Selective Cholinergic Denervation of the Uterine Artery in the Guinea-Pig

It has been reported recently that the parametrial arteries of the guinea-pig, dog, pig and human, but not those of a number of other mammalian species, are innervated by cholinergic nerves[1-3]. Physiological studies have shown that, at least in guinea-pig and dog, these nerves are vasodilator[1,4] and in the guinea-pig they can exert their effect only during the latter half of pregnancy or after administration of oestrogens, due to insensitivity of the arterial muscle to acetylcholine under other circumstances[1,5]. This situation has led to the suggestion that tonic neurogenic dilatation of the parametrial vessels during pregnancy is concerned in the production of the uterine hyperaemia which is necessary for foeto-placental growth[1,6,7].

In order to test the veracity of this hypothesis it is necessary to be able to study the course of pregnancy in the absence of dilator nervous influence. A histochemical investigation has therefore been carried out in order to delineate the peripheral course and ganglionic synapses of the cholinergic fibres, and to develop a surgical technique for their selective destruction.

Virgin female guinea-pigs weighing between 300 and 500 g were anaesthetized with sodium pentobarbitone 38 mg/kg i.p. Under aseptic conditions a midline laparotomy was performed and various nerve trunks and vessel surfaces in the pelvic region were coagulated using a small cotton wool swab dipped in 80% aqueous phenol. The field of operation was dusted with antibiotic powder (Cicatrin®), the muscle and skin incisions closed with blanket sutures and the suture lines sprayed with surgical adhesive (Nobecutane®). The animals were treated immediately post-operatively with oxytetracycline (Terramycin®) 10 mg i.m.. 7 days to several months after operation the animals were killed by cervical dislocation and the parametrial vasculature was cleared of surrounding fat, fixed overnight in 10% formol-sucrose buffered with $CaCO_3$ and stained for the demonstration of acetylcholinesterase (AChE) by the method of Karnovsky and Roots[8], using acetylthiocholine iodide substrate. The tissues were preincubated with 8×10^{-6} *M* iso-OMPA (tetramono-isopropyl pyrophosphortetramide) to prevent staining of pseudo-cholinesterase. Following staining the tissues were dehydrated, cleared and mounted in DePeX for microscopic examination.

The sites and results of regional coagulation are shown in the Table and in the Figure. Bilateral destruction of the hypogastric trunks below the inferior mesentric ganglion, or of the first and second sacral nerves lateral to the rectum, had no effect on the cholinergic nervous plexus which surrounded the parametrial artery. Coagulation of the surface of the common iliac artery (site 1 in Figure) the internal iliac artery below the origin of the uterine artery (site 2) or the uterine artery at the level of its vaginal branch (site 3) similarly had no effect. In contrast, coagulation of the surface of the uterine artery above the level of the cervix (site 4) resulted in complete degeneration of the cholinergic nervous supply to the artery on that side, indicating that these nerves joined the artery in the paracervical region.

Examination of carefully prepared tissues from normal animals after staining revealed the existence of a small ganglionic mass lying in the broad ligament between the uterine artery and the cervix (Figure). From this ganglion, AChE-staining nerves could be seen to run to the uterine artery above the cervix and form the AChE-positive vasomotor plexus. The origin of the cholinergic vasodilator supply in this paracervical ganglion was confirmed by the observation that its bilateral coagulation caused complete bilateral degeneration of the AChE-positive perivascular plexus. Unilateral coagulation (site 5) caused degeneration of only about two-thirds of the nerves on the ipsilateral artery, suggesting that some degree of crossover occured, and this was confirmed by the fact that complete degeneration of the ipsilateral nerves was seen if unilateral ablation was combined with coagulation of the ventral surface of the cervix (sites 5 and 6). Fluorescent microscopy for the visualization of catecholamines[9] showed that the degeneration of cholinergic vasomotor nerves after coagulation of the paracervical ganglia was not accompanied by any degeneration

[1] C. Bell, Circulation Res. *23*, 269 (1968).
[2] C. Bell, Circulation Res. *24*, 61 (1969).
[3] C. Bell, J. Reprod. Fertil. *27*, 53 (1971).
[4] M. J. Ryan, K. E. Clarke and M. J. Brody, Fednr. Proc. *31*, 588 A (1972).
[5] C. Bell, Br. J. Pharmac., in press (1974).
[6] C. Bell, Pharmac. Rev. *24*, 657 (1972).
[7] C. Bell and M. J. Brown, J. Reprod. Fertil. *27*, 59 (1971).
[8] M. J. Karnovsky and L. Roots, J. Histochem. Cytochem. *12*, 219 (1964).
[9] B. Falck and C. Owman, Acta univ. lund. *7*, 1 (1965).

The effect of phenol coagulation of various pelvic nerves and vessel surfaces on the cholinergic innervation of the parametrial artery in the guinea-pig

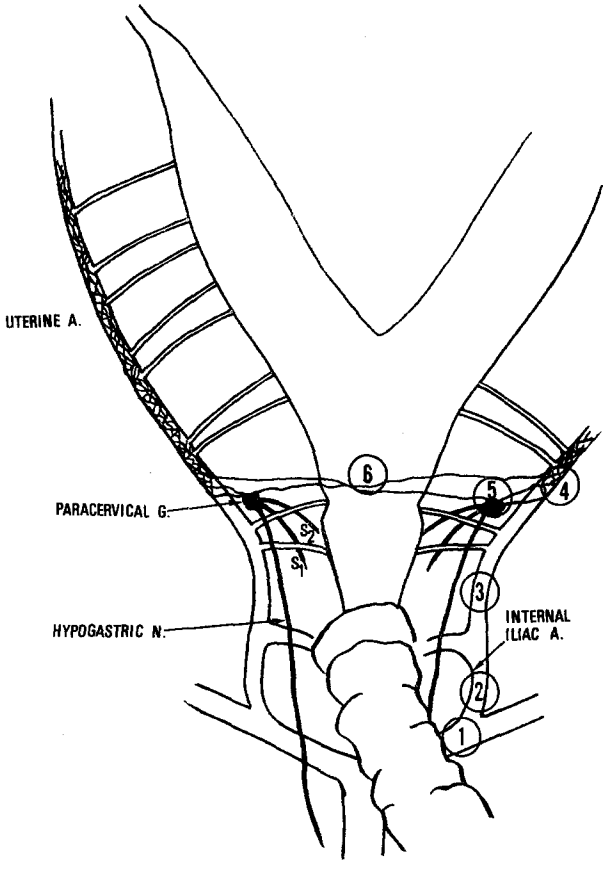
Site of lesion	No. of animals	Effect
hypogastric trunk	2	none
sacral nerves 1 and 2	3	none
1	1	none
common iliac (site 1)	2	none
internal iliac (site 2)	4	none
uterine artery (site 3)	3	none
(site 4)	4	complete ipsilateral denervation
paracervical region (site 5) bilateral	4	complete bilateral denervation
unilateral	2	$^2/_3$ denervation on ipsilateral side
unilateral + cervical surface (sites 5 and 6)	2	complete denervation on ipsilateral side

of the adrenergic vasomotor supply to the uterine artery.

These results demonstrate that the cholinergic vasodilator nerves which supply the uterine artery in the guinea-pig originate entirely from the paracervical ganglia. As no degeneration of vasomotor fibres was noted following lesion of either the hypogastric or sacral nerves it appears that these fibres arise at least in the main from paracervical ganglion cells, rather than merely travelling through the ganglion as postganglionic nerves.

The spinal origin of the nerves remains uncertain. It is well known that the hypogastric nerve synapses in pelvic ganglia[10,11] and the sparse cholinergic innervation to the vas deferens of the guinea-pig, which travels through these ganglia[12], has been thought to be hypogastric in origin[13]. On the other hand coagulation of the paracervical region with phenol, which greatly assists in visualizing fine nervous elements, reveals that the first and second sacral nerves send elements into the paracervical ganglion. Certainly a parasympathetic origin would be in accord with the majority of data for cholinergic dilator supplies[14]. In this regard it may be noted that degeneration studies on the rat paracervical ganglion have revealed the presence of presynaptic endings of both hypogastric and pelvic origins[15].

The fact that the cholinergic dilator supply to the parametrial vasculature can be selectively destroyed by ablation at a ganglionic synapse means that regeneration is unlikely. This is supported by the fact that no intact AChE-positive fibres were seen in the uterine artery of an animal killed 6 months after bilateral ganglionectomy. Thus this technique affords a method by which the progress of pregnancy in the absence of local cholinergic dilator influences can be studied.



The anatomical course of the cholinergic dilator nerves supplying the parametrial artery of the guinea-pig. Note that the preganglionic fibres may be of sacral or of hypogastric origin. The ringed numbers denote sites of phenol-induced coagulation.

Résumé. Les nerfs cholinergiques vasodilatateurs de l'artère utérine du cobaye ont leur origine dans les ganglions paracervicaux. L'ablation de ces ganglions permit d'évaluer l'importance des nerfs vasodilatateurs dans le cas d'une hyperémie de l'uterus apparaissant durant la grossesse.

CH. BELL[16–18]

University of Melbourne, Department of Physiology, Parkville (Victoria 3052 (Australia), 24 September 1973.

[10] N.-O. SJÖSTRAND, Acta physiol. scand. *65*, Suppl. *257* (1965).
[11] N.-O. SJÖBERG, Acta physiol. scand. *72*, Suppl. 305 (1967).
[12] C. BELL and J. R. MCLEAN, J. Pharmac. exp. Ther. *157*, 69 (1967).
[13] A. T. BIRMINGHAM, Br. J. Pharmac. Chemother. *27*, 145 (1966).
[14] C. BELL and G. BURNSTOCK, *Physiology and Pharmacology of Vascular Neuroeffector Systems* (Eds. J. A. BEVAN, R. F. FURCHGOTT, R. A. MAXWELL and A. P. SOMLYO; S. Karger, Basel 1971), p. 37.
[15] T. MUSTONEN and H. TERAVAINEN, Acta physiol. scand. *82*, 264 (1971).
[16] Part of the experimental work was performed in the Department of Zoology, University of Melbourne, and I thank Professor G. BURNSTOCK for his hospitality.
[17] This study was supported by the National Heart Foundation of Australia.
[18] Terramycin® was kindly donated by Pfizer Pty. Ltd.

The Package of Vesicle Content in *Octopus* Synapses as Revealed by Unbuffered Zinc Iodide-Osmium

The zinc iodide-osmium (ZIO) method was first introduced in invertebrates by MARTIN et al.[1] The method turned out to react with several types of synaptic vesicles in the *Octopus* brain, causing a homogeneously black precipitate within the vesicle membrane. The substance which reacts with ZIO has not been determined as yet. In all investigations reported so far, the ZIO-impregnation of synaptic vesicles proceeds in an all-or-none fashion: the content of a vesicle either reacts completely or not at all. This type of reaction can be modified by the use of unbuffered ZIO.

Medium-sized animals were anaesthetized in urethane (3% in seawater) and fixed by perfusion through the cephalic arteria with glutaraldehyde (3.5% in seawater, pH adjusted to 7.2 with NaOH 0.1 *N*). Small tissue slices (0.5–1 mm thick) were dissected from the perfused brain lobes and the iris and washed 3×10 min in an unbuffered stock solution containing 0.57 *M* NaCl, 0.0054 *M* $CaCl_2$ and 0.0015 *M* $MgCl_2 \cdot 6\ H_2O$, (pH 5.7). Then the tissue was ready for incubation in ZIO. 1 h before incubation 2 solutions were prepared, A) and B). A) contained 5% OsO_4 and B) 7.5% Zn-powder + 2.5% iodium bisublimate in the unbuffered stock solution. B) was shaken and

[1] R. MARTIN, J. BARLOW and A. MIRALTO, Brain Res. *15*, 1 (1969).