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J. Pharm. Pharmacol. 1988, 40: 584-585
Communicated March 29, 1988

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Brain penetration of orally administered sodium pyroglutamate

J. L. CHANAL, M. AUDRAN, M. T. SICARD, M. BRILEY*, *Faculty of Pharmacy, University of Montpellier, Avenue Charles Flahault, 34060 Montpellier, *Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81106 Castres, France*

Abstract—The absorption and brain penetration of [^{3}H]pyroglutamate was determined after oral administration to rats. Gas-liquid chromatography of the methylated derivatives followed by mass fragmentometry was used to analyse the plasma and brain levels of pyroglutamate. [^{3}H]Pyroglutamate was separated from other labelled compounds by thin layer chromatography. The administration of 500 mg kg^{-1} [^{3}H]pyroglutamate resulted in a 30-fold increase in plasma levels and a doubling in the brain levels. Over 60% of the cerebral radioactivity was present as [^{3}H]pyroglutamate demonstrating that pyroglutamate is not only well absorbed but also penetrates in significant amounts into the brain.

Pyroglutamic acid (5-oxo-l-proline, 2-pyrrolidone-5-carboxylic acid) is found as the free amino acid in a number of tissues and body fluids (Wilk & Orlowski 1975) and as the *N*-terminal amino acid of various biological active peptides. In addition to its transformation to glutamate by 5-oxo-prolinase, pyroglutamate has intrinsic activity at central glutamate receptors (Dusticier et al 1985) as an agonist (Rieke et al 1984; Antonelli et al 1984) although some antagonist activities have also been reported (Van Harreveld & Fifkova 1971; Continho-Netto et al 1981). A recent report also suggests that it may improve performance in rats in a passive avoidance memory retention task (Drago et al 1987).

Oral administration of pyroglutamate to rats has been shown to increase the endogenous levels of brain pyroglutamate and glutamate (Caccia et al 1982), although whether this was by accumulation from the plasma or an indirect stimulation of the synthesis of glutamate and subsequently pyroglutamate in the brain was not demonstrated. In view of the increasing interest in excitatory amino acids (for recent review see Trends in Neurosciences Special Issue 1987), pyroglutamate could prove to be a useful research tool as one of the fairly rare orally active glutamate receptor agonists.

We demonstrate here that orally administered [^{3}H]pyroglutamate penetrates freely into the brain where a substantial proportion of it remains for several hours as the unaltered drug and that it is subsequently metabolized to glutamate.

Correspondence to: M. Briley, Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81106 Castres, France.

Materials and methods

Materials. [^{3}H]Pyroglutamate, obtained from the Commission d'Energie Atomique (Saclay, France), had a specific activity of 85 Ci mmol^{-1}. *N*-Ethyl pyroglutamate and sodium pyroglutamate was synthesized by Pierre Fabre Research Centre, Castres, France.

Drug administration and sampling. Male Wistar rats (200-220 g), fasted for 18 h with free access to water, were administered orally sodium pyroglutamate (500 mg kg^{-1}) containing sodium [^{3}H]pyroglutamate (20 μCi/rat) as a suspension in 5% aqueous gum arabic.

Animals were killed by exsanguination through cardiac puncture 15, 30, 60, 90, 120, 240 and 480 min after drug administration. The plasma was recovered by centrifugation for 10 min at 3500 rev min^{-1} and the brains rapidly removed, frozen, and stored at -30°C.

Extraction and gas-liquid chromatography/mass spectrometric analysis. The frozen brains were homogenized in 80% aqueous ethanol (0·1 g tissue mL^{-1}) and the radioactivity of an aliquot equivalent to approximately 100 μg of brain determined in a Packard TriCarb 3255 scintillation counter. Aliquots (0·1 mL) of plasma were similarly extracted with 0·9 mL 80% aqueous ethanol. To 1 mL of each of the alcoholic suspensions of brain and plasma was added 0·1 mL of a solution of 80 μg mL^{-1} sodium *N*-ethylpyroglutamate as an internal standard for mass fragmentometry.

Ethanolic extracts of two brain samples and two plasma samples were left to stand for 30 min at 4°C before centrifuging at 3000 rev min^{-1} for 5 min. The supernatants were treated as described by Marstein et al (1973) namely, acidification to pH 1·5-2 with 6 M HCl, passage through a 0·7 × 6 cm Dowex 50W X-4 column to remove the free amino acids and amines, elution with 4 mL water-methanol (1:3 v/v) and finally lyophilization. The lyophilized extracts were methylated by the addition of 0·2 mL methanolic hydrochloric acid (prepared by adding 1 mL acetyl chlorate dropwise to 4 mL methanol). After 5 min at room temperature, the samples were evaporated to dryness.

The methyl ester residues were taken up in 100 μL ethanol and

1 μL aliquots injected onto an LKB 2091 gas chromatograph-mass spectrometer. Gas chromatography was carried out using a 25 meter capillary column of fused silica, OV 17, at a temperature of 200°C. Under these conditions the retention time for the internal standard was 1'34" and for methyl pyroglutamate, 1'4". The ions used for analysis were the molecular peaks at 143 m/z for methyl pyroglutamate and at 171 m/z for the internal standard. All analyses were carried out in duplicate. The standard curve using spiked plasma samples prepared as described above was linear from 5 to 100 μg mL^{-1}.

Thin layer chromatography. Approximately 10 μL of the final ethanolic solution of the methylated brain extracts were chromatographed on thin layer silica plates in two solvent systems:

1) methylethylketone–propionic acid–water (75:25:25 v/v/v).
2) butanol–acetic acid–water (6:2:2 v/v/v) to separate *N*-methyl [^{3}H]pyroglutamate from the other radioactive compounds present. The proportion of radioactive pyroglutamate compared with the total pool of radioactivity was thus determined.

Results and discussion

Four groups of eight animals were used. In each group one animal was used as a control. The other animals received the drug and were killed at different times. The mass fragmentometry results for the plasma and the brain are shown in Table 1 and the brain radioactivity levels in Table 2. These results are expressed, for the radioactivity data, as per cent of the administered dose per g of brain represented by the area of the chromatogram corresponding to methyl [^{3}H]pyroglutamate. The mass fragmentometry data are expressed as μg pyroglutamate per mL of plasma or g of brain.

Table 1. Plasma and whole brain levels of pyroglutamate after oral administration of 500 mg kg^{-1} pyroglutamate to rats.

Time after admin. (min)	Plasma μg mL^{-1} ± s.e.m.	Brain μg g^{-1} ± s.e.m.
0	1·6±0·4	33·4±9·8
15	3·2±0·7	34·0±9·4
30	7·3±6·5	35·4±9·8
60	36·8±23·0*	41·4±10·8
90	45·1±18·2***	54·1±10·6*
120	49·3±26·4**	66·2±14·1***
240	27·2±10·9***	58·6±7·8***
480	3·3±2·5	41·7±4·7

The values presented are the mean of four animals.
*$P<0·05$; **$P<0·02$; ***$P<0·001$ compared to predose levels using Student's *t*-test

Table 2. Brain levels of total tritium and [^{3}H]pyroglutamate after oral administration to rats of 500 mg kg^{-1} [^{3}H]pyroglutamate (20 μCi/animal).

Time after admin. (min)	Total tritium (% of admin. dose (g brain)$^{-1}$)	[^{3}H]pyroglutamate (% admin. dose (g brain)$^{-1}$)	% unchanged [^{3}H]pyroglutamate
0	—	—	—
15	0·083±0·038	0·044±0·021	53·0
30	0·109±0·050	0·061±0·034	56·0
60	0·177±0·087	0·109±0·083	61·6
90	0·204±0·084	0·129±0·070	63·2
120	0·255±0·082	0·161±0·079	63·1
240	0·270±0·055	0·159±0·043	58·9
480	0·236±0·047	0·111±0·020	47·0

The values are the means±s.e.m. of four animals at each time point.

The endogenous levels of pyroglutamate before drug administration found in the brain by mass fragmentometry are similar to those reported previously by Caccia et al (1982). The plasma levels are, however, lower.

Plasma levels of pyroglutamate increased about 45 μg mL^{-1} (30-fold) with maximum values being obtained 90 to 120 min after the administration. Brain levels were also significantly increased, the concentrations found at 120 min after administration being more than 30 μg g^{-1} above the endogenous level (100% increase).

Brain levels of total radioactivity showed a similar kinetic profile with maximal levels being obtained between 2 and 4 h after drug administration. Brain levels of [^{3}H]pyroglutamate essentially followed those of the total brain radioactivity. More than 60% of the radioactivity was present as [^{3}H]pyroglutamate for up to 4 h after drug administration.

Interestingly, the percent of unchanged [^{3}H]pyroglutamate tends to increase with brain levels. If a substantial part of the degradation of [^{3}H]pyroglutamate takes place in the brain, probably by conversion to glutamate by 5-oxo-prolinase, the above result could be the consequence of a saturation of this enzyme which becomes rate limiting at high brain levels of pyroglutamate.

Thus, orally administered pyroglutamate is well absorbed to give maximum plasma levels after 90 to 120 min. Brain levels increase in parallel to twice the endogenous levels. More than 60% of the pyroglutamate that penetrates into the brain remains as the unchanged compound for at least 2 to 4 h.

Pyroglutamate should thus be considered not only as a means of increasing cerebral glutamate but as an orally active excitatory amino acid. Further investigations of this potentially useful research tool are currently under way in our laboratories.

The authors thank M. Doucet for technical assistance and the Mass Spectrometer Center of Montpellier I University.

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