The role of the Na\(^+\),K\(^+\)-ATPase in the regulation of vascular smooth-muscle contractility, and its relationship to essential hypertension

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There has been much interest in recent years in the role of the Na\(^+\),K\(^+\)-ATPase in vascular smooth-muscle contractility in view of the suggestion that essential hypertension might be due to increased secretion of an endogenous inhibitor of Na\(^+\),K\(^+\)-ATPase (de Wardener & McGregor, 1980). For the purposes of this review I shall make the following assumptions and shall not discuss the question of whether or not these assumptions are justified.

(1) That there is an endogenous substance in man which may inhibit Na\(^+\),K\(^+\)-ATPase.

(2) That in patients with essential hypertension the substance circulates in the plasma in concentrations which are higher than normal.

In the light of these assumptions I shall ask the question: 'could such a substance cause increased vascular smooth-muscle tone such that hypertension would result?'. In asking this question I shall discuss the major hypotheses which have been evoked to explain why such a substance might cause hypertension. I shall end by offering a hypothesis suggesting that such a substance could cause hypertension, but without having to invoke an inhibitory effect on Na\(^+\),K\(^+\)-ATPase.

I shall review data from both normal animals and normotensive man, but shall not, for the most part, refer to studies in hypertension, because of the difficulties in interpretation of vascular smooth-muscle contractility in such circumstances (Folkow, 1978).

Methods of studying Na\(^+\),K\(^+\)-ATPase

The role of the Na\(^+\),K\(^+\)-ATPase in vascular smooth-muscle contractility has been studied by observing the effects of manipulations known to alter Na\(^+\),K\(^+\)-ATPase activity. Of such manipulations those most commonly used have been the addition of cardiac glycosides and the removal of potassium. Cardiac glycosides bind to Na\(^+\),K\(^+\)-ATPase with high affinity and inhibit its activity in very low concentrations. For example, the \(K_p\) for binding of digoxin to the Na\(^+\),K\(^+\)-ATPase of intact erythrocytes is around 10\(^{-8}\) mol/l (Ford et al., 1979) and the \(I_{50}\) for inhibition of rubidium transport into those cells by digoxin is around 10\(^{-8}\) mol/l (Aronson & Grahame-Smith, 1977). Concentrations of digoxin of 10\(^{-7}\) mol/l and greater cause virtually complete inhibition of Na\(^+\),K\(^+\)-ATPase. The actual concentrations of other cardiac glycosides (e.g. ouabain) at which these effects are seen, for example in erythrocytes, vary but are of a similar magnitude. However, there is virtually no information on the kinetic characteristics of the binding to and inhibition of the Na\(^+\),K\(^+\)-ATPase of vascular smooth muscle by cardiac glycosides.

The activity of the Na\(^+\),K\(^+\)-ATPase is in part dependent on the presence of potassium, the \(K_M\) for activation being about 0.5 \(\times 10^{-3}\) mol/l (Sachs & Welt, 1967). Thus Na\(^+\),K\(^+\)-ATPase activity is greatly reduced in the absence of potassium.

Hypotheses relating inhibition of Na\(^+\),K\(^+\)-ATPase to essential hypertension

It is clear that cardiac glycosides cause decreased flow in forearm blood vessels in normotensive man in the short term, after either systemic administration (Mason & Braunwald, 1964) or local intra-arterial administration (Robinson et al., 1983). Whether this effect could occur as a long-term effect of continued Na\(^+\),K\(^+\)-ATPase inhibition in man is not known, but several hypotheses have been advanced in an attempt to link possible inhibition of Na\(^+\),K\(^+\)-ATPase with essential hypertension.

(1) 'Waterlogging' of the vessel wall. If inhibition of Na\(^+\),K\(^+\)-ATPase resulted in an increase in intracellular water and increased vascular water content and increased vascular tone (Tobian, 1960). However, an increase in water content of isolated arteries has not been demonstrated in a variety of short-term experiments in vitro with ouabain or removal of potassium (see Haddy et al., 1978). When dogs were given high doses of digoxin for a month, there was a small (6%) increase in the wall water content of mesenteric arteries, but the dogs failed to become hypertensive (Overbeck et al., 1980).

(2) Depolarization of the cell membrane. The sodium pump is electrogenic (see Svedner & Goldin, 1980), and inhibition of Na\(^+\),K\(^+\)-ATPase results in depolarization of the cell membrane, which could lead to an increase in contractility either directly or indirectly. A direct effect seems unlikely since there is a considerable delay between the addition to isolated human arteries and veins of a concentration of ouabain sufficient to cause rapid inhibition of Na\(^+\),K\(^+\)-ATPase and the onset of the contractile effects (Mikkelsen et al., 1979). Depolarization could lead indirectly to increased contractility by one of two mechanisms.

(a) By an increase in calcium influx (see Haddy et al., 1978). For example, in the isolated ear artery of the rabbit contraction can be induced by membrane depolarization and the contraction is related to calcium influx. However, in the same preparation contraction could be induced by the removal of potassium but without a change in membrane potential (Droogmans & Casteels, 1977). Furthermore, even complete inhibition of Na\(^+\),K\(^+\)-ATPase is accompanied by only very small changes in membrane potential (see Lang & Blaustein, 1980), and Mulvanya et al. (1982a) were able to demonstrate progressively reduced contraction of peripheral resistance vessels in the rat during the continued absence of potassium despite persistent membrane depolarization.

(b) By enhancing sensitivity to vasoconstrictors, such as noradrenaline. The possible role of noradrenaline through this and other mechanisms is considered below.

(3) Increased intracellular calcium via increased Na\(^+\),Ca\(^2+\)-ATPase exchange. It has been suggested that a rise in intracellular sodium concentration, due to inhibition of Na\(^+\),K\(^+\)-ATPase, would lead to an increased intracellular ionized calcium concentration via Na\(^+\)/Ca\(^2+\) exchange, and hence
an increase in smooth-muscle contractility (Blaustein, 1977). A similar mechanism has been proposed to be the basis of the positive inotropic action of digitals (see Langer, 1962). This hypothesis depends on three premises:

(a) That inhibition of Na\(^+\),K\(^+\)-ATPase causes an increase in intracellular sodium. That this is so has clearly been shown in erythrocytes (see Aronson et al., 1981) but is more difficult to demonstrate in the smooth-muscle cells of peripheral resistance vessels, mainly because of problems in the accurate measurement of the extracellular space. However, recently it has been shown that the addition of ouabain or the removal of potassium causes increased sodium accumulation by rat mesenteric resistance vessels (Aalkjaer & Mulvany, 1983).

(b) That, as a result, intracellular concentrations of ionized calcium rise. Although the addition of ouabain to or the removal of potassium from smooth-muscle preparations causes contraction and calcium influx (see van Breemen, 1970), changes in intracellular ionized calcium have not been demonstrated, because of the lack of methods for measuring ionized calcium in situ. There is, therefore, no information at present on consequent measurements of sodium and ionized calcium concentrations in vascular smooth-muscle preparations in which Na\(^+\),K\(^+\)-ATPase has been inhibited. However, the recent development of an intracellularly trapped fluorescent indicator of ionized calcium, quin2 (Tsien et al., 1982), should allow such observations to be made.

(c) That an increase in the intracellular ionized concentration of calcium would increase vascular smooth-muscle contractility and that that effect would result in hypertension. The relationship between calcium and vascular smooth-muscle contractility has been reviewed elsewhere (see, for example, Fleckenstein, 1977) and there seems to be little doubt of the role that calcium plays. However, it is not clear that a change in intracellular calcium secondary to inhibition of Na\(^+\),K\(^+\)-ATPase is the chief mechanism in the long-term increase in tone of peripheral resistance vessels which is found in essential hypertension.

In support of an involvement of the Na\(^+\)/Ca\(^2+\) exchange in the mechanism of the increase in vascular smooth-muscle contractility caused by Na\(^+\),K\(^+\)-ATPase inhibition, it has been shown that contractions induced in guinea-pig aorta by the addition of ouabain or the removal of potassium depend on the ratio [Ca\(^{2+}\)]/[Na\(^{+}\)] \(^2\) even at low values of [Ca\(^{2+}\)], and that contractions do not occur in the absence of potassium until there has been a considerable rise in intracellular sodium concentrations (although the effect ofouabain was less dependent on [Na\(^{+}\)] (Özaki et al., 1978). However, in the isolated ear artery of the rabbit and in perfused rat tail arteries there is a temporal dissociation between both the contraction induced by the removal of potassium and the relaxation induced by its introduction and the likely changes in intracellular sodium, and therefore of Na\(^+\)/Ca\(^2+\) exchange (Friedman et al., 1973; Droogmans & Casteels, 1977). Furthermore, while Mulvany et al. (1982a,b) were able to show that the addition of ouabain or the removal of potassium induced contractions in guinea-pig and rat aorta and rat tail artery, confirming the results of Lang & Blaustein (1980) in perfused rat hindlimbs, they were unable to demonstrate the same effects in peripheral resistance vessels.

These last observations, indeed, make it difficult to postulate any effect of Na\(^+\),K\(^+\)-ATPase inhibition on contractility of peripheral resistance vessels of moderate size, at least in the guinea pig and rat, although, as noted above, ouabain does decrease forearm blood flow in man (Mayo & Braunwald, 1964; Robinson et al., 1983).

(4) Enhancements of the effects of vasoconstrictors. Inhibition of Na\(^+\),K\(^+\)-ATPase could cause changes in the contractile effects of noradrenaline in one of two ways: (a) depolarization, causing enhancement of the contractile effects of noradrenaline (Hermosmeyer, 1976); (b) increased release of noradrenaline from nerve-ends (Bonaccorso et al., 1977; Karaki et al., 1978; Toda, 1980). However, in experiments on the blood vessels of both animals and man the constricting effects of digitals or the removal of potassium have been reported neither to be prevented by ganglion-blocking drugs, adrenoreceptor antagonists, such as phentolamine, nor to be mimicked by phenylephrine (Ross et al., 1960; Mason & Braunwald, 1964; Belardinelli et al., 1979; Robinson et al., 1983). In addition ouabain has been reported to decrease the contractile effect of noradrenaline in rat mesenteric arteries (Mulvany et al., 1982a).

Further problems of interpretation

In addition to the difficulties in finding consistently convincing evidence in favour of any of the above hypotheses there are certain other problems to consider.

(1) The putative endogenous inhibitor of Na\(^+\),K\(^+\)-ATPase need not be 'digitalis-like'. Simply because a substance inhibits Na\(^+\),K\(^+\)-ATPase it cannot be assumed that it is in every respect like digitalis, it may lack effects of digitalis or possess effects which digitalis does not. For example, vanadate inhibits Na\(^+\),K\(^+\)-ATPase, but does so by an effect at the intracellular end of the molecule in contrast to digitalis which binds extracellularly (see Swedner & Goldin, 1980); indeed, vanadate may enhance the binding of digitalis (see Schwartz & Adams, 1980).

Again, tricyclic antidepressants may inhibit Na\(^+\),K\(^+\)-ATPase and cause release of both acetylcholine and noradrenaline from synaptosomes, but ouabain does not cause release of acetylcholine (Gilbert & Wyllie, 1980). Until any such endogenous inhibitor is isolated and characterized it will remain unclear to what extent experiments in which other manoeuvres are used to inhibit Na\(^+\),K\(^+\)-ATPase are relevant to essential hypertension.

(2) Long-term versus short-term effects. Almost all the studies discussed above have involved short-term exposure of tissues. Even in studies described as 'long-term' the expression has been used in a relative sense, e.g. for an exposure of an hour's duration compared with that of a few minutes (Mulvany et al., 1982a). Yet it is known that during long-term administration of digitals to rats and mammals and in chronic hypokalaemia, adaptive changes occur which may oppose the inhibition of Na\(^+\),K\(^+\)-ATPase (see Aronson et al., 1981). However, adaptation does not necessarily occur in all tissues (Bluschke et al., 1976) and there is little information on the long-term effects of, say, digitalis administration on vascular smooth-muscle contractility.

(3) Na\(^+\),K\(^+\)-ATPase inhibition in diseases other than essential hypertension. There is evidence of Na\(^+\),K\(^+\)-ATPase inhibition in the erythrocytes of patients with other diseases, e.g. hyperthyroidism and chronic renal failure (even in the absence of hypertension). However, this may not be a problem since inhibition has not been observed in all tissues in these disorders, and in hyperthyroidism there may be increased numbers of digitalis receptors in skeletal muscle (T. Clausen, personal communication). How the Na\(^+\),K\(^+\)-ATPase of vascular smooth muscle is affected in such disorders is not known.

An alternative hypothesis

There is good evidence that essential hypertension is associated with increased circulating concentrations of a substance which, when studied in vitro, seems to be an inhibitor of Na\(^+\),K\(^+\)-ATPase. Nonetheless, as is clear from the above discussion, it is hard to link Na\(^+\),K\(^+\)-ATPase

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inhibition convincingly to increased vascular smooth-muscle tone.

Furthermore, any hypothesis which attempts to relate such a substance to the pathogenesis of essential hypertension must take into account, in addition to the problems outlined above, the fact that while there is consistent evidence of inhibition of $\text{Na}^+\text{K}^+\text{ATPase}$ in the leucocytes of patients with essential hypertension, the body of evidence suggests that $\text{Na}^+\text{K}^+\text{ATPase}$ activity is increased in their erythrocytes (see Swales, 1982).

A suitable hypothesis is that the proposed endogenous inhibitor of $\text{Na}^+\text{K}^+\text{ATPase}$ could cause hypertension by increasing vascular smooth-muscle tone, but by mechanisms unrelated to $\text{Na}^+\text{K}^+\text{ATPase}$ inhibition. One would have to assume, however, that such a substance does possess some properties of digitalis other than inhibition of $\text{Na}^+\text{K}^+\text{ATPase}$.

While digitalis inhibits $\text{Na}^+\text{K}^+\text{ATPase}$ in the sorts of concentrations usually found in the tissues during its therapeutic use, in lower concentrations it has been found to have to assume, however, that such a substance does possess some properties of digitalis other than inhibition of $\text{Na}^+\text{K}^+\text{ATPase}$.

In rat ventricles two separate positive inotropic effects are clearly distinguishable (Grupp et al., 1984).

An endogenous substance which, on testing in vitro, appears to be an inhibitor of $\text{Na}^+\text{K}^+\text{ATPase}$ might in vivo have those actions associated with very low concentrations of digitalis, and, therefore, cause increased vascular smooth-muscle tone by a direct effect on calcium. In erythrocytes, which lack the very high-affinity binding site for digitalis suggested to be associated with the effect on calcium, the substance would produce apparent stimulation of $\text{Na}^+\text{K}^+\text{ATPase}$. In leucocytes, however, inhibition might occur, perhaps because of the much higher density of digitalis-binding sites on leucocyte membranes (see Boon et al., 1984).

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Mechanisms of the regulation of myofibrillar function in vascular smooth muscle

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The radius of a blood vessel, and hence the resistance to flow within that vessel, is a result of the dynamic balancing of the blood pressure, which tends to increase radius, against inward forces due to the stretching of the elastic walls and active contraction of the VSM. VSM exerts the force required to resist blood pressure and the shortening required to constrict the blood vessel. Contractility of the intact blood vessel is controlled by a large number of neural and hormonal agents, but at the level of the contractile elements within the cell, control is much simpler.

$Ca^{2+}$ regulates contractility

The major (perhaps only) factor which regulates contractility is the concentration of $Ca^{2+}$ in the vicinity of the contractile elements. This can be directly demonstrated in prep-