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Effect of ovulation on haem metabolism in rabbits

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Abstract. To investigate the origin of the cyclic changes in the rate of endogenous carbon-monoxide production ($\dot{n}_{CO}$) during the menstrual cycle, haem turnover was determined before and after chorion gonadotropic hormone-induced ovulation in six female rabbits.

^{14}C-labelled delta-aminolevulinic acid and glycine were administered and the excretion rate of ^{14}CO ($\dot{A}_{^{14}CO}$) was measured for determination of hepatic and bone-marrow haem turnover, respectively. Carbon-monoxide production ($\dot{n}_{CO}$) was measured to estimate total haem turnover.

After ovulation $\dot{A}_{^{14}CO}$ was increased significantly the first 2 h of the early labelled peak after ^{14}C-ALA administration and was increased also at the first determination during the early peak after ^{14}C-glycine but statistically not significantly. The total excretion of labelled CO during the period of the early labelled peak was not increased with any of these precursors for haem synthesis. On the other hand $\dot{n}_{CO}$ was increased 34% ($P < 0{\cdot}05$) during the post-ovulation period.

As the increase in 'unassigned' haem turnover was small and may be unaccompanied by a contemporary increase in bilirubin/CO production, it was concluded that the increase in $\dot{n}_{CO}$ during the post-ovulation period essentially depends on increased destruction of circulating red cells in the rabbit.

Keywords. Endogenous CO production, haem turnover, ovulation, rabbit.

Introduction

Fertile women have a regular increase in haem turnover from the oestrogen to the progesterone phase of menstruation, judged from the rate of endogenous carbon-monoxide production ($\dot{n}_{CO}$) [1–3].

As carbon-monoxide results from the breakdown of haem the cause of the variation in $\dot{n}_{CO}$ has been suggested to be either changes in haem breakdown from erythrocytes or modification of hepatic haem turnover.

As delta-aminolevulinic acid (ALA) is a precursor in hepatic and glycine in hepatic as well as haemoglobin haem synthesis, ^{14}C-labelled ALA and glycine can be used to label these haem pools [4]. Subsequent determination of the early peak in production of ^{14}CO following administration of ^{14}C-ALA and ^{14}C-glycine give an estimate of hepatic and bone-marrow haem turnover [5]. Simultaneous determination of total $\dot{n}_{CO}$ gives an estimate of the degradation of circulating red cells in addition to the hepatic and bone-marrow haem turnover.

This technique has been used before and after the administration of chorion gonadotropic hormone to female rabbits to find the origin of the variation of $\dot{n}_{CO}$ during the menstrual cycle.

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Material and Methods

The studies were performed in six white female rabbits, 1–2 years of age, weight 3–4·5 kg, before and 5–16 days after the administration of 200 IE chorion gonadotropic hormone (Gonadex, AB Leo, Helsingborg).

Ten microcuries (0·37 MBq) ^{14}C-5-delta-aminolevulinic acid (New England Nuclear) in phosphate buffer pH 7·4, was injected i.m. The resultant expired ^{14}CO was determined 2, 4, 8, 24 and 48 h later. One week later 50 μCi (1·85 MBq) ^{14}C-2-glycine was administered i.m. whereafter expired ^{14}CO was assessed 8, 24, 48, 72 and 96 h later (Fig. 1).

For determination of expired ^{14}CO the animals were placed in a tight plexiglass hood for about 120 min each time. Through the hood air was sucked with a flow rate adjusted to 1·0–1·2 l min^{-1} and passed through silica gel, soda lime and potassium hydroxide thus removing moisture and carbon dioxide (CO_2). Carbon monoxide in the remaining air was oxidized to CO_2 by a Hopkalite catalyst (Mine Safety Appliances Co.). After passing a flowmeter and a pump the CO_2 was trapped by an ethanolamine containing glass column [6]. The radioactivity of the ethanolamine was measured in a liquid scintillation counter (Packard Instrument Co.) after the addition of PPO and bis-MSB scintillators (New England Nuclear) dissolved in toluene.

The rate of excretion of labelled CO was integrated over the first 48 h after ^{14}C-ALA and the first 4 days after ^{14}C-glycine administration to calculate the area of

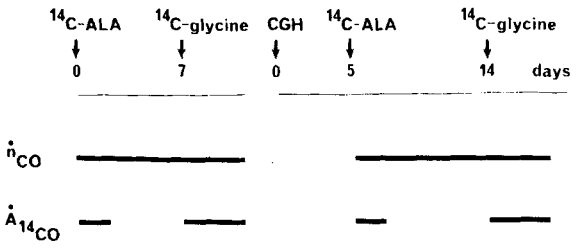

Figure 1. Design of the experiment.

the early labelled peak—i.e. the fraction of the radioactivity of the precursor recovered as labelled CO within that period.

The $\dot{n}_{CO}$ was determined immediately after the periods of ^{14}CO collection from the flow rate and the difference in CO concentration between the in- and outgoing air from the hood determined with an infrared (Maihak AG) or Hopkalite (AB Stålex, Stockholm) CO analyser.

The total amount of haemoglobin in the animal (n_{tHb}) was determined according to Tribukait [7]. During this measurement the system was changed to a closed rebreathing circuit with Hopkalite catalyst and ethanolamine column excluded. Oxygen (O_2) substance fraction in the air was kept constant at 21% by an O_2 monitor allowing addition of $O_2$when needed. After 20–30 min equilibration 10–12 ml pure CO was added to the system. After further 60 min rebreathing the partial pressures of O_2 and CO in alveolar air was calculated from the barometer pressure and the substance fraction of these gases in the rebreathing air. Haemoglobin carbon-monoxide saturation (S_{CO}) was calculated according to the Haldane equation:

$$\frac{S_{CO}}{S_{O_2}} = \frac{P_{CO} \times M}{P_{O_2}},$$

and the total amount of haemoglobin from the equation:

$$n_{tHb} = \frac{n_{CO}}{S_{CO}},$$

where M was assumed to be 220, n_{CO} is the amount of CO taken up from the rebreathing air by the animal. No correction was made for binding of CO by extravascular haem. The volume of air in the system without rabbit was 16 l. The n_{tHb} determinations were performed in duplicate before and after hormone administration.

The coefficient of variation of $\dot{n}_{CO}$ calculated from duplicate determinations was 40% ($n=14$). The $\dot{n}_{CO}$ was determined 7–14 times for each rabbit at each of the two periods.

As the same six rabbits have been examined before and after hormone administration each animal has been used as its own control. Significance of change was determined by the paired Student's t-test. Non parametric (two-tailed) sign test and analysis of variance were also carried out and showed the same significance [8]. $P=0{\cdot}05$ was chosen as significance level.

Results

The early peaks in ^{14}CO production after ^{14}C-ALA and ^{14}C-glycine administration are presented in Figs 2 and 3, respectively. Following chorion gonadotropic hormone administration ^{14}CO production was significantly increased ($P<0{\cdot}05$) 2 h after ^{14}C-ALA injection. The figures then were similar at the subsequent estimations. The same pattern was shown by the ^{14}CO determinations following ^{14}C-glycine administration but in this case the difference between the initial (8 h) figures of ^{14}CO elimination before and after hormone administration did not reach statistical significance.

The area of the early labelled peak before and after hormone administration corresponded to 6·0 and 5·2% ($P=0{\cdot}50$), respectively, of the dose of ^{14}C-ALA and 0·028 and 0·044% ($P=0{\cdot}20$), respectively, of the dose of ^{14}C-glycine injected.

The $\dot{n}_{CO}$ increased significantly from 1·04 μmol h^{-1} during the control period to 1·39 μmol h^{-1} ($P<0{\cdot}05$) after hormone administration.

No significant change in n_{tHb} was found when pre- and post-hormone determinations were compared.

Discussion

In this study we have used the CO production as a measure of total haem breakdown and ^{14}CO production following ^{14}C-5-aminolevulinic acid and ^{14}C-2-glycine administration as a measure of hepatic and hepatic as well as early haemoglobin haem turnover, respectively.

By chorion gonadotropic hormone administration to the female rabbits we tried to imitate the physiological process during female menstruation cycle and determined haem turnover before and after ovulation. Ovulation occurs about 10 h after hormone injection and this pseudo-pregnancy lasts for about 16 days [9]. An effect on haem turnover can be expected within a few days [10] and the studies were performed during the period of 5–16 days after hormone administration. As the lifespan of the red cell of the rabbit is about 60 days [11], care was taken not to allow ^{14}CO elimination from degradation of senescent red cells labelled by ^{14}C-glycine administration during the control period to influence the results of the post-hormone periods of ^{14}CO determination.

The flow rate of air through the hood must be low, so that the difference in CO concentration between in- and outgoing air can be detected, but on the other hand the flow must be kept high enough not to interfere with pulmonary gas exchange by rebreathing. A flow rate of about 1·0 l min^{-1} was chosen and was checked to be appropriate by blood gas analysis.

The results demonstrated after injection of chorion gonadotropic hormone to the rabbits a statistically significant increase in ^{14}CO production after ^{14}C-ALA

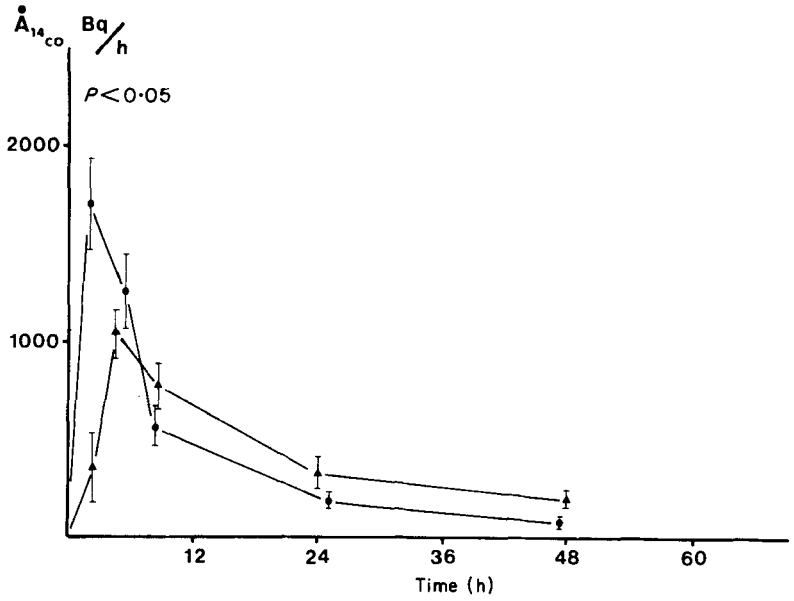

Figure 2. The rate of elimination of radioactivity of ^{14}CO ($\dot{A}_{14_{CO}}$, Bq h^{-1}) following ^{14}C-ALA administration before (▲) and after (●) chorion gonadotropic hormone administration in six female rabbits (mean ± SD).

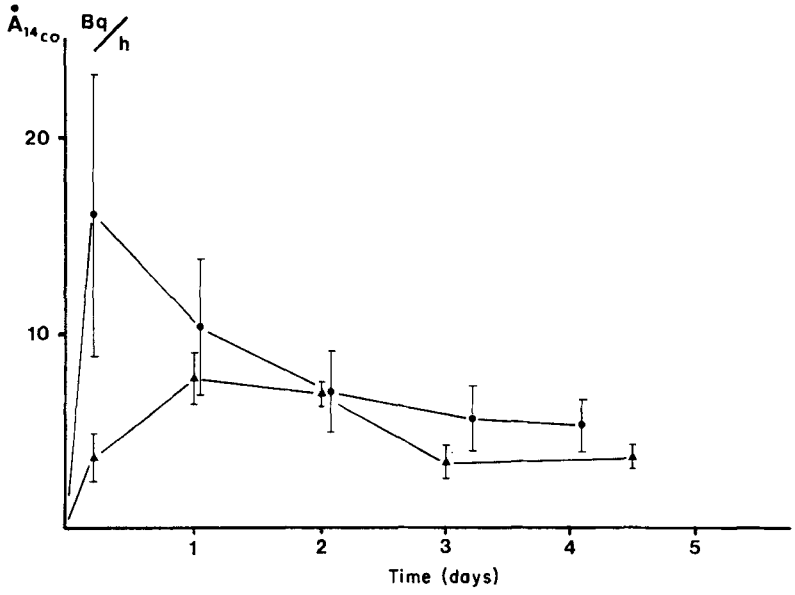

Figure 3. The rate of elimination of radioactivity of ^{14}CO ($\dot{A}_{14_{CO}}$, Bq h^{-1}) following ^{14}C-glycine administration before (▲) and after (●) chorion gonadotropic hormone administration in six female rabbits (mean ± SD).

administration. A similar increase was recorded in ^{14}CO production after ^{14}C-glycine. In both instances the increase was confined to the first few hours after administration of labelled haem precursor.

This period probably reflects the turnover of an unassigned haem pool, or of haem proteins with very short half lives that may be found both in the liver and in the bone marrow [12]. There is, however, in the early labelled peak after ^{14}C-glycine no indication of an increased bone-marrow haemolysis or exaggerated reticulocyte destruction after hormone administration.

The area of the early labelled peak after ^{14}C-ALA was unchanged after hormone administration and that after ^{14}C-glycine increased, but statistically not significantly. An increase in early labelled peak unaccompanied by an increase in total bilirubin/CO production has been demonstrated earlier [13]. The increase in $\dot{n}_{CO}$ after hormone administration corresponds to a doubled hepatic and bone-marrow haem turnover, if that fraction is 1/3 of the total $\dot{n}_{CO}$ [14]. Judged from the areas of the early labelled peak, no significant increase in hepatic or bone-marrow haem turnover occurred after hormone administration. Thus, the increase in

total $\dot{n}_{CO}$ could rather be ascribed to an increased destruction of circulating red cells, supporting the results of Mercke *et al.* [3], demonstrating a reduced filterability of red cells during the progesterone phase of the menstrual cycle.

The $\dot{n}_{CO}$ figures confirmed earlier results from human studies with increased $\dot{n}_{CO}$ during the progesterone phase of the menstrual cycle. The results are in analogy with those of Mercke *et al.* [3] concerning females on gestagen medication but the increase in $\dot{n}_{CO}$ during the progesterone phase is smaller than that reported in females not taking contraceptive drugs.

In the rabbit the increase in haem turnover after ovulation thus could be referred to increased red-cell breakdown mainly and to a smaller part of increased 'unassigned' haem turnover.

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