Regional changes in catecholamine content of the pregnant uterus

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Summary. High-pressure liquid chromatography with electrochemical detection was used to identify and measure catecholamines in rat, rabbit, sheep, guinea-pig and human uteri and follow changes with pregnancy. Noradrenaline was consistently the major catecholamine and pregnancy caused a regionally specific fall in its concentration which, in rat, rabbit and guinea-pig, was associated with a decline in total content. Adrenaline was undetectable (<10 pmol/g myometrium) in all species and at all gestational ages studied. Dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were detected at high concentrations in guinea-pig and particularly sheep uterus. In guinea-pig uterus the dopamine/DOPAC ratio fell dramatically with pregnancy, suggesting that increased quantities of dopamine were released and catabolized. The dopamine/noradrenaline ratios suggested that dopamine is stored with noradrenaline in adrenergic neurones in guinea-pig myometrium and within an additional neuronal or cellular store(s) in sheep uterus.

Introduction

The mammalian uterus receives a well developed adrenergic supply consisting of classical long neurones innervating predominantly the vasculature, and a unique system of short neurones mostly associated with the myometrium. These originate in lumbar and mesenteric ganglia, and peripheral sympathetic ganglia at and around the utero-vaginal junction respectively. The importance of short adrenergic neurones is unknown but the ability of steroid hormones to influence their transmitter metabolism may enable them to respond specifically to physiological conditions such as pregnancy (Sjoberg, 1967; Rosengren & Sjoberg, 1968; Falck, Owman, Rosengren & Sjoberg, 1969; Owman, Sjoberg & Sjostrand, 1974; Thorbert, Batra, Owman, Rosengren & Sjoberg, 1976; Thorbert, 1978). Neuronal or exogenous noradrenaline acting via α - and β -adrenoreceptors has been reported to contract (Zuspan, Cibils & Pose, 1962; Miller & Marshall, 1965; Isaac & Pennefather, 1969; Rexroad & Barb, 1978) and relax (Miller & Marshall, 1965; Bengtsson, 1978; Elmer, Alm & Thorbert, 1980) uterine smooth muscle depending on animal species and hormonal state. Adrenergic stimulation also indirectly influences myometrial contractility by increasing sensitivity to oxytocin (Russe & Marshall, 1970). However, the precise function, if any, of the sympathetic innervation in controlling myometrial contractility remains unknown.

Uterine adrenergic innervation and regional changes with pregnancy have been demonstrated by histofluorescence in many species. Fluorometric determination of myometrial noradrenaline has confirmed these data, which suggest a regionally specific depletion of neuronal noradrenaline towards term (Sjoberg, 1967; Owman, Alm, Rosengren, Sjoberg & Thorbert, 1975; Thorbert, 1978; Thorbert, Alm, Bjorklund, Owman & Sjoberg, 1979; More & Nedjar, 1984). However, because of the similar spectral characteristics of many fluorescent catechol derivatives (Shore & Olin, 1958; Iversen, 1967; Swedin & Brudin, 1968), such studies may not provide accurate identification and

measurement of specific catecholamines. Indeed, this may account for the contradictory reports on the presence of both adrenaline and dopamine within the female reproductive tract (Cha, Lee, Rudzik & Miller, 1965; Swedin & Brudin, 1968; Thorbert, Alm & Rosengren, 1978).

The physiological mechanisms underlying uterine quiescence during pregnancy and increased motility at parturition are unknown. Since adrenaline has been reported to be a powerful relaxant of uterine smooth muscle (Zuspan *et al.*, 1962) and dopamine to increase intrauterine pressure (Fishburne *et al.*, 1980; Urban, Radwan, Laudanski & Akerlund, 1982), and because both have been detected in high concentrations in the uteri of several species (Wurtman, Axelrod & Kopin, 1963; Cha *et al.*, 1965; Swedin & Brudin, 1968; Rexroad & Barb, 1978; Lackovic, Rezja & Neff, 1982), it is important to establish whether these catecholamines are potential regulators of uterine activity. Therefore, in a study of the potential significance of catecholamines in the control of myometrial contractility, changes in uterine catecholamine content during pregnancy have been measured in animals of various species.

Materials and Methods

Dated pregnant Sprague-Dawley rats and New Zealand White rabbits were purchased from Olac (Bicester, Oxfordshire, U.K.) and Ranch Rabbits (Crawley Down, Sussex, U.K.) respectively. Dated pregnant Dunkin-Hartley guinea-pigs were bred in our own animal house (Elvidge, 1972). Sheep uterine samples were obtained from non-pregnant and 125–135-day unilaterally pregnant animals. All animals were virgin or primigravid. Isthmic myometrium was obtained from normally menstruating women undergoing abdominal hysterectomy, and from patients subject to Caesarean section at 39–41 weeks gestation. All women were multiparous with 1–3 previous pregnancies. When possible, regionally specific samples were taken from animal uteri. The nomenclature previously cited (Thorbert, 1978) is used, so that myometrium from immediately around the developing fetus is termed perifetal and that from between fetuses (while distinguishable) parafetal. Tissue taken from the last 5 mm adjacent to the oviduct is termed tubal myometrium. In guinea-pig a regional study was undertaken with whole uteri sectioned serially from one tubal end to the other, with each sample analysed independently. Whole guinea-pig uteri after parturition were also analysed.

Guinea-pigs were killed by cervical dislocation, rats and rabbits by a blow to the head. Sheep were killed by use of a humane killer. Whole uteri (guinea-pig, rat, rabbit) or uterine samples (sheep, human) were excised and placed on ice, stripped of endometrium, dried by blotting, weighed and finely minced with scissors. The myometrium was homogenized in 2 volumes of ice-cold 0.4-M-perchloric acid containing 2-mM-sodium metabisulphite and a quantity of 3,4-dihydroxybenzylamine (DHBA) as internal standard (appropriate for the endogenous catecholamine concentrations) for 4×30 sec using an Ultra-Turrax homogenizer (The Scientific Instrument Centre Ltd, London, U.K.) at 20 000 rev./min. After centrifugation at 70 000 *g* for 30 min at 2°C the pellet was re-extracted with 1 volume of perchloric acid and the supernatants were pooled. The pH was adjusted to 4–5 with 10% (w/v) potassium hydroxide. Potassium perchlorate precipitate was removed and the catecholamines were extracted from 0.5 ml supernatant for 30 min with 30 mg neutral alumina (Sigma; activity grade 1) in the presence of 0.05 ml 10 mM-sodium metabisulphite and 1.0 ml 0.5 M Tris-HCl pH 8.6 containing 2% (w/v) ethylenediaminetetra-acetic acid (EDTA). Alumina was then sedimented at 1000 *g* for 15 min and washed 3 times in 1.0 ml H₂O. Catecholamines were eluted with 0.2 ml 0.1 M-perchloric acid containing 0.1 mM-sodium metabisulphite and 0.05 ml was injected directly onto the high-pressure liquid chromatography (HPLC) analytical column.

The HPLC with an electrochemical detection system consisted of a solvent pump (M45; Waters Associates Inc., Milford, MA, U.S.A.), valve injector with 0.05 ml constant volume injection loop

(Specac, Orpington, Kent, U.K.), precolumn filter (Jones Chromatography Ltd, Llanbradach, Glamorgan, U.K.) and a Spherisorb ODS (25 cm $\times$ 5 mm i.d., 5 μ m particle size) analytical column (HPLC Technology Ltd, Macclesfield, Cheshire, U.K.). Catecholamines were detected using a flow-through electrochemical cell with a glassy carbon working electrode set at +0.72 V and electronic control module (Model LC-2A, Bioanalytical Systems Inc., W. Lafayette, IN, U.S.A.). The catechols were eluted from the column at 1.0 ml/min with 1% (w/v) citric acid monohydrate, 1% (w/v) sodium dihydrogen phosphate dihydrate, 1% (w/v) disodium hydrogen phosphate, 1 mM-EDTA, 0.048% (w/v) 1-octane sulphonic acid and 5% (v/v) methanol, adjusted to pH 2.80–2.81 with phosphoric acid and degassed with helium. This system gives good resolution of catecholamines extracted by the alumina (Text-fig. 1).

Text-fig. 1. HPLC chromatogram showing separation of catecholamine standards. Peaks represent 10 pmol injected catecholamine: (1) 3,4-dihydroxyphenylglycol; (2) noradrenaline; (3) adrenaline; (4) 3,4-dihydroxyphenylalanine (DOPA); (5) 3,4-dihydroxybenzylamine (DHBA); (6) 3,4-dihydroxyphenylacetic acid (DOPAC); (7) dopamine.

Catechol concentrations were determined by standard curves based on peak heights. Recoveries were calculated and corrected for that of DHBA which was 75–85%. Each tissue extract was assayed in triplicate with intra-assay variation < 5%. The limit of detection was 0.2 pmol/0.05 ml injected, corresponding to about 10 pmol/g myometrium. The number of animals used at each gestational age is given in parentheses. Data were analysed using the Wilcoxon rank Sum test (Snedecor & Cochran, 1980) with values given as mean $\pm$ s.d. $P < 0.05$ was considered significant. The dopamine/3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine/noradrenaline ratios were derived from raw data and their values may differ slightly from those that would be obtained simply by comparing mean values for dopamine, DOPAC and noradrenaline.

Results

In no species at any gestational age was adrenaline detected (i.e. < 10 pmol/g). Noradrenaline was consistently the major catecholamine although dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were present in guinea-pig and particularly sheep uterus. In all tissues 3,4-dihydroxyphenylalanine (DOPA) was present at 20–100 pmol/g, but regional or pregnancy-related changes were not observed for any species.

Rat

Noradrenaline was the major catecholamine and during pregnancy, its concentration, but not total uterine content, fell sharply to $< 10\%$ of the non-pregnant value ($P < 0.05$) (Table 1). The largest changes occurred in perifetal myometrium while cervical noradrenaline showed greatest preservation. Total uterine content fell by about 40% in the term uterus ($P < 0.05$). Dopamine was present in the non-pregnant uterus at 43 ± 9 pmol/g (4) corresponding to a (dopamine/noradrenaline) $\times 100$ value of $2.78 \pm 0.15\%$ (4). With pregnancy dopamine levels fell rapidly until at term it was undetectable except in the cervical region (24 ± 9 pmol/g (4)). DOPAC levels were not determined.

Table 1. Influence of pregnancy on noradrenaline concentration and total content in rat uterus

Gestational age	Noradrenaline conc. (pmol/g myometrium)	Total uterine noradrenaline (pmol/uterus)
Non-pregnant	1575 ± 130.5 (4)	722 ± 24.8 (4)
12 days	745 ± 495.7 (3)	595 ± 144.3 (3)
17 days	$*239 \pm 191.5$ (4)	$*510 \pm 57.3$ (4)
21 days		$*420 \pm 111.8$ (4)
Tubal myometrium	$*93 \pm 18.5$ (4)	
Perifetal myometrium	$\dagger 43 \pm 14.7$ (4)	
Cervical	$*176 \pm 70.9$ (4)	

Values are mean $\pm$ s.d. for the no. of rats in parentheses. Full term is 21–22 days.

* Significantly less than non-pregnant value ($P < 0.05$).

$\dagger$ Significantly less than values for other uterine regions at same gestational age ($P < 0.05$).

Rabbit

The predominant catecholamine was noradrenaline although small concentrations of dopamine were detected. Whole uterine mean noradrenaline concentration fell from 1633 ± 177 pmol/g (4) in the non-pregnant uterus to 217 ± 87 pmol/g at term (4) ($P < 0.05$). This decline reflected a more rapid fall in tubal and particularly perifetal myometrium with high concentrations maintained in the cervical region (Table 2). Total noradrenaline content remained unchanged until term when, as in the rat, it was reduced by about 40% ($P < 0.05$). Dopamine was present at similar concentrations in all regions of the non-pregnant uterus with a mean concentration of 30 ± 6 pmol/g (4). With pregnancy it fell rapidly and was undetectable by term. The (dopamine/noradrenaline) $\times 100$ value, however, (while determinable) appeared constant at $2.82 \pm 1.71\%$ (22) in all regions throughout pregnancy. DOPAC was not measured.

Sheep

It was not practically possible to analyse the whole uterus of the sheep. Although noradrenaline was the major catecholamine, very high concentrations of dopamine were detected at all times

Table 2. Regional changes in noradrenaline concentration and total content in rabbit uterus with pregnancy

Gestational age and uterine region	Noradrenaline conc. (pmol/g myometrium)	Total uterine noradrenaline (nmol/uterus)
Non-pregnant		21.4 ± 2.6 (4)
Tubal	1648 ± 295.1 (4)	
Mid	1542 ± 380.3 (4)	
Cervical	1674 ± 344.3 (4)	
21 days		22.9 ± 3.5 (4)
Tubal	*543 ± 397.8 (4)	
Perifetal	*181 ± 78.7 (4)	
Parafetal	*660 ± 284.1 (4)	
Cervical	1625 ± 317.5 (4)	
27 days		18.6 ± 5.0 (3)
Tubal	*413 ± 151.1 (3)	
Perifetal	*77 ± 16.6 (3)	
Parafetal	*266 ± 216.6 (3)	
Cervical	888 ± 437.8 (3)	
31 days		*13.2 ± 4.1 (4)
Tubal	*73 ± 64.3 (4)	
Perifetal	†*12 ± 3.3 (4)	
Cervical	*716 ± 344.1 (4)	

Values are means ± s.d. for the no. of animals in parentheses. Full term is 30–32 days.

* Significantly less than non-pregnant values ($P < 0.05$).

† Significantly different from values for other uterine regions at same gestational age ($P < 0.05$).

Table 3. Concentrations of noradrenaline, dopamine and DOPAC, and relative levels of dopamine to noradrenaline and DOPAC in selected regions of non-pregnant and 125–135-day unilaterally pregnant sheep uterus

Uterine sample	Noradrenaline conc. (pmol/g myometrium)	Dopamine conc. (pmol/g myometrium)	DOPAC conc. (pmol/g myometrium)	Dopamine‡/DOPAC × 100	Dopamine‡/Noradrenaline × 100
Non-pregnant					
Tubal	3714 ± 645.8 (6)	1307 ± 619.4 (6)	5256 ± 1868.0 (6)	24.2 ± 4.0 (6)	28.1 ± 17.1 (6)
Middle	4682 ± 953.5 (6)	2139 ± 922.0 (6)	5417 ± 2328.2 (6)	40.3 ± 9.2 (6)	45.7 ± 27.6 (6)
Cervical	5045 ± 1704.9 (6)	†521 ± 205.3 (6)	†2262 ± 1232.5 (6)	35.2 ± 17.6 (6)	†9.4 ± 3.4 (6)
125–135-day pregnant (unilateral)					
'Empty' horn					
Tubal	*1028 ± 220.4 (5)	1176 ± 719.5 (5)	*2653 ± 864.5 (5)	36.2 ± 14.1 (5)	*127.7 ± 87.6 (5)
Middle	*515 ± 317.6 (5)	*731 ± 289.6 (5)	*1451 ± 412.8 (5)	47.0 ± 23.0 (5)	*152.3 ± 72.1 (5)
Cervical	*603 ± 275.3 (5)	451 ± 324.6 (5)	1341 ± 642.6 (5)	26.9 ± 13.5 (5)	*77.2 ± 32.1 (5)
Fetus-bearing horn					
Tubal	*648 ± 561.8 (5)	904 ± 475.4 (5)	*3056 ± 1454.4 (5)	*144.0 ± 12.9 (5)	*122.3 ± 62.1 (5)
Perifetal	†*151 ± 105.3 (5)	*653 ± 603.1 (5)	*1517 ± 329.2 (5)	31.0 ± 12.5 (5)	†*496.2 ± 141.7 (5)

Values represented are mean ± s.d. for the no. of animals in parentheses. Full term is 142–147 days.

‡ Relative values of dopamine derived from raw data for each experiment.

* Significantly less than respective non-pregnant value ($P < 0.05$).

† Significantly different from values for other uterine regions at same stage ($P < 0.05$).

(Table 3). Noradrenaline concentrations, that were similar in all regions of non-pregnant uterus, fell sharply by 125–135 days in all areas, particularly in the perifetal myometrium ($P < 0.01$) (Table 3). Dopamine concentrations were greater than in any other species studied, the non-pregnant values representing nearly one-half that of noradrenaline, except in the cervix. By 125–135 days of pregnancy dopamine concentrations were unchanged except in the middle uterine regions where a fall of 66% had occurred ($P < 0.05$). The (dopamine/noradrenaline) × 100 values were much

greater than those observed in other species and were markedly higher in all pregnant uterine regions than non-pregnant (Table 3). DOPAC was present in all myometrial samples at high concentrations (Table 3).

Human

Noradrenaline was the major catecholamine in the isthmic myometrium and by term fell to <10% of its non-pregnant value (Table 4). Dopamine and DOPAC were present at low levels in the non-pregnant isthmus but undetectable at term.

Table 4. Noradrenaline, dopamine and DOPAC concentrations, and relative levels of dopamine to noradrenaline and DOPAC in non-pregnant and term human isthmic myometrium

Isthmic sample	Noradrenaline conc. (pmol/g myometrium)	Dopamine conc. (pmol/g myometrium)	DOPAC conc. (pmol/g myometrium)	$\frac{\text{Dopamine}^\dagger}{\text{DOPAC}} \times 100$	$\frac{\text{Dopamine}^\dagger}{\text{Noradrenaline}} \times 100$
Non-pregnant	775 $\pm$ 381.7 (7)	39 $\pm$ 25.9 (7)	50 $\pm$ 27.0 (7)	82.3 $\pm$ 33.9 (7)	5.2 $\pm$ 1.1 (7)
Term	*67 $\pm$ 68.1 (5)	*N.D.	*N.D.	—	—

Values are mean $\pm$ s.d. for the no. of women in parentheses. N.D. = not detectable.

* Significantly less than non-pregnant value ($P < 0.01$).

† Values derived from raw data.

Guinea-pig

Guinea-pig uterus contained largely noradrenaline with moderately high concentrations of dopamine and DOPAC. Noradrenaline concentrations appeared constant throughout the oestrous cycle, but during pregnancy fell progressively between 10 and 40 days, and by 50 days were about 4% of the non-pregnant value ($P < 0.01$) and remained low until term (Text-fig. 2a). By comparison total noradrenaline content was not decreased significantly until 51 days ($P < 0.05$), and at term represented about one-half the value before pregnancy (Text-fig. 2b).

From the regional study it was apparent that the fall in noradrenaline concentration was related to the position of the developing fetus, decay being more rapid and extensive within perifetal tissue, with tubal and cervical values remaining relatively high even by term. The greater the number of fetuses within a uterine horn, the more rapid the decline of concentration in perifetal myometrium. Text-figure 3 shows noradrenaline changes with pregnancy along the length of the developing uterine horn carrying 2 fetuses. Term perifetal myometrium was nearly devoid of noradrenaline with values of < 50 pmol/g along the entire length.

Dopamine concentrations appeared unaffected by the oestrous cycle, and, as for rabbit and rat, but unlike sheep, during pregnancy they fell in line with those of noradrenaline (Text-fig. 2c). The term mean concentration of 5 $\pm$ 4 pmol/g (8) reflected higher values in cervical and tubal myometrium (often > 25 pmol/g), with dopamine undetectable in perifetal tissue. Total dopamine content was unchanged until late pregnancy when it fell to a term value around 10% of that of the non-pregnant uterus ($P < 0.05$) (Text-fig. 2d). The relative levels of dopamine and noradrenaline were similar throughout pregnancy except for a reduced value near term (Table 5). Guinea-pig uterus also contained large quantities of DOPAC, the (dopamine/DOPAC) $\times$ 100 value falling dramatically with pregnancy (Table 5).

Post-partum recovery of uterine noradrenaline and dopamine was such that tissue concentrations increased initially, but recovery was incomplete by 11 weeks ($P < 0.05$). Total noradrenaline content was unchanged from term values at 10 days *post partum* but at 11 weeks it was indistinguishable from the value in non-pregnant uterus. Total dopamine content was similar to values for non-pregnant uteri by 11 weeks (Text-figs 2a–d). The (dopamine/DOPAC) $\times$ 100

Text-fig. 2. Changes in concentration (a,c) and total content (b,d) of noradrenaline and dopamine in guinea-pig uterus with pregnancy. Full term is 66–68 days. Vertical bars represent s.d. of the mean of 6–8 determinations. *Significantly less than value at dioestrus ($P < 0.05$).

Table 5. Relative levels of dopamine to noradrenaline and DOPAC in guinea-pig uterus during pregnancy

Gestational age	$\frac{\text{Dopamine}}{\text{Noradrenaline}} \times 100$	$\frac{\text{Dopamine}}{\text{DOPAC}} \times 100$
Non-pregnant	4.24 $\pm$ 1.30 (6)	109.0 $\pm$ 11.4 (6)
10 days	3.48 $\pm$ 0.51 (6)	108.3 $\pm$ 17.0 (6)
21 days	3.93 $\pm$ 0.83 (6)	95.4 $\pm$ 54.9 (6)
31 days	2.66 $\pm$ 0.17 (6)	*35.2 $\pm$ 25.3 (6)
40 days	3.83 $\pm$ 0.75 (7)	**10.9 $\pm$ 7.0 (7)
51 days	4.05 $\pm$ 0.83 (6)	**5.5 $\pm$ 3.1 (6)
57 days	2.52 $\pm$ 1.00 (7)	**2.5 $\pm$ 2.5 (7)
64 days	*1.89 $\pm$ 0.92 (8)	**1.8 $\pm$ 2.3 (8)
Immediately post partum (< 6 h)	*2.22 $\pm$ 0.90 (6)	**4.8 $\pm$ 1.7 (6)
5 days post partum	3.40 $\pm$ 1.19 (7)	**20.5 $\pm$ 10.8 (7)
10 days post partum	3.01 $\pm$ 0.97 (7)	**32.4 $\pm$ 7.8 (7)
10 weeks post partum	2.64 $\pm$ 0.58 (6)	100.0 $\pm$ 17.4 (6)

Values represent means $\pm$ s.d. derived from raw data for each experiment. No. of animals in parentheses. Full term is 66–68 days.

Values significantly different from non-pregnant value: * $P < 0.05$, ** $P < 0.01$.

Text-fig. 3. Selected data from the guinea-pig study showing variation in noradrenaline concentration along the length of a uterine horn bearing 2 fetuses at the gestational ages indicated. Values are mean $\pm$ s.d. for 3 or 4 guinea-pigs. *Significantly greater than values for other uterine regions at same gestational age ($P < 0.05$).

value increased in the post-partum period to values similar to those of non-pregnant myometrium by 11 weeks (Table 5).

Discussion

Fluorescence histochemistry and fluorometric determinations in combination with denervation experiments revealed that uterine noradrenaline is contained within neurones (Sjoberg, 1967; Thorbert, 1978). Uterine concentration is therefore used as an index of adrenergic innervation density, whereas total content reveals change in innervation independent of smooth muscle mass.

The majority of previous studies on uterine catecholamine content utilized histofluorescence-staining techniques and fluorometric measurements which lack adequate catecholamine specificity (Shore & Olin, 1958; Iversen, 1967; Swedin & Brudin, 1968). This has led to discrepancies. For instance high concentrations of adrenaline have been found in the uterus of rat (Wurtman *et al.*, 1963), sheep (Rexroad & Barb, 1978) and rabbit (Cha *et al.*, 1965), and in human uterus at levels above those of noradrenaline (Cha *et al.*, 1965), while others have reported the uterus to be devoid of adrenaline (Sjoberg, 1967; Rosengren & Sjoberg, 1968; Thorbert *et al.*, 1978). Similarly, dopamine has been detected in (Swedin & Brudin, 1968; Lackovic *et al.*, 1982) and reported absent

from (Sjoberg, 1967; Thorbert, 1978; Thorbert *et al.*, 1978) uteri of various species. To resolve some of these inconsistencies the present study using HPLC with electrochemical detection permitted accurate identification and measurement of uterine catecholamines (Text-fig. 1).

The changes in uterine noradrenaline presented here are in general agreement with previous studies, which is as expected since the large quantities of noradrenaline limit problems of measurement (Sjoberg, 1967; Owman *et al.*, 1975; Thorbert, 1978; Thorbert *et al.*, 1979; More & Nedjar, 1984). However, rat total uterine noradrenaline content has previously been reported as unchanged (Oskarsson, 1960) or increased (Cha *et al.*, 1965) during pregnancy. Also, the reported increase in total noradrenaline content during early-mid pregnancy in guinea-pig (Owman *et al.*, 1975) and rabbit (Rosengren & Sjoberg, 1968) uteri was not observed here. Finally, by comparison with other reports (Owman *et al.*, 1975) the cervical noradrenaline concentrations observed at term, especially in the rabbit are relatively high. The present observations therefore indicate that a regionally specific fall in uterine noradrenaline content, and therefore neuronal inactivation or degradation, is a consistent feature of pregnancy in animals of various species. This depletion is probably due both to steroid action on noradrenaline metabolism within short adrenergic neurones (Sjoberg, 1967; Rosengren & Sjoberg, 1968; Falck *et al.*, 1969; Owman *et al.*, 1974; Thorbert *et al.*, 1976; Thorbert, 1978) and disintegration of sympathetic nerves (Sporrong, Alm, Owman, Sjoberg & Thorbert, 1978; Thorbert, 1978). The physiological significance of such a sympathetic withdrawal from the uterus, especially in perifetal myometrium, is unknown, but it is likely that the ability of the adrenergic innervation to elicit a contractile response is considerably limited. Another consequence is likely to be the beta-adrenergic supersensitivity response observed with pregnancy (Nakanishi, McLean, Wood & Burnstock, 1969; Elmer, Alm & Thorbert, 1980), resulting from reduced uptake and breakdown of catecholamines and potentially increased beta-receptor coupling and/or number (Harden, 1983).

An important distinction between the present measurements and those made by others is the absence of detectable adrenaline in any uterine tissue studied (Wurtman *et al.*, 1963; Cha *et al.*, 1965; Rexroad & Barb, 1978). Adrenaline is able to relax the pregnant uterus (Zuspan *et al.*, 1962) and could therefore be an important regulator of myometrial quiescence. Although a possible highly localized storage site cannot be eliminated, intrauterine adrenaline is unlikely to be a regulator of myometrial contractility.

Peripheral dopamine has been assumed to be only a precursor of noradrenaline. There is now growing evidence that it may function as a neurotransmitter and/or cotransmitter in some peripheral tissues (Lackovic & Neff, 1983). For instance, dog kidney and paw pad appear to receive dopaminergic vasodilatory neurones (Bell & Lang, 1973, 1979; Bell, Lang & Laska, 1978; Dinerstein *et al.*, 1979). Despite reports of uterine dopamine (Swedin & Brudin, 1968; Lackovic *et al.*, 1982) there is limited information on its potential functional significance. Infusion of dopamine into pregnant sheep at pharmacological ($> 20 \mu\text{g/kg}$ per min) but not at physiological ($< 2 \mu\text{g/kg}$ per min) doses increased intrauterine pressure (Fishburne *et al.*, 1980). In man, similar effects were observed at physiological rates of infusion ($0.4 \mu\text{g/kg}$ per min) (Urban *et al.*, 1982).

The evaluation of the physiological significance of uterine dopamine requires assessment of whether it is purely a precursor, is located within neurones or some other cellular store, and whether functional dopamine receptors are present. In rat brain the concentration of dopamine metabolites DOPAC and homovanillic acid are dependent upon impulse frequency in dopaminergic neurones (Bacopoulos, Hattox & Roth, 1979). Further, in some peripheral tissues of rat and human these catabolite levels are high and it was suggested that this reflected release of dopamine in preference for use in noradrenaline synthesis (Lackovic *et al.*, 1982). Therefore, if dopamine present within guinea-pig and sheep uteri is the result of local synthesis, a potential transmitter function may be indicated by the high concentration of DOPAC. The precipitous fall in dopamine/DOPAC ratio in guinea-pig uterus with pregnancy (Table 5) is consistent with increased release and metabolism of dopamine.

However, non-neuronal cell types such as platelets (Olverman & Sharman, 1983), small

intensely fluorescent (SIF) 'chromaffin' cells (Bertler, Falck, Hillarp, Rosengren & Torp, 1959) and mast cells (Falck, Nystedt, Rosengren & Stenflo, 1964) have been shown to contain dopamine and may at least contribute to the observed tissue levels. Indeed, ruminant mast cells have been shown to store large quantities of dopamine (Falck *et al.*, 1964) and if the 'chromaffin' cells observed by Bertler *et al.* (1959) in cow liver capsule were mast cells similar to those of the sheep uterus, then a myometrial mast cell incidence of 0.005% (v/v) (10^5 cells/g myometrium) would entirely account for the dopamine concentrations observed here. In addition, if the contribution of SIF cells to uterine dopamine is significant, the higher cervical concentrations in rat and guinea-pig reported here may be due to SIF cells resident in peripheral sympathetic ganglia around the utero-vaginal junction (Owman *et al.*, 1974). At present, therefore, there is insufficient information on the origin and function of uterine dopamine. However, the relative levels of myometrial dopamine and noradrenaline in the rat, rabbit and guinea-pig are very similar to those published for other tissues (Bell & Gillespie, 1981) and are no greater than would be expected for dopamine associated with noradrenaline biosynthesis within adrenergic neurones (Bell & Gillespie, 1981). This is not inconsistent with the DOPAC levels in guinea-pig uterus if dopamine is released as a cotransmitter with noradrenaline in a proportion equal to their axonal storage ratio. In comparison, the extremely high levels of dopamine relative to noradrenaline in sheep myometrium (Table 3) may reflect independent storage sites for dopamine and noradrenaline or possibly a species difference in the rate-limiting step for noradrenaline synthesis.

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