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L-METHIONINE ORDINARILY DOES NOT INTERFERE WITH THE AMINOPYRINE BREATH TEST: STUDIES IN DOGS AND RATS*

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Abstract—The influence of L-methionine on $^{14}CO_2$-exhalation resulting from administration of [^{14}C]aminopyrine was studied in rats and dogs. L-Methionine given i.v. one hour after i.v. [^{14}C]aminopyrine modified $^{14}CO_2$ output neither in normal rats nor in normal dogs. Only in methionine depleted rats subjected to enzyme induction by phenobarbital, and to subtoxic doses of [^{14}C]aminopyrine, L-methionine increased $^{14}CO_2$ production. These results are consistent with the idea that excess or deficiency of L-methionine ordinarily does not interfere with the aminopyrine breath test.

The aminopyrine breath test is now a well established noninvasive procedure to assess hepatic aminopyrine demethylation *in vivo*. It has been validated experimentally in the rat [1, 2], and clinically in normal subjects, and in patients with various diseases affecting hepatic drug metabolism [3–6].

Since the test has been applied also for the diagnosis of alcoholic liver disease the question may be raised, whether or not nutritional deficiencies, for instance of amino acids often observed in alcoholics [7], may interfere with the procedure. Within this context it appeared particularly pertinent, that Waydhas [8] investigated the contribution of L-methionine to the oxidation of formate to CO_2. Utilizing *in vitro* perfused rat livers they showed that concentrations above 0.1 mM of L-methionine in the perfusate were needed to assure maximal rates of $^{14}CO_2$ production from [^{14}C]formate. In analogy, when [^{14}C]formaldehyde was formed from [^{14}C]aminopyrine, its oxidation to $^{14}CO_2$ was assured only if L-methionine was present in the perfusion medium. Applied to the aminopyrine breath test these results could imply, that changes in nutrition with L-methionine might influence the formation of $^{14}CO_2$ from [^{14}C]aminopyrine [9], thereby invalidating $^{14}CO_2$ exhalation as a measure of [^{14}C]aminopyrine demethylation.

Experiments were therefore carried out in dogs and rats in order to evaluate whether or not L-methionine deficiency or excess might influence the aminopyrine breath test. Since ordinarily in man $^{14}CO_2$ output is assessed as specific activity of exhaled $^{14}CO_2$, studies were performed in dogs using the procedure applied in man. In order not to overlook possible changes in $^{14}CO_2$ formation, $^{14}CO_2$ output was also measured in rats where continuous collection of CO_2 is easily accomplished.

The results suggest that the aminopyrine breath test may be influenced by L-methionine only under extreme experimental conditions.

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MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (Süddeutsche Versuchstierfarm, Tuttlingen, F.R.G.), weighing approximately 50 g, were purchased. All animals had free access to food and water. They were housed at constant temperature (24°), and air moisture (47%), and at an artificial 12 hr day–night rhythm. For experiments with dogs, trained female boxer dogs, weighing 20 to 27 kg, were used. They had not been used for experiments and had not been given drugs for at least 4 weeks prior to the studies.

Chemicals. (Dimethylamine-^{14}C)aminopyrine (The Radiochemical Centre, Amersham, England), sp. act. 82.0 mCi/mmole, was diluted with 0.9% saline to obtain a radioactivity of 2 μCi/ml, and this stock solution was stored at 4° in the dark. The loading dose of unlabelled aminopyrine (Bayer-Pharma, Zürich, Switzerland), and L-methionine (Sigma, St. Louis, U.S.A.) were freshly dissolved in 0.9% saline for each experiment. Phenobarbital (Siegfried, Zofingen, Switzerland) was also diluted in 0.9% saline immediately prior to injection.

Experiments in rats. Rats were either given a normal laboratory chow (1324 Altromin, Lage, G.F.R.), or a diet low in methionine (C1012 Altromin, Lage, G.F.R.) for at least 5 weeks prior to the studies. Serum methionine levels, determined in representative animals by ionexchange chromatography according to the method described by Benson [10], were 40.7 ± 5.3 μmoles/l ($\bar{x}$ ± S.E.M., n = 7) in rats fed a diet low in methionine, and 80.2 ± 15.8 μmoles/l (n = 6) in normally fed animals.

As detailed in Table 1, unlabelled aminopyrine was given either in a dose of 39 or 400 μmoles/kg body weight together with 2 μCi[^{14}C]aminopyrine in a total volume of 3 ml of 0.9% saline. These doses were infused within 5 min into a tail vein at the beginning of each experiment. A dose of 670 μmoles/kg body weight of L-methionine, dissolved in 3 ml of 0.9% saline, or saline alone, was infused 60 min after administration of aminpyrine via the same route, the infusion lasting 5 min. This

Table 1. $^{14}CO_2$ Exhalation from [^{14}C]aminopyrine in rats ($\bar{x}$ + S.E.M.)

	Group				
Group Nr	1	2	3	4	5
Number of animals	6	3	6	6	5
Body weight (g)	234 ± 15	292 ± 38	194 ± 7	255 ± 2	264 ± 4
Experimental conditions*					
Aminopyrine dose (μmoles/kg)	39	39	39	400	400
Methionine depletion	−	−	+	+	+
Enzyme induction	−	−	−	+	+
Methionine infusion	+	−	+	+	−
$^{14}CO_2$ Output					
Peak time (min)	8.3 ± 2.1	11.6 ± 3.3	10.0 ± 2.2	18.3 ± 2.1†	19.0 ± 2.4†
Peak output (% dose/min)	0.76 ± 0.09	0.95 ± 0.09	1.16 ± 0.16	0.48 ± 0.03	0.62 ± 0.04
Output 0–60 min (% dose)	32.7 ± 1.5	40.6 ± 2.7	49.7 ± 6.1	26.2 ± 1.5	31.8 ± 1.5
Output 60–120 min (% dose)	8.9 ± 0.5	10.0 ± 1.2	14.7 ± 1.8	13.7 ± 0.5	11.7 ± 0.3
Ratio $\frac{\text{Output 60–120 min}}{\text{Output 0–60 min}}$	0.28 ± 0.03	0.24 ± 0.02	0.29 ± 0.02	0.53 ± 0.04†	0.36 ± 0.01‡

* For details see Methods.
† Different from group 1 and 3 ($P < 0.01$).
‡ Different from group 4 ($P < 0.01$).

amount of L-methionine covers approximately the daily requirement [11], and if distributed within the extracellular volume should provide a concentration of about 3 mmoles/l, whereas only 0.1 mmole/l was needed to maximally stimulate formate oxidation *in vitro* [8].

In some rats, enzyme induction was performed by daily i.p. injection of 314 μmoles/kg body weight of sodium phenobarbital, dissolved in 2 ml of 0.9% saline, for three days prior to the aminopyrine studies which were carried out on the forth day.

$^{14}CO_2$ collection was performed according to the method described by Lauterburg [1] except that the 1:4 ethanolamine–methanol mixture was replaced by 20 ml of a 1:1 hyamine–ethanol mixture (Hyamine 1m: Koch-Light Laboratories Ltd, Colnbrook Bucks, England), and that the sampling periods were 10 min. $^{14}CO_2$ radioactivity was determined by counting a 4 ml aliquot of the hyamine–ethanol mixture after addition of 10 ml of a scintillation cocktail consisting of toluol 800 ml, Triton-x-100 200 ml, PPO 5g, and POPOP 100 mg with a Packard Tri-Carb liquid scintillation spectrometer 3380. $^{14}CO_2$ output was expressed in %dose/min.

Experiments in dogs. Unlabelled aminopyrine in a dose of 39 μmoles/kg body weight together with 2 μCi[^{14}C]aminopyrine, dissolved in 20 ml of 0.9% saline, were infused into a vein of a hind leg within 5 min. In the experimental studies, L-methionine in a dose of 335 μmoles/kg body weight, dissolved in 50 ml of 0.9% saline, was infused 60 min after administration of aminopyrine via the same route within 5 min. In control experiments, L-methionine infusion was replaced by 50 ml of 0.9% saline.

$^{14}CO_2$ collection and counting of $^{14}CO_2$ radioactivity was performed as described by Küpfer [12]. Sampling intervals were 15 min. Results are expressed as specific activity of exhaled $^{14}CO_2$ in % dose kg/mmoles CO_2.

Calculations. Student's *t*-tests were applied for statistical comparisons and $P < 0.01$ was taken as statistically significant [13].

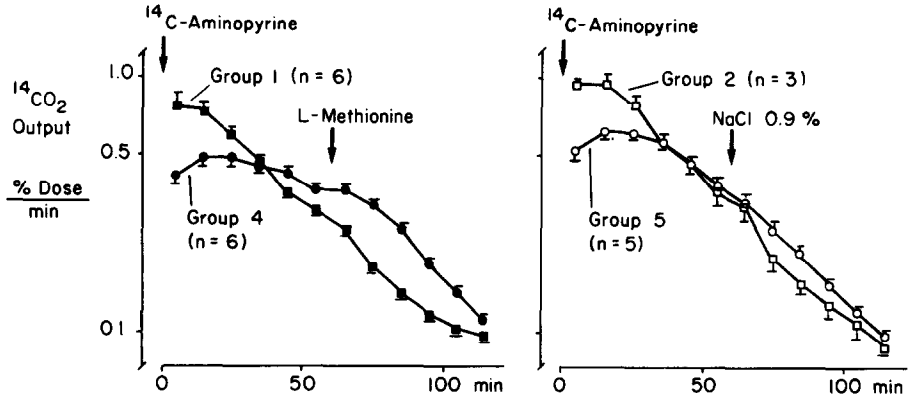

Fig. 1. Effect of L-methionine in physiological saline, or physiological saline alone, on $^{14}CO_2$ exhalation in rats which had received [^{14}C]aminopyrine. Groups 1 and 2 were normal rats receiving [^{14}C]aminopyrine in a dose of 39 μmoles/kg. The rats in group 4 and 5 were methionine-depleted, subjected to enzyme induction with phenobarbital and the aminopyrine dose was 400 μmoles/kg ($\bar{x}$ ± S.E.M.).

RESULTS

Figure 1 shows $^{14}CO_2$ exhalation curves after administration of labelled aminopyrine to rats. In agreement with the data published by Lauterburg [1], $^{14}CO_2$ exhalation rates rapidly reached a peak, and declined thereafter. Administration of L-methionine to rats without pretreatment did not visibly modify the time course of $^{14}CO_2$ exhalation. An effect of L-methionine appeared to be visible only in methionine depleted rats after phenobarbital induction, and with high doses of aminopyrine (group 4).

As detailed in Table 1, peak $^{14}CO_2$ output expressed as per percentage of the dose per minute was lower, and occurred significantly later in rats receiving the higher doses of aminopyrine. Similarly, the fraction of the dose exhaled as $^{14}CO_2$ within the first 60 min tended to be smaller in rats receiving the higher doses. $^{14}CO_2$ exhalation within the 60 min after L-methionine or saline infusion varied between 9 and 14 per cent of the dose. There were no significant differences between experimental and control groups.

Thus, the effect of L-methionine infusion was not impressive. In order to analyse the effect of L-methionine in more detail, the ratio of $^{14}CO_2$ exhalation after L-methionine (i.e. 60 to 120 min after aminopyrine injection) to $^{14}CO_2$ exhalation before L-methionine (i.e. 0 to 60 min after aminopyrine injection) was formed. This ratio showed a significant difference ($t = 5.4$ $P < 0.01$) due to L-methionine only in rats subjected to methionine depletion, to enzyme induction, and to a submaximal dose of aminopyrine (groups 4 and 5).

Experiments in dogs. Figure 2 shows specific activity of $^{14}CO_2$ following administration of [^{14}C]aminopyrine to normal fed dogs. Peak specific activity occurred in the 15 or 30 min sample and, thereafter, seemed to disappear as a first order process. The curves were not different whether L-methionine in saline, or saline alone, was infused. In Table 2, mean $^{14}CO_2$ specific activity of the samples taken in the period between administration of aminopyrine, and infusion of L-methionine, or saline respectively (i.e. 0 to 60 min after aminopyrine injection), and in the 60 min following infusion of L-methionine, or saline (i.e. 60 to 120 min after aminopyrine injection) are indicated. No effect of L-methionine can be detected. Also, the ratio of $^{14}CO_2$ specific activity of the second to the first hour was not modified by L-methionine.

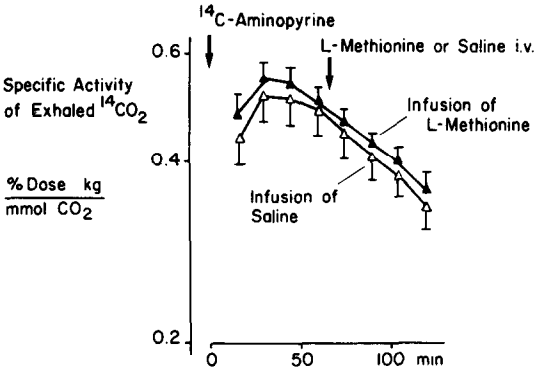

Fig. 2. Effect of L-methionine in physiological saline, or physiological saline alone, on specific activity of exhaled $^{14}CO_2$ in the dog. No effect of L-methionine is apparent. The aminopyrine dose was 39 μmoles/kg ($\bar{x}$ ± S.E.M., n = 6).

DISCUSSION

The data shown in Fig. 1 and Table 1 confirm results obtained by Waydhas [9] who demonstrated that under suitable experimental conditions administration of L-methionine may increase $^{14}CO_2$ formation from [^{14}C]aminopyrine. Compared to the findings obtained with the perfused liver, the *in vivo* effect of L-methionine, however, seemed to be weak. It could not be observed in healthy rats (group 1 and 2) or dogs receiving pharmacologic doses (40 μmoles/kg body wt) of aminpyrine. Furthermore, L-methionine depletion resulting from a diet deficient in L-methionine for at least 5 weeks (group 3) was not followed by a decrease in $^{14}CO_2$ output. Only when L-methionine deficiency was combined with an experimental procedure designed to achieve a very high rate of formaldehyde production from aminopyrine, did L-methionine administration result in an increased $^{14}CO_2$ output (group 4). Presumably only these circumstances led to a limitation of formate oxidation which was responsive to L-methio-

Table 2. $^{14}CO_2$ Exhalation from 39 μmoles/kg body wt of [^{14}C]aminopyrine in dogs($\bar{x}$ ± S.E.M.)

	L-Methionine infusion	Saline infusion
Body weight (kg)	22.3 ± 0.6	23.5 ± 0.8
Number of animals	6	6
Mean $^{14}CO_2$ sp. act. (% Dose kg/mmoles CO_2)		
0–60 min	0.515 ± 0.028*	0.486 ± 0.046
60–120 min	0.411 ± 0.020†	0.391 ± 0.033
Ratio $\frac{60\text{–}120 \text{ min}}{0\text{–}60 \text{ min}}$	0.805 ± 0.040‡	0.817 ± 0.040

* Statistically not different from control experiments ($P > 0.5$).
† Statistically not different from control experiments ($p > 0.6$).
‡ Statistically not different from control experiments ($P > 0.8$).

nine repletion. Since urinary formate excretion has not been examined, it is not possible to known whether or not the ^{14}C-label not appearing in breath might have been excreted as [^{14}C]formate [14].

When analysing results of the aminopyrine breath test in man, it was noted that the first order rate constant for the decrease in specific activity of $^{14}CO_2$ in breath was the best indicator of aminopyrine metabolism as evaluated by the aminopyrine plasma disappearance [15]. It appeared important, therefore, to evaluate whether L-methionine could influence the time course of $^{14}CO_2$ exhalation, particularly during the phase of apparent first order disappearance. The doses of L-methionine were, therefore, administered one hour after [^{14}C]aminopyrine. Furthermore, at this time it could be expected that substantial amounts of [^{14}C]formate should have been formed from [^{14}C]aminopyrine. Since *in vitro* L-methionine accelerated $^{14}CO_2$ production from formate within 5 to 10 min, an effect of the infused amino acid should have been detectable during the ensuing 60 min period of observation, particularly since several samples were taken.

The use of $^{14}CO_2$ specific activity determinations as indices for $^{14}CO_2$ output—the practice applied in man—has the disadvantage that changes in production of unlabelled CO_2, which serves as standard of reference, will influence the results. It is reassuring, therefore, that the data obtained in normal dogs are consistent with the ones obtained in normal rats in showing that L-methionine does not modify the time course of $^{14}CO_2$ output resulting from a single dose of [^{14}C] aminopyrine.

If these results are extrapolated to patients it appears likely that only with a combination of severe nutritional deficiency, enzyme induction, and a high dose of aminopyrine, could formate oxidation be altered to an extent that might interfere with the aminopyrine breath test. Although such conjectures always remain uncertain, the use of tracer doses of [^{14}C] aminopyrine ensures that the formaldehyde and formate pools are not overloaded during aminopyrine demethylation.

Under these circumstances the disappearance curve of $^{14}CO_2$ in breath is likely to reflect demethylation *in vivo* [16].

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