SELECTIVE LIGHT MICROSCOPIC DEMONSTRATION OF THE SPECIFIC GRANULATION OF THE RAT ATRIAL MYOCARDIUM BY LEAD-HEMATOXYLIN-TARTRAZINE

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ABSTRACT. MacConaill's lead-hematoxylin as modified by Solcia et al. was found to be a highly selective stain for the specific granulation of atrial cardiocytes in the rat. The specific atrial granules were stained blue-black. Contrast was enhanced by counterstaining in a saturated solution of tartrazine in Cellosolve. The stain is compatible with several fixatives and may be used with paraffin or Epon-embedded material.

Solcia et al. (1969) have recently modified MacConaill's lead hematoxylin and successfully applied the stain to selectively demonstrate secretory granules in several endocrine cells. In the present work it was found that the stain also demonstrated selectively the specific granules of the rat atrium. These organelles form part of a structural complex in mammalian atrial cardiocytes which is morphologically similar to that found in a variety of secretory cell types (Jamieson and Palade 1964).

MATERIALS AND METHODS

Tissue samples from the right atrium and left ventricle were obtained from Sprague-Dawley rats.

The following fixatives were used: 1) glutaraldehyde 3% and 6%; 2) formaldehyde (from paraformaldehyde) 4% and 8%. All these aldehydes were buffered to pH 7.3 with 0.1 M Sorensen's phosphate buffer; 3) formalin, 10%, and 4) Zenker-formol. Fixation time was varied from 1.5 to 24 hours for the aldehydes. Fixation in Zenker-formol was 24 hours.

Tissue was embedded in paraffin or Epon. Paraffin-embedded tissues were routinely sectioned at 3–4μ. Semithin (0.5μ) sections of Epon-embedded material were obtained in a MT-2 Porter-Blum microtome using glass knives. Epon was removed by placing sections mounted on glass slides in a 1:1 mixture of ethanol-toluene containing 1% NaOH for 2 hours, followed by two ten-minute changes in 1:1 ethanol-toluol, one five-minute change of absolute ethanol and running tap water for 3 minutes (Erlandsen et al. 1968).

After carrying the sections to water they were stained with lead-hematoxylin (hematoxylin puriss. crist., Chroma-Gesellschaft, Schmidt & Co.) for 1 hour at 45°C according to Solcia et al. (1969). The sections were counterstained (1–5 min) in a saturated solution of tartrazine (Acid Yellow 25, C.I. 19140, Matheson Coleman and Bell) in Cellosolve, dehydrated in 100% ethanol, cleared and mounted.
RESULTS

The specific atrial granules stained blue-black whereas others sarcoplasmic structures appeared yellow. All fixation procedures tested as well as paraffin or Epon-embedding were found compatible with the stain. The results obtained are illustrated in Figure 1.

The atrial granules were usually found in the paranuclear zones of atrial cardiocytes although in some instances the cells showed two or more sarcoplasmic “pockets” filled with granules. A number of granules were also observed in the subsarcolemmal space.

Lead-hematoxylin staining of ventricular cardiocytes showed that the sarcoplasm of these cells do not contain structures with affinity for the stain comparable to that of the granules in atrial cardiocytes (Fig. 2). After tartrazine counterstaining the sarcoplasm of ventricular cardiocytes is yellow.

Nuclei and especially nucleoli showed affinity for lead-hematoxylin in both atrial and ventricular cardiocytes.

DISCUSSION

The uniform size of the granules demonstrated by lead-hematoxylin-tartrazine in the rat atrium as well as the absence of lead-hematoxylin-positive structures in the sarcoplasm of ventricular cardiocytes attest to the selectivity of the stain for the specific atrial granules. This selectivity appears to result from a high affinity for some storage granules on the one hand and poor affinity for mitochondria and lysosomes on the other (Solcia et al. 1969).

Myofibrils show some affinity for lead-hematoxylin but the atrial granules stain long before this becomes a problem. Therefore, loss of selectivity due to interference by myofibrils is in fact a result of overstaining. Moreover, tartrazine quenches to a large extent the bluish background normally obtained

![Fig. 1. (Left) Rat atrial myocardium stained with lead-hematoxylin-tartrazine. Oblique section of centrally located cardiocyte shows heavily granulated sarcoplasmic core. Zenker-formol fixation. × 950.](image)

![Fig. 2. (Right) Rat ventricular myocardium stained with lead-hematoxylin-tartrazine. The sarcoplasm of ventricular cardiocytes shows no structure with affinity for lead-hematoxylin comparable to that shown by the specific atrial granules. Zenker-formol fixation. × 950.](image)
with lead-hematoxylin alone, but does not interfere with the granule stain; the result is greatly enhanced contrast.

Visualization of atrial granules after lead-hematoxylin-tartrazine in laboratory species other than the rat is not satisfactory with the technique described (unpublished observations). Hot hydrochloric acid pretreatment does not improve the result in these cases. Undoubtedly, the smaller average size and number of atrial granules in most species as compared to the rat help make difficult their visualization with the light microscope. Another factor appears to be a low affinity of these granules for the stain. This would suggest that there are physicochemical differences between the granules of different species.

That some protein-containing endocrine granules show high affinity for lead-hematoxylin appears to be due to a high density of side-chain carboxyl groups (Solcia et al. 1969) and to an inherent or induced random coil conformation of polypeptide precursors (Pearse 1969). Catecholamine (Solcia et al. 1969) does not seem of primary importance in the lead-hematoxylin staining of rat atrial granules. We have recently found (de Bold and Bencosme 1973) that the catecholamine content of isolated granule fractions is too small to ascribe a catecholamine-storing function to these organelles. The findings reported in the present work are compatible with the view that the atrial granules are a site of protein storage.

The selectivity and simplicity of lead-hematoxylin-tartrazine should make it possible to perform hitherto impractical histological studies on the atrial granules i.e., to determine their exact distribution within the rat atria and to critically evaluate changes in the granule population in different situations.

REFERENCES


