Introduction

This short Review presents a summary of present research on the receptor function of Na,K-ATPase (EC 3.6.1.3) and indicates references to some selected publications and reviews on the topic.

The cell surface Na,K-ATPase is one of the most versatile membrane systems as it combines receptor function, coupled Na⁺:K⁺ transport and ATPase activity in a single molecule composed of only two different polypeptide chains (α and β) and lipids (Wallick et al., 1979; Robinson & Flashner, 1979; Sweadner & Goldin, 1980; Cantley, 1981; Schuurmans Stekhoven & Bonting, 1981; Jørgensen, 1982), as outlined schematically in Fig. 1.

It has been known for some time that cardioactive steroids inhibit the coupled Na⁺:K⁺ exchange process (Skou, 1960). Reconstitution experiments with purified Na,K-ATPase incorporated into phosphatidylcholine liposomes (Goldin & Tong, 1974; Hilden et al., 1974; Anner et al., 1977) confirm the findings that have been previously observed in intact cells (Glynn, 1964), i.e. that the cardioactive steroids (see Fig. 2 for some typical structures) inhibit Na⁺:K⁺ transport at an extracellular receptor site that is separated from the intracellular catalytic ATPase region by the lipid bilayer. The extracellular binding region for cardioactive steroids and their putative endogenous analogues, as well as the functional aspects related to ligand interaction with the binding site, are defined in the present review as ‘the receptor function of Na,K-ATPase’.

The receptor function of Na,K-ATPase has been analysed in detail by studying the specific binding of ³H-labelled cardioactive steroids to the Na,K-ATPase molecule (Schwartz et al., 1975; Akera, 1981). This assay titrates the number of active sodium pump molecules in tissues and cells, or in crude or purified Na,K-ATPase preparations (Erdmann, 1981; Jørgensen, 1980).

The discovery of endogenous peptides that bind to opiate receptors and produce opiate-like effects (Hughes et al., 1975; Pasternak et al., 1975) encouraged the search for endogenous, cardiac glycoside-like substances. By now, a whole series of candidates has been presented and reviewed (Song Kim & La Bella, 1981; La Bella, 1982; Haupert, 1983; Hamlyn & Blaustein, 1984). Therefore, the various endogenous inhibitors are discussed only briefly here. Similarly, the putative action mechanisms of exo- and endogenous Na,K-ATPase inhibitors, implicating the involvement of calcium ions, have been discussed in several recent reviews (Lüllmann & Peters, 1981; Akera & Brody, 1982; Schwartz et al., 1982; Repke & Schönfeld, 1984) and are therefore not discussed in detail here. The localization of the receptor site on the Na,K-ATPase molecule, the extremely interesting aspect of the appearance of cardiac glycoside-resistant enzyme forms, as well as some aspects of receptor regulation, are considered here in more detail.

Receptor topography

The activity and affinity of the Na,K-ATPase receptor for cardioactive steroids is regulated by the same ligands that allosterically modify the conformation of the α-subunit. Combinations of ligands that promote phosphorylation (e.g. Na⁺ ions) also favour and stabilize the binding of cardioactive steroids to the receptor site, whereas ligands leading to the dephosphorylated state (e.g. K⁺ ions) decrease the binding rate of the cardioactive steroids (Schwartz et al., 1975; Repke & Schönfeld, 1984). The interaction is mutual, since occupancy of the extracellular receptor site by a cardioactive steroid immediately and drastically inhibits the K⁺-promoted dephosphorylation step.

There is much evidence that the key conformational changes induced by the pump ligands take

Abbreviation used: Na, K-ATPase, Na⁺, K⁺-activated adenosine triphosphatase
place in the α-subunit (Cantley, 1981; Schwartz et al., 1982; Jørgensen, 1983). In addition, Rhee & Hokin (1979) show that antibodies to the α-subunit, but not antibodies to the β-subunit, decrease ouabain binding. Taken together, such experiments suggest that both the pump and the receptor functions are properties of the α-subunit.

Quantitative binding studies reveal that the receptor site, the phosphorylation site and the ATP-binding site (Munson, 1981, 1983; Rempeters & Schoner, 1981; Ponzio et al., 1983; Bobis et al., 1983) are present in a 1:1:1 ratio on the active Na,K-ATPase molecule (Wallick et al., 1979; Robinson & Flashner, 1979; Sweek & Goldin, 1980; Cantley, 1981; Schuurmans Stekhoven & Bonting, 1981; Jørgensen, 1982), which means that each active pump molecule contains a potential receptor site. Whether there are (i) two phosphorylation and two receptors sites per pump molecule functioning with a flip–flop type mechanism (Stein, 1979; Repke & Dittrich, 1979) or (ii) one permanent high-affinity ATP binding site and one permanent low-affinity, regulatory, ATP binding site per two associated α-subunits (Robinson, 1980; Fritzsch & Koepsell, 1983) or (iii) only a single α-subunit monomer containing one ATP site with changing affinity during the turnover cycle and one cardiac glycoside receptor site (Kyte, 1981; Moczydlowski & Fortes, 1981; Peters et al., 1981) is a much discussed question. These three possibilities are shown schematically in Fig. 3.

Evidence for the localization of the receptor site on the α-subunit comes from photoaffinity labeling studies with covalently binding cardiac glycoside derivatives (Ruoho & Kyte, 1974; Hegyvary, 1975; Forbush, 1983). Depending on the localization of the label on the various cardioactive steroid molecules used for photoaffinity labelling,
Fig. 2. Structures of cardioactive steroids and their chemically modified derivatives
(a)–(e) Five typical cardioactive steroids (a, digitoxin; b, digoxin; c, ouabain; d, cymarin; e, scillaren A). (f)–(i)
Examples of chemically modified cardioactive steroids used to label covalently the receptor site: f, 3'-diazomalonyl
digitoxin (3m-DAM digitoxin) (Hall & Ruoho, 1980); g, p-nitrophenyltriazene ouabain (NPT-ouabain) (Rossi et
al., 1980); h, aryldiazonium ouabain (ABD-ouabain; Goeldner et al., 1983); i, iodoazidocymarin (IAC-cymarin); (Lowndes et al., 1984). f, h, and i are photoactivatable compounds, g is an alkylating derivative.

Fig. 3. Three theoretical configurations that could rationalize the expression in the Na,K-ATPase molecule of
binding sites with low (○) or high (□) affinity
(a) Two binding sites per (αβ)2 unit functioning with flip-flop mechanism; (b) one permanent low- affinity and one permanent high-affinity site per (αβ)2 unit; (c) an (αβ)1 unit containing a single site changing the affinity during the turnover cycle.

a 12kDa proteolipid (Forbush et al., 1978; Rogers & Lazdunski, 1979a; Lowndes et al., 1984) or the α-
and the β-subunits (Hall & Ruoho, 1980; Lowndes et al., 1984) or 12kDa and 15kDa proteolipid components as well as the α- and the β-subunits
(Lowndes et al., 1984) are labelled, so that it was suggested that the binding site for cardioactive steroids could be formed between the α- and the β-subunits, perhaps with the aid of proteolipids. However, by using very specific ouabain-triazene alkylating (Rossi et al., 1980) or photolabelling compounds (Rogers & Lazdunski, 1979b; Rossi et al., 1982; Goeldner et al., 1983) only the α-subunit is labelled. The receptor site is then found on an N-terminal 41kDa tryptic fragment of the α-subunit (Goeldner et al., 1983). Four examples of derivatives of digitoxin, ouabain and cymarin are shown in Fig. 2.

Measurements of energy transfer between a fluorescent derivative of ouabain (anthroylouabain) and a fluorescent label of the ATP site, fluorescein mercuric acetate (Jesaitis & Fortes, 1980) or fluorescein 5'-isothiocyanate (Carilli et al., 1982) indicate that the distance between the receptor and the ATP site is around 7nm. This distance exceeds by about 2.5nm the lipid bilayer thickness of 4.5nm. This large distance between the catalytic and the receptor sites can be explained by the fact that catalytic ATP site is located on the 5nm (Zampighi et al., 1984)
cytoplasmic protrusion of the α-subunit on a 60kDa C-terminal tryptic fragment (Karlish, 1980; Jørgensen et al., 1982) and may well be 2–3 nm away from the internal surface of the bilayer. In contrast, the protein protrusion on the extracellular side of the membrane is small (Ting-Beall et al., 1984; Zampighi et al., 1984) so that the receptor site is probably close to the lipid bilayer surface. That lipids could be a component of the receptor site, in analogy to opiate and cholera toxin receptors (Brady & Fishman, 1979; Lee & Smith, 1980; Deber & Behnam, 1984), is suggested by (i) the slower K⁺-induced release of lipophilic cardioactive steroids compared with that of more hydrophilic compounds (Akera et al., 1979); (ii) the lipid-specific modulation of the receptor affinity (Abeywardena & Charnock, 1983); and (iii) a postulated role of sulphotides in the K⁺ and ouabain binding sites at the cell surface (Karlsson, 1976; Gonzales & Zambrano, 1983). As yet, however, there has been no unequivocal experimental demonstration of a direct involvement of lipids in the functioning of the receptor at the molecular level.

The ouabain-resistance gene

In 1856, Vulpian discovered that toads are poorly sensitive to the toxic effects of digitalis. Later, Repke et al. (1965) showed that the toad is about 400 times and the rat about 30 times less sensitive to cardioactive steroids than is, for instance, the cat (short review by Detweiler, 1967). The question arises whether the ouabain-sensitive and the ouabain-resistant Na,K-ATPase forms are produced by two different genes or whether they can be converted after translation. There is evidence for both alternatives, i.e. for isoenzymes appearing at distinct developmental stages, or in various tissues, or in different animal species, as well as for conversion in situ of the receptor by ligand binding. In the following paragraphs a few experiments illustrating both possibilities are presented.

The fact that, in embryonic rat hearts, the Na,K-ATPase is still ouabain-sensitive but becomes 40-fold less sensitive in adult rats illustrates the receptor variation occurring during development (Inturissi & Papaconstantinou, 1974). Similarly, in embryonic chick heart cells the amount of high-affinity receptor decreases during development in ovo or in culture, while the expression of low-affinity receptors remains constant (Kazazoglou et al., 1983); both forms are present at all embryonic stages studied (Lazdunski et al., 1984). However, even in the adult rat heart some high affinity receptors persist (Adams et al., 1982) and some can be revealed by Ca²⁺-free perfusion (Mansier & Lelievre, 1982). Interestingly, the high-sensitivity form induced by Ca²⁺-free perfusion can be converted to a low-sensitivity form by addition of K⁺ ions (Mansier et al., 1983). A similar K⁺-induced conversion can occur post-translationally, as demonstrated by Hansen (1976), who converted isolated ox brain Na,K-ATPase from a heterogenous form to a homogenious low-affinity form by the addition of K⁺ ions. This is a typical ligand-induced conversion. For detailed descriptions of ligand-induced receptor conversion consult, for instance, Schwartz et al. (1975), Erdmann (1984), Hansen (1984), Repke et al. (1984), Repke & Schönfeld (1984).

Evidence for Na,K-ATPase isoenzymes associated with different tissues comes from the following observations. In the rat, the ouabain resistance seems to be limited predominantly to cardio toxic effects, because this animal can be used to study cardiac glycoside neurotoxicity (Detweiler, 1967). Such tissue-specific ouabain-sensitivity is in agreement with Sweedner's (1979) work reporting that rat brain axolemmal Na,K-ATPase is genuinely ouabain-sensitive, whereas the enzyme from other tissues (kidney, superior cervical ganglion, brain astrocytes) is ouabain resistant. Sweadner (1979) also reported that the ouabain-sensitive form [α(+)] contains an extra 2 kDa piece as compared with the ouabain-resistant (α)-form. However, it would be an over-simplification to assume that the [α(+)] and (α) subunit forms correspond generally to ouabain-sensitive and ouabain-resistant Na,K-ATPase isoenzymes, respectively (McDonough & Schmitt, 1984).

That ouabain-resistant Na,K-ATPase forms can be produced via biosynthesis is also demonstrated by the observation that the resistance is maintained in the purified, ligand-free, enzyme (Periyasamy et al., 1979). Na,K-ATPase purified from the heart or the kidney of an ouabain-resistant animal, the rat, is about 1000-fold less sensitive to ouabain inhibition than is enzyme purified from canine kidney or heart (Periyasamy et al., 1979). The I₅₀ remains around 100 µM throughout purification of the rat enzymes, indicating that at least one type of the ouabain-resistance lies within the structure of the Na,K-ATPase molecule.

The K₀,₅ for ouabain inhibition is 80 µM for purified rat kidney Na,K-ATPase but only 40 nM, i.e. 2000-fold less, for purified dog kidney enzyme, whereas there is no difference between these two enzymes in their affinities for ATP, Na⁺, K⁺, vanadate, oligomycin, or p-nitrophenylphosphate (Periyasamy et al., 1983). These results demonstrate that the structural difference between ouabain-sensitive and ouabain-resistant enzyme forms is limited to the ouabain-binding domain.
The functional role of two receptor forms is not yet clear. Forest et al. (1982) reported that in preadipocyte cell lines a Na,K-ATPase with high-affinity receptors binds ouabain but does not mediate active $^{86}$Rb uptake, whereas a low-affinity enzyme binds ouabain and also transports $^{86}$Rb. However, there is no evidence so far to indicate that this finding is typical.

Ouabain-resistant diploid mutants can be isolated from human lymphoblast cells (Lever & Seegmiller, 1976), HeLa cells (Robbins & Baker, 1977), or from Madin–Darby canine kidney cells (Soderberg et al., 1983) when these cells are cultured in the presence of ouabain. The mutation occurs spontaneously and with increased frequency when the cells are cultured in the presence of mutagens (Choy & Littlefield, 1980). The cell lines are stable and maintain their resistant phenotype for years.

The isolated mouse ouabain-resistance gene (ouaR) has now been transferred to CV-1 African green monkey cells by DNA-mediated gene transfer, with the result that these originally highly ouabain-sensitive cells become ouabain-resistant (Levenson et al., 1984). When the transfected CV-1 cells are grown in the absence of ouabain, they display a normal ouabain-sensitive Na$^+$:K$^+$ pump. However, when 10$\mu$m ouabain is present in the culture medium, a ouabain-resistant pump is induced which transports only K$^+$ ions and no Na$^+$ ions (English et al., 1984). This abnormal pump results from a message coded by the ouaR gene which is expressed only in the presence of ouabain. Although the induction of a pump exclusive for K$^+$ ions must be interpreted with caution before extensive confirmatory work has been done, a (r)evolution in the field of membrane transport systems, caused by genetic engineering, is previsible.

It is interesting that partial proteolysis of the Na,K-ATPase $\alpha$-subunit followed by reconstitution of the modified enzyme into liposomes also produces an abnormal pump with intact K$^+$ transport but lowered, vanadate-resistant, Na$^+$ transport (Anner & Jørgensen, 1979). The amino acid sequence of the $\alpha$-subunit being unknown, it is not yet possible to localize Na$^+$- and K$^+$-transport regions on the polypeptide to check whether the defect in the pump produced by ouaR gene-transfer or by selective proteolysis is caused by similar structural alterations.

A transport ATPase exclusive for K$^+$ ions is found in bacteria (Rhoads & Epstein, 1977; Laimins et al., 1978). Sequence homology between the K-ATPase of Escherichia coli and the Ca-ATPase of sarcoplasmic reticulum has been reported. On the other hand, the sequence of Ca-ATPase resembles that of the $\alpha$-subunit of Na, K-ATPase (Kyte, 1981). It can be speculated that during evolution monofunctional transport systems 'fuse' to multifunctional ones. In this sense, the ouaR gene would induce a more 'primitive' transport system.

**Exo- and endogenous factors**

The cloning of the ouaR gene (Levenson et al., 1984) has opened a new avenue for studying the regulation of the Na,K-ATPase receptor by exogenous and endogenous factors. Pressley et al. (1984), on the basis of recent results, propose that the ouabain affinity of the Na,K-ATPase is genetically regulated, i.e., that a regulatory gene product controls the ouabain affinity of the Na,K-ATPase. The authors state: "This presumed ouabain-regulatory protein (ORP) appears to be inducible by ouabain. Differential expression of ORP may account for the 500-fold difference in sensitivity to ouabain seen in various tissues from the same animal. In vivo, ORP may function in tandem with an endogenous ouabain to regulate the degree of activity of Na,K-ATPase in various physiological states."

Four categories of substances modulating the receptor indirectly, and two categories acting directly at the receptor site, can be distinguished. The four groups of indirect modulators are (i) physiological ligands of Na,K-ATPase, e.g. ATP, ADP, P$_i$, Mg$^{2+}$, Na$^+$ and K$^+$ (Schwartz et al., 1975; Repke et al., 1984; Repke & Schönfeld, 1984); (ii) chemical and enzymic modifiers, e.g. SH-blocking agents, proteolytic enzymes (Wallick et al., 1979; Cantley, 1981; Schurmans Stekhoven & Bonting, 1980; Jørgensen, 1982); (iii) intracellular proteins (Charlemagne et al., 1980; Geby et al., 1982); and (iii) the genetically induced ouabain-regulatory protein (Pressley et al., 1984).

The two categories of substances interacting directly with the receptor site comprise (i) the cardioactive steroids and related substances (Akera et al., 1981; Erdmann, 1981; Buckalew & Gruber, 1984) and (ii) the endogenous factors (Song Kim & La Bella, 1981; La Bella, 1982; Buckalew & Gruber, 1984).

A whole series of endogenous factors extracted from serum, urine, kidney, heart, brain, and skin has been described. They can be separated into three major categories: (i) 'endogenous digitalis' (endigin) or 'ouabain-like factors' (OLF), reviewed by La Bella (1982) and Schwartz (1983); (ii) natriuretic factors (Marx, 1981; Haddy, 1982a,b; de Wardener & MacGregor, 1982; Buckalew & Gruber, 1984); and (iii) inhibitory factors circulating in hypertensive patients (MacGregor & de Wardener, 1981; Glynn & Rink, 1982; Haddy, 1982a,b). The common feature of the factors is that
they decrease the Na,K-ATPase activity and displace bound ouabain from the receptor site (Song Kim & La Bella, 1981; La Bella, 1982; Schwartz, 1983; Hamlyn & Blaustein, 1984). With regard to physiological effects, a ‘ouabain-like factor’ extracted from toad skin or sheep brain has recently been shown to increase the contractility of frog and guinea pig atria (Shimoni et al., 1984).

It remains to be elucidated whether ‘ouabain-like factors’, ‘natriuretic factors’, and hypertensive factors are one and the same compound, variation of one compound, or whether they are entities of an entirely different origin. Indeed, a number of the ‘endogenous factors’ have turned out to be artifacts of the extraction procedures (see, e.g., Schwartz et al., 1982; Kracke, 1983). This aspect awaits chemical characterization and comparison of all factors observed. Preliminary reports show that the molecular mass of the factor(s) is below 2kDa (Haupert & Sancho, 1979; Kelly et al., 1984). Reverse-phase h.p.l.c. of desalted and deproteinized plasma extracts yields at least three fractions with ouabain-like activities, but only one of the fractions crossreacts with polyclonal anti-digoxin antibody (Kelly et al., 1984). Schoner and collaborators (Kuske et al., 1984a,b,c) report that a ‘ouabain-like factor’ isolated from haemodialysates of uremic patients or from urine is destroyed byashing and is extracted under conditions similar to those used to extract unsaturated fatty acids and prostaglandins, but does not migrate like these compounds during t.l.c. The authors feel that it could well be a peptide.

Lazdunski’s group (Bidard et al., 1983) extracted from the electrical organ of Electrophorus electricus a mixture of unsaturated fatty acids (linoleic, arachidonic, linolenic, and docosahexaenoic acids) which inhibit Na,K-ATPase activity, [³H]ouabain binding and ⁸⁸⁸Rb⁺ (K⁺) transport at μM concentrations (ED₅₀ 20–30μM). Although these substances are not necessarily the much searched-for ‘ouabain like factors’, their interaction with the ouabain receptor opens exciting perspectives for an additional regulation of the Na,K-ATPase receptor function by local modification of the lipid microenvironment. It remains to be discovered whether such a regulatory mechanism involving unsaturated fatty acids exists in vivo. Thus, although the endogenous factors are an exciting and potentially very important aspect of the field, much uncertainty remains at present.

Once the physiological and pharmacological roles of the endogenous and exogenous factors regulating the cardioactive steroid receptor and the associated sodium pump activity are understood, particularly in pathological conditions of salt metabolism, the development of highly specific receptor agonists and antagonists by modern immunological and genetic techniques may become a possibility. In addition, the cloning and successive comparison of the genes that code for the regulatory factors produced by plants, animals and men will give new clues to the co-evolution of receptors, hormones, and drugs. The genes will teach us that what is a hormone or a factor to one is a drug to the other. Thus, in the future, fields such as molecular biology, cell biology, endocrinology, physiology, and pharmacology will fuse.

The calcium-link

Some 30 years ago, Wildbrandt (1955) suggested that inhibition of the sodium pump by cardioactive steroids leads to transient increases in intracellular concentrations of Ca²⁺ ions, which in turn restore the contractility of the fatigued heart muscle. This hypothesis was then refined by Repke (1964) and Langer (1965). But what mechanism links the sodium pump to calcium fluxes?

Reuter & Seitz (1968) discovered that the heart muscle contains a calcium–sodium exchange system, which was also found in other muscles (Blaustein, 1976) and in nerve (Baker et al., 1969).

This system reversibly exchanges Na⁺ and Ca²⁺ ions across the membrane at a 2–4 Na⁺: 1 Ca⁺ stoichiometry (Mullins, 1979). In view of the roughly 10000-fold difference in the concentrations of free intracellular Na⁺ and Ca²⁺ ions, the Na⁺:Ca²⁺ exchange stoichiometry means that a negligible increase in the intracellular concentration of Na⁺ ions brings about a considerable increase in intracellular concentration of Ca²⁺ ions. This putative calcium-mediated mechanism of action of cardioactive steroids has been discussed in detail in many reviews (e.g. Schwartz et al., 1975; Akera, 1981; Erdmann, 1981; Schwartz, 1983; Repke & Schönfeld, 1984).

Blaustein (1977) evokes the same mechanism to rationalize the effect of circulating Na,K-ATPase inhibitors in hypertension. Upon inhibition of vascular smooth muscle sodium pumps, the intracellular free Ca²⁺ pool increases and therefore also the vascular muscle tone, resulting in hypertension with an intensity proportional to the concentration of the circulating Na,K-ATPase inhibitor (Hamlyn et al., 1984).

Although the chemical nature of the Na⁺:Ca²⁺ exchanger is not yet known, the functional demonstration of its presence in cardiac sarcolemmal vesicles (Reeves & Sutko, 1979; Pitts, 1979; Caroni et al., 1980; Miyamoto & Racker, 1980; Philipson & Nishimoto, 1980), and co-reconstitution of the brain Na⁺:Ca²⁺ exchanger and Na,K-ATPase in liposomes (Eckert & Grosse, 1982) gives hope for its early isolation and characterization.
It has been speculated that the link between Na,K-ATPase receptor function and calcium fluxes could be more direct in that cardioactive steroids change the affinity of calcium-binding sites located on or associated with the Na,K-ATPase molecule (Schwartz, 1976; Lüllmann & Peters, 1981). Although such an ouabain-induced modification of the binding of Ca\(^{2+}\) ions to the lipid portion of purified renal Na,K-ATPase has been demonstrated (Gervais et al., 1977), the precise role of such an enzyme-associated calcium pool is not yet understood. A similar mechanism has been invoked for Ca\(^{2+}\)-mobilizing hormones stimulating the Na,K-pump (Capiod et al., 1982). It is proposed that such hormones, for example noradrenaline, displace an inhibitory Ca\(^{2+}\) pool located near the internal part of the enzyme.

Hormone-regulated processes often imply Ca\(^{2+}\) and/or cyclic AMP as messengers (review by Cohen, 1982). In view of the important role that the Na,K-ATPase seems to play in hormone-controlled processes, it is crucial to understand the precise nature of the link between Na,K-ATPase activity and other hormone-controlled events. It is known that protein phosphorylation–dephosphorylation by protein kinases is often an essential control mechanism of cellular events by external stimuli (Cohen, 1982). An essential step towards understanding the link between hormone action and ion-transport has been made by the demonstration that the Na,K-ATPase protein is phosphorylated by a cyclic AMP-independent protein kinase in vitro (Mardh & Zetterquist, 1972; Mardh, 1979; Yeh et al., 1982) and in vivo (Mardh, 1979; Yeh et al., 1982; Ling & Cantley, 1984) at three locations on a C-terminal 35 kDa fragment of the \(\alpha\)-subunit that is different from the target fragment of the phosphorylation process involved in pump turnover (Ling & Cantley, 1984).

It remains to be examined, firstly, whether the Na,K-ATPase hormone-receptor is connected to the protein kinase-mediated phosphorylation and, secondly, whether there is a link between protein phosphorylation and the Na\(^{+}\):Ca\(^{2+}\) exchange system. Such an additional chemical link could provide a more complete control of the internal Ca\(^{2+}\) concentration by the cell-surface Na,K-ATPase.

**Receptor recycling**

Many cell-surface receptors have been shown to be internalized subsequent to ligand binding by an endocytic process (Catt et al., 1980; Willingham et al., 1981). They are first concentrated in an intermediate compartment, called endosomes, where the ligand is in some cases dissociated from the receptor by low pH. The ligand is then carried to lysosomes, eventually degraded, and excreted by exocytosis, while the receptor may be recycled to the plasma membrane up to 100 times (Hopkins, 1980; Brown et al., 1983; Helenius et al., 1983; Pastan & Willingham, 1983; Rothman & Lenard, 1984). Alternatively, in the case of acid-stable receptor–ligand interactions, the receptor may be processed to lysosomes and be degraded together with the ligand, resulting in down-regulation of cell-surface receptors (Rothman & Lenard, 1984).

Which pathways up- or down-regulate the Na,K-ATPase-receptor? At present, relatively little is known about this topic. Concerning up-regulation, it has been shown that thyroid hormones in mammals (Lo & Edelmann, 1976; Lin & Akera, 1978), aldosterone in amphibia (Geering et al., 1982, 1984) or salt stress in marine ducks (Hossler et al., 1978; Lingham et al., 1980) stimulate the biosynthesis of Na,K-ATPase molecules and their insertion into the plasma membrane.

In HeLa cell cultures, it was demonstrated that ligand binding using \(^{3}\text{H}\)ouabain as a tracer leads to internalization of Na,K-ATPase (Vaughan & Cook, 1972). The \(^{3}\text{H}\)ouabain is found in a lysosomal fraction and is then released unchanged from the cells, presumably by exocytosis, with a half-time of about 70h (Pollack et al., 1981a; Cook et al., 1982). The inactivated, internalized, pumps are replaced (Boardman et al., 1981) at a rate which is at least 4-fold higher than the turnover rate in the absence of ouabain (Lamb & Ogden, 1982) but slower than the internalization rate (Lamb & Ogden, 1982). As a result, the receptor are down-regulated in the presence of cardioactive steroids (Aiton et al., 1981). Ouabain accumulates in the cell due to the high repair rate compared with the ouabain-detoxification rate (Will et al., 1977; Aiton et al., 1981; Pollack et al., 1981b). A recent communication reports that the internalization half-time of ouabain-blocked pumps may be below 30min, indicating that the expression of Na,K-ATPase molecules at the cell surface may be regulated by short-term and long-term mechanisms (Lamb et al., 1984).

In view of the possibility that the number of cell-surface Na,K-ATPase molecules can be rapidly up- and down-regulated, it will be most interesting to study whether the receptor occupied by various endogenous ligands (e.g. 'natriuretic hormones', 'hypertensive' inhibitors, 'ouabain-like factors') is internalized at the same rate as when cardioactive steroids are bound. It may then be possible to know whether the recycling rate of Na,K-ATPase, and of membrane receptors in general, is determined by the chemical nature of the ligand which is bound to the receptor or whether the recycling kinetics are determined purely by a pH-regulated...
association and dissociation rate of the inhibitor, be it hormone, drug or toxin. This information will give insight into the molecular mechanism by which the transmembrane signal produced by receptor occupancy is modulated by the extracellular ligand.

Summary and conclusion

Fig. 4 summarizes the current picture of the receptor function of Na,K-ATPase as outlined in the present Review. Fig. 4(a) illustrates the ligand-dependent conversion of receptor forms with high (h) or low (l) affinity for cardioactive steroids taking place at the plasma membrane. Ligands comprise the substances affecting the conformation of the enzyme, for instance ATP, ADP, Pi, Mg^{2+}, Na^{+}, or K^{+}.

Fig. 4(b) depicts schematically the transformation by mutation of high-affinity receptors into low-affinity ones following cell culture in the presence of high ouabain concentrations. Such a mutation may explain the presence of two receptors in cells and organs as well as the development of ouabain-resistant species in evolution.

Figs. 4(c) and 4(d) illustrate hypothetical links between Na,K-ATPase activity and calcium metabolism to rationalize the calcium-mediated positive inotropic affect of cardioactive steroids. Fig. 4(c) implies a link via phosphate metabolism, i.e. via the ATP concentration near the inner membrane face. Upon inhibition of the ATPase by occupancy of the external receptor site, the internal ATP pool increases and stimulates a protein kinase leading to phosphorylation of the enzyme at sites different from the sites phosphorylated during the normal pump turnover. The protein kinase-phosphorylated pump produces either a localized increase in Ca^{2+} permeability leading to Ca^{2+} influx or release of a bound Ca^{2+} pool within the cell. Both mechanisms produce an increase of the internal Ca^{2+} concentration which stimulates muscle contraction.

Fig. 4(d) implies a hypothetical calcium link via changes in the Na^{+} ion concentration following pump inhibition. The increased internal Na^{+}
concentration augments the internal Ca^{2+} concentration either by release of membrane-bound Ca^{2+} ions or by stimulation of the Na^{+}:Ca^{2+} exchange system.

Fig. 4(e) presents receptor recycling as an additional mechanism for the regulation of the cardioactive steroid receptor. Upon ligand binding, the receptor is taken up to endosomes and the ligand is excreted via lysosomes and exocytosis. Whether the ligand dissociates from the receptors in the endosomes and is recycled to the plasma membrane or whether it is taken up by lysosomes together with the receptor is not yet known.

In conclusion, the Na,K-ATPase system, which contains (i) a receptor function for drugs and hormones; (ii) an ion-pump; and (iii) an ATPase function, presents a complete research tool to study complex regulatory and communicative processes in a single molecule.

I wish to thank Professors M. Lazdunski and W. Schoner for critical reading of the manuscript, and Ms. Sylvianne Bonnet for text editing. My work is supported by the Swiss National Science Foundation, grant no. 3.536-0.83.

References


Vol. 227
Na⁺, K⁺-activated adenosine triphosphatase

Rogers, T. B. & Lazdunski, M. (1979b) *Biochemistry* 18, 135–140
Sweadner, K. J. (1979) *J. Biol. Chem.* 254, 6060–6067
Vulpius (1856) *C. R. Mem. Soc. Biol.* (cited by Detweiler, 1967)