METHIONINE AND THYROID HORMONE EFFECTS ON $^{14}CO_2$ EXHALATION FROM [*DIMETHYLAMINO*-^{14}C]AMINOPYRINE IN INTACT PHENOBARBITAL-PRETREATED RATS

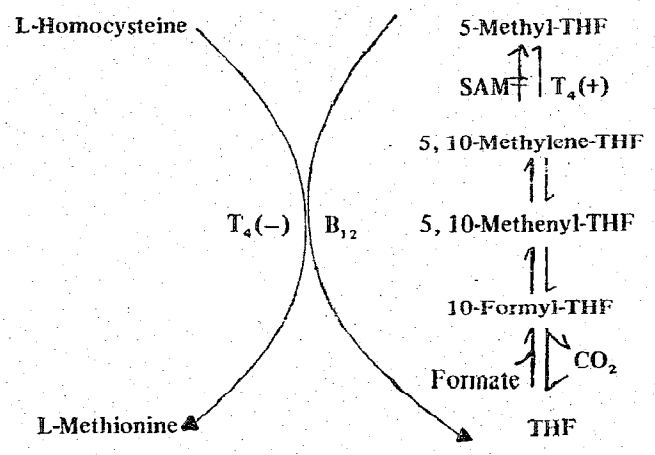
Christian WAYDHAS, Helmut SIES and E. L. Robert STOKSTAD+

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, Goethestr. 33, D-8000-München-2, FRG and *+Department of Nutritional Sciences, University of California, Berkeley CA 94720, USA*

Received 22 May 1979

1. Introduction

In a study of drug *N*-demethylations in isolated hepatocytes and perfused rat liver, we have observed a substantial stimulation of $^{14}CO_2$ formation from [*dimethylamino*-^{14}C]aminopyrine upon addition of L-methionine [1]. This is in agreement with the results of studies on the role of methionine in the metabolism of C_1-units derived from histidine [2–4] and formate [4]. An explanation of the methionine effects is provided by its conversion to *S*-adenosyl methionine (scheme 1) which reduces the formation of methyl-tetrahydrofolate by inhibition of methylene tetrahydrofolate reductase [5]. This provides an increased proportion of tetrahydrofolate which functions in the degradation of forminoglutamate and the oxidation of formate to carbon dioxide by folate-dependent pathways [6] (scheme 1).



Scheme 1
THF, tetrahydrofolate

The thyroid state has been shown to affect folic acid and vitamin B_{12} metabolism [7]. The effect of hyperthyroidism in increasing the excretion of forminoglutamic acid and decreasing the oxidation of [2-^{14}C]histidine to $^{14}CO_2$ has been identified as due mainly to an increase in methylenetetrahydrofolate reductase [8,9] which would increase the production of methyl-tetrahydrofolate and thereby decrease the non-methyltetrahydrofolates which can function in formate oxidation by the folate-dependent pathway (scheme 1) and in the degradation of forminoglutamic acid. Production of hypothyroidism by either feeding of thyroid inhibitors (thiouracil) or by thyroidectomy increases histidine oxidation to CO_2 and reduces excretion of forminoglutamic acid [8,9].

The exhalation of $^{14}CO_2$ derived from [*dimethylamino*-^{14}C]aminopyrine [10] has been evaluated as a non-invasive assay of the drug-metabolizing capacity of the liver in animals and in man in the so-called 'breath test' [11–14]. In order to assess the possible significance of the above-mentioned observations [1–8] on the aminopyrine breath test, we have examined the effects of low methionine diet and of thyroid status on $^{14}CO_2$ exhalation rates in rats given [*dimethylamino*-^{14}C]aminopyrine. The results show that in phenobarbital-treated animals there is a marked stimulatory effect due to methionine and, further, an increased rate of $^{14}CO_2$ exhalation is produced by thyroidectomy or feeding thiouracil.

Elsevier/North-Holland Biomedical Press

2. Methods

Male Wistar or Sprague Dawley rats (90–130 g) as indicated which had been on the experimental diets for 3–4 weeks were used in the respiration studies, which were carried out in a small respiration chamber (desiccator, 1.6 l). Air was drawn through the chamber and the trapping liquid (40 ml ethanolamine: ethyleneglycol monoethylester, 1:2) at ~ 300 ml/min. Radioactivity was measured using the scintillation mixture [15] which is especially well suited for use with alkaline solutions containing large amounts of CO_2. [*dimethylamino*-^{14}C]Aminopyrine, 12 Ci/mol (Radiochemical Centre, Amersham) was diluted with unlabeled aminopyrine (gift from Hoechst AG, Frankfurt) and used with a spec. act. 6 mCi/mol. L-[2-^{14}C]Histidine and [^{14}C]formate were diluted with unlabeled material and used with a spec. act. 2 mCi/mol. These materials were injected i.p. to provide ~ 0.2 μCi radioactivity/animal and either 0.15 mmol aminopyrine, 1 mmol histidine, or 1 mmol formate/kg body wt.

Phenobarbital pretreatment was performed by addition of sodium phenobarbital to the drinking water (1g/l) for at least 7 days prior to the experiment.

The animals were kept on different dietary regimens for 3–4 weeks prior to the experiment. Two series of experiments were carried out: the first consisting of groups 1–4 with Wistar rats; the second of groups 5–8 with Sprague Dawley rats, 6 months after the first series. Group 1 received regular laboratory diet (Altromin), whereas all other groups were kept on a 20% soy protein diet low in methionine [2] supplemented with vitamin B_{12} (50 μg/kg diet) and folic acid (2 mg/kg diet). Group 3 received L-thyroxine (20 mg/kg diet) in order to produce thyrotoxicity, whereas group 4 received thiouracil (1 g/kg diet) to produce hypothyroidism.

In the second series (table 2) the animals of groups 6–8 were thyroidectomized at weaning and placed on experimental diets 2 days later.

3. Results

Table 1 presents the results of an experiment comparing the effects of methionine on the formation of $^{14}CO_2$ from [*dimethylamino*-^{14}C]aminopyrine in phenobarbital-pretreated rats kept on stock diet (group 1) or on a low-methionine diet (groups 2–4). $^{14}CO_2$ production was diminished by ~ 25% when the animals were kept on a diet low in methionine as

Table 1
$^{14}CO_2$ exhalation from [*Dimethylamino*-^{14}C]aminopyrine

Group no.	Diet	Supplement per kg diet	$^{14}CO_2$ exhaled (% of Dose of [^{14}C]aminopyrine)						%Δ due to methionine at 1.0 h
			L-Methionine at 0.5 h		L-Methionine at 1.0 h		L-Methionine at 3.0 h		
			–	+	–	+	–	+	
1	Stock diet	None	22.0 (3) ±0.6	24.4 (3) ±2.1	32.9 (3) ±0.8	37.9 (3) ±2.1	49.3 (6) ±2.5	55.7 (3) ±1.2	15
2	Low methionine	None	12.4 (4) ±0.7	26.2 (4) ±5.8	25.5 (4) ±1.2	40.9 (3) ±5.3	39.9 (7) ±1.3	55.0 (3) ±3.3	60
3	Low methionine	L-thyroxine 20 mg	12.2 (3) ±1.5	21.0 (3) ±6.4	24.0 (3) ±0.7	38.6 (3) ±7.0	33.7 (6) ±2.8	53.2 (3) ±3.5	62
4	Low methionine	Thiouracil 1 g	15.5 (4) ±2.7	28.6 (4) ±0.5	30.4 (4) ±2.1	42.2 (4) ±0.9	45.6 (6) ±2.2	53.7 (4) ±1.5	39

Pretreatment of the rats was performed as in section 2. Data are means ± SEM. Number of animals in parentheses. L-Methionine was injected 30 min before start of experiment (1 mmol/kg body wt). Aminopyrine (0.15 mmol/kg body wt) was injected at zero time. All animals received phenobarbital (1 g/l) in drinking water beginning 7 days before metabolism tests were made

Table 2
Effect of methionine on $^{14}CO_2$ exhalation from ^{14}C-labeled formate, aminopyrine and histidine in normal and thyroidectomized rats pretreated with phenobarbital

Group no.	Treatment	$^{14}CO_2$ exhaled (% of Dose)					
		[^{14}C]Formate[a] L-methionine		[*dimethylamino*-^{14}C]-Aminopyrine[a] L-methionine		[2-^{14}C]Histidine[b] L-methionine	
		–	+	–	+	–	+
5	Control	28.5 ±0.8	33.4 ±1.0	32.1 ±1.1	42.8 ±0.9	2.9 ±0.3	18.7 ±0.6
6	Thyroidectomized	38.0 ±0.3	41.0 ±1.9	35.6 ±1.2	40.2 ±1.7	30.4 ±1.0	41.3 ±0.7
7	Thyroidectomized; 5 μg T_4/day[c]	30.4 ±1.4	30.7 ±0.9	22.7 ±1.1	48.4 ±1.8	2.5 ±0.3	16.9 ±4.1
8	Thyroidectomized; 3 g thyroid powder/kg diet	32.4 ±2.1	37.0 ±2.1	34.3 ±2.9	50.7 ±1.7	1.9 ±0.1	12.1 ±2.6

[a] Values for 1 h in respiration chamber
[b] Values for 2 h in respiration chamber
[c] T_4 (10 μg) injected i.p. every other day; this represents replacement level of T_4

Values are percentage of injected dose oxidized to respiratory carbon dioxide in time indicated (± SEM, n=5–6). L-Methionine (2 mmol/kg body wt) was injected 20–30 min before injection of the labeled compound. Amounts of injected compound (mmol/kg body wt): formate, 1.0; aminopyrine, 0.3; histidine, 1.0. Phenobarbital-pretreatment was as indicated in table 1

compared to that observed with the stock diet. The injection of methionine (1 mmol/kg body wt) 30 min before start of the experiment led to an increase (60%) in animals kept on the diet low in methionine. Methionine injection had a greater stimulatory effect in animals kept on the low-methionine diet than on the stock diet. $^{14}CO_2$ exhalation reached similar values after methionine injection irrespective of the prior methionine supply with the diet (table 1).

A further comparison of the effect of methionine on the $^{14}CO_2$ formation from [^{14}C]formate, [*dimethylamino*-^{14}C]aminopyrine and [2-^{14}C]histidine is shown in table 2. Clearly, there is an increase in $^{14}CO_2$ exhalation from [*dimethylamino*-^{14}C]aminopyrine due to methionine in all the dietary treatments. The increase in formate oxidation in the same groups was smaller. These in vivo stimulatory effects of methionine injection may be compared with the much larger increases (~ 3-fold) in [*dimethylamino*-^{14}C] aminopyrine oxidation and (~4-fold) in [^{14}C]formate oxidation observed in perfused liver [1] and (~2-fold) in [*dimethylamino*-^{14}C]aminopyrine oxidation [1] and [^{14}C]formate oxidation [4] in isolated hepatocytes. The stimulatory effect of methionine on $^{14}CO_2$ production from [2-^{14}C]histidine (table 2) was quite marked in the in vivo system, similar to the previous findings with isolated hepatocytes and perfused rat liver [2–4].

Thiouracil feeding increased $^{14}CO_2$ exhalation from labeled aminopyrine by 20% at 1 h (table 1: group 2 versus group 4), and the increase observed after thyroidectomy was 11% (table 2: group 5 versus group 6). Under the same conditions thyroidectomized rats showed a 10-fold increased $^{14}CO_2$ formation from [2-^{14}C]histidine in these phenobarbital-pretreated animals, and formate oxidation was also increased by 33% (table 2: group 5 versus group 6).

4. Discussion

These observations indicate that in phenobarbital-pretreated animals fed a diet low in methionine there is a restricted capacity to form $^{14}CO_2$ from [*dimethylamino*-^{14}C]aminopyrine, [^{14}C]formate and [2-^{14}C]-histidine. This restriction can be overcome by injec-

tion of methionine. Thus, the nutritional status relative to methionine intake or to vitamin B_{12} status which can affect methionine synthesis, could lead to alterations in the extent of formate–CO_2 conversion. This could be of relevance in the so-called 'breath test' in man. Evidence that the formate–CO_2 conversion is the rate limiting step in $^{14}CO_2$ exhalation has been presented by the urinary excretion of [^{14}C]formate in intact rats [10]. The present observations also indicate that hypothyroidism may increase $^{14}CO_2$ exhalation from [*dimethylamino*-^{14}C]aminopyrine in the 'breath test' similar to its effect in promoting oxidation of [2-^{14}C]histidine to $^{14}CO_2$.

Acknowledgement

Part of the experiments were carried out in the Institut für Biochemie, Universität Köln; we thank Professor Dr L. Jaenicke for his hospitality. These studies were supported by Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 51 'Medizinische Molekularbiologie und Biochemie', grant D/8) and by the National Institutes of Health (USPHS grant AM08171).

References

[1] Waydhas, C., Weigl, K. and Sies, H. (1978) Eur. J. Biochem. 89, 143–150.
[2] Buehring, K. U., Batra, K. K. and Stokstad, E. L. R. (1972) Biochim. Biophys. Acta 279, 498–512.
[3] Shin, Y. S., Buehring, K. U. and Stokstad, E. L. R. (1975) Mol. Cell. Biochem. 9, 97–108.
[4] Krebs, H. A., Hems, R. and Tyler, B. (1976) Biochem. J. 158, 341–353.
[5] Kutzbach, C. and Stokstad, E. L. R. (1967) Biochim. Biophys. Acta 139, 217–220.
[6] Kutzbach, C. and Stokstad, E. L. R. (1968) Biochem. Biophys. Res. Commun. 30, 111–117.
[7] Stokstad, E. L. R., Webb, R. S. and Shah, E. (1966) Proc. Soc. Exp. Biol. Med. 123, 752–754.
[8] Stokstad, E. L. R., Chan, M. M. and Bloomer, J. E. (1979) in: Chemistry and Biology of Pteridines (Kislick, R. L. and Brown, G. M. eds) p. 603, Elsevier/North-Holland, Amsterdam, New York.
[9] Stokstad, E. L. R., Chan, M. M., Watson, J. E. and Brody, T. (1979) Proc. 3rd Eur. Symp. Vitamin B12 and Intrinsic Factor, Zurich, 1979. (Zaglack, B. ed) De Gruyter, in press.
[10] Aebi, H. and Roggen, G. (1958) Pharm. Acta Helv. 33, 413–428.
[11] Lauterburg, B. H. and Bircher, J. (1973) Gastroenterology 65, A-32/556.
[12] Hepner, G. W. and Vesell, E. S. (1974) N. Engl. J. Med. 291, 1384–1388.
[13] Lauterburg, B. H. and Bircher, J. (1976) J. Pharm. Exp. Ther. 196, 501–509.
[14] Willson, R. A., Hart, F. E. and Hew, J. T. (1979) Gastroenterology 76, 697–703.
[15] Jeffay, H. and Alvarez, J. (1961) Anal. Chem. 33, 612–615.