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Immunohistochemical localization of S-100 protein in human cerebral and cerebellar cortices

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Summary. S-100 protein, a highly acidic protein specific to the nervous system, is immunohistochemically localized exclusively in glial cells, but not in any type of neuron in human cerebral and cerebellar cortices.

The biological role of S-100 protein, an extremely acidic protein unique to the nervous system, is still speculative, although physicochemical properties are well known[1,2]. The presence of S-100 protein in glial cells is generally accepted, but its presence in or absence from neurons currently remains controversial. Although a considerable number of studies exist regarding the immunohistochemical localization of S-100 protein in mammalian brains[3-8], no report has described the cellular distribution of S-100 protein in human brain. The present communication is concerned with the immunohistochemical localization of S-100 protein in human cerebral and cerebellar cortices.

Materials and methods. For light microscopy, 5 adult human cerebral and cerebellar cortices obtained at autopsy were fixed with 10% neutral formalin, dehydrated and embedded in paraffin. 8-µm-thick paraffin sections were processed for the immunohistochemical staining of S-100 protein by Sternberger's PAP method[9] using monospecific rabbit antiserum against bovine S-100 protein which had been shown to be antigenically identical to human S-100 protein[10]. As a control, the sections were treated with normal rabbit serum instead of the antiserum against S-100 protein. For immunoelectron microscopy, 5 adult human cerebral cortices (frontal lobes) were obtained at lobectomy for internal decompression to inaccessible tumors. Blocks of fresh cerebral tissue (approximately 50 mm^3) were fixed by immersion in PLP solution[11] for 4 h at 4 °C immediately after removal, washed with 3 changes of 10% sucrose in phosphate buffered saline overnight, and quickly frozen after embedding in O.C.T. (Ames Co.). 6-µm-thick sections cut on a cryostat were processed for immunoelectron microscopy by the direct immunoperoxidase method using either rabbit Fab' conjugate against bovine S-100 protein or normal rabbit Fab' conjugate as detailed elsewhere[12]. The ultrathin sections cut on a ultramicrotome were examined and photographed in a Hitachi HS-8 electron microscope without any counterstain.

Results and discussion. By light microscopy, the positive immunohistochemical staining for S-100 protein, a dark accumulation of diaminobenzidine reaction precipitates, is localized exclusively in both the astrocytes and oligodendrocytes, but not in any type of neuron in human cerebral cortex (fig. 1,a and b). The precipitates from enzymic reaction are evenly distributed through the glial cells and are occasionally faintly seen in the nucleus (fig. 1b). The degree of staining intensity for S-100 protein in glial nuclei varies from cell to cell. The nuclear localization of S-100 protein suggests this highly acidic protein to be an integral constituent of the chromatin acidic proteins[13] involved in the genomic regulation of glial cell differentiation, because the appearance of S-100 protein in developing mammalian brains is a relatively late ontogenic event[14,15]. In the cerebellar cortex, the positive immunoperoxidase staining for S-100 protein is observed in the Bergman's glial cells which

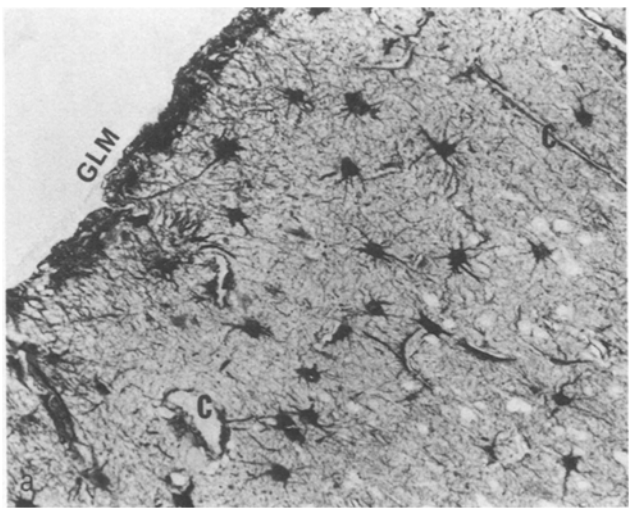
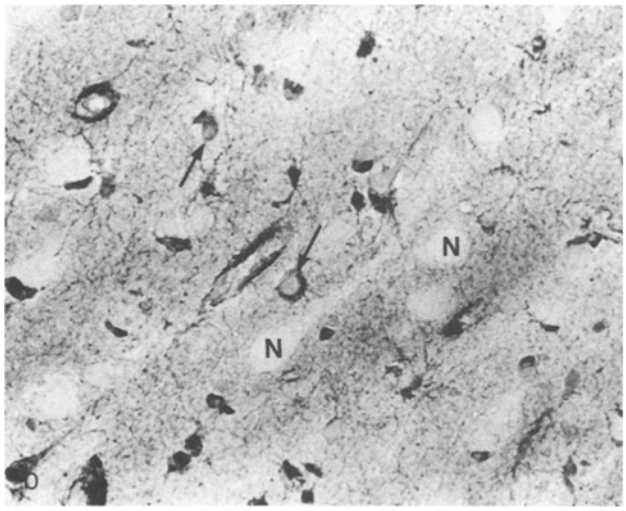
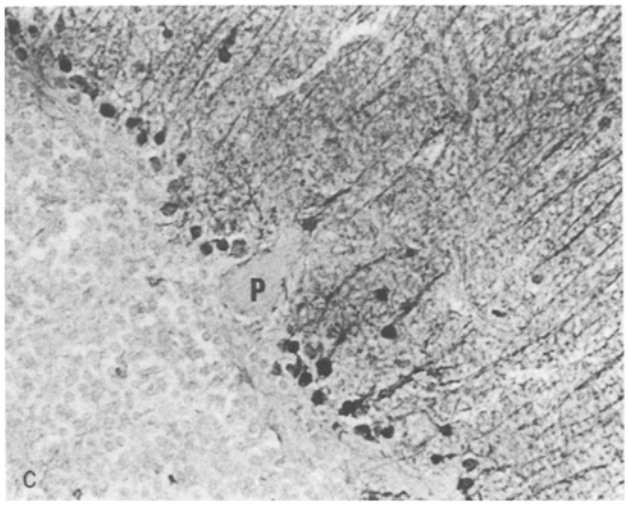


Figure 1. *a* By light microscopy, the positive immunoperoxidase staining for S-100 protein is seen as dark accumulation of diaminobenzidine reaction precipitates in both the astrocytes and oligodendrocytes, but not in any type of neuron in the human cerebral cortex. The glial limiting membrane (GLM) and the glial cytoplasmic processes surrounding capillaries (C) are also stained intensely. × 82.



b The positive staining precipitates for S-100 protein are evenly distributed throughout the glial cells with an occasional faint or negative reaction in the nuclei (arrows). The neurons (N) are devoid of specific reaction precipitates. × 165.



c In the cerebellar cortex, the positive staining for S-100 protein is observed in the Bergman's glial cells which extend their long radial cytoplasmic processes. Any type of neuron including Purkinje cell (P) lacks positive reaction. × 165.

extend their long radial cytoplasmic processes to the external glial limiting membrane across the molecular layer (fig. 1c). The astrocytes and oligodendrocytes in the granular and molecular layers are also stained positive. Neurons, including Purkinje cells, in the cerebellar cortex seem to be devoid of a positive reaction of S-100 protein. The cellular distribution of S-100 protein is essentially the same in brains obtained at autopsy and at lobectomy. By electron microscopy, the electron-dense reaction products of the positive immunoperoxidase staining for S-100 protein are observed solely in the glial cells. The positive reaction products appear to be uniformly distributed over the cytoplasm and nucleus except for the mitochondria and nucleolus of an astrocyte (fig. 2a). No positive staining is seen in any part of the neurons such as synaptic junctions (fig. 2b), in which the neuronal presence of this protein has been reported[16]. Control sections for both light and electron microscopy did not reveal any specific reaction. The immunohistochemical results presented clearly show that S-100 protein exists exclusively in glial cells in human cerebral and cerebellar cortices, which confirms some earlier observations[4,7,8] of S-100 protein in mammalian brains other than human, but is in contrast to other reports[3,5,6] of the neuronal presence of S-100 protein. We have no real explanation for this discrepancy except that the inconsistent cellular localization of S-100 protein in mammalian brains may partly be ascribed, in view of the recent review article by Eng et al.[17], to technical aspects of the immunohistochemical procedures employed. Moreover, the cytoplasmic processes of astrocytes or oligodendrocytes surrrounding capillaries in the brain are also stained strongly (fig. 1a). This localization indicates the possible participation of S-100 protein in the permeability characteristics of the nervous capillaries known as the blood-brain barrier, since S-100 protein is reported to increase transmembrane transport of cations in the presence of Ca^{2+}[18].

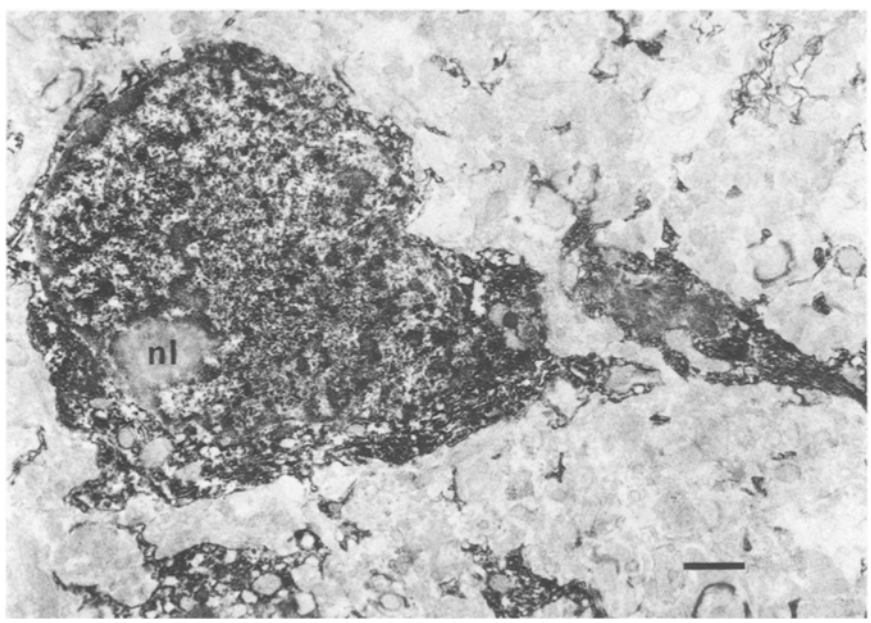
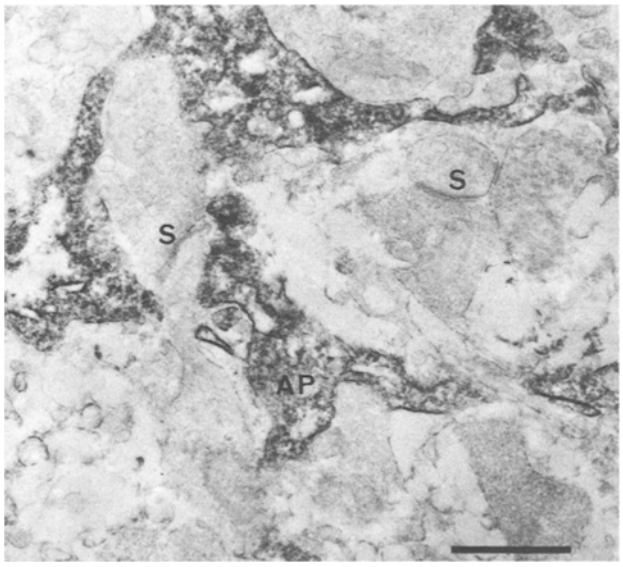


Figure 2. *a* By electron microscopy, the electron-dense reaction products of the positive immunoperoxidase staining for S-100 protein are localized exclusively in glial cells. The positive staining is uniformly distributed throughout the protoplasm of the astrocyte except for mitochondria and nucleolus (nl). Bar = 1 μm.



b Immunoelectron microscopically, no specific reaction precipitate is found in any part of neuron such as synaptic junctions (S). AP: Astrocytic processes. Bar = 1 µm.

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α-Particle track autoradiography for localization of a ^{211}At-astatinated drug

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Summary. A potential endoradiotherapeutic drug, 6-^{211}At-astato-2-methyl-1,4-naphthoquinol bis (diphosphate salt), incorporating the α-emitting radio-halogen astatine-211 of half-life 7.2 h, is shown to be valuable for localization studies by means of α-particle track autoradiography in malignant and normal cells and tissues in the mouse with transplanted adenocarcinoma of the rectum.

Attempts have been made since 1953 to develop radioactive anti-tumor drugs which target selectively to neoplastic cells and if possible to their stem cells, as an alternative approach to the treatment of patients with advanced and disseminated malignant tumors[3,4]. More effective isotopes providing high LET radiation are desirable. The compound incorporating radio-iodine-125, 6-^{125}I-iodo-2-methyl-1,4-naphthoquinol bis (diammonium phosphate) – abbreviated 6-^{125}-iodo-MNDP – has been synthesized[5] and appears promising in laboratory studies[6–8]. Much more precise information is required about the distribution of these compounds in the cells of malignant tumors and normal tissues[4,9–11].

This information can now be obtained by the use of the ^{211}At analogue, 6-^{211}At-astato-2-methyl-1,4-naphthoquinol bis (diphosphate salt), abbreviated 6-^{211}At-astato-MNDP (see fig. 1). Auto-radiography with ^{211}At was first used by Hamilton et al.[12] in studies of its uptake into the thyroid gland after i.v. injection of inorganic ^{211}At$^-$.

We now report studies of the distribution of the ^{211}At labeled compound, in malignant and normal cells and tissues of the mouse with transplanted adenocarcinoma of the rectum by means of α-particle track autoradiography on frozen sections.

Materials and methods. ^{211}At was prepared by the ^{209}Bi (α, 2n)^{211}At nuclear reaction using the Nuffield 1.52 m cyclotron at Birmingham University, by bombarding bismuth metal, melted on to supporting copper foils, with a 28 MeV α-particle beam.

The ranges of the α-particles of ^{211}At in unit density tissue are either 55 µm for energy 5.87 MeV for 42% of the disintegrations or 80 µm for energy 7.45 MeV for approximately 58% of the disintegrations[13–15].

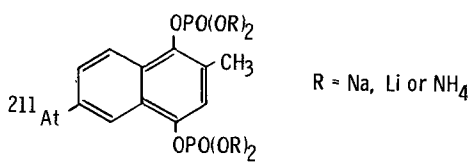

Figure 1. 6-^{211}At-astato-2-methyl-1,4-naphthoquinol bis (diphosphate salt).