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PLASMA KININ AND KININOGEN LEVELS IN THE FEMALE RAT DURING THE OESTROUS CYCLE, PREGNANCY, PARTURITION AND THE PUERPERIUM

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1 Plasma kininogen concentration of the female rat did not change during the oestrous cycle but increased two-fold during gestation. Plasma kininogen did not change during parturition, rose in the first puerperal day and then rapidly declined to non-pregnant levels.

2 Free kinin levels in the blood of non-pregnant female rats were low and inconstant. Free kinin was undetectable in the blood of pregnant rats, but was found in four out of ten parturient rats.

3 After administration of the kininase inhibitor dimercaprol (30 mg/kg i.p.), free kinin was present in the blood of six parturient rats. Dimercaprol caused appearance of free kinin in the blood of only one out of six non-pregnant rats.

4 It appears that the plasma kinin system is activated to a small degree during parturition.

Introduction

Hawker, Walmsley, Roberts, Blackshaw & Downes (1961) described the occurrence in extracts of blood taken from pregnant and parturient women of oxytocic activity that could not be attributed to oxytocin. Gomes (1958) demonstrated a polypeptide in the urine of pregnant women that was active on smooth muscle, and had the same chemical and pharmacological properties as bradykinin.

Following the work of Armstrong, Jepson, Keele & Stewart (1960), who observed a 95% reduction of glass activated kinin precursor in the plasma of parturient women, Martinez, de Carvalho & Diniz (1962) showed that 25% of the plasma kininogen stores of women were mobilized during labour, but free plasma kinin could not be demonstrated. Periti & Gasparri (1966) found that there was an increase of plasma kininogen during pregnancy, a fall during labour, and a return to prelabour levels during the early puerperium. Similar results were obtained by Wiegershausen, Paegelow, Neumayer & Walter (1967) and Porter, Shennan & Smith (1972).

In an attempt to find a suitable animal model for further studies, Wiegershausen, Klausch, Hennighausen & Sosat (1968) showed that plasma kininogen levels in rats and rabbits rose with advancing gestation and fell during parturition, a similar pattern to the human one.

This study describes the plasma kininogen and free kinin levels found during the oestrous cycle, pregnancy, parturition and the puerperium in the rat.

Methods

Animals

Mature, female albino rats (200-250 g) of the C.S.E. strain were used. They were allowed free access to food and water.

Vaginal smears were taken daily from non-pregnant rats, and the day of the oestrous cycle designated by the criteria of Long & Evans (1922).

For the experiments involving pregnant animals, female rats were housed six to a cage with a male rat. Vaginal smears were taken daily in the morning, and the first day of pregnancy was defined as the day on which spermatozoa were first found in the vagina. Only maternal rats producing six or more young were subsequently used.

Parturition occurred on the night of day 22 or in the early morning of day 23. 'Late day 22' pregnant rats refers to animals which had not delivered their young by the morning of day 23. The beginning of parturition was indicated by the

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rat stretching at frequent intervals. Parturition itself was arbitrarily divided into sections depending upon the number of pups delivered. The group designated 'immediately post partum' consisted of maternal rats taken immediately after delivery to about 2 h after delivery, and the group designated 'day 1 post partum' contained rats which had littered on the night of day 22, used on day 23. On day 1 post partum the number of young per litter was adjusted to eight in lactating rats. In another experiment all the young rats were removed on day 1 post partum (non-lactating rats).

Preparation of apparatus

All syringes (polypropylene), syringe needles and glassware coming into contact with either blood or bradykinin were silicone coated by immersion in a 5% v/v solution of silicone concentrate (Siliclad; Clay Adams, New York).

Collection of blood samples

Rats were lightly anaesthetized with diethyl ether and blood was taken by cardiac puncture into a polypropylene syringe. In the experiments concerning estimation of plasma kininogen, the syringe contained 20 units of heparin. Rats were bled once only.

Plasma kininogen determination

The plasma kininogen level was measured by the method of Diniz & de Carvalho (1963), involving denaturation of plasma and the conversion of the kininogen to free kinin, which is then assayed on the rat isolated uterus preparation with synthetic bradykinin used as the standard. The plasma kininogen concentration is expressed in μg/ml, 1 μg referring to the amount of liberated free kinin being equivalent to 1 μg of synthetic bradykinin. The plasma kininogen level was determined in blood samples taken from rats during the oestrous cycle, pregnancy, parturition and the puerperium.

With the same strain of rat and the same weight range, changes in maternal blood volume during gestation and early puerperium have previously been reported (McCormick & Senior, 1972). These values are used in this study to estimate the total plasma kininogen in groups of pregnant and puerperal rats. The total plasma kininogen is the product of the mean maternal blood volume and the mean kininogen level per ml of plasma.

Free kinin determination

The method used for the estimation of free kinin in the blood of the rat was that of Brocklehurst & Zeitlin (1967). The final dry extract was assayed on the rat isolated uterus preparation with synthetic bradykinin used as standard. The free kinin level was determined in blood samples taken from rats during the oestrous cycle, pregnancy and parturition.

The effect of the kininase inhibitor dimercaprol was studied on the free kinin level of non-pregnant and parturient rats. Six non-pregnant rats were injected with dimercaprol (30 mg/kg i.p.) and 10 min after the injection blood samples were taken and the free kinin content determined. A similar experiment was performed with six parturient rats, dimercaprol (30 mg/kg i.p.) being administered after the delivery of two pups.

Isolated rat uterus preparation

Diethylstilboestrol (500 μg/kg in arachis oil) was administered subcutaneously into a virgin female rat 18 h before use. The uterus was removed and one horn suspended in a tissue bath containing de Jalon's solution at 32° C. The de Jalon's solution used had the following composition (g/l): NaCl, 9; KCl, 0.42; $CaCl_2$, $6H_2O$, 0.12; $NaHCO_3$, 0.5; $MgCl_2$, $6H_2O$, 0.0025; and dextrose, 0.25, and contained atropine sulphate (1 μg/ml) and 2-bromolysergic acid diethylamide (BOL 148; 0.5 μg/ml). Uterine responses were recorded with a Statham tension transducer and a tension on the uterus of 2 g. The record was displayed on a Devices single channel recording unit, Type R2.

In the plasma kininogen assays the de Jalon's solution was aerated with 95% oxygen : 5% carbon dioxide. In the free kinin assays air or oxygen was substituted. Though much less stable the preparations were then much more sensitive to bradykinin.

In all assays of kininogen a four point bioassay was performed, in which the effects of two doses of test solution were compared with those of two doses of synthetic bradykinin. In most of the free kinin assays there was not enough kinin in the extract to perform a four point bioassay so bracketing or dose matching was used.

Frequently, after assay, samples were incubated with chymotrypsin at 37° C for 20 min and tested for residual activity. None was ever detected. As an additional precaution some samples were reassayed on the rat isolated duodenum preparation. Invariably no difference in result was obtained between the two assays.

Drugs used

Atropine sulphate (May and Baker); bradykinin (Synthetic bradykinin, Sandoz); 2-bromolysergic acid diethylamide (BOL 148, Sandoz); diethylstilboestrol (B.D.H.); dimercaprol (B.D.H.); heparin

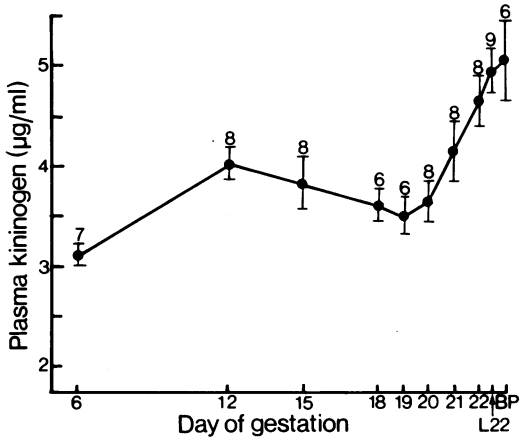

Fig. 1 Plasma kininogen concentration of female rats during pregnancy. L22, late day 22 (see methods section); BP, immediately before parturition. The number of observations and standard errors are indicated.

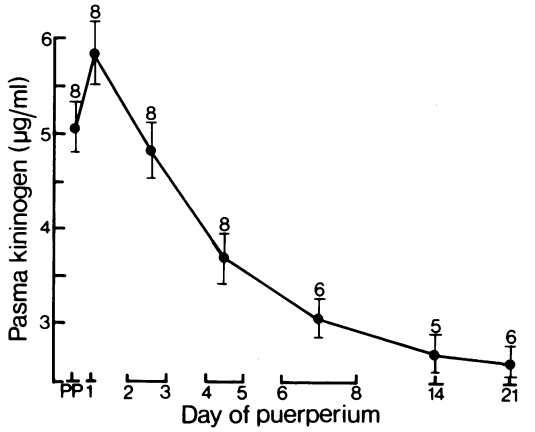

Fig. 2 Plasma kininogen concentration of lactating rats during the puerperium. PP, immediately post partum. The number of observations and standard errors are indicated.

(Pularin, Evans Medical); trypsin (Tryptar, Armour); α-chymotrypsin (B.D.H.).

Results

Plasma kininogen

There was no difference in the plasma kininogen concentration of rats in pro-oestrus (2.63 μg/ml, s.e. ± 0.15), oestrus (2.65 μg/ml, s.e. ± 0.11) and dioestrus (2.52 μg/ml, s.e. ± 0.11).

The plasma kininogen level rose almost two-fold with advancing gestation (Figure 1). The increase appeared to be biphasic. There was a rise between day 6 and day 12 from 3.11 μg/ml plasma (s.e. ± 0.11) to 4.02 μg/ml (s.e. ± 0.17, $P < 0.01$). The plasma kininogen concentration then levelled out until day 20 of gestation. After day 20 there was a second more rapid rise ($P < 0.05$) from 3.66 μg/ml (s.e. ± 0.02) on day 20 to 5.04 μg/ml (s.e. ± 0.38) at the beginning of parturition.

The kininogen level of plasma samples taken at intervals from the onset of parturition until immediately post partum did not show any change from the level at the beginning of parturition.

Figure 2 shows the plasma kininogen concentration of groups of post partum lactating rats. There was a rise ($P < 0.05$) in the plasma kininogen level from immediately post partum (5.08 μg/ml, s.e. ± 0.26) to day 1 post partum (5.89 μg/ml; s.e. ± 0.30). After day 1 post partum there was a rapid decline in the plasma kininogen concentration until around days 6 to 8 post partum, when normal non-pregnant plasma kininogen levels had been attained. We were unable to demonstrate a difference in the plasma kininogen level of lactating and non-lactating rats during the puerperium.

Figure 3 shows that there was a rise in the estimated total plasma kininogen content of the rats with advancing gestation, and this rise appeared to be biphasic, resembling that in Figure 1. There was a very rapid rise from day 19 to term from 42.5 μg to 68.2 μg of plasma kininogen.

Free kinin

The level of free kinin found in the blood of non-pregnant female rats was very low, and in many samples no free kinin could be detected. In other samples levels varied from 0.11 ng/ml blood to 3 ng/ml blood. There was no correlation between the stage of the oestrous cycle and the level of free kinin in the blood.

In blood samples taken from 20 rats at various stages of pregnancy free kinin could not be detected, irrespective of the day of pregnancy.

When blood samples were taken from 10 parturient rats after the delivery of at least two pups, levels of free kinin were found to be low and inconstant. In the blood of six of the rats no free kinin could be detected. In the other four rats the free kinin levels were 0.23 ng/ml, 0.78 ng/ml, 0.81 ng/ml and 1.37 ng/ml.

In blood samples taken from six non-pregnant rats pretreated with dimercaprol (30 mg/kg i.p.)

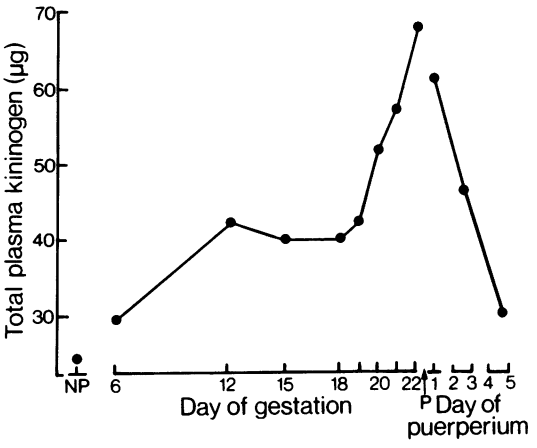

Fig. 3 Total plasma kininogen content of pregnant and puerperient rats. NP, non-pregnant rats; P, parturition.

free kinin was detected in the blood of only one, the level being 1.71 ng/ml blood. When six parturient rats were similarly pretreated, free kinin was detected in the blood of all the rats, the mean free kinin level being 2.10 ng/ml blood, s.e. ± 1.21.

Discussion

The increase in the plasma kininogen level in the rat during gestation agrees with the results of Wiegershausen *et al.* (1968). Morgan (1964) demonstrated that the rat differs from the human, there being no increase in the α_2 globulin fraction of plasma during gestation. It therefore appears that the hyperkininogenaemia of rat pregnancy is not a simple reflection of a raised α_2 globulin concentration.

Fluctuations in the level of plasma kininogen during pregnancy, parturition and puerperium in the rat are similar in many respects to those found in the human (Martinez *et al.*, 1962; Periti & Gasparri, 1966; Wiegershausen *et al.*, 1967). The only difference appears to be that in the human there is mobilization of plasma kininogen stores during parturition, whereas in the rat the consumption of plasma kininogen during parturition is markedly lower. This could be partly explained by the difference in stress and duration of labour between the human and the rat. Plasma kininogen levels during pregnancy show the same tendency in man and the rat, and the rat might be a suitable model for studies on the elucidation of the kinin system in reproductive physiology. This is in contrast to the guinea-pig where the level of plasma kininogen has been shown to decrease during pregnancy (Da Silva, 1971).

McCormick & Senior (1972) demonstrated that the blood volume of the maternal rat rises with advancing gestation up to day 20 of pregnancy, and then levels out to term. As the plasma kininogen concentration is also rising this means that the plasma kininogen concentration is being effectively diluted by the increasing blood volume. In order to obtain a more accurate picture of the actual increase in the plasma kininogen stores, the total plasma kininogen content of the animal was calculated (Figure 3).

Increase in the plasma kininogen concentration may be due to an increased rate of synthesis of kininogen by the liver, a change in the body distribution of kininogen or a decrease in the rate of activation of the plasma kinin system. No free kinin was detected in the blood of rats during pregnancy, and this was not due to an increased blood kininase activity, as up to day 20 of pregnancy the kininase activity of rat blood is lowered (McCormick & Senior, 1972). Therefore, during pregnancy there is probably some reduction of activity in the processes leading to activation of the plasma kinin system.

The rapidity of the elevation of the plasma kininogen concentration from day 20 of gestation to parturition may be partially explained by the fact that the blood volume reaches a peak on day 20, and then levels out to term (McCormick & Senior, 1972). Hence from day 20 to term, blood volume does not affect kininogen levels.

The possibility that the hyperkininogenaemia of pregnancy is due to hormone effects has been studied by McCormick & Senior (1971). They found that daily administration of oestrogens to non-pregnant female rats caused an elevation in the plasma kininogen concentration. However, Yoshinaga, Hawkins & Stocker (1969) and Waynforth, Pope & Hosking (1972) found that the secretion rate of oestrogen from the ovaries of rats during pregnancy was generally much lower than during the oestrous cycle. On day 20 of pregnancy there was a surge of oestrogen from the ovaries reaching a climax on day 22.

During parturition there was no change in plasma kininogen concentration. However, it would appear from experiments in which the free kinin content of parturient rat blood was measured that there is some degree of cleavage of plasma kininogen during parturition. Samples of blood taken from rats during various stages of pregnancy invariably contained no free kinin, whereas in some of the blood samples taken from parturient rats free kinin could be detected. Free kinin could easily be demonstrated in the blood of parturient rats which had been treated with

dimercaprol, though it appears unlikely that there is a gross activation of the plasma kinin system during parturition in the rat.

The elevation in the plasma kininogen concentration on day 1 post partum is associated with a rapid decline in the maternal blood volume after parturition (McCormick & Senior, 1972). Figure 3 shows that there is in fact a fall in total plasma kininogen stores after parturition. After day 1 post partum there is a rapid decline in plasma kininogen concentration.

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