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IMMUNOHISTOCHEMICAL LOCALIZATION OF ADENOSINE DEAMINASE IN PRIMARY AFFERENT NEURONS OF THE RAT

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Adenosine deaminase (ADA) was detected immunohistochemically in neuronal cell bodies of dorsal root ganglia (DRG) of the rat. ADA-immunoreactivity was confined exclusively to small type B ganglion neurons in cervical, thoracic and lumbar sensory ganglia; large type A neurons in sensory ganglia were devoid of immunostaining for ADA. It was consistently found that only a small proportion of type B neurons in DRG contain immunohistochemically detectable ADA. It is suggested that the expression of high ADA levels is a distinguishing feature of a subpopulation of type B DRG neurons and, further, that ADA in these neurons may reflect their utilization of purines (adenosine or adenine nucleotides) as transmitters or cotransmitters.

Several subpopulations of small type B neurons in dorsal root ganglia (DRG) of the rat have now been identified on the basis of immunocytochemical and histochemical localization of their constituent peptides and enzymes. These subpopulations include type B neurons containing substance P (SP) [8], somatostatin [7], vasoactive intestinal polypeptide (VIP) and gastrin/cholecystokinin (CCK)-like peptide [13], bombesin/gastrin releasing peptide-like peptide (GRP) [5] and fluoride-resistant acid phosphatase (FRAP) [11]. The term 'subpopulation' as used here is meant to infer that these peptides and proteins are found in some but not all type B neurons. The extent to which various combinations of these substances are found in entirely different, partially overlapping or the same subpopulations of neurons is a separate issue. To date, SP, somatostatin and FRAP have been found in three separate subpopulations of type B neurons [8,14]. A CCK-like peptide has been found exclusively in all SP-containing DRG neurons [3]. A GRP-like peptide has been found in some [4] but not all [17] type B neurons containing SP.

In addition to the possibility that some of these peptides might have some sort of transmitter role in type B neurons, there have been suggestions that purines (adenosine and/or adenine nucleotides) may also serve as primary afferent transmit-

ters. Holton [9] originally demonstrated that antidromic stimulation of sensory nerves induced the release of adenosine-5′-triphosphate (ATP) at peripheral sites of nerve termination. More recently, electrophysiological studies have shown that ATP selectively excites certain populations of dorsal horn and sensory ganglia neurons grown in dissociated cell culture [10] and DRG neurons in vivo [12]. ATP was also found to increase the firing rate of neurons receiving primary afferent input in cuneate and caudal trigeminal nuclei [6,19]. These and other studies indicating a potentially important role for purines in regulating central neuronal activity [18] prompted us to investigate the regional CNS distribution of enzymes directly involved in the metabolism of adenosine and its nucleotides. These studies were based on the premise that a selective localization of such enzymes may reveal those neural systems in which purines have a special role in neuronal function distinct from their involvement in intermediary metabolism. One such enzyme is adenosine deaminase (ADA), which promotes the conversion of adenosine to inosine. We report here the immunohistochemical localization of ADA in a subpopulation of type B DRG neurons.

Rats were deeply anesthetized with chloral hydrate and perfused transcardially with 50 ml of 0.9% saline followed by 100 ml of 4% paraformaldehyde containing 0.1 M sodium phosphate buffer at pH 6.5 followed by 200 ml of 4% paraformaldehyde in the same concentration of phosphate buffer but at pH 9.0. Cervical, thoracic and lumbar sensory ganglia were excised, post-fixed in the latter fixative for 1.5 h and kept overnight in 0.1 M sodium phosphate buffer (pH 7.5) containing 30% sucrose. Sections of ganglia (20 μm) were cut on a cryostat and collected on slides or were obtained using a sliding microtome and incubated either free-floating or on the slide for 24–48 h at 4°C in a solution containing 0.9% saline, 0.1 M sodium phosphate buffer (pH 7.5), 0.04% normal goat IgG, 0.3% Triton X-100 (PBSG) and rabbit anti-calf intestinal ADA antisera at a dilution of 1:500. Control sections were similarly incubated but with antisera which had been preabsorbed with purified ADA. The purification of ADA and the preparation of specific antibodies to this enzyme has been previously described [2].

It was shown that antibodies to calf intestinal ADA cross-react with ADA derived from rat tissues (P.E. Daddona, unpublished observations). Following incubation with primary antisera, sections were washed at room temperature for 45 min in a solution containing 0.1 M sodium phosphate buffer (pH 7.4) and 0.9% saline (PBS), and incubated for 45 min at 37°C in PBSG containing goat anti-rabbit antiserum (Miles Laboratories, Ltd.). The sections were then washed in several changes of PBS as before and incubated for 45 min at 37°C in PBSG containing rabbit horseradish peroxidase–antiperoxidase (PAP) (Miles Laboratories, Ltd.) diluted 1:100. Sections were then washed in 50 mM Tris-HCl buffer, pH 7.4 (Tris-buffer) for 45 min at room temperature, and incubated for 15 min in Tris-buffer containing 0.02% diaminobenzidine (DAB) and 0.005% H_2O_2. Free-floating sections were mounted on slides from a gelatin solution, dehydrated, and coverslipped

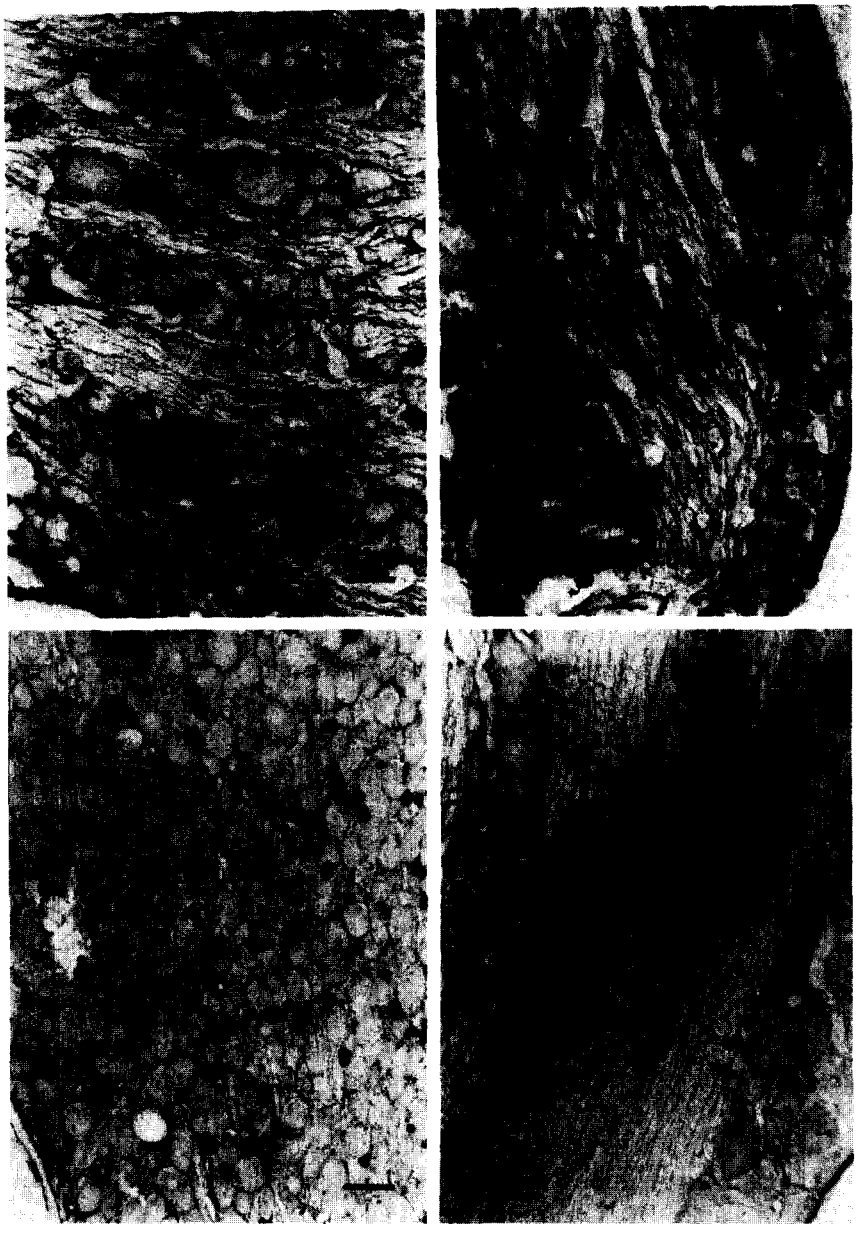

Fig. 1. Sections of cervical (a), thoracic (b) and lumbar (c) sensory ganglia of the rat demonstrating adenosine deaminase (ADA)-immunoreactivity in small type B neurons by the PAP method. Arrowheads indicate examples of ADA-positive cells. Note that no large neurons (arrow in c) and not all small neurons exhibit immunostaining for ADA. As shown in d (arrows indicate unlabeled small cells), ADA-immunoreactivity was abolished in sections processed by the PAP method following incubation with ADA antisera (0.5 ml diluted 1:500) preabsorbed with purified ADA (6 ng). Scale bars for a, b and d (shown in a) = 50 μm; and in c = 100 μm.

with Permount. Absorption controls were conducted by incubating tissue sections with ADA antisera diluted 1:500 and preabsorbed with 6.0 ng of purified ADA.

The above procedure led to the formation of DAB reaction product in neurons of cervical, thoracic and lumbar DRG (Fig. 1). No reaction product was observed when the ADA antisera was omitted in the first incubation or when the ADA antisera employed in this incubation had been preabsorbed with purified ADA (Fig. 1d). Therefore, the immunohistochemical staining can be considered to represent the localization of immunoreactive ADA. Large and small sensory ganglia neurons, classified as type A and type B, respectively, by Andres [1] exhibited differential staining for ADA. At all craniospinal levels examined, ADA-immunoreactivity was restricted to type B ganglion neurons and was not observed in type A cells. It was further evident that only a subpopulation of type B neurons were ADA-positive. In cervical, thoracic and lumbar sensory ganglia, it was estimated that about 7%, 9% and 13%, respectively, of all the neurons stained positively for ADA. That ADA is confined to a subpopulation of type B neurons is supported by observations of only small variations in the density of reaction product in ADA-positive neurons, thereby allowing a clear distinction to be made between stained and unstained cells. No ADA-containing fibers were found in sensory ganglia. It is presently uncertain whether this failure to detect ADA in fibers associated with ADA-positive neurons is due to low levels of this enzyme in axons or to inappropriate fixation procedures. It is perhaps noteworthy in this regard that using fixation methods similar to that described above, ADA-containing axons have been observed in brain in association with neurons exhibiting intense ADA-immunoreactivity (J.I. Nagy, unpublished observations).

Two issues arise from the observation that a subpopulation of type B DRG neurons contains relatively greater quantities of ADA than type A cells or the majority of type B cells in which ADA was immunohistochemically undetectable. The first is whether ADA is contained in neurons separate from those containing SP, somatostatin and FRAP. Based on the neurotoxic actions of capsaicin, evidence previously presented indicates that SP-, somatostatin- and FRAP-containing type B cells give rise to unmyelinated fibers [16]. It is likely that ADA-containing cells are also associated with unmyelinated axons since we have recently found that ADA-positive cells were depleted in DRG of adult rats which had been treated neonatally with a dose of capsaicin which leads to the depletion of only unmyelinated fibers in dorsal roots [15]. Future studies may further reveal the relationship of neurons containing ADA to other DRG neuronal subpopulations.

The second issue is the reason for the expression of ADA in greater abundance in some type B neurons than in type A or other type B cells. The view presently favored is that an amplification of ADA is required in those neurons in which purines (adenosine and its nucleotides) subserve a special role distinct from their ubiquitous function in intracellular metabolism. Such a role may include the utilization of these purines as neurotransmitters. Thus, ADA could be intimately involved

in the control of the availability and disposition of purine transmitters. This would be consistent with observations on the physiological effects of altering ADA activity [18].

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