CHANGES IN THE INNERVATION OF THE UTERUS DURING PREGNANCY

By Greta J. E. David

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Cardiovascular Neuroscience Group, Cardiovascular Medicine and

Centre for Neuroscience, Flinders University of South Australia

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written except where due reference is made in the text.

Greta J. E. David

March 2009

I believe that this thesis is properly presented, conforms to the specifications of the thesis and is sufficient standard to be, *prima facie*, worthy of examination.

Associate Professor Ida J. Llewellyn-Smith

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ABSTRACT

Constriction of blood vessels has been linked to reduced blood flow in pre-eclampsia, a hypertensive disorder of pregnancy. This finding suggests that nerves controlling blood vessel diameter may contribute to the pathophysiology of pre-eclampsia. Pregnancy significantly reduces the sympathetic innervation of the uterus and there is some data suggesting that the sensory innervation is also affected. Since sympathetic nerves control blood vessel diameter, a good understanding of the sympathetic innervation of the uterus and how this innervation changes during normal pregnancy is critical for investigating the involvement of sympathetic nerves in the development of pre-eclampsia.

Previous histochemical and immunohistochemical studies on uterine innervation have been done on sections. Sections allow only a small sample of the nerves in an organ to be studied whereas whole mounts are capable of providing a picture of all nerves. We therefore developed a method to assess innervation in full-thickness whole mount preparations of non-pregnant uterine horns from rats. Uteri were removed from virgin female rats, bubbled in Ca^{2+}-free Krebs solution, cut open, pinned flat and fixed in 4% formaldehyde. Whole mount preparations of intact, full-thickness uterine horns were stained with an immunoperoxidase method to localize neurochemicals in nerves. Stained whole mounts were dehydrated through graded acetone solutions and propylene oxide. A wire mesh with weight was designed to ensure the whole mounts remained flat during dehydration. After dehydration, the whole mounts were embedded in resin on glass slides under Aclar and polymerised. Uterine whole mounts processed with this method were immunostained throughout their thickness so that all nerves were revealed.

Using this whole mount technique, I stained uterine horns from non-pregnant rats in late estrous/early diestrous and rats at day 20 of pregnancy for six neurochemicals that identify different populations of nerves. Tyrosine hydroxylase (TH) and neuropeptide Y (NPY) were considered to be markers for sympathetic nerves; vesicular acetylcholine transporter (VAChT) and nitric oxide synthase (NOS), for parasympathetic nerves; and substance P (SP) and calcitonin gene-related peptide (CGRP), for sensory nerves. The non-pregnant uteri showed significant densities of axons immunoreactive for TH, NPY, SP, CGRP, VAChT and NOS. Dense plexuses of TH- and NPY-containing axons and a few SP-, CGRP-, VAChT- and NOS-containing axons occurred around blood vessels. All six types of axons were present in the muscle.. In non-pregnant rats, the linea uteri, dense bands of longitudinal muscle on the anti-mesometrial side of each uterine horn, contained higher densities of TH, NPY, CGRP and VAChT fibers than the rest of uterine smooth muscle, Uteri from 20-day pregnant rats had very significantly reduced numbers of TH-, NPY-, VaChT- , NOS-, SP- and CGRP-immunoreactive axons. Rare nerves immunoreactive for each of these markers were present on the mesometrial side of the pregnant uterus whereas there were no nerves on the anti-mesometrial side.

This thesis describes for the first time the complete innervation of the non-pregnant rat uterus and shows that pregnancy causes sympathetic, parasympathetic and sensory denervation. This information will provide a foundation for future studies on the involvement of sympathetic nerves in the development of pre-eclampsia.

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