Biochemical Pharmacology, Vol. 25, pp. 2237–2240. Pergamon Press, 1976. Printed in Great Britain.

PROTEIN METABOLISM IN THE RAT CEREBRAL CORTEX *IN VIVO* AND *IN VITRO* AS AFFECTED BY THE ACQUISITION-ENHANCING DRUG PIRACETAM

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(*Received* 24 *February* 1976; *accepted* 29 *April* 1976)

Abstract—The effect of Piracetam on rat cerebral protein metabolism *in vivo* and *in vitro* was studied. It was found that the drug stimulates the uptake of labelled leucine by cerebral cortex slices, has no effect on the incorporation of leucine into cerebral protein, neither in slices nor *in vivo*, but inhibits the breakdown of newly formed protein in slices.

In the literature conflicting opinions have been presented about the nature of the acquisition-enhancing effect of 2-pyrrolidon-*N*-acetamide (Piracetam®). Based on detailed behavioural and training studies in the rat, Wolthuis [1] presented evidence for an action primarily on the first phase of the information processing in the brain. These findings were supported by subsequent studies of the effect of Piracetam on visual evoked responses in the rat [2]. In contrast with this idea, Giurgea [3] concluded that Piracetam mainly improves consolidation. He based this conclusion on effects obtained with Piracetam together with metabolic blockers and amnesic agents in a spinal model of learning and in intact animals.

It is generally thought that the consolidation of new information in the brain is closely associated with the synthesis of cerebral proteins [4]. The finding by Platt *et al.* [5] that Piracetam enhanced the incorporation of labelled leucine into cerebral protein therefore lent strength to the conclusion of Giurgea [3]. Burnotte *et al.* [6], however, studing the effect of Piracetam on cerebral polysomal profiles in rats of various ages, found effects only in old rats. Since training studies are generally carried out with young or adults rats, these results do not support the idea that the acquisition-enhancing effect of Piracetam is based on an elevated protein synthesis. It seemed worthwhile, therefore, to re-examine the effects of Piracetam on the incorporation of labelled leucine in more detail.

MATERIALS AND METHODS

Male Small–Wistar (WAG) rats weighing 180–200 *g* were used.

Incorporation of labelled leucine in vivo. A stainless steel intracerebroventricular cannula was implanted stereotactically into anaesthesized rats. To allow recovery from the operation the animals were kept for 5–7 days, one to a cage. Thirty min before the intraventricular injection of 10 μCi of L-[5-^{3}H]leucine (1 mCi/ml) the rats received 150 mg/kg Piracetam subcutaneously and 150 mg/kg intraperitoneally. Control rats received saline (0.9% NaCl). In the first experiment rats were placed together after the injection of labelled leucine (leu). In the second experiment they were returned to their individual cages after the injection of [^{3}H]leu. At regular intervals after the injection of labelled leu rats were decapitated. Identical parts of both cerebral hemispheres were dissected with the aid of an Araldite mold, and subsequently homogenized in 14 vol of ice-cold 50 mM Tris–HCl buffer, pH 7.0, in a Teflon–glass homogenizer (clearance 0.25 mm).

Incorporation of labelled leu into protein in vitro. Slices of rat cerebral cortex were prepared according to McIlwain and Rodnight [7], stored in phosphate Krebs medium [8] at room temperature for 5–20 min and transferred into incubation vessels. They were pre-incubated for 30 min at 37° under continuous shaking in bicarbonate Krebs medium [8] containing 25 mM K^+ [9]. Unlabelled amino acids were added to the medium in the concentrations given by Ham [10]. During incubation the medium was equilibrated with 95% O_2 and 5% CO_2. After pre-incubation the medium was replaced by medium containing 1 μCi/ml [^{3}H]leu. The final concentration of leu was 0.05 mM. Twenty min later the slices were washed three times with unlabelled medium. At regular intervals the medium was refreshed, and slices were removed and homogenized in 2 ml of Krebs medium at 0°. Piracetam was continuously present at a concentration of 10 mM.

Uptake of [^{3}*H*]*leu* in vitro. Slices were prepared as described above. They were not stored but pre-incubated immediately in bicarbonate Krebs medium. After 30 min, [^{3}H]leu was added. Twenty min later the slices were washed and homogenized. Piracetam was continuously present at concentrations of 0, 0.1, 1 or 10 mM.

TCA precipitation. Homogenates were precipitated with 6.25% (w/v) trichloroacetic acid (TCA) containing 1 mM unlabelled leu. TCA-soluble and insoluble fractions were separated by centrifugation. After washing of the precipitate with ethanol and ether and solubilization in 0.2 N NaOH the protein content and the radioactivity were measured. After incubation of the precipitate with trypsine or pronase, more than 95 per cent of the TCA-insoluble radioactivity became TCA-soluble. The TCA-soluble fraction was separated into an amino acid fraction and a non-amino acid fraction by chromatography on Dowex 50 W X 12 as described by Schotman [11].

Determination of protein. Protein was determined following the method of Lowry *et al.* [12]. Crystallized bovine serum albumin was used as a standard.

Determination of radioactivity. Samples (usually 1–1.5 ml) were mixed with a scintillation cocktail (10–15 ml) of the following composition: 330 ml Triton-X100, 1000 ml toluene, 0.1 g 2,2′-*p*-phenylene-bis(4-methyl-5-diphenyloxazole) and 4 g 2,5-diphenyloxazole. Radioactivity was assayed in a Nuclear Chicago Mk II liquid scintillation counter. Corrections for quenching were made by the external standard-channel ratio method.

Materials. L-[5-^{3}H]leucine, sp. act. 58 Ci/m-mole, was obtained from the Radiochemical Centre, Amersham, G.B. and Piracetam was a gift from the Union Chimique Belge, Brussels, Belgium.

RESULTS

The uptake of [^{3}H]leu by rat cerebral cortex slices appeared to increase with the dose of Piracetam in a dose-dependent way (Fig. 1). Since differences in uptake of added labelled leu cause differences in the specific radioactivity of the leu available for incorporation into protein, incorporation values were expressed as relative specific radioactivities, i.e. the ratios of the radioactivity incorporated and the radioactivity initially taken up by the slices. In this way a correction is made for the influence of changes in the uptake of the labelled precursor on the amount of incorporated radioactivity. The incorporation of [^{3}H]leu in protein of rat cerebral cortex slices pre-incubated for 30 min is shown in Fig. 2. Thirty min after the addition of labelled leu the amount incorporated into TCA-insoluble material (which consisted for more than 95 per cent of protein) was equal in slices

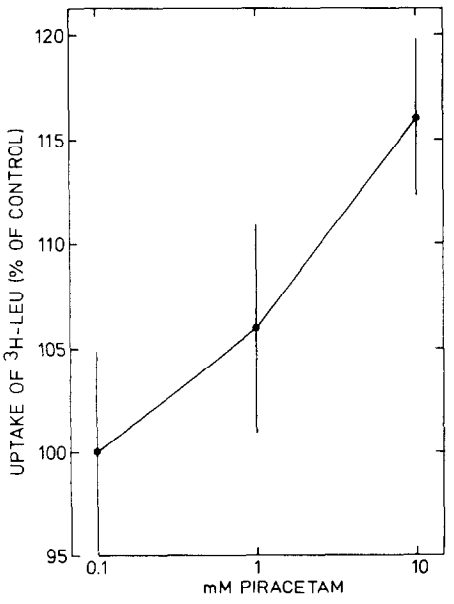

Fig. 1. The dose-dependent stimulation by Piracetam of the uptake of [^{3}H]leu by rat cerebral cortex slices *in vitro*. Values are expressed as percentages of the value found in the absence of Piracetam. Means of 6 values ± S.E.M.

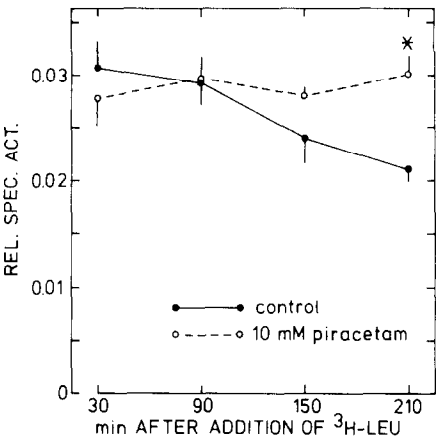

Fig. 2. The effect of Piracetam on the incorporation of [^{3}H] leu into protein in rat cerebral cortex slices *in vitro*. The relative sp. act. is the ratio of radioactivity incorporated and the amount of radioactivity initially taken up by the slices. X = significantly different from the control value, $P_2 < 0.05$. Means of 6 values ± S.E.M.

incubated in the presence and slices incubated in the absence of Piracetam. After prolonged incubation of the tissue the amount of incorporated radioactivity went down in the control slices but remained constant in the slices treated with Piracetam.

When the pre-incubation in the presence or the absence of Piracetam was extended to 180 min, again no effect of the drug on the incorporation of labelled leu could be demonstrated. The relative specific activity in the control slices was 0.014 ± 0.002 (n = 6) under these conditions and in slices treated with Piracetam 0.014 ± 0.001 (n = 6). Comparison of these data with those in Fig. 2 shows that the capacity of the tissue to incorporate labelled leu had declined as a result of the prolonged pre-incubation. In contrast, the uptake of added, labelled leu was not changed.

In Table 1 the fate of i.v. injected [^{3}H]leu in rat cerebral cortex *in vivo* is shown. Thirty min after the injection about 70 per cent of the total radioactivity was incorporated into protein. The relative specific activity, i.e. the ratio of incorporated radioactivity in the amino acid fraction, was about 3.3. Both the percentage of radioactivity incorporated and the relative specific activity increased during the subsequent period. This was mainly due to a decrease of the amount of total TCA-soluble radioactivity and that in the amino acid fraction since the amount of incorporated radioactivity remained fairly constant. Examination of the percentages of [^{3}H]leu incorporated and the relative specific activities shows that treatment with Piracetam had no effect on the incorporation of labelled leu into cerebral protein *in vivo*. In this series of experiments the animals were highly active during the period in which the labelled leu became incorporated, since they were placed together after a few days of isolation. In a second series of experiments the animals were kept quiet after the injection of labelled leu by placing them back in their individual home cage. Under the latter conditions the percentage of the radioactivity incorporated in the control rats after 30 min was significantly ($P_2 < 0.001$) higher than in the former experiments, i.e.

Table 1. Effect of Piracetam on the incorporation of intraventricularly injected [^{3}H]leu into cerebral cortex proteins *in vivo*

	n	Time after injection of [^{3}H]leu (min)	^{3}H incorporated (dpm)	Total ^{3}H in TCA-soluble fraction (amino acid + non-amino acid) (dpm)	% of total incorporated	RSA
Control	6	30	12000 ± 3300	1300 ± 1300	69 ± 2.0	3.4 ± 0.28
	6	90	9000 ± 1600	1900 ± 300	82 ± 1.2	7.4 ± 0.75
	6	150	10000 ± 1400	1200 ± 100	88 ± 1.2	13.6 ± 1.88
	7	210	8000 ± 1600	900 ± 100	89 ± 1.5	15.2 ± 1.74
Piracetam	7	30	14000 ± 3200	6200 ± 1600	70 ± 1.5	3.3 ± 0.17
	6	90	11000 ± 3000	2300 ± 500	81 ± 1.0	8.2 ± 0.57
	7	150	6300 ± 400	1000 ± 100	85 ± 0.9	13.4 ± 1.48
	7	210	7300 ± 1700	800 ± 100	89 ± 1.0	16.8 ± 2.13

The relative sp. act. (RSA) is the ratio of incorporated radioactivity and radioactivity in the amino acid part of the TCA-soluble fraction. n = number of animals. Means ± S.E.M.

81 ± 0.6 vs 69 ± 2.0, n = 6 in the previous experiments. Again, the value found for experimental rats simultaneously treated with Piracetam (81 ± 1.2%, n = 6) was not different from the control value. After 180 min an identical picture was obtained. The percentage of labelled leu which was converted into labelled non-amino acid metabolites 210 min after the application of [^{3}H]leu was about 10 in the *in vitro* experiments and about 50 in the *in vivo* experiments. Neither *in vitro*, nor *in vivo* Piracetam affected the metabolism of labelled leu.

DISCUSSION

The present results show that Piracetam does not affect, neither in highly activated, nor in quiet rats the incorporation of labelled leu into cerebral protein *in vivo*. This finding agrees with that of Burnotte *et al.* [6] who found that in young rats the polyribosome/ribosome ratio in the brain, which is indicative of the level of protein synthesis, is not changed by Piracetam. Moreover, since cerebral protein synthesis seems to be closely associated with the process of memory consolidation [4], this observation may be compatible with the view of Wolthuis [1] and Oglesby and Winter [13] that the enhancement of acquistition by Piracetam is not based upon an improvement of consolidation.

Also, *in vitro* the incorporation of labelled leu into cerebral protein appeared to be unaffected by Piracetam. On the other hand, it was found that prolonged incubation of control slices resulted in a decrease of the amount of labelled leu which had initially been incorporated, a phenomenon which may have been caused by breakdown of newly formed radioactive protein. This decrease was not observed when Piracetam was present during incubation. The observation that even after prolonged pre-incubation Piracetam had no effect on the incorporation of labelled leu makes it unlikely that there was a 'late' stimulatory effect of Piracetam on the rate of incorporation of labelled leu but leaves the possibility that Piracetam inhibited the breakdown of newly formed radioactive protein. This would be compatible with the observation of Platt *et al.* [14] that after treatment with Piracetam the activity of free lysosomal enzymes in rat brain, e.g. the proteinase Cathepsin D, is much lower than normal. It is not clear whether this effect is related with the enhancement of acquisition by Piracetam.

Similarly, the observed stimulation of the uptake of labelled leu is very difficult to relate to effects on acquisition, particularly since preliminary experiments indicate that the uptake of other amino acids like glutamic acid, glutamine, glycine, proline and γ-aminobutyric acid is not influenced by the drug. The stimulation of the uptake of labelled leu may however be responsible for the observation by Platt *et al.* [5] that Piracetam increased the incorporation of labelled leu into cerebral protein. These authors did not correct their values for differences in uptake of the labelled precursor by determining relative specific activities but based their conclusions simply on the amounts of radioactivity incorporated.

Acknowledgements—The authors are grateful to the Union Chimique Belge for the gift of Piracetam. M.E.R. Martens and A. Verkerk are gratefully acknowledged for their participation in this work during their training in biochemical pharmacology. This work was supported by the Foundation for Medical Research FUNGO which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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