Short Communications

Localization of brain type creatine kinase in kidney epithelial cell subpopulations in rat

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Summary. Immunoperoxidase studies of rat kidney using antibody to brain type isoenzyme of creatine kinase (BB) revealed a specific staining in the epithelial cells of the thick ascending limb of the Henle's loop and collecting tubule. Occasional epithelial cells in cortical tubules that lack brush border were also positive for BB. Renal glomeruli and proximal tubules showed no immunoreactivity to this enzyme.
Key words. Creatine kinase; brain type isoenzyme; immunohistochemistry; renal tissue; thick ascending limb of the Henle's loop.

Creatine kinase (CK; ATP: creatine *N*-phosphotransferase, EC 2.7.3.2) is a key ATP-regulating enzyme and plays an important role in energy metabolism of vertebrates [1]. The enzyme has a dimeric structure composed of two immunologically distinct subunits, B-CK and M-CK [2]. Two isoenzymes, largely specific for skeletal muscle (MM) and brain (BB), are the most abundant forms of the enzyme [2]. A third form with mixed heterodimer (MB) is found in heart and other smooth muscle tissues. A fourth isoenzyme (MiMi) appears to be confined to mitochondria of cells that express either B-CK or M-CK [3]. Although the BB form is found in especially high concentrations in brain, the isoenzyme has been known to have a wide tissue distribution in man [4]. Recent studies of Kato et al., employing a highly sensitive enzyme immunoassay, confirmed this finding in rat [5]. As in human tissue, the BB form was abundant in brain and various tissues containing smooth muscle in rat. However, it was noted that the isoenzyme was also present, though in lower concentrations, in other tissues such as kidney, spleen and adipose tissue, where the smooth muscle component is minimal. This observation prompted me to examine the cellular localization of this isoenzyme by immunohistochemistry in tissues other than muscle and brain. In this communication I report the results of immunoperoxidase studies of rat kidney using affinity purified antibody to BB and MM, and show that isoenzyme containing B-CK subunit, presumably BB, is mainly localized in the epithelial cells of the thick ascending limb of the Henle's loop and collecting tubule, and in the certain epithelial cells of renal tubules in cortex.

Material and methods. Adult Wistar rats were used. The animals were anesthetized with ether and bled by heart puncture. Kidneys were removed and cut in pieces. They were placed in a solution consisting of ethanol and acetic acid (3:1) at 4 °C for 72 h as described for fixing brain tissue [6]. They were dehydrated and embedded in paraffin. Sections were cut at 8 µm. For immunohistochemistry, sections were deparaffinized and pretreated with 0.1% nonionic detergent NP40 in phosphate-buffered saline (PBS) and 20% fetal calf serum in PBS for 30 min, respectively. Sections were then incubated with affinity purified rabbit immunoglobulin raised against BB or MM isoenzyme of rat CK at a concentration of 2–5 µg/ml overnight and peroxidase-conjugated immunoglobulins of goat anti-rabbit IgG (Dako, Copenhagen, Denmark) diluted at 1:200 for 2 h. Between each incubation, sections were washed in PBS. After final washing, the reaction product was visualized with 0.04% diaminobenzidine hydrochloride and 0.01% hydrogen peroxide. Sections were briefly osmificated and counterstained with methyl green. The antibodies to the isoenzymes of creatine kinase were kindly provided by Dr K. Kato and were well characterized [5, 7].

Results and discussion. Immunoperoxidase staining of renal sections using antibody to BB brought about a macroscopic brown coloration which was confined to the zone of renal medulla, sparing cortex and papilla. Microscopically, the positive reaction products were seen in the epithelial cells of the thick ascending limb of the Henle's loop and collecting tubule (fig. 1). Staining pattern was cytoplasmic and granular. Thin segments of the loop and blood vessels in medulla were virtually devoid of staining. In cortex, cells positive for CK were scattered singly or in groups. They were epithelial cells of the tubules that lack brush border, corresponding to either distal convoluted segment or arched collecting tubule (fig. 2). In these cells, strong staining was occasionally observed at the basal margin of the cells. Renal glomeruli and proximal tubules, which occupy most of the renal cortex, lacked reaction products. Smooth muscle cells of arterial wall and rare slim, fibroblastic cells between cortical tubules also showed staining. Antibody to MM or normal rabbit immunoglobulin used as control gave no appreciable staining in any of the renal sections examined.

Thus, it is clear that renal tissue of rat exhibits immunoreactivity to brain type subunit of creatine kinase, which is main-

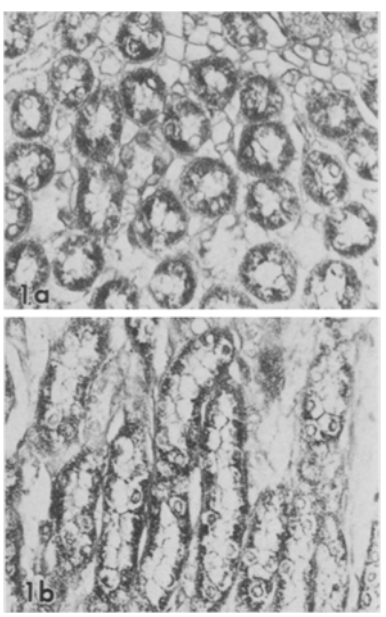

Figure 1. Renal medulla. Immunoperoxidase staining for BB. ×256. *a* and *b* Cross-cut and longitudinal section of medullary tubules, respectively. Most of the epithelial cells of the thick ascending limb of the Henle's loop and collecting tubule are positively stained.

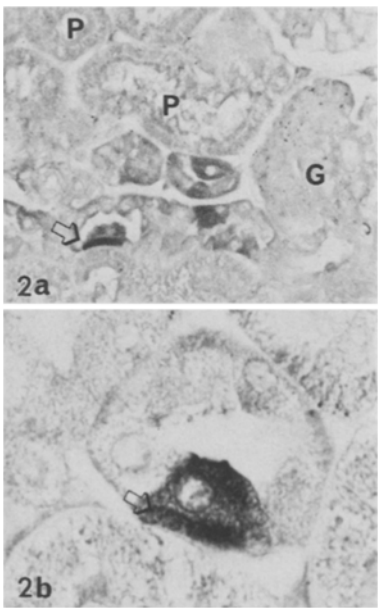

Figure 2. Renal cortex. Immunoperoxidase staining for BB. *a* ×200; *b* ×1000. Strong staining can be seen at the cellular basal part (arrow). G, glomerulus; P, proximal tubule.

ly localized in the epithelial cells of the thick ascending limb of the Henle's loop and collecting tubule, and certain epithelial cells in cortical tubules. Although the present immunohistochemistry does not discriminate BB and MB isoenzymes, both containing B-CK, it is likely that immunoreactivity to CK observed here largely corresponds to that of BB, since this isoenzyme is predominant (97%) in renal tissue homogenate [5]. Preliminary studies on the limited sections of human and mouse kidney revealed a similar distribution of immunoreactivity to B-CK. It is, therefore, highly likely that brain type of creatine kinase shows a defined cellular localization in mammalian renal tissue. In muscle, sperm cells, electric organ of *Torpedo marmorata*, or retina, CK has been postulated to be functionally coupled to the ATP-producing system in mitochondria and also to the ATP-regenerating system of intracellular components that require immediate supply of ATP [1, 8 – 11]. In heart cells, for example, a close functional coupling of CK with Na^+/K^+-ATPase has been demonstrated on their plasma membrane [12]. The present results, thus, raise the possibility that CK may also take a part in certain renal tubular function(s), by supplying ATP to the membrane-bound ATPase(s) present in these cell cubpopulations.

Note added in proof: After submission of the manuscript, I encountered an immunohistochemical study of human tissues for CK-B (Wold, L. E., Li, C.-Y., and Homburger, H. A., Am. J. clin. Path. *75* (1981) 327), in which the presence of the BB form in renal epithelial cells has been briefly mentioned.

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A comparison of the activity of flight interneurones in locusts, crickets, dragonflies and mayflies

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Summary. The activity patterns of interneurones in the flight systems of dragonflies and mayflies were investigated using standard intracellular recording and staining techniques, and were compared with those of crickets and locusts. The results show several basic similarities in the operation of a central motor pattern generator for flight in all four groups of insects. These similarities can be explained as resulting from conservative evolution of flight pattern generating circuitry within the central nervous system.
Key words. Insect; flight; evolution.

Many features of nervous systems defy adequate explanation in terms of optimal design for some presumed function. One reason for this is that some features exist due to nonadaptive determinants of the current form and function of nervous systems [1]. A means of attacking this problem is to study homologous systems to distinguish adaptive features from those related to a common history. Several studies have shown that the identification of putative homologues in nervous systems is feasible [2 – 4] although dissimilar characteristics, in the absence of developmental evidence, could obscure the homologous relationship of neurones [5, 6].

There is now considerable evidence to support the conclusion that the insect wing is monophyletic and that it originated as a primitively articulated wing appendage [7]. Although the evolutionary precursor to the behaviour of flight is still unclear [8 – 11], similarities in the structure and organization