quantity of the enzyme. A fall of the AldDH/ADH-E ratio may lead to increased production of acetaldehyde accompanied by its delayed utilization to acetic acid. However, a fall in the AldDH/ADH-AA ratio can be regarded as evidence of stimulation of the reductase pathway of utilization of acetaldehyde into ethanol. As was shown previously [4], in the liver perfused with ethanol only 2% of acetaldehyde is reduced to ethanol. On the basis of our observations it is logical to suggest a more significant contribution of the fall in AldDH activity than of activation of the reductase reaction of ADH in the process of acetaldehyde accumulation. The biphasic action of alcohol on the brain can be explained by antagonism of the inhibitory effect of alcohol itself and the excitatory effect of acetaldehyde [9]. The degree of narcotic action of ethanol will evidently depend on the ethanol/acetaldehyde ratio.

During repeated contact of the body with ethanol conversion of alcohol is thus intensified and utilization of acetaldehyde is delayed in the liver; this may be one mechanism of the development of metabolic tolerance, which is accompanied by diminution of the narcotic action of ethanol on the brain.

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EFFECT OF PIRACETAM ON SENSITIVITY TO PAIN, BLOOD β-ENDORPHIN LEVEL, AND CEREBRAL CORTICAL *c*AMP LEVEL IN RATS

O. P. Vakulina, V. V. Yasnetsov, V. A. Isachenko, I. N. Krylova, I. P. Tishkova, E. O. Bragin, and E. V. Popkova

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Piracetam is a classical member of the nootropic group, whose antiamnesic effect has been well studied [12]. However, the mechanism of this effect is not yet clear. An important role in the regulation of learning processes is played by cyclic nucleotides. It has been shown, for example, that administration of dibutyryl-*c*AMP or of the adenylate cyclase stimulator, forskolin, improves defensive conditioning in poorly trained animals [3] and prevents memory disturbances in hypoxia [7]. Data on the effect of piracetam on the cyclic nucleotide system are virtually absent. One of the aims of this investigation was accordingly to study the effect of piracetam on the *c*AMP level in the cerebral cortex of rats.

Central Research Institute of Reflex Therapy, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.)

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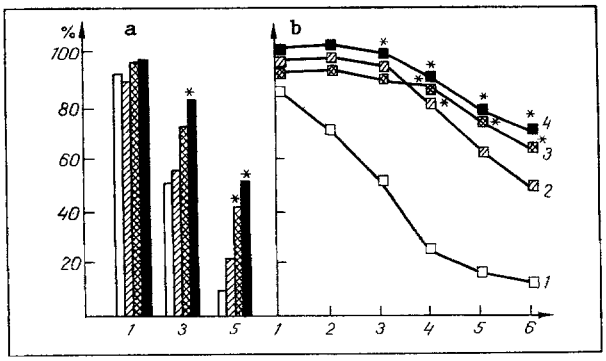

Fig. 1. Effect of single intraperitoneal injection (a) and long-term consumption with drinking water (b) of piracetam on weakening of PACR. Abscissa, days after learning; ordinate, percentage of reactions performed. 1) Control, 2) 10 mg/kg,10 mg/kg daily; 3) 100 mg/kg, 100 mg/kg daily; 4) 400 mg/kg, 1000 mg/kg daily. *$p < 0.05$.

TABLE 1. Effect of Single Intraperitoneal Injection of Piracetam on Behavior of Rats in Open Field and on Sensitivity to Pain ($M \pm m$)

Dose of piracetam, mg/kg	Motor activity	Investigative activity	TPS, time after placing in constraining				TPS, in
			20	45	70	90	110
Control	36,1±2,6	0,38±0,12	10,89±0,89	9,31±0,39	8,97±0,99	8,74±0,77	3,94±0,25
10	37,8±3,1	0,75±0,39	10,05±1,21	8,76±0,72	9,09±0,69	9,24±0,81	2,91±0,39*
100	31,8±2,0	1,10±0,40	9,76±0,51	6,91±0,98*	6,27±0,83*	8,01±0,97	2,80±0,28*
400	43,7±3,8	1,63±0,25	9,28±0,87	5,83±0,74*	6,13±0,79*	7,35±0,62	2,64±0,31*

Legend. Here and in Table 2 motor activity means number of squares crossed; investigative activity means number of holes sniffed in floor of chamber. *$p < 0.05$.

TABLE 2. Effect of Chronic Consumption of Piracetam on Behavior in Open Field, Sensitivity to Pain, and Blood β-Endorphin and Cerebral Cortical *c*AMP Levels in Rats ($M \pm m$)

Dose of piracetam, mg/kg/day	Motor activity	Investigative activity	β-endorphin, fmoles/ml	cAMP, pmoles/mg tissue	TPS, time after placing in constraining cages, min				TPS, in 100
					20	45	70	90	
Control	34,0±2,6	0,7±0,3	46,7±9,8	2,94±0,35	3,86±0,49	3,67±0,29	3,44±0,28	3,26±0,24	6,25±0,12
10	42,7±3,5	0,9±0,2	36,8±1,7	2,69±0,34	2,89±0,28	2,58±0,25*	2,31±0,21*	2,73±0,29	5,71±0,19*
100	44,7±4,0*	1,1±0,1	29,1±4,3	3,98±0,41*	3,17±0,28	2,41±0,25*	2,52±0,27*	2,91±0,33	5,44±0,25*
1000	36,4±3,0	3,2±0,6*	18,2±2,1*	3,06±0,28	2,97±0,36	2,44±0,23*	2,61±0,32*	2,59±0,26	4,30±0,16*

Another aim of this investigation was to study the action of piracetam on the threshold of pain sensitivity (TPS) and the plasma level of β-endorphin, a peptide characterizing the response of the body to stressors, including nociceptive [11]. The motivation for this type of investigation was provided by data [6, 10] indicating that administration of β-endorphin impairs learning in animals; the peptide, moreover, was given in doses not affecting sensitivity to pain. It is likewise not known whether TPS is changed by the action of piracetam. However, facilitation of memory consolidation during recall of conditioned avoidance reflexes may be due, besides to other effects, to piracetam and its influence on the sensitivity of animals to pain.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 220-280 g. In the experiments of series I a single injection of piracetam ("Olaine," USSR) in doses of 10, 100, and 400 mg/kg, was given intraperitoneally. In an open field test 40 min after

the injection, the animals' motor and investigative activity and grooming were recorded and a passive avoidance conditioned reflex (PACR) in response to a single presentation. For this purpose, in the course of 3 min the animal's paws were stimulated electrically in a chamber (30 × 40 cm) with a platform insulated from the electrically conducting floor (15 × 15 cm) [8]. On the following days, the effectiveness of recall was tested for 1 min. TPS was determined by two methods, enabling the action of the drug to be analyzed at the level of the spinal cord and brain: the latent period of the tail-flick test and the strength of the current used for electrodermal stimulation of the tail in order to obtain a vocal response were measured. During determination of TPS the rats were kept for 2 h in plastic constraining cages. The rats of one group were adapted to the conditions of measurement of TPS for 1 week daily.

In the experiments of series II rats of group 3 (n = 8-10) were given piracetam in doses of 10, 100, or 1000 mg/kg daily for 12 days with their drinking water. On the 6th day from the beginning of the experiment, a PACR was formed in animals of these groups and the control group by an analogous method, and TPS and activity in the open field were determined by the methods described above on the 10th-11th days. At the end of the experiment the rats were guillotined and the β-endorphin concentration determined in the blood plasma by radioimmunoassay using kits from Star Corporation Inc. (USA) and the *c*AMP level in the cerebral cortex also was determined. *c*AMP was extracted from brain tissue on ice with 0.5 N perchloric acid (*w/v* 1:10); the homogenate was centrifuged and the supernatant neutralized with 5 N NaOH and lyophilized. The *c*AMP concentration was determined by radioimmunoassay, using antibodies to *c*AMP obtained previously in V. A. Isachenkov's laboratory. The lyophilized product was dissolved in 0.05 M acetate buffer with 0.1% ovalbumin (pH 6.2) and incubated with antibodies, using *c*AMP from "Reanal" (Hungary), as the standard, and using dibutyryl-8-^{3}H-*c*AMP ("Izotop," USSR) as the labeled compound. The bound form was precipitated with 50% ammonium sulfate and radioactivity was measured on a Beta-2 beta-spectrometer (Sweden).

The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

A single dose (Fig. 1a) and chronic administration (Fig. 1b) of piracetam considerably improved learning and preservation of the memory trace during recall of the PACR. The remaining results are given in Tables 1 and 2. In rats taking piracetam for a long time in a dose of 100-150 mg/kg daily, an increase in the *c*AMP concentration was found in the cerebral cortex (Table 2). This result is in agreement with data showing an increase in the *c*AMP concentration in the brain of guinea pigs receiving piracetam by mouth for 16 days in a dose of 35 mg/kg [14]. The absence of any changes in the *c*AMP level during consumption of larger quantities of piracetam (Table 2) may be due to balancing of the processes of adenylate cyclase and *c*AMP phosphodiesterase activation at a new level. It must be noted, however, that a considerable increase in activity of the cyclic nucleotide system under the influence of piracetam could not have taken place because we know that an increase in the *c*AMP concentration in the brain is accompanied by a definite behavioral syndrome and, in particular, by motor depression and grooming [13]. In the present experiments the animals' motor activity was unchanged (Table 1) or increased (Table 2); grooming was not increased.

The mechanism of the improvement of long-term memory may include a change in the meaning of the external signals for the animal under the influence of piracetam. First, attention is drawn to the dose-dependent increase in investigative activity of the rats following a single and repeated doses of the drug. Second, piracetam led to a reduction of TPS by 25-30%. The increase in sensitivity to pain, measured by the tail-flick test, developed gradually, 20-40 min after the rats had been placed in the constraining cages (Tables 1 and 2). Short-term hypokinesia is known to induce a stressor reaction in rats and the onset of an opioid form of stress-induced analgesia [11]. It has also been shown that piracetam counteracts the analgesic effects of agonists of opiate receptors [5]. It was therefore interesting to discover whether the change in TPS was due to the effect of piracetam on the development of stress-induced analgesia. It was found that TPS also was reduced under the influence of piracetam in animals adapted to the conditions of measurement. This is evidence that the mechanism of increased sensitivity to pain under the influence of piracetam is triggered independently of activation of the antinociceptive component of the stress reaction.

In this connection we consider that data obtained during measurement of TPS by the animal vocalization method are important. By the use of this method it was possible to detect lowering of TPS of the experimental rats 2 h after being placed in constraining cages, when differences found by the tail-flick method had already disappeared (Tables 1 and 2). We know that during the vocal reaction, suprasegmental brain structures are involved in the process of evaluation of nociceptive information, whereas the tail-flick response is realized at the spinal level [9]. Consequently, under the influence of piracetam the activity of these structures is probably changed, and this leads to a change in perception and to an increase in the meaningfulness of the pain signal for rats.

The lowering of TPS observed in the present experiments may be connected with a fall in the β-endorphin concentration in the blood: during chronic piracetam consumption in a dose of 1 g/kg the content of the peptide fell by almost two-thirds (Table 2). This hypothesis is not in conflict with the view that the main function of β-endorphin in physiological concentrations is not analgesia, but the fine regulation of behavior [1]. The behavioral effects of β-endorphin may include a change in the assessment of nociceptive information. It has been reported that in rats with a low β-endorphin level in the hypothalamus and pituitary gland as a result of active immunization to this peptide, manifestations of the stress reaction are weakened [4]. Our own data enable the spectrum of the stress-protective action of piracetam [2] to be supplemented by a neurohumoral aspect, namely the effect of piracetam on the endorphinergic system.

The fall in the blood level of β-endorphin may also be one cause of the improvement of PACR recall, for β-endorphin is a factor accelerating the weakening of PACR [6].

The results are thus evidence that the effect of piracetam on preservation of PACR is accompanied by a fall of the plasma β-endorphin concentration and by a small rise of the *c*AMP level in the cerebral cortex of rats and by an increase in sensitivity to pain.

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