Submucosal plexus of terminal ileum: a study of the cholinergic and noradrenergic nerves in rats with streptozotocin-induced diabetes

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Summary. In order to study the type and degree of the alterations in the innervation of the intestine in experimental diabetes, a histochemical study on the cholinergic and noradrenergic nerves of the submucosal plexus of terminal ileum from rats with streptozotocin-induced diabetes was performed. The results obtained suggest that the diabetic animals keep the cholinergic activity undamaged 20 weeks after the induction of the illness, while the number of the catecholaminergic nerves appears to be markedly reduced.

Key words: Diabetic neuropathy, Rats, Adrenergic, Cholinergic. Submucosal plexus, Small intestine

Introduction

Autonomic neuropathy is a frequent complication in diabetes mellitus and often causes gastrointestinal dysfunction revealed as alterations of the esophageal motility, gastroparesis, diarrhea or chronic constipation (Hosking et al., 1978; Clarke et al., 1979). Although the pathogenesis of these alterations remains unknown, it is generally accepted that there exists a multifactorial origin, in such a way that metabolic and vascular changes, modifications of the hormonal control and an increased susceptibility to infections could play an important role in the development of the syndrome.

Histochemical and electrophysiological studies of the enteric nervous system in several models of experimental diabetes have shown different types of alterations (Lincoln et al., 1984; Nowak et al., 1986; Belai et al., 1988; Hoyle et al., 1988; Cuervas-Mons et al., 1990), but there are very few studies on submucosal plexus and they only refer to colon in experimental models (Nelson et al., 1976; Schmidt et al., 1984) or in man (Schmidt et al., 1984; Belai and Burnstock, 1990) with contradictory results. All this justifies a detailed study of preparations of ileum «in toto» to observe the type and degree of the alteration of the submucosal plexus in experimental diabetes.

Materials and methods

Diabetes was induced in 10 male Wistar rats, initial weights of 250-300 g, by intraperitoneal injection of streptozotocin (STZ, 65 mg/kg, in citrate buffer 0.05 M, pH 4.5). The same number of age-weight-matched control animals received citrate buffer alone. All the animals were kept for 20 weeks under the same conditions and fed with food and water ad libitum. 48 h after the injection, plasma glucose was controlled weekly with VISIDEX strips (Ames).

Method for Cholinesterase. The method used was a combination of those of El-Badawi and Schenck (1967) and Qayyum and Fatani (1985). Segments of terminal ileum were cut open lengthwise, stretched out and pinned on thin cork sheets, and immersed in 2% glyoxylic acid solution, pH 7.2, for 5-30 min at room temperature. After that, the segments were dehydrated in alcohols of increasing concentration, cleared in xylol and mounted in DPX.

Method for Adrenergic fibres (Furness and Costa, 1974). Dissection, fixation and delamination proceed as described above. The segments were exposed to
paraformaldehyde vapours at $80^\circ$ C for 1 h., mounted and examined through a fluorescence microscope (Leitz-Orthoplan).

**Results**

All diabetic animals showed loss of corporal weight and levels of plasma glucose within 400-550 mg/100 ml. On opening the abdomen, marked distention of both small and large bowel was observed, with occurrence of thick and amorphous faeces.

Acetylcholinesterase histochemistry showed, in both diabetic and control animals, a similar distribution of the submucosal plexus, this being constituted by a thin tridimensional network of cholinergic fibres arranged in a polygonal way with the ganglia localized in the junctions (Fig. 1). The ganglia were smaller than those of the myenteric plexus and appeared to be constituted by 3 to 5 AChE-positive neurons, although there can also be isolated neurons in the connective fibres (Figs. 2a,b,c,d). These results suggest that the diabetic animals keep the cholinergic activity undamaged after 20 weeks of illness.

On the contrary, the catecholaminergic fluorescence showed a notorious decrease in the noradrenergic innervation of the diabetic animals, which could be particularly seen in the perivascular plexus. In the control animals (Fig. 3a), abundant fluorescent varicose catecholaminergic fibres were observed all along the route of the blood vessels, while in the diabetic animals (Fig. 3b) a marked reduction in the number of these type of fibres was noted. Also, in the diabetic rats a diminished number of fluorescent varicosities around the ganglia of the submucosal plexus was observed, these being, nevertheless, bigger and intensely fluorescent in comparison with the control rats (Figs. 4a,b).

The results suggest the occurrence of autonomic neuropathy in animals with induced diabetes.

**Discussion**

The pathology observed with the catecholaminergic fluorescence induced by vapours of paraformaldehyde in the submucosal plexus of the terminal ileum of animals with streptozotocin-induced diabetes coincides with that observed by Schmidt et al. (1981) in the submucosal plexus of the distal colon in rats after 4 to 7 months of diabetes. This suggests that the noradrenergic innervation of both colon and ileum is diminished as an effect of the diabetes mellitus.

However, Belai and Burnstock. (1990) describe the absence of changes in the immunoreactivity of the noradrenergic fibres of the submucosal plexus. The explanation of such contradictory results could be in the different periods of evolution of the illness.

The intensely fluorescent varicosities observed around the submucosal ganglia probably correspond to the dystrophic axons with high content of TOH (tyrosine hydroxylase) and DBH (dopamine-$\beta$-hydroxylase) described by Schmidt et al. (1981), who also observed these axons in the margins of the myenteric ganglia and in the interconnective network of these ganglia.

The noradrenergic fibres inhibit the secretion of water and electrolytes by their action on the cellular bodies of the secretomotory neurons of the submucosal ganglia (Furness and Costa, 1987). The loss of such fibres would then produce a shortage of the stimulation of the $\alpha$-2-receptors with the result of an altered

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*Fig. 1. Acetylcholinesterase staining of the submucosal plexus of the ileum of the diabetic rat. $\times 16$*
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Fig. 2. Acetylcholinesterase staining (a) Ganglia and connective fibres. (b) and (d) ganglia with 3 to 5 AChE-positive neurons in the junctions. (c) Isolated neuron in the interconnective fibres. × 100. (b) and (d) ganglia with 3 to 5 AChE-positive neurons in the junctions. × 100. (b) and (d) ganglia with 3 to 5 AChE-positive neurons in the junctions. × 200

Water and electrolyte transport in the enterocyte (Chang et al., 1985). This could be a factor to take into account to explain the diarrhoea present in diabetes mellitus within the alterations of the intestinal motility.

At the same time, the combined occurrence of similar alterations in the submucosal perivascular plexus of the ileal wall and in those plexus innervating the submucosal ganglia makes us think that the origin of all observed alterations in the postganglionic sympathetic fibres is probably a consequence of degenerative changes in the prevertebral sympathetic ganglia.

The absence of alterations in the cholinergic innervation of the submucosal plexus of the terminal ileum, revealed by acetylcholinesterase histochemistry, does not agree with that reported by Schmidt et al. (1981) who studied this plexus in frozen sections, i.e. without being delaminated.

The similarity of these findings both in the cholinergic and in the catecholaminergic innervation of the submucosal plexus with those of Cuervas-Mons et al. (1990) on the myenteric plexus, using the same techniques for preparations «in toto», points to the occurrence of autonomic neuropathy in the experimental diabetes affecting evenly both plexus as well as the perivascular plexus of all vessels of the ileal wall.

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References


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Fig. 3. Catecholaminergic fluorescence of the perivascular plexus of ileum submucosal from control (a) and diabetic (b) rats. × 200

Fig. 4. Catecholaminergic fluorescence in the ileal submucosal ganglia of diabetic (a) and age-matched control (b) rats. × 200.

stores of noradrenalin and 5-hydroxytryptamine in whole mounts. Histochemistry 41, 335-352.

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