Protein synthesis in rat tissues during lactation

No effect of diethyl ether anaesthesia

David A. SAMPSON,* Marc MASOR and G. Richard JANSEN

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523, U.S.A.

(Received 28 August 1984/Accepted 10 October 1984)

1. Rates of protein synthesis were measured in mammary gland, liver, intestinal mucosa and muscle of lactating rats by using a flooding dose of [^3H]phenylalanine that was injected into diethyl ether-anaesthetized dams via a lateral vein or into undisturbed dams via a jugular cannula. 2. Ether anaesthesia did not alter rates of protein synthesis significantly in any tissue.

Protein synthesis in the lactating mammary gland is likely to be crucial for milk secretion, providing intracellular protein for tissue maintenance as well as secretory protein for milk (Sampson & Jansen, 1984a). The flooding-dose method of Garlick *et al.* (1980) appears to be particularly appropriate for measuring rates of protein synthesis *in vivo*. An advantage of this method is that export proteins such as milk proteins are included in the measurement of synthesis rates (Waterlow *et al.*, 1978; Waterlow & Stephen, 1981). In the flooding-dose method, a bolus of 150 mM-phenylalanine radiolabelled with ^3H is injected via a lateral tail vein, and tissues are removed 2 or 10 min later for analysis of radioisotope incorporation into tissue protein. To inject the radioisotope solution, the rat must be restrained or lightly anaesthetized, either of which stresses the animal. Stress and anaesthesia both decrease mammary blood flow (Linzell, 1974) and thus both potentially affect measurement of protein synthesis in that tissue during lactation. Hanwell & Linzell (1972a) reported that pentobarbital anaesthesia did not alter total cardiac output in rats during lactation, although they did not measure mammary blood flow itself. However, injection of pentobarbital requires handling the animal to be anaesthetized. Elsewhere Hanwell & Linzell (1972b) reported that merely removing lactating rats from litters and restraining them

gently by hand decreased cardiac output to mammary tissue by 60%.

The objective of this study was to determine if diethyl ether anaesthesia affected rates of protein synthesis measured by the flooding-dose method of Garlick *et al.* (1980) in mammary gland and other tissues of the lactating rat. We compared rates of protein synthesis in anaesthetized dams at mid-lactation given radioisotope via a lateral tail vein with rates in undisturbed dams given radioisotope via an indwelling jugular cannula. The results indicate that fractional rates of protein synthesis were not significantly affected by ether anaesthesia in any tissue, including mammary gland.

Experimental

Some 22 female rats (Charles River CD; Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) weighing 205 ± 2 g at breeding were mated with males of the same age and strain. Animals were offered stock diet (Wayne Lab Blox; Continental Grain Co., Chicago, IL, U.S.A.) and water *ad libitum* throughout the experiment. Animal rooms, controlled for temperature (18–22°C) and humidity (45–55%), provided light from 07:00 to 19:00 h. Dams were housed in wire-bottomed stainless-steel cages until day 18 of gestation, when they were moved to plastic cages lined with wood chips. Litters were culled to ten and eight pups on days 1 and 3 of lactation respectively. At day 12 of lactation, a cannula was implanted in the right jugular vein of 11 randomly selected dams as described below. At day 15 of lactation, dam weights were the same in cannulated and non-cannulated dams (293 ± 3 g). At this

* To whom correspondence should be sent. Present address: Western Human Nutrition Research Center, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 29997, Presidio of San Francisco, CA 94129, U.S.A.

time, a solution containing 150 mM-L-phenylalanine (Sigma, St. Louis, MO, U.S.A.) and 50 μ Ci of L-[4- 3 H]phenylalanine (Amersham, Arlington Heights, IL, U.S.A.)/ml was injected at 1.0 ml/100 g body wt., via a jugular cannula in the cannulated group or via a lateral tail vein in the non-cannulated group. Dams given radioisotope via a tail vein were lightly anaesthetized for 1–2 min with diethyl ether to facilitate the injection. Tail-vein injection was done with 26-gauge needles fitted to disposable 5 ml syringes. Dams given radioisotope via a jugular cannula were unanaesthetized during injection. Cannula injection was done with a 5 ml disposable syringe connected to the distal end of the cannula with a blunt 21-gauge needle. During radioisotope injection, unanaesthetized (i.e. cannulated) dams did not become agitated, leave their suckling pups or show other signs of stress. Radioisotope was injected into both groups in 3–5 s once syringes were in place. Dams were decapitated 2 min ($n = 5$) or 10 min ($n = 6$) after injection of radioisotope. Left inguinal–abdominal mammary gland, median lobe of liver, calf muscle (including gastrocnemius, extensor digitorum longus, plantaris and soleus plus surrounding connective tissue) and duodenal–jejunal mucosa were quickly removed, rinsed in 0.9% (w/v) NaCl and frozen in liquid N_2 . Intestinal mucosa was obtained by the procedure described by McNurlan *et al.* (1979). Fractional rates of protein synthesis were estimated from incorporation of [3 H]phenylalanine into tissue protein by the method of Garlick *et al.* (1980). The fractional synthesis rate (J_s), expressed as the percentage of protein mass synthesized per day, was calculated as:

$$K_s = \frac{S_B}{S_A \cdot t} \times 100$$

where S_A is the average specific radioactivity of free phenylalanine during the incorporation period, estimated from free phenylalanine specific radioactivity at 2 and 10 min after injection (Sampson & Jansen, 1984b), S_B is the specific radioactivity of protein-bound phenylalanine at 10 min, and t is the time in days between radioisotope injection and decapitation. Data from the anaesthetized (non-cannulated) and unanaesthetized (cannulated) groups were compared by Student's t test.

From day 5 until day 11 of lactation, rats to be cannulated were exposed at close quarters to the sound of Velcro pieces being separated for 5 min each day. Rats in this group were cannulated on day 12 of lactation by the procedure described by Axelson & Bruot (1982). Briefly, 30 cm of Silastic tubing (0.64 mm internal diam., 1.19 mm outer diam.) was implanted in the right jugular vein and

exteriorized in the dorsal cervical region of ether-anaesthetized rats. Velcro hook and pile pieces (Velcro U.S.A. Inc., Manchester, NJ, U.S.A.) measuring 5.0 cm $\times$ 2.5 cm were separated and a small hole was cut in the centre of one half. This piece was sutured on to the rat's dorsal neck so that the cannula's distal end exited through the hole. The exteriorized cannula was coiled and secured between the sutured Velcro and its matching piece. The cannulas and Velcro 'back-packs' did not interfere with dam mobility or pup nursing behaviour. Because of preoperative conditioning described above, the top piece of Velcro could be removed and the cannula utilized without agitating the dam or her pups.

Results and discussion

Diethyl ether anaesthesia did not significantly affect fractional synthesis rates of protein in mammary gland, liver, intestinal mucosa or calf muscles (Table 1). It should be emphasized that cannulated dams in the unanaesthetized group showed no signs of agitation or stress during the procedure in which upper Velcro pieces were removed and cannulas unfastened and utilized for radioisotope administration. The results suggest that light ether anaesthesia and the associated animal handling used to facilitate radioisotope injection into tail veins did not affect protein-synthesis rates in mammary gland, liver, intestine or muscle measured by the flooding-dose method of Garlick *et al.* (1980).

The flooding-dose method has been used previously to measure fractional synthesis rates of protein in liver, muscle and intestine (McNurlan *et al.*, 1979; Garlick *et al.*, 1980; Goldspink *et al.*, 1984). Sampson & Jansen (1984b) validated this method for use in lactating mammary gland of the rat. One technique for restraining rats during administration of the flooding dose of radio-labelled amino acid is to roll the animals in a towel

Table 1. Fractional synthesis rates of protein during lactation in ether-anaesthetized or unanaesthetized rats (%/24 h)

For experimental details, see the text. Anaesthetized dams received a flooding dose of [3 H]phenylalanine via a lateral tail vein; unanaesthetized dams received the flooding dose via a jugular cannula. Values are means $\pm$ S.E.M. Means for the anaesthetized and unanaesthetized groups did not differ significantly at $P = 0.05$ for any tissue.

Tissue	Anaesthetized	Unanaesthetized
Mammary gland	124 $\pm$ 11	100 $\pm$ 6
Liver	70 $\pm$ 3	65 $\pm$ 2
Intestinal mucosa	98 $\pm$ 12	124 $\pm$ 16
Calf muscle	3.1 $\pm$ 0.2	3.3 $\pm$ 0.4

(McNurlan *et al.*, 1979; Garlick *et al.*, 1980). We have found that this technique causes the rat to struggle, with the intensity of struggle varying considerable from rat to rat. As an alternative, we have used light ether anaesthesia during radioisotope injection in studies of maternal diet and tissue protein synthesis during lactation (Sampson & Jansen, 1984*b,c*). In our hands, this technique has the advantage of inducing much more uniform conditions for radioisotope injection than is obtained by using towel restraint. However, this approach requires subjecting the animals to brief ether anaesthesia. Results of the study reported here suggest that use of light diethyl ether anesthesia does not affect subsequent incorporation of [³H]phenylalanine into tissue protein. Using this method, we have reported that protein synthesis in the mammary gland during lactation is highly correlated with milk yield ($r^2 = 0.72$; $P < 0.001$; $n = 39$; Sampson & Jansen, 1984*c*).

We acknowledge the reliable and expert technical assistance of Mr. Harry Hunsaker, Mr. Earle Horton and Ms. Kelly Eager. This study was funded in part by the U.S. Department of Agriculture Competitive Grants Program in Human Nutrition, Grant no. 59-2081-1-1-648-0.

References

- Axelson, J. E. & Bruot, B. C. (1982) *Physiol. Behav.* **29**, 949–951
- Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980) *Biochem. J.* **192**, 719–723
- Goldspink, D. F., Lewis, S. E. M. & Kelly, F. J. (1984) *Biochem. J.* **217**, 527–534
- Hanwell, A. & Linzell, J. L. (1972*a*) *J. Endocrinol.* **53**, lvii–lviii
- Hanwell, A. & Linzell, J. L. (1972*b*) *J. Physiol. (London)* **226**, 24*P*–25*P*
- Linzell, J. L. (1974) in *Lactation* (Larson, B. L. & Smith, V. R., eds.), vol. 1, pp. 143–225, Academic Press, New York
- McNurlan, M. A., Tomkins, A. M. & Garlick, P. J. (1979) *Biochem. J.* **178**, 373–379
- Sampson, D. A. & Jansen, G. R. (1984*a*) *Annu. Rev. Nutr.* **4**, 43–67
- Sampson, D. A. & Jansen, G. R. (1984*b*) *J. Pediatr. Gastroenterol. Nutr.* **4** in the press
- Sampson, D. A. & Jansen, G. R. (1984*c*) *J. Nutr.* **114**, 1470–1478
- Waterlow, J. C. & Stephen, J. M. L. (1981) *Nitrogen Metabolism in Man*, Applied Science, London
- Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, North-Holland Publishing Co., New York