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Chronic Stress and Memory: Implication of the Central Cholinergic System

ROBERT ZERBIB AND HENRI LABORIT[1]

Laboratoire d'Eutonologie, Hôpital Boucicaut, Paris, France

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ZERBIB, R. AND H. LABORIT. *Chronic stress and memory: Implication of the central cholinergic system.* PHARMACOL BIOCHEM BEHAV 36(4) 897–900, 1990.—Restraint stress for ten days (two times two hours daily) induces a hypersensitivity of the central cholinergic system, reflected by antagonism to amnesia induced by scopolamine at 0.1 mg/kg in a passive avoidance test and by hypersensitivity to the hypothermic effect of oxotremorine at 1 mg/kg. A restraint stress for 30 days, on the other hand, diminishes animal retention in the passive avoidance test and causes a hyposensitivity to oxotremorine-induced hypothermia, reflecting a hypoactivity of the central cholinergic system. An acute 24-hour stress causes no change. The relationship between chronic stress and associated memory deficits is discussed.

Chronic restraint stress Memory Cholinergic system Scopolamine Oxotremorine

THE involvement of the septo-hippocampal cholinergic system in stress phenomena has been shown in a large number of studies [see (8) for review]. The septo-hippocampal system also plays a very important role in the processes of learning and memorization [see (2) for review], in particular in situations of behavioral inhibition (10–12). A number of authors consider that the deficit in memory processes encountered in senile degeneration and Alzheimer's disease results from the deterioration of central cholinergic neurons (1).

In recent work (13), we have shown that a relationship exists among the chronicity of stress caused by electric footshocks (EF), the capacity to control stress and the memorization of a passive avoidance test. The daily application of an inescapable electric footshock (IEF) for 10 days inhibited scopolamine-induced amnesia in a passive avoidance test, while 30 days of IEF led to a deterioration in the retention of the passive avoidance and an augmentation of the amnesic action of scopolamine.

If the EF could be avoided, then no modification was detected. These results thus highlighted the consequences of chronic unavoidable stress on the retention of a passive avoidance test. The application of chronic unavoidable stress, however, involves what we have termed the action inhibition system (AIS) (10) which includes the median septal area, the dorsal hippocampus, the lateral amygdala and the ventromedian hypothalamus. Since synaptic conduction in this system is primarily cholinergic, it was decided to measure the activation state of the cholinergic system in the course of chronic restraint stress, using the test of hypothermia induced by the muscarine agonist oxotremorine (3,4). The effect of chronic restraint stress on the retention of passive avoidance was also studied.

METHOD

Animals

Male OF1 mice with mean bodyweight of 30 g were used. Animals were obtained from Iffa Credo (France) and were randomly distributed and housed in groups of 10. They were left for one week in standard animal facility conditions before starting experiments.

Restraint Stress

Mice were placed in 25 mm inner diameter plastic tubes with suitable ventilation at one extremity, the other closed off. Animals were kept in restraint conditions for two hours between 9 a.m. and noon and again for two hours between 2 and 5 p.m. for one, 10 or 30 days, five times per week. Controls underwent no manipulation during this time.

Passive Avoidance

Twenty-four hours after the last stress, the animals were subjected to a passive avoidance test training.

Passive avoidance test equipment. The training and test of passive avoidance were conducted with two Plexiglas cages, one black, one white. The white compartment (40 × 40 cm) was illuminated by a 100 W bulb. The black compartment (30 × 40 cm) had an electrified floor composed of 2 mm diameter bars 1 cm apart. A 0.3 mA DC current (DC Shock Source: 20 V, Apelex) was delivered to the bars of the black compartment. A 7 × 7 cm opening in the partition between the two halves of the cage could be closed by a sliding guillotine door.

[1]Requests for reprints should be addressed to Dr. H. Laborit, Laboratoire d'Eutonologie, Hôpital Boucicaut, 78, rue de la Convention, 75015 Paris, France.

TABLE 1

PASSIVE AVOIDANCE TEST

Groups	Latency Time to Penetrate Black Compartment 24 Hours After Training (seconds)			
	Not Stressed + Saline (n = 10)	Not Stressed + Scopolamine[a] (n = 10)	Stressed + Saline (n = 10)	Stressed + Scopolamine [a] (n = 10)
Acute Stress	177 ± 2.0	17.6 ± 3.00	179 ± 0.7	26.8 ± 5.70
		<——————————> NS		<——————————> NS
	<——————————> NS			
Ten-Day Stress	178.7 ± 0.70	14.1 ± 2.20	179.1 ± 0.60	102.1 ± 23.60
		<——————————> †		<——————————> †
	<——————————> NS			
Thirty-Day Stress	175.8 ± 2.20	79 ± 24.8	104.8 ± 21.40	25.3 ± 11.50
		<——————————> *		<——————————> *
	<——————————> *			

Results are expressed as mean ± standard error of the mean (SEM) and statistical analysis is made with the Mann-Whitney U-test: *$p<0.05$; †$p<0.01$; NS: nonsignificant.

[a]Scopolamine dose was 0.1 mg/kg for the "Acute" and "ten-day" stress groups and 0.05 mg/kg for the "thirty-day" stress group.

Training methods and the test. The mice were subjected to a training session 24 hours after the last stress and were tested for the acquisition of the passive avoidance 24 hours later, i.e., 48 hours after the last stress. The training and the test were conducted between 9 a.m. and noon. On the day of the training, the mice were individually placed in the illuminated white compartment opposite the black compartment. When the mouse entered the black compartment, the door was closed and a 5-second shock was given. Each mouse was again placed in the white compartment opposite the black compartment 24 hours later and the time required for the mouse to enter the black compartment and the time spent in that compartment were measured for three minutes. Mice not entering the black compartment received a delay time of 180 seconds and a duration score of 0.

On the day of the training, controls and stressed animals were injected intraperitoneally (IP) with either isotonic saline or scopolamine hydrobromide (Sigma Chemical Company) at 0.1 mg/kg in the case of animals stressed for 1 or 10 days, and at 0.05 mg/kg for those stressed for 30 days. The low dose of scopolamine for animals stressed 30 days was used in order to be able to detect any potentiation of the amnesic action of scopolamine by the chronic stress, since the scopolamine dose of 0.1 mg/kg already causes considerable amnesia (13).

Oxotremorine Test

After the passive avoidance test, both stressed and nonstressed animals that had not received scopolamine in the passive avoidance test were subjected to the oxotremorine test. Thirty minutes before the IP injection of oxotremorine base (Sigma Chemical Company) at 1 mg/kg, atropine methyl nitrate (Sigma Chemical Company) was injected IP at 1 mg/kg in order to block the peripheral effects of oxotremorine (3,4). Rectal temperature of the animals was recorded before and 15, 30, 60, 90 and 120 minutes after injecting oxotremorine. The results are expressed as the mean difference in rectal temperature between t_o and the other time points. The animals were tested 48 hours after the last stress since it had been shown that disturbances of the cholinergic system caused by a chronic stress lasted for at least 48 hours after the last stress (3,6).

Statistics

The statistical study was carried out with the Mann-Whitney U-test in the case of passive avoidance and with the Student *t*-test for the oxotremorine test.

RESULTS

Acute Stress

Passive avoidance (Table 1). After an acute stress, there was no change in retention of the passive avoidance, with or without scopolamine in comparison to controls. No difference was observed on the day of training between the stressed and nonstressed groups. All the animals entered the black compartment in 1 to 10 seconds.

Oxotremorine test (Table 2). There was no difference in oxotremorine-induced hypothermia between the controls and animals receiving an acute stress.

Chronic 10-Day Stress

Passive avoidance (Table 1). After a restraint stress lasting 10 days, there was no change in retention of the passive avoidance. It did, however, antagonize amnesia induced by scopolamine at 0.1 mg/kg. No difference was observed on the day of training between the stressed and nonstressed groups. All the animals entered the black compartment in 1 to 10 seconds.

Oxotremorine test (Table 2). Animals stressed for 10 days were more sensitive to oxotremorine-induced hypothermia, which was highly significant during the first hour of observation.

Chronic 30-Day Stress

Passive avoidance (Table 1). A 30-day restraint stress led to a significant deficiency in the retention of the passive avoidance test, while there was no change on the day of training. In addition, stressed animals were significantly more sensitive to amnesia induced by a low dose of scopolamine (0.05 mg/kg). No difference was observed on the day of training between the stressed and nonstressed groups. All the animals entered the black compartment in 1 to 10 seconds.

Oxotremorine test (Table 2). Animals subjected to a 30-day restraint stress manifested a significant hyposensitivity to oxotremorine-induced hypothermia.

TABLE 2

OXOTREMORINE TEST

Groups	Mean Rectal Temperature Decrease After Injection of Oxotremorine Base 1 mg/kg IP (°C) Not Stressed (n = 10)						Stressed (n = 10)					
Time After Injection of Oxotremorine (minute)	15	30	45	60	90	120	15	30	45	60	90	120
Acute Stress	2.3±0.2	3.8±0.3	5.0±0.3	5.8±0.3	5.5±0.5	4.1±0.8	2.5±0.1 NS	3.6±0.2 NS	5.2±0.5 NS	6.0±0.4 NS	5.0±0.4 NS	3.9±0.4 NS
Ten-Day Stress	2.2±0.2	3.4±0.2	4.2±0.2	5.0±0.3	4.9±0.3	1.8±0.4	3.5±0.2 ‡	5.6±0.2 ‡	6.5±0.2 ‡	7.0±0.3 ‡	6.1±0.4 *	2.9±0.5 NS
Thirty-Day Stress	4.2±0.2	6.5±0.3	7.8±0.3	8.4±0.3	8.4±0.5	7.4±0.6	3.6±0.1 *	5.5±0.2 *	6.7±0.5 †	7.0±0.1 ‡	5.7±0.4 ‡	3.5±0.4 ‡

The results are expressed as the mean ± SEM and statistical analysis is made at each time interval with the Student *t*-test: *$p<0.05$; †$p<0.01$; ‡$p<0.001$; NS: nonsignificant. (No statistical difference in rectal temperature is noted between stressed and not stressed groups before oxotremorine injection.)

DISCUSSION

The present results obtained by the use of restraint stress confirm those reported when IEF were given (13), i.e., a chronic 30-day stress disturbs the retention of a passive avoidance test, whereas the same stress but for only 10 days antagonizes scopolamine-induced amnesia. In addition, the hypersensitivity of 10-day stressed animals to oxotremorine-induced hypothermia, reflecting a hypersensitivity of the cholinergic system, agrees with published data (3,4) obtained by the use of forced swimming or IEF stress.

There are relatively few published studies on stress continuing for a relatively long period. The present results show that stress lasting 30 days induces a hyposensitivity of the cholinergic system as shown by an accrued sensitivity of the animals to amnesia induced by a low dose of scopolamine in the passive avoidance test and by their hyposensitivity to oxotremorine-induced hypothermia.

Thus, although there is apparently an up-regulation of muscarine receptors during a chronic stress lasting 5 to 10 days (6), a down-regulation of muscarine receptors or a decrease in their coupling with second messenger systems would occur in the course of a chronic stress lasting 30 days. These behavioral results are to be compared to a pharmacological study (14), in which the authors observed a deficit in the retention of a passive avoidance test after the chronic administration of physostigmine, an acetylcholinesterase (ACHE) inhibitor. Similarly, it has also been shown (7) that the chronic administration of another ACHE inhibitor, diisopropylfluorophosphate (DFP), induces a decrease in the density of cortical muscarine receptors and diminishes the performance of animals in a passive avoidance test.

Numerous anatomical (15), neurochemical (9) and pharmacological evidence (5) supports the hypothesis of a hypoactivity of the central cholinergic system which is responsible for memory deficits. Our work has shown that hypoactivity of the cholinergic system occurs in the course of a chronic stress, suggesting an intimate relationship between the chronic involvement of the action inhibition system and the appearance of memory disorders.

REFERENCES

1. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
2. Bartus, R. T.; Dean, R. L.; Flicker, C. Cholinergic psychopharmacology: An integration of human and animal research on memory. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:219–232.
3. Dilsaver, S. C.; Snider, R. M.; Alessi, N. E. Stress induced supersensitivity of the cholinergic system in rats. Biol. Psychiatry 21:1093–1096; 1986.
4. Dilsaver, S. C.; Alessi, N. E. Chronic inescapable footshock produces cholinergic system supersensitivity. Biol. Psychiatry 22:910–914; 1987.
5. Drachman, D. A. Memory and cognitive function in man: Does the cholinergic system have a specific role? Neurology 27:783–790; 1977.
6. Finkelstein, Y.; Koffler, B.; Rabey, J. M.; Gilad, G. M. Dynamics of cholinergic synaptic mechanisms in rat hippocampus after stress. Brain Res. 343:314–319; 1985.
7. Gardner, R. R.; Ray, J.; Frankenheim, K.; Wallace, M.; Loos, M.; Ribochaud, R. A possible mechanism for diisopropylfluorophosphate-induced memory loss in rats. Pharmacol. Biochem. Behav. 21: 43–46; 1984.
8. Gilad, G. M. The stress-induced response of the septohippocampal cholingeric system. A vectorial outcome of psychoneuroendocrinological interactions. Psychoneuroendocrinology 18(3):167–184; 1987.
9. Jaffard, R.; Ebel, A.; Destrade, C.; Burkin, T.; Mandel, P.; Cardo, B. Effects of hippocampal electrical stimulation on long-term memory and on cholinergic mechanisms in three inbred strains of mice. Brain Res. 133:277–289; 1977.
10. Laborit, H. Proposition d'un modèle intégré des comportements normaux et anormaux à partir de données biochimiques, neurophysiologiques, éthologiques, cliniques et sociologiques. Ann. Med. Psychol. 1(1):47–63; 1974.
11. Laborit, H.; Zerbib, R. Phorbol esters antagonize scopolamine-induced amnesia. Res. Commun. Psychol. Psychiatr. Behav. 12: 105–117; 1987.
12. Laborit, H.; Zerbib, R. Role of various second messengers in the memorization of passive or active avoidance. Res. Commun. Psychol. Psychiatr. Behav. 12:193–204; 1987.
13. Laborit, H.; Zerbib, R. Different environmental situations modify scopolamine-induced amnesia in mice assessed with a passive avoidance test. Res. Commun. Psychol. Psychiatr. Behav. 14(2):143–156; 1989.
14. Louillis, C. C.; Dean, R. L.; Lippa, A. S.; Meyerson, L. P.; Beer, B.;

Bartus, R. T. Chronic administration of cholinergic agents: Effects on behavior and calmodulin. Pharmacol. Biochem. Behav. 18:601–604; 1983.

15. Scoville, W. B.; Milner, B. Loss of recent memory after bilateral hippocampus lesions. J. Neurol. Neurosurg. Psychiatry 20:11–21; 1957.