Releasable GABA in tubular epithelium of rat kidney

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Summary. The distribution of gamma-aminobutyric acid (GABA) in the rat kidney was examined by immunocytochemical techniques. GABA-like immunoreactivity (GABA-LI) was predominantly confined to the renal tubules, including the ascending parts of the distal tubules, and the loops of Henle, the collecting tubules and ducts, and the connective parts of the convoluted tubules. In GABA-positive cortical tubules, about half of the epithelial cells were labelled. The labelled cell type showed the ultrastructural features of principal cells. Depolarizing stimulation by ouabain and high K^+ concentration evoked the efflux of endogenous GABA from kidney slices. The present findings, along with previous results, suggest that GABA released from renal tubular epithelium, and transported with the urine, might be involved in the modulation of contractility in the urinary tract.
Key words. Kidney; gamma-aminobutyric acid (GABA); immunocytochemistry; release.

Biochemical results indicate that gamma-aminobutyric acid (GABA), a known inhibitory neurotransmitter in the vertebrate central nervous system, is also present in remarkable concentrations in various peripheral tissues [1–5]. It has long been recognized that the mammalian kidney contains GABA and related enzymes [6–12]. However, attempts to identify the metabolic relevance of the renal GABA system have not been successful [11,12]. Here we provide immunocytochemical and biochemical evidence that GABA is localized in epithelial cells of some renal tubules, from which it can be released by depolarizing stimuli.

Methods

Animals. Adult male CD rats (300–350 g, Charles River Wiga GmbH) were used.

Immunocytochemistry. Renal GABA was visualized by two different polyclonal antisera (No. 483a/2 and No. 7; gifts from Drs P. Petrusz and P. Somogyi, respectively). These antisera have been found to be highly specific for GABA conjugated to proteins via glutaraldehyde [13–15]. Rats were anesthetized with ether and transcardially perfused first with 150–200 ml phosphate buffered saline (0.9% NaCl in a 10 mM phosphate buffer, pH 7.4), then with 300–350 ml of a fixative containing 5% glutaraldehyde in 0.12 M phosphate buffer. Kidneys were cut on a freezing microtome to give 75-µm sections, and these were processed for the immunocytochemical localization of GABA using the PAP technique of Sternberger [16]. Incubations were carried out at 37 °C in the following sequence: (1) in normal goat serum for 1 h; (2) in anti-GABA serum No. 483a/2 diluted 1/1000-1/9000, or No. 7 diluted 1/2000-1/18 000 in a mixture of 90% normal goat serum and 10% normal rat serum (GRS) for 16 h; (3) in goat anti-rabbit IgG (Jackson) diluted in GRS for 1–1.5 h; (4) in rabbit PAP (Jackson) diluted 1/400 in RGS for 1-1.5 h. Sites of immunoreactions were visualized by 3,3'-diaminobenzidine (0.05%, Sigma) in the presence of $NiCl_2 \cdot 6\ H_2O$ (0.1%) and H_2O_2 (0.005%) for 10–12 min. For electron microscopy, samples were washed in 0.13 M phosphate buffer, postfixed in phosphate buffered 1% OsO_4 for 1 h, dehydrated and embedded in Araldite (Durcupan ACH, Fluka) on slides.

Endogenous GABA release. Rats were killed by a blow on the head, decapitated and the kidneys quickly dissected out. Tissue slices (0.5 mm diameter, 1 mm thickness) were obtained by punching from both the cortical and medullary parts of the kidney, and superfused with a Krebs-Henseleit bicarbonate buffer (pH 7.4) in a chamber of 0.5 ml volume, at a constant rate (0.3 ml/min). After 20 min equilibration a nearly constant GABA outflow was detected. Subsequently, 5-min fractions of the superfusate were collected. GABA efflux was induced during the 5th fraction collection period by 5 min superfusion with a similar medium containing 2.5 mM ouabain, or 56 mM K^+ (osmotically balanced by equimolar reduction of Na^+ concentration). GABA contents were determined by a HPLC/fluorometric technique [17] in the superfusates and in fresh tissue samples.

Results and discussion

The same pattern of intense GABA-like immunoreactivity (GABA-LI) was encountered with both antisera in the medulla and cortex of the kidney (fig. 1 A). Medullary structures, including the ascending parts of the distal atuabules, and the loop of Henle were immunostained. In the renal cortex, the immunoreaction was restricted to collecting tubules, the connective parts of convoluted distal tubules, and the ascending parts of distal tubules. In control sections no reaction product appeared (not illustrated).

Under the light microscope, GABA-LI proved to be present in about half of the epithelial cells of collecting ducts (fig. 1 B). Electron microscopic examination revealed a very characteristic distribution pattern of GABA-LI. The reaction product was exclusively seen in

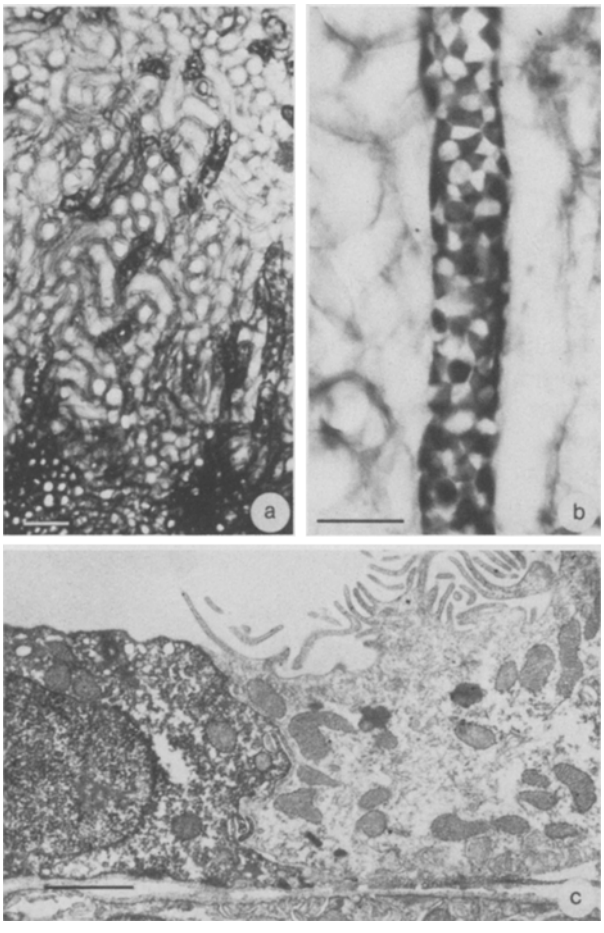

Figure 1. Light and electron microscopic visualization of GABA-LI in rat kidney. *A* The greater part of the GABA-positive immunoreaction was almost homogeneously distributed in the medulla, while in the cortex some tubules showed selective immunoreaction. Scale bar 100 µm. *B* As shown at higher magnification, in collecting tubules, GABA-LI occurred in a particular population of the tubular epithelial cells only. Scale bar 50 µm. *C* Electron microscopic analysis revealed that the labeled cells in the collecting tubules are identical with the principal cells of the epithelium. Scale bar 1 µm.

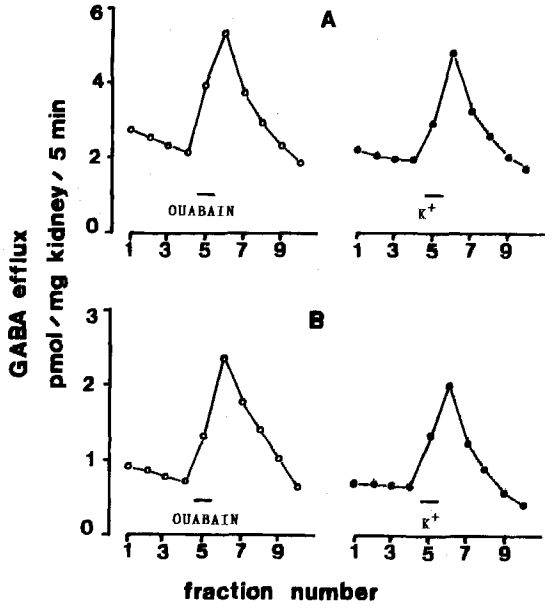

Figure 2. Ouabain- and potassium-evoked release of endogeneous GABA from slices of rat renal medulla (*A*) and cortex (*B*). Points represent mean values obtained in three repeated experiments with less than 14% SD. GABA concentrations in the renal cortex and medulla were 19 ± 2 and 92 ± 7 nmol/g, respectively (mean ± SD; n = 5).

cells which had few cell organelles and could be identified as the principal cells of the epithelium. The other major cell type of the duct, the intercalated 'dark' cells, with numerous mitochondria in their cytoplasm, did not contain GABA-LI (fig. 1 C).

It is well documented that (Na^+K)-ATPase is a specific marker of principal cells in the tubular epithelium [18,19]. The localization of GABA in this cell population suggested that GABA can be released from the kidney by the inhibition of (Na^+K)-ATPase or, alternatively, by other depolarizing stimuli. Therefore, the effects of ouabain, a specific inhibitor of the (Na^+K)-ATPase, and of a high external potassium concentration, on the efflux of endogenous GABA from superfused tissue slices obtained from the renal medulla and cortex, were separately examined.

The basal release of GABA was more intense from the medulla (fig. 2A) than from the cortex (fig. 2B). Ouabain and a high external potassium concentration evoked a remarkable increase in the efflux of GABA from slices of both renal regions (fig. 2). The stimulus-evoked release of GABA from the kidney indicates that there may be an extramural function for the renal GABA. The release of renal GABA may account for the higher concentration of GABA in the urine than in the blood [4,20]. Thus, one can speculate that the efflux of GABA from the kidney, along with the renal GABA uptake systems demonstrated earlier [21,22], may regulate the GABA concentration in the urine. In fact, the urinary GABA concentration, known to be in the µM range [20], is high enough to affect the high-affinity GABA receptors (K_d values around 50 nM) which are widely distributed in the lower urinary tract [23]. These GABA receptors are known to modulate the motility of the bladder and its sphincters [24–26]. In the light of the above findings, it seems possible that the GABA released from the tubular epithelium of the kidney may have a role in the regulation of extramural targets in the lower urinary tract. However, further lines of evidence are needed to verify this putative function of renal GABA in vivo.

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A novel exocrine gland inside the thoracic appendages ('gemmae') of the queenless ant *Diacamma australe*

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Summary. In the queenless ant *Diacamma australe*, all workers eclose with a pair of tiny thoracic appendages ('gemmae'). These are sac-like and exhibit a distinct cuticular sculpturing, with minute pores opening on the outer surface. These pores are connected to glandular cells which completely fill the appendages, and thus an exocrine signal is likely to be released. We discuss the social context of this signal: only one worker in each colony retains the gemmae.
Key words. Ant; Ponerinae; reproduction; exocrine glands; queenless.

The dorsal appendages found on the thorax of workers of *Diacamma* do not exist in any other ant genus. Although they were originally described as vestigial wings[1], they differ from the wing rudiments which W. M. Wheeler[2] has described in rare abnormal worker forms. In order to eliminate confusion, we advocate that the dorsal appendages of *Diacamma* be termed 'gemma' (pl. 'gemmae'), meaning a bud or jewel. This neutral description leaves aside the question of whether the gemma is homologous with the forewing or the tegula.

In *Diacamma* and in various other ponerine species, queens have disappeared and gamergates (= mated workers) reproduce instead[3–5]. In *D. australe* as well as *D. rugosum*, there is a single gamergate per colony, which can be recognized externally because it is the only individual which retains the gemmae[4,5]. In all the other workers of a colony, the gemmae are pulled off ('mutilated') soon after the cocoons eclose. The gemmae have an important social function, because their mutilation determines an individual's behaviour, reproductive activity and opportunity to mate[4]. The gemmae in *D. australe* are filled with glandular cells which open to the outside via minute ducts. Here we describe the external and internal morphology of gemmae, using scanning and transmission electron microscopy.

Material and methods

A colony of *D. australe* was excavated near Townsville, north Queensland, Australia, in March 1989. The ants were taken to Sydney and distributed into several experimental groups. When cocoons eclosed in the absence of a worker with gemmae, one of the callows would remain unmutilated. Callows with gemmae were kept alive for a few days before they were sacrificed. Strips of cuticle with the gemmae attached were cut away and fixed in cold 2% glutaraldehyde, buffered with 50 mM sodium cacodylate and 150 mM saccharose. Tissues were kept in buffer until arrival in Belgium. Postfixation in 2% osmium tetroxide in the same buffer preceded dehydration in a graded