RENAL PHARMACOLOGY: COMPARATIVE, DEVELOPMENTAL, AND CELLULAR ASPECTS

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Understanding the mechanism of action of drugs requires a penetrating insight into the functioning not only of the organ itself but also each of its cells. Therefore, in this review the findings of biochemical and biophysical investigations as well as the results of studies on the ultrastructure of nephron cells and their functional analogs have been used. The data on the kidney comparative pharmacology and physiology are of primary importance for comprehending tendencies in the development of the renal transport systems and evolution of the kidney.

The comparative approach, in the broad sense, suggests a comparison of the kidney function in animals at different evolutionary stages, work of cells from various nephron parts and other organs specially adapted for ion transport (amphibian skin and urinary bladder, gills, etc). Comparison of the data on the function of kidneys in phylogenesis, with the results of study of changes in the renal activity in ontogenesis, permits discussion of principles of development of various renal functions.

This review is confined to an analysis of the effect of diuretics and some hormones on kidneys in various classes of vertebrates, in animals in postnatal ontogenesis, and on isolated biological membranes, the functional analogs of renal tubules. In this connection interesting reviews and monographs have appeared in the last few years (1–5).

NEW DATA ON VERTEBRATE NEPHRON STRUCTURE

Morphological investigations of recent years have made alterations in classical concepts of the vertebrate kidney structure (6, 7). In nephrons of the lampreys *Lampetra fluviatilis* (8, 9) and *Petromyzon marinus* (10) there are not only proximal tubules and collecting ducts, as has been previously suggested, but also intermediate and distal tubules. Cytochemical characteristics (enzymes, mucopolysaccharides) of

lamprey renal tubules are similar to respective parts in kidneys of other vertebrates (8, 11). Distal tubules have been found in kidneys of a few marine teleosts *Spicara smaris* L., *Mugil cephalus* L., *Myoxocephalus scorpius* L. (12, 13). A short distal tubule has been detected even in the aglomerular *Nerophis ophidion* (14) but is absent in kidneys of *Pleuronectes platessa* (15) and *Lophius piscatorius* (16). Such features of the kidney anatomy (and involved functional properties) as the medulla in birds and mammals, and the renoportal system in fishes, amphibians, reptiles, and birds, are of great importance for assessing the action of various drugs on the kidney.

ELEMENTS OF TRANSPORT SYSTEM IN KIDNEY CELL

Studies of the kidney of mammals and amphibians, and the skin and urinary bladder of amphibians are aimed at elucidating functional organization of the ion transport system and especially that of Na in the cell. Filtered Na enters the nephron lumen. Cells of the proximal tubule reabsorb Na against a small gradient: in *Necturus* at a concentration over 66 meq/liter, in rats, over 95 meq/liter (17). The essential difference between lower and higher vertebrates is the volume of their proximal reabsorption. At the proximal convoluted tubule sites accessible to micropuncture, (TF/P) in [(tubular fluid/plasma) inulin] makes up 2.61 (18) and 2.53 (19) in rats and 1.75 (20) in dogs, whereas in the frog *Rana temporaria* even in final urine (u/p) in equals 1.3 (21). The lower level of filtration and the lesser volume of proximal reabsorption are typical of cold-blooded vertebrates (22).

During reabsorption Na enters the cell from the lumen through the apical membrane, passes across the cell, and discharges to blood with the aid of pumps located in the lateral and basal plasmatic membranes. Analysis of properties of various elements of the Na transport system is of particular significance for renal pharmacology, because their difference enables us to choose appropriate drugs for each element.

In accord with widespread opinion, Na entrance into the cell from lumen is a passive process (23). The same explanation is offered for Na entrance through the apical cell membrane of amphibian urinary bladder and skin (24). However, there are different points of view as to how this occurs (25, 26). Thus it is suggested that the apical membrane of the active layer of frog skin cells contains a sodium pump (27). According to another hypothesis, Na moves along the outer membrane through the intercellular junction (28). The latter hypothesis is at variance with the data presented on the half-period of Na wash-out from the transport pool at the action of ouabain (29). These evidences show that Na is transferred to the cell cytoplasm.

It is believed (23, 24, 30) that in the renal tubule cell and its functional analogs, Na channels and ion pumps are spatially separated. This is also evidenced by the difference in chemical properties of the apical and basal plasmatic membranes (31, 32) and the tubular cell ultrastructure. These findings corroborate the assumption that Na channels localize in the apical membrane and ion pumps in the basal plasmatic membrane. There are many infoldings and a great number of mitochondria at the base of the nephron proximal tubule cell of mammals (33), in the

distal segment of fishes (7), amphibians (22), and mammals (34). The basal infoldings and mitochondria in the proximal tubule are not numerous in lamprey and frogs, which show a low level of proximal reabsorption, but are abundant in the nephron proximal and distal tubules of animals with intensive Na transport (22). The ultrastructure of mitochondria depends not only on the amount of Na reabsorbed but also on the gradient against which Na is transferred. In the cells of the distal tubles of lamprey (9), frogs, and rats (22), where Na is transferred in lesser amount but against a larger gradient, each mitochondria contains more crysts than the proximal tubule cell. The ultrastructure of the nephron cell correlates with the Na transport level, this fact speaking in favor of transcellular rather than paracellular Na transport in the kidney. The high permeability of tubular walls, however, must affect the activity of the nephron. In this context, the paracellular transport of substances from the tubular lumen to the peritubular space and in the opposite direction is discussed (23, 35, 36).

The question of the size and meaning of the Na transport pool is open to argument. The Na transport pool constitutes a lesser portion of the total amount of Na in the cell (26, 29, 37). Cell Na content changes at the action of hormones and diuretics. As shown by X-ray microanalysis, the intracellular content of Na, Cl (but not K) increases in both limbs of the loop of Henle from the cortex to the medulla. The Na intracellular content is higher in the thin descending limb than in the thick ascending one. This difference disappears during furosemide diuresis (38).

Nephron cells have several types of sodium pumps, among them an Na/K exchange pump and an electrogenic pump (39). It is also suggested that Na reabsorption is provided by the neutral NaCl pump rather than by the electrically coupled Cl^- transport following Na^+ (40). Bicarbonate plays the important part in Na reabsorption (41), as in its absence NaCl reabsorption is nearly as small as that after kidney poisoning with ouabain (42).

The K transport mechanism in the tubules differs from that of Na. There are considerable specific variations of K reabsorption in the proximal tubules of various animals. The use of selective liquid ion-exchange microelectrodes shows that TF/P_k in the last convolution of the proximal tubule is 0.8 ± 0.01 in rat (43) and 1.9 ± 0.2 in *Necturus maculosis* (44). In *Amphiuma,* filtered K does not reabsorb in the proximal tubule (45), as it does in rats.

Cells of the distal tubule play a decisive role in K excretion. The interrelations between K active transport to the cell on the luminal and peritubular membrane and K passive secretion through the apical membrane determine the amounts of K excreted (39, 44, 46). The K transport pool of the distal tubule cell makes up a smaller portion of the cellular K. The K transport pool is enhanced significantly upon transition from K reabsorption to secretion (45). A considerable K secretion is observed at the action of acetazolamide or after the maintenance of *Amphiuma* (45) and *Rana temporaria* (47) in a medium with high K concentration. Under K load the activity of the ion in the distal tubule cell of the rat kidney increases from 46.5 ± 9.6 m*M* to 60.5 ± 2.1 m*M* (48).

The transport systems of organic acids are probably similar in kidneys of most vertebrates. PAH is secreted by the kidneys in lamprey (49), cartilaginous (50) and

bony (51) fishes, and amphibians (52). Probenecid decreases PAH transport in kidneys of rabbits (53, 54) and that of mercurial diuretics in kidneys of mammals and fishes (55). Probenecid inhibits the effect of arginine vasotocin in the kidneys of water snakes and frogs as well as the transport of organic acids. It fails to inhibit the hydroosmotic effect of theophylline on the frog urinary bladder, affecting it probably at the level of the tissue receptors of neurohypophyseal peptides (56). In the chick kidney PAH transport may be blocked with novobiocin. Its action is similar (though not identical) to that of probenecid (57).

COMPARATIVE STUDY OF DIURETIC ACTION

Ethacrynic acid decreases Na reabsorption with kidneys of rats (58), dogs (59, 60), chickens (61), frogs (21), marine fishes (62), and lampreys (63). In rabbit, ethacrynic acid affects both kidney and intestine (64), and influences the ionic content of the inner ear endolymph (65).

Furosemide inhibits Na reabsorbtion in the kidneys of various mammals (66), birds (63), amphibians (21, 67), fishes (62, 68), and cyclostomes (63). Hydrochlorothiazide, mercaptomerin, aminophylline, and ouabain decrease ion excretion by the chick kidney (61). The natriuretic effect of hydrochlorothiazide in rats is much higher than in hens (69). Brinaldix induces Na excretion in rats (70), dogs (71), *Oncorhynchus nerka, Salvelinus malma,* and *M. scorpius* (72). After the 1–10 mg/kg brinaldix injection the Na excretion fraction (EF_{Na}) increases from 1.6 to 5.1% in rat and from 9 to 24% in *S. malma* (72). Acetazolamide inhibits Na reabsorption in the kidneys of mammals, birds, alligators, frogs, some fishes, and lampreys (3, 73, 74), in the gills of fishes (75, 76), and in the turtle urinary bladder (77).

Amiloride enhances Na excretion by the kidneys of rat (78), dog (79), and man (80), and inhibits Na transport in the colon of amphibians (81), in erythrocytes (82), and in cells of salivary gland ducts (83). At the same time amiloride does not produce any effect on Na transport in the gills of goldfish, while in eel Na leakage is increased (84). Triamterene induces natriuresis and decreases K excretion in man (85, 86), the water snake *Natrix cyclopion* (87), and others.

Thus components of the Na reabsorption system sensitive to the action of various types of modern diuretics are found in representatives of all classes of vertebrates. As a rule diuretics increase EF_{Na} in lower vertebrates more sharply than in mammals, despite the fact that Na excretion is lower when counted by weight or body surface. This is caused by a lesser glomerular filtration rate (GFR) and consequently a lesser load on the nephron.

Experiments on amphibian skin and urinary bladder membranes are of particular interest for studying cellular action mechanisms of diuretics. The response of cells of these organs and the nephron cells to hormones is similar (88), whereas their reaction to diuretics may vary. As demonstrated in experiments with frog skin, hydrochlorothiazide, furosemide, and salyrgan increase short circuit current (SCC) (89). In skin of the toad *Bufo bufo,* furosemide and salyrgan decrease SCC (90). In the skin of *Rana temporaria,* furosemide does not affect SCC while mercusal and

ethacrynic acid inhibit it (91). In toad skin ethacrynic acid enhances but furosemide decreases Na transport (81). Mercurial diuretics increase Na transport in the frog skin (89, 92) and reduce it in the toad urinary bladder (93). Furosemide and ethacrynic acid diminish SCC and increase dc resistance of membrane in the toad urinary bladder (94). As the effects of furosemide and ouabain are not additive (95) it is supposed that their action mechanisms are similar. One cannot admit it without reserve, because in the skin of *Rana temporaria* SCC is inhibited by various cardiac glycosides (96) but is not changed upon addition of furosemide (91, 97). In the frog cornea, furosemide inhibits SCC. The effect of furosemide is probably determined by its action on Cl transport (97). Brinaldix inhibits SCC of the frog skin (98) by increasing the osmolarity induced by polyethylenglycol 400, which has been used as a clopamide solvent in the ampule with brinaldix.

SODIUM PERMEABILITY: ITS ACTIVATION AND INHIBITION

Among saluretics, amiloride and triamterene differ qualitatively by the sites of their action on the cell (99). They inhibit Na transport when added to the solution at the outer surface of the skin and urinary bladder cells. They begin to act immediately and Na transport is restored after the washout (100, 101). The same degree of inhibition is observed after the addition of $4 \cdot 10^{-7}$ *M* amiloride and 10^{-4} *M* triamterene (100). Amiloride interacts with a certain component in the cell apical membrane characterized by saturation kinetics (102). As a result Na influx to the cell is decreased (103, 104). Pumps, however, continue discharging Na from the cell so long as the transport pool is exhausted (105). The percentage of Na transport inhibition induced by amiloride is not dependent on the original level of SCC (106) being observed at any Na concentration in the mucosal solution (107). As has been demonstrated on erythrocytes, amiloride inhibits passive influx of Na to the cell without affecting the efflux (82). After amiloride is added, the lowering O_2 consumption is mainly caused by the decrease of Na transport (108, 109). Amiloride itself does not influence O_2 consumption in concentrations required for Na transport inhibition (110). It appears that amiloride prevents Na entrance to the Na transport compartment without affecting the pump (37). The interaction of amiloride with the membrane receptor increases in the presence of bivalent ions in solution near the outer surface of the frog skin and decreases in a solution without Ca. It is probable that amiloride, Ca, and membrane receptor make up a complex preventing Na entrance to the cell (111). In the toad urinary bladder the membrane component to which amiloride is bound is removed with amphotericin B (112).

In the presence of amiloride, hormones (aldosterone, insulin, vasopressin) enhance Na and water transport through the wall of the toad urinary bladder and skin (112). The action of vasopressin (104) and other hormones is connected with the increase of Na permeability of the cell apical membrane. Amphotericin (113) and nistatine (114) also increase K permeability of the cell membrane of the frog skin. The distinguishing feature of amiloride and triamterene effects is their ability to enhance natriuresis at the same or diminished level of K excretion (85, 115). These drugs reduce Na reabsorption in the distal tubule (116) and K secretion (117).

Amiloride decreases the potential difference in the distal tubule. This blocks K passive secretion to the nephron lumen. Guignard & Peters (115) suggest that amiloride and triamterene exert two independent effects, one of which is displayed as K sparing, and the second as natriuresis and H^+ inhibition of secretion.

ACTION OF DRUGS ON CELL METABOLISM AND ION PUMPS

This section is of particular importance for investigation of the action mechanisms of diuretics. Despite numerous works on the topic, the achievements are not great. Studies of the action of diuretics on key enzymes of the Na transport system and cell energy metabolism have shown that these drugs affect a number of metabolic processes. As has been established with certainty, the effect of acetazolamide is produced by carbonic anhydrase inhibition (118). The problem of the biochemical action mechanism of many diuretics is more complicated. Changes in the enzymatic activity from the action of diuretics are not the same in different parts of the kidney. The activity of malic dehydrogenase decreases notably in the rat kidney cortex after polythiazide and furosemide administration. The latter reduces while ethacrynic acid enhances the activity of this enzyme in the kidney medulla. Furosemide increases the succinic dehydrogenase activity in the cortex and decreases it in the medulla (119). In guinea pigs furosemide lowers the activity of acid phosphatase, succinic dehydrogenase, and leucinaminopeptidase in the nephron cell which involves changes in the mitochondrial structure (120). By contrast, in rats, at a maximum of furosemide diuresis, no sharp changes occur in the structure of mitochondria from the cells of the proximal and distal tubules (121). According to Schmidt & Dubach (122) furosemide inhibits Na,K-ATPase in the cells of the distal segment. Nechay et al claim that in the kidneys of dogs and rats Na,K-ATPase are the receptors of ethacrynic acid (123) and mercurial diuretics (124). The inhibition of Na,K-ATPase has been found in microsomes of the kidney cortex cells of guinea pigs (125, 126). Landon & Fitzpatrick, however, hold a contrary opinion (127). These authors have shown that in rabbits and rats, both in vivo and in vitro, the diuretic effect of ethacrynic acid does not involve changes in the mitochondrial O_2 consumption and inhibition of Na,K-ATPase. Ethacrynic acid and furosemide have no effect on the Na,K-ATPase activity of plasmatic membranes of the rat kidney, but amiloride decreases it (128).

It would be not reasonable to cite here numerous data on alteration in the activity of various enzymes after diuretic administration. The diversity of effects does not permit the suggestion that changes in activity are determined by the key role of a given enzyme in reaction to the test diuretic.

To sum up the literature concerning the change of the Na,K-ATPase activity from natriuretic effects of various drugs (1, 128–130) we may conclude that only the effect of cardiac glycosides is completely determined by inhibition of the enzyme (131, 132). At least on the strength of the available data, the effect of a great number of other substances on Na transport cannot be directly related to the level of Na,K-ATPase activity measured in the kidney.

In the kidney cortex and urinary bladder cells there is an ouabain-insensitive transport of electrolytes (133, 134). It depends on the energy metabolism and is completely blocked by uncouplers or anaerobiosis (135, 136). This transport system responsible for regulation of the cell volume is related to pH and Ca concentration in the external solution (133). Experiments with kidneys of toads (137), guinea pigs (136), and dogs (138) show that nephron cells contain two pumps. One is Na/K and is inhibited by ouabain; the other provides Na and Cl transport and is inhibited by ethacrynic acid. In the nephron cells Na and Cl transport proceeds in the presence of high concentrations of ouabain when Na,K-ATPase is completely inhibited (139, 140). Na reabsorption may be independent of K entrance to the cell from peritubular fluid (141). The relative role of each pump in Na reabsorption in vivo in cells from different nephron parts is still uncertain. Both pumps seem to be localized in one cell but not in different cell populations (142). However, the hypothesis of ethacrynic acid inhibition of the second Na pump is not shared by all authors, since ethacrynic acid affects various metabolic processes and possibly reduces the energy supply of the Na pump (133). In the light of the hypothesis about the existence in kidney of a Na reabsorption mechanism independent of Na,K-ATPase, of primary importance are the data of Kessler suggesting that the election transport system provides energetically the reabsorption of Na without participation of ATP as an intermediate agent (143).

Ethacrynic acid and furosemide increase natriuresis but do not change the kidney O_2 consumption (144). Consequently, the effect of some diuretics does not depend on the inhibition of cell energy metabolism but may be due to other causes. It has been assumed that furosemide increases Na permeability of the basal plasma membrane; Na passive transport to the cell from peritubular fluid increases and, although the total amount of transported Na (and O_2 consumption involved) does not change, the intensity of Na reabsorption from the tubule is diminished (145).

According to another hypothesis the above saluretics enhance inner membrane resistance, which increases the expenditure of energy for Na reabsorption. As O_2 consumption remains unchanged at the action of furosemide, Na reabsorption decreases (145). Moreover, it may be suggested that saluretics influence membranes, disturbing their interaction with cell energy systems. Ethacrynic acid inhibits renal membrane stimulation of mitochondrial respiration, and mercurial diuretics decrease membrane stimulation of glycolysis. Cellular effects of some diuretics may be caused by uncoupling the oxydative metabolism from ion transport systems (1, 146).

One of the most important problems in the study of the diuretics action mechanism is to answer the question whether drugs reduce reabsorption of all ions or affect only Na transport. In the latter case the enhancement of excretion of other ions must depend on the degree of their transport coupling with Na transfer. In man and higher vertebrates, after administration of furosemide, chlorothiazide, acetazolamide, and metolazone, the excretion of Na and of Ca, Mg phosphates increases. This may be due to the reduction of proximal reabsorption (147–149). In amphibians brinaldix does not affect diuresis. It inhibits Na reabsorption and increases K

excretion but does not influence Ca and Mg excretion (98). Unlike the kidneys of mammals, birds (150), and amphibians (151), those of hagfishes (152) and marine cartilaginous and bony fish kidneys are capable of secreting magnesium after $MgCl_2$ injection (7, 153–158). A direct correlation has been found between Mg secretion and Na reabsorption in *O. nerka* (159). In the marine teleosts *Scorpaena porcus* there is a reverse correlation between Na and Mg concentration in urine (158). In *O. kisutch,* after injections of brinaldix and $MgCl_2$, Mg secretion and Na reabsorption decreases equivalently (159). Injections of furosemide, ethacrynic acid, and brinaldix in the partially aglomerular fish *M. scorpius* enhance Na concentration in urine and lower its Mg content at a stable level of diuresis (62). It is necessary to consider the formation mechanism of "primary urine" in aglomerular fishes since it has become known that at the action of floridzine glucose concentration in the urine of *Lophius americanus* increases from 1.31 to 16.7 mg% (160). Thus in the case of coupled cation transport (for instance, Mg secretion) diuretics cease to transfer ions to the lumen as a result of Na reabsorption inhibition. In the case of independent reabsorption of each cation the Na reabsorption decreases mainly under the effect of diuretics. This conclusion is true for instances of saluretic administration when no sharp changes occur in diuresis. This is observed in lower vertebrates, which show comparatively low fluid reabsorption and whose kidneys are not capable of accomplishing osmotic diuresis (22) and NaCl excretion after the injection of NaCl hypertonic solutions (161).

The Na reabsorption system plays an essential role in the transport of various substances in the renal tubule cell (162). Na transport in the nephron cell is related to the transport of K (46), Mg (158), PAH, and glucose (163, 164). In the isolated kidneys of *Rana ridibunda,* furosemide, convallotoxin, and mersalyl inhibit Na and glucose reabsorption as well as PAH secretion (67). In the kidneys of *Rana temporaria,* furosemide, cthacrynic acid, brinaldix, etc inhibit fluorescein secretion without affecting its storage in the proximal tubule cell (21). Dissociation of the ouabain effect on Na and PAH transports has been noted. In dogs this inhibitor causes prolonged inhibition of Na reabsorption and gradual return of PAH secretion to the control level (165).

REGULATION OF KIDNEY FUNCTION

We consider here only data on cellular action mechanisms of hormones and drugs that either activate or inhibit hormonal effects. The question of hormonal regulation of water-salt metabolism and kidney function in vertebrates is reviewed in a few recent publications (2, 3, 166–172). The findings of comparative endocrinology are of exceptional interest for studying regulation principles of cellular processes in animals at different developmental levels.

The system of Na balance regulation is different in lower and higher vertebrates. In lampreys aldosterone does not influence the kidney electrolyte excretion (73). The kidneys of cyclostomes and cartilaginous fishes contain no renin (173, 174). The appearance of the renin-angiotensin system and aldosterone as factors of Na balance regulation by the kidneys (2) is likely to be followed by the formation of some

natriuretic factors, at least in higher vertebrates. Substances with natriuretic activity can be secreted to mammalian blood (175–178). By now peptides of high natriuretic activity have been synthesized (175). The prostaglandins PGA_2 and PGE_2 are considered to play a role in regulation of Na excretion (179, 180).

Changes in Na reabsorption are regulated in the cell in at least two ways: via protein synthesis de novo (e.g. in the aldosterone case) and via formation of 3',5'-AMP from ATP. Vasopressin enhances water permeability and Na transport in the urinary bladder cells stimulating adenylcyclase (181–183). 3',5'-AMP and theophylline, which inhibits the phosphodiesterase that degrades 3',5'-AMP, act like vasopressin. Dopamine (184) and epinephrine inhibit the effect of vasopressin on water permeability; epinephrine does not change cellular reactions to 3',5'-AMP. The inhibiting effect of epinephrine is probably localized at an adenylcyclase level (181). It is eliminated by phenoxybenzamine (α-adrenergic blocking agent) (181). Small doses of noradrenalin increase water permeability and Na transport in the isolated frog skin epithelium. Both these effects disappear after the skin has been treated with the β-blocking agent propranolol (185). It is conceivable that β-receptors contribute to the increase of the 3',5'-AMP level; α-receptors produce the opposite effect (186). Administration of epinephrine to man blocks the antidiuretic effect of vasopressin (187). Another factor regulating adenylcyclase activity is prostaglandin E_1. This substance inhibits the vasopressin effect reducing the intracellular 3',5'-AMP content (188). Prostaglandin E_1 does not affect adenylcyclase isolated from the urinary bladder. It appears that prostaglandins are natural regulators of cell function (182). Indomethacin blocks prostaglandin synthesis and release and enhances response of the toad urinary bladder cells to vasopressin (189). Cu^{2+} lowers the effects of oxytocin and theophylline on osmotic permeability (190).

The question of mechanisms increasing permeability to water and Na transport at the action of 3',5'-AMP is still to be answered. The increase of Na transport under the influence of neurohypophyseal hormone is ascribed both to the increase of permeability of the apical plasmatic membrane (191–193) and to Na pump stimulation (194). Most convincing is the hypothesis of double vasopressin effect on Na permeability of the membrane and Na pump (194, 195).

The question of mechanisms increasing permeability to water is even more arguable. According to one hypothesis vasopressin increases the diameter of pores in the apical membrane through which water flows along the osmotic gradient (191, 196). The hypothesis of Hays (197) is that vasopressin increases the number but not the size of the pores. Eggena claims that under the influence of vasopressin the osmotic water flow occurs through narrow nonpolar channels in the membrane (198). A third hypothesis states that vasopressin increases permeability to intercellular space water (199–201). The antidiuretic reaction is observed only when the renal artery is injected with large amounts of hyaluronidase (199). Small amounts, however, lead to antidiuresis when the enzyme is introduced from ureter to collecting ducts (202).

In rats, aldosterone increases Na reabsorption in the proximal and distal tubules (203, 204); in aquatic snakes it is mainly in the proximal segment (205). Aldosterone penetrates the cell nucleus where it is bound by chromatine stereospecific for mineralcorticoids (206). Spirolactone blocks the binding of ^3H-aldosterone with chroma-

tine in the rat kidney (207). The primary aldosterone binding seems to be with 4S nonhistone chromosomal protein. About 600 aldosterone molecules are bound by the kidney cell nucleus (207). Aldosterone stimulates synthesis in the RNA nucleus (208), as well as protein synthesis, providing the increase of Na transport (209). It is likely that aldosterone influences the synthesis of two types of proteins that increase *(a)* Na permeability of the apical membrane of amphibian urinary bladder and skin cells (210, 211) and *(b)* work of the Na pump (212). Aldosterone enhances the action of vasopressin, p ssibly decreasing the decay rate of 3',5'-AMP (213). An aldosterone antagonist, SC 19886, inhibits the aldosterone-stimulated Na transport through the frog urinary bladder (214). The spirolactone SC 14266 can inhibit Na transport in the frog skin even in the absence of aldosterone in solution (215).

Angiotensin II enhances permeability to the urinary bladder water in the toads *Bufo parachemis* and *B. arenarum,* and potentiates the 3',5'-AMP effect (216). In toad kidneys, angiotensin II induces antinatriuresis and antidiuresis (217). In rats, small doses reduce Na excretion while large doses lead to natriuresis (218). The vasoconstrictory effect of angiotensin may account for its diuretic action in rats (219). Na and K reabsorption in the isolated dog kidney increases at the action of insulin (220). The effect of this hormone seems to be caused by Na pump activation (221, 222).

The effect of methylxanthines is partly due to alteration in the response of cells to hormones and mediators. Theophylline is an inhibitor of phosphodiesterase and in its presence 3',5'-AMP inactivation decreases and vasopressin action becomes more durable (181). In the case of moderate dehydration in man, aminophylline increases reabsorption of osmotically free water. In patients with diabetes insipidus, aminophylline produces no effect on water and Na excretion by the kidney (223). The aminophylline inhibition of Na reabsorption is increased during hydration or after vasopressin injection and is diminished during hyperhydration (224). Theophylline also affects proximal reabsorption (225). It must be taken into account that the action of theophylline on epithelial cells may differ from their reaction to 3',5'-AMP and neurohypophyseal hormones (226, 227).

ONTOGENESIS

The kidneys of newborn mammals (199, 200, 228) and birds immediately after hatching (229) are functionally immature, indicated by the lower GFR (222–230), less effective secretory ability (PAH, phenolred, excretion) (199, 231), reduced effectiveness of amino acid reabsorption (232), and decreased ability of osmotic concentration (200). Salt loads, such as NaCl (233), KCl (234), $CaCl_2$ and $MgCl_2$ (235) are discharged more slowly.

In postnatal ontogenesis along with functional (236), morphological (237) and biochemical maturation of the kidney occurs (238). In the kidneys of newborn rats during the first month the activity of alkaline phosphatase, carbonic anhydrase (239) succinic, α-ketoglutaric, and glutamic dehydrogenase (8) increases and the content of acid mucopolysaccharides in the medulla also increases (199). Mitochondria of adult rat kidneys show much higher activity of cytochromoxidase and succinic cytochromreductase as compared with the 10 day old rats (240). This indicates

qualitative changes of the kidney membrane system in the course of development. In 3 week old rats the activity of Na,K-ATPase of the microsomal fraction does not differ from that of adult rats (241). The kidneys of newborn babies and animals differ considerably from adult kidneys in their response to various drugs. In early ontogenesis of rats and dogs the reaction of the kidneys to vasopressin is lowered (242, 243) and there is no response to epinephrine (243). In newborn rats aldosterone does not decrease Na concentration in urine and spirolactone does not influence the ratio Na/K in urine (244). The inability of ontogenetically immature kidneys to develop osmotic diuresis after administration of Na_2SO_4, urea, glucose, and NaCl is typical (233). Due to the low secretory ability of the renal cell, diuretics (cyclopenthiazide, acetazolamide) are excreted more slowly by young rat kidneys (245). This probably explains the more prolonged action of some diuretics in young rats (246). In newborn rats theophylline increases GFR after the fifth day, whereas cyclopenthiazide, acetazolamide, mersalyl, etc do not affect GFR. In rats T_{mPAH} decreases after acetazolamide administration and increases at the action of mersalyl. The other drugs are not effective (247). After aminophylline administration the maximum value of EF_{Na} is 3.9% in young calves and 15.2% in cattle; for hydrochlorothiazide it is 4.2 and 3.9% respectively (248).

Furosemide, triamterene, and other diuretics exert a natriuretic effect on the kidneys of newborn children (249) and animals (246, 250). After the injection of 1 mg/kg furosemide to 8 and 90 day old babies Na excretion increases during the first hour 39- and 131-fold respectively (249). This may be a result not so much of a lower effectiveness of the cellular action as of a still smaller loading of nephrons due to reduced GFR.

The available data does not permit as yet a quantitative estimation of changes in some of the transport systems of nephron cells in phylogenesis and ontogenesis. Since saluretics (furosemide, ethacrynic acid, etc) are efficient in all classes of vertebrates and even in early postnatal ontogenesis of mammals, this is evidence for the presence of the main cellular systems of Na transport. By contrast, the response to some hormones differs in lower and higher vertebrates and develops gradually during postnatal ontogenesis. A considerable increase of GFR and proximal reabsorption levels is characteristic of the kidney development in ontogenesis. The GFR and the proximal reabsorption are higher in warm-blooded animals. These factors provide more efficient excretion of substances and create a possibility for vigorous osmotic diuresis.

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