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COGNITIVE DEFICIENCY INDUCED BY THE ACUTE STRESS IN RATS: A POSSIBLE ROLE OF BRAIN CATECHOLAMINERGIC SYSTEMS

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Summary - *The evaluation of the male albino rats cognitive capacity was performed by placing the animals in glass cylinder plundged into water (22 °C.) In order to escape from that stress situation the rat should dive under the cylinder's edge, solving by this way the extrapolatory escape task (EET). Two groups of rats (A and B) have been selected and characterized within the total laboratory population. The A rats have shown a smaller number of attempts to achieve successful escape and a shorter latency of that reaction in comparison with the B animals. The latter displayed a higher behavioral activity in «open field» test. The combination of 100 mg/kg L-DOPA with 25 mg/kg of benzerazide (DOPA-BENZ) was shown to impair dramatically the cognitive capacity of the two groups of animals. DOPA-BENZ treated rats failed to demonstrate any correct solution of EET. The rats of the two groups were distinguished clearly in terms of dopamine contents and turnover rate measured in three brain areas by the HPLC technique. Among psychotropic drugs studied only haloperidol and fluphenazine were found to restore partially the animals capacity to cope with the EET after DOPA-BENZ administration.*

Riassunto (Deficienze cognitive indotte da stress acuto nel ratto: un possibile ruolo dei sistemi catecolaminergici cerebrali). - *Sono state valutate le capacità cognitive di ratti albini maschi mettendoli in un cilindro di vetro immerso in una bacinella di acqua (22 °C). Al fine di evitare tale situazione di stress i ratti: devono immergersi sotto acqua e così uscire fuori dal cilindro di vetro. Tale comportamento di fuga è conosciuto come «extrapolatory escape task» (EET). Sono stati selezionati due gruppi di ratti: il gruppo A (emotivamente stabili) e quello B (emotivi). I ratti del gruppo A si sono rivelati più capaci di uscire dal cilindro come documentato sia dal minor numero di tentativi effettuati che dal periodo di latenza più breve richiesto per uscire dal cilindro. Inoltre nel test comportamentale dell'*open field *i ratti del gruppo B hanno dimostrato una più intensa attività comportamentale, espressione di una maggiore «emotività». In entrambi i gruppi di animali un trattamento intraperitoneale con DOPA-BENZ (L-DOPA + benzerazide, 100 + 25 mg/kg) ha inibito drammaticamente la capacità di apprendimento nel suddetto test. I ratti del gruppo A e quelli del gruppo B, tuttavia, hanno dimostrato variazioni diverse a carico delle concentrazioni e del turnover di dopamina in alcune aree cerebrali. Fra vari psicofarmaci usati, soltanto l'aloperidolo e la flufenazina sono stati in grado di antagonizzare almeno in parte gli effetti inibitori del DOPA-BENZ sulle capacità cognitive di ratti nel test studiato (EET).*

Introduction

Experimental modelling of behavioral «pathology» including the cognitive deficiency seems to be of interest as a procedure for the evaluation of the therapeutic effect of different types of psychotropic drugs. We focused on modelling the pathologic states with a stress-situation, as a pathogenic factor, inducing in certain conditions the cognitive activity disturbances in animals. The dopaminergic neurotransmission is known to play a significant role in the pathogenesis of psychotic states. The dopamine (DA) receptors agonists such as L-DOPA, amphetamine, apomorphine and some others are capable of aggravating psychopathologic symptomatology in schizophrenics, provoking in some cases an acute psychotic state in healthy persons or patients receiving L-DOPA [1]. Stress is also known to be one of the factors predisposing to the development or display of psychopathology.

The injection of L-DOPA at low doses is followed by the increase of blood catecholamine level, i.e. the effect of this agent proves similar to the action of the situational stress-factors. It is of interest to note that according to the clinical observations the response to the L-DOPA injection of healthy subjects differs from that of psychotic patients [2, 3].

The aim of the present work was to assess behavioral disturbances developing as a response to L-DOPA injection in animals during an acute stress-situation modelled by putting them into a cylinder with water. In addition, the purpose of the present work was to study possible changes in the content and turnover rate of brain catecholamines in rats with developed cognitive deficiency. The effects of some psychotropic drugs, mainly neuroleptics, on such behavioral pathology were also assessed.

Methods

The experimental animals were outbred albino male rats weighing 180-200 g. The «extrapolatory escape» method [4] was employed to evaluate the cognitive functions of the animals, their ability to find a way out in an acute stress-situation. A rat put into a cylinder plunged in water with the temperature of 22 °C must solve an «extrapolatory task», i.e. to dive under the cylinder's edge thus escaping the stress-situation (the animal is taken out of water immediately after the solution of the task). The time during which the animals were in water did not exceed 2 min. The following parameters were registered: a) the total latency of the motor reaction; b) the number of abortive attempts to escape; c) the latency of an escape reaction, i.e. the time of solving the extrapolatory task. In ordinary conditions from 75 to 90% of rats of the total laboratory population solve the task using a different number of abortive attempts. The latter alongside with the latency of an escape reaction is regarded to be the extent of the cognitive deficiency in an animal. Three days after a single testing 0.9% NaCl solution (control group) or the suspension in Tween 80 of a mixture of L-DOPA (100 mg/kg) + benzerazide (25 mg/kg) (DOPA-BENZ, inhibitor of aromatic amino acids peripheral decarboxylase), were administered to the animals intraperitoneally. Neuroleptics were also injected 10 min prior to DOPA-BENZ.

To evaluate the emotional-behavioral reactivity of the animals «open field» was applied with the registration for 4 min of the following parameters: a) the number of rearings in the centre of the field; b) the number of holes explored; c) the number of grooming acts; d) the number of squares crossed by the rat.

The L-DOPA, dopamine (DA), noradrenaline (NA) and dihydroxyphenylacetic acid (DOPAC) contents in the three brain areas, i.e. striatum, *nucleus accumbens* and hypothalamus, were measured by the HPLC technique with electrochemical detection using the «BAS» chromatograph [5]. Statistical evaluation of all data was done using Student's t-test.

Results

Most animals first exposed to the stress-situation showed correct behavior solving the extrapolatory escape task (EET) with the average latency of 23 ± 1.2 s. Two groups of animals, the «steady» (A) and «emotional» (B) ones, were singled out in the total population of rats according to their emotional reactivity. The investigations carried out have revealed essential differences between the two groups as far as some of their behavioral characteristics are concerned (Table 1).

Table 1. - *Effect of DOPA-BENZ on behavior of rats belonging to A and B groups*

Method	Parameters		A	B
Open field test	Number of rearings in the central zone	C	1.8 ± 0.04	$2.8 \pm 0.1(a)$
		D	$0.2 \pm 0.001(c)$	$0.1 \pm 0.003(c)$
	Number of holes explored	C	9.3 ± 1.1	$13.2 \pm 1.2(a)$
		D	$0.7 \pm 0.2(c)$	$0.9 \pm 0.2(c)$
	Number of grooming acts	C	0.6 ± 0.15	$2.8 \pm 0.3(a)$
		D	$4.7 \pm 0.9(c)$	$5.1 \pm 1.4(c)$
	Number of crossed squares	C	61.4 ± 2.4	$54.4 \pm 1.8(a)$
		D	$33.2 \pm 1.5(c)$	$31.0 \pm 2.1(c)$
Extrapolatory escape task (EET)	Latency of motor reaction (s)	C	4.9 ± 0.6	5.7 ± 0.4
		D	$3.5 \pm 0.8(c)$	$3.6 \pm 0.3(c)$
	Number of abortive attempts of escape	C	2.3 ± 0.8	$28.3 \pm 3.1(a)$
		D	$120.0 \pm 5.4(c)$	$117.0 \pm 9.2(c)$
	Latency of escape (s)	C	8.8 ± 0.5	$43.2 \pm 2.4(a)$
		D	none	none

The data are expressed as means ± SEM; C): Control group (0.9% saline); D): DOPA-BENZ, 125 mg/kg of body weight; *a*): $p < 0.01$, significant differences between «A» and «B» control rats; *c*): $p < 0.01$, significant differences between Control and DOPA-BENZ groups.

Thus, the B animals display a higher behavioral activity in «open field» in terms of the number of rearings in the central zone, the number of holes explored, the grooming intensity, yet their horizontal locomotor activity appeared to be slightly decreased. In the EET the emotional animals distinguished by taking much more time to solve the task making a considerable number of abortive attempts to escape (28.3 and 2.3 for B and A groups, respectively). There are also some biochemical differences between the groups (Table 2). A higher DA content in the striatum of the emotional rats is correlated to a decreased turnover rate of this amine (evaluated by the DOPAC/DA ratio), and NA in the *nucleus accumbens*. At the same time, the DOPA content and the DOPAC/DA ratio in the striatum and *nucleus accumbens* of the A rats were higher then those of the B rats.

The intensity of L-DOPA metabolism (measured as DA/DOPA ratio) appeared considerably lower in all the brain areas of the A group rats (by 2.1 times in the striatum, 3.3 times in the *nucleus accumbens*, 1.4 times in the hypothalamus). These differences increased considerably in conditions of the DOPA-BENZ treatment and this indicates a higher DA turnover in the animals of A group. As for the DA content, it increased in these conditions in an approximately similar manner for the two groups of rats.

DOPA-BENZ (50 mg/kg) protracted the latent period of an escape reaction, and, at a dose of 125 mg/kg, completely impaired the ability of the rats of both groups to escape from the stress-situation. The «open field» behavior of the animals also changed. There was a sharp decline in the exploring activity of the animals both in terms of the number of rearings in the central zone and the number of holes explored. The number of grooming acts grew considerably while the differences between the groups disappeared.

It is noteworthy that after DOPA-BENZ medication the latency of motor activity in the EET situation decreased in both groups, while the number of abortive attempts to escape increased dramatically. As already observed, the animals of both groups were totally unable to cope with the EET. The movements of the animals acquired a stereotyped character.

Table 2 shows that DOPA-BENZ produced significant changes in DOPA, catecholamine and DOPAC contents in the brain structures, and the differences between the groups varied considerably. DOPA content drastically increased, particularly in the A animals, whereas NA changed only slightly. The DOPAC level and the DOPAC/DA ratio increased in the three brain areas of A and B rats with persisting differences between the groups. The DOPAC content increased in the three brain structures. In the A rats this was far more pronounced than in the B ones, DA content also increased, while differences between the two groups disappeared.

Psychopharmacological drugs of different classes (haloperidol, fluphenazine, sulpiride, clozapine as neuroleptics, imipramine as an antidepressant, phenazepam as one of the most potent benzodiazepine tranquillizers) were used in order to correct the cognitive pathology caused by DOPA-

Table 2. - *Effect of DOPA-BENZ on neurochemical parameters of rats belonging to A and B groups*

Treatment	Brain area	Group of rats	Content µmol/g of tissue DOPA	NA	DA	DOPAC	DOPAC/DA
Control (0.9% saline)	Striatum	A	0.32 ± 0.04*(a)*	1.2 ± 0.1	37.0 ± 4.2*(a)*	5.4 ± 1.2	0.12 ± 0.07*(a)*
		B	0.21 ± 0.04	1.2 ± 0.1	51.6 ± 8.8	4.6 ± 1.6	0.058 ± 0.001
	nucleus accumbens	A	1.18 ± 0.13*(a)*	2.2 ± 0.6*(a)*	41.4 ± 3.8	6.2 ± 1.4	0.164 ± 0.09*(b)*
		B	0.43 ± 0.04	2.9 ± 0.2	49.4 ± 9.0	6.3 ± 0.8	0.128 ± 0.01
	Hypothalamus	A	1.45 ± 0.7*(b)*	7.6 ± 1.3	4.7 ± 1.8	1.7 ± 0.5	0.243 ± 0.01*(a)*
		B	0.69 ± 0.3	7.7 ± 0.5	3.1 ± 0.6	2.3 ± 0.2	0.493 ± 0.027
DOPA-BENZ 125 mg/kg	Striatum	A	44.3 ± 5.9*(a,c)*	1.2 ± 0.3	130.8 ± 27.6*(c)*	72.8 ± 21.6*(a,c)*	0.450 ± 0.02
		B	17.8 ± 4.4*(c)*	1.2 ± 0.2	122.4 ± 17.4*(c)*	26.8 ± 2.6*(c)*	0.216 ± 0.019*(c)*
	nucleus accumbens	A	58.2 ± 5.9*(a,c)*	2.0 ± 0.4	86.0 ± 26.2*(c)*	79.0 ± 18.4*(a,c)*	0.940 ± 0.37*(a,c)*
		B	29.6 ± 6.1*(c)*	3.0 ± 0.5	84.4 ± 14.8*(c)*	32.0 ± 6.6*(c)*	0.368 ± 0.072*(c)*
	Hypothalamus	A	68.6 ± 11.4*(a,c)*	4.5 ± 0.6*(c)*	14.7 ± 4.6*(c)*	37.5 ± 11.4*(a,c)*	2.6 ± 0.18*(a,c)*
		B	30.7 ± 6.3*(c)*	6.2 ± 0.6	12.0 ± 1.4*(c)*	9.4 ± 0.9*(c)*	0.920 ± 0.11*(c)*

The data are expressed as means ± SEM *a*): $p < 0.01$; *b*): $p < 0.05$, significant differences between A and B control rats; *c*): $p < 0.01$, significant differences between control and treated rats of the same group.

Table 3. - *Effect of psychotropic drugs on extrapolatory behavior impairment induced by DOPA-BENZ in rats*

Drugs, doses, mg/kg	The number of attempts to escape the stress situation	(%) of animals with successful escape response
DOPA-BENZ, 125	140 ± 8	0
Haloperidol, 0.1 + DOPA-BENZ, 125	61 ± 7*(a)*	50*(a)*
Fluphenazine, 0.5 + DOPA-BENZ, 125	128 ± 6*(b)*	40*(b)*
Sulpiride, 75 + DOPA-BENZ, 125	109 ± 3*(b)*	0
Clozapine, 25 + DOPA-BENZ, 125	110 ± 8*(b)*	0
Imipramine, 12.5 + DOPA-BENZ, 125	73 ± 2*(a)*	0
Phenazepam, 0.5 + DOPA-BENZ, 125	160 ± 13*(a)*	0

a) $p < 0.01$ versus DOPA-BENZ values; *b)* $p < 0.05$.

BENZ. The results of these experiments are presented in Table 3. The neuroleptics and imipramine decreased the number of abortive attempts to escape, haloperidol and imipramine were the most effective in this respect while phenazepam exerted an opposite effect. If, however, we evaluate the drugs efficacy in terms of restoring the ability to solve the extrapolatory task, only the classical neuroleptics, i.e. haloperidol and fluphenazine, were active; their effect was, however, incomplete, consisting in only 40-50% restoration. The atypical neuroleptics - sulpiride and clozapine, as well as psychotropic drugs of other groups, i.e. imipramine and phenazepam, were found uneffective.

Discussion

Placing a rat in a cylinder with water can be regarded as a laboratory behavioral test of an acute stress-situation. In order to escape from this situation i.e. to solve a task the rat should dive under the cylinder's edge. Open field is a milder stress-situation which can be used to reveal more subtle behavioral differences between animals both in control conditions and when treated with DOPA-BENZ.

In fact, «emotional» B rats proved to be much more active in «open field» as far as the number of rearings, holes explored and grooming intensity are concerned. The latter is usually believed to suggest a conflict between the two motivations, those of exploration and anxiety. Intensive exploring activity causes a decline in the total locomotor activity in these animals which is proved by a lower number of crossed squares. The animals of the two groups displayed «dramatic» differences in their behavior as shown in the extrapolatory escape test. That makes itself conspicuous in the two behavioral characteristics of the rats: the number of abortive attempts to escape proved about 10-fold higher in the B group as compared to that in the A group. The escape latencies correlations were also similar (Table 1). These data indicate a high reactivity of the B group animals to novelty.

It appeared also of interest to compare the biochemical differences in the rats of the two groups. The indicator of the metabolic rate of L-DOPA (DA/DOPA ratio) has proved much lower in all the brain areas of the A rats. In the B rats DA content was higher in the striatum, and that of NA in the *nucleus accumbens*, i.e. the brain areas functionally related to the locomotor and emotional activity.

DOPA-BENZ at the dose of 125 mg/kg brought about equal behavioral disturbances in the animals of the two groups. The suppression of every type of the exploring activity was observed in «open field» though the total locomotion decreased slightly and the grooming intensity even rose due to a stereotyped character of this activity. DOPA-BENZ impaired the ability of both groups of animals to escape from the stress-situation in EET. The individual differences in cognitive capacity of rats treated with DOPA-BENZ were eliminated. Biochemical findings show the marked increase of DA content in these animals, especially in the striatum and hypothalamus. These changes were present in the two groups and paralleled the behavioral effects of DOPA-BENZ. The differences between the groups persisted only for DOPA and DOPAC (Table 2) i.e. in the DA biosynthesis and metabolism rate.

The preservation of the ability to explore the surface in the «open field» test can be accounted for by the supposition that L-DOPA does not disturb the forms of behavior habitual for the animal, at the same time the behavior associated with the exploring activity, the solution of the tasks posed by changes of these or those factors of the environment appears to be strongly disturbed. The animals are no longer able to control the situation, their adaptive reactions and cognitive functions prove to be impaired and the ability to change the strategy of behavior lost.

Among the psychotropic drugs studied only haloperidol and fluphenazine did partially restore the ability of animals to solve the EET (up to 50 and 40%, respectively), simultaneously decreasing the number of abortive attempts to escape the stress-situation (Table 3). Sulpiride and clozapine, atypical neuroleptics, as well as imipramine and phenazepam failed to restore the ability to escape, though they did change the number of the attempts.

Thus, the work carried out has revealed that the exploring and cognitive activity disturbances in animals modelled with L-DOPA load can be regarded as valuable from the viewpoint of the evaluation of drugs with a potential antipsychotic activity.

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