The influence of drugs on the kinin-forming system in relation to pregnancy and parturition in the rat

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Summary. The duration of normal gestation and parturition in the rat can be changed by treatment with drugs which alter the equilibrium of the kallikrein–kinin system. The kallikrein inhibitor, aprotinin, when given from Days 19–22 of pregnancy prolongs gestation. Treatment with aprotinin from Days 20–22 of pregnancy prolongs the parturient process, as does a single dose given on the morning of Day 22. Kallikrein, when administered from Days 19–22 of pregnancy, results in a prolongation of gestation and abolishes the pre-parturient behaviour ('labour'). Parturition is prolonged and many fetuses are stillborn. Soya bean trypsin inhibitor when given from Days 19–22 of pregnancy delays and prolongs parturition; maternal haemorrhage occurs during birth and many fetuses are born dead or are abandoned at birth.

It is suggested that the kallikrein–kinin system plays a functional role in the normal process of parturition in the rat.

Introduction

In the pregnant rat there is an increase in plasma kinin precursor as gestation advances, so that at term the kininogen values are double those found in the non-pregnant animal (McCormick & Senior, 1974). Similar results have been reported by Weigerhausen, Klausch, Hennighausen & Sosat (1968); these workers showed a fall in kininogen values during parturition. This change in the plasma kininogen concentration during late pregnancy may be related to changes in the oestrogen to progesterone ratio in the maternal plasma (Yoshinaga, Hawkins & Stocker, 1969; Pepe & Rothchild, 1974), since oestrogen increases and progesterone decreases the plasma kininogen content in the female rat (Senior & Whalley, 1974).

The work reported in this paper is an attempt to elucidate the functional significance of the changes in the plasma kinin precursor levels during late pregnancy in the rat by using compounds which are known to alter the equilibrium of the kallikrein–kinin system. The substances used were aprotinin and soya bean trypsin inhibitor (SBTI) which inhibit the enzyme kallikrein, thus preventing the formation of kinin from kininogen.

Materials and Methods

Mature, female rats (200–250 g) of a Wistar-derived strain were used. They were allowed free access to food and water and were housed in plastic cages in light- (07.00–19.00 hours) and temperature-controlled rooms.

Pro-oestrous females were caged separately overnight with a male of proven fertility and the vaginal smear was checked for the presence of spermatozoa the following morning. The day on which spermatozoa were found in the smear was designated Day 1 of pregnancy and was accurate to within 12 hr. The rats were housed individually during pregnancy. Parturition was observed for each rat and the measurements followed the criteria detailed in a previous study (McCormick, Senior &

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Whalley, 1974). Briefly, these consist of the division of the birth process into two sections. The first, termed 'pre-parturient behaviour', is characterized by abdominal stretching and the adoption of a characteristic posture on the hind limbs with the head down. The second section is termed parturition and refers to the time between the delivery of the first fetus and the last placenta.

Kininogen determinations were performed on separate groups of pregnant rats using a modified method of Diniz & Carvahlo (1963), as described by McCormick & Senior (1974); animals were used once only for kininogen determinations. The liberated free kinin was assayed against synthetic bradykinin (BRS 640, Sandoz A.G.) using either the isolated rat uterus or the isolated guinea-pig ileum preparations.

Statistical analysis of the results was performed using Student's *t* test (Bailey 1959).

Results

Aprotinin

Subcutaneous administration of 25,000 k.i.u. aprotinin/kg twice daily had no apparent toxic effects and all the young were viable and sucked normally. Treatment was started on each day from the morning of Day 19 of gestation onwards and the last dose was given on the morning of Day 22. The length of gestation and the parturient process were noted (Table 1). The control (saline-treated) animals are shown as one group because there were no significant differences between the saline-treated animals when treatment was started on different days of gestation. The number of young born did not differ significantly in the control and treated groups of animals. Only when treatment was started on Day 19 was any significant effect seen on the duration of gestation; there was a delay in the onset of the pre-parturient behaviour and therefore of the onset of parturition. The duration of the former was significantly increased when aprotinin treatment was started on Day 19 but the duration of parturition was not affected. When treatment with aprotinin was begun on Day 20 the duration of pre-parturient behaviour and of parturition was increased, but a single injection of aprotinin on the morning of Day 22 prolonged only the duration of parturition. After treatment for 1 day the plasma kininogen values on Day 20 were significantly higher ($P < 0.05$) in the control group (3.8 ± 0.3 (S.E.) $\mu\text{g/ml}$; $N = 6$) than in the treated group (2.8 ± 0.2 $\mu\text{g/ml}$; $N = 6$). However, on subsequent days there were no significant differences in the kininogen concentrations in the aprotinin-treated and control groups.

Table 1. The effect of 25,000 k.i.u. aprotinin/kg twice daily on the mean $\pm$ S.E.M. length of gestation and parturition in the rat (times are taken from 12.00 hours on Day 22 of pregnancy)

Day treatment started	No. of rats	Mean no. of fetuses/rat	Pre-parturient behaviour (hr)		Parturition (hr)		Time/fetus (min)
			Onset	Duration	Onset	Duration	
19	6	11.0	$36.4 \pm 4.5^{**}$	$2.8 \pm 0.5^{**}$	$39.3 \pm 4.0^{**}$	1.4 ± 0.1	8.4 ± 0.9
20	6	12.6	10.6 ± 3.7	$2.1 \pm 0.1^*$	12.5 ± 3.8	$1.7 \pm 0.2^*$	9.0 ± 1.1
21	6	11.0	16.8 ± 3.1	1.4 ± 0.1	18.2 ± 3.0	1.4 ± 0.2	7.9 ± 1.1
22	6	11.3	12.1 ± 3.9	1.6 ± 0.1	13.7 ± 3.9	$1.7 \pm 0.2^*$	8.6 ± 0.9
Control	18	11.6	9.6 ± 4.1	1.3 ± 0.3	10.9 ± 4.2	1.2 ± 0.1	6.6 ± 0.7

Significantly different from control values, * $P < 0.05$; ** $P < 0.001$.

Kallikrein

Seven rats were each given an i.p. injection of 30 ku. kallikrein/kg twice daily from Day 19 up to and including the morning of Day 22. It can be seen from the results in Table 2 that the pre-parturient behaviour was abolished and gestation was prolonged in most of the animals. Parturition was prolonged in all rats in the group and excessive bleeding appeared to have occurred during parturition

as blood was present on the floor of the cages. Many of the fetuses were born dead, and only 15% of the total number were alive and sucking on Day 24. There were no differences in the plasma kininogen concentrations on Days 20, 21 and 22 compared to the control values on those days of pregnancy.

Table 2. The effect of kallikrein administered i.p. to pregnant rats on the length of gestation, parturition and fetal survival

	Treatment with kallikrein		
	30 ku./kg twice daily, Days 19–22	120 ku./kg twice daily, Day 21	120 ku./kg twice daily, Day 22
No. of rats/group	7	5	7
Parturition within normal limits	2	3	5
Prolonged gestation (delivery after 12.00 hours Day 23)	5	2	2*
Pre-parturient behaviour	0	0	5
Prolonged parturition	7†	1	0
Haemorrhage present	7	2	3
Fetuses born alive/dead	16/35‡	54/3	74/0
Fetuses <i>in utero</i> alive/dead	0/19	0/0	0/15*
% of total fetuses alive and sucking on Day 24	15	14	50

* 1 rat died without giving birth.

† 1 rat died during parturition.

‡ 1 litter not included.

A larger dose of kallikrein (120 ku./kg) was given intraperitoneally to a group of 5 rats at 09.00 hours and again at 21.00 hours on Day 21 of gestation. Another 7 rats were injected i.p. with the same dose on Day 22 of gestation. A second injection of kallikrein was given to those rats in this group which had not started to give birth by 16.00 hours on Day 22. The results of both treatments are shown in Table 2. No pre-parturient behaviour was observed in rats treated with kallikrein on Day 21 of pregnancy; most of the fetuses were born alive, but they subsequently died because of lack of maternal care. Treatment with kallikrein on Day 22 prolonged gestation in only 2 rats of the 7, but one died before it could give birth. Only 50% of the total number of fetuses were alive and sucking on the following day.

Soya bean trypsin inhibitor

The inhibitor (SBTI) was injected s.c. at a dose of 40 mg/kg daily from Days 19 to 22 of gestation. One rat died on the afternoon of Day 22 without giving birth and so has not been included in the results. Times were not recorded for the onset and duration of the pre-parturient behaviour. The onset of parturition was significantly ($P < 0.001$) delayed in the rats treated with SBTI (24.3 ± 2.2 hr) when compared to that in the saline-treated control group (see Table 1), and the duration of parturition (3.8 ± 0.6 hr) was also significantly prolonged ($P < 0.001$). No fetuses were retained *in utero* following this treatment but 12% were born dead. The mean number of fetuses/rat was 11.6, as in the control animals, but the parturition time/fetus (18.7 ± 4.9 min) was significantly longer ($P < 0.001$). Only 44% of the total number of fetuses born were given maternal care and were surviving 24 hr after birth. Excessive blood was present on the floor of the cage of the rats giving birth after treatment with SBTI.

Discussion

These experiments have shown that alteration in the components of the kallikrein–kinin system during pregnancy in the rat can alter the course of the pregnancy. Aprotinin delays the onset of parturition or prolongs the pre-parturient behaviour or parturition without having a deleterious

effect on the fetus. The effect is, however, dependent on the time when treatment is started. Only when treatment is started on Day 19 is there a pronounced prolongation of gestation. This would suggest that the biochemical processes leading to parturition are triggered after Day 19, confirming the work of Dukes, Chester & Atkinson (1974), although the prolongation of gestation is only of the order of 1 day, not 3 days as would be anticipated. After the trigger has occurred, aprotinin, in the dose investigated, is incapable of prolonging gestation. The prolonged duration of pre-parturient behaviour after treatment with aprotinin from Day 19 suggests that this stage of the birth process is controlled by events which occur 2–3 days earlier. It is difficult to define the processes which are involved during pre-parturient behaviour in the rat but prolongation of this stage may be due to uterine inertia or inadequate cervical dilatation. Because aprotinin is a kallikrein inhibitor, the effects produced could result from a lack of free kinin being produced from kininogen. The inhibition of kallikrein by aprotinin only produces a significant increase in kininogen on Day 20 when treatment is started on Day 19, indicating that kininogen synthesis after this time is inhibited for the precursor levels to return to control values. The two pharmacological actions of kinins that may be involved in the events leading up to the onset of parturition and the parturient processes are the local vasodilator and smooth muscle stimulating effects. It seems possible that lack of either or both of these effects could influence parturition in the rat.

Similarly, kallikrein inhibition from Days 19 to 22 with SBTI produces a prolongation of gestation but the duration of parturition is also protracted. With SBTI treatment, however, maternal haemorrhage occurs and many fetuses are born dead or are abandoned by the mother, suggesting that this treatment does have an effect on the contractile ability of the uterine smooth muscle. Whereas aprotinin has been shown to inhibit kallikrein activity in both plasma and tissues, SBTI is only active against plasma kallikrein (Vogel & Werle, 1970), implicating tissue and plasma kallikrein in the parturient processes in the rat.

The kallikrein–kinin system has been studied during labour and parturition in women (Martinez, Carvalho & Diniz, 1962; Periti & Gasparri, 1966; Porter, Shennan & Smith, 1972). A rise in maternal plasma kininogen values occurs in late pregnancy and it has been postulated that a large amount of bradykinin is produced during expulsion of the fetus. Aprotinin treatment has been shown to arrest labour temporarily in women (Konopka, Afchain & Senèze, 1973) and has also been shown to arrest premature labour successfully (Goisis & Lami, 1973).

As inhibition of kallikrein activity with aprotinin or SBTI prolongs gestation or produces abnormal maternal behaviour during the parturient processes in the rat, it would be expected that treatment with the enzyme itself would speed up the process of parturition. Injection of kallikrein to rats during the late stages of pregnancy does initially produce abdominal contractions which are similar to those seen in the pre-parturient behaviour period, but these could result from the nociceptive effect of liberated kinin. When treatment with kallikrein is started on Day 19 of pregnancy and continued until Day 22, gestation is usually prolonged, as it is after aprotinin treatment for the same period. With kallikrein treatment, however, all pre-parturient behaviour is abolished and parturition is prolonged. The fact that many of the fetuses are born dead and there is excessive maternal haemorrhage after kallikrein treatment could suggest that the uterus is failing to contract or that the contractions are unphysiological. Although plasma levels of kininogen are not depleted by treatment with kallikrein, the levels of kininogen in the uterus may be affected, thus promoting the production of free kinin to produce a local effect. The effect produced by bradykinin on the rat uterus *in situ* is confused in the literature. Some workers report that the uterus contracts to kinin (Stürmer & Berde, 1963) but others report that the stimulant action of oxytocin is antagonized (Bisset, Haldar & Lewin, 1966). It may be that the effect of kinin on uterine muscle depends on the hormonal environment.

Earlier studies in this laboratory have shown that the treatment of the pregnant rat with the kininogen-depleting agent, cellulose sulphate, from Days 19 to 22 of gestation results in the prolongation of pregnancy and the pre-parturient behaviour (McCormick, Senior & Whalley, 1974). The results from these earlier studies and from the present experiments involving administration of kallikrein and its inhibitors suggest that these substances may involve changes in the uterine blood flow or in the contractile ability of the uterus.

The changes in maternal behaviour produced by modification of the kinin-forming system are of interest, but no explanation is yet available.

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References

- BAILEY, N.Y.J. (1959) The use of t-tests for small samples. In *Statistical Methods in Biology*, pp. 43–51. English Universities Press, London.
- BISSET, G.W., HALDAR, J. & LEWIN, J.E. (1966) Actions of oxytocin and other biologically active peptides on the rat uterus. In *Endogenous Substances Affecting the Myometrium*, pp. 185–197. Eds V. R. Pickles & R. J. Fitzpatrick. Cambridge University Press.
- DINIZ, C.R. & CARVALHO, I.F. (1963) A micromethod for the determination of bradykininogen under several conditions. *Ann. N. Y. Acad. Sci.* **104**, 77–89.
- DUKES, M., CHESTER, R. & ATKINSON, P. (1974) Effects of oestradiol and prostaglandin $F_{2\alpha}$ on the timing of parturition in the rat. *J. Reprod. Fert.* **38**, 325–334.
- GOISIS, M. & LAMI, V. (1973) Nuovi indirizzi eziopatogenetici e terapeutici in tema di minacce d'aborto. *Minerva Ginec.* **25**, 481–492.
- KONOPKA, P., AFCHAIN, J. & SENÈZE, J. (1973) Essai d'explication du mécanisme du travail. *Rev. franc. Gynec.* **68**, 7–12.
- MARTINEZ, A.R., CARVALHO, I.F. & DINIZ, C.R. (1962) The bradykininogen content of plasma from women in labour. *J. Obstet. Gynaec. Br. Commonw.* **69**, 1011–1014.
- MCCORMICK, J.T. & SENIOR, J. (1974) Plasma kinin and kininogen levels in the female rat during the oestrous cycle, pregnancy, parturition and the puerperium. *Br. J. Pharmac. Chemother.* **50**, 237–241.
- MCCORMICK, J.T., SENIOR, J. & WHALLEY, E.T. (1974) Changes in plasma kininogen levels induced by cellulose sulphate during pregnancy in the rat. *Br. J. Pharmac. Chemother.* **52**, 533–537.
- PEPE, G.J. & ROTHCHILD, I. (1974) A comparative study of serum progesterone levels in pregnancy and in various types of pseudo-pregnancy in the rat. *Endocrinology* **95**, 275–279.
- PERITI, P. & GASPARRI, F. (1966) Bradykininogen in the blood of women during pregnancy, labour and the puerperium. In *Hypotensive Peptides*, pp. 536–539. Eds E. G. Erdos, N. Back, F. Sicuteri & A. F. Wilde. Springer, Berlin.
- PORTER, J.F., SHENNAN, A.T. & SMITH, S. (1972) Plasma kinin and kininogen levels in women during pregnancy and labour. *J. Reprod. Fert.* **30**, 247–254.
- SENIOR, J. & WHALLEY, E.T. (1974) Variation in the oestrogen–progesterone ratio and its effect on plasma kininogen levels in the rat. *J. Reprod. Fert.* **41**, 425–433.
- STÜRMER, E. & BERDE, B. (1963) A comparative pharmacological study of synthetic eledoisin and synthetic bradykinin. *J. Pharmac. exp. Ther.* **140**, 349–355.
- WEIGERHAUSEN, B., KLAUSCH, B., HENNIGHAUSEN, G. & SOSAT, R. (1968) Der Kininogenspiegel von Ratten und Kaninchen während der gestation. *Experientia* **24**, 1128–1129.
- VOGEL, R. & WERLE, E. (1970) Kallikrein inhibitors. In *Handbook of Experimental Pharmacology XXV*, Chap. 3, pp. 213–239. Ed. E. G. Erdos. Springer-Verlag, Berlin.
- YOSHINAGA, K., HAWKINS, R.A. & STOCKER, J.F. (1969) Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and pregnancy. *Endocrinology* **85**, 103–112.

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