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Immunohistochemical Localization of Alcohol Dehydrogenase in Human Kidney, Endocrine Organs and Brain

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BÜHLER, R., D. PESTALOZZI, M. HESS AND J.-P. VON WARTBURG. *Immunohistochemical localization of alcohol dehydrogenase in human kidney, endocrine organs and brain.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 55–59, 1983.—Antibodies against human liver alcohol dehydrogenase (ADH) were produced in rabbits. Peroxidase-labeled protein-A with diaminobenzidine as substrate was used to detect anti-ADH binding in human tissue thin sections. In the kidney, ADH was localized in the epithelia of the tubuli; glomeruli and collecting tubules appeared negative. In prostata and epididymis, the epithelia stained strongly. In the testes, the seminiferous epithelium and the Leydig cells stained strongly. In the ovary and uterus only faint staining was observed. The ADH-content of the adrenal gland was low, but was higher in the cortex than in the medulla. In the pancreas, the Langerhans islets exhibited particularly high ADH concentrations. In the brain, ADH was localized in neurons of the cerebral cortex, hypothalamus, infundibular stalk of the pituitary, and Purkinje cells of the cerebellum. In summary, ADH could be localized primarily in cells known as targets of ethanol toxicity.

Human alcohol dehydrogenase Localization Immunohistochemistry Protein-A peroxidase Kidney Reproductive system Endocrine organs Central nervous tissue Alcohol abuse Cellular damage

ALCOHOL dehydrogenase (ADH), the principle enzyme in ethanol elimination, converts ethanol to acetaldehyde which is very toxic and believed to play a significant role in the development of tissue damage caused by alcohol abuse [18]. The concomitant production of large amounts of NADH during ethanol oxidation changes the cellular redox potential which in turn leads to disturbances of cellular metabolism. In addition, oxidation of ethanol may compete with physiological, hitherto unknown functions of ADH. Hence, preferential damage of cells containing ADH should be expected to occur in states of chronic alcohol abuse. Therefore, an exact topographic localization of this enzyme in various tissues is of great interest.

Conventional histochemical methods and animal tissues were predominantly used to localize ADH [1, 11, 12, 16, 25]. Our own studies carried out on sections of human tissues, are based on an immunohistochemical method which is both more specific and more sensitive than conventional histochemistry. With the aid of a specific antiserum directed against human liver ADH, the enzyme could be demonstrated in human liver cells, gastrointestinal mucosa, and cultured fibroblasts and HeLa cells [3,21]. Preliminary reports in which anti-horse and anti-rat ADH antisera were used on rat tissues have been published recently [6, 13, 14].

In this report we describe the immunohistochemical localization of ADH in the kidney, in a series of human endocrine organs, and in parts of the brain.

METHOD

The mixture of pyrazole-sensitive human liver ADHs was purified and characterized as described [3]. Antibodies against this mixture of ADH-isoenzymes were raised in rabbits. The specificity of the antisera was tested by double immunodiffusion, enzyme-linked immunosorbent assay (ELISA), immunoprecipitation of enzymatic activity, and specific binding to immobilized ADH [3,4].

Anti-ADH antibodies were purified by affinity chromatography on ADH coupled to Ultrogel AcA 44 by glutardialdehyde [3] or to CNBr-activated Sepharose CL-4B [10]. The breakthrough fractions of the affinity columns, i.e., antiserum depleted of specific anti-ADH antibodies, were concentrated to the original volume, and this concentrate was used as a control.

Human tissue specimens were fixed in 4% phosphate buffered formaldehyde, pH 7.4, embedded in paraplast, sectioned at a thickness of 5 μm and mounted on slides. Kidney, brain, adrenal, prostate, epididymis, ovary, and uterus specimens were obtained at autopsy. Tissue samples of pancreas and testes were derived from biopsy material.

Immunohistochemical staining was performed as described before [3]. In some cases, sections were counterstained with Meyer's haematoxylin (1:5 diluted in water) for 10 min before mounting. Antiserum depleted of specific anti-ADH antibodies by affinity chromatography and preimmuneserum were routinely used as negative controls. Both antisera and control sera were used in a dilution of 1:100 or 1:10.

RESULTS

ADH could be detected in the cytoplasm of all organs and

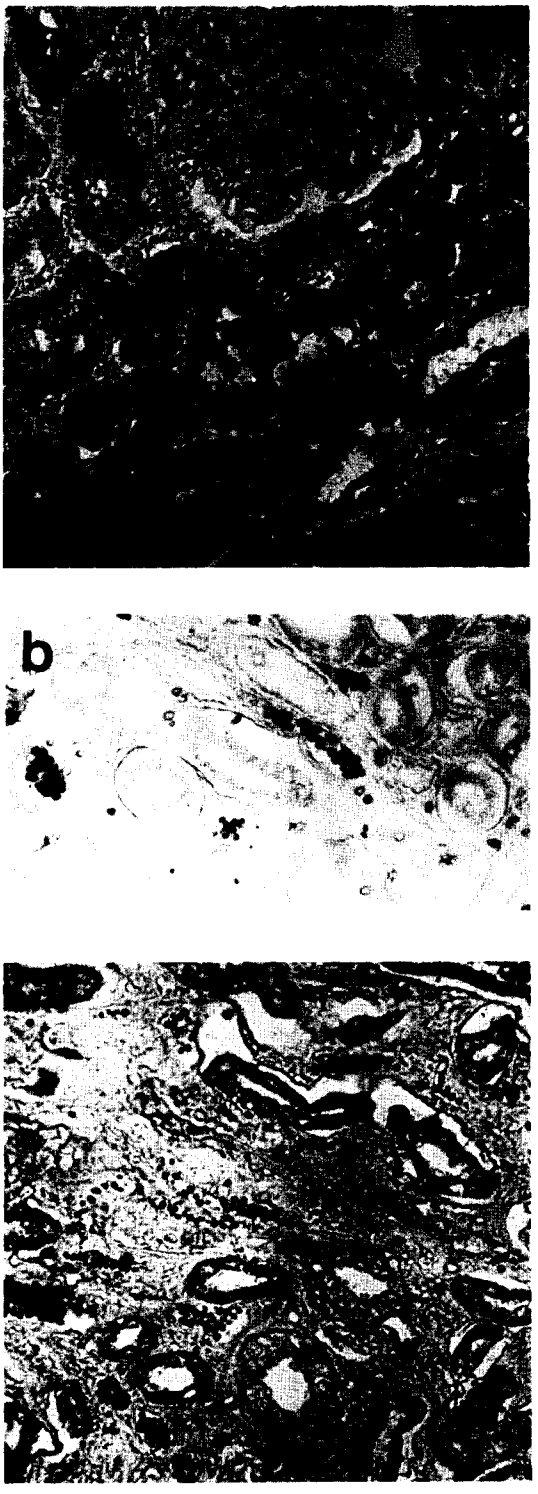

FIG. 1. Immunohistochemical staining of ADH in sections of human kidney. (a,c) Sections were incubated with anti-ADH antiserum and the bound antibodies detected with protein-A peroxidase followed by incubation with diaminobenzidine. (b) Control. The section was incubated with immunoadsorbed antiserum (depleted of anti-ADH antibodies by affinity chromatography on ADH-Ultrogel). The antiserum as well as the immunoadsorbed antiserum was diluted 1:100. (×200).

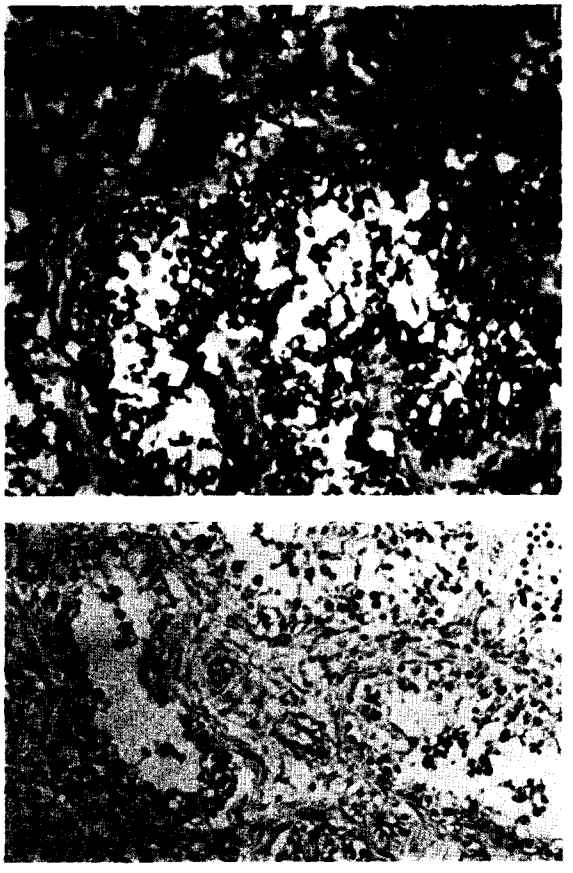

FIG. 2. Immunohistochemical staining of ADH in sections of human testes. (a) The section was incubated with anti-ADH antiserum. The arrowhead points to a group of Leydig cells. (b) Control. The section was incubated with immunoadsorbed anti-ADH antiserum (depleted of anti-ADH antibodies by affinity chromatography on ADH-Sepharose). The antiserum as well as the control serum was diluted 1:10. (×250). The sectons were counterstained with Meyer's haematoxylin.

tissues investigated in this study. No difference in the staining pattern was observed between anti-ADH antiserum and purified anti-ADH antibodies. The control sera were always negative. Certain cell types exhibited much stronger staining than others within the same organ, indicating that ADH is unevenly distributed in many organs.

In the kidney, the epithelial lining of the tubuli stained strongly, glomeruli and collecting tubules appeared negative (Fig. 1a, 1c). The staining intensity of the proximal sections of the tubuli appeared to be more intense than the more distal parts; however, a more extensive study on serial sections ought to be performed. No staining was observed after incubation with control sera (Fig. 1b).

In the testes, the Leydig cells appeared as conspicuous, dark cell clusters (Fig. 2a, arrowhead) amidst the surrounding interstitial cells that stained much weaker. As judged by staining intensity, spermatogonia as well as the epithelia of the seminiferous ducts contained more ADH than the interstitial cells, but less than the Leydig cells (Fig. 2a). A section incubated with control serum is shown in Fig. 2b. The cytoplasm of epithelial cells of both prostate and epididymis exhibited strong positive staining (not shown). With the tech-

FIG. 3. Immunohistochemical staining of ADH in sections of human adrenal gland. (a) The section was incubated with anti-ADH antiserum. (b) Control. The section was incubated with immunoadsorbed anti-ADH antiserum. The sera were diluted 1:10. (×150).

nique used, only weak staining was detectable in the ovary and in the uterine endometrium (not shown).

The overall staining of the adrenal gland was weak, the cortex showing a slightly more intense staining than the medulla (Fig. 3a). The control is shown in Fig. 3b.

All cells of the pancreas stained for ADH. Quite strikingly, cells of the islets of Langerhans were much more heavily stained than the surrounding exocrine pancreas, indicating a larger amount of ADH in the endocrine cells (Fig. 4c, arrows pointing to Langerhans islets). Figure 4a depicts a Langerhans islet at greater magnification. Figures 4b and 4d are the respective control sections. To date we have not been able to differentiate between insulin and glucagon producing cells with respect to their ADH-content.

When sections of cerebral cortex, cerebellum, and hypothalamus were investigated, it was remarkable that many but not all neurons in particular some Purkinje cells in the cerebellum stained very strongly (Figs. 5a and 6a show sections through cerebral cortex and cerebellum, respectively; Figs. 5b and 6b are the respective controls). At present it is not possible to say which type of neurons exhibit high ADH-content. Also, in the hypothalamus and the infundibular stalk of the pituitary gland, some neurons stained strongly (not shown). We could not obtain the pituitary gland proper for immunohistochemical experiments.

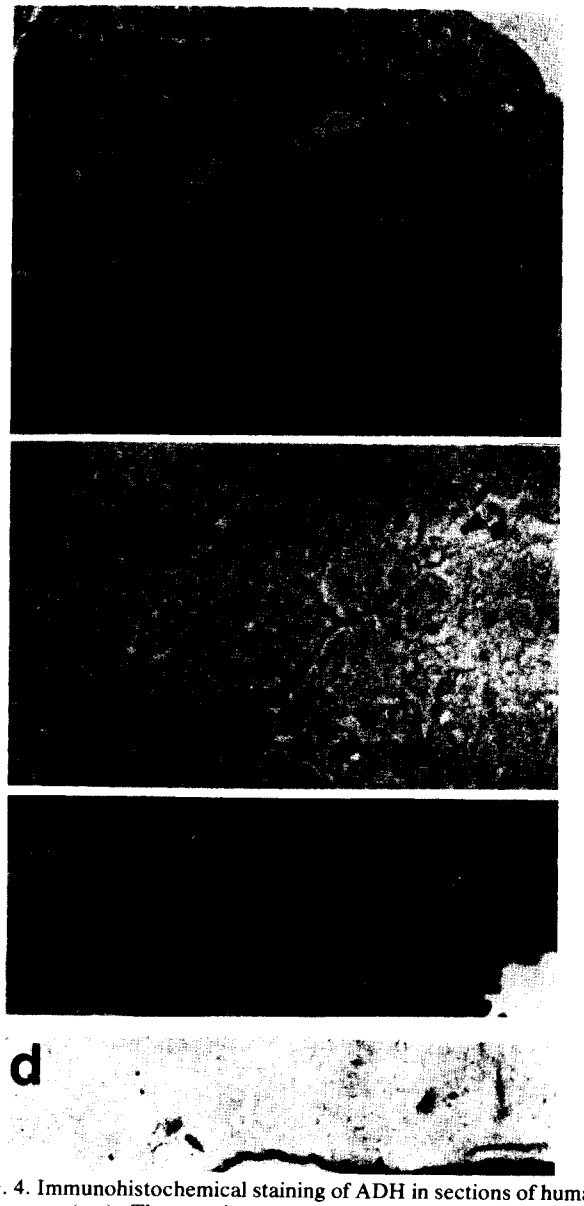

FIG. 4. Immunohistochemical staining of ADH in sections of human pancreas. (a,c) The sections were incubated with anti-ADH antiserum. (a) Langerhans islet at high magnification (×500). (b) Control to (a). (c) Low magnification, illustrating the more intensely stained Langerhans islets (arrows) amidst the exocrine cells (×35). (d) Control to (c). The serum dilutions were 1:100 (a,b) and 1:10 (c,d).

DISCUSSION

Ethanol acts directly on rat renal structure and function [29]. Whether ethanol or its metabolites are the effective agents was not studied. ADH-activity has been demonstrated in human kidney tissue [20,24]. Our own results confirm the histochemical demonstration of ADH in the epithelia of rat kidney tubuli [23]. Therefore, it cannot be excluded that ADH may be involved in the generation of toxic effects of ethanol in this organ.

The effects of alcohol abuse on the reproductive system of the human male are similar to those observed in experimental animals: hypogonadism, hypoandrogenization, testicular atrophy, and sterility [27,32]. Indications exist that testes have to be considered a direct target of ethanol intoxi-

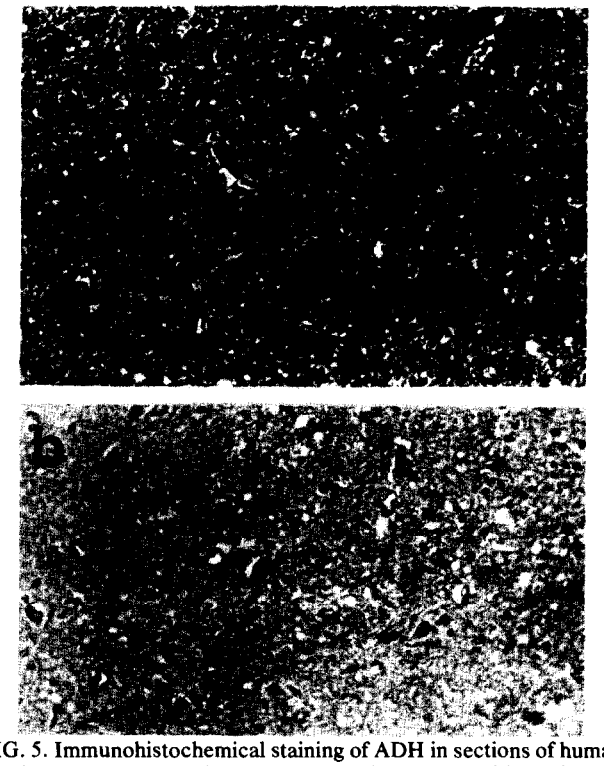

FIG. 5. Immunohistochemical staining of ADH in sections of human cerebral cortex. (a) The section was incubated with anti-ADH antiserum. The arrows point to some strongly stained neurons. (b) Control. The section was incubated with immunoadsorbed anti-ADH antiserum. The sera were diluted 1:10. (×1500).

FIG. 6. Immunohistochemical staining of ADH in human cerebellum. (a) The section was incubated with anti-ADH antiserum. The arrows point to some strongly stained Purkinje cells. (b) Control. The section was incubated with immunoadsorbed anti-ADH antiserum. Both sera were diluted 1:10. (×300).

cation and the possible involvement of ADH in the pathology of tissue changes has been suggested [7, 19, 30, 33]. Our present results support the suggestion that the toxic effects of ethanol can be attributed, at least in part, to the action of ADH which appears to be present in considerable amounts in the testicular tissue. Toxic effects on the female reproductive organs are less clear [27], and ADH does not seem to be involved in their pathogenesis [22]. Again, these observations are supported by the observation that neither ovary nor uterus exhibited noticeable staining for ADH. However, since our observations are based solely on experiments with post menopause tissue, the results cannot be generalized.

Studies with perfused rat adrenals indicate that acetaldehyde seems to be the effective toxic agent [9]. In view of the fact that ADH could be directly localized in the human adrenal, predominantly in the cortex, as our experiments suggest, the direct action of acetaldehyde may have to be considered a possibility in man as well.

The pancreas is a major target of the toxic effects of ethanol. In vitro experiments with rat tissues showed that acetaldehyde and acetate have a marked influence on both insulin and glucagon release as well as on the function of the exocrine pancreas [5,26]. Published observations suggest the presence of ADH in the pancreas and corresponding enzyme activities have been measured [8,24]. Our own results demonstrated the presence of ADH in both exocrine and endocrine compartments of the pancreas. Indeed, the staining in the endocrine cells was much stronger, indicating a higher ADH-content. Thus, our results are consistent with the suggestion that ADH may be involved in the genesis of alcoholic pancreas insufficiency.

Ethanol easily penetrates the brain and severe abnormalities of neuronal structures have been demonstrated in the central nervous system of the rat [17]. Both ethanol and acetaldehyde can interact with neurotransmitters [15] and alkaloids may be generated by condensation of acetaldehyde with biogenic amines [31]. The presence of ADH in neurons, therefore, could, independent of blood acetaldehyde levels, lead to sufficiently high acetaldehyde concentrations at critical sites of the central nervous system. We could localize ADH predominantly in the neurons with a marked heterogeneity of staining intensity which might indicate a variable ADH content of individual neurons. Mouse Purkinje cells have been shown to react to ethanol intoxication with distinct and individually different electrophysiological responses [2]; a correlation of this finding with variable ADH contents of Purkinje cells might be of interest.

CONCLUSION

With the immunohistochemical methods developed it was possible to localize ADH in human kidney, endocrine organs, and brain (this study), liver [3], gastrointestinal tract [21], fibroblasts and HeLa cells [3]. In many of these organs

ADH was predominantly present in certain cell types. These observations suggest that, even if the overall ADH-activity in a given organ is very low, specialized cells within an organ may contain particularly high amounts of ADH. Since ADH containing cells are probably most affected by alcohol toxicity, most of the organs studied so far could be direct targets of the toxic effects of ethanol even at low blood acetaldehyde concentrations.

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