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Physiological disposition of oral piracetam in Sprague-Dawley rats

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The distribution and fate of piracetam (2-oxo-1-pyrrolidine acetamide, Nootropil), the prototype 'nootropic drug', was examined in rats given 100–1000 mg kg^{-1} by gavage, with or without [^{3}H]piracetam as a tracer. Peak serum concentrations were attained after 60 min. Its half-life of disappearance from serum was about 2 h during the initial 8 h after administration and then about 6·4 h for the next 16 h. Brain piracetam concentrations equilibrated with those of serum at about 4 h, after which they fell exponentially but remained about twice those of serum; piracetam concentrations in the brainstem were lower (by 30–40%) than those in the cortex, olfactory bulb, and colliculi. No evidence could be obtained for significant piracetam metabolism, either in-vivo or when incubated with liver homogenates. No specific binding of [^{3}H]piracetam to any of various subcellular fractions was observed after its administration along with unlabelled carrier. Repeated daily doses of piracetam (7 days, 100 mg kg^{-1}) failed to elevate serum or brain concentrations beyond those observed after a single dose.

Little is known about the biochemical mechanism through which piracetam (2-oxo-1-pyrrolidine acetamide. Nootropil), a 'nootropic' drug used to treat memory impairment due to cerebral ischaemia (Herrschaft 1978), alcoholism (Dencker et al 1978; Marks 1977), senile dementia or Alzheimer's disease (Stegink 1972; Friedman 1981) modifies brain functions. The compound reportedly enhances brain energy metabolism (Nickolson & Wolthius 1976; Woelk & Peiler-Ichikawa 1978), hippocampal acetylcholine release (Wurtman et al 1981) and the firing of the locus coeruleus neurons (Olpe & Steinmann 1981). Some reports have claimed that piracetam becomes concentrated in brain tissue (Gobert 1972) [especially rat cortex (Ostrowsky et al 1975)] with respect to plasma values; however, relatively few data are available on its distribution or fate in animals. The piracetam doses used clinically tend to be large (2–6 g day^{-1}), suggesting that its effect results either from the delivery of small proportions of administered material to active sites in the brain, or from piracetam's metabolism to an active compound via a minor pathway. This report explores the fate of large, oral piracetam doses in rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats housed 4 per cage had free access to food (Charles River Laboratories; RMH 3000) and water, and were kept under light (Vita Lite, Duro-Test Co., North Bergen, NJ) between 7 am and 7 pm. Piracetam was given orally by gavage (100 mg ml^{-1}) at 9 am, and food was withheld thereafter until the rats were killed. In some experiments [^{3}H]piracetam (10 mCi mg^{-1}, UCB, Brussels, Belgium) was added to obtain a specific activity of 1 and 100 μCi mg^{-1}. Unlabelled piracetam was assayed by gas chromatography (Hesse & Schultz 1979) after its extraction from tissue (200–400 mg) of serum (200 μl) into 4 ml of 0·15 M formic acid/acetone, containing 50 μg of 2-oxo-1-pyrrolidine propionamide (PPA) as a reference compound. After centrifugation, an aliquot (3·5 ml) of the supernatant fluid was evaporated to dryness, resuspended in 1 ml of water, and washed with hexane; the hexane phase was then discarded and the water evaporated to dryness. Samples from serum were then redissolved in 50 μl of water, of which 1–2 μl portions were injected into the chromatographic apparatus. Samples from brain and liver were further purified on tlc (LK6D silica gel plates, Whatman, n-propanol–acetic acid–water, 80 : 10 : 10); the drug was then eluted with methanol, which was evaporated to dryness. After resuspension with water, samples were treated as for serum.

Chromatography was with a Packard 430 with an electronic integrator, Tenax 60–80 column (1·9 m long, i.d. 2 mm); temperatures of oven, injector and detector (FID) were 220, 280, 280 °C, respectively. Carrier gas: nitrogen, flux 20 ml min^{-1}.

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Samples of serum and brain from untreated animals, to which piracetam and PPA were added in ratios of 0·25:1, 0·5:1, 1:1, 2:1, were run as external standards through all the experiments. Data from the resulting standard curve were used to normalize those obtained (from serum or tissue samples) by measuring the areas under piracetam curves, and the concentration of piracetam in each sample was calculated by the peak area ratio technique, using PPA as an internal standard. Recoveries, examined by adding [^{3}H]piracetam to serum and tissue extracts, were found to be 93 ± 2% for serum and 84 ± 2% for brain. Tissue, serum, and homogenate extracts from rats that had received [^{3}H]piracetam were extracted with formic acid/acetone as described above; 2·5 ml of the acetone phase was then evaporated to dryness and redissolved in water to which Aquasol had been added, after which the radioactivity was counted directly. To affirm the purity of the radioactive material, 0·025 μCi of stock solution was run on the tlc using the solvent mixture described above. Six bands of silica gel (1 × 2 cm), running from the origin to the front, were scraped into scintillation vials to which methanol (1 ml) and Betafluor had been added: the radioactivity was found only in the area corresponding to the retention factor (Rf) of unlabelled piracetam run as a standard. The subcellular fractionations of rat liver and brain homogenates were performed as described by Hogeboom (1955) and Dodd et al (1981) respectively.

RESULTS

Among rats decapitated 0·5, 1, 2, 4, 6 or 8 h after receiving unlabelled piracetam (1 g kg^{-1}) by gavage, peak drug values were reached in serum after 1 h and in brain after 4 h (Table 1); 8 h after treatment brain concentrations were approximately twice those in plasma. The $t_{\frac{1}{2}}$ of piracetam's disappearance from serum, obtained by plotting the data, was 120 min.

Table 1. Piracetam concentrations in rat serum and brain. Groups of 4 animals were killed at the times indicated after receiving 1 g kg^{-1} of unlabelled piracetam orally. Data in this and following Tables are given as mean ± s.e.m.

Time (min)	Serum (μg ml^{-1})	Brain (μg g^{-1})
30	455 ± 16	56 ± 2
60	535 ± 17	113 ± 1
120	457 ± 4	171 ± 7
240	340 ± 29	368 ± 4
360	175 ± 12	208 ± 5
480	75 ± 12	176 ± 3

To determine whether the piracetam was metabolized in-vivo, the radioactive compounds in sera and brains of rats receiving [^{3}H]piracetam 4 h earlier (100 mg kg^{-1} by gavage; sp. act. 1 μCi mg^{-1}) were fractionated as described in Methods. At least 98% of the radioactivity was found in a spot corresponding to the reference standard; this was the only locus with radioactivity greater than background. When [^{3}H]piracetam (1·5 × 10^{-5} mol litre^{-1}, sp. act. 1 μCi μmol^{-1}) was incubated with a 5% liver homogenate in 0·05 mol litre^{-1} Tris buffer, pH 8·8, with or without added *S*-adenosylmethionine (10^{-4} mol litre^{-1}) for 60 min at 37 °C, radioactivity remained associated with unchanged piracetam (Table 2).

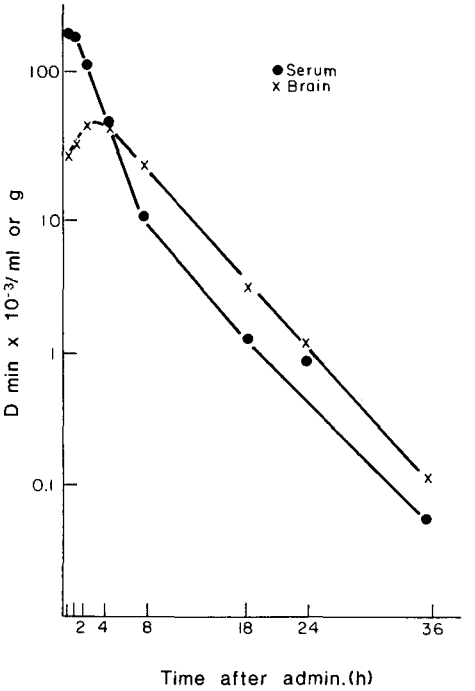

FIG. 1. Disappearance of [^{3}H]piracetam from rat serum and brain. Groups of 4 animals were killed at the times indicated after receiving [^{3}H]piracetam (100 mg kg^{-1}, 100 μCi kg^{-1}) by gavage. Unchanged [^{3}H]piracetam, representing almost all of the radioactivity, was extracted into formic acid/acetone, evaporated to dryness and counted. Each point represents the mean of 4 samples. The best-fit equation for the serum curve was obtained by nonlinear least square methods: this was

$$y = 4496\, e^{-0.108x} + 314\,099\, e^{-0.39x}$$

The calculated half-life of disappearance was 1·8 h, for the initial 8 h, and 6·4 h thereafter. The initial half-life did not differ from that obtained in a study using unlabelled piracetam (Table 1). The disappearance of [^{3}H]piracetam from brain (8–36 h) after its administration paralleled that from serum.

Table 2. Recovery of [^{3}H]piracetam after incubation with liver homogenates. [^{3}H]piracetam ($1{\cdot}5 \times 10^{-5}$ M; 1 μCi mol^{-1}) was incubated with liver homogenates (2–4 mg protein) with and without *S*-adenosylmethionine (SAM), 10^{-4} M, as described in the text, after which the identity of the radioactivity as unchanged piracetam was confirmed by solvent chromatography followed by thin-layer chromatography (tlc) of the aqueous phases. (Numbers 1–6 indicate particular bands of silica gel scraped from the tlc plates and counted.)

			[^{3}H]Piracetam (d min^{-1} mg^{-1} liver)									
		Time of incubation		After washing			After tlc					
Piracetam	SAM	(min)	Initial	After acetone	Hexane phase	Aqueous phase	1	2	3	4	5	6
+	−	0	11 500	10 400 ± 230	50 ± 7	10 000 ± 360	18	12	18	81	10 950	43
+	−	60	11 500	10 660 ± 300	75 ± 7	8 100 ± 390	12	12	20	129	10 866	92
+	+	0	11 700	10 100 ± 80	61 ± 6	10 200 ± 340	11	11	15	89	9 904	68
+	+	60	11 700	10 700 ± 80	60 ± 5	10 600 ± 230	14	11	16	103	10 144	93

Table 3. Regional distribution of piracetam in brain. Brains were obtained from 6 animals killed 4 h after receiving piracetam (500 mg kg^{-1}) orally; tissues from 2 animals were pooled for assay.

	Piracetam μg g^{-1}
Olfactory bulb	56·8 ± 1·3
Colliculi	54·2 ± 1·3
Cortex	52·0 ± 5·0
Hypothalamus	51·2 ± 5·0
Hippocampus	49·4 ± 0·5
Striatum	46·7 ± 0·3
Cerebellum	42·0 ± 2·4
Thalamus	39·8 ± 2·0
Medulla	36·1 ± 1·1
Pons	31·1 ± 4·0

In another experiment designed to follow piracetam's time-course of disappearance for longer periods, animals were given 100 mg kg^{-1} (100 μCi kg^{-1}) of [^{3}H]piracetam and killed at intervals from 0·5–36 h; food was withheld for 36 h before death. Peak serum values were attained about 60 min after administration; thereafter drug concentrations dropped exponentially, with a $t_{\frac{1}{2}}$ of 1·8 h for the initial 8 h (similarly as in the previous experiment using unlabelled drug) and a $t_{\frac{1}{2}}$ of 6·4 h thereafter. Brain and serum piracetam concentrations equilibrated in about 4 h; brain values then decreased exponentially, remaining approximately twice those of serum. Virtually all the radioactivity continued to be associated with unchanged piracetam (Fig. 1).

The regional distribution of piracetam in the rat brain was examined in animals given the unlabelled drug (500 mg kg^{-1}, by gavage) 4 h previously. Areas from two brains were pooled and assayed. Highest values were found in the olfactory bulb, the colliculi, the hypothalamus, and cortical structures; lowest values were in the brainstem (Table 3).

The subcellular distribution of piracetam was examined in brains obtained from rats 2 h after [^{3}H]piracetam (1 and 100 mg kg^{-1}; 100 μCi kg^{-1}). Samples from fractions containing 2–4 mg protein were extracted as described above; their radioactivity was measured and compared with that present in samples of total homogenate. (To control for non-specific absorption of the compound, we assessed its subcellular distribution after adding 10 μg/0·1 μCi ml^{-1} to 10% brain homogenates prepared from untreated rats.) About 87% of the drug was recovered from the cytosol; when calculated per mg protein, cytosol concentrations were 4-fold those in total homogenate (1249 ± 139 vs 316 ± 37 d min^{-1} mg^{-1} of proteins); a similar distribution was seen with animals receiving [^{3}H]piracetam diluted 100-fold with unlabelled piracetam. The pattern of subcellular distribution observed in liver extracts resembled that observed in brain, i.e. most of the radioactivity remained in the cytoplasm (Table 4).

To determine whether piracetam accumulated in the brain after its repeated administration, we treated rats with the drug (100 mg kg^{-1} day^{-1} by gavage) for 7 days. On the last day rats also received 100 μCi kg^{-1} of [^{3}H]piracetam and were killed 2 or 24 h later. Other animals, treated in parallel, received only a single dose of the mixed unlabelled and labelled drug. There were no differences in tissue values after single or multiple doses (Table 5).

DISCUSSION

These studies affirm that piracetam is able to enter the brain from the serum (Table 1) and that it becomes more concentrated in some brain regions, including the cortex and hippocampus (Table 3), than in others. However, the data fail to provide an explanation of why relatively large doses of the drug are required clinically. No evidence could be found for the formation of a piracetam metabolite that might be mediating its effects (Table 2), nor for the selective binding of the unchanged drug to a particular subcellular organelle (Table 4). The stability of

Table 4. Subcellular distribution of piracetam in rat liver and brain.

Dose mg kg^{-1}	Specific activity μCi mg^{-1}	Time after treatment (min)	[^{3}H]Piracetam as % of total radioactivity in homogenate					
			Nuclei	Mitochondria	Microsomes	Synaptosomes	Myelin	Cytosol
Liver								
1	100	60	2·6 ± 0·1	2·2 ± 0·1	2·2 ± 0·4	—	—	92·2 ± 0·7
1	100	240	2·8 ± 0·2	2·6 ± 0·2	4·6 ± 0·5	—	—	89·8 ± 0·6
Brain								
1	100	120	3·5 ± 0·5	2·2 ± 0·3	1·7 ± 0·1	2·7 ± 0·3	2·3 ± 0·3	87·6 ± 0·8
100	1	120	3·3 ± 0·4	3·1 ± 0·1	1·6 ± 0·1	2·8 ± 0·4	1·8 ± 0·2	87·3 ± 0·5
Brain homogenate*			3·90	0·001	0·77	0·002	0·007	95·3

Groups of 3 animals were killed after receiving 1 or 100 mg kg^{-1} of piracetam, containing 100 μCi kg^{-1}, orally.
* In studies on the non-specific binding of [^{3}H]piracetam, 10 μg ml^{-1} containing 0·1 μCi ml^{-1} was added to 1 ml samples of 10% brain homogenates.

Table 5. Serum and brain piracetam concentrations 2 or 24 h after rats received single doses, or the last of 7 daily doses. Animals received 100 mg kg^{-1} of piracetam per day, supplemented on the last day with 100 μCi kg^{-1} of isotopically labelled drug.

Days of treatment	Hours post treatment	Serum		Brain	
		d min^{-1} ml^{-1}	μg ml^{-1}	d min^{-1} g^{-1}	μg g^{-1}
1	2	104 000 ± 12 000	49 ± 7	26 000 ± 2600	20 ± 6
7	2	107 000 ± 20 000	58 ± 10	27 000 ± 300	18 ± 2
1	24	1300 ± 50	<0·1	1500 ± 50	<0·1
7	24	1300 ± 80	<0·1	1400 ± 110	<0·1

the dose of piracetam used to attain desired behavioural or neurological effects is compatible with the observation that brain concentrations are not greater several hours after the seventh daily dose than at a similar interval after a single dose (Table 5).

The disappearance of piracetam from the serum, estimated using isotopically labelled or unlabelled drug, followed a double-exponential curve compatible with a two-compartment model, i.e. a central compartment, including serum and tissues into which the drug diffuses easily, with a volume of distribution like that of body water (0·69 litre kg^{-1}) and an elimination constant of 0·39 h^{-1}, and another compartment with a lower diffusion rate. Brain might be a part of the second compartment. Since piracetam has a chloroform–water partition coefficient of 0·04 it is likely that it meets some difficulty in crossing the blood-brain barrier; this would explain the relatively long period that is required for it to equilibrate with serum (4 h) and its lower elimination rate (compared with that of serum) thereafter. The relatively high polarity of the compound is compatible with its accumulation in the cytosols of brain and liver, instead of in subcellular organelles. When we tested the possibility that piracetam might bind to soluble proteins in brain cells, we were unable to detect such specific binding (data not shown).

Our data fail to provide an explanation for the unusually high piracetam dose apparently needed for clinical effects, nor do they favour a particular biochemical mechanism underlying those effects.

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