Excitatory response of guinea-pig myometrium to intravenous noradrenaline declines towards term

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Summary. Guinea-pigs were anaesthetized at three stages of pregnancy. Intrauterine pressure was recorded for a 1-h control period and during 10-min intravenous infusions of noradrenaline at rates of 1.0 and 10 $\mu\text{g}/(\text{min}\cdot\text{kg})$. The mean and maximum amplitude of contractions occurring during the infusions was compared with that of contraction cycles registered in the control period. At 18–21 and 35–43 days *post coitum*, noradrenaline invariably evoked a rapid and sustained rise in intrauterine pressure, the amplitude of the contractions being greater than during spontaneous contraction cycles recorded in the control period. In late pregnancy, 59–68 days *p.c.*, infusion of 1.0 μg noradrenaline/(min·kg) failed to elicit a clear response; contractions occurring during infusion of 10 μg noradrenaline/(min·kg) had amplitudes similar to those of the control period and were without a sustained contracture. The absence of denervation hypersensitivity, despite the occurrence of sympathetic denervation in the course of pregnancy, may be due to a generalized effect on excitation–contraction coupling, possibly caused by relaxin.

Introduction

The sympathetic innervation of the uterus is profoundly affected by pregnancy (Marshall, 1981). In guinea-pigs, a reduction in the ability to synthesize new transmitter (Alm, Björklund, Owman & Thorbert, 1979) leads to a fall in the noradrenaline content of the uterus (Sjöberg, 1968; Bell & Malcolm, 1978) and degeneration of the noradrenergic nerve terminals is said to occur (Thorbert, Alm, Owman, Sjöberg & Sporrang, 1978). Denervation of a tissue usually renders it hypersensitive, both to the neurotransmitter and to other agonists (Fleming, McPhillips & Westfall, 1973). Thus myometrial strips from pregnant uteri relax at lower concentrations of noradrenaline than comparable strips from non-pregnant guinea-pigs, and the response is greater (Elmér, Alm & Thorbert, 1980). Similarly, the uterine vasculature, which also is subject to denervation during pregnancy (Bell & Malcolm, 1978), shows increased sensitivity to the vasoconstrictor action of noradrenaline towards the end of gestation (Mårtensson & Carter, 1982). In contrast, preliminary results suggested that the excitatory component of the myometrial response to noradrenaline was greatly reduced in late pregnancy (Mårtensson & Carter, 1982). The present study was undertaken to extend these observations by measuring the changes in intrauterine pressure that occur during the intravenous infusion of noradrenaline in guinea-pigs at three different stages of pregnancy, each of which has been carefully characterized with respect to the adrenergic innervation of the uterus (Thorbert *et al.*, 1978).

Materials and Methods

Guinea-pigs of outbred stock (Ssc:AL) were mated in our laboratories. Coitus was assumed to occur at the onset of oestrus. The day on which rupture of the vaginal membrane occurred was

therefore designated Day 0 of pregnancy. The studies were made at three stages of pregnancy: 18–21 days *post coitum* (N = 8), 35–43 days *p.c.* (N = 9), and 59–68 days *p.c.* (N = 7). Anaesthesia was initiated and maintained by i.p. pentobarbitone sodium. Body temperature was maintained at $\sim 38^{\circ}\text{C}$ with the aid of a heating pad. Polyethylene catheters were inserted in a carotid artery for continuous registration of arterial blood pressure, and in the ipsilateral jugular vein for the administration of drugs. To prevent the oxidation of noradrenaline, it was dissolved in 2.5% (w/v) dextrose and 0.45% (w/v) NaCl immediately before infusion.

Intrauterine pressure was recorded from a sponge-tipped, open-ended polyvinyl catheter (Bengtsson, 1968) situated between the fetal membranes and the uterine wall. The catheter was inserted at laparotomy through a small incision in the uterine wall, which was closed by a purse-string suture. The catheter was filled with isotonic saline solution and connected to a pressure transducer (Model 746, Siemens-Elema, Solna, Sweden). The intrauterine pressure was recorded for a control period of 1 h to establish the pattern of spontaneous uterine activity. An intravenous infusion of noradrenaline was then given for 10 min at a rate of $1.0\text{ }\mu\text{g}/(\text{min}\cdot\text{kg})$. After 20 min, when the response had subsided, a second infusion of noradrenaline was given at a rate of $10\text{ }\mu\text{g}/(\text{min}\cdot\text{kg})$ for 10 min. At the end of the experiment the animal was killed by an overdose of pentobarbitone sodium and the fetuses were weighed. Fetal weight was subtracted from total body weight to calculate the amount of noradrenaline given per kg maternal weight. At the lower rate of infusion this was $1.0\text{--}1.1\text{ }\mu\text{g}/(\text{min}\cdot\text{kg})$ at 35–43 days *p.c.* and $1.2\text{--}1.4\text{ }\mu\text{g}/(\text{min}\cdot\text{kg})$ at 59–68 days *p.c.*

To compare noradrenaline responses with spontaneous bursts of uterine activity, the mean pressure amplitude was measured over a 10-min period from the start of noradrenaline infusion or from the start of a spontaneous contraction cycle. If present, two or more contraction complexes were analysed and the results averaged to yield a control value. The area under the curve was determined planimetrically, using a digitizer (Hipad Digitizer, Houston Instruments, Austin, Texas, U.S.A.) and a suitable computer programme. The intrauterine pressure before the contractions was taken as the baseline value. The reproducibility of this method was tested by making 10 repeat measurements of selected pressure complexes. The coefficient of variation varied from 5.9% for a contraction of small mean amplitude (85 Pa) to 0.3% for a response with a large mean amplitude (1.86 kPa). The maximum pressure attained within each contraction cycle was also noted, but no attempt was made to analyse the frequency of the pressure peaks.

In the text and tables all values are expressed as means $\pm$ s.e.m. The significance of differences occurring within and between groups, respectively, was analysed by Student's *t* test for non-independent and independent observations.

Results

Early in pregnancy the spontaneous activity of the uterus was characterized by continual fluctuations in pressure but with regular bursts of greater intensity (Text-fig. 1a). In middle and late pregnancy the pressure complexes were separated by quiescent periods. In one animal (64 days *p.c.*) no alterations in intrauterine pressure occurred during the 1-h control period. The mean and maximum amplitudes of the pressure complexes were greater early in gestation than in middle or late pregnancy (Text-figs 1a, d & g; Table 1). As shown by Porter (1971b), this is because the uterine lumen is small in relation to the thickness of the uterine wall.

Infusion of $1.0\text{ }\mu\text{g}$ noradrenaline/(\text{min}\cdot\text{kg}) in early, middle and late gestation, respectively, evoked a rise in mean arterial blood pressure from 6.60 ± 0.45 to 8.18 ± 0.45 kPa (+ 26%), 7.97 ± 0.49 to 9.55 ± 0.56 kPa (+ 20%) and 6.38 ± 0.52 to 7.94 ± 0.45 kPa (+ 26%). There was no significant difference in the pressor response between the three groups of animals. In early and middle pregnancy, infusion of noradrenaline invariably caused a rapid and sustained rise in pressure (Text-figs 1b & e). In late pregnancy, noradrenaline failed to elicit a clear response, and contraction complexes recorded during the infusion resembled those of the control period (Text-fig. 1h).

Text-fig. 1. Representative tracings of intrauterine pressure complexes recorded from pregnant guinea-pigs during a control period and in response to the intravenous infusion of 1.0 or 10 µg noradrenaline/(min·kg). The recordings were made at 18 days (a-c), 40 days (d-f) and 68 days (g-i) *post coitum*.

Table 1. Amplitude of spontaneously occurring contraction complexes and of contractions occurring during the intravenous infusion of noradrenaline at three stages of pregnancy in the guinea-pig

	18–21 days <i>p.c.</i> (N=8)	35–43 days <i>p.c.</i> (N=9)	59–68 days <i>p.c.</i> (N=7)
Spontaneous contractions			
Maximum amplitude† (kPa)	1.63 ± 0.13	0.37 ± 0.06	0.29 ± 0.07
Mean amplitude‡ (kPa)	0.53 ± 0.04	0.10 ± 0.01	0.06 ± 0.02
Noradrenaline 1.0 µg/(min·kg)			
Maximum amplitude† (kPa)	1.76 ± 0.16	0.70 ± 0.10**	0.34 ± 0.09
Mean amplitude‡ (kPa)	0.61 ± 0.12	0.21 ± 0.06	0.09 ± 0.03
Noradrenaline 10 µg/(min·kg)			
Maximum amplitude† (kPa)	2.42 ± 0.27*	1.05 ± 0.15**	0.37 ± 0.04
Mean amplitude‡ (kPa)	1.15 ± 0.18*	0.41 ± 0.10**	0.08 ± 0.01

Values are mean ± s.e.m.

† In relation to the intrauterine pressure before the contraction.

‡ Obtained by measuring the area under the curve (see text).

Significantly different from control: * $P < 0.05$; ** $P < 0.01$.

There was a greater blood pressure response to infusion of noradrenaline at the higher rate of $10 \mu\text{g}/(\text{min}\cdot\text{kg})$. In early, middle and late gestation, respectively, mean arterial blood pressure rose from 7.39 ± 0.37 to 11.58 ± 0.33 kPa (+ 59%), 7.66 ± 0.40 to 12.22 ± 0.24 kPa (+ 62%), and 6.34 ± 0.31 to 11.04 ± 0.58 kPa (+ 76%). The magnitude of the pressor response was similar at all three stages of pregnancy. In contrast, the myometrial response appeared to differ. In early and middle pregnancy, there was an immediate rise in intrauterine pressure (Text-figs 1c & f). Both the maximum amplitude attained and the mean amplitude were distinctly greater during noradrenaline infusion than during pressure cycles that occurred in the control period (Table 1). In late pregnancy, there was only a moderate response to noradrenaline (Text-fig. 1i), with no tonic component, and neither the mean nor the maximum amplitude of contractions during the drug infusion were significantly different from those of spontaneous contraction complexes (Table 1).

Discussion

The myometrial response to adrenergic stimulation has two components, excitation and inhibition, mediated by two separate populations of receptors (Ahlquist, 1948). Relaxation of myometrial strips from the guinea-pig is enhanced late in pregnancy, and it has been suggested that this is an example of denervation hypersensitivity (Elmér *et al.*, 1980). The endogenous sympathetic transmitter disappears from the guinea-pig uterus in the course of pregnancy and degeneration of the nerve terminals may occur (Sjöberg, 1968; Bell & Malcolm, 1978; Thorbert *et al.*, 1978). In contrast, the results of the present study show that the excitatory response to noradrenaline is present two-thirds of the way through pregnancy (35–43 days *p.c.*), when noradrenergic nerves are still present in the parafetal segments of the uterus (Thorbert *et al.*, 1978), yet is greatly reduced closer to term (59–68 days, *p.c.*), when endogenous transmitter can be demonstrated only at the tubal end of the uterus and in the cervix (Thorbert *et al.*, 1978).

Noradrenaline was given intravenously and account must therefore be taken of the change in plasma volume that occurs during pregnancy. Blood volume corresponds to 7.5% of maternal weight until Day 40 of pregnancy and 8.3% in the last 2 weeks of gestation (van Dijk, van Kreel & Heeren, 1983). Towards term there is also a fall in haematocrit from 0.38 to 0.34. Compared with early pregnancy, it can be estimated that plasma volume was 5% greater in the midpregnant group, reflecting maternal growth, and 18% greater near term. This variation is not sufficient to explain the difference in myometrial response. In terms of maternal weight, there was a corresponding variation in the amount of noradrenaline administered.

Due care must be taken when comparing intrauterine pressure tracings from different stages of pregnancy, since there is a complex relation between mechanical tension, wall thickness, and lumen diameter (Porter, 1971b). The amplitude of contractions was greater at 10–21 days *p.c.*, when the fetus was still small, than at later stages, when the uterine wall had been distended by growth of the conceptus (Table 1). Responses to noradrenaline should therefore be seen in relation to the amplitude of the pressure complexes associated with spontaneous myometrial contractions. Even taking this into account, there can be little doubt that the uterine response to noradrenaline alters towards the end of gestation. In early and middle pregnancy noradrenaline evokes a tonic contraction, and both the mean and the maximum amplitudes are greater than those recorded during spontaneous contraction cycles (Text-fig. 1; Table 1). Towards the end of pregnancy the response evoked resembles a spontaneous contraction cycle (Text-figs 1g–i). The intrauterine pressure rises slowly, to an amplitude that is slightly higher than in a spontaneous contraction complex, and there is no sustained contracture. The overall impression is that noradrenaline causes premature initiation of the next contraction cycle.

Since denervation hypersensitivity does not affect the α -receptor-mediated contractile response to noradrenaline in the guinea-pig myometrium, the loss of sympathetic innervation must be counteracted by other mechanisms that influence the pregnant uterus. These presumably bear upon

the initiation of the contractile process, since the inhibitory response is subject to denervation hypersensitivity (Elmér *et al.*, 1980). Oestrogens favour the synthesis of α -adrenergic receptors (Roberts, Insel, Goldfien & Goldfien, 1977), and the concentration of oestradiol-17 β in the guinea-pig uterus undergoes a moderate fall from midpregnancy to term (Batra, Sjöberg & Thorbert, 1980). A reduction in the density of α -adrenergic receptors can also occur under the influence of progesterone (Williams & Lefkowitz, 1977), thereby rendering the myometrium less sensitive to adrenergic excitation. However, plasma and uterine tissue concentrations of progesterone peak at mid-pregnancy in the guinea-pig (Challis, Heap & Illingworth, 1971; Batra *et al.*, 1980), whereas the present results show a reduction in sensitivity to noradrenaline nearer term.

Myometrial quiescence in the guinea-pig is probably due to relaxin rather than progesterone (Porter, 1971a, 1972). Relaxin is synthesized within the endometrial gland cells (Pardo, Larkin & Renegar, 1984) and the uterine content of relaxin continues to rise from 45 days to term of pregnancy (Pardo & Larkin, 1982). Relaxin is thought to act at some point in the excitation-contraction coupling sequence (Porter, Downing & Bradshaw, 1979) and has been shown to interfere with myosin light-chain phosphorylation (Nishikori, Weisbrodt, Sherwood & Sanborn, 1982). This action of relaxin on the pregnant myometrium would be consistent with the observed reduction in sensitivity to the excitatory action of noradrenaline.

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