Perikaryal proteins that react with an antibody against the 220 K component of axonal neurofilaments

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ABSTRACT

Perikarya of bovine spinal ganglion cells were dissected out from freeze-dried sections and their protein composition was examined by two-dimensional gel electrophoresis and immunoblotting. New components which reacted with an antibody against the axonal 220 K neurofilament protein were found as two skewed bands on the gel. They showed high pI and low molecular weight compared with the axonal counterpart. In the previous work (14), we found the following neurofilament proteins in perikarya: a 150 K protein and a group of proteins ranging from 150 K to 170 K, in addition to a trace amount of the 170 K component. Thus two of the three neurofilament proteins, high and intermediate molecular weight components, are different between axons and perikarya. Post-translational modifications of neurofilament proteins may explain the differences.

Axonal neurofilaments consist of three polypeptides, (4, 8), with apparent molecular weights of 76,000, 170,000 and 220,000 in the bovine nervous tissue (6, 14), while the protein composition of perikaryal neurofilaments has not been clarified. We reported in the previous paper that the perikaryal intermediate molecular weight component was more alkaline and smaller in size than the axonal 170 K component, and that the high molecular weight component could not be detected in perikarya (14). In this paper, the presence of high molecular weight components in the perikaryon was revealed by immunoblotting with an antibody against the axonal 220 K component.

Bovine spinal ganglia and anterior roots were obtained at a local slaughter house and frozen in dry ice/n-hexane within 30 min after decapitation. Freeze-dried sections of 30 μm thickness were prepared as described previously (15) by the method of Lowry and Passonneau (7). About 200 perikarya of ganglion cells and adjacent anterior roots were dissected out from these sections in a dry atmosphere at room temperature.

Neurofilament proteins of the bovine spinal cord were prepared as described previously (14). The 170 K component purified by ion exchange column chromatography (13) with DEAE-Sephacel (Pharmacia) was injected to a Japanese white rabbit as an antigen. Anti-170 K antibody thus raised cross-reacted with the 220 K component, and an anti-220 K antibody was isolated from this anti-170 K antibody by affinity column chromatography with Sepharose 4B coupled with the 220 K component (1).

Two-dimensional gel electrophoresis of proteins of perikarya and anterior roots was carried out by the method of O'Farrell (10), with modifications described previously (15). Gels were stained with silver (15). Proteins separated in two-dimensional gels were blotted onto a nitrocellulose paper and allowed to react with the anti-220 K antibody. Bound antibody was stained with the peroxidase-antiperoxidase system (2, 14).

Fig. 1a shows a two-dimensional gel electrophoretic pattern of the anterior root, where triplet neurofilament proteins are marked by arrowheads. The anti-220 K antibody prepared by affinity chromatography reacted with both
Fig. 1  Two-dimensional electrophoretic pattern of bovine anterior root proteins (1 μg) stained with silver (a), and immunoblot with an anti-220 K antibody (b). Arrowheads indicate triplet neurofilament proteins.

170 K and 220 K components (Fig. 1b). Immunoblotting of perikaryal proteins with the anti-220 K antibody showed three diffuse and skewed bands (Fig. 2b). Proteins corresponding to these three bands could be traced on the silver-stained gel (Fig. 2a). Bands 1 and 2 may be related to the axonal 220 K component, since another anti-170 K antibody used in the previous work (14) reacted with band 3 but not with bands 1 and 2. Band 2 was more alkaline and smaller in molecular weight than band 1. Band 2 was stained more heavily by silver (Fig. 2a), but equally by horseradish peroxidase compared with band 1 (Fig. 2a), which may reflect a difference between these two components in their antigenicity against the anti-220 K antibody used in the present work.

In Fig. 3, relative positions in two-dimensional gel of the 220 K axonal protein and their perikaryal counterparts are illustrated. Some of these features of newly found perikaryal proteins were similar to those of another perikaryal neurofilament proteins related to the axonal 170 K component (14). But the difference between new perikaryal polypeptides and the axonal 220 K component was so large that the new perikaryal polypeptides could be detected only by immunoblotting.

Bands 1 and 2 are unlikely to represent proteolytic products of the 220 K polypeptide, since the ganglia were quickly frozen after decapitation of the animal. Furthermore, their gel-electrophoretic pattern clearly differed from that of human neurofilament proteins degraded by cathepsin D (9).

Intracellular distribution of neurofilament proteins has been studied by immunohistochemical techniques. With polyclonal antibodies, several authors showed that the amount of the high molecular weight component was small in perikarya (3, 11). Sternberger et al. (12) obtained two populations of monoclonal antibodies against the high molecular weight component of the neurofilament protein: one group was specific to phosphorylated proteins and stained axons, whereas the other was specific to dephosphorylated proteins and stained perikarya and dendrites in the rat cerebellar cortex. Julien and Mushynski (5) reported that the 200 K component of the rat axonal neurofilament changed its location on a two-dimensional gel to a more alkaline and lower molecular weight position when the tissue was treated with alkaline phosphatase. Their electrophoretic patterns closely resembled those shown in Fig. 2, a and b.

The remarkable difference between perikarya and axons in their intermediate and high molecular weight components of the neurofilament proteins may be due to post-translational modifi-
Fig. 2. Two-dimensional electrophoretic pattern of proteins (about 2 μg) from 200 perikarya dissected out from bovine spinal ganglia (a), and immunoblot with an anti-220 K antibody (b). The antibody stained two proteins related to the axonal 220 K component (bands 1 and 2), and one protein related to the axonal 170 K component (band 3).

Fig. 3. Composite figure based on Figs. 1b and 2b

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