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MICROPUNCTURE TECHNIQUES AS A TOOL IN RENAL PHARMACOLOGY[1]

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INTRODUCTION

Although renal micropuncture (MP) techniques are available in many laboratories, they have not been extensively used in answering the cardinal questions of pharmacology: Where does a drug act, how does it act, why does it act, and what does it become? Only diuretics and endogenous substances such as steroid hormones, parathyroid hormone, vasopressin, and vasoactive autacoids have been extensively investigated by MP techniques. The renal fate of most drugs or their metabolites has not yet been studied by these techniques, which comprise free-flow micropuncture, in situ microperfusion of tubules and/or peritubular capillaries, tubular or peritubular capillary microinjection, and in vitro perfusion of isolated tubular segments. Each technique has its own limitations (1). Most micropuncture techniques are carried out in the rat or the dog, while isolated tubules are most readily obtained from the rabbit. Unfortunately, observations on renal effects or the fate of drugs cannot easily be extrapolated from one species of mammals to others or to man. The same nephron segments of different species differ anatomically (2) and functionally (3, 4). Even within a given species, such as the rat, different strains may differ with respect to the tubular handling of urinary constituents, such as uric acid (5) or phos-

[1]The following abbreviations are used throughout this review: C_x, urinary clearance of substance *x*; FD_x, fractional delivery (fraction of filtered load reaching a given tubular segment) of substance *x*; FE_x, fractional excretion (C_x/GFR); FL, filtered load; FR_x, fractional reabsorption ($P_x \times GFR - FE_x$); GFR, glomerular filtration rate; MP, micropuncture; PAH, *p*-aminohippurate or *p*-aminohippuric acid; PG, prostaglandin(s); P_x, concentration of substance *x* in plasma or in plasma water; RBF, renal blood flow; SNGFR, single nephron GFR.

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phate (6). Furthermore, only a few segments of a fraction of the superficial nephrons and other segments of a few juxtamedullary nephrons are accessible to MP. The functions of these segments may differ from those of the same structures in other nephrons: Major functional differences have been shown to exist between different nephron subpopulations (7, 8), as well as between different parts of the same nephron (9). Finally, the anesthesia and surgery required for in vivo MP may modify renal functions (10–12), although anesthesia does not appear to influence the relative contributions of superficial and deep nephrons to total GFR (13).

General MP methodology (10, 14), as well as particular aspects, such as free-flow sample collection (15, 16), measurement of SNGFR (17, 18) or measurement of hydrostatic pressure (11), has been reviewed by specialists in the respective fields. Only a few substances of pharmacological interest on which reliable micropuncture data are available are discussed in this review.

EXCRETION OF DRUGS

Many drugs are organic acids or bases. A variable fraction of these drugs is present, at body fluid pH, as organic anions or cations.

Organic Anions

Clearance studies revealed that the urinary excretion of weak acids depends on glomerular filtration and two types of transtubular transport. Organic anions are thought to be *secreted*[2] into proximal tubular fluid (TF) by a nonspecific mechanism thought to handle anions chemically as different as *p*-aminohippurate (PAH), penicillin G, and phenol red, but not all known organic anions. The fraction of such substances present as nondissociated acid in lower nephron TF is then assumed to *diffuse back* into peritubular blood by "nonionic diffusion," insofar as it is liposoluble. Non-ionic diffusion is usually thought to be confined to distal tubules and collecting ducts because of the pH profiles found in TF, but also because diffusion equilibrium of the nondissociated moiety is not often attained in pelvic urine (19). The micropuncture data are not entirely consistent with this overall picture.

PAH PAH is pharmacologically relevant as a prototype of a strong organic acid (pK = 3.7), whose nondissociated moiety is only weakly liposoluble. PAH extraction from renal blood of mammals varies from 30 to 90% and is usually between 70 and 90%. Incomplete extraction is an inherent property of cortical proximal secretion since proximal $FD_{PAH/In}$ is equal to $C_{PAH/In}$ (20). The glomerular filtration of PAH is restricted by minor protein binding varying from 6.5 to 15% in rats (21, 22) but reaching 32%

[2]The terms *secretion* and *reabsorption* designate the direction of fluxes only.

in hamsters (23). Extraction from blood is mainly due to secretion into TF of the late convoluted (20–22, 24–26) and straight parts (27) of proximal tubules. The contribution of pars recta to total PAH secretion appears to be smaller in urea (21) or expansion (22) diuresis than in the nondiuretic rat (25). In contrast to older conclusions (24) a definite number of secretory sites appears to limit PAH secretion (26). There is some controversy about flow, and SNGFR, dependency of proximal PAH secretion (22, 24–26). No PAH secretion occurs in distal tubules. Proximal PAH secretion is blocked by large doses of probenecid (28). When both proximal tubules and peritubular capillaries are microperfused, however, neither lack of Na^+ nor the presence of ouabain causes a major block of PAH secretion (29, 30). PAH uptake into proximal tubular cells, which is the most important step of secretion (27), could occur as a countertransport coupled to part of the (hypothetical) OH-ion extrusion from these cells (29). The (presumably carrier-mediated) transport from cells to TF could be determined by H^+-ion secretion into TF (29). Microperfusion (24) and microinjection (31) experiments suggest, however, that only very small amounts of PAH are lost from proximal TF, possibly by diffusion. The lower nephron segements of the rat appear to be impermeable to PAH (31, 32). In hamsters, high PAH concentrations in vasa recta blood have been attributed to back diffusion from collecting ducts (23).

PENICILLINS Penicillin derivatives, which are rapidly cleared into the urine, are assumed to be handled by the kidney by the same mechanisms as PAH. PAH is known to interfere with tubular penicillin G secretion, while probenecid has been developed as a penicillin G–retaining agent. In the only published micropuncture study (33), penicillin concentrations in all fluids were evaluated microbiologically. Penicillin G was 8% protein-bound and excreted into the urine approximately 2.5 times more rapidly than inulin. Net secretion, smaller than that of PAH, occurred along the whole length of the convoluted proximal tubules. Between distal tubules, apparently impermeable to the compound, and pelvic urine some penicillin G was lost by back diffusion or by inactivation; the loss increased with decreasing urine flow rates. Carbenicillin, which was 20% protein bound, was excreted into the urine at a rate approximately half that for penicillin G. Carbenicillin was secreted into proximal convoluted TF at a lower rate than penicillin G. Distal FD did not differ significantly from FE. In man, the urinary clearance of carbenicillin is equal to GFR; this fact suggests tubular secretion since the drug is 47% protein-bound (34).

DIURETICS Many diuretic agents are known to be concentrated in tubular cells or to reach the urine by tubular secretion (35–38). Yet, only furosemide appears to have been investigated by MP; it is secreted into

convoluted proximal TF more slowly than PAH and leaves tubular fluid by non-ionic diffusion from distal nephron segments at a very slow rate thought to be explained by the low pK_a of 3.9 (39).

SALICYLATE Salicylate, a prototype of drugs whose excretion depends to a great extent on the pH of the urine, has been thought to be excreted by glomerular filtration, proximal tubular secretion, and non-ionic backdiffusion. A micropuncture study (40) in rats in which salicylate was found to be protein-bound at 70% at low plasma levels demonstrated that considerable amounts of salicylate are secreted into the first part of the proximal convoluted tubules which is not accessible to MP (40). $FD_{salicylate}$ to puncturable superficial proximal convoluted tubules was larger in rats made alkalotic by bicarbonate infusion than in mannitol-infused controls. Since protein binding was equal in both groups, this difference denotes either enhanced secretion or depressed reabsorption in alkalosis. $FD_{salicylate}$, along proximal convoluted tubules, remained constant in alkalotic and in nonalkalotic rats. This constancy presumably reflects secretion and (active?) reabsorption of equal magnitude. Proximal net reabsorption of salicylate was demonstrated at high $P_{salicylate}$ levels (and in the presence of inhibitors of salicylate secretion). Large amounts of salicylate were reabsorbed between late proximal convoluted tubules and early distal sites in nonalkalotic rats while, under bicarbonate infusion, no salicylate was reabsorbed from the pars recta or Henle's loops. As a result, $FD_{salicylate}$ to distal tubules was much larger in the alkalotic animals than in controls; this difference is responsible for the enhancement of salicylate excretion. Though absorption from distal tubules was of the same magnitude in controls and in alkalotic rats, the (calculated) constant of reabsorption appeared to be decreased in alkalosis. No significant reabsorption of salicylate occurred between superficial late distal tubules and pelvic urine. The enhancement of salicylate excretion in bicarbonate-induced alkalosis thus appears to be due mainly to depressed reabsorption from (or to enhanced secretion into) both parts of proximal tubules and/or Henle's loops, rather than to distal non-ionic backdiffusion.

cAMP At normal plasma levels (concentrations measured by radioimmunoassay), amounts of cAMP equivalent to 170% of FL were secreted into proximal TF (41). Secreted cAMP may have been partly generated in the tubular cells because the secretion was depressed, though not abolished, by parathyroidectomy, which is known to depress cAMP generation (41). No net movements occurred across the pars recta and Henle's loops. FE_{cAMP} was smaller than distal FD_{cAMP} indicating reabsorption from the distal nephron. FE_{cAMP} was 190% of FL in normal rats but close to FL in

parathyroidectomized animals. Tubular cAMP transport did not appear to be linked to phosphate transport.

PROSTAGLANDINS Only the intrarenal transport of ^{3}H-labeled PGE_2 has been studied by microinjection techniques (42). No attempt was made to identify possible metabolites. PGE_2 was not reabsorbed from proximal or distal convoluted tubules, but was reabsorbed extensively between late proximal and early distal sites. The major fraction of PGE_2, reabsorbed from these sites after proximal microinjection, appeared to be returned into loop or distal TF and appeared in pelvic urine with some delay. Prostaglandins of the renal medulla thus could reach the luminal side of distal tubules via TF.

ANIONS OF HIGHLY LIPID SOLUBLE ACIDS The drugs belonging to this group comprise an anticonvulsant, dimethadione (DMO), a few antibacterial (sulfasymazine, sulfamerazine) or blood sugar–lowering (glymidine, previously called glycodiazine) sulfonamides and barbiturates, such as phenobarbital (19, 43, 44). The overall tubular fate of these drugs characteristically is net reabsorption, though net secretion may occur for sulfasymazine at very high urine pH (19). That an important part of the reabsorption of such drugs could occur by diffusion of the nonionic moiety from proximal TF was first suggested by the rapid disappearance of sulfamerazine and phenobarbital from proximal tubular perfusate; the rate of reabsorption depends on the pH of the perfused fluid and the liposolubility of the nondissociated acids. Nonliposoluble acids, such as PAH or 1-*p*-aminophenyl-sulfonylurea, did not diffuse out of the perfused fluid (44). Though highly lipid-soluble as an acid, N^4-acetylsulfamerazine failed to be reabsorbed from the perfusate. This fact suggests a transport mechanism more complicated than simple diffusion (44). The relative diffusion rates of all these drugs out of fluid perfused through distal tubules were similar, but the permeability constants through distal tubular walls were about five times smaller than for proximal tubules (32). The proximal tubular absorption of glymidine, which is a buffer with a pK_a (5.7) close to that of the carbonic acid system, has been extensively investigated (29, 30, 43, 45). Glymidine is reabsorbed from proximal perfusate, against an electrochemical gradient, more rapidly than HCO_3^-. The reabsorption depends on H^+ secretion and is inhibited by a specific inhibitor of H^+ ion transport, 4-acetamido-4'-isothiocyanato-stilbene-2'-disulfonic acid (SITS) (30). It thus appears to be coupled to H^+ secretion and may be used as a measure of the rate of the latter (30).

Proximal glymidine reabsorption was partially inhibited by high concentrations of carbonic anhydrase inhibitors (43% for acetazolamide) or of

furosemide (22%) (45), but not by peritubular ouabain, even in the highly glycoside-sensitive hamster (30). Replacement of peritubular sodium by Li^+ or choline strongly reduced glymidine reabsorption (45). Sodium, water, and HCO_3^- reabsorption from perfused proximal tubules is inhibited if their capillaries are perfused with HCO_3^- free solutions. Glymidine or sulfamerazine ($pK_A = 7.1$) and to a lesser extent butyrate ($pK_a = 4.8$), propionate ($pK_a = 4.9$), or acetate ($pK_a = 4.8$) may substitute for HCO_3^- in the peritubular perfusate (29, 43). This finding supports the assumption of an important role of H^+ or OH^- ion transports in proximal sodium and water reabsorption (29, 30). Possible secretory fluxes do not appear to have been investigated.

AMINO ACIDS AND THEIR METABOLITES The prevalent transport of α-amino acids is active proximal reabsorption (46), coupled to sodium reabsorption for some of them (29, 30). Metabolically nonutilized compounds, such as cycloleucine are equally rapidly reabsorbed, at different rates from different levels of proximal tubules (47). β-Alanine or γ-aminobutyric acid (GABA) and the aminosulfonic acid taurine are reabsorbed from rat proximal tubules at a slower rate by a common transport mechanism independent of α-amino acid transport (48). L-Tryptophan is secreted into as well as reabsorbed from proximal TF by an α-amino acid transport system [stop-flow experiments in dogs, isolated rabbit tubules (49) and microperfusion in rats (50)]. D-Tryptophan is secreted by a probenecid-sensitive mechanism into proximal TF (50), but is reabsorbed from distal segments at a slow rate, as is 5-OH-D,L-tryptophan (51). 5-OH-Tryptamine (HT) is equally secreted into proximal TF but does not appear to be reabsorbed subsequently; its secretion is insensitive to probenecid (50). 5-Hydroxyindoleacetic acid (HIAA) is secreted by a probenecid-sensitive mechanism into the TF of lower nephron segments. The reabsorption of HIAA, but not of other tryptophan derivatives from proximal perfusate, is enhanced by lowering the pH, i.e. by depressed ionization (51). Levodopa is rapidly reabsorbed from proximal TF or perfusion fluid by a process insensitive to decarboxylase or monoamine oxidase inhibition, but sensitive to cyanide. The 3-methoxy derivatives of levodopa, L-tyrosine and L-phenylalanine, are reabsorbed even more rapidly, but neither L-methyldopa nor dopamine is reabsorbed (52).

Organic Cations

From clearance and stop-flow experiments, it had been known that organic cations are secreted into proximal TF in various species of mammals by an apparently nonspecific transport system, entirely distinct from that for organic anions, and inhibited by other drugs (e.g. the cyanine dye No. 863).

The cations secreted comprise quaternary ammonium compounds, amines, various aliphatic and aromatic substances, and a few compounds with more than one positive charge (53). Cations secreted proximally may undergo (diffusional?) reabsorption in lower nephron segments. Cations, which can be transformed into more or less liposoluble bases by losing a proton, may be reabsorbed by non-ionic diffusion. Amphetamine is the prototype of a drug, the excretion of which is considerably enhanced by acidifying the urine. Organic cations comprise many pharmacologically active compounds. Unfortunately, very few MP studies are available.

N^1-Methylnicotinamide in the rat is secreted into proximal TF, but not into lower nephron segments, by a process inhibited by mepiperphenidrol, a quaternary ammonium compound. Reabsorption from proximal, but not from distal, TF was demonstrated by microinjection experiments. The compound is not transported across the walls of Henle's loops, distal tubules, or collecting ducts (54). Choline, a quaternary compound, is reabsorbed from proximal tubules, Henle's loops, but neither from distal tubules nor from collecting ducts of rats at its low physiological plasma concentration. If $P_{choline}$ is raised thirty times, net secretion can be demonstrated to occur in the first part of the proximal tubules, while no net movements (possibly secretion and reabsorption at the same rate) occur in the lower proximal convoluted tubule and an additional amount may be secreted before the TF reaches early distal sites (presumably into pars recta TF). Metabolic utilization of choline may play a role in the reabsorptive process (55). (Although biogenic amines are bases, they are discussed under amino acids.)

Nonpolar Compounds

Very few MP studies have been published. The renal excretion of only one cardiac glycoside has been studied by MP (56); ^{3}H-labeled digoxin in rats is reabsorbed from proximal TF, but not from Henle's loops (56). Distal $FD_{digoxin}$ did not differ significantly from $FE_{digoxin}$, although the mean value of the latter does not exclude the possibility of an entry into collecting duct fluid (56). In microinjection experiments in rats, the urinary recovery of digoxin was 62% after early proximal and 87% after late proximal injection, suggesting reabsorption from the proximal convoluted tubule. It should be remembered that, as judged from clearance studies, nonpolar compounds such as creatinine may be transported by anion or cation transport systems or both (53).

PEPTIDES Proximal microinfusion studies in rats demonstrated that 80–90% of infused labeled angiotensin II (57), angiotensin I (58), or bradykinin are reabsorbed from tubular fluid. Since the radioactive material appearing in urine after tubular injection of the peptides contains mainly smaller

peptide fragments, the proximal reabsorption of angiotensins and kinins is assumed to occur after cleavage by brush border enzymes, by reabsorption of amino acids or peptide fragments. When infused into distal tubules of rats, angiotensin II and bradykinin are recovered nearly completely and unsplit in the urine (57–59). The data do not exclude the possibility of a proximal tubular reabsorption of unsplit bradykinin or angiotensin II. Proximal infusion of excess unlabeled bradykinin, or of angiotensin I, depresses the reabsorption of labeled bradykinin to the same extent, while only excess unlabeled bradykinin depresses the intratubular hydrolysis of the labeled compound (60). Bradykinin is also split and reabsorbed by isolated perfused straight segments of rabbit proximal tubules in vitro (61), but oxytocin is not. Since peptide fragments have been found in the urine after infusion of oxytocin into the renal artery, the peptide may be split elsewhere in the kidney.

In constrast to the rapid tubular cleavage of 8 to 10 amino acid peptides, sheep growth hormone, a large peptide, like proteins, appears to be reabsorbed by a mechanism involving prolonged endocytosis and intracellular lysosomal degradation (62).

Conclusions

Many drugs are transported through the walls of proximal convoluted tubules in both directions. Proximal net secretory or net reabsorptive transports observed under a given set of conditions may generally represent the sum of simultaneous absorption and secretion, as demonstrated for uric acid (1) or for choline (55). Transport in either direction may be diffusional (and may furthermore be restricted to non-ionized acids or bases) or carrier mediated. Transport against an electrochemical gradient may depend on coupling with, or countertransport against, "active" transport of H^+ (or OH^-), or Na^+ ions. Some compounds are secreted, or reabsorbed, predominantly in the first part, or else in the pars recta of proximal tubules. MP studies reinforce doubts about the exclusive role of two putatively nonspecific proximal secretory mechanisms for anions and cations (53). Transtubular movements of all ionic drugs could, however, depend on primary movements of H^+ or OH^- ions (29). This working hypothesis could eliminate the uncomfortable assumption of transport mechanisms for nonphysiological drugs, but could not easily explain transports of nonpolar compounds. Henle's loops may absorb drugs or concentrate them in medullary tissue by countercurrent exchange. Distal tubules and collecting ducts tend to be less permeable to drugs than the upper nephron. Eight to ten residue peptides may be split at the luminal surface of tubular cells. Other drugs may enter these cells from either TF or the blood and may then be metabolized or deposited in the cells.

EFFECTS OF DRUGS ON TRANSTUBULAR TRANSPORTS OF ORGANIC COMPOUNDS

Uric Acid

Urate is actively transported into and out of proximal TF; secretory transport in some species extends into the pars recta. Many drugs interfere with these transports (reviewed in 1). Proximal reabsorption in the rat is inhibited by benziodarone, benzbromarone, indanylacetic acid, pyrazinoic acid, PAH, and probenecid, but not by chlorothiazide (1). The secretory flux is inhibited by probenecid (63), as well as by furosemide, pyrazinoic acid, and PAH (1). In the Dalmatian coach hound, pyrazinamide inhibits net secretion of urate in convoluted and straight proximal tubules. In monkeys, the prevalent reabsorption of urate from proximal TF is inhibited by derivatives of probenecid, and the secretory flux by pyrazinamide (1). Many drugs investigated therefore appear to inhibit the secretory as well as the reabsorptive transport, the net effect in each nephron segment depending on the species investigated and on experimental conditions. The apparent absence of an effect on uric acid transport may conceal a simultaneous inhibition of both fluxes (1). Either the carriers or the mechanisms of coupling to other more basic transport systems appear to be quite similar for the secretion and the reabsorption of urate.

Oxalic Acid

Oxalate has been shown by various MP techniques to be both secreted into and reabsorbed from proximal TF, while lower nephron segments appeared to be impermeable. The secretory flux which may be localized in the very first part of the proximal convoluted tubules is inhibited by PAH (64) but apparently not by large doses of probenecid (28).

Sugars

Only the well known inhibitory effects of phlorrhizin analogues on glucose reabsorption have been investigated by MP (29).

EFFECTS OF DRUGS ON GLOMERULAR FILTRATION

MP techniques, and a new method for measuring intracapillary pressure applied to glomeruli at the surface of certain strains of rats or monkeys, permit a precise description of the process of glomerular filtration. This has been extrapolated from indirect evidence to the glomeruli of other species. Methods for assessing the determinants of GFR and for elucidating the mechanism of action of drugs are now available (65, 66). Glomerular filtra-

tion is driven by the net ultrafiltration pressure, that is, the difference between the intracapillary hydraulic pressure of approximately 45 mm Hg in normally hydrated normal rats (higher in a strain of rats with hydronephrosis) and the sum of the opposing hydraulic pressure in Bowman's space (10 to 12 mm Hg) and the intracapillary oncotic pressure. The fall of the intracapillary hydraulic pressure from one to the other end of the glomerular capillaries is very small; constriction of efferent arterioles, therefore, cannot be expected to raise the intracapillary pressure (unless it results in complete closure of the efferent arteriole). From the afferent to the efferent pole the oncotic pressure increases from 20 to 35 mm Hg by the abstraction of filtrate; filtration equilibrium, thus, is reached before the end of the glomerular tuft in normal hydropenic rats. A two- to threefold increase of the plasma flow rate (normally 75 nl/min·glomerulus) induces filtration disequilibrium; at the end of the tuft, the net ultrafiltration pressure increases from approximately 5 to 10 mm Hg. Consequently, SNGFR rises with blood flow within this range; further increases of blood flow fail to cause proportional increases of SNGFR. At filtration disequilibrium, it becomes possible to measure the glomerular ultrafiltration coefficient, that is, the product of the capillary water permeability and the surface area available for filtration; the normal value in rats is approximately 0.08 nl per second per mm Hg per glomerulus. Inserting a value for the filtering surface obtained by morphological measurements one finds that the water permeability of glomerular capillaries is 1 to 2 orders of magnitude higher than values reported for other blood capillaries. Glomerular filtration, thus, is a process driven by a small pressure head through a highly water-permeable filter (65, 66).

Drugs or control mechanisms may modify SNGFR by altering one of its determinants. Increases of glomerular blood flow induced by plasma volume expansion or isovolemic reduction in hematocrit will increase SNGFR. Increases of glomerular blood flow by vasodilator drugs, such as acetylcholine, PGE_1, or bradykinin, fail to increase SNGFR (66), because these agents depress the ultrafiltration coefficient by an unknown mechanism. Decreases of plasma protein concentration should increase net ultrafiltration pressure and SNGFR. In fact, plasma dilution induces only minor increases of SNGFR because hypoproteinemia depresses the ultrafiltration coefficient by an equally unknown mechanism. Conversely, increases of blood protein concentrations from low to normal values entail a smaller than expected fall of SNGFR, presumably because plasma proteins increase the ultrafiltration coefficient (66). Other high molecular substances such as dextran apparently do not have the latter effect. Changes of the glomerular ultrafiltration coefficient, of course, may represent either changes of the water permeability or of the filtering surface, as would occur, for example,

by constriction or dilatation of branches of the capillary network. Antidiuretic agents such as arginine-vasopressin, (1-deamino, 4-val)-8-D-arginine-vasopressin, or dibutyryl-cAMP increase net ultrafiltration pressure by depressing Bowman's space hydraulic pressure (an effect opposite to that of hydronephrosis) but fail to increase SNGFR because they simultaneously depress the ultrafiltration coefficient (67). Among the drugs known to depress SNGFR, gentamycin at mean doses in rats depresses SNGFR by lowering the ultrafiltration coefficient (68). Very high doses of the drug also depress glomerular blood flow rate and the transcapillary hydraulic pressure difference.

Drugs may, of course, modify GFR by interfering with extrinsic control systems. SNGFR also appears to be controlled by intrinsic feedback regulation within each individual nephron (reviewed in 69); an increased saline flow rate through Henle's loops and the early distal tubule depresses SNGFR. The sensory element of this control system appears to be located in the early distal tubule, possibly in the macula densa. The signal perceived by the sensor is hypothesized to be the chloride flux through the walls of the early distal tubules and bromide appears to be as effective as chloride. Among the chlorides tested, those of divalent cations, lithium and choline, induced feedback depression of SNGFR less reliably than other chlorides. A decrease of SNGFR in response to increased TF flow through the early distal tubule is generally thought to be due to afferent arteriolar constriction and the consequent decrease of glomerular intracapillary pressure and plasma flow. A depression of the glomerular ultrafiltration coefficient, possibly due to a reduction of the number of parallel channels in the glomerular tuft, could also play a role (69). The feedback transducer of the intrinsic control system has been suggested to involve a mysterious activation of intrarenal renin inducing an increased concentration of angiotensin II around afferent arterioles. Though a number of observations support this concept, the majority of the data available do not appear to be compatible with this assumption (69). The utter complexity of the methodology involved, however, creates so many possibilities of error that a definite judgment becomes difficult. Other intrarenal autacoids that could be involved are calcium, cAMP (a vasodilator), adenosine (a renal vasoconstrictor), as well as renal prostaglandins (69).

The decrease of SNGFR, in response to increased early distal TF flow rate is inhibited by a number of diuretic agents that depress loop or early distal chloride reabsorption, such as furosemide (71), triflocin, or cyanide (70). Amiloride, though interfering with sodium and chloride reabsorption at these sites, does not block feedback depression of SNGFR. Acetazolamide, ethacrynic acid, or mercurial diuretics are also inactive, presumably because they fail to influence chloride reabsorption, either at the specific

sensor site, or in the rat nephron (70). The feedback control system of SNGFR is, furthermore, said to be blocked by antagonists of angiotensin (which, however, are also partial agonists) and by propranolol. Verapamil, a vasodilator drug known to block the entry of calcium into smooth muscular cells, also interferes with the feedback response (69). Theophylline and 3-isobutyl-1-methylxanthine, when added to loop perfusion fluid, block the feedback depression of SNGFR, although they do not interfere with chloride reabsorption; dibutyryl-cAMP in large concentrations, but not cAMP, has a similar, though smaller effect (72). Inhibitors of prostaglandin synthesis may also inhibit feedback depression of SNGFR (69). Substances interfering with the feedback depression of SNGFR should, of course, increase SNGFR and GFR, particularly under conditions of reduced GFR due to enhanced tubular flow or enhanced chloride reabsorption from the ascending part of Henle's loops.

DRUG EFFECT ON TRANSTUBULAR SODIUM, CHLORIDE, AND WATER MOVEMENTS

MP data on diuretics have been reviewed elsewhere (35–38, 73, 74). MP technique as applied to pharmacology of diuretics (75) and the use of diuretics as tools for interpreting electrophysiological data on Na transport (76) were equally reviewed, as was the depression of Na and water reabsorption by many metabolic inhibitors and drugs reacting with $-NH_2$ or –SH groups (77). SH-reagents of small molecular size such as N-ethyl-maleimide, *p*-chloromercuribenzoate and p-chloromercuriphenylsulfonate (non-diuretic mercurials) and mersalyl (a mercurial diuretic) inhibited Na reabsorption from perfused rat proximal tubules into perfused capillaries more effectively from the capillary than from the luminal side, while large molecule –SH inhibitors including *p*-chloromercuribenzoate-dextran and benzoxanthene-3,4-decarboxylic-N-iodoacetyloligoprolyl-2-aminoethylimide were ineffective, as was a specific COOH-reagent and two substances specifically reacting with amino groups, dansyl chloride and SITS (78). Enzymes involved in Na reabsorption, therefore appear to contain important SH groups and to be located in the depth of the basolateral membrane (78).

Several mechanisms are thought to participate in proximal Na reabsorption: (*a*) coupling to H^+ ion secretion, (*b*) electrogenic Na^+ transport independent of the presence of substrates for cotransport, (*c*) electrogenic transport dependent on the intratubular presence of glucose or amino acids, and (*d*) nonelectrogenic transport of a fraction of proximal TF Na. Electrophysiological evidence suggests that acetazolamide incompletely inhibits H^+ secretion and HCO_3^- reabsorption, that the proximal effect of furose-

mide may be due to inhibition of the H^+-coupled Na reabsorption, that mersalyl may primarily inhibit the glucose-coupled fraction of sodium reabsorption, and that ouabain blocks the glucose-independent electrogenic Na^+ transport (79). Microperfusion experiments imply that only 25% of Na^+ transport is ouabain-sensitive (80). As shown by free-flow MP, ouabain injected into the renal artery inhibits the distal reabsorption of Na *and* of K (81). The hallucinogenic drug, harmaline, has been suggested to block specifically Na^+-coupled cotransport mechanisms in the luminal cell membrane of the small intestine and of proximal tubules. When applied from the capillary side in double-microperfusion experiments, the drug blocks active Na^+ transport and possibly H^+ secretion, but neither competes with Na for transport sites nor specifically inhibits brush border cotransport systems (82).

A number of recent micropuncture studies on water diuresis induced by lithium salts (83–85) and diuretic effects of calcium (86–91) or magnesium (92–95) cannot be reviewed here because of lack of space.

RENAL EFFECTS OF HORMONES

These effects have recently been reviewed (96).

Vasopressin

Both the antidilutional and antidiuretic effect of small doses of vasopressin and the more obscure natriuretic effect of large doses of the hormone have been investigated by MP techniques [(97–99), reviews: (100–102)]. A number of drugs, namely vasopressin analogues, nicotine, narcotic analgesics (mainly morphine), chlorpropamide and possibly other blood sugar–lowering sulfonylureas and biguanides, clofibrate, tricyclic antidepressive agents, neuroleptics and antineoplastic agents such as vincristine or cyclophosphamide, possess vasopressin-like antidilutional effects (103). The mechanism of action of these agents, and of drugs capable of inducing nephrogenic diabetes insipidus (104), such as tetracyclines, methoxyfurane, propoxyphene, or the vasopressin antagonist vasopressinoic acid, have not been investigated by MP.

Adrenocortical Hormones

Renal effects of gluco- and mineralocorticoids have been studied by MP [reviews: (102, 105)]. Spironolactone has been shown to enhance urinary Na excretion of adrenal-enucleated, salt-loaded rats by depressing Na reabsorption from either collecting ducts or from juxtamedullary nephrons, while dexamethasone increased the filtered Na load (106).

Parathyroid Hormone

Effects of this hormone on the renal excretion of phosphate, calcium, and sodium have been reviewed elsewhere (107, 108).

Vasoactive Hormones and Autacoids

CATECHOLAMINES The discovery that β-adrenergic agonists, in contrast to α-adrenergic agents, depress urine flow and sodium excretion (109), and investigation of the particular renal effects of dopamine, renewed interest in this field and led to MP studies.

Systemic norepinephrine increases the urinary excretion of solute free water in man, dogs, and rats (blocked by α-adrenergic blocking agents); systemic isoprenaline exerts the opposite effect (blocked by propranolol) (110). In man and in dogs, both effects appear to be mediated by changes in pituitary vasopressin secretion (110). In unanesthetized water-loaded rats, norepinephrine, but not epinephrine, increases C_{H_2O}. This effect is *not* suppressed by phentolamine or propranolol (111). Isoprenaline has the opposite effect. Epinephrine, however, has an antivasopressin effect in anesthetized rats: It depresses solute free water reabsorption (112). In isolated rat kidneys perfused at constant pressure, both epinephrine and norepinephrine increase C_{H_2O}, and the effect is blocked by propranolol but not by phenoxybenzamine. Curiously enough, isoprenaline and phenylephrine had similar effects (113). Antivasopressin effects of norepinephrine could be explained by the finding that in the isolated papilla, norepinephrine blocks the stimulation of cAMP production induced by vasopressin (114). This effect is inhibited by phentolamine. Unfortunately, in the same experiments, neither norepinephrine nor isoprenaline had any influence on the water permeability of collecting ducts (114). The Na excretion of dogs is not modified by norepinephrine, epinephrine, or isoprenaline infused into the renal artery (110). Mixtures of either phenoxybenzamine + isoprenaline or propranolol + noradrenaline failed to affect Na excretion, SNGFR, proximal Na and fluid reabsorption, or the intrarenal distribution of GFR (115, 116). In dogs, i.v. phenoxybenzamine appears to be natriuretic without affecting RBF or GFR and without depressing proximal Na and fluid reabsorption (117). The diuresis could be unrelated to the α-receptor blocking effect of the drug. In rats, pressor doses of epinephrine or of norepinephrine are consistently natriuretic (111, 118). Proximal reabsorption is not impaired (118) but Na reabsorption from the ascending limb is depressed (112). This natriuretic response depends on an increase of systemic blood pressure and can be prevented by an aortic clamp (112), as can be the natriuretic effect of acute hypertension induced by other means in dogs

(119). Pressor doses of norepinephrine in rats bring about a fall of glomerular capillary and efferent arteriolar pressure while equipressor doses of epinephrine have no such effect (120). Systemic isoprenaline in rats is antinatriuretic (111, 121) and depresses GFR and proximal reabsorption (121). This decrease is entirely compensated for by increased reabsorption from the loop. The antinatriuretic effect appears to be due to an enhancement of distal Na reabsorption, which has been demonstrated to occur when orciprenaline (111) or isoprenaline (121) was added to droplets placed into distal tubules. Isoprenaline has also been reported to redistribute GFR from superficial to deep nephrons (122). The effect of norepinephrine secreted by renal nerve endings could be the opposite of that induced by pressor doses of systemic norepinephrine: Acute denervation induces diuresis and natriuresis by inhibiting sodium and fluid reabsorption from proximal tubules and possibly also from Henle's loops (123, 124).

Dopamine infused i.v. into dogs increases Na^+ and water excretion but neither GFR nor proximal reabsorption. It has, therefore, been postulated to act on the lower nephron (125). In rats, the diuretic effect of dopamine also is due mainly to a distal effect (126); dopamine increases GFR but also depresses proximal reabsorption by a luminal effect that is abolished by luminal propranolol (126). In rats, dopamine at doses that do not influence systemic blood pressure increases glomerular capillary and efferent arteriolar pressure (120).

ANGIOTENSINS Angiotensins exert a natriuretic effect prevalent at higher doses with extracellular expansion, an antinatriuretic effect with depression of GFR prevailing with extracellular contraction and in some species (dog), and a vasopressin-like antidiuretic effect; they also induce proteinuria [review: (127)]. Diuresis induced by pressor doses of angiotensinamide in expanded rats is due to diversion of GFR to juxtamedullary nephrons and possibly to an inhibition of proximal tubular reabsorption in these deep nephrons (128). Other investigators (129) also found an inhibition of proximal and ascending limb reabsorption. In contrast to older negative results (130), capillary and tubular microperfusion experiments demonstrated an inhibition of proximal fluid reabsorption by peritubular val^5-or $ileu^5$-angiotensin II (131, 132). The inhibitory effect could be related to a simultaneous increase of hydraulic pressure in the peritubular capillaries (131) due to a decrease of their diameter (133). Smaller doses of angiotensin may stimulate proximal sodium reabsorption (132). Subpressor, but nor pressor, doses of angiotensin II may depress SNGFR. Glomerular plasma flow is depressed, but the hydraulic pressure gradient across glomerular capillary walls increases, while the glomerular water permeabil-

ity coefficient is considerably depressed (134). Angiotensin-induced proteinuria appears to be due to increased glomerular filtration; this is partly explained by the increased hydraulic pressure, but also by protein permeability changes of the glomerular membrane (135, 136).

ACETYLCHOLINE The natriuretic effect of acetylcholine in dogs is accompanied by a minor increase of GFR, and a large increase of SNGFR, and is due to a decrease of proximal reabsorption (137). When proximal reabsorption is blocked by blood volume expansion, acetylcholine depresses reabsorption from distal tubules (138). In rats, acetylcholine-induced natriuresis is also accompanied by delayed proximal reabsorption, as measured by the split drop technique; other investigators (139), however, found no depression. GFR in acetylcholine-diuretic rats was depressed by 50% (140). In rats, nondiuretic doses of acetylcholine that did not depress SNGFR increased glomerular plasma flow rate so that filtration equilibrium was not reached at the end of glomerular tufts, while the capillary ultrafiltration coefficient was significantly reduced (141).

BRADYKININ In dogs, bradykinin is natriuretic and depresses SNGFR in some but not in all nephrons (142). Bradykinin does not depress proximal reabsorption (139, 142); its diuretic effect must be due to changes in superficial distal or deep nephron reabsorption. The effect on SNGFR is the same as that of acetylcholine (141).

OTHER PEPTIDES Substance P infused into the abdominal aorta of rats is natriuretic and depresses proximal reabsorption without influencing SNGFR or intrarenal pressures (143). Secretin markedly increases renal blood flow in dogs but is only slightly natriuretic. GFR or blood pressure are not altered while peritubular capillary and free-flow intratubular pressure are increased (144).

PROSTAGLANDINS PGE_1, PGE_2, $PGF_{2\alpha}$, but not PGA_2, induce natriuresis when infused into the renal artery of rats or dogs while prostaglandin synthetase inhibitors have no consistent effect (145–148). Proximal reabsorption is not inhibited by PGE_2 in rats or dogs (147) but is inhibited by PGE_1 in dogs (148). PGE_2-diuresis thus could be due to an inhibition of distal or collecting duct reabsorption (147, 148). An inhibition of Na reabsorption was observed in rats after microinjection of PGE_2 and radioactive Na into late distal tubules (149). PGE_2, $PGF_{2\alpha}$, or PGA_2 do not affect Na reabsorption from isolated perfused thick ascending limbs or collecting tubules of rabbits (146). This observation does not exclude an effect at this level in the rat kidney. In dogs PGE_1 and PGE_2 increase peritubular capil-

lary hydraulic pressure and depress peritubular oncotic pressure (148). The effects of PGE_2 on glomerular filtration are similar to those of bradykinin (141).

Vasodilator hormones and autacoids (and perhaps other vasodilator substances) therefore increase renal plasma flow, depress the filtration fraction, and exert natriuretic effects either proximally or distally or on collecting ducts that may be related to changes of intrarenal "physical forces."

DRUGS AND HORMONES AFFECTING K^+ AND H^+ (HCO_3^-) TRANSPORTS

The field is covered by recent reviews on potassium homeostasis (150, 151), on corticosteroids (102, 105), on the effect of diuretics on K^+ excretion (152), and on drug effects on bicarbonate reabsorption (153).

CONCLUSION

The use of MP techniques, including microperfusion and microinjection, in addition to other methods of renal physiology is a prerequisite for understanding the mechanisms of the renal excretion of drugs and their metabolites, as well as of the actions of drugs on the renal excretion of water and solutes. Yet, because of their complexity, MP methods have as yet been applied only to the solution of a very few problems of renal pharmacology. Recognition of the need for more micropuncture studies has induced the establishment of a large number of new groups of micropuncture workers.

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