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Effect of progesterone on the metabolism of noradrenaline in rabbit uterine endometrium and myometrium

J. A. Kennedy and I. S. de la Lande

Department of Clinical and Experimental Pharmacology, The University of Adelaide, Adelaide, South Australia 5000, Australia

Summary. 1. The metabolism of (−)-^{3}H-noradrenaline was examined in the endometrium and the myometrium from rabbits which had received 17β-oestradiol, either alone (oestrogen-dominated) or with progesterone (progesterone-dominated).

2. The progesterone treatment resulted in a 2.5-fold increase in ^{3}H-NMN formation in the endometrium, with no change in ^{3}H-DOPEG, ^{3}H-DOMA or ^{3}H-OMDA formation. In the myometrium, progesterone caused a 5-fold increase in ^{3}H-NMN formation and a 2.5-fold increase in ^{3}H-OMDA formation, but did not affect ^{3}H-DOPEG or ^{3}H-DOMA formation.

3. In the progesterone-dominated endometrium, both ^{3}H-NMN and ^{3}H-OMDA formation were strongly inhibited by cocaine 30 µmol/l. When O-methylation was inhibited by a COMT inhibitor, cocaine prevented the resultant increases in deamination of noradrenaline to ^{3}H-DOPEG and in the accumulation of ^{3}H-noradrenaline by the tissue. The ^{3}H-noradrenaline which accumulated in endometria, in which both MAO and COMT were inhibited, was firmly bound; desipramine 3 µmol/l and (+)-amphetamine 10 µmol/l were equieffective with cocaine 30 µmol/l in inhibiting the accumulation.

4. Cocaine 30 µmol/l was without effect on ^{3}H-NMN and ^{3}H-OMDA formation in the progesterone-dominated myometrium, nor did it prevent the increase in ^{3}H-DOPEG formation produced by COMT inhibition.

5. Fluorescent histochemical analysis of the endometrium indicated that the epithelial cells of the endometrial glands were the site of cocaine-sensitive noradrenaline accumulation.

6. It is concluded that progesterone stimulates O-methylation in the endometrium and myometrium in different ways. In the endometrium, it increases the activity of an unusual cocaine-sensitive extraneuronal uptake system associated with endometrial glands, presumably by causing the glands to proliferate. In the myometrium, it increases the activity of a cocaine-insensitive extraneuronal O-methylating system.

Key words: Endometrium – Myometrium – Noradrenaline – Uptake – O-Methylation – Cocaine

Introduction

Uterine changes produced by progesterone in rabbits receiving oestrogen include, in the endometrium, proliferation of glands, stroma and blood vessels (Finn and Porter 1975), and in the myometrium, a decrease in the density of the sympathetic innervation when assessed by monoamine fluorescent histochemistry (Falck et al. 1969b).

In an earlier study from this laboratory, it was shown that the ovarian hormones influence the metabolism of noradrenaline in rabbit uterine slices in a manner which is consistent with their effects on the sympathetic innervation (Kennedy et al. 1984). However, progesterone also produced changes in the extraneuronal metabolism of noradrenaline, which appeared to be independent of those in neuronal metabolism. The most pronounced was a 3- to 4-fold increase in NMN formation. This was attributed to a direct stimulant effect of the progesterone treatment on the metabolising systems involved in the extraneuronal uptake and O-methylation of noradrenaline (Kennedy et al. 1984).

Since the metabolic studies were carried out on slices of whole uterus, it was of interest to establish whether the effect of progesterone on O-methylation occurred in the endometrium or myometrium, or in both regions. In the present study, we have investigated the metabolism of exogenous noradrenaline in endometrial slices and myometrial slices taken from rabbits receiving oestrogen alone (termed oestrogen-dominated) or oestrogen plus progesterone (termed progesterone-dominated).

Methods

Treatment of animals. Mature nulliparous female rabbits (semi-lop eared), weighing approximately 3 kg, were treated with 17β-oestradiol (1 µg/kg) subcutaneously daily for 14 days. For the last 7 days of treatment rabbits were either treated with progesterone 2 mg/kg subcutaneously daily, or with the peanut oil vehicle. The rabbits were killed by stunning and bleeding.

Preparation of tissues. The uterus was removed from the rabbit, cleaned of fat and mesentery and cut open longitudinally along the mesometrial border. The endometrium

Send offprint requests to Dr. J. A. Kennedy at the above address

Abbreviations. DOMA, 3,4-Dihydroxymandelic acid; DOPEG, 3,4-dihydroxyphenylethyleneglycol; NMN, normetanephrine; OMDA, O-methyl deaminated metabolite fraction comprising vanillyl-mandelic acid plus the 3-methoxy derivative of DOPEG; MAO, monoamine oxidase; COMT, catechol-O-methyl transferase; MeOISO, 3-methoxy isoprenaline

was separated from the myometrium using fine forceps. Mean uterine weights, and weights of endometria expressed as percentages of uterine weights, were: oestrogen treatment 5.1 ± 0.6 g, $19 \pm 3\%$, $n = 5$; oestrogen plus progesterone treatment 6.1 ± 0.8 g, $31 \pm 1\%$, $n = 18$.

Endogenous noradrenaline contents. The noradrenaline contents of the endometrium and myometrium were estimated by the radioenzymic method of Da Prada and Zürcher (1976).

Metabolism of (−)-^{3}H-noradrenaline. The procedure was identical with that previously used for studying the metabolism of ^{3}H-noradrenaline in whole uterine slices (Kennedy et al. 1984). Small segments of endometrium and 1 mm slices of myometrium, each approximately 30 mg wet weight, were taken from the mid-portion of the uterine horn and incubated for 30 min in 5.0 ml of Krebs solution (termed preincubation) at 37° C gassed with 95% O_2:5% CO_2. The tissues were then incubated with (−)-2,5,6-^{3}H-noradrenaline in 1.0 ml of Krebs solution for 30 min, after which they were rinsed in noradrenaline-free Krebs solution for 10 s.

The composition of the Krebs solution in mmol/l was NaCl 120, KCl 4.7, $NaHCO_3$ 25, glucose 5.5, KH_2PO_4 1.0, $CaCl_2$ 2.5, $MgCl_2$ 1.1, ascorbic acid 0.29 and EDTA 0.01.

The ^{3}H-noradrenaline and ^{3}H-metabolite contents of the tissues and the ^{3}H-metabolite contents of the incubating media were estimated by the method of Graefe et al. (1973) Metabolite formation was estimated from the sum of the metabolite contents of the tissue and of the incubating medium, and expressed as nmol/g.

Effects of drugs. Cocaine 30 µmol/l or the COMT inhibitor, U-0521 55 µmol/l were added to the Krebs solution at the commencement of preincubation, so that the tissues were exposed to these agents for 30 min prior to, and during, incubation with ^{3}H-noradrenaline. In some experiments, prior to exposure to U-0521 the tissues were incubated with nialamide 350 µmol/l for 60 min in order to achieve inhibition of both, MAO and COMT. Evidence that inhibition of MAO persists after nialamide wash-out was presented in an earlier study (de la Lande et al. 1970).

Tissue accumulation of ^{3}H-noradrenaline. To determine whether the noradrenaline which accumulated in the tissue was firmly bound, the 10 s wash was replaced by a 30 min wash. The wash was achieved by incubating the tissue for a further 30 min in 5.0 ml of noradrenaline-free Krebs solution at 37° C. In the present experiments, the 30-min wash procedure was carried out only on tissues in which MAO and COMT were inhibited and the ^{3}H-contents only of the tissue and the wash solution were determined; i.e., ^{3}H-noradrenaline and its metabolites were not separated. In these experiments, the effects of desipramine 3 µmol/l and (+)-amphetamine 10 µmol/l were compared with those of cocaine 30 µmol/l. The drugs were added 30 min before the ^{3}H-noradrenaline and were present also in the wash solution.

Fluorescent histochemistry. Uptake of unlabelled noradrenaline by the endometrium was visualised, using the histochemical method of Falck (1962), with the minor modifications of Waterson and Smale (1967). The procedure was applied only to tissues in which MAO and COMT were inhibited. Portions of endometrium weighing approximately 50 mg were incubated for 15 min with (−)-noradrenaline 50 µmol/l in 2.0 ml of Krebs solution at 37° C, after which they were washed for either 10 s or 30 min, as described above. The tissue was then snap-frozen in acetone-dry ice, freeze dried, exposed to formaldehyde vapour and vacuum-embedded in paraffin. 7 µm sections were examined by fluorescent microscopy. In some experiments, the tissues were incubated with Krebs solution in the absence of noradrenaline, and in others, cocaine 30 µmol/l was present during incubation with the noradrenaline. In all experiments, a small segment of the ear artery from the same rabbit was subjected to fluorescence histochemistry. Since the artery has dense sympathetic innervation, it provided a positive control.

Metabolism of ^{3}H-isoprenaline. Progesterone-dominated endometria from four rabbits were incubated with (±)-^{3}H-isoprenaline 0.18 µmol/l instead of ^{3}H-noradrenaline, but otherwise under conditions identical to those described under "Metabolism of ^{3}H-noradrenaline". ^{3}H-isoprenaline and ^{3}H-methoxy isoprenaline were separated by the same column chromatographic method used for separating ^{3}H-noradrenaline and ^{3}H-metabolites, but using the first stage only; i.e., a single passage through alumina and Dowex 50, followed by elution of the O-methylated metabolite from the Dowex with HCl ethanol. Details of the method were presented in an earlier study from this laboratory (Head et al. 1980).

Treatment of data. The data are presented as means and SE's. Tests of significance were performed by two-tailed Student's *t*-tests, with a critical value of $P = 0.05$.

Drugs and chemicals. (−)-2,5,6-^{3}H-noradrenaline, specific activity 44 Ci/mmol (New England Nuclear, Boston, MA, USA); (±)-7-^{3}H-isoprenaline hydrochloride, specific activity 11.9 Ci/mmol (Amersham, Buckinghamshire, UK); cocaine hydrochloride (MacFarlane-Smith, Edinburgh, UK); desipramine hydrochloride (Ciba-Geigy, Australia); (+)-amphetamine sulphate and (−)-noradrenaline bitartrate (Sigma, St. Louis, MO, USA); nialamide (Pfizer, Groten, CT, USA); 3,4-dihydroxy-2-methyl propiophenone, U-0521 (Upjohn, Kalamazoo, MI, USA); 17β-oestradiol, progesterone (Steraloids Inc., Wilton, NH, USA).

Results

Endometrium: ^{3}H-noradrenaline metabolism

Endometria from rabbits receiving 17β-oestradiol alone (i.e., oestrogen-dominated) or 17β-oestradiol plus progesterone (i.e., progesterone-dominated) were incubated with ^{3}H-noradrenaline 0.18 µmol/l for 30 min and washed for 10 s. Following either hormone treatment, the content of ^{3}H-noradrenaline in the tissue (abbreviated to ^{3}H-noradrenaline accumulation) was small compared with the quantity converted to ^{3}H-metabolites (Table 1). In the oestrogen-dominated endometrium, the ^{3}H-OMDA fraction constituted 41% of the total ^{3}H metabolite formation; the remaining metabolites, as percentage of total ^{3}H metabolites, comprised ^{3}H-NMN (26), ^{3}H-DOMA (23), and ^{3}H-DOPEG (10). In the progesterone-dominated endometrium, there was a 2.5-fold increase in ^{3}H-NMN formation; as a

Table 1. Metabolism of ^{3}H-noradrenaline 0.18 μmol/l in endometrium[a]

	Hormone treatment			
	Oestradiol ($n = 5$)		Oestradiol + progesterone ($n = 9$)	
Presence of cocaine 30 μmol/l	−	+	−	+
^{3}H-NA[b] content of tissue	0.13 ± 0.02	0.11 ± 0.04	0.11 ± 0.02	0.08 ± 0.01
^{3}H-metabolites formed[a]				
− ^{3}H-NMN	0.19 ± 0.04	0.09 ± 0.01[c]	0.48 ± 0.06**	0.04 ± 0.01*
− ^{3}H-OMDA	0.30 ± 0.08	0.14 ± 0.02[c]	0.47 ± 0.09	0.12 ± 0.03*
− ^{3}H-DOPEG	0.07 ± 0.02	0.05 ± 0.01	0.09 ± 0.03	0.03 ± 0.01
− ^{3}H-DOMA	0.17 ± 0.05	0.20 ± 0.07	0.10 ± 0.03	0.09 ± 0.02
− Σ ^{3}H-metab.[d]	0.72 ± 0.08	0.48 ± 0.07*	1.13 ± 0.18	0.27 ± 0.06**

[a] Data in nmol/g
[b] ^{3}H-noradrenaline
[c] The decrease in the sum, ^{3}H-NMN + ^{3}H-OMDA, is significant; $P < 0.05$, paired t-test
[d] Σ ^{3}H metab. refers to the sum of the ^{3}H-metabolites
* Effect of cocaine significant, $P < 0.05$, paired t-test
** Effect of progesterone significant, $P < 0.05$, unpaired t-test

result, in the progesterone-dominated tissue, the O-methylated metabolites (^{3}H-NMN and ^{3}H-OHDA) together constituted 84% of the total ^{3}H-metabolites.

The effects of cocaine 30 μmol/l were examined in the expectation that these would reveal the importance of neuronal uptake in metabolism. In the oestrogen-dominated endometrium, cocaine caused a small decrease in total metabolite formation, the decrease being in the O-methylated metabolite fraction (Table 1). In the progesterone-dominated endometrium, the effect of cocaine was much more pronounced, ^{3}H-NMN formation being decreased by 92% and ^{3}H-OMDA formation being decreased by 74%.

Further experiments were confined to the progesterone-dominated endometrium. These showed that the COMT inhibitor, U-0521 55 μmol/l virtually eliminated ^{3}H-NMN and ^{3}H-OMDA formation, but, unlike cocaine, caused a marked increase in ^{3}H-noradrenaline accumulation (4-fold increase) and in ^{3}H-DOPEG formation (7-fold increase). These increases were prevented by cocaine 30 μmol/l (Fig. 1 A).

At a higher concentration of ^{3}H-noradrenaline, 1.2 μmol/l, ^{3}H-NMN remained the major metabolite, but the percentage of deaminated catechols in the total metabolites was higher than at the lower concentration (26 ± 3% cf. 13 ± 1%, $P < 0.05$, paired t-test, $n = 5$). Cocaine 30 μmol/l inhibited ^{3}H-DOPEG formation as well as O-methylated metabolite formation (Fig. 1 B), indicating that it was able to inhibit deamination of ^{3}H-noradrenaline when COMT was active. The effects of COMT-inhibition, and the modification of these effects by cocaine (Fig. 1 B), resembled those at the lower concentration of ^{3}H-noradrenaline (Fig. 1 A).

Endometrium: Metabolism of ^{3}H-isoprenaline

To assess whether cocaine inhibited O-methylation of other catecholamines besides noradrenaline in the progesterone-dominated endometrium, (±)-^{3}H-isoprenaline 0.18 μmol/l was used as substrate instead of (−)-^{3}H-noradrenaline. The quantity of ^{3}H-MeOISO formed (Table 2) was one-half of that of ^{3}H-NMN at the same substrate concentration (Table 1) and was unaffected by cocaine 30 μmol/l. U-0521 decreased ^{3}H-MeOISO formation by 90% and increased the accumulation of ^{3}H-isoprenaline by the tissue by 140%; the increase was unaffected by cocaine (Table 2). A small quantity of ^{3}H appeared in the column fraction corresponding to the O-methylated deaminated metabolite fraction for noradrenaline metabolites (Table 2). The identity of the ^{3}H was not determined.

Myometrium: Metabolism of ^{3}H-noradrenaline

Compared with the endometrium (Table 1), the oestrogen-dominated myometrium incubated with ^{3}H-noradrenaline 0.18 μmol/l accumulated more ^{3}H-noradrenaline in the tissue but generated about the same quantity of total ^{3}H-metabolites (Table 3). The composition of the total metabolites differed in that, in the myometrium, the percentage of deaminated catechols (63%) was somewhat greater than that of the O-methylated metabolites. Progesterone treatment resulted in a five-fold increase in ^{3}H-NMN formation and a 2.5-fold increase in ^{3}H-OMDA formation. As a result, under conditions of progesterone dominance, the ^{3}H-metabolite profiles of the myometrium (Table 3) were very similar to those of the endometrium (Table 1).

The effects of cocaine in the myometrium contrasted with those in the endometrium. In the oestrogen-dominated myometrium, cocaine 30 μmol/l depressed ^{3}H-noradrenaline accumulation (by 68%) and ^{3}H-DOPEG formation (by 50%). In the progesterone-dominated myometrium, cocaine was without effect on ^{3}H-metabolite formation; an apparent decrease in noradrenaline accumulation was not significant (Table 3).

The effects of U-0521 55 μmol/l and the modification of these effects by cocaine 30 μmol/l were examined only in the progesterone-dominated myometrium incubated with noradrenaline at the higher concentration of 1.2 μmol/l. At this concentration, the O-methylated metabolites still predominated, although the percentage of deaminated catechols in the total metabolites (37%) was somewhat greater than at the lower concentration (26%). Furthermore, effects of cocaine on noradrenaline accumulation (decreased

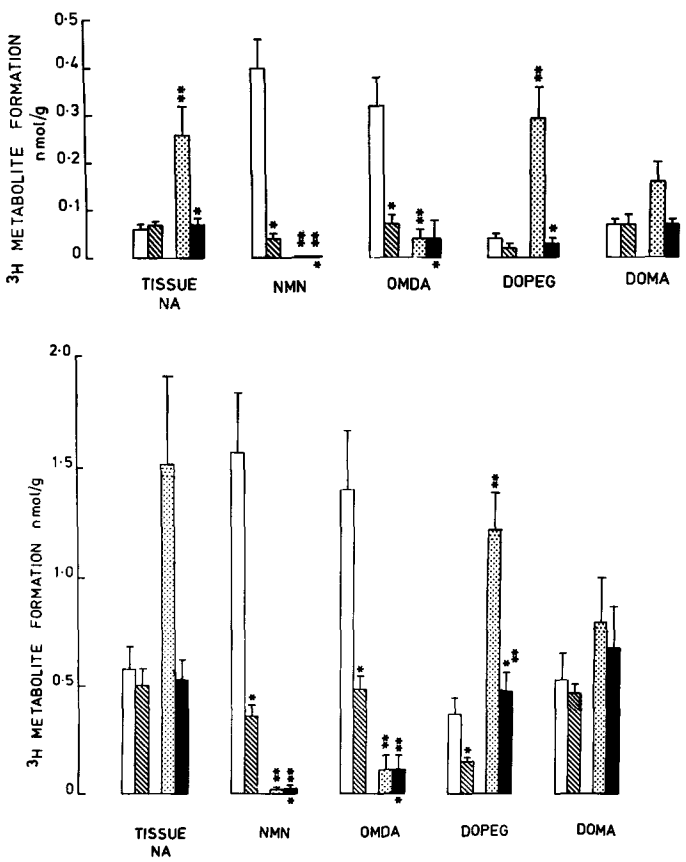

Fig. 1. Effect of cocaine 30 µmol/l on metabolism of ^{3}H-noradrenaline in the progesterone-dominated endometrium in the absence and presence of U-0521 55 µmol/l. **A** ^{3}H-noradrenaline 0.18 µmol/l $n = 5$. **B** ^{3}H-noradrenaline 1.2 µmol/l, $n = 6$. Symbols: □ cocaine and U-0521 absent; ▨ cocaine present; ▩ U-0521 present; ■ cocaine and U-0521 present; * *above column*; effect of cocaine significant, paired *t*-test, $P < 0.05$; ** *above column*; effect of U-0521 significant, paired *t*-test, $P < 0.05$; * *below column*; effect of cocaine plus U-0521 significant, paired *t*-test, $P < 0.05$. *Note on Fig. 1B:* In one of the six experiments, the accumulation of ^{3}H-noradrenaline by the tissue in the presence of U-0521 was high (3.55 nmol/g) compared with the accumulation in the remaining five experiments (1.09 ± 0.03 nmol/g). As a result, neither the effect of U-0521, nor the effect of cocaine in the presence of U-0521, on accumulation of ^{3}H-noradrenaline are significant when analysed by paired *t*-test ($0.1 > P > 0.05$). However, both effects are significant when the data are transformed into logarithms and analysed by paired *t*-tests (each, $P < 0.01$), and when the data are analysed by the non-parametric Wilcoxon matched-pairs signed rank test (each, $P < 0.02$)

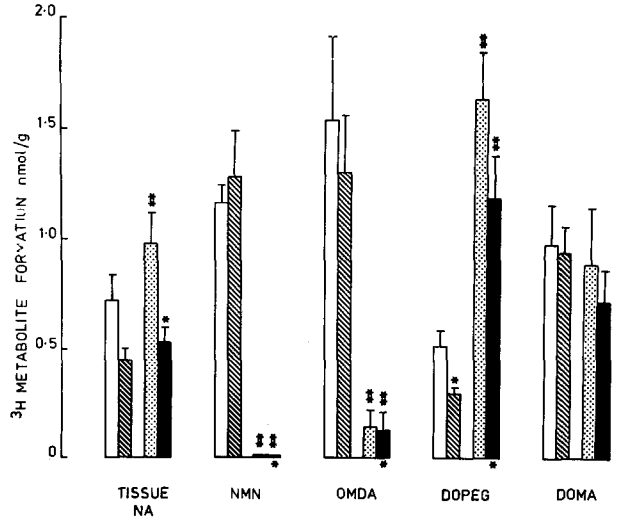

Fig. 2. Effect of cocaine 30 µmol/l on metabolism of ^{3}H-noradrenaline 1.2 µmol/l in the absence and presence of U-0521 55 µmol/l in the progesterone-dominated myometrium. Symbols: □ cocaine and U-0521 absent; ▨ cocaine present; ▩ U-0521 present; ■ cocaine and U-0521 present; * *above column*; effect of cocaine significant, paired *t*-test, $P < 0.05$, $n = 5$. ** *above column*; effect of U-0521 significant, paired *t*-test, $P < 0.05$, $n = 5$. * *below column*; effect of cocaine plus U-0521 significant, paired *t*-test $P < 0.05$, $n = 5$. *Note:* In the absence of U-0521, the effect of cocaine on ^{3}H-noradrenaline accumulation, shown above for $n = 5$, is not significant, but, as indicated in the text, is significant when $n = 6$

from 0.75 ± 0.10 to 0.45 ± 0.04 nmol/g) and DOPEG formation (decreased from 0.53 ± 0.06 to 0.29 ± 0.03 nmol/g) were significant when analysed by paired *t*-test ($n = 6$, $P < 0.05$). Five of the experiments involving cocaine included comparisons with U-0521 (Fig. 2). Elimination of O-methylated metabolite formation by U-0521 caused a three-fold increase in DOPEG formation, but had little effect on noradrenaline accumulation (22% increase). The increase in DOPEG formation appeared to be unaffected by cocaine, the values of the increases being (in nmol/g, shown in brackets, $n = 5$) cocaine absent (1.14 ± 0.26), and cocaine present (0.89 ± 0.21).

Table 2. Metabolism of (±)-^{3}H-isoprenaline 0.18 µmol/l in progesterone-dominated endometrium[a]

Drug	Content of ^{3}H-ISO[b] in tissue	^{3}H-MeISO formed	^{3}H in "^{3}H-OMDA" fraction[c]
Nil	0.08 ± 0.01	0.24 ± 0.03	0.05 ± 0.03
Cocaine 30 µmol/l	0.07 ± 0.01	0.22 ± 0.03	0.06 ± 0.03
U-0521 35 µmol/l	0.17 ± 0.01*	0.02 ± 0.01*	0.01 ± 0.01
Cocaine + U-0521	0.18 ± 0.01*	0.02 ± 0.01*	0.01 ± 0.01

[a] Data in nmol/g ($n = 4$)
[b] ^{3}H-isoprenaline
[c] Expressed as ^{3}H-isoprenaline equivalent
* Effect of U-0521 significant, $P < 0.05$, paired *t*-test ($n = 4$)

Metabolite distribution

The tissue contents of ^{3}H-metabolites at the conclusion of incubation are expressed as percentages of the quantities formed in Table 4. It will be noted that the tissue retention of deaminated catechols is considerably greater in the endometrium than in the myometrium.

^{3}H-accumulation

In uterine tissues which were exposed to both an MAO and a COMT inhibitor (nialamide 350 µmol/l and U-0521 55 µmol/l, respectively), the ^{3}H content of the tissue was used as an index of ^{3}H-noradrenaline accumulation. The uterine tissues were progesterone-dominated. Ear arteries from the same rabbits were used as an example of a relatively

Table 3. Metabolism of ^{3}H-noradrenaline 0.18 μmol/l in myometrium

	Hormone treatment			
	Oestradiol ($n = 5$)		Oestradiol + progesterone ($n = 5$)	
Presence of cocaine 30 μmol/l	−	+	−	+
^{3}H-NA content of tissue[a]	0.40 ± 0.07	0.13 ± 0.04*	0.24 ± 0.07	0.09 ± 0.01
^{3}H-metabolites formed[a]				
− ^{3}H-NMN	0.06 ± 0.03	0.04 ± 0.01	0.32 ± 0.03**	0.29 ± 0.04**
− ^{3}H-OMDA	0.17 ± 0.04	0.17 ± 0.04	0.40 ± 0.06**	0.30 ± 0.09
− ^{3}H-DOPEG	0.14 ± 0.02	0.07 ± 0.01*	0.07 ± 0.02	0.05 ± 0.01
− ^{3}H-DOMA	0.27 ± 0.10	0.25 ± 0.10	0.18 ± 0.09	0.14 ± 0.06
− Σ ^{3}H metab.[b]	0.65 ± 0.11	0.54 ± 0.09	0.98 ± 0.11	0.80 ± 0.12

[a] nmol/g
[b] Σ^{3}H-metab. refers to the sum of ^{3}H-metabolites
* Effect of cocaine significant, $P < 0.05$, paired t-test
** Effect of progesterone significant, $P < 0.05$, unpaired t-test

Table 4. Distribution of ^{3}H-metabolites between tissue and medium in progesterone-dominated uteri

Tissue	Percentage of ^{3}H-metabolite formed retained in the tissue[a]			
	^{3}H-NMN	^{3}H-OMDA	^{3}H-DOPEG	^{3}H-DOMA
Endometrium	30 ± 2	22 ± 3	25 ± 2	38 ± 4
Myometrium	39 ± 2*	24 ± 3	15 ± 1*	23 ± 3*

[a] Tissues were incubated with ^{3}H-noradrenaline 1.2 μmol/l for 30 min and washed for 10 s
* Significantly different from endometrium; $P < 0.05$, paired t-test ($n = 15$)

densely sympathetically-innervated organ. After 30 min incubation with ^{3}H-noradrenaline, the tissues were washed for 30 min by incubating in 5.0 ml of ^{3}H-noradrenaline-free Krebs solution (see Methods).

In the endometrium, at 1.2 μmol/l ^{3}H-noradrenaline, the ^{3}H-accumulation represented a 6-fold increase in tissue content (per g) above that of the bath (per ml). The proportion removed by the 30 min wash was small (less than 20%), indicating that most of the ^{3}H was firmly bound. The accumulation of this firmly bound ^{3}H was strongly inhibited by cocaine 30 μmol/l, desipramine 3 μmol/l, and (+)-amphetamine 10 μmol/l. Cocaine exerted a similar effect at higher concentrations of ^{3}H-noradrenaline (10 and 50 μmol/l) where it inhibited firmly-bound ^{3}H-accumulation by 92 and 63%, respectively (Table 5; data at 10 μmol/l not shown).

Compared with the endometrium, the myometrium incubated with ^{3}H-noradrenaline 1.2 μmol/l accumulated less ^{3}H (about one-half), of which a greater percentage (36%) was removed by washing. Cocaine 30 μmol/l inhibited the accumulation of firmly-bound ^{3}H by 60%, but was without effect at the highest concentration of ^{3}H-noradrenaline (50 μmol/l) (Table 5).

The rabbit ear artery displayed the greatest capacity to accumulate firmly bound ^{3}H (to a level 14 times that of the bath concentration). At the ^{3}H noradrenaline concentration of 1.2 μmol/l, cocaine inhibited this accumulation to about the same extent as in the endometrium. However, when the ^{3}H noradrenaline concentration was increased to 50 μmol/l, the effect of cocaine in the ear artery was not significant (Table 5).

Fluorescence histochemistry

Progesterone-dominated endometria (from each of five rabbits) which had been incubated with noradrenaline 50 μmol/l for 15 min followed by a 30 min wash showed pronounced green fluorescence in the epithelial cells of the endometrial glands. The fluorescence was particularly intense in the deeper regions of the glands (Fig. 3A). Green fluorescence was faint, or absent, in the endometrial glands (from the same rabbits) which had been incubated with noradrenaline 50 μmol/l in the presence of cocaine 30 μmol/l (Fig. 3B). Endometrial glands (from four rabbits) incubated in the absence of noradrenaline also failed to display fluorescence. The only other fluorescence was yellow, punctate, and extremely sparse in its distribution. It appeared to be unaffected by the presence of noradrenaline or cocaine. There was no evidence of a distribution of monoamine fluorescence typical of that of sympathetic nerves.

In all experiments, rabbit ear arteries (not exposed to noradrenaline) showed the intense green fluorescence at the medial-adventitial border characteristic of sympathetic nerve terminals.

Endogenous noradrenaline contents

In progesterone-dominated uteri, the endogenous noradrenaline content of the endometrium was extremely low (0.10 ± 0.03 nmol/g, $n = 5$) and approximately one-tenth of the content of the myometrium (0.96 ± 0.30 nmol/g, $n = 5$).

Discussion

Effect of progesterone

The results indicate that the stimulant action of progesterone on O-methylation of noradrenaline observed in the oestrogen-primed intact uterus by Kennedy et al. (1984) occurs in both the endometrium and myometrium. However,

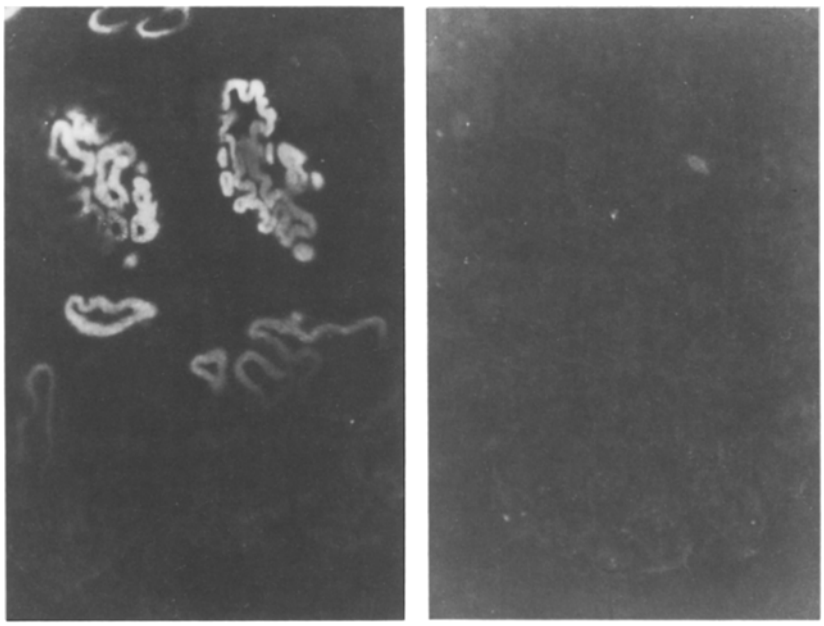

Fig. 3
Fluorescent micrographs of sections of progesterone-dominated endometrium following incubation with ^{3}H-noradrenaline 50 μmol/l in the absence (**A**) and presence (**B**) of cocaine 30 μmol/l. The more intense fluorescence in the deeper regions of the glands is evident in A. The occasional points of fluorescence in B represent the sparsely-distributed yellow punctate fluorescence referred to in the text. The lumen border is faintly visible towards the bottom of each micrograph. Scale: 100 μm

the finding that the progesterone-stimulated O-methylation is cocaine-sensitive in the endometrium, but cocaine-insensitive in the myometrium, indicates that the mechanism of stimulation is quite different in the two regions. The effects of cocaine are discussed in more detail subsequently, but it is noteworthy that, in the study of Kennedy et al. (1984), cocaine did not depress NMN formation to a significant degree in the progesterone-dominated intact uterus. The probable explanation is that the endometrium constitutes less than one-third of the uterine mass, so that, in the intact uterus, cocaine-insensitive O-methylation in the myometrium predominates.

Metabolism of noradrenaline in the progesterone-dominated endometrium

In the endometrium, noradrenaline in a low concentration (0.18 μmol/l) is metabolised mainly to the O-methylated metabolites, whereas in a higher concentration (1.2 μmol/l) there is a proportionally increased conversion to the deaminated catechols, DOPEG and DOMA. These results indicate that metabolism mediated by COMT predominates at the lower concentration, but MAO is of increased importance at the higher concentration. This shift of metabolism from O-methylation to deamination may reflect saturation of COMT at the higher concentration of noradrenaline; examples of this phenomenon in other tissues have been documented by Trendelenburg (1980). The importance of O-methylation at the lower concentration is further emphasised by the magnitude of the increases in noradrenaline accumulation (4-fold) and the increase in DOPEG formation (7-fold) produced by COMT inhibition. This result indicates that COMT activity is a major factor limiting both the accumulation and the deamination of noradrenaline at low concentrations.

A feature of endometrial metabolism is the sensitivity of NMN and OMDA formation to cocaine. Cocaine also depressed DOPEG formation, to an extent that at the lower concentration of noradrenaline (0.18 μmol/l) it eliminated the increase produced by U-0521. On the basis of these findings, it is suggested that noradrenaline is taken up by a cocaine-sensitive process into one or more extraneuronal compartments possessing COMT and MAO activity. Presumably the same compartments accumulate noradrenaline when the enzymes are inhibited, since cocaine also eliminated the U-0521-induced increase in accumulation of noradrenaline 0.18 μmol/l, and decreased by approximately 90% the accumulation of noradrenaline 1.2 μmol/l when both COMT and MAO were inhibited. Evidence for an extraneuronal location of the proposed compartment(s) is based on the apparent absence of sympathetic nerve terminals in the endometrium, as indicated both by the extremely low endogenous noradrenaline content (of the order of 0.1 nmol/g) and by our failure to detect monoaminergic nerves in the endometrium by fluorescence histochemistry. An early histochemical study of Owman and Sjöberg (1966) on the rabbit uterus also points to the absence of significant sympathetic innervation of the endometrium. Although there is evidence to suggest that there is COMT activity in the sympathetic nerves in other organs in some species (e.g., cat, Langer et al. 1972), the above histochemical findings argue against the possibility that cocaine exerts its effects on O-methylation by inhibiting neuronal uptake into COMT-containing nerves. Instead, a positive indication of the site of cocaine's extraneuronal action is provided by the histochemical finding that the epithelial cells of the endometrial glands accumulate noradrenaline and that the accumulation is prevented by cocaine. Although the relatively high concentration at 50 μmol/l noradrenaline was used in the histochemical studies, preliminary autoradiographic studies indicate that the glands are also the site of cocaine-sensitive accumulation of ^{3}H-noradrenaline when the concentration of the latter is 0.18 μmol/l (Kennedy, unpublished observations). It is assumed that the site of accumulation is also the site of O-methylation. This is based on the sensitivities of both processes to cocaine, as indicated above, but will require further histochemical studies for confirmation. In support of the assumption, the presence of

Table 5. ^{3}H-Accumulation[a] in MAO-COMT inhibited tissues[b]

^{3}H-Noradrenaline concentration in μmol/l	Drug[d]	^{3}H-Contents in nmol/g[c]								
		Endometrium			Myometrium			Ear artery		
		Tissue	Wash	*n*	Tissue	Wash	*n*	Tissue	Wash	*n*
1.2	–	7.1 ± 1.1	1.1 ± 0.1	(6)	2.7 ± 0.3	1.5 ± 0.3	5	17 ± 1	1.1 ± 0.1	5
	Cocaine	0.4 ± 0.1*	1.0 ± 0.1	(4)	1.0 ± 0.1*	1.8 ± 0.3	5	1.2 ± 0.2*	2.0 ± 0.2	3
	Desipramine	0.4 ± 0.1*	1.1 ± 0.2	(3)	–	–	–	–	–	–
	(+)-Amphetamine	0.6 ± 0.2*	0.8 ± 0.1	(3)	–	–	–	–	–	–
50	–	46 ± 8	34 ± 5	(4)	45 ± 6	54 ± 7	5	63 ± 7	80 ± 4	6
	Cocaine	16 ± 3*	38 ± 6	(4)	43 ± 7	62 ± 9	3	44 ± 11	90 ± 12	4

[a] Expressed as ^{3}H-noradrenaline equivalent
[b] Tissues from progesterone-dominated uteri
[c] Tissues were incubated with ^{3}H-noradrenaline for 30 min
[d] Cocaine 30 μmol/l, desipramine 3 μmol/l, (+)-amphetamine 10 μmol/l
n Number of rabbits
* Effect of drug significant, $P < 0.05$, unpaired *t*-test

COMT activity has been demonstrated immunohistochemically in cells lining the walls of many hollow organs, including the rat oviduct (Creveling and Hartman 1982) and the pregnant rat uterus (Inoue et al. 1980).

The preliminary characterisation of the cocaine-sensitive uptake system indicates that the noradrenaline is firmly retained by the tissue and that its accumulation is prevented by two other inhibitors of neuronal uptake, namely, desipramine and (+)-amphetamine. Comparison with the rabbit ear artery, a relatively densely sympathetically-innervated organ, indicated that the inhibitory effect of cocaine persisted in the endometrium under conditions (a substrate concentration of 50 μmol/l) where its inhibitory effect in the rabbit ear artery was largely abolished. The latter effect is in accord with evidence that cocaine is a competitive inhibitor of the noradrenaline transmembrane transport system in the sympathetic nerve terminal (Graefe et al. 1978). Hence the greater potency of cocaine in the endometrium suggests that the uptake system in the glandular epithelium may differ kinetically from that in the nerve terminal.

The high sensitivity to neuronal-uptake inhibitors distinguishes the uptake system from the corticosteroid-sensitive extraneuronal uptake systems in sympathetically innervated organs such as rat heart, rat submaxillary gland, and vascular smooth muscle in rabbit and dog, the properties of which were reviewed by Trendelenburg (1980). Isoprenaline is commonly used as a model substrate for the corticosteroid-sensitive extraneuronal O-methylating system, with an affinity for this system (based on estimates of half saturating outside concentrations) which is of a similar order of magnitude to that of noradrenaline in rat heart and dog saphenous vein (Trendelenburg 1980; Paiva and Guimaraes 1984; Grohmann and Trendelenburg 1985). Hence the present finding that the endometrial O-methylation of isoprenaline occurs at a considerably lower rate than O-methylation of noradrenaline and is insensitive to cocaine points to a possible further distinction between the cocaine-sensitive O-methylating systems in the endometrium and the corticosteroid-sensitive O-methylating system in other organs. It remains to be tested whether isoprenaline is O-methylated in the same compartment(s) as noradrenaline. This possibility is not completely excluded by the differential sensitivities to cocaine, since there is evidence in other tissues that isoprenaline diffuses more rapidly into cells than does noradrenaline (Bryan et al. 1984).

Finally, it should be noted that an extraneuronal transport system which is highly sensitive to neuronal uptake inhibitors is unusual but not novel. From the studies of Nicholas et al. (1974), Gillis (1976), and Junod and Ody (1977), it appears that the pulmonary endothelium of rat, rabbit and pig possesses a catecholamine uptake and metabolising system which is inhibited by neuronal uptake inhibitors. The results of a separate study in the present authors' laboratory indicate that rabbit dental pulp also possesses an extraneuronal O-methylating system which is highly sensitive to cocaine (Parker and de la Lande, unpublished observations). Hence the possibility exists that such a system is widespread in distribution.

Metabolism of ^{3}H-noradrenaline in the myometrium

The metabolism of noradrenaline in the oestrogen-dominated myometrium was characterised by a somewhat greater conversion to deaminated catecholamines than to O-methylated metabolites, and an inhibitory effect of cocaine on both DOPEG formation and noradrenaline accumulation. Since the oestrogen-dominated myometrium is sympathetically innervated (Falck et al. 1969a), the pattern of metabolism and its modification by cocaine is compatible with evidence that, as in other sympathetically innervated organs, exogenous noradrenaline is taken up by the nerve terminals and deaminated by intraneuronal MAO (Trendelenburg 1977).

In the progesterone-dominated myometrium, the conversion of noradrenaline to O-methylated metabolites was 3-fold greater than in the oestrogen-dominated myometrium. The absence of an effect of cocaine on O-methylated metabolite formation shows that progesterone stimulates extraneuronal systems involved in O-methylation. The possibility that the increased O-methylation is secondary to a decrease in the neuronal metabolism is raised by histochemical findings that progesterone decreases the apparent density of sympathetic nerve terminals in the

myometrium (Falck et al. 1969b). However, this possibility is excluded by the finding that NMN formation in the oestrogen-dominated myometrium is unaffected when neuronal metabolism is eliminated by cocaine.

In the progesterone-dominated myometrium, the effects of cocaine on metabolism were confined to a decrease of between 40% and 50% in DOPEG formation. Since DOPEG plus DOMA represented only a minor proportion of the total metabolites, it would appear that the sympathetic nerves play a relatively minor role in metabolism. However, it can be argued that neuronal deamination may have been limited by diversion of substrate to extraneuronal sites of metabolism. This argument is not supported by the effects on deamination of inhibiting O-methylation. Although U-0521 increased DOPEG formation three-fold, the increase was unaffected by cocaine, indicating that it was due to increased deamination at extraneuronal, and not at neuronal, sites of metabolism.

Cocaine-sensitive accumulation of noradrenaline by the progesterone-dominated myometrium, when estimated under conditions where metabolism is prevented, is quite small and of the order of one-tenth of that of a representative blood vessel (rabbit ear artery). However, the difference accords fairly well with the difference between the densities of sympathetic nerve terminals in the two tissues, as assessed by endogenous noradrenaline contents, namely, endometrium, 1.0 nmol/g (this study); ear artery, 19 nmol/g (Head et al. 1977). Hence, it appears that the accumulation of noradrenaline in the myometrium is due, in part at least, to uptake of noradrenaline by the sympathetic nerve terminals.

Some implications

The study has provided an insight into the sites and mechanism of progesterone's actions on uterine noradrenaline metabolism. In the endometrium, one of the classical morphological responses to progesterone is development of the secretory glands (Finn and Porter 1975). Since the present results have shown that the endometrial glands represent the site of cocaine-sensitive accumulation of noradrenaline, it seems probable that the marked stimulation of cocaine-sensitive noradrenaline metabolism by progesterone is simply a consequence of endometrial gland proliferation. The properties of the glandular uptake system remain to be characterised, but the results described here draw attention to the possibility that the epithelial cells possess a transport system resembling that in the sympathetic nerve terminal membrane. The factors responsible for the apparently firm binding after uptake of noradrenaline into the gland cell also await investigation.

Most of the published studies on progesterone's actions on the myometrium relate to its effects on muscle cell excitability (e.g., Wynn 1977). There do not appear to be any documented actions which can explain its profound stimulatory effect on noradrenaline O-methylation. It remains to be established whether an increased activity of the extraneuronal amine transport system, or of COMT, is responsible.

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