ELSEVIER

Comparative Biochemistry and Physiology Part C 129 (2001) 211–216

CBP

www.elsevier.com/locate/cbpc

Importance of sulfate, cysteine and methionine as precursors to felinine synthesis by domestic cats (*Felis catus*)

W.H. Hendriks*, S.M. Rutherfurd, K.J. Rutherfurd

Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11 222, Palmerston North, New Zealand

Received 2 February 2001; received in revised form 11 April 2001; accepted 17 April 2001

Abstract

There is conflicting evidence in the literature on the utilization of cysteine and methionine as precursors to the urinary sulfur-containing amino acid felinine in cats. Three entire domestic short-haired male cats, housed individually in metabolism cages, were injected intraperitoneally with either [^{35}S]-sulfate, [^{35}S]-cysteine, or [^{35}S]-methionine. Daily urine samples were collected quantitatively for up to 9 days after injection. Each cat was injected once with each compound after observing an appropriate interval for [^{35}S] to be depleted between injections. All the urine samples were analysed for felinine content and total radioactivity. Felinine was isolated from each urine sample and analysed for radioactivity. No radioactivity was found in felinine from cats injected with [^{35}S]-sulfate. The mean ($\pm$S.E.M.) cumulative recovery of radioactivity in the urine of the [^{35}S]-sulfate injected cats was 90.6 $\pm$ 6.1% after 4 days. The mean ($\pm$S.E.M.) cumulative incorporation rate of radioactivity into felinine by the cats receiving the [^{35}S]-cysteine and [^{35}S]-methionine were 11.6 $\pm$ 1.6 and 8.6 $\pm$ 0.6%, respectively, after 9 days. The mean ($\pm$S.E.M.) cumulative recoveries of radioactivity in the urine were 58.1 $\pm$ 3.7 and 36.0 $\pm$ 8.0%, respectively. Cysteine and methionine, but not sulfate, are precursors to felinine, with cysteine being a more quantitatively important precursor compared to methionine.

Keywords: Cats; Felinine; Cysteine; Methionine; Sulfate; Precursors; Radioactivity; Urine; *Felis catus*

1. Introduction

Felinine is a little known amino acid found in the urine of certain members of the Felidae family. First discovered by Datta and Harris (1951) and characterized by Westall (1953), this sulfur-containing branched chain amino acid can be detected in the urine of domestic cats as young as 2 months of age. During development to adulthood, the urinary felinine concentration of male kittens steadily increases, while female cats show little or no increase (Tarttelin et al., 1998). Felinine concentrations in adult male cats are extraordinarily high (0.4–3.6 g/l), while female cats have concentrations that are 0.2–0.25 that of entire male cats (Hendriks et al., 1995a). Although several hypotheses have been put forward for the biological role of felinine, its function as a precur-

* Corresponding author. Tel.: +64-6-3505065; fax: +64-6-3505631.

E-mail address: w.hendriks@massey.ac.nz (W.H. Hendriks).

1532-0456/01/$ - see front matter
PII: S1532-0456(01)00196-X

sor to a pheromone seems likely, but remains to be confirmed.

Studies into the biosynthesis of felinine have shown that the radioactivity of DL-[2-^{14}C]-leucine, DL-[2-^{14}C]-mevalonic acid and [2-^{14}C]-acetate can be incorporated into felinine (Avizonis and Wriston, 1959; Shapiro, 1962; Wang, 1964). Contradictory evidence, however, exists on the use of cysteine and methionine as precursors of felinine. Avizonis and Wriston (1959) were able to increase urinary felinine concentrations by supplementation of the diet with cystine. Shapiro (1962) incorporated 4% of administered [^{35}S]-cystine into felinine within 24 h after injection. Roberts (1963), however, was unable to detect any radioactively labeled felinine after intravenously injecting [^{35}S]-cystine into a male cat. Even after supplementing the diet of a cat with [^{35}S]-yeast, Roberts (1963) was unable to detect any radioactivity in felinine, and subsequently concluded that cystine and methionine are not immediate precursors to felinine. Wang (1964) found some support for the latter conclusion by isolating radioactive felinine from the urine of cats after administration of [2-^{14}C]-leucine. The radioactivity was found to be present in the alanine moiety of the felinine molecule.

The present study aimed to determine the relative importance of sulfate, cysteine and methionine as precursors to felinine synthesis by adult domestic cats.

2. Materials and methods

The study reported here was approved, and followed the procedures set out by the Massey University Animal Ethics Committee (Anonymous, 1992).

2.1. Animals and diet

Three entire male domestic short-haired cats (*Felis catus*), 4–7 years old from the Feline Unit at Massey University (Palmerston North, New Zealand) were used as experimental animals. The body weights of the cats at the start of the experiment ranged from 3.52 to 4.11 kg. Throughout the study, the cats were fed a moist canned cat food that passed a minimum feeding protocol for proving an adult maintenance claim for a cat food (AAFCO, 2000). The diet provided the following nutrients (g/100 g dry matter): crude protein 52; crude fat 29; methionine 1.2; cysteine 1.8; taurine 0.2; and calculated metabolisable energy 461 kcals/100 g dry matter. All cats had been vaccinated against feline rhinotracheitis, calicivirus and panleukopenia using a modified live vaccine (Felocell CVR, Norden Laboratories, München, Germany). Feline leukemia and feline immunodeficiency virus have not been detected in the colony since its establishment in 1976.

2.2. Experimental procedures

The study was conducted in three parts. In the first part (period 1), the three cats were injected with [^{35}S]-sulfate. On completion of part 1 and after a further 2-week 'cooling down' period (to allow radioactivity to be eliminated from the body of each cat), one cat was randomly selected and injected with [^{35}S]-cysteine while the other two cats were injected with [^{35}S]-methionine (period 2). The latter two treatments were reversed following a 5-month 'cooling down' period (period 3). Overall the experiment was completed over a 6-month period. The levels of radioactivity in the body of the cats at the start of each experimental period were confirmed by measurement of the radioactivity in the urine of the cats. At the start of each experimental period, each cat was weighed and given an accurately weighed amount (approx. 2 ml) of sterile isotonic (9 g/l) saline containing 250 μCi (9.25 MBq) of either [^{35}S]-sulfate, [^{35}S]-cysteine or [^{35}S]-methionine (Amersham Corporation, Arlington Heights, IL, USA) by intraperitoneal injection. The cats were then housed individually in metabolism cages, which had been previously validated to allow total collection of uncontaminated urine from cats (Hendriks et al., 1999), where they had free access to food and fresh water. During experimental period 1, urine was collected daily for 4 days, while during experimental periods 2 and 3 urine was collected daily for 9 days. Urine collection was as described by Hendriks et al. (1999) with urine volumes calculated as 1.025 × urine weight. Daily urine samples were analysed within 6 h after collection each morning. Food intake was measured daily during each experimental period and body weights were recorded at the end of each experimental period.

2.3. Measurement of felinine and radioactivity

Felinine and [^{35}S]-felinine from the urine were quantified as described below. The urine was filtered through a 0.22-μm filter before being loaded onto a cation-exchange column (Waters Amino Acid Analysis Column P/N 80002) using a Waters HPLC system (Waters Corporation, Milford, MA, USA). Buffer A contained 67 mM sodium citrate, pH 3.4 and 0.1% (w/v) phenol. Buffer B contained 40 mM borate, pH 9.6 and 250 mM sodium nitrate. Compounds were eluted using a pH gradient of 100% buffer A to 80% buffer B/20% buffer A in 40 min. Following this, the column was washed with buffer B for 30 min before being re-equilibrated in buffer A for 20 min. The flow rate was 0.4 ml/min. Baseline separation was achieved between the main sulfur-containing compounds: felinine, cysteine, methionine, taurine, and sulfate. Felinine and other primary amino group-containing compounds were detected using post-column derivatisation with *o*-phthalaldehyde. Felinine levels were quantified by comparison of the peak area of felinine in the urine with the peak area of felinine in a standard synthetic felinine solution (Hendriks et al., 1995b).

All the major peaks present in the chromatogram of each urine sample, and the baseline regions between peaks, were collected manually into preweighed tubes, after which the tubes were re-weighed for determination of the quantity of fluid collected. The specific gravity of the buffer/*o*-phthalaldehyde solution was used to convert collected weights to volumes. Radioactivity present in the original urine, and in the collected fractions from the HPLC, was determined following the addition of a 0.5-ml sample to 0.5 ml glacial acetic acid and 4 ml PBS II™ (Amersham Corporation, Arlington Heights, IL, USA). The radioactivity present in the scintillation cocktail was counted using a Wallac 1414 liquid scintillation spectrometer (Wallac OY, Turku, Finland). Radioactivity present in the [^{35}S]-sulfate, [^{35}S]-cysteine and [^{35}S]-methionine injection solutions was determined as described above except that the solutions were diluted 1:800 with demineralised water. The mean counting efficiency of the [^{35}S] was 84%.

Table 1
Mean (±S.E.M.)[a] daily felinine concentration and excretion and the cumulative incorporation of [^{35}S] into felinine by entire male cats[b]

Injected compound	Felinine		Cumulative incorporation (%)
	Concentration (g/l)	Excretion (g/day)	
[^{35}S]-cysteine	1.71 ± 0.39	0.34 ± 0.03	11.6 ± 1.5
[^{35}S]-methionine	1.73 ± 0.14	0.30 ± 0.03	8.6 ± 0.6
[^{35}S]-sulfate	1.70 ± 0.28	0.38 ± 0.01	0

[a] Mean of 9 individual days for cysteine and methionine, and 4 individual days for SO_4.
[b] $n = 3$.

3. Results

The cats all remained healthy and maintained their body weight throughout the study period.

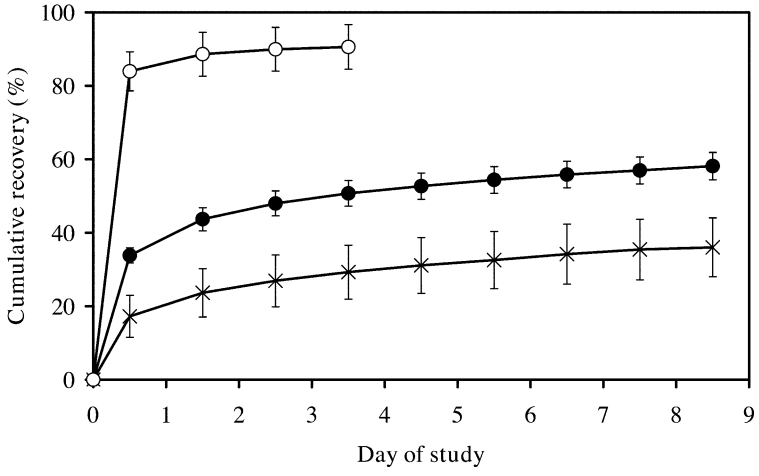

Fig. 1. Cumulative recovery of [^{35}S] from the urine of entire male cats injected with [^{35}S]-cysteine (●), [^{35}S]-methionine (×) and [^{35}S]-sulfate (○).

There was no significant difference between the average daily urinary feline concentrations of cats injected with labeled sulfate, methionine, or cysteine, and values ranged from 1.70 to 1.73 g/l (Table 1). The average daily felinine excretion rate ranged from 0.30 g when [^{35}S]-methionine was injected, to 0.38 when [^{35}S]-sulfate was injected (Table 1).

A graph showing the cumulative recovery of radioactivity from [^{35}S]-sulfate, [^{35}S]-cysteine and [^{35}S]-methionine in the urine of the entire male cats is shown in Fig. 1. Twenty-four hours after the intraperitoneal injection of the [^{35}S]-sulfate, [^{35}S]-cysteine and [^{35}S]-methionine, 83.9, 33.8 and 17.2% of the total injected radioactivity, respectively, had been recovered from the urine of the entire male cats. Within 4 days of injection 90.6% of the injected [^{35}S]-sulfate had been recovered from the urine, after which time urine collection was ceased. In contrast only 58.1% of the injected [^{35}S]-cysteine and 36.0% of the injected [^{35}S]-methionine had been recovered in the urine within the 9 days after injection.

The cumulative incorporation of radioactivity from [^{35}S]-sulfate, [^{35}S]-cysteine and [^{35}S]-methionine into felinine from the urine of the entire male cats is shown in Fig. 2. Injection of [^{35}S]-sulfate did not result in any incorporation of the [^{35}S] into felinine over the 4-day urine collection period. The intraperitoneal injection of [^{35}S]-cysteine resulted in an incorporation of 5.2% of the [^{35}S] into felinine in the urine after 24 h. Within 9 days of injection a total of 11.6% of the injected [^{35}S] had been incorporated into felinine (Table 1). Injection of [^{35}S]-methionine resulted in a lower level of [^{35}S] incorporation into felinine in the urine with 3.1% of the injected [^{35}S] being incorporated into felinine within the first 24 h after injection and a total of 8.6% incorporated into felinine within the first 9 days after injection (Table 1). The cumulative urinary excretion of [^{35}S]-cysteine and [^{35}S]-methionine in cats injected with these compounds, respectively, was less than 0.8% over the 9-day experimental period.

4. Discussion

In order to determine the importance of sulfate, cysteine and methionine as precursors to felinine synthesis, entire male cats were administered intraperitoneal injections of [^{35}S]-sulfate, [^{35}S]-cysteine or [^{35}S]-methionine. The level of incorporation of the [^{35}S] into felinine in the urine, as well as the recovery of [^{35}S] in the urine was determined over a period of 4 days for [^{35}S]-sulfate and 9 days for [^{35}S]-cysteine and [^{35}S]-methionine.

Urinary felinine concentration was relatively constant regardless of the sulfur compound being injected (Table 1). Furthermore, felinine concentrations were similar to previously reported felinine excretion levels in entire male cats of 0.4–3.6 g/l (Hendriks et al., 1995a). The cumulative incorporation rates of [^{35}S]-cysteine and [^{35}S]-

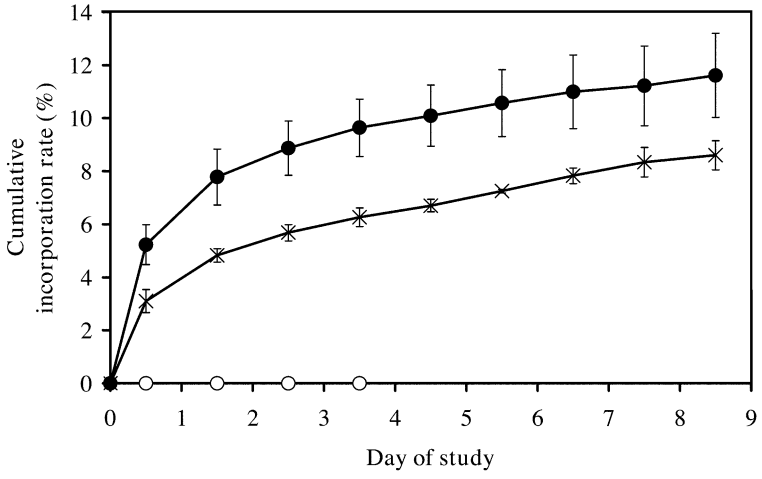

Fig. 2. Cumulative incorporation rates of [^{35}S] into felinine by entire male cats injected with [^{35}S]-cysteine (●), [^{35}S]-methionine (×) and [^{35}S]-sulfate (○).

methinine over the 9-day study were 11.6 and 8.6%, respectively, with no radioactivity being incorporated into felinine from [^{35}S]-sulfate. Shapiro (1962) injected a male cat with L-[^{35}S]-cystine and found 4% of the injected radioactivity present in felinine within the first 24 h. In the present study, similar values of 5.2 and 3.1% were found for the incorporation of [^{35}S]-cysteine and [^{35}S]-methionine into felinine, respectively. Roberts (1963), injecting a 2.6-kg male cat with 316 μCi (11.7 MBq) of [^{35}S]-cystine, and using paper chromatography to isolate felinine from urine, found no incorporation of the radioactivity into felinine. Roberts (1963) was also unable to detect radioactivity in felinine from a cat fed milk containing 2.5 mCi of [^{35}S]-labeled yeast. In both experiments, Roberts (1963) was able to detect radioactivity in other sulfur-containing amino acids such as cystine, cysteic acid and cysteine but not in felinine. The reason Roberts (1963) failed to incorporate [^{35}S]-cystine and [^{35}S]-methionine (from yeast) into felinine is not clear. However, Roberts (1963) was also unable to detect the incorporation of [2-^{14}C]-mevalonic acid into felinine while Avizonis and Wriston (1959) and Shapiro (1962) demonstrated that mevalonic acid is a precursor to felinine. Like Avizonis and Wriston (1959) and Shapiro (1962), who incorporated DL-[2-^{14}C]-leucine into felinine, Roberts (1963) was able to detected radioactivity in felinine after administration of DL-[4,5-^{3}H]-leucine to a cat. The present study shows clearly that cysteine and methionine but not sulfate, are precursors to felinine and that cysteine is quantitatively more important as a precursor compared to methionine.

In the present study, the cumulative recovery of radioactivity in the urine of the cats when [^{35}S]-sulfate was injected was 90% after 4 days while the cumulative recovery of the radioactivity from cysteine and methionine were 58 and 36%, respectively, after 9 days. Sulfate is not metabolized in the body of cats, and as a result most radioactivity was excreted in the urine of the cats within the first 4 days. Cats mainly use methionine to synthesise proteins/peptides, as a source of cysteine and as a methyl donor, while cysteine is mainly used to synthesise proteins/peptides, gluthathionine and felinine. It appears that only very small amounts of absorbed dietary cysteine and methionine passed through the cats unmetabolised as the cumulative urinary excretion of [^{35}S]-cysteine and [^{35}S]-methionine injected was less than 0.8% over the 9-day experimental period. The cumulative recovery of radioactivity from methionine and cysteine over the 9-day collection periods was, as expected, well below 100%, since both compounds would have been incorporated into body proteins, which exhibit variable biological half-lives ranging from a few minutes to several months (Mathews et al., 1999). The level of [^{35}S] recovery from methionine was lower compared to that of cysteine. The latter is understandable, as some methionine would have been incorporated into body protein, and not all the methionine would have been used to synthesize cysteine via metabolism to homocysteine and cystathionine. Due to the low activity of the enzyme cysteine dioxygenase in the liver of cats (Knopf et al., 1978), and the efficient transamination of any formed cysteinesulfinic acid to β-sulfinylpyruvate (Edgar et al., 1998), taurine synthesis from cysteine in cats is limited. Cysteine, however, seems to be efficiently used to synthesize felinine in cats.

The site of felinine synthesis in vivo remains unknown. There is convincing evidence that felinine is synthesized from the same isoprenoid pool as cholesterol (Hendriks et al., 1995c) and from the present study it is apparent that the sulfur atom originates from cysteine and methionine. The formation of felinine can occur through a condensation reaction of an allylic carbonium ion and cysteine. Free felinine, however, can only be found in the kidney, urine and bladder of cats, and cannot be found in the free form in the: liver; skin; blood; intestines; pancreas; or spleen of cats (Hendriks, unpublished). This indicates that felinine is either synthesized in the kidney and excreted directly, or synthesized in other tissues and transported in the blood as part of a larger molecule. Studies are currently underway to determine the site of felinine synthesis in domestic cats.

References

AAFCO, 2000. Official Publication of the Association of American Feed Control Officials Inc., Atlanta, GA.

Anon, 1992. Code of Ethical Conduct for the Use of Live Animals for Teaching and Research. Rev. ed., Massey University, Palmerston North, New Zealand.

Avizonis, P.V., Wriston, J.C., 1959. On the biosynthesis of felinine. Biochim. Biophys. Acta 34, 279–281.

Datta, S.P., Harris, H., 1951. A convenient apparatus for paper chromatography. Results of a survey of the urinary amino acid patterns of some animals. J. Physiol. 114, 39P–41P.

Edgar, S.E., Kirk, C.A., Rogers, Q.R., Morris, J.G., 1998. Taurine status in cats is not maintained by dietary cysteinesulfinic acid. J. Nutr. 128, 751–757.

Hendriks, W.H., Tarttelin, M.F., Moughan, P.J., 1995a. Twenty-four hour felinine excretion patterns in entire and castrated cats. Physiol. Behav. 58, 467–469.

Hendriks, W.H., Woolhouse, A.D., Tarttelin, M.F., Moughan, P.J., 1995b. Synthesis of felinine, 2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid. Bioorg. Chem. 23, 89–100.

Hendriks, W.H., Moughan, P.J., Tarttelin, M.F., Woolhouse, A.D., 1995c. Felinine: a urinary amino acid of Felidae. Comp. Biochem. Physiol. 112B, 581–588.

Hendriks, W.H., Wamberg, S., Tarttelin, M.F., 1999. A metabolism cage for quantitative urine collection and accurate measurements of water balance in adult cats (*Felis catus*). J. Anim. Physiol. Anim. Nutr. 82, 94–105.

Knopf, K., Sturman, J.A., Armstrong, M., Hayes, K.C., 1978. Taurine: An essential nutrient for the cat. J. Nutr. 108, 773–778.

Mathews, C.K., van Holde, K.E., Ahern, K.G., 1999. Biochemistry, 3rd ed. Addison Wesley Longman, San Francisco, CA, p. 1186.

Roberts R.N., 1963. A study of felinine and its excretion by the cat. PhD., State University of New York, Buffalo.

Shapiro I.L., 1962. In vivo studies on the metabolic relationship between felinine and serum cholesterol in the domestic cat. PhD., University of Delaware, Delaware.

Tarttelin, M.F., Hendriks, W.H., Moughan, P.J., 1998. Relationship between blood testosterone and urinary felinine in the growing kitten. Physiol. Behav. 65, 83–87.

Wang T.M., 1964. Further studies on the biosynthesis of felinine. PhD., University of Delaware, Delaware.

Westall, R.G., 1953. The amino acids and other ampholytes of urine. 2. The isolation of a new sulphur-containing amino acid from cat urine. Biochem. J. 55, 244–248.