ESSAYS ON APS CLASSIC PAPERS

The use of mammalian cortical kidney slices for the study of tubule secretion: a pioneering step toward understanding organic anion transport

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This essay examines the historical significance of an APS classic paper that is freely available online:


The year was 1950, and although secretion of organic substances by the renal tubules had been conclusively demonstrated more than 25 years earlier (18, 19), almost nothing was known about the transport mechanisms involved. This was a result primarily of a lack of techniques to study them. In vivo techniques did not permit detailed analysis of the secretory transport steps. In vitro approaches were not much better. For example, Chambers and Kempton (6) had observed the secretion of phenol red by cysts of embryonic chick renal tubules, but this approach was not adequate for more a detailed analysis of transport steps. Forster (12) had found that thin slices and, especially, isolated bunches of renal tubules from fish kidneys (primarily flounder), could be used to study the kinetics of secretory transport. John Taggart had teamed with Forster (13, 29) to exploit this technique and to examine the effect of decoupling oxidative phosphorylation on transport of phenol red and its analogs. However, he noted that this technique required frequent visualization of the tubules and was limited to the study of colored compounds. Moreover, it was not readily applicable to the mammalian kidney. But Taggart also noted that Forster (12) had shown that slices of renal tissue could be used for the study of renal transport and that Stern and colleagues (28) had shown that brain slices incubated in a Warburg apparatus could take up glutamate against a considerable gradient. He decided to build on these approaches to examine organic anion transport in slices from rabbit kidney cortex. With Richard Cross, he performed the classic studies reported in their 1950 paper, “Renal tubular transport: accumulation of p-aminohippurate by rabbit kidney slices” (9).

The development of this technique marked a major advance in the study of tubular secretion in the mammalian kidney. For the first time, investigators were able to examine the process of transport into the tubule cells across the basolateral membrane in a relatively simple in vitro preparation. Indeed, despite the development of the isolated, perfused renal tubule technique by Burg and his colleagues in 1966 (3) and the development of techniques for studying transport in basolateral membrane vesicles isolated from renal tubules in the early 1970s (e.g., Ref. 1), this simple slice preparation continued to play a significant role in physiological and, particularly, pharmacological studies of basolateral membrane transport for over 30 years. Moreover, the basic technique, with modifications, is still used in some studies today, more than 50 years after its development (e.g., Ref. 17).

In this classic paper (9), Cross and Taggart evaluated some of the factors influencing the transport of para-aminohippurate (PAH), the prototypical substrate for what then appeared to be a single organic anion secretory system. Although this study did not lead to any important breakthroughs in the understanding of the basolateral transport step for organic anion secretion, it did show that transport of PAH into the cells was against an electrochemical gradient and required energy (9), confirming for mammals the findings of Chambers and colleagues (5) for embryonic chick kidney tubules. Of far greater importance, Cross and Taggart’s (9) initial evaluation of the possible requirements for organic anion transport with this slice preparation set the stage for future studies by focusing attention on two areas: 1) requirements for inorganic ions and 2) requirements for organic substrates. The later studies in these areas, with many techniques (including renal cortical slices), eventually led to our current understanding of the physiological

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mechanism for the transport of organic anions across the basolateral membrane of renal proximal tubule cells (see below).

Although studies with this renal cortical slice preparation demonstrated that transport was not directly coupled to the hydrolysis of ATP (i.e., there was no evidence for an organic anion ATPase) (20, 24), investigators also recognized [as a direct follow-up of this classic paper (9)] that transport was inhibited, not just by a reduction in energy production but also by removal of Na\(^+\) and K\(^+\) from the medium bathing slices or isolated tubules and by specific inhibition of Na\(^+\)-K\(^+\)-ATPase. These observations suggested that Na\(^+\), in particular, might be important to the transport process (4, 21). This possibility was reinforced by the observation that the inwardly directed Na\(^+\) gradient, not just the presence of Na\(^+\), was the critical factor (22, 25). However, direct coupling of organic anion transport across the basolateral membrane to the Na\(^+\) gradient could not be demonstrated (1, 14, 15, 30).

In studies growing out of the attempt by Cross and Taggart (9) to examine requirements for organic substrates, various investigators demonstrated that 1) basolateral PAH/PAH exchange could occur (1, 14, 15); 2) basolateral PAH uptake could be inhibited by the anion exchange inhibitor SITS (10); 3) a number of anionic organic substrates, whose entry across the basolateral membrane was directly coupled to the Na\(^+\) gradient, could inhibit or stimulate basolateral PAH uptake (16, 30, 31); and 4) the combination of an outwardly directed PAH or OH\(^-\) gradient coupled with an inwardly directed Na\(^+\) gradient could produce a brief uptake of PAH above equilibrium in basolateral membrane vesicles (11, 14). As these data accumulated, a number of investigators postulated that the uptake of PAH (or similar substrates for the organic anion transport system) across the basolateral membrane might involve exchange for some intracellular anionic organic metabolite (1, 14, 26). In 1987, Pritchard (23) and Burchardt (27) simultaneously but independently extended this idea by proposing that PAH transport into the tubule cells across the basolateral membrane occurred against an electrochemical gradient by exchange for a dicarboxylate moving out of the cells down an electrochemical gradient and that this dicarboxylate was, in turn, taken up into the cells by a Na\(^+\) cotransport process. They demonstrated that this was the case and that α-ketoglutarate (α-KG) was the physiological dicarboxylate involved, using a preparation of basolateral membrane vesicles (23a, 27).

According to this model, transport of PAH (or any other organic anion that shares this system) into proximal renal tubule cells across the basolateral membrane is a tertiary active transport system, the terminal step in which is the countertransport of PAH against its electrochemical gradient in exchange for α-KG moving out of the cells down its electrochemical gradient. The outwardly directed gradient for α-KG is maintained, in turn, both by metabolism and by transport into the cells across the basolateral membrane via the Na\(^+\)-dicarboxylate cotransport system. The inwardly directed Na\(^+\) gradient driving α-KG uptake is maintained in its turn by the primary energy-requiring step in this tertiary process, the transport of Na\(^+\) out of the cells by Na\(^+\)-K\(^+\)-ATPase in the basolateral membrane. This process has generally been confirmed with isolated, intact renal tubules from every species studied (e.g., 2, 7, 8). As indicated, the studies that led to the development of this model grew out of the initial approach of Cross and Taggart in this classic paper (9). Moreover, in retrospect, the possibility of PAH countertransport for α-KG was actually suggested by these classic studies using kidney slices (9), for Cross and Taggart observed that a high concentration (10 mM) of α-KG completely inhibited PAH uptake by the slices whereas a low concentration (~100 μM) slightly stimulated PAH transport.

REFERENCES


