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Substance P-like immunoreactivity in the frog dorsal root ganglia

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Summary. The distribution of substance P-like immunoreactivity was studied in the thoracic dorsal root ganglia of the frog *Rana esculenta* by immunohistochemistry. Substance P-like immunoreactivity was contained in approximately 50% of primary sensory neurons. The immunoreactive fibers arising from the cell bodies are collected in small bundles within the ganglia neuropil before entering the central and peripheral roots.
Key words. Substance P; immunohistochemistry; spinal ganglia; frog.

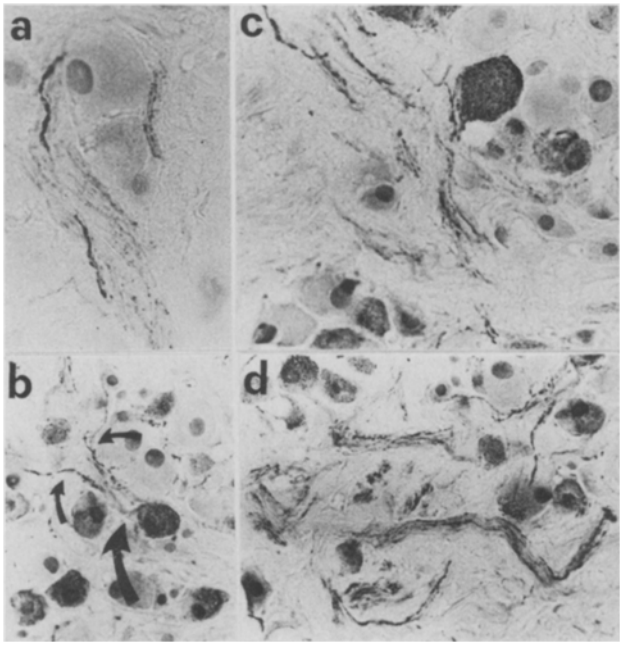
Substance P (SP) is a polypeptide which is thought to play a role in sensory systems, particularly in those involved in the processing of pain information (for review see Nicoll et al.[1]). In fact, immunohistochemical studies have demonstrated the presence of SP in primary sensory neurons of mammalian dorsal root ganglia (DRG)[2,3] as well as in the dorsal horn of the spinal cord of cats[3], rats[2], monk[4] and frogs[5,6]. There is also experimental evidence that immunoreactive SP in the dorsal originates from DRG[7-10] and physiological studies have provided evidence for the role of SP as a transmitter of primary sensory neurons in rats[11] and in frogs[12].

In view of the above and of the fact that the morphological features of immunoreactive SP elements in DRG of the frog have not yet been shown, we investigated immunohistochemically the distribution of SP in DRG in the frog *Rana esculenta*. Two frogs, anesthetized with tricaine methanesulphonate (MS 222, 1:3000), were perfused transcardially with 10 ml of saline (NACl 0.64%) followed by 15 ml of fixative (0.1 M phosphate-buffered 4% formaldehyde, pH 7.4) at 4 °C. The DRG (thoracic 1–2) were removed, placed in the fixative for an additional 3 h and then incubated for a few days at 4 °C in buffer containing 20% sucrose. The ganglia were cut into transverse sections 12 µm thick in a cryostat and put on slides pretreated with potassium chrome sulphate gelatine. Sections were incubated with SP antiserum, diluted 1:50 to 1:500, for 20 h at 4 °C and later processed according to the unlabeled peroxidase-antiperoxidase technique[13] using diaminobenzidine as chromogen. Control sections were processed in parallel but incubated with diluted antiserum preabsorbed (1 mg/ml) with SP or without being exposed to SP antiserum.

The DRG of the frog consist of large neuronal cells and small satellite cells[14]. Although the existence of post-ganglionic sympathetic neurons 'without central processes' has been reported[15], recent anatomical results do not support these data[14]. Presumably all DRG neurons of the frog are primary sensory neurons.

In our results substance P-like immunoreactivity was localized in some of the large neuronal cells (figure, b and c) suggesting that it is contained only in a portion of the ganglion cell population. In 10 sections 200 SP positive neurons were counted of a total number of 400 neurons. We may say that substance P-like immunoreactivity is contained in approximately 50% of primary sensory neurons. Since in the frog thoracic spinal cord somatostatin-like immunoreactivity appeared to be contained in primary afferents[6], some of the neurons which have negative immunoreactivity to substance P may contain somatostatin.

The process arising from the SP-like immunoreactive cell body is clearly visible in the figure, c, where the unipolar or pseudounipolar nature of the neuron is apparent. We presume that the diverging fiber branches appearing in the figure, b (arrows) originate both from the same single process arising from the nearby dark neuron which is positive to SP-like immunoreactivity.



Frog dorsal root ganglion showing the distribution of substance P-like immunoreactivity. *a* Fibers showing a positive SP-like immunoreactivity collected in bundles run between non-immunoreactive cells. ×250. *b* The small arrows indicate SP-like immunoreactive diverging fibers both belonging to the process originating from the SP positive cell (large arrow). ×180. *c* SP-like immunoreactive neuron is apparent close to other neurons negative to SP. The process emerging from one pole of the cell runs in a bundle of fibers which show SP-like immunoreactivity. × 220. *d* SP-like immunoreactive bundles of fibers are shown in the neuropil of the spinal ganglion. ×180.

We believe that these cells are the cells of origin of the SP-like immunoreactive fibers entering the frog spinal cord through the dorsal root and distributing to the dorsal horn[6].
The SP-like immunoreactive fibers, before reaching the place where central and peripheral roots emerge from the spinal ganglion, collect in small bundles of several fibers within the ganglion neuropil (figure, a and d). This suggests that cells containing the same putative transmitter have their processes assembled together and may maintain their group individuality when they run in the roots where other fibers with other neurotransmitters are also running.
Our results demonstrate that substance P is present in approximately 50% of the population of primary sensory neurons in thoracic DRG of the frog.

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Changes in brush-border enzyme activities of intestinal epithelial cells isolated from the villus-crypt axis during the early phase of alloxan diabetes in rats

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Summary. The sucrase activity in enterocytes isolated from the villus crypt axis was found to increase in all regions of the villus from day 2 after induction of diabetes, and the increase continued until day 4. In contrast, alkaline phosphatase activity increased mainly in the apical one-third of the villus-crypt column, and the increase occurred abruptly on day 4 with increase in food intake.
Key words. Rat; alloxan diabetes; intestinal villus; alkaline phosphatase; sucrase; epithelial cells; villus-crypt axis.

The activities of hydrolytic enzymes in the intestinal brush-border membrane are known to be increased in diabetic animals[1-5]. However, the site of these increased activities along the villus-crypt axis of diabetic animals has not been investigated. In this study, we investigated the sites and time-courses of increase in alkaline phosphatase and sucrase activities in enterocytes from the villus-crypt axis during the early phase of induction of alloxan diabetes in rats.
Materials and methods. Male Sprague-Dawley rats weighing 160–190 g were kept in individual stainless-steel cages in an air-conditioned room at 23 ± 2 °C with lighting from 08.00 to 20.00 h. Experimental diabetes was induced by intramuscular injection of 120 mg of alloxan monohydrate (Wako Junyaku Co., Osaka, Japan) per kg b.wt (4% solution in saline). Control rats received an injection of saline instead. Food and water were freely available except in some experiments, in which from days 3 to 5 after alloxan treatment diabetic rats were given a restricted diet, equivalent in amount to that consumed by control rats.
The semisynthetic diet consisted of 20% casein, 45% α-corn starch, 23% sucrose, 5% oil (soybean oil:cod liver oil, 4:1, v/v), 4% salt mixture[6], 1% vitamin mixture[6], 1.85% cellulose powder (40–100 mesh), and 0.15% choline-Cl. Food intake and b.wt were recorded between 09.00 and 10.00 h every day.
All experiments were started at 10.00 h to avoid circadian changes[7]. Blood was withdrawn from the inferior vena cava of rats under ether anesthesia, and then the animals were killed by decapitation. The entire small intestine was quickly removed, rinsed thoroughly with ice-cold saline and divided into eight equal lengths. The second segment from the pyrolus was everted and its enterocytes were isolated along the villus-crypt axis by the methods of Weiser[8] and Raul et al.[9], except that the incubation times used were 10, 3, 4, 5, 6, 7, 8, 9, 10, 15 and 20 min with shaking (100 oscillations per min). Alkaline phosphatase was assayed by the method of Forstner et al.[10]. Sucrase activity was determined by the method of Dahlqvist[11]. Protein was measured by the method of Lowry et al.[12] with bovine serum albumin as a standard. Enzyme activities were expressed as amounts of substrates hydrolyzed per mg protein per min at 37 °C in μmoles for alkaline phosphatase, and in nmoles for sucrase. Blood sugar was determined by the method of Ashwell[13]. Animals with a plasma glucose level of more than 250 mg per 100 ml of plasma were regarded as diabetic. Data were analyzed by Student's t-test and a value of $p < 0.05$ was considered as significant.
Results and discussion. The gradients from the tip of the villus to the crypt of alkaline phosphatase and ^{3}H-thymidine incorporation after a 3-h pulse in vivo were examined by reported methods[8,9].
As shown in figure 1, alkaline phosphatase activity had decreased in all regions of the villus on the day after induction of diabetes. Food intake of the diabetic rats also decreased for the first few days after alloxan treatment. During this period, the activity of alkaline phosphatase remained low. Sucrase activity began to increase on day 2 and continued to increase until day 4 in the entire villus-crypt column. In contrast, alkaline phosphatase activity increased in the apical one-third of the villus-crypt column on day 4, when the diabetic rats became hyperphagic. Thus the localizations of the increased activities of alkaline phosphatase and sucrase along the villus-crypt axis of diabetic rats were different.
The times of increase in the activities of alkaline phosphatase