potent effect on the acquisition of the shuttle-box behavior and on the retention of passive avoidance reaction was found in animals treated subchronically with the ergot derivative (0.05 and 0.1 mg/kg for 10 days). These rats also showed a facilitation of acquisition and an inhibition of extinction of pole jumping behavior.

Dihydroergocristine Learning and memory Active avoidance Passive avoidance

THE loss in learning and memory ability occurring in old animals and humans has been related to the age-related alterations in central neurotransmission. In particular, changes in acetylcholine neurotransmission have been associated with these behavioral deficits [14b,26]. Also dopamine neurotransmission has been concerned with these changes [11]. Evidence has been presented that central neurotransmission, particularly acetylcholine [8b,29] and dopamine neurotransmission [4,28], plays an important role in learning and memory processes and appears to be altered in aging brain [9, 12, 13, 30].

To restore learning and memory ability in aged subjects, the treatment with dopaminergic drugs has been proposed. The ergot alkaloid dihydroergocristine (DHECS) possesses a potent dopaminergic activity that has been shown both in vitro and in vivo [22, 24, 25]. The central actions of this ergot alkaloid include an inhibitory influence on the secretion of anterior pituitary hormones (i.e., prolactin and growth hormone), an emetic action [14], the induction of stereotyped behavior [23], changes in the sleep-wakefulness cycle [7] and a reduction in brain hypoxia in rats [3]. Like other dihydroderivatives of ergot alkaloids, DHECS interferes also with noradrenergic and serotonergic neurotransmission [21]. DHECS can be used, alone or in combination with other ergot derivatives, in pathological situations characterized by a deficit of central dopamine neurotransmission, such as Parkinson's disease [6], dyskinesia [27], hyperprolactinaemia [10] and behavioral deficits of aged patients, mainly of cerebrovascular origin [18,19]. However, DHECS could be potentially effective also in primary learning and memory disability.

This prompted us to study the effects of DHECS on primary learning and memory deficits occurring in aged rats. These animals are normally devoid of serious cerebrovascular alterations [21] and hence, may serve as useful model for learning and memory deficits of the primary type. In the present experiments DHECS was not associated with other ergot alkaloids in order to characterize its profile of action on these behavioral deficits of aged rats.

METHOD

Animals

One hundred and twelve male rats of Sprague-Dawley strain (purchased from Charles River, Italy), weighing 600±20 g, 26 months old, were used throughout all experiments. A group of 32 male rats of the same strain, weighing 310±10 g, 2 months old, was also used in some experiments. The animals were housed two–three to a cage under a constant light-dark cycle (lights on between 0800 and 2000) at 21°C. Food and water were available ad lib.

All animals were used only once in the behavioral experiments.

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TABLE 1

EFFECTS OF ACUTE AND SUBCHRONIC ADMINISTRATION OF DIHYDROERGOCRISTINE METHANESULFONATE (DHECS) ON THE ACQUISITION OF SHUTTLE-BOX ACTIVE AVOIDANCE BEHAVIOR OF AGED MALE RATS (26 MONTHS OLD)

Experimental Groups	CARs (mean ± SEM)	Learners (percentage)
	Acute Treatment	
Young Animals		
Vehicle (8)	14.5 ± 1.2	75.0
Old Animals		
Vehicle (8)	4.2 ± 0.4	0.0
DHECS 0.05 mg/kg (8)	8.1 ± 0.3*	25.0
DHECS 0.1 mg/kg (8)	8.6 ± 0.4*	50.0†
	Subchronic Treatment	
Young Animals		
Vehicle (8)	15.2 ± 1.0	75.0
Old Animals		
Vehicle (8)	4.5 ± 0.6	0.0
DHECS 0.05 mg/kg (8)	9.1 ± 0.5*	37.5
DHECS 0.1 mg/kg (8)	14.6 ± 0.8*	50.0†

Acute treatment was made 1 hr prior to behavioral testing. Subchronic treatment was made for 10 days, the last administration being made 1 hr prior to behavioral testing. In parentheses is shown 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 ($p<0.05$, Fischer exact probability test).

Drugs

Dihydroergocristine methanesulfonate (provided by Poli, Italy) was dissolved in saline and injected subcutaneously (SC) at the doses of 0.05 and 0.1 mg/kg. These doses have been found to be fully active in animal experiments [16]. The injection was made either acutely or subchronically for 10 days. Control animals received an injection of saline alone with the same procedure.

Behavioral Tests

Active avoidance behavior was studied in a shuttle-box and a pole-jumping situation. Shuttle-box acquisition was studied in a single test as described elsewhere [2]. Briefly, the rats were trained to avoid the unconditioned stimulus (US) of a scrambled electrical footshock (0.20 mA) delivered through the grid floor. The conditioned stimulus (CS) was a buzzer presented for 3 sec prior to the US. If no escape occurred within 20 sec of CS/US presentation, the shock was terminated. A maximum of 30 conditioning trials was given 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 number of learners per group.

Acquisition and extinction of a pole jumping avoidance response were studied in the apparatus described by De Wied [8]. The rats were conditioned to avoid the US of an electrical floor shock (0.25 mA) by jumping onto a pole located vertically in the center of the conditioning apparatus. The CS was a light (40 W) provided above the pole and presented for 5 sec. The US was applied if an avoidance response had not occurred within 5 sec of CS presentation, and lasted 20 sec. The acquisition trials were given daily for 4 days with variable intervals averaging 60 sec. Ten nonreinforced extinction trials per day were presented on the next 3 successive days.

Passive avoidance behavior was studied in a step-through type of passive avoidance situation [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. Adaption 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 (0.20 mA) 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 re-enter the shock compartment was recorded up to a maximum of 300 sec.

Animals were killed by decapitation at the end of the behavioral procedure. Data were used only from those animals appearing physically healthy during the tests and which showed no gross abnormalities on post-mortem examination. Twelve animals out of 100 animals were found unhealthy and were discarded.

All experiments were performed blind to treatment between 9.00 and 14.00.

Experimental Design

In the first experiment, the effect of acute or subchronic administration of DHECS on the acquisition of the shuttle-box performance was studied. Acute injection of DHECS was made 1 hr before the test. The subchronic treatment lasted 10 days, the last administration being made 1 hr before the test. Control animals received injections of saline with the same procedure. With the same treatment schedule, the effects of DHECS on the acquisition and extinction of a pole jumping response and on the retention of passive avoidance behavior were also studied. In the experiments concerning the effects of DHECS on shuttle-box active avoidance behavior and on passive avoidance response, a group of young rats were also tested after acute or subchronic administration of saline.

Statistical Analysis

The Dunnett's test for multiple comparisons was used for statistical analysis of data from multiple active groups compared to a control group. Paired comparisons have been made using the Student's t-test. The Fisher exact probability test was used for the frequency analysis of data on the percentage of learners. Data of nonparametric systems were analysed with the Mann-Whitney U-test. ANOVA with replication was also used for statistical analysis of data of parametric systems. A level of 0.05 or less was accepted as indicative of significant difference.

TABLE 2

EFFECT OF SUBCHRONIC ADMINISTRATION OF DIHYDROERGOCRISTINE METHANSULFONATE (DHECS) ON THE ACQUISITION AND EXTINCTION OF POLE-JUMPING ACTIVE AVOIDANCE BEHAVIOR OF AGED MALE RATS (26 MONTHS OLD)

	Vehicle (N=10)	DHECS 0.05 mg/kg (n=8)	DHECS 0.1 mg/kg (n=8)
Days of Acquisition			
1	0.20 ± 0.1	1.00 ± 0.2	1.26 ± 0.1
2	1.00 ± 0.2	2.32 ± 0.3	2.90 ± 0.2
3	2.20 ± 0.3	4.60 ± 0.4	6.20 ± 0.4
4	3.10 ± 0.3	6.80 ± 0.4	6.90 ± 0.6
Days of Extinction			
1	1.30 ± 0.1	4.56 ± 0.4	4.60 ± 0.3
2	0.20 ± 0.3	4.44 ± 0.4	4.32 ± 0.4
3	0.10 ± 0.1	1.40 ± 0.3	2.20 ± 0.1

Subchronic treatment was made for 10 days, the last administration being made 1 hr prior to behavioral testing. Values are mean ± SEM. In parentheses is shown the number of animals per each group. ANOVA with replication revealed a significant interaction treatment/days, $F(2,25)=3.90$, $p<0.05$. The Newman-Keuls post hoc test revealed a significant difference between each treatment group and the controls ($p<0.05$), but not between the two treatment groups, both in the acquisition and the extinction session.

TABLE 3

EFFECT OF ACUTE AND SUBCHRONIC ADMINISTRATION OF DIHYDROERGOCRISTINE METHANENSULFONATE (DHECS) ON THE RETENTION OF PASSIVE AVOIDANCE BEHAVIOR OF AGED MALE RATS

Experimental Groups	Latency (in sec)
Acute Treatment	
Young Animals	
Vehicle (8)	88.0
Old Animals	
Vehicle (10)	18.0
DHECS 0.05 mg/kg (8)	36.0*
DHECS 0.1 mg/kg (8)	74.0†
Subchronic Treatment	
Young Animals	
Vehicle (8)	92
Old Animals	
Vehicle (8)	12.0
DHECS 0.05 mg/kg (8)	66.0*‡
DHECS 0.1 mg/kg (10)	98.0†‡

Values are median. Acute treatment was made 1 hr prior to behavioral testing. Subchronic treatment was made for 10 days, the last administration being made 1 hr prior to behavioral testing. In parentheses the number of animals per each group.

*Significantly different as compared to controls ($p<0.05$, Mann-Whitney U-test). †Significantly different as compared to controls and to animals treated with DHECS 0.05 mg/kg ($p<0.05$, Mann-Whitney U-test). ‡Significantly different as compared to the acute treatment group ($p<0.05$, Mann-Whitney U-test).

RESULTS

Table 1 shows the effects of acute subchronic treatment with DHECS on the acquisition of shuttle-box active avoidance behavior of aged rats. The number of CARs and the percentage of learners were significantly lower in acutely or subchronically saline-treated aged rats as compared to those of young animals ($p<0.01$, Student's t-test). Acute injection in aged rats of 0.05 or 0.1 mg/kg of DHECS made 1 hr before the test was followed by an increase in the number of CARs and in the percentage of learners. The effect of 0.1 mg/kg of the drug was greater than that of the lower dose. Subchronic treatment with DHECS also facilitated the acquisition of shuttle-box active avoidance behavior.

The pole-jumping test revealed that acute treatment with DHECS was followed by an increase in CARs that was statistically significant only at the first acquisition trial, made 1 hr after the injection of the drug ($p<0.05$, Dunnett's test for multiple comparisons). In the subsequent acquisition and extinction trials, no effect of the acute treatment was observed (data are not shown).

The subchronic treatment with the drug was followed by a facilitation of acquisition and an inhibition of extinction of pole-jumping active avoidance behavior. An increase in CARs was found in all trials of acquisition and extinction session in DHECS-treated animals as compared to control rats (Table 2).

The retention of passive avoidance behavior was lower in acutely or subchronically saline-treated old rats than in young rats ($p<0.01$, Mann-Whitney U-test). However, the retention of this behavior in old rats was higher when the animals were acutely treated with DHECS than in control animals (Table 3). The effect was more potent with 0.1 than with 0.05 mg/kg. The subchronic treatment with DHECS was also followed by a facilitation of retention of passive avoidance behavior and this effect was higher than that observed after acute treatment (Table 3).

DISCUSSION

Evidence for a dopaminergic activity of DHECS and other ergot alkaloids and derivatives has been presented in various animal models [14, 22–25] and in pathological situations in humans which are characterized by a loss of dopamine neurotransmission in the brain. These include Parkinson's disease [6], dyskinesia [27], and hyperprolactinaemia [10]. Dihydroergocristine has also been used, together with other ergot alkaloids and derivatives, in the treatment of behavioral deficits of aged patients, mainly of cerebrovascular origin [18,19]. However, a primary loss in learning and memory ability occurring in old animals and humans has been related to age-related alterations in central neurotransmission, including dopamine neurotransmission [11]. Evidence has been presented that central neurotransmission, particularly acetylcholine [8b,29] and dopamine neurotransmission [4,28], plays an important role in learning and memory processes and appears to be altered in aging brain [9, 12, 13, 30].

The present data provide experimental evidence that DHECS can improve the learning and memory deficits of aged rats. Interestingly, a similar effect of dihydroergotoxine, a mixture of dihydrogenated ergot alkaloids also containing DHECS, has been described on maze learning of the rats [15].

An interesting problem concerns the possible change in effectiveness of DHECS after repeated administration.

Here, we show that acquisition of pole-jumping avoidance behavior was facilitated and the extinction of this behavior was inhibited only after subchronic, and not acute administration of the drug. Also, the acquisition of shuttle-box active avoidance behavior and the retention of passive avoidance reactions were facilitated more in subchronically-treated rats than in animals given an acute injection of the drug. In most studies where ergot alkaloids or derivatives were administered subchronically, an increased effect was found in behavioral tasks (reviewed in [16]). In contrast, it has been shown that six days after bromocriptine treatment, a decrease of the number of striatal binding sites and of cAMP responses to dopamine in striatal slices develops [5]. This subsensitivity at neuronal receptor sites, however, may correspond to a supersensitivity in behavioral measures [16]. Furthermore, other studies have shown an increased response in noradrenaline-induced cAMP elevations in the rat cerebral cortex after repeated administration of dihydroergotoxine [20]. Thus, at receptor sites other than striatal dopamine receptors, prolonged administration of an ergot derivative has been shown to produce an increased neurochemical response. Interestingly, chronic treatment with DHECS was unable to change the stereotypy response of rats to apomorphine, suggesting that this treatment did not interfere with the sensitivity of the striatal dopaminergic system to apomorphine [17]. However, a higher effect of chronic dihydroergotoxine as compared to acute treatment was found in learning-related tests such as the maze learning [15].

The present findings suggest that DHECS may be useful in further studies on memory because of the intense effects on several aspects of performance in operant tasks associated with memory and cognition.

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