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CHANGES IN PLASMA KININOGEN LEVELS INDUCED BY CELLULOSE SULPHATE DURING PREGNANCY IN THE RAT

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1 Cellulose sulphate (1 mg/kg) produced a 30-40% depletion of plasma kininogen in rats.

2 The time course of repletion of kininogen in the plasma was compared in rats in the oestrous and dioestrous stages of the cycle and in 22 day pregnant animals. A partial repletion occurred, 3 h after the cellulose sulphate injection, which was followed by a secondary fall in plasma kininogen. Plasma kininogen values were back to control levels 10 h after the treatment in all groups.

3 Treatment of rats from days 19-22 of pregnancy with cellulose sulphate resulted in 40% depletion of plasma kininogen and in prolongation of pre-parturition behaviour.

4 It is suggested that the increase which normally occurs in plasma kininogen levels towards the end of pregnancy in the rat may play a role in the process of parturition.

Introduction

In the pregnant rat there is an increase in plasma kininogen values as gestation advances, so that at full term the kininogen values are twice those found in the non-pregnant animal (McCormick & Senior, 1974). Similar results have been reported by Wiegerhausen, Klausch, Hennighausen & Sosat (1968); these workers showed a fall in kininogen values during parturition. The increase in the kininogen level in late pregnancy may be related to the increase in circulating reproductive hormone levels. Oestrogen in optimal doses produces an increase in plasma kininogen values (McCormick & Senior, 1971).

The level of plasma kininogen may be involved in the process of normal pregnancy in the rat, or could be just an unimportant consequence of the pregnant state. The increase in kininogen values found in late pregnancy may be a result of an increase in rate of synthesis. To investigate these hypotheses we decided to examine the effect of the kininogen depletor, cellulose sulphate, on plasma kininogen. Cellulose sulphate has been shown to deplete rat plasma kininogen levels (Rothschild, 1968) without giving rise to toxic effects. By using cellulose sulphate we hoped to deplete plasma kininogen, compare the rate of repletion in pregnant and non-pregnant rats, and observe any effects on pregnancy and parturition.

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Methods

Mature female rats of the CSE strain, weighing 200 to 250 g, were used and were housed in plastic cages. The animals were allowed free access to food and water and were housed in a light (07 h 00 min to 19 h 00 min) and temperature controlled room.

The stage of the oestrous cycle was determined by the criteria of Long & Evans (1922). Pregnancy was induced by caging a pro-oestrus female between 20 h 00 min and 08 h 00 min on the following day with a male of known fertility. Vaginal smears were taken the following morning and if spermatozoa were present the onset of pregnancy was known to have occurred within the preceding 12 hours. From this time until the end of the experiment these pregnant animals were housed separately.

Cellulose sulphate was prepared from Whatman ashless cellulose paper by the method of Astrup, Galsmar & Volkert (1944). The cellulose sulphate thus prepared was dissolved in 0.9% w/v NaCl solution (saline) and injected intravenously in a dose volume not exceeding 0.2 ml. The initial studies involving non-pregnant rats were performed by the injection of cellulose sulphate into the femoral vein of rats under ether anaesthesia. In all the subsequent work involving pregnant rats the cellulose sulphate was administered intravenously into the tail vein of restrained animals. Plasma kininogen extraction was performed using essentially the method of Diniz & Carvalho

(1963). A larger volume of alcohol (10 ml) was used and the temperature was maintained at 70°C for 15 min, to inhibit any trypsin activity which might alter the final result. The free kinin released by this method was assayed against synthetic bradykinin (Sandoz) on the rat isolated uterus or guinea-pig ileum preparations. The plasma kininogen concentration is expressed in μg/ml, 1 μg referring to the amount of liberated free kinin equivalent to 1 μg of bradykinin.

Experiments involving the duration of pregnancy and parturition were performed by continuously observing the animals from 12 h 00 min on day 22 of pregnancy until delivery had taken place. The onset of preparturient behaviour was indicated by the occurrence of regular abdominal contractions and the adoption of a characteristic posture on the hind limbs with the head down. The time from the onset of abdominal contractions to the delivery of the first foetus was noted. The time between the delivery of the first foetus and the last placenta was also noted and was termed 'parturition'. Blood loss was noted, as was the general condition of the young and the mother. In these experiments involving pregnant animals the last treatment was given at 09 h 00 min on day 22.

Results

Dose of cellulose sulphate

In non-pregnant female rats kininogen concentration in the plasma 20 min after injection of various doses of cellulose sulphate was compared with that of groups which had received saline intravenously. The results in Table 1 are expressed as the percentage depletion of kininogen, comparing each test group with a similar control group. Injection of cellulose sulphate (1 mg/kg) produced an approximately 30% depletion of plasma kininogen and maximal depletion (43%) occurred with a dose of 6 mg/kg. Higher doses of cellulose sulphate failed to produce any further depletion. A dose of 1 mg/kg cellulose sulphate was used in subsequent experiments; this dose produced no obvious toxic effect on the rat; appetite and behaviour were normal. At autopsy following this treatment there was no evidence of any gross change in either the pregnant or non-pregnant animal.

Table 1 Effect of dose of cellulose sulphate on depletion of plasma kininogen levels in the female rat

Dose of cellulose sulphate (mg/kg)	*Number of rats*	*% Kininogen depletion*
1	2	33
3	4	38
6	4	43
20	4	43
40	2	36

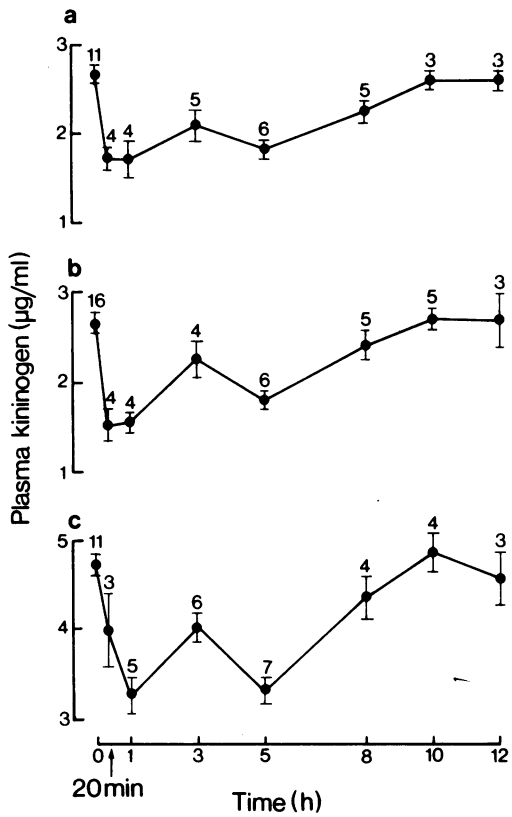

Fig. 1 Depletion and repletion of plasma kininogen levels (as bradykinin equivalent) in (a) oestrous (b) dioestrous and (c) pregnant rats, 22 days gestation, following cellulose sulphate (1 mg/kg i.v.). Number of rats and s.e. mean are indicated.

The rate of kininogen synthesis after treatment with cellulose sulphate was compared in oestrous, dioestrous and pregnant rats. Groups of animals were injected intravenously with cellulose sulphate (1 mg/kg) and blood samples were taken by cardiac puncture at intervals following the injection. Each animal was bled only once to minimize the danger of haemorrhage caused by the anticoagulant activity of cellulose sulphate. The results are shown in Figure 1. When rats were treated during oestrus (Fig. 1a) the plasma kininogen value had fallen significantly ($P < 0.001$) within 20 min of the injection. This

low level was maintained until 1 h after the injection and was followed by a gradual repletion of the plasma kininogen concentration. There appeared to be a partial repletion of the kininogen content around 3 h following injection, but this was not statistically significant. After 10 h the plasma kininogen concentration had returned to normal values.

Figure 1b shows a similar experiment performed on rats in the dioestrous phase of the cycle. The pattern was similar to that obtained with oestrous rats. Depletion of plasma kininogen was significant ($P < 0.001$) 20 min after the injection of cellulose sulphate and complete repletion occurred in 10 hours. A partial repletion in kininogen content occurred 3 h after the injection of cellulose sulphate and this repletion was significant ($P < 0.01$) when compared to the 1 h value.

The effect of cellulose sulphate on the plasma kininogen values for day 22 pregnant rats is shown in Figure 1c. Once again the pattern was similar to that obtained with non-pregnant rats. A significant depletion of plasma kininogen occurred within 1 h of the injection of cellulose sulphate ($P < 0.001$), the value at 20 min after the injection showing only partial depletion. There was a significant partial repletion of the plasma kininogen content when the 3 h value was compared to the 1 h value ($P < 0.05$); this repletion was followed by a further significant depletion when the 3 h and 5 h values were compared ($P < 0.01$). Normal plasma kininogen values for this day of pregnancy had been regained by 10 h after the injection of cellulose sulphate.

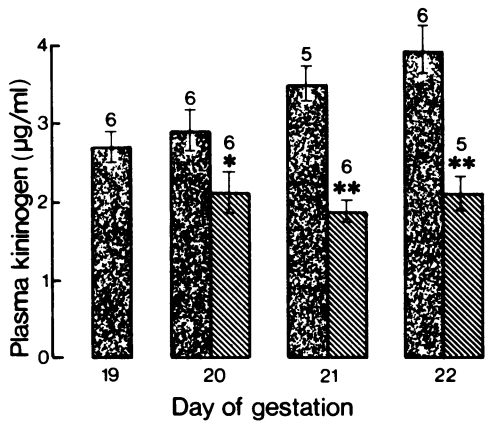

Fig. 2 Effect of cellulose sulphate (1 mg/kg i.v.) on plasma kininogen concentration (as bradykinin equivalent) during late pregnancy. Control groups are indicated by stippled columns and cellulose sulphate groups by hatched columns. Numbers of rats and s.e. mean are indicated. * $P < 0.05$; ** $P < 0.001$.

Plasma kininogen concentration during pregnancy

Treatment with cellulose sulphate (1 mg/kg twice daily) was started on day 19 of gestation and the effect of this treatment on the kininogen concentration in plasma was measured on days 20, 21 and 22. The results are shown in Figure 2. It can be seen that on day 20 the plasma kininogen level in the treated group had fallen significantly ($P < 0.05$) when compared with the normal level for day 20 of pregnancy. On days 21 and 22 after

Table 2 Effect of cellulose sulphate (1 mg/kg i.v.) on duration of gestation (from midnight on day 0-1 to delivery of 1st foetus), pre-parturient behaviour (from onset of abdominal contractions to delivery of 1st foetus) and parturition (from delivery of 1st foetus to expulsion of last placenta) in the rat

Treatment	*Saline (0.1 ml i.v.) twice daily*	*Cellulose sulphate (1 mg/kg i.v.) once daily*	*Cellulose sulphate (1 mg/kg i.v.) twice daily*
Number of rats	6	6	6
Duration of gestation	21 d, 22.9 h (range 21 d, 14.5-36.4 h)	21 d, 27.9 h (range 21 d, 18.8-30.3 h)	21 d, 32.6 h (range 21 d, 16.5-40.7 h)
Duration of pre-parturient behaviour (h)	1.3 ± 0.3	9.0 ± 2.9**	3.6 ± 1.4*
Duration of parturition (h)	1.2 ± 0.1	2.4 ± 0.5*	2.3 ± 0.1*
Average time between foetuses (min)	6.6 ± 0.7	13.0 ± 2.8*	11.8 ± 1.0*
Foetuses (live/dead)	68/0	63/3	64/6

Means with s.e. mean.
* $P < 0.01$; ** $P < 0.001$.

treatment with cellulose sulphate twice daily from day 19, the fall in plasma kininogen concentration was significant ($P < 0.01$).

Duration of pregnancy and parturition

The effect of cellulose sulphate treatment on the length of pregnancy and delivery of the foetuses is shown in Table 2. In our laboratories no rats having a normal pregnancy deliver before noon on day 22. Treatment with cellulose sulphate was commenced on day 19 of gestation and it can be seen from Table 2 that the duration of pre-parturient behaviour was significantly longer in the treated groups. When cellulose sulphate was administered twice a day (1 mg/kg, i.v.) the duration of gestation appeared to be increased. In both treated groups the duration of parturition and consequently the average time between the delivery of each foetus was significantly greater than in the normal delivery. In the treated groups 3 out of 66 (1 mg/kg i.v., daily) and 6 out of 70 (1 mg/kg i.v., twice daily) foetuses were stillborn but all 68 foetuses in the control group were born alive. At autopsy it was revealed that no foetuses or placentas had been retained in the uterus in any of the animals (control or treated). We noted significant bleeding after delivery in 4 of the 12 rats given cellulose sulphate but in none of the 6 saline controls. In all groups the mothers appeared well and did not neglect the offspring after birth.

Discussion

The plasma kininogen depletion obtained in this work was usually between 30-40% with a dose of cellulose sulphate 1 mg/kg; the maximal depletion obtained was 43%, with a high dose of cellulose sulphate (6 mg/kg). Earlier work by Rothschild (1968) showed that a 70% depletion of plasma kininogen could be obtained with cellulose sulphate 3 mg/kg in male rats. Previous work has shown (McCormick & Senior, unpublished observation) that the male rat has a significantly lower plasma kininogen concentration than that found in the female; it could be that the greater depletion found in male rats by Rothschild (1968) is a sex difference. The modification of the extraction procedure for kininogen seems unlikely to have influenced the depletion produced by cellulose sulphate, as the samples of plasma from untreated and treated animals were subjected to the same process. A dose of 1 mg/kg cellulose sulphate was chosen as a suitable kininogen depleting agent as higher doses produced only a slightly greater effect. At this dose level no deleterious effects were observed in any of the animals, and it is unlikely that the results recorded were due to a toxic effect of the drug.

Comparison of the depletion of plasma kininogen evoked by cellulose sulphate in oestrous and dioestrous rats shows that the results are similar; the maximal depletion (40%) occurred within 1 h of treatment and was followed by a gradual repletion within 10 hours. In dioestrous rats there was a secondary depletion about 5 h after treatment, perhaps due to haemodilution or activation of a kinin-releasing system. Eisen & Vogt (1970) have suggested that two major enzyme systems may be involved in the release of kinin from kininogen; the kininogenase enzymes and the plasmin enzymes in the fibrinolytic system. Cellulose sulphate may cause depletion of plasma kininogen through the action of one or both of these systems (Rosa, Rothschild & Rothschild, 1972); it could be that a secondary activation of one of these systems occurs between 3 and 5 h after treatment with cellulose sulphate. In the pregnant animal one dose of cellulose sulphate (1 mg/kg) caused a depletion of approximately 30% of the plasma kininogen on day 22 of gestation. At this stage of pregnancy the plasma kininogen level is almost double that in the non-pregnant rat and the total circulating kininogen is very high because the blood volume is almost twice the normal volume (McCormick & Senior, 1972). Consequently, the lower depletion of kininogen by cellulose sulphate is not unexpected. The repletion of kininogen stores in pregnant rats was completed within 10 h of treatment, suggesting that the rate of kininogen formation in the day 22 pregnant rat is faster than in the non-pregnant rat, where the total plasma kininogen content is lower.

The observations that gestation was prolonged by a few hours, that duration of pre-parturient behaviour was longer and that the interval between delivery of the foetuses was increased, following treatment with cellulose sulphate, suggest an impairment of uterine action. This effect could have resulted from a direct action of cellulose sulphate, a decrease in the circulating kininogen levels or a release of free kinin. The groups of rats studied were of course small and the problem needs examination using direct recordings of uterine activity. The treatment used in this experiment was completed on the morning of day 22 of gestation and this resulted in a lengthening of gestation by several hours; it would be interesting to see if continuation of the treatment would further prolong gestation. The bleeding noted after delivery of foetuses in some cellulose sulphate treated rats might also be accounted for by the failure of the uterus to contract or by an anticoagulant action of the drug. The latter is

suggested by the occurrence of stillbirths in the treated group but again, the problem needs investigation by direct methods.

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