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Intramural distribution of neuron specific enolase (NSE) in the human gastrointestinal tract

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Summary. NSE concentrations were measured by radioimmunoassay in the main separated layers of the human gastrointestinal tract. At all levels, a similar pattern of distribution of this protein was found, primarily parallel to that of nerve elements. Lower amounts of NSE were detected in the separated mucosal epithelium, containing the endocrine cells.

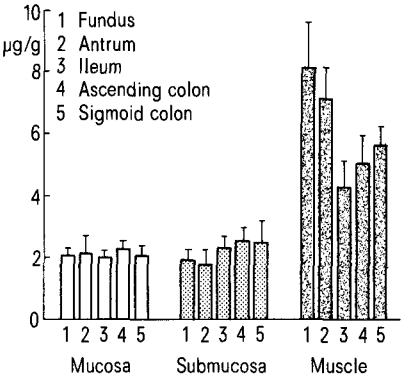
NSE is the γ-γ form of the glycolytic enzyme enolase[3-5], exclusively localized in neurons in the CNS[6]. More recently, NSE was also demonstrated in peptide- and/or amine-producing endocrine cells[7], including all endocrine cells types of the gut and pancreas[8], and in gut neurons[8,9]. In this study we have examined the quantitative intramural distribution of NSE immunoreactivity at various levels of the human gastrointestinal tract.

Materials and methods. Specimens of histologically normal stomach (fundus and antrum, n = 5), small and large bowel (ileum, n = 4, ascending colon, n = 4, and sigmoid colon, n = 7) were obtained fresh at surgery (from resections of carcinomas) and processed as previously described[10]. In brief, a sample from each specimen was taken as full-thickness control and another sample was microdissected into mucosa, submucosa and external muscle layer. A 3rd sample was injected with a 5 mmoles/l solution of sodium EDTA in the submucosa, incubated in the same solution (1 h) and vigorously shaken (20 sec). An epithelial pellet was collected by centrifugation, while the lamina propria was removed from the remainder of the wall by microdissection. This procedure proved to be highly effective in removing the epithelium from ileum and colon, but not from gastric samples. Its use in the stomach was therefore abandoned. Samples were extracted in 5 vols of 10 mmoles/l tris-PO_4, pH 7.3, containing 1 mmole/l $MgSO_4$. The polytroned homogenates were centrifuged at 100,000 × g for 1 h. NSE concentrations were measured by radioimmunoassay, as previously described[11]. The sensitivity was 100 pg per assay tube and the effective detection limit was 0.5–1 ng/ml. Samples of the separated layers were taken for routine histology, in order to check the quality of the separation obtained.

Results. In all cases, histology showed a clean separation between mucosa and submucosa, which took place at the level of the muscularis mucosae. After removal of the muscularis externa, only a few muscle fibers occasionally remained attached to the submucosa. No detectable contamination by structures from the other layers was found in the epithelial preparations.

The total recovery of NSE (sum of recoveries from the 3 separated layers) compared to the full-thickness samples was 101 ± 5% (mean ± SEM, n = 25). Of the total content of NSE, the largest part was recovered from the muscle layer (71.0 ± 2.4%, mean ± SEM, n = 25), while smaller amounts were found in the mucosa and submucosa (16.4 ± 1.6% and 12.6 ± 1.4%, respectively). When the mucosa was separated into epithelium and lamina propria, these 2 layers were found to contain 1.6 ± 0.2% and 15.0 ± 4.3%, respectively, of the NSE content (mean ± SEM, ileum and colon only, n = 15). The concentrations of NSE in the various layers are summarized in the figure.

Discussion. The separation procedure used in this study proved to be accurate and reproducible, as confirmed by histology, while the excellent recovery of NSE from the separated layers confirms its suitability for a quantitative study.



NSE concentration (µg/g of wet tissue weight) in the 3 separated layers at the various levels of the gastrointestinal tract. Bars represent means ± SEM; fundus and antrum; n = 5, ileum and ascending colon: n = 4, sigmoid colon: n = 7.

The differential distribution of NSE consistently shown at all levels of the gut primarily parallels the distribution of the innervation. In fact, the major amounts were found in the muscle layer, containing the large ganglia of Auerbach's plexus, while the lower concentrations measured in the submucosa parallel the looser network of Meissner's plexus. In the lamina propria of the mucosa no neurons are present, but nerve fibers form a very rich network[12]. Our findings are in keeping with previous investigation, which showed much higher concentrations of NSE to be present in the 'muscle' (muscle layer and submucosa) compared to the mucosa in the rat intestine[13].

The possibility that the low amounts of NSE found in the separated epithelium are due to contamination from the other layers cannot be ruled out in our study. However, in a previous investigation[10], we detected less than 0.5% of the total content of vasoactive intestinal polypeptide and substance P (exclusively neural peptides in the human gut) in the epithelium, while the adjacent lamina propria contained the highest concentration of both, thus indicating the negligible extent of contamination of the separated epithelium by nerve structures when this separation procedure is used.

By immunocytochemistry, endocrine cells were found to be the only source of NSE in the gut epithelium[8]. In the distal intestine they showed a lower immunostaining intensity[8], thus suggesting that their content of NSE may decrease distally. The finding of a low, but clearly detectable proportion of the NSE content in the separated ileal and colonic epithelium fits well with this finding, while indicating that the NSE concentration in the individual endocrine cells is likely to be significantly lower than that in enteric neurons.

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Immobilization of rat brain synaptic vesicles on positively-charged glass microspheres

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Summary. Synaptic vesicles from rat brain were immobilized on glass microspheres covalently coated with poly-L-lysine. Using a potassium tartrate perfusion medium, the vesicular accumulation and methamphetamine-induced release of (L)-^{3}H-norephinephrine could be conveniently monitored in a flow experiment.

Synaptic vesicles from mammalian brain display a large, negative surface charge at pH 7.4[2]. It was felt that if this property could be utilized to electrostatically immobilize vesicles, then drug-induced release of neurotransmitters from vesicles could easily be monitored in a perfusion experiment. Such a method would eliminate such isolation procedures as membrane filtration, where separation of vesicles from the incubation medium precludes further use of the tissue. It had been reported[3] that glass microspheres of 25 µm diameter could be covalently functionalized with poly-L-lysine to provide a bead with a strong positively-charged surface at pH 7.4. This elegant technique was used to immobilize and expose the cytoplasmic surface of erythrocyte membranes[4]. The current report details the immobilization of synaptic vesicles from rat brain on glass microspheres functionalized with poly-L-lysine, and the methamphetamine-induced release of accumulated (L)-^{3}H-norephinephrine (NE) from perfused vesicles.

Methods. Tartrate buffer was prepared from neutral potassium tartrate (110 mM; pH 7.4)[5]. Ascorbic acid (10^{-5} M) and iproniazid (10^{-5} M) were included.

Glass beads (Ferro Corp., 25 µm diameter) were functionalized with poly-L-lysine (Sigma Chem. Co. mol.wt > 80,000) as described by Jacobson et al.[3]. Briefly, this procedure involved sequential treatment of 20 g portions of beads with nitric acid (to expose free surface silol groups), triethoxyaminopropylsilane (Pierce Chem. Co.) to covalently link aminopropyl residues via siloxy bridges, succinic anhydride (to amidate the aminopropyl residues), and poly-L-lysine (amidation via dicyclohexylcarbodiimide). Beads prepared in this manner were stable for several months at −90 °C.

Crude vesicles were isolated from rat brain by the method of Seidler et al.[6]. Sprague-Dawley rats of either sex (150–200 g) were decapitated, and the brain tissue (less cerebellum) quickly removed and weighed. The tissue was homogenized in 4 vols of cold 0.32 M sucrose by 6 up-down strokes of a hand-held Duall glass/glass homogenizer. The resulting suspension was sequentially centrifuged (3000 × g for 20 min; 20,000 × g for 30 min; 100,000 × g for 30 min) to yield a crude vesicle pellet (2.73 mg protein/g wet wt of brain). The pellet was resuspended in 500 µl of 0.32 M sucrose at 4 °C using slow up-down strokes of a hand-held teflon-glass homogenizer.

The resuspended pellet was transferred to a microcolumn (5 × 30 mm) containing 250 mg of beads supported on a small plug of glass wool, and the slurry allowed to stand for 5 min at 4 °C. The jacketed column was then warmed to