THE HALF-LIFE OF HUMAN PLACENTAL LACTOGEN IN NON-IMMUNE AND IMMUNE RHESUS MONKEYS*

J. SWANSON BECK, JENNIFER M. O. MELVIN AND G. HEMS

*Department of Pathology, University of Dundee, and
Departments of Pathology and Social Medicine,
University of Aberdeen, Scotland*

(Received 7th January 1974)

Summary. A dose of human placental lactogen (HPL), calculated to produce an initial plasma concentration ten times the maximal levels found in pregnant women, was injected intravenously into adult non-pregnant female rhesus monkeys (*Macaca mulatta*). The disappearance curves in non-immune animals can be best explained by a double exponential function: the half-life of HPL in these animals was approximately 10 min in the first phase and approximately 50 min in the second phase.

Circulating HPL could not be demonstrated in the serum of monkeys immunized previously with HPL.

INTRODUCTION

The polypeptide hormone, human placental lactogen (HPL), otherwise known as human chorionic somato-mammotrophin, can be detected in the trophoblast about 12 days after fertilization (Beck, 1970) and is present in the placenta throughout pregnancy (Currie, Beck, Ellis & Read, 1966); the plasma level rises progressively during pregnancy and at term reaches a maximal level which is approximately 1000 times greater than that of the related hormone, human growth hormone (Beck, Parker & Daughaday, 1965). Several different physiological functions have been suggested for this hormone, namely lactogenic (Josimovich & Maclaren, 1962), lutetrophic (Josimovich, Atwood & Goss, 1963) and somatotrophic (Kaplan & Grumbach, 1964), but its exact biological rôle in the pregnant woman has not been established. An antigenically related hormone, monkey placental lactogen (MPL), is found in the monkey placenta (Kaplan & Grumbach, 1964) and presumably fulfills the same physiological function in this species, in view of the close similarity of reproductive processes within the primates.

In our experiments on the rôle of endogenous MPL in pregnant monkeys, we have immunized non-pregnant female rhesus monkeys with HPL (MPL was not available at that time) in the hope that we would induce the production of circulating anti-HPL antibodies that would cross-react with MPL produced

* Reprint requests to: Professor J. Swanson Beck, Department of Pathology, University of Dundee, Dundee DD1 4HN, Scotland.

during pregnancy and so achieve biological neutralization of this hormone. The first step in this programme was to determine whether immunization with HPL modified the rate of disappearance of HPL administered by intravenous injection into non-pregnant adult female monkeys.

MATERIALS AND METHODS

Monkeys

Six healthy adult non-pregnant female rhesus monkeys (*Macaca mulatta*) were studied. Three of the animals had not been immunized.

The other three animals had been immunized with a Friesen (1965) extract of HPL emulsified in Freund's incomplete adjuvant: the immunization schedule and the methods used for determination of the specificity and for titration of their antisera have been reported previously (Beck, Melvin & Masson, 1971).

Administration of HPL and collection of blood samples

The monkeys were restrained in a handling box (Melvin & Beck, 1969) during these experiments. Approximately 20 mg HPL (Lederle PPP(H), Batch No. 717240) dissolved in 5 ml sterile 0.15 M-NaCl were injected into a saphenous vein of each animal: this dose was calculated to give an initial concentration of approximately 100 μ g HPL/ml plasma. In control experiments, 5 ml sterile 0.15 M-NaCl was injected instead of the HPL solution. Blood samples were removed at timed intervals after the intravenous injection (5, 15, 30, 45, 60 and 120 min for non-immune animals; 5, 15 and 30 min for immune monkeys). In order to minimize disturbance of the animal, the same saphenous vein was used for administration of HPL and collection of blood. Blood samples were allowed to clot for 24 hr at 4°C: the serum was separated and then stored at -20°C.

Since immune animals are liable to anaphylaxis on re-exposure to the immunizing antigen, every monkey, irrespective of its immune status, was given an intramuscular injection of 25 mg promethazine hydrochloride ('Phenergan') daily for 3 days before the start of the experiment and also 30 min before the intravenous injection.

Radioimmunoassay of HPL levels

The HPL (Lederle) and an extract (Raben, 1957) of human growth hormone (HGH) were labelled with ^{125}I (Beck *et al.*, 1971), using a modification of the method of Hunter & Greenwood (1964). The labelled antigen usually had specific activity of 80 to 100 $\mu\text{Ci}/\mu\text{g}$: each batch was stored at 4°C and discarded after 2 weeks. A standard solution of HPL (10 ng/ml) in 0.05 M-phosphate buffer, pH 7.5, was stored at -20°C. Goat anti-HPL and anti-HGH antisera (Beck *et al.*, 1971) were both used at a dilution of 1:64,000 in the radioimmunoassay. The diluent solution was 0.2% bovine serum albumin in 0.05 M-phosphate buffer, pH 7.5.

The experimental design for the radioimmunoassay was similar to that used by Hunter & Greenwood (1964). The incubation mixtures were prepared in a series of tubes, to each of which was added sequentially: (i) 0.2 ml of appro-

priate dilution of monkey serum, or diluent solution, or dilution of standard solution containing 0.625, 1.25, 2.5, 5.0 or 10.0 ng HPL/ml; (ii) 0.05 ml antiserum dilution or diluent solution, and (iii) 0.05 ml of a solution containing 4 ng labelled antigen/ml. The tubes were covered with 'Parafilm' and left for 48 hr at 4°C.

Bound and free antigen were separated on wicks of Whatman 3MC chromatography paper as described by Ørskov, Thomsen & Yde (1968). The wicks were cut in half and counted in a Tracerlab Gamma/Guard II automatic gamma counter. The ratios of free/bound antigen were then calculated. The standard curve was used to determine the HPL concentrations in the monkey serum samples.

Serum samples were assayed at three different dilutions, each in triplicate: each sample was assayed on at least two separate occasions. For each sample, the HPL concentration was expressed as the arithmetic mean of these observations.

Samples from non-immune monkeys were assayed using [125 I]HPL and goat antiserum to HPL, but sera of the immune animals could not be measured with this system, since the high-titre monkey antibody to HPL would interfere with the assay. Since monkey antisera to HPL do not cross-react with HGH (Beck *et al.*, 1971), it was possible to measure free HPL levels in the serum of immune monkeys in a radioimmunoassay by using goat antiserum to HGH (which does cross-react with HPL) and [125 I]HGH.

RESULTS

Non-immune monkeys

Serum concentrations of HPL in each animal measured directly with the anti-HPL radioimmunoassay after intravenous injection of approximately 20 mg HPL were plotted on an arithmetic scale against time (Text-fig. 1). From these curves, it seemed that the rate of disappearance of HPL in non-immune monkeys during the first 60 min of the experiment was different from that during the second 60 min. Substantially similar results were obtained when these samples were assayed indirectly with the anti-HGH radioimmunoassay.

Since it is probable that the mixing and distribution time will be less than 5 min after intravenous injection of HPL, the observed HPL levels were expressed as percentages of that of the 5-min sample for each animal (Table 1). The mean of the percentage values were calculated and plotted on a logarithmic scale against time. The data followed a fairly smooth curve (Text-fig. 2), and could be expressed as the sum of two exponential functions (Riggs, 1963) with half-lives of about 10 min and 50 min respectively.

After injection of 0.15 M-NaCl, HPL was not detected in the serum of the non-immune monkeys.

Immune monkeys

Using the indirect anti-HGH radioimmunoassay, HPL was not detected in any serum sample removed after intravenous injection of approximately 20 mg HPL into any of the three monkeys that had been immunized previously with

TEXT-FIG. 1. The serum concentration of HPL in three rhesus monkeys (Δ , Monkey 18; $\square$, Monkey 26; $\circ$, Monkey 27) at intervals up to 2 hr after intravenous injection of approximately 20 mg HPL.

Table 1. The disappearance of injected HPL from the circulation of non-immune rhesus monkeys

Monkey No.	HPL concentration expressed as % of 5-min value at time (min):						
	5	15	30	45	60	90	120
18	100	48.3	24.9	—	2.6	1.8	2.1
26	100	43.0	22.5	7.8	4.1	2.2	1.1
27	100	52.0	36.9	6.4	3.2	2.2	0.4
Mean	100	47.7	28.1	7.1	3.3	2.1	1.2

HPL, human placental lactogen.

HPL. The rapid removal of HPL is almost certainly the result of an immunological reaction in which circulating antibody to HPL combines with the injected hormone. The half-life of HPL in the immune monkeys is therefore zero.

After intravenous injection of 0.15 μ -NaCl, HPL was not detected in the serum of the immune monkeys.

TEXT-FIG. 2. The disappearance of injected HPL from the circulation of rhesus monkeys. The serum concentrations are expressed as the mean of the percentages of the 5-min level.

DISCUSSION

The mode of disappearance of HPL from the monkey circulation has not been reported previously. In this study, the serum levels of HPL were assayed in adult female non-pregnant monkeys after intravenous injection of a relatively large dose calculated to produce an initial blood concentration approximately ten times the maximal levels found in pregnant women. The observed disappearance curves are better explained by a double exponential function than by a

single one, and we have calculated that the half-life ($t_{1/2}$) of HPL is approximately 10 min in the early rapid phase and approximately 50 min in the later slower phase.

In a detailed study on human subjects, Kaplan, Gursipide, Sciarra & Grumbach (1968) deduced that the disappearance curve of HPL could be resolved into at least two exponential functions and they concluded that the failure of Beck *et al.* (1965) and Samaan, Yen, Friesen & Pearson (1966) to demonstrate a double exponential curve could be explained by the shortness of the sampling period which was restricted to the early part of the disappearance curve. Kaplan *et al.* (1968) calculated that the half-life of HPL following delivery of the placenta ranged from 10.0 to 15.2 min in the first exponential phase and ranged from 32.7 to 61.3 min in the second exponential phase. Our estimates of the corresponding half-lives for the rhesus monkey are consistent with those obtained by Kaplan *et al.* (1968) for man: it is possible that minor discrepancies could be explained by species differences in metabolism of HPL, but we do not believe that they should necessarily be regarded as significant.

Meyer & Knobil (1969) have shown that HGH had a double exponential disappearance curve in the rhesus monkey and that the half-life values of injected HGH and monkey growth hormone were similar in the first exponential phase. These experiments, like those reported in this paper, suggest that the metabolism of the analagous polypeptide hormones is similar in man and monkey.

Immune monkeys

Since the indirect anti-HGH radioimmunoassay detected HPL quantitatively in the sera of non-immune monkeys, it seems reasonable to deduce from our failure to detect HPL in the sera of the immune monkeys that there was no free HPL in these sera. This implies that the immune monkeys rapidly removed large quantities of injected HPL from the circulation. Since the preparation of HPL that we used has been shown to be biologically active (Florini, Tonelli, Breuer, Coppola, Ringler & Bell, 1966), we can deduce that this preparation has probably been biologically neutralized in these animals. The serum of monkeys immunized with HPL does not, however, appear to cross-react with MPL (Beck *et al.*, 1971) and so it is highly improbable that immunity to HPL would have any effect on the half-life or on the biological activity of endogenous MPL.

ACKNOWLEDGMENTS

This work was supported by a grant to Professor A. R. Currie and J.S.B. from the Cancer Research Campaign. We wish to thank Dr A. Glick (Lederle Laboratories, Pearl River, N.Y., U.S.A.) for a gift of Purified Placental Protein (Human) and Dr P. Mary Cotes (Division of Biological Standards, National Institute for Medical Research, London) for the Raben preparation of HGH. We are grateful to Mr R. Robertson for the care of the monkeys.

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