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Effects of Piracetam on Learned Helplessness in Rats[1]

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CAVOY, A., A. ENNACEUR AND J. DELACOUR. *Effects of piracetam on learned helplessness in rats.* PHYSIOL BEHAV 42(6) 545–549, 1988.—In rats, the effects of Piracetam (P), the prototype of nootropic drugs, were studied on a very widely used model of behavioral disturbance: the learned helplessness (LH) phenomenon. In this model, exposure to uncontrollable and unsignalled shocks impairs subsequent escape-avoidance learning. In a first experiment, this deficit was abolished by 200 mg/kg of P, and to a lesser extent, by a 100 mg/kg dose, administered before the training session. In non-stressed animals, no dose of P was able to have a facilitatory effect on escape-avoidance. In a second experiment, the administration of P, not before the training session as in Experiment I, but before the stress, had no effect on the LH phenomenon regardless of the dose.

Nootropic drugs Piracetam Learned helplessness Escape-avoidance Rats Depression

FACILITATORY effects of nootropic drugs on learning, memory and other cognitive processes [7,8], while frequently reported, remain controversial.

Most of these effects were observed in deficient organisms. Nootropic drugs improve learning and memory performances in aged animals [2,25] and in animals subjected to hypoxia, hypercapnia, electroconvulsive shocks, ischemia, amnestics drugs such as scopolamine and antibiotics [3, 6, 14, 21]; similar effects were observed in senile humans and in patients suffering from brain pathologies [9, 11, 13, 16, 19, 20, 24].

These data raise an important question: is this normalizing action restricted to the effects of purely organic deficiencies such as those mentioned above or does it also have a significant role in "functional" disturbances produced in normal organisms by environmental factors. In the latter case, the field of application of nootropic drugs would be considerably increased since the second type of disturbance is at least as frequent as the first. So far, the only evidence supporting this point is the fact that "behavioral dispair," a behavioral test sensitive to anti-depressant drugs [18], is significantly reduced in mice by nootropic administration [22].

We tried to obtain further information by the study of the effects of Piracetam (P), the prototype of nootropic drugs, (Nootropil, 2-oxo-1-pyrrolidine acetamide, a cyclic derivative of GABA), on one of the most popular models of behavioral disturbances: the "learned helplessness," LH [17]. In this model, exposure to uncontrollable and unsignalled shocks impairs subsequent escape-avoidance learning. Whatever be the involvement of cognitive factors in LH, it is generally considered as a model of depression.

METHOD

General Methods

Animals were male Wistar rats weighing 250–300 g. They were housed in individual cages and maintained on a 12:12 light-dark schedule (0700:1900 hours). Ambient temperature was 23°C±1. Standard rodent food as well as water were available ad lib throughout the experiments.

Behavioral tests were performed in shuttle-boxes which were 60×30×50 cm (height) Plexiglas cages divided into two compartments (30×30 cm) by a 5 cm barrier. The floor and the barrier were made of brass bars, 4 mm in diameter and 15 mm apart. The crossing of the barrier was detected by an infrared system. Each compartment was equipped with a 15 W bulb fastened to the end wall, 35 cm above the floor. The apparatus was placed in a sound-attenuated cabinet measuring approximately 1 m^3, in which sound-attenuation was aided by a masking noise of 70 dB above the human threshold. A 15 W bulb fastened to the ceiling of the cabinet provided a constant illumination of 10 lux at the level of the floor of the shuttle-box. The programming and recording apparatus were located outside the cabinet and the animals were observed by means of a closed-circuit television. Four identical shuttle-boxes located in different cabinets permitted the simultaneous testing of four animals.

The animals were handled daily for at least a week prior to behavioral testing.

Control (physiological saline) and experimental (Piracetam) injections were administered IP 30 min before sessions in the volume of 1 ml/kg. Piracetam (P) injections were prepared from a solution of Gabacet (Carrion, France)

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TABLE 1
RESULTS OF EXPERIMENT I

Groups	NSP1	NSP2	NSP3	NSC	SP1	SP2	SP3	SC
CAR	16.3	17.4	14.6	16.8	4.3	7.9	12.6	4.8
	±2.7	3.1	3.3	2.9	1.5	1.9	2.4	1.3
Fr	2.6	2.1	3.1	2.4	7.1	3.8	1.6	11.1
	±0.9	0.6	1.0	0.9	2.1	1.6	0.8	4.4
ITC	41.2	39.3	35.7	40.8	11.5	23.4	32.2	12.3
	±10.4	11.0	8.9	11.2	5.9	10.1	9.8	6.1

*Mean values ± S.E.M.
CAR: Conditioned Avoidance Responses, Fr: "Freezing," ITC: Intertrial Crossings, during session 2.

diluted in an approximate volume of physiological saline.

As the design of experiments was a 2×4 (Stress × Drug) factorial plan, two-way ANOVAs were computed according to Winer [26].

Experiment I

Eighty rats were submitted to two sessions separated by a 24 hr interval. During the first session, forty animals (stressed, S) were placed in the shuttle-boxes and subjected to 40 unsignalled and uncontrollable electrical shocks of 1 mA and 10 sec in duration, separated by a mean interval of 70 sec (range: 40–90). The other forty rats were placed for the same duration in the same apparatus and according to the same conditions but received no shocks (non stressed, NS). Before this session, all the animals received a control injection.

During session II, in the same apparatus, all the animals were submitted to an escape-avoidance conditioning. The session consisted of 40 trials separated by an interval of 70 sec (range: 40–90). The signal was the simultaneous illumination of the two bulbs fastened to the interior of the shuttle-box; intensity of this signal at floor level was 200 lux. Five seconds after the onset of the signal, an electric shock of 1 mA was delivered to the floor and the barrier by means of a scrambler-equipped generator. A complete crossing of the barrier during the signal-shock interval terminated the signal and no shock was delivered (conditioned avoidance response, CAR). A crossing made more than 5 sec after signal onset simultaneously ended the signal and the shock (escape response). Maximum shock duration was 10 sec. When no avoidance or escape response were made during a trial, the behavior of the rat was classified as "freezing" (Fr). Three measurements were considered: the numbers of CAR, Fr and intertrial crossings (ITC). The number of Fr may be considered as a global index of escape-avoidance behavior to which it was inversely related.

Before escape-avoidance training, S (stressed) animals were randomly divided into four groups (n=10), one receiving a control injection (SC group), the other three, 50 mg, 100 mg and 200 mg/kg of P (SP1, SP2 and SP3 groups respectively). In a parallel fashion, NS (non-stressed) rats were randomly divided into four groups (n=10): NSC, NSP1, NSP2 and NSP3 receiving respectively a control injection, 50, 100 or 200 mg/kg of P before session II.

Experiment II

It was performed on another batch of animals in exactly the same conditions as Experiment I, except that the experimental injections to the appropriate groups were administered before session I instead of session II: Before session I, forty animals were randomly divided into four groups (n=10), one receiving a control injection (CS group), the others, 50, 100 or 200 mg/kg of P (groups P1S, P2S and P3S respectively). After injection, these four groups were submitted to uncontrollable and unsignalled shocks as in Experiment I. In a parallel fashion, forty other animals were divided into four groups: CNS, P1NS, P2NS and P3NS. Each of these groups was subjected to the appropriate injection (saline, 50, 100 and 200 mg/kg of P respectively) and then placed in the shuttle-boxes where no shock was delivered.

Before session II, a control injection (physiological saline) was administered to all the animals then they were submitted to an escape-avoidance training as in Experiment I.

RESULTS

Experiment I (Table 1)

(1) Number of CAR. The factor Stress was significant, $F(1,72)=10.71$, $p<0.01$, as well as the factor Drug, $F(3,72)=5.08$, $p<0.01$, but not the interaction Stress × Drug, $F(3,72)=2.17$, $p>0.05$. The main results of the two by two comparisons were as follows.

The SC group made significantly ($p<0.01$) less CAR than the NSC which means that the avoidance behavior in session II was impaired in the control animals stressed during session I.

The SP3 group made significantly ($p<0.05$) more CAR than the SC and did not differ from the NSC. These two results show that the avoidance deficit produced by the stress was abolished by the 200 mg/kg dose of P. However, the 50 mg and 100 mg/kg doses were ineffective since the SP1 and SP2 groups were significantly inferior ($p<0.05$) to the NSC and did not differ from the SC.

The NSP1, NSP2 and NSP3 groups were not significantly different from the NSC which means that in animals not previously stressed no dose of P was able to modify the acquisition of CAR.

(2) Number of freezing. The factor Stress was significant, $F(1,72)=8.39$, $p<0.01$, as well as the factor Drug,

TABLE 2
RESULTS OF EXPERIMENT 2

Groups	P1NS	P2NS	P3NS	CNS	P1S	P2S	P3S	CS
CAR	15.1	12.7	13.2	14.5	4.2	4.6	4.0	3.8
	± 3.3	3.4	2.8	4.1	1.2	1.4	1.6	1.7
Fr	3.4	3.9	2.7	3.7	12.3	7.2	13.2	14.0
	±1.1	1.4	1.0	1.3	5.0	4.1	4.8	6.2
ITC	47.6	51.3	44.2	49.8	27.7	15.4	18.5	17.9
	±9.8	12.3	10.7	14.5	11.5	6.1	6.8	5.9
CROS	52.8	49.4	57.6	55.1	75.4	69	80.2	71.6
	±11.9	7.5	10.4	8.8	16.2	14.3	15.7	12.8

*Mean values ± S.E.M.
CAR: Conditioned Avoidance Responses, Fr: "Freezing," ITC: Intertrial Crossings, during session 2. CROS: Crossings during session 1.

$F(3,72)=3.43$, $p<0.02$, but not the Stress × Drug interaction, $F(3,32)=2.47$, $p>0.05$. The results of the two by two comparisons were mainly the mirror image of the preceding ones.

The SC group made significantly more Fr ($p<0.02$) than the NSC: the stress in session I globally impaired the escape-avoidance behavior of stressed controls in session II.

The SP3 group made significantly less Fr ($p<0.05$) than the SC but did not differ from the NSC: the 200 mg/kg dose of P abolished the escape-avoidance deficit in the stressed animals. However, the 50 mg/kg dose was ineffective since the SP1 group made significantly ($p<0.05$) more Fr than the NSC and was not different from the SC; the 100 mg dose had intermediate effects (the SP2 group was not significantly different from the NSC and SC groups).

The number of Fr was not modified by P administration in non-stressed animals since the NSP1, NSP2 and NSP3 groups were comparable to the NSC.

(3) Intertrial crossings. The factor Stress was significant, $F(1,72)=12.46$, $p<0.01$, as well as the factor Drug, $F(3,72)=7.17$, $p<0.01$, but not the interaction Stress × Drug, $F(3,72)=2.04$, $p>0.05$. The SC group made significantly less ($p<0.01$) ITC than the NSC, which means that the deficit in escape-avoidance behavior in control stressed rats was associated with a reduction of ITC. This reduction was antagonized by the 200 mg/kg dose of P since the SP3 group made significantly ($p<0.02$) more ITC than the SC but did not differ significantly from the NSC. The 50 mg dose was ineffective (the SP1 group was inferior to NSC ($p<0.02$) and not different from the SC group) and the 100 mg dose had intermediate effects (the SP2 group was not significantly different from the NSC and SC groups).

As for avoidance and freezing, none of the three doses of P had a significant effect on ITC in non-stressed animals.

Experiment II (Table 2)

(1) Number of CAR. The stress factor is significant, $F(1,71)=13.39$, $p<0.01$: As a whole, the stressed animals (groups CS, P1S, P2S and P3S) made less CAR than the non-stressed (groups CNS, P1NS, P2NS, and P3NS). The number of CAR of the control stressed (CS) group was significantly inferior ($p<0.01$) to that of the control non-stressed (CNS): as in Experiment I, the stress of session I impaired avoidance behavior during session II.

On the other hand, the Drug factor, $F(3,72)=1.21$, and the Stress × Drug interaction, $F(3,72)=0.91$, were not significant ($p>0.05$). There were no differences between the stressed groups, especially between the experimental groups (P1S, P2S and P3S) and their control group (CS). Thus, no dose of P administered before the stress (session I) was able to improve subsequent avoidance behavior (session II). As in Experiment I, there was no difference between non-stressed groups, that is, no effect of P.

(2) Number of Fr. The Stress factor, $F(1,72)=7.97$, $p<0.01$, was significant: As a whole, the stressed animals (groups CS, P1S, P2S and P3S) made more Fr than the non-stressed (groups CNS, P1NS, P2NS and P3NS). The number of Fr of the CS group was significantly higher ($p<0.02$) than that of the CNS: as in Experiment I, escape-avoidance behavior was globally impaired in control stressed animals.

On the other hand, the Drug factor, $F(3,72)=0.58$, and the Stress × Drug interaction, $F(3,72)=1.04$, were not significant ($p>0.05$). There were no significant differences between the stressed groups, especially between the experimental groups and the controls: thus the freezing behavior during session II (avoidance training) was not decreased by P administered before session I (stress). As in Experiment I, there were no differences between the non-stressed groups.

(3) Number of ITC. The Stress factor was significant, $F(1,72)=5.34$, $p<0.02$. As a whole, the stressed animals made less ITC than the non-stressed ($p<0.05$) and the number of ITC in the CS group was significantly lower than in the CNS ($p<0.05$).

On the other hand, the Drug factor, $F(3,72)=1.11$, and the Stress × Drug interaction, $F(3,72)=0.51$, was not significant ($p>0.05$). There was no difference between the stressed groups: no dose of P administered before session I had an effect on ITC during session II. There was also no difference between the non-stressed groups.

(4) Number of crossings during session I. Since the experimental injections were administered before session I instead of session II as in Experiment I, a fourth measurement was considered in the results of Experiment II: the number of crossings during session I. The Stress factor, $F(1,72)=5.31$,

$p<0.02$, was significant. As a whole, the stressed animals made more crossings than the non-stressed ($p<0.02$) but the interpretation of this result is difficult since most of the crossings of the stressed animals were elicited by electrical shocks while the non-stressed made only spontaneous crossings. More important is the fact that the Drug factor was not significant, $F(3,72)=0.69, p>0.05$: this means that no dose of P was able to modify the motor activity either in stressed or in non-stressed animals. The Stress × Drug interaction also did not reach significance, $F(3,72)=0.86, p>0.05$.

DISCUSSION

Results of Experiment I show the following:

(1) The procedure we used was able to produce a learned helplessness phenomenon as defined in the introduction, that is an impairment in escape-avoidance behavior: compared to non-stressed controls, stressed controls made less CAR and showed more freezing.

(2) This deficit was abolished by a 200 mg/kg dose of P administered just before the escape-avoidance training and to a lesser extent, by a 100 mg/kg dose.

(3) In non-stressed animals, no dose of P was able to have a facilitatory effect on avoidance conditioning, which confirms the data from Ennaceur and Delacour [4].

In Experiment II, the learned helplessness phenomenon was still present as indicated by the differences between stressed and non-stressed controls. However, the administration of P, not before escape-avoidance training (session II) as in Experiment I, but before the stress (session I), had no effect on the LH phenomenon regardless of the dose.

The facilitatory action of P on escape-avoidance behavior in stressed animals may have several causes.

(1) It can be considered as a performance effect. P could, as amphetamine [10], facilitate escape-avoidance behavior by increasing locomotor activity and decreasing freezing. However, this interpretation is not supported by our data. P had no effect on the motor activity of either stressed or non-stressed rats (Experiment II, session I) nor on the number of intertrial crossings during avoidance conditioning in the non-stressed rats (Experiment I, session II). Stressed rats receiving 200 mg/kg of P made more ITC than stressed controls but did not differ from non-stressed controls; thus the facilitation of escape-avoidance behavior by this dose of P was not associated with "supra-normal" levels of ITC.

(2) According to a cognitive hypothesis, P might have facilitated the detection of differences in reinforcement contingencies between session I (unsignalled and uncontrollable shocks) and session II (shocks preceded by a visual signal and controllable by escape or avoidance responses).

(3) The facilitation of escape-avoidance behavior of stressed rats by P could be due to a normalizing action of the drug on the brain. Uncontrollable and unsignalled shocks deplete brain catecholamines and this decrease is perhaps the cause of the LH phenomenon [1]. P could restore normal levels of catecholamines. There is no direct proof in favor of this hypothesis but several data suggest that P may act on brain catecholaminergic mechanisms [5, 12, 15, 23]. It may also modulate other neurotransmitters through its interaction with the two main second-messengers, cAMP and phosphotidylinositol [12].

Whatever be the nature of this action, these experiments confirm that the facilitation of learning by P is more detectable in disturbed organisms than in normal ones. Our results give also a new and potentially important information: these beneficial effects may be significant not only in animals impaired by organic pathologies but also in organically normal animals whose behavior is disturbed by environmental factors. Thus, the normalizing effects of P could have a much larger range than that presently assumed.

Finally, although it was not within the scope of this series of experiments to study the mechanisms of depression, our results, as well as those of Schmidt [22], may be suggestive in this respect, since the LH phenomenon was used as a model of behavioral disturbance. According to the second hypothesis considered above, the fact that a nootropic drug decrease LH could provide an argument in favor of cognitive theories of depression [1].

However, this argument is not decisive since the relevance of LH to depression as well as the relationship between the effects of P on learning tasks and a facilitation of cognitive abilities remain controversial.

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