Carbonic Anhydrase Activity in Axon Terminals of Sensory Corpuscles

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Summary. The distribution of carbonic anhydrase (CA) activity was studied by electron microscopic histochemistry in rat Pacinian corpuscles, Meissner corpuscles and Merkel cell-neurite complexes using the cobalt bicarbonate method. The distribution of CA activity in these axon terminals was compared to the activity in sciatic nerve axons. An intense enzymatic CA activity was demonstrated in axon terminals of both Pacinian and Meissner corpuscles, while a weak activity was found within the axoplasm of terminals abutting Merkel cells. Some large- and medium-sized axons in sciatic nerves exhibited an intense activity. These findings indicate that large- or medium-diameter sensory axons innervating corpuscular endings have an intense CA activity extending from their somata to their sensory terminals. Axons to Merkel-neurite complexes differ in CA activity from those innervating Meissner and Pacinian corpuscular endings.

Carbonic anhydrase (CA) is known to be an enzyme with intense activity in mammalian erythrocytes. CA activity in addition has been demonstrated in the central nervous system (CNS), i.e. spinal cord (SAPIRSTEIN et al., 1978; TRACHTENBERG and SAPIRSTEIN, 1980; CAMMER et al., 1985a), brain (YANDRASITZ et al., 1976; SAPIRSTEIN et al., 1978; TRACHTENBERG and SAPIRSTEIN, 1980), and isolated myelin sheaths from CNS (CAMMER et al., 1976; SAPIRSTEIN and LEES, 1978), by biochemical as well as histochemical methods. CA activity has also been demonstrated immunohistochemically in glial cells (GHANDOUR et al., 1980; LINSER and MOSCONA, 1981; CAMMER et al., 1985b; LINSER, 1985).

Recent cytochemical studies have shown that the CA activity is found in large- and medium-sized neurons in dorsal root ganglion (DRG) cells of rat (RILEY et al., 1982, 1984; WONG et al., 1983; TOHYAMA et al., 1984), mouse (SAPIRSTEIN and LEES, 1978) and chick (KAZIMIERCZAK et al., 1986). In addition, approximately 60% of thick myelinated axons in the dorsal root exhibit CA activity (RILEY et al., 1982, 1984; WONG et al., 1983; RILEY and LANG, 1984; KAZIMIERCZAK et al., 1986). Similarly, thick axons of the peripheral nerves (e.g., intramuscular nerve bundles of rats) usually exhibit CA activity (RILEY et al., 1984). These studies indicate that there is a CA activity at least in large axons and their cell bodies in neurons involved in somatosensory perception. We thus questioned whether CA activity might be present in axon terminals of mechanoreceptive sensory corpuscles which are innervated by large diameter axons. To our
knowledge, however, there have been no cytochemical studies of CA activity in axon terminals of sensory nerve endings such as Pacinian and Meissner corpuscles.

The present study was carried out to examine the histochemical localization of CA activity at the ultrastructural level in the axon terminals of rat sensory corpuscles such as Pacinian corpuscles, Meissner corpuscles and Merkel cell-neurite complexes.

MATERIALS AND METHODS

Fifteen Wistar rats (both sexes, weighing 200-280 g) were anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbiturate, 50 mg/kg body weight) and perfused with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Segments of sciatic nerves, toe pad skin (for Meissner and Merkel-neurite complexes), and the periosteum at the distal fibula (for Pacinian corpuscles) were dissected and immersed

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**Fig. 1.** A medium-sized myelinated fiber (about 7 μm in diameter) in sciatic nerve. a. Reaction product is found within axoplasm (arrows) and more prominent beneath the axolemma (double arrow). Faint reaction (arrowheads), which is non-specific, is found in the myelin sheath. b. Control experiment. Enzymatic activity was inhibited by sodium acetazolamide. No reaction product is found within axoplasm. Faint reaction (arrowheads) is found in the myelin sheath, indicating this reaction is non-enzymatic. ax Axon, S Schwann cell, M myelin sheath. ×20,000

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**Fig. 2.** Pacinian corpuscle. Both the axon terminal (ax) and inner core cells (IC) contain reaction product, whereas no reaction product is found in outer core cells (OC). Mitochondria in axoplasm (arrowheads) are usually devoid of the reaction, in contrast, mitochondria in inner core cells (arrows) contain reaction product. ×5,400

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**Fig. 3.** Higher magnification of a part of Fig. 2. Reaction product can be found abundantly in the axoplasm (ax), whereas it can be found scattered in the cytoplasm of inner core cells (arrows). Mitochondria (m) have no definite reaction product. ×39,000
Fig. 2 and 3. Legends on the opposite page.
in the same fixative for 1.5-2 hrs at 4°C. The specimens were rinsed with 0.1 M cacodylate buffer containing 8% sucrose and sectioned 15-40 μm thick on a freezing microtome. The sections were put on a nylon mesh, and incubated by floating on Hansson's medium (HARPER and LAWSON, 1985) for 10 min at room temperature. The nylon mesh was used to keep the whole surface of the sections in contact with the air to secure a homogenous reaction over the entire surface of the section. Hansson's medium used in this study was prepared by mixing two different solutions immediately before use: one of them was a mixture of 1 ml of 0.1 M CoSO₄, 10 ml of 1/15 M KH₂PO₄, and 6 ml of 0.5 M H₂SO₄, and the other contained 0.75 gm NaHCₐO₃ in 40 ml of distilled water. For the control, the specimens were preincubated in sodium acetazolamide (Diamox) at a concentration of 10⁻³-10⁻⁴ M in order to inhibit the enzyme activity. After incubation, the sections were rinsed and treated with 2% ammonium sulfide for 1 min and postfixed with 1% OsO₄ in 0.1 M phosphate buffer (pH 6.0) for 3 -5 min at 4°C (SUGAI and ITO, 1980). These specimens were dehydrated through a series of ethanol concentrations and embedded in Epon 812. Thin sections were cut with a diamond knife on an LKB Ultrotome. The sections, which were non-stained or double-stained with uranyl acetate and lead citrate, were observed using a JEM 100B or Hitachi H-700 electron microscope.

RESULTS

By light microscopy, a dark brown reaction product was found in the axon terminals of all sensory corpuscles examined in the present study. In addition, the non-neuronal inner core of Pacinian corpuscles exhibited a weakly positive reaction.

By electron microscopy, some of large- and medium-size myelinated axons in sciatic nerves contained electron dense reaction product within the axoplasm. The reaction product was clearly confined in small spaces between neurofilament zones and was prominent beneath the axolemma (Fig. 1a). Myelin sheaths of such axons had a few fine electron dense deposits (Fig. 1a), but such deposits were also found in the control specimens (Fig. 1b), suggesting that these deposits were due to non-specific reactions as reported by RILEY et al. (1982).

The axon terminals of Pacinian corpuscles contained a high density of reaction product (Fig. 2, 3) which was diffusely scattered throughout the axoplasm. In addition, reaction deposit was sporadically distributed in clusters within the cytoplasm of inner core lamellar cells (Fig. 3). Mitochondria in inner core cells showed a definite reaction product (Fig. 2, 3). In contrast, the mitochondria in axon terminals had no definite reaction product (Fig. 2, 3). The central area of axons containing abundant neurofilaments was largely devoid of reaction product.

Fig. 4. A Meissner corpuscle which is located in the dermal papilla of the epithelium (Ep) is shown. Axon terminal (asterisk) which contains reaction product, is surrounded by reaction-free lamellar cells (LC). Reaction product also can be seen in some mitochondria in lamellar cells (arrows). ×5,400

Fig. 5. Higher magnification of a part of Fig. 4. An axon terminal (ax) and lamellar cells (LC) which have many characteristic caveoles, are shown. The reaction product is prominent in the axoplasm. In contrast, no definite reaction product can be found in lamellar cells. Mitochondria in the axoplasm (arrowheads) have no reaction product, whereas some mitochondria in lamellar cells (arrow) have reaction product. ×20,000
Fig. 4 and 5. Legends on the opposite page.
In Meissner corpuscles (Fig. 4, 5), axon terminals contained a reaction product in the axoplasm similar to that of Pacinian corpuscles. No reaction product was present in areas of axoplasm containing neurofilaments (Fig. 5). Mitochondria in axon terminals had no reaction product (Fig. 5). Lamellar cells in Meissner corpuscles also exhibited no reaction product.

In Merkel cell-neurite complexes, there was a small deposit of reaction product in the axoplasm as well as some mitochondria (Fig. 6). No definite reaction product was found in the cytoplasm of Merkel cells.

In the control experiment no reaction product was found in axon terminals of these three sensory endings. Inner core lamellar cells of Pacinian corpuscles also exhibited no reaction product.

DISCUSSION

The present study has demonstrated the histochemical localization of CA activity by electron microscopy in axon terminals of rat sensory endings such as Pacinian corpuscles, Meissner corpuscles and Merkel cell-neurite complexes. Previous studies on the distribution of this enzyme have concentrated on DRG and large neurons. This is the
first report that could demonstrate the presence of CA activity in axon terminals of mammalian sensory corpuscles at the electron microscopic level. The axon terminals of sensory corpuscles are derived mainly from Aα (II) fibres of 6-12 μm in diameter (WILLIS and GROSSMAN, 1981). CA activity in DRG was detected in large neurons. Recently, HARPER and LAWSON have suggested that neuronal somata giving rise to Aα (I) and Aα (II) fibres are large DRG cells by a combined technique of intracellular recording and dye-injection (HARPER and LAWSON, 1985). They also indicated that Aα (III) fibres probably are derived from the medium-sized DRG neurons. CA activity labels a population of large DRG neurons and large sciatic nerve axons. The assumption is thus reasonable that the same neuron is labeled from somata to sensory terminal (RILEY et al., 1982, 1984; WONG et al., 1983; KAZIMIERCZAK et al., 1986).

Yet the distribution of CA activity is not uniform in cutaneous receptors. The CA activity of nerve terminals associated with Merkel cell-neurite complexes was sparse, while axon terminals of the other two corpuscles had intense CA activity. This finding indicates that axon terminals abutting Merkel cells are distinctly different neurons in terms of CA activity from those of innervating other corpuscular endings.

The presence of CA activity in sensory axons as well as the absence of CA activity in α-motor axons are of importance as these differences indicate enzymatic differences between motor and sensory nerves. However some motor neurons do contain CA activity as RILEY et al. (1982) reported that, although less reactive than sensory endings, γ-motor endings have some CA activity. Thus with the exception of γ-motor nerves, we can conclude that CA activity is mainly found in large- and medium-sized sensory neurons and their sensory endings. However, we must express concern as to possible species differences as in our experience Hansson’s method yielded only a faint CA activity in feline DRG cells (unpublished). KAZIMIERCZAK et al. (1986) have reported that CA activity in chicken DRG cells might be transitory rather than permanent in nature.

The CA enzyme is thought to be membrane-bound. However in the present study, the localization of reaction product was not always associated with membranous structures in axons. A similar pattern of localization of CA activity was reported in the annulospiral sensory endings of muscle spindles (RILEY et al., 1984) and in parietal cells of mouse gastric mucosa (SUGAI and ITO, 1980) using Hansson’s method. These findings indicate that the reaction product detected by Hansson’s method tends to localize not only in membrane-bound compartments.

The histochemical findings of CA activity in peripheral nerve as revealed by Hansson’s method are in accordance with immunohistochemical studies using anti-CA-II (CA-C) antibody (WONG et al., 1983; LINSER, 1985; KAZIMIERCZAK et al., 1986). The localization of the CA activity histochemically demonstrated in the present study is, therefore, thought to be related to that of CA-II. In addition to peripheral nerves, immunohistochemical studies have shown the presence of CA-II activity in mouse spinal cord (CAMMER et al., 1985b), rat cerebellum (GHANDOUR et al., 1980) and chick retina (LINSER and MOSCONA, 1981) by light microscopy.

Many hypotheses has been proposed as to the physiological significance of CA activity in the nervous system (SAPIRSTEIN et al., 1978; GHANDOUR et al., 1980; LINSER and MOSCONA, 1981; PARTHE, 1981; SAPIRSTEIN, 1983; WONG, 1983; CAMMER et al., 1985b.) CA is thought to be concerned with controlling of the volume of water in the CNS. In the peripheral nervous system CA also could control the intracellular concentration of H+ and Cl- in some CA positive neurons in DRG. The significance of the CA activity, however, remains obscure in the peripheral nervous system. The positive CA
reactivity of inner core lamellar cells of Pacinian corpuscles indicates that these cells might play an important role in the control of the ionic environment of axon terminals. Recently, abundant gap junctions were found between the inner core lamellar cells using freeze fracture methods (Ide and Hayashi, 1987). Gap junctions in the inner lamellae would imply electrotonic coupling of each block of hemi-lamellae in Pacinian corpuscles. The presence of CA activity in inner core lamellar cells thus might be related to the control of the ionic environment of the central axon and mechano-electric transduction in Pacinian corpuscles.

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