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## Dopamine-containing cells in rabbit nodose ganglia

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*Summary.* Microspectrofluorometry of rabbit nodose ganglia exposed to formaldehyde vapor revealed that the intraganglionic fluorescent cells (SIF-cells) contain dopamine.

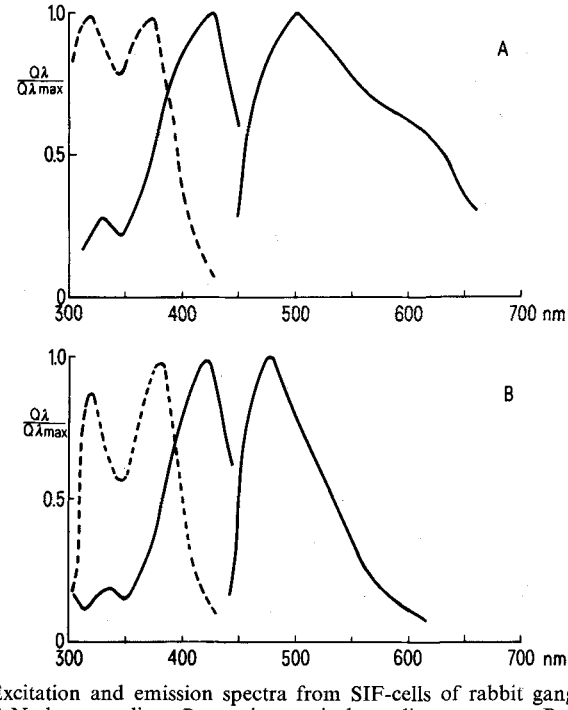
It has been reported that mammalian nodose ganglia contain small, intensely fluorescent cells (SIF-cells)[1-4], though the fluorescent monoamine in these cells has not yet been identified. The SIF-cells in mammalian sympathetic ganglia, on the other hand, have been studied by microspectrofluorometry[5] and shown to contain either a primary (dopamine or noradrenaline)[6-8] or a secondary catecholamine (adrenaline)[9]. The superior cervical ganglion of the rabbit, in particular, is well known to be endowed with SIF-cells characterized by dopamine fluorescence[10].

In the present investigation, the nodose ganglion of the rabbit has been studied using microspectrofluorometry and liquid chromatography to elucidate the biochemical characteristics of its SIF-cells in comparison with those of the superior cervical ganglion.

Nodose and superior cervical ganglia of 20 mature rabbits were used. Histochemical procedure; the ganglia were freeze-dried immediately after isolation and processed according to a minor modification of the Falck-Hillarp method[11] as described elsewhere[12]. The formaldehyde vapor treatment was performed, using paraformaldehyde stored at 75% humidity, at 80 °C for 30-60 min. Some freeze-dried ganglia were heated alone without exposure to formaldehyde vapor. The sections (10 µm thick) used for microspectrofluorometry were deparaffinized in xylene and exposed to concentrated HCl vapor for 1-3-10 min at room temperature according to Björklund et al.[5]. A Nikon fluorescence microscope (improved SPM-RFL system)[13] and a Zeiss fluorescence microscope (MPM01 system) were employed for measurements of excitation and emission spectra, respectively.

Biochemical determinations; the ganglia were freed from connective tissues and nerve trunks under a dissecting microscope, and stored at −80 °C. 14-20 nodose and 2 superior cervical ganglia were used for each determination. Catecholamine assay was made by a high-performance liquid chromatograph (Yanagimoto, L-2000L) with a high-sensitivity electrochemical detector (Yanagimoto, VMD-101). The practical assay procedure followed the method of Refshauge et al.[14] modified by Kojima et al.[15].

The chemical analyses showed that the nodose and superior cervical ganglia contained both dopamine and noradrenaline. Dopamine amounted to 51 ± 13 ng/g in the nodose




Excitation and emission spectra from SIF-cells of rabbit ganglia. *A* Nodose ganglion, *B* superior cervical ganglion. ———, Before exposure to HCl vapor; ----, after exposure to HCl vapor for 10 min.

ganglia (n=4) and 2.0±0.1 μg/g in the superior cervical ganglia (n=5), and noradrenaline to 38±3.0 ng/g and 7.7±0.3 μg/g, respectively. The adrenaline content was not significant in either ganglion. Examination of sections for formaldehyde-induced fluorescence revealed that nodose ganglia except for SIF-cells and greenish fluorescent axons (possible source of noradrenaline) around the blood vessels and among the ganglion cells, showed no specific fluorescence, whereas superior cervical ganglion cells exhibited a characteristic greenish fluorescence. SIF-cells in both ganglia were often seen in close apposition to blood vessels. The number of SIF-cells in the nodose ganglion was, however, much smaller than in the superior cervical ganglion. It was noted that the SIF-cells in the nodose ganglion were devoid of any prominent processes protruding from their somata, whereas single SIF-cells with a process were often observed in the superior cervical ganglion. Microspectrofluorometrically, the excitation peak of a SIF-cell cluster (7 cells) in the nodose ganglion was 420 nm with a secondary peak at 325 nm, and the peak of emission was 470–490 nm (fig. A). After a short exposure (60 sec) of the section containing the SIF-cells to HCl vapor, the excitation spectrum peak shifted to 370–380 nm. This peak persisted during continued exposure to HCl vapor (10 min) (fig. A). The shift in the excitation spectrum of the SIF-cells agrees with that found for the fluorophore of authentic dopamine[5].

As shown in the figure B, the SIF-cell in the superior cervical ganglion, which contains dopamine[10], showed similar excitation characteristics, whereas the excitation spectrum maximum of the sympathetic ganglion cells in the same section, which contain noradrenaline[10], was changed from the original 420 nm over 370 nm to 320 nm with increasing exposure to HCl vapor.

The observed low concentrations of catecholamines in the nodose ganglion as compared to those in the superior cervical ganglion is in keeping with the histofluorescent finding that the number of catecholamine-containing elements such as SIF-cells and adrenergic terminals was much smaller in the nodose ganglion[4] than in the superior cervical ganglion[10]. In the nodose ganglion, the dopamine content was higher than that of noradrenaline. This suggests that certain dopaminergic elements exist independently from noradrenergic elements in the ganglion. Indeed, the close similarity in excitation spectrum between the SIF-cells in the nodose and the superior cervical ganglion indicates that the SIF-cells of the nodose ganglion store dopamine exclusively.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## Calcification of the deep zone in pig femoral head cartilage[1]


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*Summary.* X-Ray diffraction shows an almost random arrangement of collagen fibrils in the region of uncalcified pig femoral head cartilage furthest from the articular surface. The characteristic radial orientation of the deep zone of articular cartilage is revealed in the underlying tissue after decalcification.


X-Ray diffraction shows that the deep zone of articular cartilage coating the femoral head may be masked by calcification. It is generally accepted that uncalcified articular cartilage consists of three zones: a) in the surface zone collagen fibrils are oriented roughly parallel to the articular surface, b) in the transition zone there is nearly random fibril orientation and c) in the deep zone fibrils tend to be oriented radially i.e. perpendicular to their orientation near the surface[2]. We have used X-ray diffraction to confirm this pattern of fibril orientations in adult human patellar cartilage; the zones gradually merge into each other without discontinuity[3]. (The advantages of X-ray diffraction for this purpose are discussed elsewhere[3,4].) Radial orientation was observed in the deep zone of human and dog femoral head cartilage in the SEM by McCall[5] and by Speer and Dahners[6]. Using the same technique Clarke[7] observed a random orientation in human tissue. Random orientation was also observed in the deep zone of human and mouse femoral head cartilage in the TEM[8,9].

We have measured the orientation of collagen fibrils as a function of depth from the articular surface in femoral head cartilage of an adult pig (fully grown Göttingen minipig, less than 1-year-old). Split lines were made to indicate the preferred alignment of collagen fibrils in the plane of the articular surface[10].

The femoral head was sliced in two along a line passing mediolaterally through the fovea, a block was cut from the most superior aspect and fixed in formol saline (9.5 g NaCl in 900 $cm^3$ distilled water and 100 $cm^3$ of formalin); fixation in formol saline does not appear to affect the