Different Behaviour of ^{3}H-Glycine and ^{14}C-Glycine in Studies of Mammalian Tissue Metabolism

In investigations of purine nucleotide synthesis de novo, using radioactive glycine[1,2], the time dependent changes of the intracellular glycine pool had to be determined. In order to obtain these data in a simple way the application of a double labelling method[3,4] (simultaneous use of ^{3}H- and ^{14}C-glycine in each experiment) seemed to be most promising, provided glycine metabolism does not result in a different distribution of radioactivity from ^{3}H- and ^{14}C-glycine. To our knowledge a comparison of the distribution of radioactivity from ^{3}H- and ^{14}C-glycine has not been made as yet, though tritium labelled glycine was already applied in investigations of glycine metabolism[5] and protein synthesis[6–11].

Sprague Dawley rats (200–220 g) fasted for 12 h were i.v. injected (tail vein) with 0.5 ml sodium chloride (0.9% w/v) containing 150 µCi 2-^{3}H-glycine (specific activity 500 mCi/mM) and 50 µCi 2-^{14}C-glycine (specific activity 21.8 mCi/mM) or 50 µCi 1-^{14}C-glycine (specific activity 41.4 mCi/mM), respectively. 1 h after the injection kidney, liver, brain, heart and skeletal muscle were rapidly removed and immediately frozen in liquid nitrogen. In vitro studies were performed with kidney slices (0.2 mm thickness) prepared and incubated for 1 h as described elsewhere[2]. The incubation medium (glycine concentration 0.25 mM) contained 25 µCi 2-^{3}H-glycine as well as 8 µCi 1-^{14}C-glycine.

The frozen tissues were ground under liquid nitrogen and extracted with 0.3N $HClO_4$ for 5 min at 0 °C. After centrifugation and neutralization (3N KOH) aliquots of the extracts (0.25 ml) were placed on columns (Spherix XX 907-10-OPKU, Phoenix Precision Instrument Company, Philadelphia). Elution from the columns was carried out according to the glycine separation procedure of Shih et al.[12]. The eluates were collected in 1 and 5 ml portions, respectively. 3 radioactive fractions were eluted with citrate buffer: Fraction I between 1 and 15 ml, fraction II (serine) between 40 and 50 ml, fraction III (glycine) between 65 and 80 ml. The remaining radioactivity was eluted as fraction IV with 30 ml NaOH.

The radioactivity was determined in a Packard Tricarb scintillation spectrometer using Triton scintillator[13]. The simultaneously counted ^{3}H- and ^{14}C-activities were calculated according to the method of Koch[14].

Data regarding the ^{3}H- and ^{14}C-activity in various organs after application of ^{3}H- and ^{14}C-glycine are summarized in Table I. Obviously the extract activity ratios ^{3}H:^{14}C as well as the distribution pattern of ^{3}H- and ^{14}C-activities differ from organ to organ, thus indicating differences in the organ specific rate of glycine utilization and conversion. Most probably the smaller ^{14}C-extract activity in experiment A results from a loss of radioactivity from 1-^{14}C-glycine due to its decarboxylation. The most remarkable result concerns the extraordinary shift of ^{3}H-activity from the glycine fraction to fraction I in all organs analyzed. Since it is known that the α-hydrogen atoms of the glycine molecules become liberated as water during the rapid interconversion of glycine and serine[15,16] one could already suppose the high ^{3}H-

Table I. Distribution of extract radioactivity (dpm/g wet weight) between glycine, serine and two other fractions in different rat organs in vivo after i.v. application of 2-^{3}H-glycine and 1-^{14}C-glycine (experiment A) and 2-^{3}H-glycine and 2-^{14}C-glycine (experiment B)

		Experiment A		Experiment B	
		^{3}H	^{14}C	^{3}H	^{14}C
Kidney					
Extract activity	dpm/g	1,700,000	644,000	1,440,000	1,210,000
Fraction I	%	73	17	70	14
Fraction II (serine)	%	4	10	4	12
Fraction III (glycine)	%	8	33	8	38
Fraction IV	%	15	40	18	36
Liver					
Extract activity	dpm/g	597,000	366,000	453,000	630,000
Fraction I	%	92	17	82	10
Fraction II (serine)	%	1	15	6	28
Fraction III (glycine)	%	1	19	7	26
Fraction IV	%	6	49	5	35
Brain					
Extract activity	dpm/g	1,270,000	52,000	1,000,000	226,000
Fraction I	%	97	31	97	34
Fraction II (serine)	%	1	21	1	30
Fraction III (glycine)	%	1	39	1	25
Fraction IV	%	1	9	1	11
Heart					
Extract activity	dpm/g	832,000	98,000	865,000	246,000
Fraction I	%	91	20	92	23
Fraction II (serine)	%	2	27	2	38
Fraction III (glycine)	%	4	38	4	24
Fraction IV	%	3	12	2	18
Skeletal muscle					
Extract activity	dpm/g	745,000	130,000	1,010,000	324,000
Fraction I	%	75	7	72	7
Fraction II (serine)	%	2	18	3	24
Fraction III (glycine)	%	21	62	24	67
Fraction IV	%	2	3	1	2

activity in fraction I to be due to tritium water originating during glycine metabolism. Actually the radioactivities present in water distillates from liver and brain extracts were found to be almost the same as those in the respective fractions I (92% and 97%).

The shift of ^{3}H-activity from glycine to fraction I occurs also in kidney slices, though to a lesser extent (Table II). This can be explained by taking into consideration that tritium water originating in the slices exchanges with the excess of water of the incubation medium. The data concerning the distribution of radioactivity of the medium before and at the end of the incubation are in favour of this view.

From all these findings it is evident that the use of ^{3}H-labelled glycine seems not to be advisable in studies of glycine metabolism and in investigations concerning the role of glycine as a precursor substance[17].

Table II. ^{3}H- and ^{14}C-activity of medium (dpm/ml) before and after the incubation of kidney slices and percentage distribution of extract radioactivity (dpm/g wet weight) between glycine, serine and two other fractions

		^{3}H	^{14}C
Activity of medium prior to incubation		4,230,000	1,290,000
Activity of medium after incubation		4,000,000	880,000
Extract activity	dpm/g	9,270,000	4,046,000
Fraction I	%	36	13
Fraction II (serine)	%	9	14
Fraction III (glycine)	%	37	43
Fraction IV	%	19	29

Zusammenfassung. Die Radioaktivitätsverteilung von ^{3}H und ^{14}C nach Gabe von ^{3}H- und ^{14}C-Glycin weist sowohl in vivo als auch in vitro erhebliche Unterschiede auf. Unsere Resultate zeigen, dass die Aufnahme von Glycin und seine metabolischen Umsetzungen nicht mit 2-^{3}H-Glycin bestimmt werden können.

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Conformations of Amino Acids Calculated from Molecular Orbital Theory

The forces that govern the folding of a polypeptide chain in aqueous solution are poorly understood, although it has been suggested that the major influence may be conformation of individual amino acid residues with interaction between adjacent residues being of lesser importance[1]. We have used a quantum mechanical method developed by HOFFMANN[2] (extended Hückel molecular orbital) to calculate the energetically preferred conformation of amino acid residues and reported results for glycine, alanine, phenylalanine, and proline which agree with experimental data[3]. Previous calculations of minimum energy conformation of small molecules[4–6] using this method have in general correlated well with experimental data. A report of calculations of 2 amino acid residues using an extended Hückel approach has recently appeared[7], and here we report the results of calculations of 4 additional amino acid residues.

Methods. The N-acetyl amides of amino acids were the molecules considered as these simulate peptide bonds at each end of the amino acid residue. The bond lengths and angles of the backbone atoms were those of PAULING and COREY[8] and of the side chain were taken from X-ray crystallographic data compiled by SCHERAGA[9]. The molecular orbital parameters were those previously used[3]. The energy was calculated as a function of varying geometry of all freely rotating bonds until the energy minimum was obtained. Angles near the ϕ, minimum were refined to $\pm 15°$. The calculated minimum energy conformations (using the Ψ convention of EDSALL et al.[10]) of leucine, isoleucine, valine, and serine are shown in the Table along with secondary conformations having energies within 1 kcal/mole.

The ϕ, Ψ values of the calculated minimum energy conformations of amino acid residues can be compared