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IMMUNOHISTOCHEMICAL IDENTIFICATION OF ENDOGENOUS SOURCES OF CARDIAC GLYCOSIDE BIOSYNTHESIS

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As a result of progress in the development of immunohistochemical methods of investigation it is now possible to identify sources of biosynthesis and storage of chemical substances with antigenic properties, including hormones, proteins, enzymes, and so on, in man and animals. The data thus obtained have considerably widened our ideas on pathways of synthesis and metabolism of many biologically important products, and compelled a re-examination of some existing views, and some new and hitherto unknown compounds have been discovered [1, 5, 6].

This paper describes an attempt to discover endogenous sources of synthesis of substances with physiological and pharmacological properties characteristic of cardiac glycosides. The theoretical grounds for such a search were provided by data on synthesis of endorphins – substances pharmacologically similar to opiates of plant origin, in animals and man [3, 4].

EXPERIMENTAL METHOD

Material for investigation was taken from various parts of the brain (hypothalamus, cerebellum, brain stem, medulla), the atrial wall, the superior vena cava where it enters into the right atrium (the zone of the sinoatrial node), the lungs, liver, pancreas, stomach, different parts of the intestine, and the adrenals and kidneys of dogs. Pieces of the above-mentioned organs were fixed in 10% neutral formalin and Bouin's fluid and embedded in paraffin wax. Dewaxed sections were stained with hematoxylin and eosin and by the argyrophilic method of Grimelius. Sections cut from material fixed in Bouin's fluid were treated immunohistochemically with the use of antiserum against digoxin from the DIGOCTK-125 kit (from CIS, France). To confirm the specificity of the immunohistochemical reaction, the usual controls were set up, including exhaustion of the antiserum with a pharmacopoeial solution of digoxin. Luminescent donkey serum against rabbit globulins, labeled with fluorescein isothiocyanate, was used as the label. The preparations were examined in the LYUMAM I-3 luminescent microscope (LOMO, USSR) with FS 1-2 and SZS 7-2 filters.

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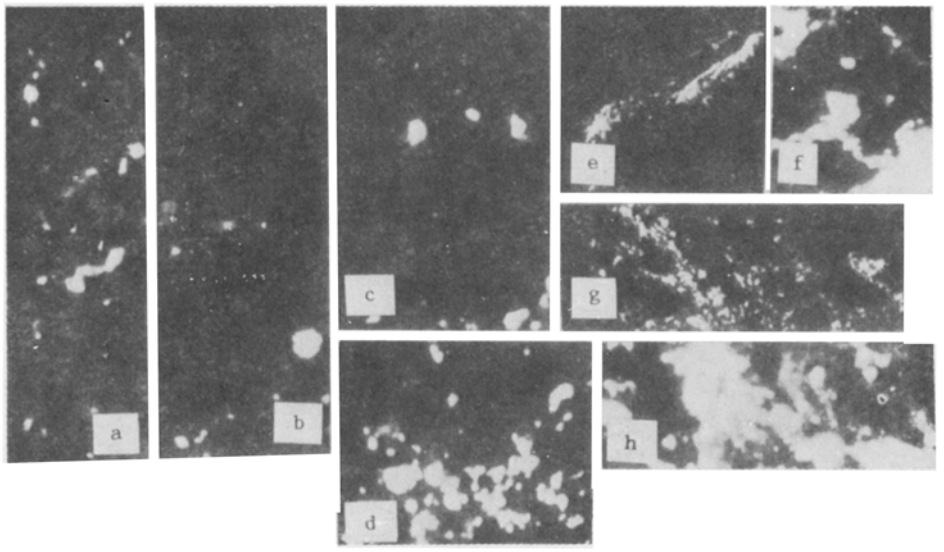

Fig. 1. DCC in various organs of dogs: a) hypothalamus, b) brain stem, c) liver, d) pancreas, e, f, g) zone of sinoatrial node, h) atrial wall. a-h: Treated with antiserum against digoxin. Magnification: a-d, f, h) 280 ×, e, g) 120 ×.

EXPERIMENTAL RESULTS

The investigation demonstrated the presence of cells immunoreactive to digoxin in the hypothalamus, brain stem, liver and pancreas (Fig. 1a-d). Concentrations of small cells and thin nerve fibers reacting positively to treatment with antidigoxin serum also were found in the zone of the sinoatrial node (Fig. 1e-g). In the atrial wall digoxin-containing cells (DCC) also were identified (Fig. 1h). No DCC were found in the lungs, gastrointestinal tract, adrenals, and kidneys.

In some serial sections of the same organs stained by Grimelius' method argyrophilic cells were detected, but they did not correspond either in location or number to DCC.

Data on the presence of endogenous cellular sources of synthesis of substances resembling cardiac glycosides in the living organism were thus obtained for the first time; by analogy with endorphins, endogenous analogs of morphine of plant origin, the substances resembling cardiac glycosides synthesized in the body can be called "endocarsides" (endogenous cardiac glycosides). The negative Grimelius' argyrophilic reaction is evidence that these endocarsides are not contained in endocrine cells of the APUD system, most of which react positively with silver nitrate [2]. The DCC are evidently an independent cell population whose function, biosynthesis of endocarsides, is determined by the current conditions of homeostasis.

Isolation of the endocarsides and the elucidation of their chemical nature will be topics for special research. The discovery of endogenous sources of possible synthesis of cardiac glycosides opens up a new and promising direction in research in general pathology and pharmacotherapy.

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