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Actions of Adrenergic and Cholinergic Drugs on Renal Tubular Cells*

LAL C. GARG†

Department of Pharmacology and Therapeutics and Division of Nephrology, Hypertension and Transplantation, University of Florida College of Medicine, Gainesville, Florida

I. Introduction

IN this review, I will analyze the sites at which and mechanisms whereby adrenergic and cholinergic drugs affect specific functions in their respective target cells along successive nephron segments. Views regarding the mechanisms of actions of drugs in the kidney have changed during the past several years because of the development of micromethods to study the individual nephron segments and general advances in our understanding of signal transduction and second messenger systems.

The focus will be a description of the actions of adrenergic and cholinergic drugs on the cells of the main nephron segments, namely, the proximal tubule, the thick ascending limb of the loop of Henle, and the collecting duct. Where appropriate, references will be made to the effects of these drugs on the other nephron segments and on cultured cells of renal tubular origin. All cells in the renal tubules are of epithelial cell type, and the epithelial cells in the kidney are present in renal tubules, papillary epithelium, and a few cells in the glomerulus. In this paper, the term "tubular" is used for tubular cells and the phrase "epithelial cells" is used to indicate tubular cells of the mammalian kidneys and nontubular epithelial cells of the amphibian bladder that have been used as a model for renal tubular cells. As is apparent from the title, this review will not include a discussion of the actions of adrenergic and cholinergic drugs on the renal circulation.

In the first part of the review, the general mechanisms of drug action will be described briefly and the general

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† Address: University of Florida, Health Science Center, Department of Pharmacology, P.O. Box 100267, Gainesville, FL 32610–0267.

physiological properties of the kidney and renal tubular epithelial cells summarized. In the following two sections, the actions of adrenergic and cholinergic drugs on various nephron segments and cultured renal tubular cells will be described. Finally, the role of medullary interstitial hyperosmolality with respect to the actions of drugs in the kidney will be discussed.

In view of the vast literature pertaining to the subject, I have restricted the reference citations to articles published during the last 10 years from which the reader may obtain lists of older references. Whenever available, review articles are cited rather than individual references. Original articles are selected either for historical reasons or because of the details of the topic being discussed.

II. General Considerations

A. General Mechanisms of Drug Action

The biological response to drugs is dependent upon the concentration of drug at the receptor site, receptor number and affinity, and postreceptor events. Based on their molecular mechanisms of actions, receptors can be grouped into four classes: (*a*) ion channel receptors such as the nicotinic type cholinergic receptor, (*b*) G protein‡ coupled receptors such as the muscarinic type cholinergic receptor, (*c*) receptors with intrinsic tyrosine protein kinase activity such as the insulin receptor, and (*d*) cytosolic receptors such as the aldosterone receptor. The discussion will be limited to G protein-coupled receptors in this review.

As a group, the G protein-coupled receptors have seven hydrophobic membrane-spanning regions (Lefkowitz et al., 1989; Dohlman et al., 1991). These are connected by extracellular and cytoplasmic loops. In general, extracellular loops and the membrane-spanning domains determine the binding of the ligands, whereas the cytoplasmic loop(s) determine the interaction with the effector system (such as G proteins).

The interaction of a drug/hormone (first messenger) with its specific receptor stimulates the production of an intracellular second messenger. Sutherland and coworkers (1965) were the first to propose that cAMP serves as a second messenger for the action of many hormones. It is now well established that agonist-receptor interaction stimulates the adenylate cyclase system through a G protein (fig. 1). A large number of G proteins have been identified (Freissmuth et al., 1989; Birnbaumer, 1991).

The G proteins that stimulate the activity of adenylate cyclase have been called G_s. In some cases, the activation of receptors results in a decrease in basal or hormone-stimulated levels of cAMP. In these cases, binding of agonists to their receptors activate the G_i proteins which in turn depress the activity of adenylate cyclase (fig. 1). Classical G proteins are heterotrimers composed of three subunits termed α, β, and γ. Agonist binding to external receptors induces a conformational change in the G protein that enables GTP to replace GDP on the α subunit. The α-GTP complex then dissociates from the β and γ subunits and activates an effector (an enzyme or a channel) to produce a physiological response. The intrinsic GTPase activity of the α subunit subsequently hydrolyzes GTP to GDP, and the α-GDP complex reassociates with the β and γ subunits to terminate the response.

The α subunit of G proteins can be ADP ribosylated

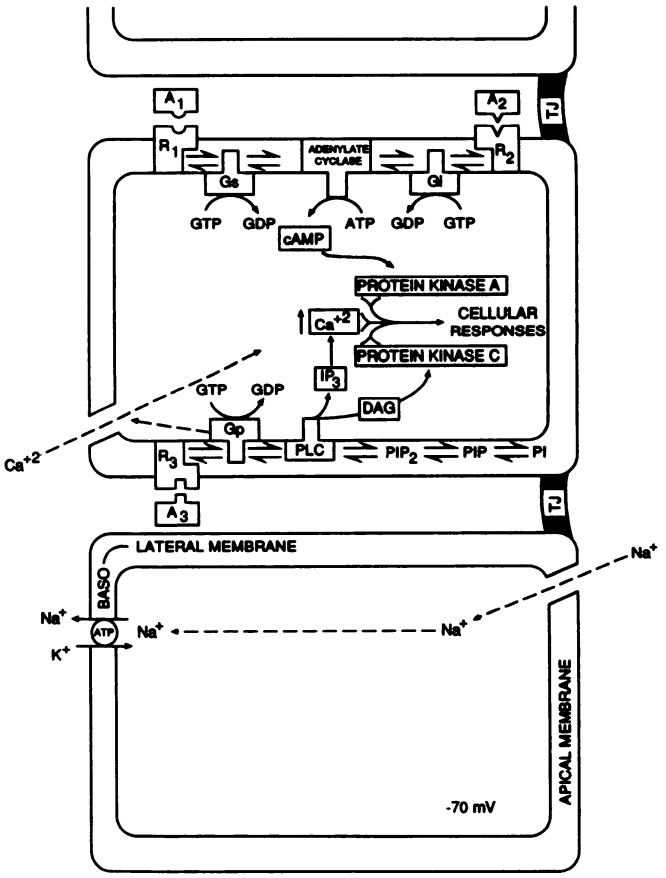

FIG. 1. Top, Representation of the mechanism of action of adrenergic and cholinergic (muscarinic) drugs in an epithelial cell (modified from Lefkowitz et al., 1990). A_1 to A_3, agonists; R_1 to R_3, receptors; G_s, G_i, and G_p, guanine nucleotide regulatory proteins; PLC, phospholipase C; TJ, tight junction between the two cells. Bottom, Simplified model of Na^+ transport from apical side to basolateral side in an epithelial cell.

‡ Abbreviations: GTP, guanosine 5′-triphosphate; G protein, GTP-binding protein; G_s, G protein that stimulates the activity of adenylate cyclase; G_i, inhibitory G proteins; AMP, adenosine 3′,5′-monophosphate; GMP, guanosine 3′,5′-monophosphate; cAMP (cGMP), cyclic AMP (cyclic GMP); GDP, guanosine 3′,5′-diphosphate; ATP, adenosine 3′,5′-triphosphate; PI, phosphatidylinositol; PIP, phosphatidylinositol-4-phosphate; IPP_2, phosphatidylinositol 4,5-bisphosphate; DAG, 1,2-diacylglycerol; IP_3, inositol 1,4,5-triphosphate; IMCD, inner medullary collecting duct; CCD, cortical collecting duct; CNT, connecting tubule; OMCD, outer medullary collecting duct; DCT, distal convoluted tubule; PCT, proximal convoluted tubule; PAC, *p*-aminoclonidine; PST, proximal straight tubule; PTH, Parathyroid hormone; MTAL, medullary thick ascending limb; CTAL, cortical thick ascending limb; ADH, antidiuretic hormone; QNB, 1-quinuclidinyl(phenyl-4)benzilate.

by bacterial toxins which block its GTPase activity. In general, cholera toxin ribosylates G_s, whereas pertussis toxin (also known as islet-activating protein) ribosylates G_i. ADP ribosylation of G_s by cholera toxin results in persistent activation of adenylate cyclase and an increase in cAMP. ADP ribosylation of G_i by pertussis toxin blocks the G_i function and thereby also increases cAMP. It has been reported that pertussis toxin treatment blocks the opening of a K channel induced by muscarinic receptors in the mammalian heart (Yatani et al., 1987). The addition of G_i protein reverses the effect of pertussis toxin. Therefore, it has been suggested that ion channels can be regulated directly by G proteins. In addition, the activity of cGMP phosphodiesterase in the retina is also regulated by G proteins (Freissmuth et al., 1989; Birnbaumer, 1991).

cAMP activates protein kinase A. The activated kinases phosphorylate several types of proteins (Walaas and Greengard, 1991) that ultimately produce a metabolic or physiological response. The phosphorylated proteins are dephosphorylated by phosphatases. Like cAMP, cGMP also has been shown to be one of the fundamental messenger systems (Waldman and Murad, 1987). Unlike adenylyl cyclase, which is located in the plasma membrane, guanylyl cyclase is present in both plasma membranes and cytosol.

It has been suggested for some time that cytosolic calcium, both by itself and with calmodulin, may act as a second messenger (Rasmussen and Barrett, 1984), and recent studies have provided evidence that an increase in cytosolic calcium concentration may occur by hormone receptor-mediated changes in membrane inositol phospholipids. Various aspects of inositol phospholipid turnover and signal transduction in biological systems have een reviewed in detail elsewhere (Abdel-Latif, 1986; Berridge, 1987; Pfleilschifter, 1989).

Cell membranes contain three major inositol phospholipids that are present in the inner leaflet of the lipid bilayer. PI is phosphorylated to form PIP, which is further phosphorylated to form PIP_2 by the action of PI and PIP kinases. The bulk of evidence indicates that PIP_2 is the immediate target of signal-dependent hydrolysis by phospholipase C (fig. 1). Recently, we examined the distribution of PIP_2 by immunochemical localization of PIP_2-specific antibodies in the kidney. Intense staining was observed primarily in the collecting duct cells, whereas staining in other cells was very low (Garg and McArdle, 1990).

The hydrolysis of PIP_2 gives rise to two products: DAG and IP_3, both of which have been shown to have many properties of classical second messengers. The IP_3 releases Ca^{2+} from intracellular stores (fig. 1). Like the adenylate cyclase-cAMP system, G proteins are also involved in the PI messenger system via their interaction with phospholipase C. However, much less is known about the characteristics of G proteins interacting with phospholipase C. As is the case with the cAMP system, DAG also activates a protein kinase called protein kinase C. The activated protein kinase C phosphorylates several proteins that subsequently produce a metabolic or physiological response. Protein kinase C is present both in the cytosol and in plasma membranes. It is also activated by tumor-promoting phorbol esters.

The generation of one of these second messengers may affect the production, release, degradation, or action of the other messengers. In some cases, receptor-mediated decrease in second messenger as well as the interaction of one second messenger with the others may be explained on the basis of coupling of receptors to more than one G protein.

B. Special Characteristics of the Kidney

When the kidney is coronally sectioned, the cortex (outer portion) and medulla (inner portion) can be distinguished with the naked eye. The medulla can be further divided into outer and inner medulla. There is a steep osmotic gradient between the cortex, where the osmolality is 300 mOsm/kg, and the inner medulla, where it is >1200 mOsm/kg. Because of the differences in the interstitial osmolality in the different zones, the division of the kidney into these zones is of considerable physiological and pharmacological importance (see section V).

The functional unit of the kidney is the nephron. It has been estimated that each human kidney contains more than one million nephrons. It is now well established that each nephron is made up of successive segments of diverse structure and function. The common names and partly highlighted location of some of the major segments of the rabbit nephron are shown in fig. 2. In addition to the intranephron heterogeneity, there is an internephron heterogeneity. Nephrons have been classified into at least two groups according to the cortical location of their glomeruli and/or the lengths of their loops of Henle. Superficial nephrons have their glomeruli located in the outer part of the cortex and usually have short loops of Henle that complete their turn at the junction of outer and inner medullae. Juxtamedullary nephrons have their glomeruli located immediately above the corticomedullary junction and have long loops that complete their turn deep in the inner medulla. The structural characteristics, permeabilities, transepithelial voltages, and solute and fluid transport characteristics of the different segments of various types of nephrons have been reviewed by Tisher and Madsen (1991) and Sands et al. (1992).

There are more than 30 cell types in the whole kidney, at least 20 of which are epithelial cells belonging to different segments of the nephron. If drug receptors are localized to one or two types of cells that constitute a small but critical portion of each nephron, the receptor or receptor-activated actions may not be detected in the whole kidney, in spite of well-known renal actions of the

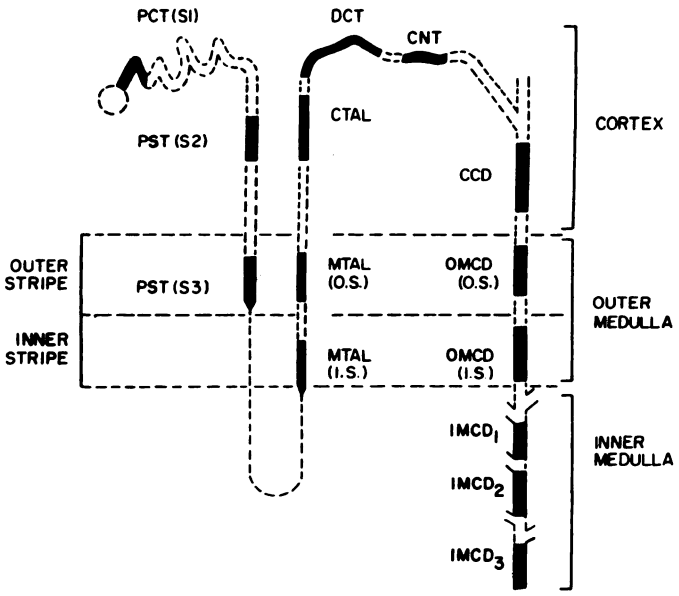

FIG. 2. Diagram of the nephron to show location of microdissected segments (reproduced from Garg, 1991). PCT (S1), initial portion of PCT; PST (S2), PST from cortex; PST (S3), PST from medulla; OMCD (O.S.), OMCD from outer stripe; OMCD (I.S.), OMCD from inner stripe; $IMCD_1$, initial one-third of IMCD; $IMCD_2$, middle one-third of IMCD; $IMCD_3$, terminal one-third of the IMCD.

drug. For example, although cholinergic agonists were known to produce a diuretic action, cholinergic receptors could not be detected in whole kidney homogenates but were detected in the isolated IMCD cells (see section IV.B).

Similarly, in spite of the well-known effect of physiological concentrations of aldosterone on Na^+ reabsorption in the kidney, no change in Na-K-ATPase activity could be detected in renal cortical preparations. However, examination of Na-K-ATPase activity in individual nephron segments revealed that physiological concentrations of aldosterone produced a 200% increase in Na-K-ATPase activity in the CCD (Garg et al., 1981). Recently, it was possible to detect H-K-ATPase activity in the microdissected CNT, CCD, and OMCD (Garg and Narang, 1988), which was not possible in whole kidney preparations.

Finally, recent studies have demonstrated that chronic treatment of rats with hydrochlorothiazide decreases Na-K-ATPase activity in the DCT and increases the enzyme activity in the CCD (Garg and Kapturczak, 1987), suggesting that renal compensation to the natriuretic effect of thiazides occurs by an increase in Na^+ reabsorption in the CCD, a segment distal to the site of action of these drugs.

One of the most important properties of renal tubular cells is their capacity to transport electrolytes and other solutes from the lumen to the plasma. The transport of solutes and water through renal tubular cells is limited by three barriers in series: the apical membrane, the cytosol, and the basolateral membrane. Although cytosolic contents and cell organelles may play a significant role in the regulation of transcellular transport, their role in this transport has not been studied. At present, it seems that the rate-limiting steps for transtubular transport lie mostly in the membranes.

Our current concept regarding transcellular transport in the nephron is based on the model of Koefoed-Johnsen and Ussing (1958). According to this model, the asymmetric arrangement of membrane proteins and of the lipid composition in the two membranes of epithelial cells provide a unidirectional transport of solutes. For example, sodium is transported from the apical to the basolateral side of the renal tubule as a consequence of active transport of this ion across the basolateral membrane by the sodium pump and facilitated transport of Na^+ across the apical membrane (fig. 1). From the pharmacological point of view, the relatively higher content and turnover rates of phospholipids (Molitoris and Simon, 1985) makes the basolateral membrane, where most receptors are located, better suited for the interaction of drugs and hormones than the apical membrane. Of course, this does not exclude the presence of some drug receptors in the apical membrane.

The transcellular transport of most of the solutes across renal tubular cells is directly or indirectly coupled to the intracellular negative voltage and low Na^+ concentration (about 15 mM as compared to 140 mM in the plasma) that is maintained by the basolateral Na-K-ATPase (fig. 1). In fact, most of the energy in the renal tubules is used by Na-K-ATPase (Gullans and Hebert, 1991). The mammalian kidney is rich in Na-K-ATPase, so much so that some renal tubular cells (e.g., MTAL cells) have as many as 40 to 50 million pump sites per cell as compared to only 500 sites per red blood cell (Jorgensen, 1986).

Depending upon the functional requirements, the enzyme activity varies a great deal in various parts of the nephron. We have demonstrated that there is a good relationship between Na-K-ATPase activity and net Na^+ reabsorption in various segments of the rabbit nephron (fig. 3). Hormonal treatment of the animal selectively increases Na^+ transport in certain nephron segments, which is associated with a similar increase in Na-K-ATPase activity in these segments (Garg et al., 1981).

Although, most of the transcellular transport processes in the renal tubule are dependent on low intracellular Na^+ (and Na-K-ATPase), the reabsorption of filtered bicarbonate and urine acidification are dependent, at least in part, on two types of proton-translocating ATPases, an electrogenic H-ATPase and an electroneutral H-K-ATPase, which are present in the apical membranes (Garg, 1991).

There are both quantitative and qualitative differences between the same nephron segment of various animal species. For example, Na-K-ATPase and H-ATPase activity in the rat PCT is approximately twice as high as in the rabbit (Garg et al., 1982; Garg, 1991). This is

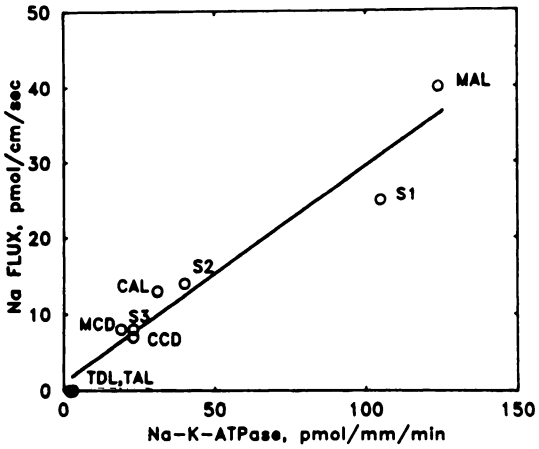

FIG. 3. Relation between net sodium flux and Na-K-ATPase activity in microdissected rabbit nephron segments. CAL, cortical ascending limb; MCD, medullary collecting duct; TDL, thin descending limb; MAL, medullary ascending limb; TAL, thin ascending limb. Reproduced from Garg et al., 1981, and used with permission.

probably related to the greater single nephron glomerular filtration rate, Na^+ reabsorption, and H^+ secretion in the rat than in the rabbit. Species differences in the response of specific nephron segments to adrenergic drugs will be discussed in later sections.

III. Pharmacology of Adrenergic Drugs

Ten years ago, the actions of catecholamines on sodium transport in the kidney were reviewed in this journal (Kim et al., 1980). The authors concluded: "It seems likely, but not totally proven, that the effects of α- and β-adrenergic stimulation directly influence renal tubular sodium transport. It would appear that the development of microdissection techniques for the isolation of specific renal tubular segments will allow for direct examination of the biochemical and the physiological responses of the kidney to the adrenergic system." Although all the answers regarding the biochemical pathways and the involvement of specific nephron segments in adrenergic mediated renal effects are not yet available, much progress has been made in this field in the last decade. The results of these studies are discussed in this section.

A. Actions on the Whole Kidney

The kidney is innervated by sympathetic neurons. Adrenergic nerve terminals have been identified on almost all major blood vessels, juxtaglomerular apparatus, and glomerular afferent and efferent arterioles. The morphological evidence indicates that almost all segments of the mammalian nephron are under the influence of some degree of adrenergic innervation (Barajas et al., 1984). The role of renal sympathetic nerves in the regulation of tubular sodium reabsorption and renin secretion was reviewed recently (DiBona, 1989; Kopp and DiBona, 1992). Adrenergic stimulation affects glomerular filtration rate, renin release, and tubular sodium reabsorption. An increase in low-frequency renal nerve sympathetic activity increases Na^+ reabsorption and decreases Na^+ excretion in the absence of hemodynamic changes in the kidney. The effect is mediated by α_1-adrenoceptors, located at neuroeffector junctions along the nephron. The nerve-stimulated antinatriuresis is also accompanied by a decrease in urinary bicarbonate excretion (Osborn and Harland, 1988). Like the antinatriuretic effect, the antibicarbonaturic effect is mediated by α_1-adrenoceptors because it is blocked by prazosin, an α_1-adrenoceptor antagonist. Renal nerve stimulation also causes a decrease in urinary excretion of calcium which is mediated by activation of α_1-adrenoceptors, as it is blocked by prazosin but not by calcium channel blockers (Johns and Manitius, 1987).

During normal sodium intake, adrenergic nerves play a minor role in sodium conservation (DiBona, 1989). However, animal data suggest that intact renal innervation is essential for sodium conservation during diuretic treatment (Petersen et al., 1991) and during sodium restriction (DiBona, 1989). Studies of patients with idiopathic autonomic insufficiency have demonstrated that restricting their sodium intake to 17 mEq/day does not decrease sodium excretion <50 to 60 mEq/day (Wilcox et al., 1977). These results suggest that normal renal innervation is necessary to conserve sodium during sodium restriction.

Whereas α_1-adrenoceptors are probably located on the neuroeffector junctions and are the target for neuronally released norepinephrine, α_2-adrenoreceptors are located extrajunctionally and are not activated by renal nerve stimulation (Smyth et al., 1985). However, circulating epinephrine and norepinephrine can activate both α_1- and α_2-adrenoceptors in the kidney. In contrast to the antidiuretic effect of α_1-adrenergic agonists, selective α_2-adrenergic agonists administered in vivo cause diuresis (Gellai, 1990). This effect is probably due to: (*a*) α_2-adrenergic-mediated inhibition of vasopressin release in the central nervous system; (*b*) inhibition of the effect of vasopressin in cortical and medullary collecting ducts by α_2-adrenergic agonists via a decrease in cAMP formation (see below); and (*c*) α_2-adrenergic-mediated decrease in renin release (Keeton and Campbell, 1980; McPherson and Summers, 1983), leading to a decrease in angiotensin-mediated Na^+ reabsorption in the PCT (Schuster et al., 1984).

In addition to the action of adrenergic agents on sodium and water excretion, intravenous infusion of epinephrine or norepinephrine is associated with a decrease in urinary excretion of potassium (Johnson and Barger, 1981). An effect of epinephrine on potassium excretion has also been demonstrated in the isolated perfused rat kidney (Katz et al., 1984). This suggests that epinephrine influences potassium excretion by a direct effect on the kidney rather than through the release of some other hormones. In contrast to the effects of adrenergic agents

on sodium excretion which are mediated by α_1-adrenoceptors, the antikaliuretic effect seems to be mediated by β-adrenoceptors because isoproterenol, a β-adrenergic agonist, mimics and propranolol, a β-adrenergic antagonist, blocks this effect of epinephrine.

B. Adrenergic Receptors

In general, adrenergic receptors have been classified into four types: α_1, α_2, β_1, and β_2 (table 1). These receptors are classified on the basis of differences in the rank order of potency to a series of adrenergic agonists and reversal of their effects by selective antagonists (Lefkowitz et al., 1990). For example, α_1-adrenoceptors have high affinity for methoxamine (agonist) and prazosin (antagonist), and α_2 receptors have high affinity for clonidine (agonist) and rauwolscine (antagonist).

Activation of α_1-adrenoceptors stimulates PI hydrolysis, whereas activation of α_2-adrenoceptors produces a decrease in cAMP formation (table 1). On the other hand, activation of both β_1- and β_2-adrenoceptors stimulates cAMP formation.

Recently, both α_1- and α_2-adrenoceptors were further divided into several subtypes. The pharmacological evidence for two subtypes of α_1-adrenoceptors, α_{1A} and α_{1B}, was reviewed by Minneman (1988). α_{1A}-Adrenoceptors have high affinity for WB4101, whereas α_{1B}-adrenoceptors are inactivated by chloroethylclonidine. The pharmacological characteristics of two subtypes of α_2-adrenoceptors, α_{2A} and α_{2B}, have also been discussed in detail by Bylund et al. (1988). In brief, both subtypes of α_2-adrenoceptors have high affinity for rauwolscine, but prazosin, the selective α_1 antagonist, has high affinity for α_{2B}, whereas oxymetazoline is selective for the α_{2A}-adrenoceptor. Molecular biology data also provide evidence for heterogeneity of α_1- and α_2-adrenoceptors (Dohlman et al., 1991).

The earlier data concerning the binding of α-adrenoceptor ligands in renal preparations were summarized by Summers (1984). Recent data regarding the binding of α-adrenoceptor ligands in renal preparations are summarized in table 2. Autoradiographic as well as ligand-binding experiments indicate that both α_1- and α_2-adrenoceptors are present in renal cortex with the highest concentration of adrenoceptors in the PCT (Kusano et al., 1984; Stephenson and Summers, 1985; Calianos and Muntz, 1990). In general, the density of α_2-adrenoceptors in the PCT is more than twofold greater than that of α_1-adrenoceptors (table 2). The variation in actual values of K_d and B_{max} for α_1- and α_2-adrenoceptors may be due to the type of preparation, type of ligand used, and/or different subtypes of α_1 and α_2 receptors.

Recently, it was demonstrated that renal tubule cells isolated from the rat kidney have a 60:40 mixture of α_{1A} and α_{1B} subtypes of adrenoceptors (Han et al., 1990). These results were confirmed in the rat renal cortex and outer medulla (Feng et al., 1991). In addition, it was demonstrated that the α_{1B}-adrenoceptor subtype predominates (85%) in the inner stripe of the outer medulla. Our recent observations also indicate that α_1-adrenoceptors in IMCD cells isolated from the rabbit are the α_{1B} subtype (Clarke and Garg, 1991).

Marked differences in the affinity of prazosin for displacing [^{3}H]rauwolscine from the renal membranes of various animal species have been reported (Neylon and Summers, 1985). Prazosin has a high affinity not only for α_1-adrenoceptors but also for α_{2B}- and α_{2C}-adrenoceptors (Bylund et al., 1988; Loomasney et al., 1990). Therefore, some of the species differences in the affinity of prazosin may be due to the presence of different subtypes of α_2-adrenoceptors.

Species differences in the densities of rauwolscine-binding sites have also been reported (Neylon and Summers, 1985). The densities of rauwolscine binding sites were, in increasing order: humans < dog < rabbit < rat < mouse (table 2).

Although studies of the binding of yohimbine (an α_2 antagonist) and clonidine (an α_2 agonist) indicate that there is only one type of α_2-adrenoceptor in the kidney, the binding of PAC to renal membranes suggests the presence of two subtypes of α_2-adrenoceptors: those having high affinity (K_d 1.7 nM) and those having low affinity (K_d 14 nM) for PAC (Sripanidkulchai et al., 1987). The number of low-affinity PAC-binding sites is more than four times greater than the high-affinity ones (table 2). Recent evidence indicates that PAC and other α-adrenergic ligands with an imidazoline structure (e.g.,

TABLE 1
*Major classes of adrenergic receptors**

	α_1†	α_2†	β_1	β_2
Agonist	Methoxamine	Clonidine	Isoproterenol	Isoproterenol, terbutaline
Antagonist	Phentolamine, prazosin, HEAT	Phentolamine, rauwolscine, yohimbine	Propranolol, ICP, atenolol	Propranolol, ICP, α-methyl propranolol
Effector system	IP_3/DAG	↓ cAMP	↑ cAMP	↑ cAMP
G protein	G_p	G_i	G_s	G_s
Distribution	Renal vessels, renal tubules	Renal vessels, renal tubules, glomeruli	JGA, renal tubules	JGA, renal tubules

* Abbreviations: ICP, iodocyanopindolol; HEAT, 2-β-4-hydroxy-3-iodophenyl ethylamino methyltetralone; JGA, juxtaglomerular apparatus.
† Several subtypes of α_1- and α_2-adrenergic receptors have been identified (see text).

TABLE 2
α-Adrenergic receptors in renal preparations

Nephron segment (animal species)	Ligand	K_d (nM)	B_{max} (fmol/mg protein)	Reference
Renal cortical membranes	Rauwolscine	2.5	340	Insel et al., 1985
(rat)	Yohimbine	18	355	Insel et al., 1985
Whole kidney membranes				
Human	Rauwolscine	1	730*	Neylon and Summers, 1985
	Yohimbine	1.6	95	Dickinson et al., 1986
Dog	Rauwolscine	3	4,480*	Neylon and Summers, 1985
Rabbit	Rauwolscine	2.7	5,450*	Neylon and Summers, 1985
	Yohimbine	6.6	149	Dickinson et al., 1986
Rat	Rauwolscine	2.3	8,780*	Neylon and Summers, 1985
Mouse	Rauwolscine	2.8	12,800*	Neylon and Summers, 1985
Renal cortex				
Rat	Rauwolscine	2.7	120	Cheung et al., 1986
Rabbit	Rauwolscine	5.6	76	Cheung et al., 1986
Whole kidney membranes	PAC	1.7	47	Sripanidkulchai et al., 1987
(rat)		14	219	Sripanidkulchai et al., 1987
Whole kidney (rat)	Yohimbine	8	330	Periyasamy, 1988
PCT enriched (rat)	HEAT	0.07	364	Matsushima et al., 1986
	Yohimbine	9	1,130	Matsushima et al., 1986
PCT enriched (rat)	Prazosin	0.1	100	Sundaresan et al., 1987
	Rauwolscine	1.5	250	Sundaresan et al., 1987
PCT enriched (rat)	Prazosin	12	49	Jensen and Berndt, 1988a
	Clonidine	22	113	Jensen and Berndt, 1988a
PCT enriched (rabbit)	Rauwolscine	5	1,678	Nord et al., 1987
MTAL (rabbit)	Rauwolscine	2.6	244	Mohuczy-Dominiak and Garg, 1990
IMCD (rabbit)	Prazosin	0.9	30	Clarke and Garg, 1991
IMCD (rabbit)	Rauwolscine	4.2	170	Clarke and Garg, 1990b
OK cells	Rauwolscine	0.08	135	Murphy and Bylund, 1988
	Yohimbine	0.23	124	Murphy and Bylund, 1988

* fmol/g wet weight.

idazoxan) bind to sites that are not competed for by catecholamines. This suggests that these additional sites are not adrenoceptors. The nonadrenergic binding sites have been called "imidazoline-preferring binding sites" (Lehmann et al., 1989). The presence of imidazoline-preferring binding sites has been demonstrated in several organs including the kidney (Michel et al., 1989). However, neither the complete pharmacological and biochemical characteristics nor the endogenous ligand(s) for these binding sites are known at this time.

Both ligand-binding and autoradiographic studies indicate the presence of β-adrenoceptors in the glomerulus, thick ascending limb, DCT, and CCD (table 3). There is conflicting evidence regarding the presence of β-adrenoceptors in the PCT. Ligand-binding studies of tubules prepared from collagenase-treated rat renal cortex showed the presence of a small number of β-adrenoceptors in the PCT (Struyker-Boudier et al., 1986; Sundaresan et al., 1987) (table 3). However, autoradiographic studies failed to demonstrate β-adrenergic receptors in this segment of the nephron (Munzel et al., 1984; Summers et al., 1985). Based on adenylate cyclase data (Murayama et al., 1985; Morel and Doucet, 1986), it seems that β-adrenoceptors may be present in the PST but not in the PCT (see discussion below). However, this remains to be established.

C. Actions on Renal Tubular Cells

Relatively few studies have been performed to determine the direct effects of adrenergic agonists and antagonists on specific nephron segments. The sites of adrenergic actions in the kidney have mostly been identified from indirect experiments using autoradiographic localization of receptors, ligand binding to tubule enriched preparations, and pharmacological experiments on the whole kidney. These are described below.

1. Proximal tubule. In the proximal tubule, Na-K-ATPase in the basolateral membrane energizes several Na-dependent cotransport (Na-glucose, Na-amino acids, Na-phosphate) and countertransport (Na/H) processes in the S1 segment of the PCT (fig. 4). The rate of transport of fluid, Na^+, and other solutes decreases from the S1 to the S3 segment in both superficial and juxtamedullary nephrons. The rate of active secretion of acidic drugs increases from S1 to S2 and then decreases again in S3 in both types of nephrons. On the other hand, the rate of active secretion of basic drugs decreases from S1 to S2 to S3 in superficial nephrons and remains the same in S1 and S2 but decreases in S3 in juxtamedullary nephrons (McKinney, 1988). Proximal tubule cells metabolize drugs, synthesize ammonia, and produce glucose by gluconeogenesis.

The data from in vivo microperfusion of the rat PCT

TABLE 3
β-Adrenergic receptors in renal preparations

Nephron segment (and animal species)	Ligand (receptor)	K_d (nM)	B_{max} (fmol/mg protein)	Reference
Membranes from whole kidney (rat)	Dihydroalprenolol (β_1)	7	70	Gavendo et al., 1980
PCT enriched (rat)	Iodocyanopindolol ($\beta_1 + \beta_2$)	0.042	15	Struyker-Boudier et al., 1986
DCT enriched (rat)	Iodocyanopindolol ($\beta_1 + \beta_2$)	0.049	29	Struyker-Boudier et al., 1986
PCT enriched (rat)	Iodocyanopindolol ($\beta_1 + \beta_2$)	0.01	9	Sundaresan et al., 1987
Glomeruli (rat)	Iodocyanopindolol ($\beta_1 + \beta_2$)	0.01	88	Sundaresan et al., 1987
DCT enriched (rat)	Iodocyanopindolol ($\beta_1 + \beta_2$)	0.01	52	Sundaresan et al., 1987

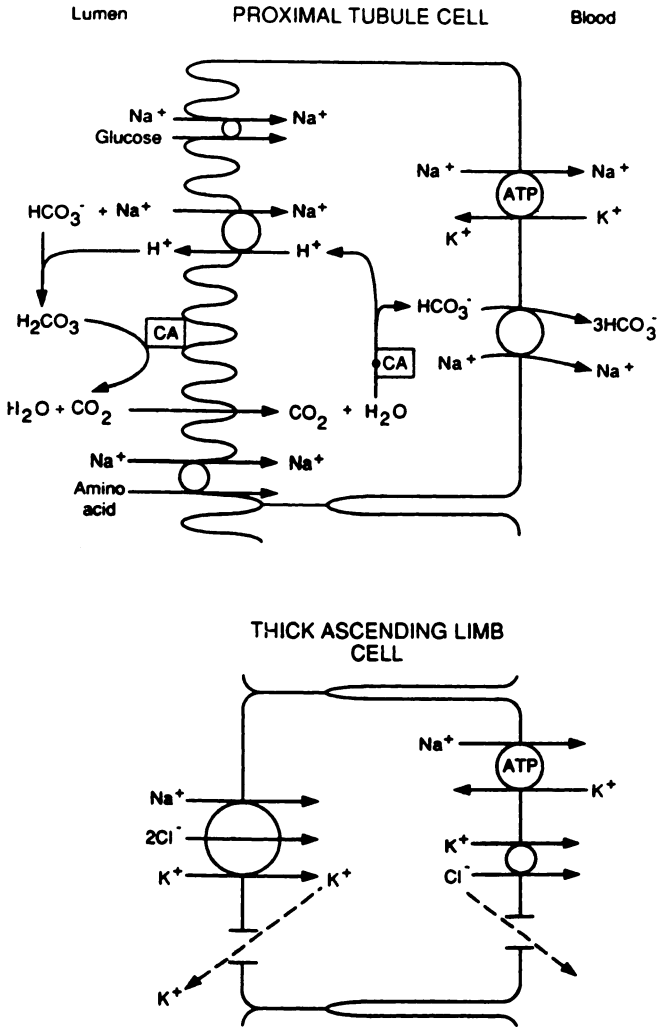

FIG. 4. Top, Sodium absorption in the PCT. CA, carbonic anhydrase. Bottom, Sodium absorption in the MTAL/CTAL. See text for explanation of various transport processes.

indicate that both α- and β-adrenergic agonists stimulate fluid transport in this segment (Chan, 1980; Weinman et al., 1982). On the other hand, it has been shown in the rabbit PCT perfused in vitro that adrenergic stimulated fluid reabsorption is mediated by β-adrenergic receptors (Bello-Reuss, 1980; Rouse et al., 1990). The mechanism by which β-adrenergic agents stimulate fluid reabsorption in the PCT is not known, and the data concerning their cellular effects are conflicting. Isoproterenol did not stimulate cAMP formation in the microdissected PCT of rat or rabbit (Morel and Doucet, 1986). On the other hand, isoproterenol produced a significant stimulation of cAMP formation in rat proximal tubules isolated by centrifugation (Jacobs and Chan, 1986; Sundaresan et al., 1987). This stimulation is probably due to the presence of PSTs which have been shown to be responsive to β-adrenergic agonists (Murayama et al., 1985).

The stimulatory effect of isoproterenol on fluid reabsorption in the rabbit PCT is blocked by the α_2 agonist, clonidine, but not by the α_1 agonist, methoxamine (Rouse et al., 1990). These results suggest that the effect of isoproterenol on fluid reabsorption in the PCT may be mediated by adenylate cyclase. However, cAMP itself has not been shown to stimulate fluid reabsorption in the PCT. Therefore, the exact role of the adenylate cyclase system in β-adrenergic mediated stimulation of fluid reabsorption in the PCT remains to be established.

PTH also stimulated cAMP formation in the PCT. The major effect of PTH is to inhibit phosphate reabsorption. An additional effect of PTH is that it inhibits bicarbonate reabsorption in the PCT (Puschett and Zurbach, 1976). It has been suggested that both PTH and cAMP produce bicarbonaturia by inhibition of carbonic anhydrase in the PCT. We have shown that neither PTH nor cAMP inhibits renal carbonic anhydrase (Garg, 1975, 1976). On the other hand, PTH and cAMP inhibit the Na/H antiporter in brush border vesicles (Kahn et al., 1985). Therefore, it is likely that PTH and cAMP produce bicarbonaturia by inhibition of Na/H exchange in the PCT.

Although epinephrine did not inhibit PTH or other hormone-stimulated adenylate cyclase activity in renal cortical preparations (Snavely and Insel, 1982), norepinephrine has been shown to inhibit PTH-stimulated adenylate cyclase activity in PCT suspensions and in microdissected PCTs (Guder and Rupprecht, 1975; Jeffries and Pettinger, 1989). α_2-Adrenergic agonists stim-

ulate Na/H exchange in the PCT (Nord et al., 1987; Gesek et al., 1989), and PTH and cAMP inhibit the Na/H antiporter in the brush border vesicles (Kahn et al., 1985). Taken together these results suggest that the stimulatory effect of α_2-adrenergic agonists on Na/H exchange is mediated by inhibition of adenylate cyclase activity in the PCT.

Recent studies have demonstrated that α_1-adrenergic agonists stimulate $^{22}Na^+$ uptake in rat PCT (Gesek et al., 1989). The effect was blocked by 5-(N-ethyl-N-isopropyl) amiloride and was, therefore, ascribed to an increase in Na/H antiporter activity in the PCT. Activation of protein kinase C by phorbol esters has been shown to increase Na:H exchange in brush border membranes of the rabbit kidney (Weinman and Shenolkar, 1986) and in the rat PCT (Gesek et al., 1989), indicating that DAG may be the physiological mediator of the stimulation of the Na/H exchanger in the PCT. It has been demonstrated that norepinephrine stimulates PI hydrolysis in rat renal cortical slices (Neylon and Summers, 1987; Jeffries and Pettinger, 1989), in rat isolated PCT (Garg and Wozniak, 1991), and in microdissected mouse PCT (Wirthensohn and Guder, 1985). Taken together, these observations suggest that the effect of α_1-adrenergic agonists on Na/H exchange is mediated by an increase in PI hydrolysis and activation of protein kinase C by DAG.

In addition, α_1-adrenergic agonists stimulate gluconeogenesis in the rat PCT, and the effect is blocked by prazosin (Nakada et al., 1986). In canine PCT, gluconeogenesis has been shown to be dependent on an increase in cytosolic calcium, suggesting that the effect is mediated by PI hydrolysis (Goligorsky et al., 1987). On the other hand, the concentration-response curves of agonist-stimulated PI hydrolysis lie one order of magnitude to the right of those for gluconeogenesis (Neylon and Summers, 1987), suggesting that PI hydrolysis may be dependent on α_1-adrenoceptor-stimulated gluconeogenesis. Studies using the isolated perfused rat kidney have demonstrated that gluconeogenesis is required for stimulation of α_1-adrenoceptor-mediated increase in Na^+ reabsorption in the PCT (Baines et al., 1987). Further investigations are needed to determine the exact relationship among α_1-mediated PI hydrolysis, gluconeogenesis, and Na^+ transport in the PCT.

Both α_1- and α_2-adrenergic agonists produced a similar degree of stimulation of $^{22}Na^+$ uptake in the PCT (Gesek et al., 1989). Submaximal, but not maximal, effective concentrations of α_1- and α_2-adrenergic agonists produced an additive effect on $^{22}Na^+$ uptake in the PCT. These results suggest that both types of α-adrenergic agonists stimulate Na^+ uptake in the PCT by a final common pathway. It should be remembered that adrenoceptors are present in the basolateral membranes, whereas the Na/H antiporter is present in the brush border of the apical membranes of the PCT. The steps involved between the receptor-mediated signal transduction and the activation of the Na/H antiporter are not known at the present time.

There are contradictory data regarding the effects of adrenergic drugs on Na-K-ATPase activity in the PCT. Norepinephrine has been shown to increase Na-K-ATPase in membrane fragments of the PCT but not in a purified preparation of Na-K-ATPase from the PCT (Beach et al., 1987). Epinephrine and norepinephrine also increase Na-K-ATPase activity in basolateral membrane vesicles prepared from the rat PCT (Jensen and Berndt, 1988a). Recent data concerning isolated rat proximal tubules suggest that norepinephrine stimulates Na/H exchange by activation of DAG-sensitive, protein kinase C, whereas stimulation of Na-K-ATPase occurs by activation of DAG-insensitive protein kinase C (Baines et al., 1990). In another study, norepinephrine and other adrenergic agonists failed to affect Na-K-ATPase in rabbit kidney cortex homogenates, separated PCTs, and basolateral membranes (Podevin and Parini, 1989). In addition, adrenergic agonists had no effect on ouabain-sensitive rubidium uptake in PCTs. The reason for differences between the results of Podevin and Parini (1989) and other studies is not known. Only future investigations will clarify whether or not adrenergic drugs produce an effect on Na-K-ATPase activity.

Epinephrine and norepinephrine enhance *p*-aminohippurate transport into basolateral membrane vesicles prepared from rat proximal tubules. The effect is inhibited by phentolamine or yohimbine but not by similar concentrations of prazosin or atenolol (Jensen and Berndt, 1988b). These results suggest that the effect is mediated by α_2-adrenoceptors.

2. Loop of Henle. The thin descending or ascending limbs of the loop of Henle are not believed to be sites of active transport. α_2-Adrenergic agonists inhibit prostaglandin-stimulated cAMP formation in the thin descending limb of the rat. These results are consistent with the observation that α_2-adrenergic activation reverses arachidonic acid-induced diuresis in the rat (Pettinger et al., 1987). Because there is no evidence for active Na^+ transport in this segment, the permeability to water may be dependent on local prostaglandin production, and α_2 agonists may produce their effect by blocking prostaglandin-dependent osmotic water permeability. Adrenergic drugs do not produce any effect on the thin ascending limb.

The MTAL and CTAL cells are impermeable to water and have high capacity (MTAL > CTAL) for sodium transport. As is the case in the PCT, the primary energy-consuming process in the MTAL and CTAL is basolateral Na-K-ATPase activity that mediates the transport of Na^+ out of the cytosol. The resulting Na^+ concentration gradient between the tubular lumen and the cytosol provides the driving force for Na:K:2Cl transport across the apical membrane (Greger, 1985). Cl^- is transported

across the basolateral membrane passively via a Cl^- channel (fig. 4). K^+ is recycled back to the tubular lumen through apical K^+ channels. The stimulation of any one of these ionic pathways may produce a secondary increase in the activity of other pathways, resulting in a net increase in NaCl reabsorption in these segments.

In vivo microperfusion data have demonstrated that an increase in low-frequency renal nerve stimulation increases and acute renal denervation decreases the reabsorption of NaCl in the loop of Henle (DiBona and Sawin, 1982; Bencsath et al., 1985). This is most likely due to an effect on the MTAL and/or CTAL where Na^+ is actively reabsorbed. It is possible that renal nerve stimulation may produce its effect via α_1-adrenoceptors, as is the case in the PCT.

There is evidence that β-adrenergic agents stimulate adenylate cyclase activity in the CTAL of the rat and mouse but not of the rabbit (Morel and Doucet, 1986). Recently, it was shown that isoproterenol stimulates adenylate cyclase activity in both the CTAL and MTAL of the mouse (Baily et al., 1990). In vitro microperfusion of the mouse CTAL with isoproterenol (10^{-7} M) increased Ca^{2+} and Mg^{2+} reabsorption, whereas NaCl reabsorption was increased by isoproterenol both in the CTAL and MTAL. These results indicate that β-adrenoceptors play an important role in the thick ascending limb of the mouse.

cAMP increases basolateral Cl^- conductance in the in vitro perfused mouse MTAL (Greger, 1985). It has been demonstrated with patch-clamp techniques that cAMP also increases Cl channel activity in the mouse CTAL (Paulais and Teulon, 1990). Therefore, the primary action of β-adrenergic agonists and other hormones that increase cAMP may be activation of a basolateral Cl^- channel and subsequent increase in NaCl reabsorption in the thick ascending limb (fig. 4).

Our preliminary observations indicate that specific α_2-adrenoceptors are present in MTALs isolated from the rabbit kidney (Mohuczy-Dominiak and Garg, 1990). The pharmacological profile of these receptors is that of subtype 2A with low affinity for prazosin and high affinity for oxymetazoline (K_i ratio >1600). It has been reported that epinephrine does not inhibit hormone-stimulated cAMP formation in MTAL or CTAL of the rat, although α_2-adrenoceptor activation inhibited the diuretic effect of furosemide in the isolated perfused rat kidney (Jeffries and Pettinger, 1989). Therefore, further investigations are necessary to clarify the mechanism of signal transduction by α_{2A}-adrenoceptors in the MTAL.

3. Distal convoluted tubule. The distal tubule, extending from the macula densa to the first junction with another tubule, is a heterogenous tubule. It includes the "true" DCT (early portion), CNT (middle portion), and initial collecting duct (late portion). These subsegments can be easily demarcated in the rabbit but not in the rat. As in other segments, Na-K-ATPase in the basolateral membrane is responsible for energizing the reabsorption of Na^+ in the DCT. It has been suggested that Na^+ enters these cells via a neutral NaCl cotransporter. The CNT consists of CNT cells that secrete potassium and intercalated cells that are involved in the reabsorption and secretion of bicarbonate (see below).

Autoradiographic studies indicate the presence of β-adrenoceptors in the DCT and CNT of the rat (Healy et al., 1985; Summers et al., 1985). Furthermore, it has been shown that isoproterenol stimulates adenylate cyclase activity in the rat DCT, including the CNT, and in the rabbit CNT (Morel and Doucet, 1986). The effect of isoproterenol has been localized to the intercalated cells of the CNT in the rabbit (Koseki et al., 1988).

Denervation of the rat kidney decreases bicarbonate and water reabsorption in the DCT (Wang and Chan, 1989), suggesting that renal nerves influence bicarbonate and water transport in this segment. The mechanism of action of adrenergic nerves and adrenergic drugs in the DCT and CNT has not been established. The CNT is believed to behave like the CCD. Therefore, the following discussion of the effects of adrenergic drugs on the CCD may also be applicable to the CNT.

4. Collecting duct. The CCD consists of two main cell types, principal cells and intercalated cells. The principal cells are the predominant cell type (>60%), and they are responsible for Na^+ reabsorption and K^+ secretion. They have Na^+ and K^+ conductive pathways in the apical membrane and Na-K-ATPase in the basolateral membrane (fig. 5). The Na^+ channel in the apical membrane is sensitive to very low concentrations of amiloride.

The intercalated cells are responsible for H/HCO_3 transport. There are two subtypes of intercalated cells in the CNT and CCD (Tisher and Madsen, 1991). One cell type (A cells) has a hydrogen pump in the apical membrane and a $Cl:HCO_3$ exchanger in the basolateral membrane (fig. 5). These cells are responsible for H^+ secretion and bicarbonate reabsorption. The other type of intercalated cell (B cell) is involved in bicarbonate secretion. These cells have a hydrogen pump in their basolateral membrane and a $Cl:HCO_3$ exchanger that is distinct from the anion exchanger of the A cell in their apical membrane (fig. 5). Recent studies have also provided evidence for the presence of a hydrogen-potassium pump in the apical membrane of intercalated cells (Garg, 1991).

β-Adrenoceptors are present in the CCD (Healy et al., 1985; Summers et al., 1985). It is well established that isoproterenol stimulates adenylate cyclase activity in the CCD (Morel and Doucet, 1986), an effect that has been localized to the intercalated cells (Fejes-Toth and Naray-Fejes-Toth, 1989). Isoproterenol increases both Cl^- reabsorption (Iino et al., 1981) and bicarbonate secretion in the CCD (Schuster, 1985), and the effect of isoproterenol on bicarbonate secretion can also be produced by cAMP. Recent evidence suggests that both isoproterenol and cAMP increase bicarbonate secretion by stimulation of

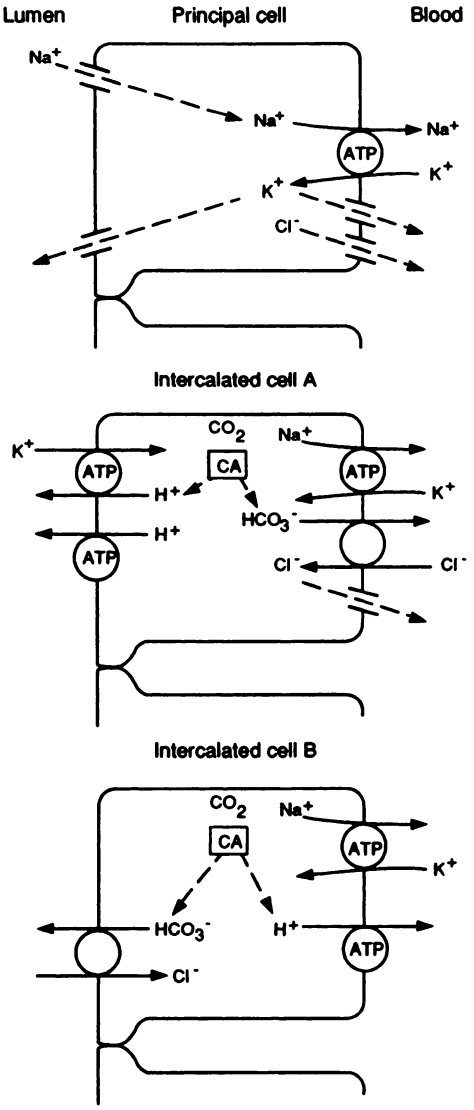

FIG. 5. Top, Sodium absorption and potassium secretion in principal cell; middle, acid secretion in type A intercalated cell; bottom, bicarbonate secretion in type B intercalated cell. CA, carbonic anhydrase. See text for explanation of various transport processes.

Cl/HCO_3 exchange (Hayashi et al., 1991) in the apical membrane of type B intercalated cell in the CCD (fig. 5).

The OMCD has been shown to be an important site of H^+ secretion. The number of intercalated cells in the OMCD are less than in the CCD, but they are all acid-secreting (type A) cells. Isoproterenol has also been shown to stimulate cAMP formation in the OMCD (Morel and Doucet, 1986) and in immunodissected intercalated cells from the rabbit OMCD (Burnatowska-Hleddin and Spielman, 1988). Although the effect of isoproterenol on fluid transport in the OMCD has not been investigated, cAMP has been shown to increase bicarbonate reabsorption in the OMCD (Hays et al., 1986). Therefore, it is likely that β-adrenergic drugs stimulate not only type B intercalated cells but also type A intercalated cells.

In addition to their effects on bicarbonate transport, isoproterenol and cAMP also reduce net K^+ secretion in the rabbit CCD (Kimmel and Goldfarb, 1984). The cellular localization of the effect of isoproterenol on K^+ transport in the CCD is not known. Because β-adrenoceptors are present in the intercalated cells, it is likely that isoproterenol produces its effect on K^+ transport in the intercalated cells also. This is consistent with the observation that ADH, which also stimulates cAMP formation in the CCD, an effect localized to principal cells, does not affect K^+ transport (Kimmel and Goldfarb, 1984). Although K^+ secretion occurs via the principal cells (Giebisch et al., 1991), there is evidence that K^+ reabsorption may occur via the intercalated cells. This is based on the demonstration of H-K-ATPase activity in the CCD (Garg and Narang, 1988) and the results of immunocytochemical studies indicating that the enzyme is located in intercalated cells (Wingo et al., 1990). Furthermore, studies of the isolated perfused OMCD indicate that K^+ reabsorption is mediated by an H-K-ATPase (Wingo, 1989). Therefore, it is likely that K^+ is also reabsorbed in the CCD via intercalated cells, and isoproterenol increases K^+ reabsorption by stimulation of the H-K-ATPase, resulting in decreased net K^+ secretion.

In addition to β-adrenoceptors, there is functional evidence that α_2-adrenoceptors also are present in the rat CCD. However, there is controversy regarding the effects of α_2-adrenergic agonists in the rabbit CCD. The α_2-adrenergic agonist clonidine blocks ADH-induced cAMP formation in the rat CCD but not in the rabbit (Chabardes et al., 1988). Similarly, clonidine inhibits ADH-dependent osmotic water permeability and transepithelial voltage in the rat but not in rabbit CCD (Chen et al., 1991). However, there are also reports indicating that α_2 agonists inhibit the hydroosmotic effect of ADH in the rabbit CCD (Krothapalli and Suki, 1984). The reasons for the differences in the results in the rabbit CCD are not known. It is possible that under certain conditions α_2-adrenergic agonists may antagonize the hydroosmotic effect of the ADH in the rabbit CCD by releasing prostaglandins that do block ADH-induced cAMP formation and ADH-induced increase in water permeability in the rabbit CCD (Chabardes et al., 1988; Chen et al., 1991). Clonidine stimulates PI hydrolysis in the rabbit IMCD cells (our unpublished results) which can lead to release of arachidonic acid and prostaglandins. Whether this happens in the rabbit CCD remains to be tested.

Pertussis toxin treatment in the rat not only prevented the α_2-mediated inhibition of ADH action in the CCD but also increased urine flow in the isolated perfused kidney (Jeffries et al., 1988), indicating that G_i may tonically modulate the response to ADH in addition to being required for signal transduction by α_2-adrenoceptors. Because α_2-adrenoceptor stimulation does not in-

hibit isoproterenol-stimulated cAMP formation in the CCD that takes place in the intercalated cells, it is likely that α_2-adrenoceptors are present in the principal cells. However, this remains to be established.

Traditionally, nonintercalated cells in the terminal two-thirds of the IMCD were considered to represent principal cells. However, morphological reexamination of the IMCD indicate that the cells in this segment have different characteristics from the principal cells of the CCD and OMCD, and they have, therefore, been called IMCD cells (Madsen et al., 1988). The nonintercalated cells in the terminal two-thirds of the IMCD respond to ADH (Knepper and Star, 1990). Activation of α_2-adrenoceptors in the rat inhibits ADH-stimulated cAMP formation not only in the OMCD (Pettinger et al., 1987) but also in the IMCD (Edwards and Gellai, 1988). The effect of α_2-adrenergic agonists is pertussis toxin sensitive. Ligand-binding experiments have shown the presence of specific and high-affinity α_2-adrenoceptors in IMCD cells isolated from the rabbit kidney (Clarke and Garg, 1990b). Activation of α_2-adrenoceptors inhibits both ADH-stimulated and forskolin-stimulated cAMP formation in IMCD cells (Clarke and Garg, 1990c). Taken together, these results indicate that the OMCD and IMCD are sites of the diuretic effect of α_2-adrenergic agonists (Gellai, 1990).

Not much is known about the presence of α_1-adrenoceptors in the CCD and OMCD segments. Recently, we examined α_1-adrenoceptors in IMCD cells isolated from the rabbit kidney by [^{3}H]prazosin binding and found that high-affinity, saturable and specific binding sites for prazosin are present in the IMCD (Clarke and Garg, 1991). We have also demonstrated that epinephrine and norepinephrine stimulate PI hydrolysis in IMCD cells (Clarke and Garg, 1990a). The effect was blocked by phentolamine and prazosin but not by yohimbine, indicating that the effect is mediated by α_1-adrenoceptors. The physiological role of α_1-adrenoceptors in the IMCD probably is to increase Na^+ reabsorption during Na^+ restriction, as is the case in other nephron segments.

5. Cultured cells. In addition to microdissected nephron segments and isolated cells, renal tubular cells in culture have been used to study adrenoceptors and receptor-mediated signal transduction. It should be mentioned that the nephronal site of origin of a cell line cannot be established with certainty. In addition, mutations occur frequently in continuous cell lines, and their properties may change with time (Handler and Kreisberg, 1991). The cells may dedifferentiate and/or redifferentiate, and this may lead to loss or gain of drug receptors and/or function in these cells. Nevertheless, several cell lines of renal tubular origin express differentiated functions and regulatory mechanisms that have been confirmed in specific nephron segments freshly isolated from the mammalian kidney. Therefore, established cell lines derived from the kidney have been used to obtain valuable insights into the renal tubular cell functions. The sources, probable nephronal site of origin, hormone responsiveness, transport and metabolic functions, and electrical properties of established cell lines were reviewed recently (Gstraunthaler, 1988; Handler and Kreisberg, 1991).

α_2-Adrenoceptors are expressed in OK cells (opossum kidney-derived cell line) which retain several properties of PCT cells. The α_2-adrenoceptors in these cells are negatively coupled to adenylate cyclase (Murphy and Bylund 1988).

There is functional evidence that α_1-adrenoceptors are present in LLC-PK_1 cells (Garg and Wozniak, 1991), a cell line that was obtained from porcine kidney and has several properties of the PCT (Gstraunthaler, 1988). Norepinephrine stimulates PI hydrolysis in LLC-PK_1 cells, and the effect is blocked by phentolamine and prazosin but not by yohimbine. However, the effect of norepinephrine on PI hydrolysis was detectable at 10^{-10} M in LLC-PK_1 cells, whereas the effect in the PCT is produced with 1 μM or higher concentrations of norepinephrine (Neylon and Summers, 1987). These results suggest that not only are α-adrenoceptors present in LLC-PK_1 cells but they are more sensitive to norepinephrine than in the microdissected PCT or in renal cortical slices (Garg and Wozniak, 1991). The reason for this difference is not known. It may be due to upregulation of the receptors because of lack of adrenergic innervation in cultured cells.

Both α_1- and β-adrenoceptors are expressed in MDCK cells (Slivka and Insel, 1987), a cell line that was obtained from canine kidney and has many properties of collecting duct cells (Gstraunthaler, 1988). There is some evidence to suggest that α_1-adrenoceptors in these cells are coupled in parallel to both phospholipase C (for PI hydrolysis) and phospholipase A_2 (for prostaglandin production) probably through separate G proteins (Slivka and Insel, 1987). Epinephrine stimulates K^+ conductance and hyperpolarizes the membrane of MDCK cells, and this effect is mediated by α_1-adrenoceptors (Paulmichl et al., 1986).

In the future, these cells and primary cultures of cells from specific nephron segments may prove to be useful in elucidating the link between adrenergic mediated signal transduction and the ultimate physiological response of the renal tubular cells.

IV. Pharmacology of Cholinergic Drugs

A. Actions on the Whole Kidney

It has long been known that infusion of cholinergic drugs into the renal artery of animals increases urinary excretion of sodium, potassium, and water (Vander, 1964). This effect is accompanied by an increase in renal plasma flow, and a decrease in filtration fraction, urine osmolality, and medullary tissue sodium concentration. The effect on glomerular filtration rate is variable. The

renal actions of cholinergic drugs can be reversed or prevented by atropine, a muscarinic type cholinergic antagonist, suggesting that the actions are mediated by muscarinic receptors. The sites and the mechanism of action of cholinergic drugs in the kidney have not been established.

Because an increase in renal plasma flow by itself produces natriuresis, it has been suggested that the cholinergic stimulated natriuresis is an indirect consequence of the increased renal plasma flow produced by these drugs (Early and Friedler, 1965). However, it has been suggested that the natriuretic effect of cholinergic agonists is due to an increase in papillary plasma flow rather than an increase in total renal plasma flow (Fadem et al., 1982). This is based on the observation that secretin, which increases total renal plasma flow but not papillary plasma flow does not produce natriuresis. In addition, it has been demonstrated that acetylcholine-induced vasodilation does not produce natriuresis in the absence of an increase in renal interstitial hydrostatic pressure (Hartupee et al., 1982).

Prior administration of meclofenamate, a prostaglandin inhibitor, blocks the vasodilatory and diuretic effects of acetylcholine (Lameire et al., 1980). The renal effects of acetylcholine are restored if cAMP is infused into the renal artery of animals in which prostaglandin synthesis is inhibited by indomethacin (Dehkordi et al., 1989). These results suggest that the acetylcholine effects are dependent on the synthesis of prostaglandins that stimulate cAMP formation. The role of prostaglandins in the renal response to acetylcholine was recently investigated in more detail (Salom et al., 1991). Salom et al. found that both prostaglandins and endothelium-derived relaxing factor mediate the acetylcholine-induced increase in renal blood flow and urine flow but not the acetylcholine-induced increase in sodium excretion.

Some investigators have proposed a direct effect of cholinergic drugs on sodium reabsorption in the renal tubule (Williams et al., 1965; Parmalee and Carter, 1968). This was based, in part, on the demonstration of an increase in urine flow and sodium excretion after infusion of cholinergic agonists into the renal portal system of hens. Because the urinary concentration mechanism is poorly developed in the chicken, it was suggested that cholinergic agonists produce diuresis and natriuresis by their direct effect on sodium reabsorption in renal tubules (Parmalee and Carter, 1968). In addition, it has been shown that acetylcholine produces natriuresis in water-hydrated dogs (Martinez-Maldonado et al., 1972). These results cannot be explained by medullary washout produced by the drug because the solute concentration in the medulla was equally low in control and experimental animals. On the other hand, the results can be explained if acetylcholine inhibits sodium reabsorption in certain segments of the nephron under these conditions.

There is controversy regarding the cholinergic innervation in the mammalian kidney. Barajas and Wang (1983) demonstrated the presence of acetylcholinesterase near the adrenergic nerves but found no evidence of cholinergic innervation. The acetylcholinesterase disappeared following treatment of the animal with 6-OH-dopamine which destroys adrenergic nerves. In addition, physostigmine, an inhibitor of acetylcholinesterase, does not affect urine flow or sodium excretion in the innervated dog kidney (Vander, 1964). However, in one report it was suggested that the dog kidney is innervated by cholinergic nerves. This was based on the synthesis of acetylcholine and the presence of muscarinic receptors in the kidney (Pirola et al., 1989). There is evidence to suggest that acetylcholine may be synthesized in non-neuronal renal tissue. In addition, the synthesized acetylcholine can be released in a calcium-dependent manner in response to physiological concentrations of urea (Evans, Garg, and Meyer, unpublished observations, 1991). The presence of cholinergic receptors in the renal tissues has been reported by several investigators (see below), but there is no direct evidence for cholinergic innervation in the kidney.

B. Cholinergic Receptors

Cholinergic receptors are of two types: nicotinic and muscarinic. In general, the nicotinic type cholinergic receptors are present in the autonomic ganglia and at neuromuscular junctions. On the other hand, the muscarinic receptors are present on the cells innervated by postganglionic fibers of the parasympathetic nervous system. In addition, nicotinic receptors are ligand-gated ion channels, whereas muscarinic receptors are coupled to G proteins. Because the renal actions of cholinergic drugs are blocked by atropine, a muscarinic type cholinergic antagonist, the cholinergic stimulated natriuresis must be mediated by muscarinic receptors.

Very few studies have been done to examine the cholinergic receptors in the mammalian kidney. In one study, specific cholinergic receptors were reported to be lacking in rat kidney homogenates (Yamamura and Snyder, 1974). In another study, specific muscarinic binding sites were reported to be present in the rat kidney (Yamada et al., 1986). Recently, we characterized muscarinic receptors in IMCD cells isolated from the rabbit kidney (McArdle et al., 1989) and demonstrated that high-affinity specific binding sites for a muscarinic receptor antagonist [^{3}H]QNB are present in these cells. On the other hand, two types of binding sites for [^{3}H]QNB have been reported in the dog kidney cortex (Pirola et al., 1989). One type of binding site has a high affinity for the ligand and is present in the outer cortex (table 4). The other type has a low affinity for [^{3}H]QNB and is present in the inner cortex. Our preliminary experiments indicate that high-affinity specific binding sites for [^{3}H]QNB are also present in MDCK cells, a renal cell line from dog kidney (Mohuczy-Dominiak and Garg, 1991b). Overall

TABLE 4
Muscarinic receptors in renal preparations

Tissue	Ligand	K_d (nM)	B_{max} (fmol/mg protein)	Reference
Kidney (rat)	[^{3}H]QNB	0.14	3.5*	Yamada et al., 1986
Kidney (dog)				Pirola et al., 1989
Outer cortex	[^{3}H]QNB	0.11	4.1	
Inner cortex	[^{3}H]QNB	3.70	9.0	
IMCD cells (rabbit)	[^{3}H]QNB	0.27	27.5	McArdle et al., 1989
MDCK cells	[^{3}H]QNB	0.09	1464	Mohuczy-Dominiak and Garg, 1991b

* fmol/mg tissue.

TABLE 5
*Subtypes of muscarinic receptors**

	M_1	M_2	M_3		
Antagonist	Pirenzepine Telenzepine	AF-DX 116 Methoctramine	4-DAMP FHHSiD		
Effector system	IP_3/DAG	↓ cAMP	IP_3/DAG		
Cloned species	m_1	m_2	m_3	m_4	m_5
Distribution	Brain	Heart	Kidney		

* Abbreviations: AF-DX 116, 11-2[2-[(diethylamino)methyl]-1-piperidinyl]-5,11-dihydro-6H-pyridol[2,3-b][1,4]benzodiazepin-6-one; FHHSiD, *p*-fluoro hexahydrosiladifenidol; 4-DAMP, 4-diphenylacetoxy N-methyl piperidine.

the affinities of [^{3}H]QNB for the high-affinity binding sites in all of these renal tubular cells are comparable (table 4). It is possible that the low-affinity muscarinic receptors demonstrated in the inner cortex of the dog kidney (Pirola et al., 1989) may be present in the blood vessels in this zone of the kidney.

The density of muscarinic receptors in MDCK cells is 50 times that of IMCD cells, which, in turn, is three times that of other renal cell preparations (table 4). The MDCK cell line may be a useful model for the characterization and elucidation of the function of muscarinic receptors in renal tubular cells.

Pharmacologically, muscarinic receptors have been classified into three subtypes (table 5). M_1 receptors (in brain) have a high affinity for pirenzepine, whereas M_2 receptors (in heart) have a low affinity for pirenzepine and a high affinity for 11–2[2-[(diethylamino) methyl]-1-piperidinyl]-5,11-dihydro-6H-pyridol[2,3-b][1,4]benzodiazepine-6-one and methoctramine (Hulme et al., 1990). M_3 receptors (in submandibular gland) have a low affinity for pirenzepine but a high affinity for hexahydrosiladifenidol and 4-diphenylacetoxy N-methylpiperidine. Up to five different molecular forms of muscarinic receptors (denoted by m_1 to m_5) have been cloned from rat and human tissues (Bonner et al., 1987; Liao et al., 1989; Ashkenazi et al., 1989). The receptor subtypes m_1, m_2, and m_3 have characteristics similar to those of M_1, M_2 and M_3, respectively. The molecular types m_4 and m_5 have an intermediate affinity for pirenzepine and can be grouped with pirenzepine-sensitive M_1 receptors (Buckley et al., 1989). However, further studies are needed to establish other pharmacological properties and functions of the cloned muscarinic m_4 and m_5 receptors.

Our data concerning [^{3}H]QNB binding to isolated IMCD cells indicate that there is only one subtype of muscarinic receptors in these cells (McArdle et al., 1989). Although our results regarding the effects of pirenzepine on carbachol-stimulated PI hydrolysis indicate that renal muscarinic receptors are not M_1 type (McArdle et al., 1988), the muscarinic receptors in the IMCD have not been fully characterized as to their subtype. Our data concerning MDCK cells also indicate that muscarinic receptors in this cell line are homogeneous (Mohuczy-Dominiak and Garg, 1991b). These data suggest that muscarinic receptors in the MDCK cells are of the M_3 subtype because they have a high affinity for 4-diphenylacetoxy N-methylpiperidine and a low affinity for pirenzepine and methoctramine.

C. Signal Transduction

Depending on the receptor subtype and the tissue, activation of muscarinic receptors has been shown to produce an increase in PI hydrolysis or a decrease in cAMP. In general M_1 and M_3, receptors are coupled to PI hydrolysis, whereas M_2 receptors are coupled to inhibition of the adenylate cyclase system (Hulme et al., 1990). The stimulation of PI hydrolysis by muscarinic receptor activation is sustained (McArdle et al., 1988; Batty and Nahorski, 1989) and is probably resistant to desensitization. Depending on the cell type, the activation of M_1 and M_3 receptors may also increase cAMP formation, release arachidonic acid, or activate K^+ or Cl^- channels (Hulme et al., 1990). Similarly, the activation of M_2 and M_4 receptors may also produce weak stimulation of PI hydrolysis (Ashkenazi et al., 1989). It is possible that there is also some cross-talk between PI hydrolysis and cAMP formation. The stimulation of PI hydrolysis has been shown to affect the intracellular level of cAMP (Felder et al., 1989). More experimental data are needed to clarify the mechanism of transduction of the two signal systems by the same subtype of muscarinic receptor.

In spite of the well-established muscarinic receptor-mediated pharmacological actions of cholinergic drugs on the kidney, not much attention has been paid to investigate the muscarinic receptor-activated signal transduction. Recently, we have demonstrated that carbachol, a cholinergic agonist, stimulates PI hydrolysis both in the inner and outer medulla (McArdle et al., 1988). The effect is greater in the inner medulla than in the outer medulla. The effect of carbachol is concentration dependent and is blocked by atropine, a muscarinic antagonist, but not by hexamethonium, a nicotinic antagonist. PI hydrolysis is not stimulated by 1,1-dimethyl-4-phenylpiperazinium iodide, a nicotinic agonist. These results indicate that cholinergic stimulation of PI hy-

drolysis in the renal medulla is mediated by muscarinic receptors.

Carbachol-stimulated PI hydrolysis in the inner medulla is inhibited by high concentrations of pirenzepine (K_i 427 nM) (McArdle et al., 1988), indicating that the effect of carbachol in the inner medulla is probably not mediated by M_1 receptors. The major portion of the inner medulla is occupied by IMCDs that form >50% of its mass (Knepper et al., 1977). We have demonstrated that carbachol also stimulates PI hydrolysis in isolated IMCD cells via activation of muscarinic receptors (McArdle and Garg, 1989), indicating that the IMCD is a major site of cholinergic stimulated PI hydrolysis in the inner medulla.

Carbachol also stimulates PI hydrolysis in the outer medulla of the rabbit kidney (McArdle et al., 1988). The major portion of the outer medulla is comprised of the MTALs. Carbachol does not stimulate PI hydrolysis in the MTAL isolated from the rabbit kidney (our unpublished observations). Therefore, it is likely that the stimulation of PI hydrolysis in the outer medulla is due to an effect of the drug on the OMCD. In addition, there is evidence that activation of muscarinic receptors by cholinergic agonists increases cell calcium in the OMCD microdissected from the rat (Marchetti et al., 1990).

Recently, we examined the effect of carbachol on PI hydrolysis and cAMP formation in MDCK cells. Our preliminary results indicate that cholinergic agonists stimulate PI hydrolysis in MDCK cells. The effect is blocked, in order of potency, by: 4-diphenylacetoxy N-methylpiperidine > pirenzepine > methoctramine. These results suggest that the muscarinic receptors in these cells belong to the M_3 subtype. In addition, the same cholinergic agonists at higher concentrations decreased ADH- and forskolin-stimulated cAMP formation in MDCK cells (Garg and Mohuczy-Dominiak, 1991a). These results are consistent with those of other investigators who have reported that activation of M_1 or M_3 receptors not only stimulate PI hydrolysis but also decrease cAMP formation at higher concentrations (Ashkenazi et al., 1989).

D. Actions on Renal Tubular Cells

Carbachol inhibits Na^+ transport in the toad urinary bladder (Wiesman et al., 1978; Sahib et al., 1978), which is commonly used as an in vitro model for the mammalian collecting duct. The carbachol-induced inhibition of Na^+ transport was accompanied by an increase in uptake of calcium and was blocked by atropine. Although the effect of carbachol was not dependent on cAMP, it was accompanied by an increase in intracellular cGMP. However, exogenous cGMP or its analogs did not produce a decrease in sodium transport. Thus, the actual mechanism of action of cholinergic agonists on the toad bladder remains to be determined.

Carbachol has also been shown to inhibit ADH-stimulated water transport in the toad bladder (Arruda and Sabatini, 1980a). This effect was dependent on the uptake of Ca^{2+} by the bladder and was blocked by atropine. In addition, carbachol inhibited urinary acidification in the turtle bladder (Arruda and Sabatini, 1980b), and this effect was also dependent on Ca^{2+} and was prevented by atropine. Taken together, these results suggest that muscarinic receptor activation not only inhibits Na^+ transport but also inhibits H^+ transport and ADH-induced increase in water permeability in the amphibian bladder.

Recently, the effects of carbachol on ADH-induced water flow were studied in the in vitro perfused rabbit CCD (Snyder et al., 1991). Carbachol reduced the ADH-induced increase in water permeability but not the cAMP-induced increase in permeability. Surprisingly, carbachol pretreatment prevented the cAMP-induced increase in water permeability. The authors concluded that carbachol affects both "pre- and post-cAMP" steps. Carbachol also increased intracellular Ca^{2+}, and staurosporine pretreatment reversed the effect of carbachol. These results suggest that carbachol produces its effect by activation of protein kinase C and are consistent with cholinergic activation of PI hydrolysis in rabbit IMCD (Garg and McArdle, 1990).

Acetylcholine has been shown to hyperpolarize the plasma membrane of MDCK cells (Lang et al., 1988). From this observation, it was concluded that activation of muscarinic receptors stimulates a K^+ conductance. However, the electrophysiological effects of cholinergic agonists in combination with other hormones, such as ADH, have not been studied in this cell line, which is rich in muscarinic receptors (Mohuczy-Dominiak and Garg, 1991b).

Activation of protein kinase C inhibits sodium transport in A6 cells (Yanase and Handler, 1986), a cell line obtained from toad kidney, in LLC-PK_1 cells (Mohrmann et al., 1987), and in the frog urinary bladder (Satoh and Endou, 1990). In addition, protein kinase C activation not only inhibits net Na^+ reabsorption but also decreases K^+ secretion and transepithelial voltage in the rabbit CCD (Hays et al., 1987). There is no effect on net chloride or total CO_2 transport in the CCD. Taken together these results suggest that protein kinase C may be affecting ion transport in the principal cells of the CCD.

All of the available data in the literature are consistent with a model in which muscarinic receptor activation stimulates PI hydrolysis and protein kinase C activation, which may ultimately inhibit Na^+ reabsorption, K^+ secretion, and ADH-induced water permeability in principal cells and IMCD cells of the mammalian collecting duct. The steps involved between protein kinase C activation and inhibition of transport remain to be investigated.

V. Pharmacological Implications of Medullary Interstitial Hypertonicity

A. Effects on Renal Tubular Cells

The renal medulla is unique in that its interstitial osmolality varies widely depending on the physiological state or pharmacological intervention of the animal. For example, during dehydration, the rat renal papillary cells are exposed to osmolalities as high as 3800 mOsm/kg H_2O (Valtin, 1966), whereas in water diuresis values close to isotonicity have been observed (Atherton et al., 1968). Acute changes in environmental osmolality induce volume regulatory responses in both renal and nonrenal cells by activating several electrolyte transport processes. The mechanisms involved in epithelial cell volume regulation are discussed in several recent reviews (Larson and Spring, 1987; Volkl et al., 1988; Blumenfeld et al., 1989) and will not be discussed here.

When cells are exposed to hypertonicity on a long-term basis as is the case with renal medullary cells in vivo, they do not balance extracellular salt concentration with an equally high concentration of sodium, potassium, or urea. Instead, they accumulate small organic osmolytes (methylamines, such as glycerophosphorylcholine and betaine, and polyhydric alcohols, such as myoinositol and sorbitol) either by uptake from the surroundings or by synthesis in the cells. The signal(s) for initiating the accumulation of organic osmolytes during hypertonicity is (are) not known at this time. The mechanisms of accumulation of various intracellular osmolytes have been reviewed by several authors (Wolff and Balaban, 1990; Garcia-Perez and Burg, 1991) and will not be discussed here.

The primary focus in this review will be on the alterations that occur in the effects of drugs on renal epithelial cells with a change in the tonicity of the interstitial fluid. The extracellular hypertonicity of the renal medulla is caused by high concentrations of NaCl and urea (Bagnasco et al., 1986; Bulger, 1987; Yancy and Burg, 1989). It has been demonstrated that the removal of water and addition of urea are the two most important factors in increasing the osmolality of the inner medulla (Schmidt-Nielsen et al., 1983). Removal of water may not change the relative abundance of urea in the interstitial fluid. In contrast, the addition of urea to the renal interstitium will increase the ratio of urea to NaCl in the renal medulla. In addition, there is a significant concentration of total ammonia (20 to 40 mM) in the renal medulla (Packer et al., 1991).

B. Effects on Signal Transduction

Very few studies have examined the effects of hypertonicity on drug-induced changes in cAMP formation in the renal medulla. It has been demonstrated that an increase in osmolality (1650 mOsm) by urea reduces the cAMP content in rat inner medullary slices (Craven et al., 1980). On the other hand, an increase in osmolality produced by a mixture of urea and NaCl has a biphasic effect on ADH-stimulated cAMP accumulation in the rat IMCD (Edwards et al., 1981). At 800 mOsm, there was a higher ADH-stimulated cAMP accumulation, whereas at 2000 mOsm there was a lower cAMP formation than at 200 mOsm. In addition, phosphodiesterase activity was inhibited at all the hyperosmolar concentrations. Recently, we demonstrated that an osmolality of 1200 mOsm made up with a mixture of urea and NaCl also decreases forskolin-stimulated cAMP accumulation in the rabbit IMCD cells (Clarke and Garg, 1990c). The increase in cAMP accumulation in the IMCD at lower osmolality (800 mOsm) may be due to the inhibition of phosphodiesterase activity, whereas the decrease in cAMP accumulation at higher osmolality may be caused by inhibition of adenylate cyclase activity. However, the specific effects of NaCl, urea, and other solutes on adenylate cyclase and phosphodiesterase activity in the IMCD remain to be elucidated.

An increase in tonicity of the incubation media produced by NaCl (4 to 400 mM), sucrose (600 mM), or mannitol increases ADH-stimulated cAMP formation in intact LLC-PK_1 cells (Skorecki et al., 1987). This effect was shown to be mediated by cell shrinkage. The data concerning the direct effects of NaCl on adenylate cyclase activity in membranes of these cells indicate that NaCl stimulates the enzyme activity at low concentrations (100 mM) and inhibits it at high concentrations (450 mM). Similar effects of monovalent ions on ADH-stimulated adenylate cyclase activity have been reported in porcine renal medullary membranes. It has been suggested that anions are responsible for stimulation of the enzyme at low salt concentrations and cations are responsible for the inhibitory effects at high concentrations (Roy et al., 1977). In addition, it has been suggested that the inhibitory effect of cations on adenylate cyclase is mediated by their effect on G proteins (Jacobs and Minuth, 1984). Urea, on the other hand, blunts the stimulatory effect of NaCl in intact LLC-PK_1 cells and also inhibits adenylate cyclase activity in membrane preparations (Skorecki et al., 1987). These results, taken together, indicate that the effects of hypertonicity on drug-induced cAMP formation in the renal tubular cells may depend on the concentration of each solute in vivo.

Recently, it was demonstrated that carbachol-stimulated release of inositol phosphates in rabbit inner medullary slices is inversely proportional to the osmolality of the incubation media (Garg et al., 1988a). At an osmolality of 1200 mOsm, carbachol-stimulated release of inositol phosphates is not significantly different from unstimulated (control) release of inositol when the osmolality of the incubation medium is increased by an equimolar mixture of NaCl and urea, urea alone, NaCl alone, or KCl alone. Although there is an inverse relationship between carbachol-stimulated PI hydrolysis when the osmolality is increased by adding mannitol, the

carbachol-stimulated release of inositol phosphoates is still higher than the control at an osmolality of 1200 mOsm. Taken together, these results suggest that urea, NaCl, and KCl may have effects on carbachol-stimulated release of inositol phospholipids in addition to the effect of an increase in osmolality, as is the case with mannitol.

The effect of hypertonicity is not limited to the cholinergic stimulated PI hydrolysis in the kidney. The increase in osmolality caused by a mixture of urea and NaCl decreases ADH-stimulated PI hydrolysis in inner medullary slices and in LLC-PK_1 cells (Garg et al., 1988b; Garg and Kapturczak, 1990). Similarly, increasing the osmolality by a mixture of NaCl and urea decreases oxytocin- and norepinephrine-stimulated PI hydrolysis in LLC-PK_1 cells (Garg et al., 1990; Garg and Wozniak, 1991) and norepinephrine-stimulated PI hydrolysis in rabbit IMCD cells (Clarke and Garg, 1990a). All of these results indicate that the effect of hypertonicity on the agonist-stimulated PI messenger system is a generalized phenomenon applicable to several hormones and drugs. In addition, unlike the cAMP system in which the effect of hypertonicity is mixed, the effect on PI hydrolysis is always inhibitory with all solutes tested so far.

The mechanism by which an increase in osmolality alters agonist-stimulated signal transduction is not known at the present time. It is possible that changes in osmolality affect the availability of the substrate PIP_2 for the receptor-coupled phospholipase C. There are no changes in the incorporation of [^{3}H]inositol into phosphoinositides in inner medullary slices from the rabbit when the osmolality of the incubation media is increased to 600 mOsm/kg H_2O by addition of either urea, NaCl, KCl, mannitol, or a mixture of urea and NaCl. Increasing the osmolality of the incubation media to 900 and 1200 mOsm/kg H_2O with NaCl or KCl, but not with urea, mannitol, or a mixture of NaCl and urea, produces a decrease in [^{3}H]inositol incorporation into phosphoinositides (Garg et al., 1988a). These results suggest that hypertonicity per se does not affect PI synthesis. The decrease in incorporation of [^{3}H]inositol into total PI with high concentrations of NaCl or KCl is probably due to a specific effect of these ions.

C. Effects on Ligand Binding to Receptors

One possible explanation for the changes in agonist-stimulated signal transduction observed after increases in osmolality may be changes in the binding of the agonist to its receptors. The available data concerning the effects of various solutes on the binding of agonists and antagonists to various adrenergic receptors is confusing and contradictory. In one study, changing the osmolality by sucrose (up to 840 mM) did not change the binding of [^{3}H]prazosin, an α_1 antagonist, or [^{3}H]yohimbine, an α_2 antagonist, to rat renal cortical membranes (Snavely and Insel, 1982). On the other hand, NaCl and Na acetate, but not LiCl and KCl, increased the specific binding of [^{3}H]yohimbine in these membranes. The Na^+-mediated increase in [^{3}H]yohimbine binding was due to an increase in B_{max} without a change in K_d. In this study, there was no effect of Na^+ on [^{3}H]prazosin binding. In addition, NaCl decreased the affinity of epinephrine for both α_1- and α_2-adrenoceptors. Similar effects were produced with KCl and LiCl, but not with N-methyl-D-glucamine, on the affinity of epinephrine for α_1-receptors and with Na acetate, but not with KCl, on the affinity of epinephrine for α_2-adrenoceptors. These authors suggested that the effect of these salts on ligand binding to α-adrenergic receptors is not due to ionic strength or tonicity but is Na^+ selective. The effect of Na^+ on ligand binding to α_1-adrenoceptors is probably nonselective.

In another study, it was reported that Na^+ decreased the apparent affinity of norepinephrine for α_1-adrenoceptors in the rat renal cortical and medullary membranes (Ernsberger and U'Prichard, 1987). Similar effects were obtained with LiCl and KCl. In this study, Na^+ increased the affinity of [^{3}H]prazosin binding for α_1-adrenoceptors. Divalent cations such as Mg^{2+} increased the agonist and decreased the antagonist affinities for α_1-adrenoceptor in renal cortical membranes. The authors suggested that monovalent cations, in a manner similar to that which occurs with GTP, shift the equilibrium of α_1-binding sites to a low-affinity state.

Recently, we examined the effects of a mixture of NaCl and urea on both adrenergic and cholinergic receptors in IMCD cells isolated from the rabbit kidney. Our data indicate that hyperosmolality (1200 mOsm) decreases, but does not abolish, the specific binding of [^{3}H]prazosin to IMCD cells (Clarke and Garg, 1991). Similar results were obtained for the specific binding of [^{3}H]QNB to IMCD cells (our unpublished observations). These results suggest that at least part of the decrease in cholinergic and adrenergic stimulated PI hydrolysis in IMCD cells (Garg et al., 1988a; Clarke and Garg, 1990a) may be due to a decrease in the binding of the ligand to its receptor.

The specific binding of [^{3}H]rauwolscine to isolated MTALs and IMCD cells is also decreased by NaCl and urea (Clarke and Garg, 1990b; Mohuczy-Dominiak and Garg, 1991a,b). The order of potency of various solutes to decrease the specific binding of [^{3}H]rauwolscine to MTALs is: KCl > NaCl > urea. Mannitol had a small effect and sucrose had no effect on the binding of [^{3}H]rauwolscine to MTALs. A Scatchard analysis of the data indicates that hyperosmolality induced by NaCl and urea increased the K_d and B_{max} of [^{3}H]rauwolscine binding in both MTALs and IMCD cells, although the change in K_d was greater than the change in B_{max}. The most potent agent in changing the K_d and B_{max} of specific binding of [^{3}H]rauwolscine to MTALs was NH_4Cl (Mohuczy-Dominiak and Garg, 1991a).

All of these results indicate that the effect of renal medullary interstitial hypertonicity, produced by NaCl,

TABLE 6
*Adrenergic receptors in mammalian nephron segments and cultured cell lines**

Segment (or cell line)	α_1	α_2	β_1 or β_2
PCT	+	+	−
PST	−	−	+ (β_2)
Thin descending limb	−	+	−
MTAL	+	+	−
CTAL	+	−	+ (in rat and not in rabbit)
DCT	+	−	+
CNT (rabbit)	−	−	+
CCD	−	+	+ (β_1)
OMCD	−	+	+
IMCD	+	+	−
OK cells	−	+	−
LLC-PK$_1$ cells	+	−	−
MDCK cells	+	+	+

* Symbols: +, Present; −, absent or not investigated.

urea, and to some extent NH_4Cl, on ligand binding to adrenoceptors is quite complex and depends on the type of solute, the receptor, and the cell type. Only future investigations will clarify the mechanism of action of various solutes on the binding of ligands to receptors in various types of renal epithelial cells.

D. Implications

Although ligand binding to receptors and agonist-stimulated second messenger systems are altered by hypertonicity in nonrenal cells, the renal medulla is the only tissue in which extracellular osmolality exceeds that of systemic plasma in mammals. Therefore, it is of great physiological and pharmacological relevance. The significance of osmolar and ionic regulation of drug actions on the kidney will probably depend on the type of drug, the cell, and the composition of the extracellular fluid. I propose a general model to explain the role of medullary interstitial osmolality in the actions of adrenergic and cholinergic drugs on the kidney.

Cholinergic agonists cause an increase in renal papillary blood flow (Fadem et al., 1982) that leads to a decrease in interstitial osmolality of the inner medulla. Under these conditions, the second effect of cholinergic agonists on the kidney will be a stimulation of the PI messenger system in the collecting duct cells of the inner medulla (McArdle and Garg, 1989). The cholinergic effects on papillary blood flow increase the solute and fluid load to the collecting duct, where the activation of protein kinase C by cholinergic stimulated PI hydrolysis will inhibit solute and fluid reabsorption. In this way, cholinergic agonists produce their diuretic and natriuretic actions not only by increasing papillary blood flow but also by their effects on electrolyte transport in the collecting duct. On the other hand, adrenergic drugs, which do not cause an increase in papillary blood flow and do not stimulate PI hydrolysis under hypertonic conditions in the renal medulla, will produce their pharmacological actions only via receptors affecting cAMP production.

VI. Summary and Conclusions

There is convincing evidence to suggest that there are direct effects of adrenergic agents on renal tubules. During the last several years, considerable progress has been made in determining the type of adrenoceptors present in renal tubular cells through the use of radioligand binding and signal transduction methods. The receptor data are summarized in table 6. Almost all major nephron segments seem to have α_1- and α_2-adrenoceptors. However, there are few data describing the subtypes of α_1- or α_2-adrenoceptors in these segments. β-Adrenoceptors are present in the CNT and collecting ducts of almost all species and in the thick ascending limbs of rats and mice. Adrenergic mediated signal transduction has been examined in some nephron segments, but virtually nothing is known about the relationship between the generation of adrenoceptor-mediated second messengers and changes in phosphorylation/activity of transport proteins (ion channels, ion pumps) in different types of renal tubular cells.

There is general agreement that gluconeogenesis in the PCT is mediated by α_1-adrenoceptors through the PI and Ca^{2+} messenger system. Evidence also indicates that the increase in Na^+ transport associated with renal nerve stimulation or adrenergic agonists in the PCT or the loop of Henle is mediated by α_1-adrenoceptors. Adrenergic agents modulate the effect of other hormones, such as PTH and vasopressin, on renal tubule transport by a decrease in cAMP, and this effect is mediated by α_2-adrenoceptors. There may be some interaction between the two α subtype-mediated effects in some nephron segments. β-Adrenergic agonists stimulate cAMP formation in the PST, thick ascending limb (rat and mouse), CNT, and collecting duct segments. The physiological role of the β-adrenoceptors in the PST is not known. β-Adrenergic agonists stimulate sodium reabsorption by activation of the basolateral Cl^- channel in the thick ascending limbs of rat and mice. The activation of β-adrenoceptors in the CNT and CCD increases Cl^- reabsorption and HCO_3^- secretion by stimulation of Cl/HCO_3 exchange in the apical membrane of type B intercalated cell. The antikaliuretic effect of β-adrenergic agonists is probably due to the stimulation of K^+ reabsorption in type A intercalated cells in the CCD and OMCD.

In the case of cholinergic drugs, the data in the literature are consistent with a model in which cholinergic agents increase papillary blood flow, resulting in the washout of the hypertonic medullary interstitium. This leads to a decrease in water abstraction out of the descending limb of Henle's loop. The increase in fluid flow to the ascending limb causes an increase in sodium delivery to the distal tubule, which by itself may be partially responsible for the natriuresis. Under these conditions, cholinergic agonists may also directly inhibit sodium and water reabsorption via activation of the PI messenger system in the collecting duct.

The high solute concentration in the renal medullary interstitium affects agonist-stimulated PI hydrolysis at multiple steps: (*a*) [^{3}H]inositol incorporation into PI, (*b*) agonist-binding to receptors, and (*c*) agonist-stimulated PI hydrolysis probably acting via phospholipase C. The high concentration of Na^+ in the renal medullary interstitium affects the agonist-mediated cAMP system. Some of the effects of Na^+ may be counteracted by urea in this system. The effects of renal medullary interstitial solutes on protein kinase A and protein kinase C are not known at this time.

There is some evidence to suggest that the interstitial osmolality in the renal medulla may modulate the actions of not only adrenergic and cholinergic drugs but of almost all drugs. Renal pharmacologists have paid very little attention to the implications of this phenomenon. The above results suggest that the effect of drugs on second messenger systems in the renal medulla in vivo will depend on the tissue osmolality that itself depends on the state of hydration of the animal. Therefore, the local factors, such as high medullary interstitial osmolality and high NaCl concentration encountered in the renal medulla, have an important regulatory function on the physiological and pharmacological actions of hormones and drugs that act via the signal transduction system in the kidney.

I am aware of the simplifications that I have made to describe the sites and mechanisms of action of adrenergic and cholinergic drugs on specific nephron segments. The overall actions of drugs and hormones will not only depend on the mechanisms that I have discussed in this review but will also depend on the desensitization, turnover rates, and microenvironments of the receptors and receptor-linked proteins (such as G proteins) and their components in vivo.

In conclusion, attempts have been made to define the mechanism of action of drugs on microdissected tubules and cells isolated from different segments of the nephron. It is now clear that all nephron segments do not respond uniformly to drugs. Although at present only limited data are available, future applications of radioligand methodology, together with measurements of signal transduction and other biochemical and cell biological responses in specific renal tubular cell types, will provide information concerning the physiological and pharmacological actions of selective adrenergic and cholinergic drugs that are or will be available. More specifically, the role of G proteins and other components of signal transduction systems in the activation (or phosphorylation) of various ion channels or ion transporters by adrenergic drugs will be better understood.

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