ferences in glycosylation pattern could explain differences in molecular weight. In addition, although we accept that only one single molecule DBH-C exists this cannot be proven. Indeed, the resolution of the separation method is too low to distinguish between closely related forms, hence if different degradation products exist, their molecular weight should be within the experimental error of the method.

We therefore suggest that the presence of low molecular weight forms of DBH, DBH-B and DBH-C, are most likely the consequence of a tissue-specific, naturally occurring degradation process of DBH-A. The structure of DBH-C and its relation to DBH-A and DBH-B remain to be elucidated with further experiments which will require denaturating and deactivating conditions.

1 Rosenberg, R. C., and Lovenberg, W., Molec. Pharmac. *13* (1977) 652.
2 Grzanna, R., Nelson, M. F., Weinshilboum, R. M., Dunette, J., and Coyle, J. T., J. Neurochem. *33* (1979) 913.
3 Fraeyman, N. H., Van de Velde, E. J., De Smet, F. H., and De Schaepdryver, A. F., J. Neurochem. *39* (1982) 1179.
4 Blakeborough, P., Louis, C. F., and Turner, A. J., Biochem. biophys. Acta *669* (1981) 33.
5 Park, D. H., Kashimoto, T., Ebstein, R. P., and Goldstein, M., Molec. Pharmac. *12* (1976) 73.
6 Grzanna, R., and Coyle, J. T., J. Neurochem. *27* (1976) 1091.
7 Martin, R. G., and Ames, B. N., J. biol. Chem. *236* (1961) 1372.
8 Bradford, M. M., Analyt. Biochem. *72* (1976) 248.
9 Gagnon, C., Schatz, R., Otten, U., and Thoenen, H., J. Neurochem. *27* (1976) 1083.
10 Winkler, H., Neuroscience *2* (1977) 657.
11 Cubeddu, L. X., Barbella, Y. R., Marrero, A., Trifaro, J., and Israel, A. S., J. Pharmac. exp. Ther. *211* (1979) 271.
12 Israel, A. S., Barbella, Y. R., and Cubeddu, L. X., J. Pharmac. exp. Ther. *221* (1982) 577.
13 Sabban, E. L., and Goldstein, M., J. Neurochem. *43* (1984) 1663.
14 Brimijoin, S., and Carter, J., J. Neurochem. *38* (1982) 588.
15 Saxena, A., Hensley, P., Osborne, J. C., and Flemidng, P. J., J. biol. Chem. *260* (1985) 3386.
16 Nolan, J., Fonsenca, R., and Hogue-Angeletti, R., Archs Biochem. Biophys. *240* (1985) 257.
17 Helle, K. B., Serck-Hanssen, G., and Bock, E., Biochim. biophys. Acta *553* (1978) 396.
18 Foldes, A., Jeffrey, P. L., Preston, B. N., and Austin, L., J. Neurochem. *20* (1973) 1431.
19 Aunis, D., Miras-Portugal, M.-T., and Mandel, P., Biochim. biophys. Acta *365* (1974) 259.
20 Hogue-Angeletti, R. A., Archs Biochem. Biophys. *184* (1977) 364.
21 Okuno, S., and Fujisawa, H., Biochim. biophys. Acta *799* (1984) 260.

0014-4754/88/090746-04$1.50 + 0.20/0

Aldolase C is localized in neuroendocrine cells

H. Inagaki[a], H. Haimoto[b], S. Hosoda[b] and K. Kato[c]

[a] *Department of Gastroenterological Surgery, Aichi Cancer Center Hospital,* [b] *Laboratory of Pathology, Aichi Cancer Center Research Institute, Nagoya 464 (Japan), and* [c] *Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi (Japan)*
Received 17 March 1988; accepted 17 June 1988

Summary. To elucidate the localization of the subunit C of aldolase (aldolase C) in peripheral neuroendocrine cells, we made an immunohistochemical study with monospecific antibodies against human aldolase C. Aldolase C was found to be localized in various types of neuroendocrine cells; in the pituitary gland, thyroid, pancreas, adrenal gland, bronchus, and gastrointestinal tract.
Key words. Aldolase; subunit; isozyme; neuroendocrine cell; immunohistochemistry.

Fructose-1,6-diphosphate aldolase (EC 4.1.2.13), a glycolytic enzyme, has a tetrameric form [1] with three immunologically distinct subunits; A, B, and C [2]. Subunit A (aldolase A) is a fetal form of aldolase, and is mainly present in muscle [1], while subunit B (aldolase B) is predominantly distributed in liver and kidney [1]. Subunit C (aldolase C) was considered for a long time to be localized mainly in the brain, in a five-membered A–C hybrid set which could be separated by electrophoresis [1]. In a study employing immunohistochemical methods, it was reported that aldolase C is localized in Purkinje cells of the cerebellum and some neurons in the central nervous system [3, 4].

Recently, we developed a highly sensitive immunoassay of human aldolase C, and reported that peripheral tissues such as adrenal gland, jejunum, and other tissues contained significant amounts of aldolase C [5].

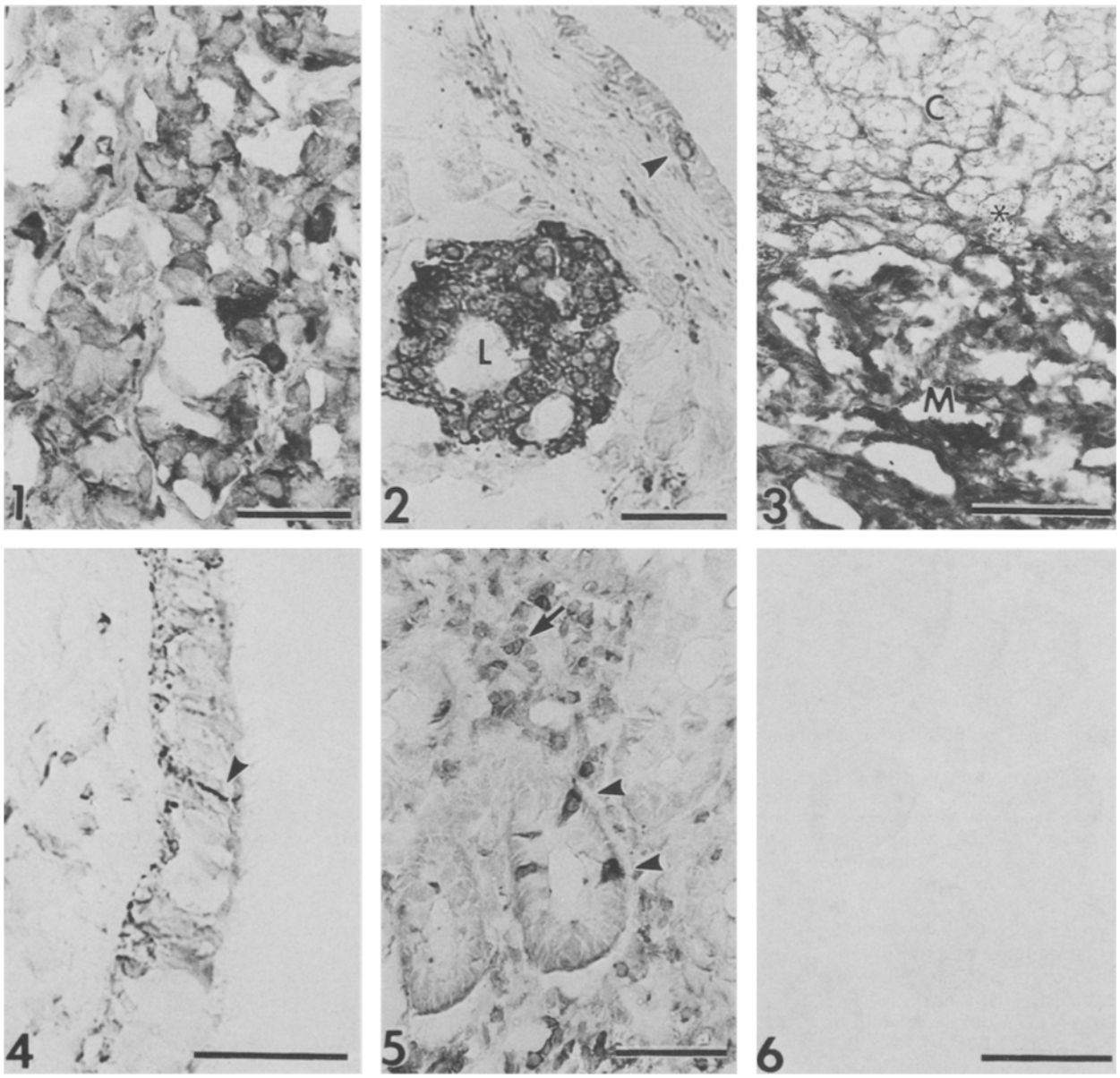
We describe here the localization of aldolase C in peripheral neuroendocrine cells, and discuss its usefulness as a biomarker for neuroendocrine cells, as compared with γ-enolase (neuron-specific enolase), another glycolytic enzyme which is known to be a specific marker for those cells [6 – 10].

Materials and methods. Antibodies. Antibodies were raised in New Zealand white rabbits by i.c. injections of purified aldolase C, and further purified by immunoaffinity chromatography as described previously [5]. The specificity of the antibodies has been reported [5]. For secondary antibodies, horseradish peroxidase(HRP)-labeled goat IgG Fab' fragments against rabbit IgG were prepared as previously described [11].

Tissue specimens and preparations. Two or three pieces of each adult tissue (thyroid, pancreas, bronchus, stomach, small intestine, and large intestine) were obtained from surgical operations. Adrenal and pituitary glands were obtained at autopsies within 2 h after the patients' deaths. They were fixed in periodate-lysine-4% paraformaldehyde (PLP) for 6 h, washed in phosphate-buffered saline (PBS, pH 7.2) containing increasing concentrations of sucrose, embedded in OCT compound (Lab-Tek Products, Miles Laboratories Inc., Naperville, Ill), and frozen quickly in dry ice and ethanol.

Normal pancreatic tissues were fixed in formalin for 12 h and embedded in paraffin in order to compare the preservation of the antigenicity under the conditions used.

Immunohistochemistry. The indirect immunoperoxidase method according to Nakane [12] was used. In brief, cryostat sections were placed on albumin-coated slides and dried at room temperature. The sections were treated with 5 mM periodic acid solution, to inactivate endogeneous peroxi-



Figures 1–6. Localization of aldolase C in peripheral tissues. *1* Endocrine cells in anterior lobe of pituitary gland are strongly stained. ×100. Bar = 50 μm. *2* Aldolase C staining is prominent in islet cells of Langerhans (L). A neuroendocrine cell found in ductal epithelia (arrow head) is also stained. ×100. Bar = 50 μm. *3* Chromaffin cells in adrenal medulla (M) are strongly stained. Cortical cells (C) are not stained. *: lipofuscin granules. ×100. Bar = 50 μm. *4* Aldolase C is strongly positive in neuroendocrine cells (arrow head) scattered in bronchial epithelia. Some basal cells are rather weakly stained. ×100. Bar = 50 μm. *5* Aldolase C immunostaining is prominent in a small number of neuroendocrine cells (arrow heads) in glands of small intestines. Lymphocytes (arrow) are also stained. ×100. Bar = 50 μm. *6* A control section of small intestine is uniformly negative. ×100. Bar = 50 μm.

dase. They were washed in PBS, and allowed to react with monospecific anti-human aldolase C antibodies IgG (1.6 μg/ml) for 120 min. For control sections, antibodies absorbed with the purified human aldolase C antigen were substituted for the primary antibodies. After washing in PBS, the sections were incubated with the HRP-labeled secondary antibodies for 60 min. After being rinsed with PBS, the sections were treated with 0.025% diaminobenzidine (DAB) solution containing 10 mM hydrogen peroxide and 10 mM sodium azide, and then counterstained with methyl green.

Results. In the pituitary gland, endocrine cells of both anterior and posterior lobes showed strongly positive staining for aldolase C (fig. 1). In the thyroid, aldolase C-positive cells corresponded to parafollicular cells in their position and number, whereas the follicular cells were negative (not shown). In the pancreas, all islet cells of Langerhans showed positive staining. A few neuroendocrine cells scattered in ductal epithelia were also stained (fig. 2). In contrast to these cells, the surrounding exocrine acinar and ductal epithelial cells were negative. In the adrenal gland, chromaffin cells in the medulla exhibited strong staining, whereas the cortical cells were negative (fig. 3). Aldolase C was observed in a small number of neuroendocrine cells located in bronchial epithelia. Basal cells showed positive staining, but bronchial columnar epithelial cells, goblet cells, and bronchial glandular cells were not labeled (fig. 4). In the stomach, some surface epithelial cells showed positive staining. Neuroendocrine cells in the intraepithelial spaces were stained

strongly (not shown). In the small and large intestines, absorptive cells, goblet cells, and Paneth cells were devoid of detectable aldolase C, but neuroendocrine cells were positive. Lymphocytes and neutrophils were stained (fig. 5). Control sections were uniformly negative (fig. 6). Formalin-fixed, paraffin-embedded sections of pancreas showed a remarkable reduction in the stainability of aldolase C.

Discussion. There are several proteins specific to or associated with neurons and neuroendocrine cells; γ-enolase (neuron-specific enolase; NSE)[6-10], chromogranin[13,14], creatine kinase BB (CK-B)[15], and α subunit of guanine nucleotide-binding protein (Goα)[16]. All of these are acidic proteins. Since their levels in the sera of patients with neuroendocrine tumors increase, the proteins could well be clinically applicable as serum markers for the diagnosis of neuroendocrine tumors. In addition, because such proteins are immunohistochemically detectable in neuroendocrine cells and the tumor cells derived from these cells, the immunohistochemical detection of these antigens is employed for differential diagnosis in the field of diagnostic pathology.

Aldolase C is also known to be distributed mainly in the central nervous system[1-5]. Sato et al. suggested that studies of the aldolase isozyme might be useful in the diagnosis of brain tumors by comparing the isozyme patterns of various brain tumors with those of normal brain[17]. Willson et al. suggested that the aldolase C level of cerebro-spinal fluid might reflect damage within the central nervous system[18]. Kumanishi et al. reported that aldolase C staining was prominent in astrocytes and Purkinje cells, although faint staining was also occasionally observed in some other neurons[4]; its localization had not been shown in the peripheral organs.

In this report, we examined the localization of the aldolase C in the peripheral neuroendocrine tissues, and demonstrated that it is localized in neuroendocrine cells of various types. The γ-enolase, another glycolytic enzyme, is a well-known marker for neuroendocrine cells[6-10]. It was initially considered to be neuron-specific, but subsequently was found to be present in non-nervous cells or tissues[10]. When the tissue distribution of aldolase C was compared with that of γ-enolase, the stainability of aldolase C in neuroendocrine cells was similar to that of γ-enolase. Aldolase C, however, was not neuron-specific like γ-enolase, since positive staining was also observed in non-neuroendocrine cells such as some surface epithelia of stomach, lymphocytes and neutrophils, with the use of PLP-fixed, cryostat sections. The distribution of aldolase C in non-neuroendocrine tissues is quite different from that of γ-enolase[10]. These findings suggest that the immunohistochemical application of aldolase C in diagnostic pathology should be undertaken with care, in order to avoid the same confusion that is seen with the use of γ-enolase[19]. In addition, the antigenicity of aldolase C in formalin-fixed, paraffin-embedded sections was found to be remarkably reduced as compared to that in PLP-fixed, cryostat sections; this might also be a disadvantage in applying aldolase C in clinical pathology.

Acknowledgments. We thank Y. Yamamura, MD and T. Kito, MD, Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, for their valuable suggestions and discussions during the course of these studies. Mrs C. Yamada of the laboratory provided excellent technical assistance.

1 Lebherz, H. G., and Rutter, W. J., Biochemistry *8* (1969) 109.
2 Penhoet, E., and Rutter, W. J., J. biol. Chem. *246* (1971) 318.
3 Thompson, R. J., Brain Res. *232* (1982) 489.
4 Kumanishi, T., Acta neuropath. (Berlin) *67* (1985) 309.
5 Haimoto, H., and Kato, K., Clin. chim. Acta *154* (1986) 203.
6 Schmechel, D., Nature *276* (1978) 834.
7 Tapia, F. J., Lancet *1* (1981) 808.
8 Bishop, A. E., Gastroenterology *83* (1982) 902.
9 Kato, K., Brain Res. *237* (1982) 441.
10 Haimoto, H., Lab. Invest. *52* (1985) 257.
11 Haimoto, H., Am. J. Path. *124* (1986) 488.
12 Nakane, P. K., Ann. N.Y. Acad. Sci. *254* (1975) 203.
13 Wilson, B., and Lloyd, R., Am. J. Path. *115* (1984) 458.
14 Hagn, C., Lab. Invest. *55* (1986) 405.
15 Wold, L., Am. J. clin. Path. *75* (1981) 327.
16 Kato, K., Cancer Res. *47* (1987) 5800.
17 Sato, S., Cancer *27* (1971) 223.
18 Willson, V. J. C. et al., Ann. clin. Biochem. *17* (1980) 110.
19 Schmechel, D., Lab. Invest. *52* (1985) 239.

0014-4754/88/090749-03$1.50 + 0.20/0

Narcotic antagonism of seizures induced by a dopamine-derived tetrahydroisoquinoline alkaloid

K. Blum

Division of Substance and Alcohol Misuse, Department of Pharmacology, The University of Texas Health Science Center, San Antonio (Texas 78284-7764, USA)
Received 2 March 1987; accepted 30 May 1988

Summary. This paper describes experiments designed to evaluate whether the narcotic antagonist naloxone significantly interferes with seizures induced by tetrahydroisoquinolines (TIQs). In these experiments we found that naloxone significantly reduced seizure scores induced by intra-cranially infusing mice with 50 μg of the dopamine-derived tetrahydroisoquinoline (TIQ) alkaloid, 6,7-dihydroxy TIQ. These findings support an opioid involvement in the actions of TIQs and may lead to further understanding of opioid-mediated novel excitatory receptors.
Key words. Tetrahydroisoquinoline; naloxone; CNS excitation; opioids; dopamine.

After scrutinizing the literature, our laboratory undertook the present study to determine whether the narcotic antagonist naloxone can alter ethanol-induced intoxicating actions in a dose-dependent fashion and whether naloxone can similarly antagonize seizures produced by simple TIQ compounds.

To date most animal research has shown that the addictive agents ethanol and opiates share common biochemical mechanisms of action[1-5]. Despite some reports to the contrary[6-8], most researchers in this field generally agree that some of ethanol's actions occur by activating opiate receptors[9-14]. Studies by Davis and Walsh[15] and others[16] postulate that benzyl TIQs share common properties with opiates. Ross's group has supported this argument[17-20], while Hamilton and co-workers have found that opiate antagonists (naloxone or naltrexone) have attenuated the opiate-