Dual Vasoconstrictor and Vasodilator Innervation of the Uterine Arterial Supply in the Guinea Pig

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ABSTRACT

The innervation of the extrinsic uterine arterial supply of the guinea pig has been studied using an isolated perfused preparation. The preparation was normally at minimal tone and responded to periarterial stimulation by a vasoconstriction which was mimicked by norepinephrine and abolished by bretylium. Constrictor responses both to stimulation and to norepinephrine were similar in both pregnant and non-pregnant states. In pregnant preparations, raising the tone of the vessels with norepinephrine revealed a powerful dilator response to periarterial stimulation. This response was mimicked by acetylcholine, potentiated by anticholinesterases, reduced by hyoscine, and abolished by local anesthetic treatment. In contrast, virgin preparations showed only a weak dilator response to stimulation, while the response to acetylcholine even at high concentrations was usually negligible. Histochemical examination of the vessels revealed a dense plexus of fine nerve fibers exhibiting high acetylcholinesterase activity along the main uterine artery. The fibers lay in close apposition to the vascular muscle layer. Nerve fibers exhibited fluorescence for catecholamines were also abundant, but were distributed along the secondary as well as the main arteries. It is concluded that the uterine arterial supply of the guinea pig is innervated by both adrenergic constrictor and cholinergic dilator fibers. The dilator fibers appear to be functional only during pregnancy. The possibility of a noncholinergic dilator innervation is also discussed.

ADDITIONAL KEY WORDS adrenergic cholinergic pregnancy vasomotor control isolated perfused artery fetal blood flow

It has been known for many years that most mammalian arterial beds are subject to the influence of adrenergic vasoconstrictor nerves (1, 2). This constrictor innervation is not confined to the arteriolar resistance vessels within a tissue, but is also distributed to the extrinsic arteries, and even to the aorta itself (3, 4). These extrinsic vessels offer convenient isolated preparations with which to study various aspects of transmission from vasoconstrictor nerves to arterial muscle, and strips or perfused segments of such arteries have been employed in both pharmacological and electrophysiological studies by a number of workers (5-8, and others).

The existence of active neurogenic vasodilatation is also well established (2, 9, 10), and it seems that several different transmitters may be involved at various sites (10-13). However, these data have all been obtained using intact animals or whole perfused limbs. This can be attributed to the fact that to date no extrinsic artery suitable for use as an isolated preparation has been found to possess a vasodilator innervation. The sympathetic cholinergic vasodilator fibers to skeletal muscle, at least, appear to supply the arteriolar bed only (14).

Recently it was found that the extrinsic arteries supplying the uterus of the guinea pig are innervated by both constrictor and dilator fibers. These arteries are readily isolated and afford an opportunity to investigate...
transmission from vasodilator nerves to arterial smooth muscle at a cellular level.

**Methods**

The Isolated Perfused Preparation.—The uterine arterial supply to the uterus of the guinea pig is illustrated in Figure 1, a. On each side, the internal iliac artery gives rise to the main uterine artery, which runs parallel to the uterine horn in the broad ligament. From this main uterine artery, numerous secondary arteries branch off along the length of the uterine horn.

After intracardiac injection of 1000 units of Heparin Sodium (Boots), female guinea pigs were killed by a blow on the head and exsanguination. The abdominal cavity was opened, and the oviducts were severed, following which the uterine horns were deflected posteriorly. The external and internal iliac arteries were ligated as shown in Figure 1, a, and a polyethylene cannula was inserted in the common iliac artery at the bifurcation of the common aorta and passed down to the origin of the internal iliac. The secondary uterine vessels were severed close to their point of entry into the uterine horn, and the entire extrinsic uterine arterial supply was isolated as shown in Figure 1, b. To avoid damaging the preparation, the extrinsic uterine veins, which run close to the arteries, and the accompanying fatty tissue were not cleared away. The perfusion and recording system used is illustrated in Figure 2. The preparation was mounted in a 50-ml bath of carbogenated McEwan's solution (NaCl, 7.6; KCl, 0.42; CaCl₂, 0.24; NaH₂PO₄, 0.143; NaHCO₃, 2.1; glucose, 2.0; sucrose, 4.5 g/liter) maintained at 37°C and was perfused with the same solution via a roller pump delivering a constant flow. The flow rate was varied between 4 to 6 ml/min in different experiments depending on the size of the preparation used.
but remained constant during any one experiment. Perfusion pressure of the preparation was recorded by a Statham P23BC transducer connected into the perfusion system distal to the roller pump, and a Grass Model 5 polygraph. Periarterial nerve stimulation was elicited with shielded platinum ring electrodes placed around the base of the main uterine artery (Fig. 1, b), and 10-second bursts of 1-msec, square-wave pulses at supramaximal voltage were delivered from a Grass S5 stimulator at intervals of not less than 4 minutes.

Drugs were administered by injection into the perfusion fluid proximal to the cannula, or by perfusion from the reservoir of McEwan’s solution. Drugs used were: acetylcholine perchlorate, B.D.H.; bretylium tosylate (Darenthin), Wellcome; cinchocaine hydrochloride (Nupercaine) Ciba; histamine acid phosphate, B.D.H.; hyoscine bromide B.D.H.; isoproterenol hydrochloride (Isopenaline), Wellcome; mecamylamine hydrochloride, Merck, Sharpe & Dohme; mepyramine maleate (Anthisan), May & Baker; 1-methyl lysergic acid butanolamide (methysergide), Sandoz; neostigmine methylsulphate (Prostigmin), Boche; norepinephrine hydrogen tartrate (Levophed) Winthrop; physostigmine sulphate, B.D.H.; pronethanol hydrochloride (Aldelin), I.C.I.; and serotonin creatine phosphate, B.D.H. All doses and concentrations cited refer to weights of the above salts. Solutions of norepinephrine contained 10⁻⁶ g/ml ascorbic acid to prevent oxidation, and in experiments in which norepinephrine infusion was utilized to give maintenance of tone in the preparation, 10⁻⁶ to 10⁻⁵ g/ml of ascorbic acid was also present in the McEwan’s solution used for perfusion. Acetylcholine was dissolved in 0.001N HCl. Neither of these acid vehicles had any effect of themselves on the preparation in the concentrations used.

Histochemical Observations.—The entire uterine vascular supply, together with the accompanying uterine horn, was dissected, pinned on a chilled paraffin wax block, and fixed overnight at 4°C in 4% formaldehyde containing 0.44M sucrose and buffered to pH 7.6 with CaCO₃. Segments of artery were removed from the fixed tissue and washed in several changes of distilled water for 1 hour at room temperature, following which they were stained for acetylcholinesterase by the method of Karnovsky and Roots (16), for the minimum period necessary to produce satisfactory staining of known cholinergic nerves in the guinea pig. In some experiments 1:5-bis (4-allyldimethylammoniumphenyl) pentane-3-one diiodide (BW 284C51) in a concentration of 5 x 10⁻⁶M was used as a specific inhibitor of acetylcholinesterase. After staining, the tissue was washed in distilled water for 10 minutes,
dehydrated and cleared, and either mounted in Canada balsam for observation as a whole mount or imbedded for paraffin wax sectioning.

Fluorescent histochemistry was performed on freeze-dried tissue by the method of Falck (17).

Classification of Animals.—The presence of a vaginal closure membrane in virgin animals was taken to indicate diestrous; its absence to indicate estrous.

**Results**

**PERFUSION EXPERIMENTS**

Under the experimental conditions used, the resting perfusion pressure of the uterine arterial tree was 20 to 30 mm Hg. Little or no spontaneous activity was observed, and the vessels were in a condition of minimal tone, as evidenced by the fact that high concentrations of papaverine and ACh produced no reduction in perfusion pressure.

(a) Responses to Norepinephrine and Acetylcholine

Injection of norepinephrine (NE) in doses of $10^{-8}$ to $10^{-6}$ g produced a rapid vasoconstriction, with up to ten-fold increases in perfusion pressure in response to the highest doses. With doses of NE of less than $10^{-6}$ g the response showed a rapid decline (Fig. 3, a). At higher concentrations the vasoconstriction was maintained at a plateau level for up to several minutes before falling off.

(b) Responses to Norepinephrine and Acetylcholine

Infusion of NE, $5 \times 10^{-7}$ to $10^{-6}$ g/ml, resulted in the maintenance of a moderate to high degree of tone (80 to 130 mm Hg) and the initiation of spontaneous rhythmic contractions. Under these conditions dilatory responses could be studied. In preparations taken from animals in mid to late pregnancy, acetylcholine (ACh) produced a vasodilata-
tion which was rapid in onset, but somewhat slower in decline than was the constrictor response to NE (Fig. 3, b). Measurable responses were produced by doses of ACh as low as $10^{-11}$ g, and doses of $10^{-7}$ g or higher reduced the perfusion pressure almost to the basal level. The pD2 for ACh in these preparations lay at about 8.5 (Fig. 4).

In contrast, preparations from virgin animals, whether in estrous or diestrous, exhibited a marked insensitivity to ACh (Fig. 4). In 10 of 16 preparations, ACh in doses of up to $10^{-4}$ g produced a decrease in perfusion pressure of only 0 to 10 mm Hg. In the remaining 6 preparations, the response to an initial dose of $10^{-7}$ to $10^{-5}$ g of ACh was considerable, but subsequent responses were very small or even absent, suggesting that tachyphylaxis occurred. Such a situation is illustrated in Figure 7.

Responses to ACh in all concentrations tested were completely abolished in the presence of hyoscine, $5 \times 10^{-7}$ g/ml.

(b) Responses to Other Neurohumors

Histamine, $10^{-7}$ to $10^{-5}$ g, caused a pure constrictor response in two late pregnant and six virgin preparations, as did $10^{-7}$ to $10^{-6}$ g of serotonin in three virgin preparations, regardless of whether the tone of the artery was basal or raised with an NE infusion; they were abolished in the presence of the selective antagonists mepyramine $10^{-6}$ g/ml, and methysergide, $5 \times 10^{-7}$ g/ml, respectively.

In three virgin preparations, $10^{-6}$ to $10^{-4}$ g of isoproterenol was injected during the maintenance of tone by NE. Doses of isoproterenol of up to $10^{-5}$ g had no effect on the tone of the preparations, though higher doses caused a weak constrictor response which was not abolished by the $\beta$-receptor blocking agent pronethalol, $10^{-5}$ g/ml.

(c) Responses to Periarterial Nerve Stimulation

Under resting conditions, electrical stimulation across the arterial wall resulted in a pure vasoconstrictor response which was rapid in both onset and decay. The vasoconstriction was abolished by infusion of bretylium, $2 \times 10^{-5}$ g/ml, (Fig. 5). This concentration of bretylium did not reduce the response to injected NE. The vasoconstrictor response

\[ \text{FIGURE 5} \]

Responses of two mid-pregnancy preparations to periarterial nerve stimulation at 20 pulses/sec (solid circles), showing vasoconstrictor and vasodilator responses. (a) Consecutive periods of stimulation before and during infusion of norepinephrine, $5 \times 10^{-7}$ g/ml. Note the biphasic response to stimulation under conditions of raised tone. (b) Basal tone, pure constriction; (c) blockade of the constriction after bretylium, $2 \times 10^{-6}$ g/ml; (d) tone raised with norepinephrine, $10^{-6}$ g/ml, pure dilatation in the absence of constrictor fiber influence.

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was frequency dependent, maximal responses being obtained at a stimulation frequency of about 70 pulses/sec (Fig. 6). Strong vasoconstrictor responses could be obtained in both estrous and diestrous virgins, and during pregnancy.

Raising the tone of the vessels with an infusion of NE revealed a marked dilator component in the response to periarterial stimulation of preparations from pregnant animals. If the vasoconstrictor response had not been previously blocked, the dilatation was seen following recovery from the constriction (Fig. 5, a). Following blockade of the vasoconstrictor response with bretylium however, stimulation produced a pure dilatation (Fig. 5, d). The vasodilator response was rapid in onset, but considerably slower in recovery than was the vasoconstriction. It was also frequency dependent, maximal responses being obtained at a stimulation frequency of about 40 pulses/sec (Fig. 6). Marked dilator responses were obtained only during pregnancy, when they paralleled the responses to injected ACh. However, dilator responses of 5 to 15 mm Hg in response to periarterial stimulation were seen in 10 out of 16 virgin preparations where ACh in doses of up to $10^{-4}$ g produced virtually no response (Fig. 7).

In two experiments performed on virgin preparations, neither the constrictor nor the dilator responses to stimulation were substantially affected by the ganglion blocking agent mecamylamine, $10^{-5}$ g/ml.

(d) Effects of Autonomic Drugs on the Vasodilator Response to Nerve Stimulation

Local Anesthetic Treatment.—The local anesthetic cinchocaine, $5 \times 10^{-4}$ g/ml, completely abolished the vasodilator response to periarterial stimulation in four pregnant and five virgin preparations. This concentration of cinchocaine did not markedly reduce the responses of the pregnant preparations to injected ACh.

Acetylcholine, Neostigmine, and Hyoscine.—In 6 pregnant preparations hyoscine, $5 \times 10^{-7}$ g/ml, reduced the duration and amplitude of the vasodilator response to stimulation by only 40 to 50% (Fig. 8, b), although responses to ACh were completely abolished. A ten-fold increase in the concentration of hyoscine produced no further blockade. Following the occurrence of maximal blockade with hyoscine, the remaining response to stimulation was abolished by cinchocaine, $5 \times 10^{-6}$ g/ml, (Fig. 8, c). The anticholinesterases physostigmine and neostigmine, $10^{-4}$ g/ml, potentiated vasodilator responses to stimulation in six and three pregnant preparations, respectively (Fig. 8, e). Subsequent infusion of hyoscine, $10^{-6}$ to $2 \times 10^{-8}$ g/ml, returned the potentiated responses to control values or less in four out of six of these experiments (Fig. 8, f).

Neither hyoscine, $5 \times 10^{-7}$ to $5 \times 10^{-6}$ g/ml,
AUTONOMIC INNERVATION OF THE UTERINE ARTERY

Responses of a virgin preparation to acetylcholine (at the arrows) and to periarterial nerve stimulation at 20 pulses/sec (solid circles) in the presence of bretylium, $2 \times 10^{-4}$ g/ml and norepinephrine, $5 \times 10^{-7}$ g/ml. The panels are continuous. Note that although the first injection of $10^{-7}$ g acetylcholine elicited a marked response, subsequent injections of up to $10^{-4}$ g were almost totally without effect. In contrast, the response to periarterial nerve stimulation did not change markedly.

(six preparations) nor physostigmine, $10^{-6}$ g/ml, (three preparations) had any effect on the weak dilator responses to stimulation seen in virgin preparations.

Other Antagonists.—Although the lack of dilator responses to histamine, serotonin and isoproterenol (Section b) made it unlikely that the hyoscine-resistant dilation in response to stimulation was due to stimulation either of histaminergic, serotonergic, or β-receptors, mepyramine, $10^{-6}$ g/ml, methysergide, $5 \times 10^{-7}$ g/ml, and pronethalol, $10^{-6}$ g/ml, were applied to six, three, and four virgin preparations, respectively. In no instance was any reduction of the dilator response to stimulation observed.

Responses of 2 mid-pregnancy preparations to periarterial nerve stimulation at 20 pulses/sec (solid circles) after bretylium, $2 \times 10^{-4}$ g/ml, in the presence of norepinephrine, $5 \times 10^{-7}$ g/ml. (a) Control; (b) in presence of hyoscine, $5 \times 10^{-7}$ g/ml; (c) after subsequent addition of cinchocaine, $5 \times 10^{-6}$ g/ml; (d) control; (e) in presence of neostigmine, $10^{-6}$ g/ml; (f) after subsequent addition of hyoscine, $2 \times 10^{-6}$ g/ml.
Histochemical localization of acetylcholinesterase (AChE) and norepinephrine in the uterine artery. (a) Whole mount of main uterine artery, stained for AChE. Note the plexus of heavily stained nerve fibers investing the arterial wall (A) and a large, heavily stained nerve bundle (B) running to the uterus. (b) Transverse section of the main uterine artery stained for AChE, showing the close relationship between the smooth muscle coat (M) and the plexus of heavily stained nerve fibers.
HISTOCHEMICAL RESULTS

Histochemical localization of acetylcholinesterase (AChE) revealed that the main uterine artery was surrounded by a dense plexus of fine nerve fibers which stained heavily for AChE (Fig. 9, a). These fibers were remote from the larger nerve bundles traveling to the uterus itself (Fig. 9, a and b), and in transverse section were seen to lie in close apposition to the muscular coat of the artery (Fig. 9, b). No evidence was seen of ganglion cells anywhere along the length of the vessel. The plexus of AChE-positive fibers extended a short way along the secondary arterial branches, but was absent from the major portion of their length (Fig. 9, c). In contrast, fluorescent histochemistry revealed that varicose fibers exhibiting fluorescence were present both along the main and secondary arteries (Fig. 9, d and e). No difference in the pattern or intensity of AChE staining was seen between immature (3 weeks postnatal) and adult animals, or between pregnant and virgin adults.

Discussion

Under the conditions of low tone of the perfused preparations utilized in this study, periartrial nerve stimulation resulted in a pure vasoconstrictor response. This response was mimicked by NE and was blocked by low concentrations of bretylium. It may therefore be concluded that the arterial tree is subject to the influence of noradrenergic vasoconstrictor fibers. This is further supported by the fact that the arteries are surrounded by a plexus of varicose nerve fibers containing high levels of fluorescent catecholamines.

Following raising of the vascular tone with an infusion of NE, periartrial stimulation of preparations from pregnant animals resulted in a marked vasodilator response. This response was abolished by local anesthetic treatment and can therefore be assumed to be neurogenic in nature. The dilatation was mimicked by ACh and potentiated by antiChE agents. Hyoscine reduced but did not completely block the response. However, the potentiation produced by antiChE treatment was reversed by hyoscine. In view of these results, it may be concluded that the uterine arterial tree is innervated by vasodilator fibers which are at least partly cholinergic in nature. Such a conclusion is supported by the fact that a dense plexus of fine nerve fibers staining intensely for AChE was closely associated with the muscular coat of the main uterine artery. These fibers are not identical with the adrenergic vasomotor nerves because they were almost entirely restricted to the main artery, while fibers exhibiting fluorescence were distributed along both the main and secondary arteries. Furthermore, electronmicroscope histochemistry has revealed that some of the terminal axons adjacent to the media of the main artery are associated with high levels of AChE, and that AChE activity is also present on the muscle cell membranes adjacent to these axons (Bell, unpublished observations). This situation is identical to that seen at known cholinergic autonomic neuromuscular junctions (19).

Responses of the uterine arteries to NE and to vasoconstrictor nerve stimulation were similar in both virgin and pregnant animals. In contrast, marked dilator responses to neither ACh nor nerve stimulation were observed.

stained nerve fibers (A). (B) is a nerve bundle supplying the uterus. (c) Transverse section of a secondary uterine artery stained for AChE. Note the complete absence of staining nerve fibers. (d) Transverse section of a secondary uterine artery close to its entry into the uterus, showing nerve fibers possessing catecholamine fluorescence (A) in close apposition to the muscular coat. (cf. panel c). There also appears to be some fluorescence in nerves of an accompanying vein (V). (e) Transverse section of the main uterine artery, showing nerve fibers possessing noradrenergic fluorescence (A) in close apposition to the muscular coat (M), and autofluorescence in the elastic intima (X). Contraction during fixation has resulted in the artery having an apparently thicker media than that illustrated in panel b. Calibrations: 100μ

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in virgin preparations. It appears, therefore, that initiation of pregnancy results in some alteration in the muscarinic receptors present on the arterial smooth muscle, leading to a dramatic sensitization to ACh of the muscle which enables the cholinergic dilator nerves to become functional. The marked response to an initial injection of ACh of some virgin preparations, with subsequent failure of responses to further injections, suggests that the mechanism underlying such a sensitization is likely to be concerned with the maintained response of existing receptors to ACh, and not to an actual induction of receptors.

The dilator response to stimulation during pregnancy was reduced, but not abolished, by hyoscine. It may be that the ineffectiveness of hyoscine was due to the resistance of the cholinergic fibers to blockade, as appears to be the case in the bladder (20-24), and has been suggested for the vasodilator fibers to the cat tongue (25). On the other hand, a considerable body of evidence exists to indicate that noncholinergic transmitters may be involved in neurogenic vasodilatory responses at some sites. Among the substances which have been implicated are histamine (10, 12, 26, 27), polypeptides (10), and prostaglandin E1 (11). In the present study, some virgin preparations which were virtually completely insensitive to injected ACh, still responded to nerve stimulation with a weak vasodilatation which was unaffected by phystostigmine and hyoscine. Thus the release of a noncholinergic dilatatory transmitter by these nerves cannot be discounted as a possibility, although the nature of such a transmitter remains obscure. Certainly the pharmacological findings suggest that it is not histamine, serotonin, or a catecholamine that stimulates β receptors.

The concept of a vasodilator innervation of the uterine blood supply was suggested as long ago as 1877 (28), although the current view is that any such innervation is functionally unimportant, and that the uterine hyperemia seen during pregnancy is due primarily to the dilation by circulating hormones (29-31). The present study has demonstrated that the uterine arterial supply of the guinea pig is innervated by vasodilator fibers which are capable of exerting an extremely powerful influence during pregnancy, but not under other circumstances. Thus it would appear likely that, at least in this species, the hyperemia of pregnancy is related to active neurogenic dilatation, although presumably the enhanced sensitivity of the vessels to ACh is itself produced by hormonal changes.

It is of interest that evidence has been obtained for a dual adrenergic and cholinergic innervation of the uterine arterial supply of the human (Bell and Townsend—unpublished observations). If a similar situation occurs here to that in the guinea pig, this raises points of interest regarding the control of fetal blood flow in various clinical conditions.

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References
EDITORIAL COMMENT

The Editors of Circulation Research, acting with the advice of the members of the Editorial Board, or special consultants, or both, usually decide that a manuscript shall be published (with or without revision) or not published. Occasionally the reviewers (or a majority of them) present cogent reasons for not publishing a manuscript, and the authors present equally cogent reasons for publishing it. In such cases, the Editors may decide to publish the manuscript (so that views other than those of experts selected by us may be read) but to publish with it a critical commentary by our experts (so that the reader shall be aware that these views have already been seriously challenged). Such an article follows. Below are some comments and questions written by the Editors.

Although stress relaxation has been observed in heart muscle, secondary to both passive stretch and active development of tension, the site and physiological significance of this phenomenon have not been defined. The following study by Hoffman, Bassett, and Bartelstone, which seeks to establish the existence of residual interactions in contractile elements in resting muscle as a further source of apparent changes in compliance, is not convincing. The presence of knots with unknown compliance, the use of high rates of stimulation which at 37°C may produce hypoxic muscle cores and possible contracture, and diastolic stresses far above physiological conditions limit the conclusions. The presence of muscle fatigue following excessive stretch must be excluded. Further, the authors have rarely shown an increase in compliance with paired stimulation, but rather a decrease (Fig. 5) such as would be induced by incomplete relaxation or after contractions. The previous claims of the authors that paired stimulation can directly increase compliance (refs. 12 and 15), apart from effects on viscous components, have not been supported.

This article also illustrates the great need for those working in the field of cardiac dynamics to agree on definitions and terminology. Should the term compliance be used only for measurements made under static conditions (to denote elastic recoil)? Does tension = force = stress? Are its units dynes/cm or dynes/cm²?
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