ULTRASTRUCTURAL AND HISTOCHEMICAL OBSERVATIONS ON INNERVATION OF DEVELOPING HUMAN URINARY BLADDER

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Urinary bladder innervation in human foetuses of 13 to 25 weeks has been studied by electron microscopy and glyoxylic acid and acetylcholinesterase histochemical methods. Cholinergic fibers appear as a faintly stained dense plexus at 13 weeks. An increase in thickness and intensity of staining of the cholinergic nerve bundles occurs over the foetal age period under study. At 25 weeks, the finer cholinergic plexuses are demonstrable on the muscle fibers. Adrenergic innervation, on the other hand, becomes apparent at 16 weeks as faintly stained plexuses along the blood vessels in the trigone and is also sparsely seen in the wall of the fundus. By 25 weeks the intensity of staining increases. plexuses appear around the ganglionic clusters in the adventitia and in the wall of the urinary bladder; intensely fluorescent neurons and individual adrenergic fibers in the muscle layers are also seen. Ultrastructurally the muscle fibers undergo maturation by 19 weeks with the development of myofibrils and increasing number of intercellular contacts. The axonal profiles in all the layers of the bladder wall of foetuses from 13 to 19 weeks contain large numbers of small granulated vesicles and some have large vesicles with a halo around them. Neuromuscular synaptic zones seen by 16 weeks do not show the typical structure and generally contain clear vesicles in the presynaptic varicosities.

Few studies are available on the ontogeny of autonomic innervation of the urinary bladder (1, 2, 10, 12, 15). Ultrastructural observations (10) reveal that the innervation of urinary bladder in early human foetuses is derived largely from the sympathetic system. Recent studies on the innervation of bladder in the early postnatal life in rabbits, indicate the presence of cholinergic innervation at birth. The adrenergic innervation has been described to be poor at this stage. Subsequently, over the first six weeks of postnatal life, the cholinergic innervation remained unchanged while the adrenergic innervation showed a progressive increase (12). These conflicting observations prompted us to study the human foetal urinary bladders by histochemical and electron microscopic methods.

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MATERIAL AND METHODS

Twenty-five fresh human foetuses, of both sexes, ranging from 13 to 25* weeks (65 mm CRL to 210 mm CRL) were obtained by hysterotomy performed for medical termination of pregnancy in multiparous cases. The foetal age was assessed on the basis of crown-rump length by the criteria of Hamilton et al (9). For histochemical studies the urinary bladder was immediately dissected and placed on a precooled metal chuck and transferred to a deep freeze at −40°C. The specimens were sectioned at 24–32 μm in a cryostat at −20°C. Alternate slides, with two or three sections each, were stained for catecholamines using the glyoxylic acid (GA) method (3) and specific acetylcholinesterase (AchE) by El Badawi and Schenk’s modification of Karnovsky and Roots method (4). Specificity of the GA method was tested by using controls which were not treated with the sucrose-phosphate-glyoxylic acid solution (SPG), by vapour steaming the SPG treated sections or omitting the heat reaction. The fluorescence was examined using a Zeiss fluorescence microscope and photographed with ORWO 125 ASA film. Specificity of AchE staining was assessed by using tetraisopropyl pyrophosphoramide as an inhibitor and by control tissues incubated in the media without substrate. The pH, temperature and duration of incubation was standardized to obtain optimal results. One section on each slide was counterstained with haematoxylin. For electronmicroscopic studies small strips of the fundic wall and trigone were fixed in ice cold Karnovsky’s fixative (pH 7.4) immediately after procuring the specimen. After 10 min of fixation, one millimeter strips were cut and left in fresh fixative for 2–4 hr. The tissues were then washed with 0.1 M cacodylate buffer and postfixed in 1 per cent buffered osmic acid for 2–3 hr, followed by dehydration in ascending grades of acetone. The tissues were embedded in araldite. 1 μm thick sections were cut with Ultracut microtome and stained with 1 per cent toluidine blue. Suitable regions were selected for ultrathin sectioning and viewed under Philips 300 EM.

RESULTS

In 13 weeks-old foetuses, cholinergic innervation identified by AchE activity was demonstrable in the musculature of the trigone and fundus of the urinary

![Figs. 1-3. Cholinergic innervation of the foetal human urinary bladder identified by AchE activity.](image)

* According to obstetric history and examination the age was assessed to be 20 weeks but calculation based on crown-rump length determined the age to be 25 weeks.
bladder as a lightly stained dense plexus of fibers. Ganglionic cells in the adventitia of the bladder wall were also stained. AchE stained cholinergic fibers were seen to invade the lamina propria (Figs. 1a, b). At 19 weeks the cholinergic nerve bundles showed an increase in thickness and intensity of staining both in the trigone and in the fundus (Figs. 2a, b). It was observed that the smooth muscle of the bladder also possessed AchE activity in the early foetuses. In a 25 weeks-old foetus, the finer fibers of the myenteric plexus were clearly visible and the muscle fibers did not take up the AchE stain (Fig. 3) at this age period. Adrenergic innervation demonstrated by the GA method became apparent at 16 weeks when occasional ganglia in the adventitia of the urinary bladder showed fluorescence. Faintly stained fluorescent fiber bundles became visible in the trigone. These were also seen but more sparsely in the fundic wall (Figs. 4a, b). At 19 weeks fluorescent fibers were found around the vesical ganglia and the intensity of staining was increased (Fig. 5). By 23 weeks the intensity of staining and density of the adrenergic fiber bundles coursing along the blood vessels increased considerably. Individual fluorescent fibers were seen in the muscular layers. More intensely stained fluorescent nerve cells were also seen (Figs. 6–8). Ultrastructurally, at 19 weeks, smooth muscle fibers developed myofibrils oriented parallel to the long axis of the muscle fiber (Fig. 9). The intercellular contacts e.g. nexuses increased in number. The interfascicular nerve bundles and those in the adventitia had the Schwann cell envelope while the intrafascicular bundles and axons were devoid of it (Fig. 10). In the urinary bladders examined between 13 and 19 weeks the axonal profiles in all the layers of the wall contained large numbers of small granulated vesicles and some had large vesicles with a halo around the core (Figs. 10, 11). Immature synaptic zones between the varicosities of the nerve fibers and the muscle cells were seen by 16 weeks. The presynaptic varicosities generally had clear vesicles (Fig. 12).
DISCUSSION

The present study illustrates that the cholinergic innervation of the bladder in human foetuses develops earlier than adrenergic innervation and subsequently the latter undergoes a progressive increase. A similar pattern of development has been histochemically described in the autonomic innervation of the foetal rabbit gut (7), human foetal gut (13) and postnatal rabbit urinary bladder (12).

The early appearance of AchE positive nerves in the submucosal layers seen in the present study may well represent the early sensory innervation of the bladder mucosa as is also indicated by silver impregnation in our previous study (15). It has been pointed out by Gosling and Dixon (8) in the adult urinary bladder that afferent nerves subserving sensory function are associated with AchE and have 40–50 nm agranular and large 100 nm granulated vesicles.

The AchE activity seen in the smooth muscle of the urinary bladder in these early foetuses reflects a pattern seen in the development of neuromuscular innervation of skeletal muscle (5, 14) where the AchE is diffusely distributed in the muscle in the early stages and later the AchE spots become localized to the neuromuscular junctions. This localization occurs when nerve endings make contact with the muscle. The final evolution and the maturation of the neuromuscular contact is linked to the stage of the muscle differentiation at the time of establishing contact with the nerve endings (11). In the present study the smooth muscle does not show the diffuse AchE activity from the age of 25 weeks indicating thereby that the neuromuscular contacts have become localized by this stage. This correlates with our earlier observation on the demonstration of silver stained neuromuscular terminal fibers at 22 weeks (15), our present ultrastructural findings and those of Hoyes et al. (9) who also demonstrated the intimate apposition of the axonal varicosities with the smooth muscle cells of the human foetal bladder between 4–5 months, i.e. 16–20 weeks of gestation. These authors, however, have indicated the possibility of a predominant sympathetic innervation of the vesical musculature on the basis of the presence of small and large dense granules in the intrafascicular nerve terminals. In the present histochemical observations it has been observed that only sparse adrenergic fibers are seen in the trigone and fundus at 16 weeks with a gradual increase in the adrenergic density by 23 weeks. It may be argued that histochemically undemonstrable adrenergic vesicles may be present in the early foetal stages. Even if so, the amount would not be such as to give an impression of predominant adrenergic innervation. The possibility of the presence of non-cholinergic, non-adrenergic vesicles has also not been considered. It is likely that this phase where mainly small granular vesicles are seen simulates a situation seen in the early developing guinea pig small intestine (6). Here varicosities have mainly small lucent vesicles before gestational day 48, with a profound change in the appearance of vesicles occurring after this day when the vesicles acquire adult variegated forms. This problem needs further clarification by using combined immunocytochemical methods for different transmitters at the ultrastructural level.

It is evident that there is a process of differentiation and maturation of the autonomic innervation of the human urinary bladder during ontogeny. This developmental and maturation process could be susceptible to the environmental
hazards occurring during pregnancy and may result in subtle neuromuscular imbalances of the urinary bladder function.

REFERENCES