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EFFECT OF THE ACQUISITION-ENHANCING DRUG PIRACETAM ON RAT CEREBRAL ENERGY METABOLISM. COMPARISON WITH NAFTIDROFURYL AND METHAMPHETAMINE

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Abstract—The effects of Piracetam, Naftidrofuryl and methamphetamine on several parameters of cerebral energy metabolism have been studied. At variance with some reports in the literature neither Piracetam nor Naftidrofuryl affected the cerebral contents of adenine nucleotides and, accordingly, both substances were without effect on the adenylate energy charge. This disagreement is explained by methodological differences. Methamphetamine also had no effect on cerebral adenine nucleotides. Piracetam increased the activity of adenylate kinase (EC.2.7.4.3) in isotonically diluted rat brain homogenates without altering the K_M of the enzyme for ADP as substrate. It is concluded that although Piracetam has no effect on the cerebral energy metabolism under normal conditions, it may have a beneficial effect under marginal conditions like those met during hypoxia, by virtue of its adenylate kinase stimulating action. It is suggested that this action is responsible for the protective effect of Piracetam against cerebral hypoxia. It may also be related to the enhancement of acquisition under training conditions where cerebral energy metabolism is disturbed.

Although the acquisition-enhancing effect of 2-pyrrolidon-*N*-acetamide (Piracetam®) in rats [1,2] and goldfish [3] is well documented, little is known about the underlying mechanism. It has been reported that Piracetam protects rats and rabbits against cerebral hypoxia [2,4] and increases the ATP content of rat brain [5]. Therefore, the question arises as to whether the enhancement of acquisition might be due to a positive effect of Piracetam on cerebral energy metabolism. In the present experiments the influence of Piracetam on the adenine nucleotide content of rat brain was studied, together with the adenylate energy charge and the activity of adenylate kinase (EC.2.7.4.3; AK) in the brain. In some experiments two other drugs, methamphetamine and the oxalate of the *N*-diethylaminoethylester of *β*(naphtyl-1)*β*′-(tetrahydrofuryl-2)isobutyric acid (Naftidrofuryl®) were included for comparison. The former drug has well known behavioural effects such as a stimulation of spontaneous motor activity and enhancement of acquisition [1,6] whereas the latter has been reported to increase the ATP content of mouse brain [7].

MATERIALS AND METHODS

In all experiments male Small–Wistar (WAG) rats, weighing 180–200 g were used.

Extraction of adenine nucleotides. Thirty min after the intraperitoneal injection of either Piracetam (150 mg/kg), Naftidrofuryl (25 mg/kg), methamphetamine (2 mg/kg) or an equivalent volume of 0.9% NaCl (saline) rats were plunged alive, head forward, into liquid N_2. Ten min later their heads were cut off and the frozen brains were chiselled out and powdered under liquid N_2. One g of the powder was homogenized at 0° in 6 ml of either 0.5 N perchloric acid (PCA) or 10% trichloroacetic acid (TCA). After centrifugation of the homogenate for 30 min at 30,000 *g* the supernatant was removed and stored (not longer than 3 hr) at 0° until the determination of the adenine nucleotides.

Determination of ATP, ADP and AMP. ATP was determined by the method of Bücher [8] and ADP and AMP according to Adam [9]. Both enzymatic methods are based on the coupling of ATP- or ADP-driven transphosphorylase reactions with dehydrogenase reactions. By the latter reactions NADH is converted into NAD, which was monitored with a Zeiss spectrophotometer at 340 nm. The adenylate energy charge was calculated as $(ATP + \frac{1}{2}ADP)/(ATP + ADP + AMP)$ [10].

Determination of adenylate kinase (AK) activity. In the *in vivo* experiments rats were treated during three consecutive days with either saline or 150 mg/kg Piracetam i.p., twice daily. On the fourth day the rats received only one injection and 30 min later they were decapitated. Cerebral hemispheres were dissected and homogenized in 10 vol of 0.32 M sucrose at 0°. One part of the homogenate was diluted 50-fold with 0.1% Triton-X100 in water (hypotonically) and another part was diluted 50-fold isotonically with 0.32 M sucrose. The AK activity was determined essentially according to Oliver [11] with the use of an automated Gilford spectrophotometer. In the *in vitro* experiments the rats were not pretreated, but various concentrations of Piracetam were added to the brain homogenates which had been diluted as described above. Diluted homogenates were then pre-incubated for 10 min at 37° before the determination of AK.

Table 1. Effects of Piracetam, Naftidrofuryl and methamphetamine on the contents of adenine nucleotides in rat brain

	ATP	ADP	AMP	Adenylate energy charge
Saline, brain extracted with PCA	2.6 ± 0.07*	0.47 ± 0.01†	0.12 ± 0.004‡	0.89 ± 0.003§
Saline, brain extracted with TCA	2.9 ± 0.12*	0.52 ± 0.01†	0.02 ± 0.002‡	0.92 ± 0.003§
Piracetam 150 mg/kg	3.1 ± 0.08	0.51 ± 0.02	0.02 ± 0.001	0.95 ± 0.023
Naftidrofuryl 25 mg/kg	2.8 ± 0.10	0.53 ± 0.02	0.02 ± 0.004	0.91 ± 0.004
Methamphetamine 2 mg/kg	3.0 ± 0.13	0.52 ± 0.02	0.02 ± 0.001	0.92 ± 0.004

For the determination of drug effects the nucleotides were extracted with TCA. Note that extraction of nucleotides from control brains with PCA yields very different results. Values are expressed as μmole/g wet wt. The adenylate energy charge is equal to (ATP + ½ADP)/(ATP + ADP + AMP). Means of 6 values ±S.E.M.

* Significantly different, $P_2 < 0.05$; † Significantly different, $P_2 < 0.01$; ‡,§: significantly different, $P_2 < 0.001$.

Materials. The chemicals used for the determination of adenine nucleotides (ATP u.v.-test and ADP/AMP u.v.-test) as well as those used for the determination of the AK activity were obtained from Boehringer, W. Germany. Methamphetamine was obtained from Burroughs Wellcome, G.B. Naftidrofuryl was a gift of Roussell Laboratoria, The Netherlands and Piracetam was a gift of the Union Chimique Belge, Belgium.

Statistics. Results were analyzed statistically using Students' *t*-test.

RESULTS

Cerebral adenine nucleotides. During the determination of ADP in extracts of rat brain it was found that the extracts made with PCA frequently contained substantial amounts of dehydrogenase activity. This activity manifested itself in a rapid conversion of added NADH into NAD in the absence of added enzymes. Since this endogenous activity would complicate the determination of adenine nucleotides, making the results unreliable, in further experiments 10% TCA was used for the extraction. No dehydrogenase activity was ever found in the TCA extracts. As can be seen from Table 1 the values for the adenine nucleotide contents and for the adenylate energy charge in TCA extracts are significantly different from those found in PCA extracts, those for ATP, ADP and adenylate energy charge being higher and that for AMP being very much lower. This shows that, in addition to the residual dehydrogenase activity, phosphatase activity was also present in the PCA extracts, an observation which is in agreement with that of Davison and Finn [12] on bacterial phosphatases.

The results in Table 1 indicate that neither Piracetam, nor Naftidrofuryl, nor methamphetamine affected the cerebral contents of ATP, ADP and AMP if measured under the right conditions. Accordingly, the adenylate energy charge remained unchanged.

Cerebral adenylate kinase activity. It was observed that rat brain homogenates diluted in isotonic sucrose showed a considerably lower adenylate kinase (AK) activity than hypotonically diluted homogenates in which AK enclosing membranes have been disrupted (Table 2). When the isotonically diluted homogenates were pre-incubated for 10 min at 37° with 40 mM Piracetam, the AK activity increased up to the hypotonic level. Substitution of Piracetam by an equivalent amount of sucrose did not result in such an increase, which excludes the possibility that the effect of Piracetam is due to a change in osmolarity. Piracetam had no effect on the AK activity in hypotonically diluted homogenates. The K_M of AK measured with ADP as a substrate was not changed by Piracetam. For control homogenates a K_M value of 0.48 ± 0.01 mM (n = 3) and for Piracetam treated homogenates a value of 0.50 ± 0.01 mM (n = 3) was observed. The stimulation of the AK activity in isotonically diluted homogenates by Piracetam was found to be dose-dependent, as is shown in Fig. 1.

If Piracetam was injected *in vivo* twice daily during three days, the activity of AK in isotonically diluted brain homogenates was significantly increased compared to that in brain homogenates of saline-treated rats (Table 2). No effect of Piracetam was seen in hypotonically diluted homogenates.

DISCUSSION

During our studies on the mechanism of action of Piracetam the possibility was envisaged that the acquisition-enhancing action of this drug might be based on an effect on the cerebral energy metabolism. If this were true it might have been expected that

Table 2. Effects of Piracetam on the activity of adenylate kinase in isotonically (iso) and hypotonically (hypo) diluted rat brain homogenates

	Hypo	Hypo + Piracetam	Iso	Iso + Piracetam
In vitro	3700 ± 110	3800 ± 130	2500 ± 90*	3600 ± 100*
In vivo	3800 ± 150	4100 ± 160	1900 ± 40*†	2100 ± 50†

Piracetam was either administered to the rats before decapitation (*in vivo*) or added to the homogenates of the brains of untreated rats which were then pre-incubated with the drug for 10 min at 37° (*in vitro*). Activity is expressed as μmoles of ATP formed per g wet wt per hr. Means of 6 values ±S.E.M.

* = significantly different, $P_2 < 0.001$; † = significantly different, $P_2 < 0.01$.

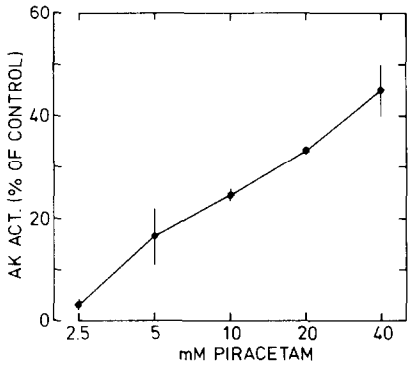

Fig. 1. Stimulation of the adenylate kinase activity in isotonically diluted rat brain homogenates by Piracetam as a function of dose.

another drug, Naftidrofuryl, which has been reported to stimulate cerebral energy metabolism [7] would also enhance acquisition. We were unable, however, to demonstrate any effect of Naftidrofurly on the rate of acquisition by rats in an Y-maze and in a drink-test system and found that another important behavioural parameter, i.e. spontaneous motor activity, was even depressed by this drug (unpublished observations). It seemed therefore useful to examine the effects of Piracetam and Naftidrofuryl on cerebral energy metabolism in more detail. Not only the adenine nucleotide contents of rat brain were measured, but also the adenylate energy charge was determined, a parameter which appears to be a more important determinant of the state of energy metabolism than the mere ATP content [10,13]. For comparison a third drug, methamphetamine, was included in the present experiments because of its well known behavioural effects. It enhances, like Piracetam, acquisiton and, unlike Piracetam, stimulates spontaneous motor activity [1].

Unexpectedly, the present results indicate that neither of the three drugs has any effect on the level of rat cerebral adenine nucleotides and, accordingly, on the cerebral adenylate energy charge. The discrepancy between the reported increase of the ATP content of mouse brain by Naftidrofuryl administration [7] and the present negative results may, of course, be due to the difference in animal species used. Another explanation, however, seems more likely. In the experiments with mice, the brain adenine nucleotides were extracted with PCA. The present experiments indicate that PCA extracts of rat brain may contain considerable amounts of dehydrogenase activity which interfere with the enzymatic determination of adenine nucleotides. Consequently, if PCA is used the ATP contents will erroneously be found too low. This may be an explanation for the low control values observed by Meynaud [7]. Since Naftidrofuryl is an inhibitor of lactate dehydrogenase [7] it might well be that the drug inhibited the interfering dehydrogenase activity in the PCA extracts of mouse brain resulting in an elevation of the observed ATP content up to a more correct, higher level.

Another point of discussion is the disagreement between the results of Gobert [5] and the present data. Apart from the fact that the control values of Gobert for cerebral adenine nucleotides differ from those normally stated in the literature [14,15], the finding of Gobert that Piracetam increases the ATP contents of rat brain was not confirmed by the present experiments. This discrepancy may be explained as follows. In the experiments of Gobert, the brains were frozen after the decapitation of the rats. This procedure renders the anoxic period during which large disturbances occur in the adenine nucleotide contents [14,15] unnecessarily long. In the present experiments the anoxic period was reduced by freezing the animals alive [14]. It may well be that during the prolonged anoxic period in the former study the protective effect of Piracetam against cerebral hypoxia (see below) resulted in a reduced decrease of the cerebral ATP content in the experimental rats compared to that in the controls.

The present results show that Piracetam enhances the activity of adenylate kinase, the enzyme which catalyzes the conversion of ADP into ATP and AMP and *vice versa*. Since the K_M of the enzyme is not affected by Piracetam, the state of equilibrium of the adenine nucleotides, which is normally maintained by the action of adenylate kinase [16], should remain unchanged by this drug, as was born out by the present experiments. The enhancement of the adenylate kinase activity might, however, offer an explanation for the protective effect of Piracetam against cerebral hypoxia. When hypoxia sets in, oxidative phosphorylation stops and the ATP content of the brain starts to decrease, whereas the AMP and, initially, the ADP contents increase [17]. In such a situation the decrease of the ATP content is slowed down by formation of ATP, catalyzed by adenylate kinase [17,18]. An increase of the adenylate kinase activity by Piracetam would accelerate this compensatory process and could thereby offer some protection against the effects of hypoxia. During the recovery from hypoxia oxidative phosphorylation is resumed. Since AMP which has accumulated during the period of hypoxia cannot enter the mitochondrion it first has to be converted into ADP by the adenylate kinase. This ADP then enters the mitochondrion where it is converted into ATP [17,18]. This process, too, might be accelerated by the increase of the adenylate kinase activity by Piracetam, which could account for the acceleration of post-hypoxic recovery caused by this drug [4].

The molecular basis of the increase of adenylate kinase activity by Piracetam is a subject of further study. Besides, the question whether the enhancement of acquisiton by Piracetam is based on a beneficial effect on disturbed cerebral energy metabolism during training [19,20] is presently under investigation.

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