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THE ELECTROGENIC NA^+,K^+-PUMP IN SMOOTH MUSCLE: PHYSIOLOGIC AND PHARMACOLOGIC SIGNIFICANCE

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INTRODUCTION

In the discussion of the electrogenic pump in smooth muscle cells, certain symbols will be used. The following list defines these symbols.

E: potential difference.
E_m: total potential across the cell membrane.
E_K: potassium equilibrium potential (potential at which there would be no net diffusion of K^+).
E_{ec}: electrochemical (diffusion) potential.
R: universal gas constant.
T: absolute temperature.
F: "Faraday" (electric charge per gram equivalent of univalent ions).
$[K]_i$, $[K]_o$: intracellular and extracellular concentrations, respectively, of K^+.
$[Na]_i$, $[NA]_o$: intracellular and extracellular concentrations, respectively, of Na^+.
$[Cl]_i$, $[Cl]_o$: intracellular and extracellular concentrations, respectively, of Cl^-.
P_{Na}, P_K, P_{Cl}: permeabilities, respectively, to Na^+, K^+, and Cl^-.
I_p: current contributed by electrogenic pump.
R_m: resistance of the cell membrane.

In many excitable cells two factors can contribute to membrane potential (1). One of these is the consequence of the electrochemical distribution of ions across the cell membrane. An approximation of the potential difference

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across the membrane due to the electrochemical gradients can be determined from the Goldman equation (2):

$$E = -\frac{RT}{F} \ln \frac{[K]_i + P_{Na}/P_K\,[Na]_i + P_{Cl}/P_K\,[Cl]_o}{[K]_o + P_{Na}/P_K\,[Na]_o + P_{Cl}/P_K\,[Cl]_i}.$$

The concentration gradient of Cl^- across the membrane is considerably less than that of Na^+ or K^+ and the permeability to Na^+ is much less than that to K^+. Consequently, K^+ makes the greatest contribution to the electrochemical potential. This electrochemical potential is also called a diffusion potential (2).

The second factor is an electrogenic component of the Na^+, K^+ pump (1). The inward diffusion of Na^+ and outward diffusion of K^+, each following its concentration gradient, requires the existence of a Na^+, K^+ exchange pump to extrude Na^+ which accumulates intracellularly in exchange with extracellular K^+. If the exchange is equal, i.e. if one Na^+ is pumped out for each K^+ pumped in, the pump is neutral; that is, it does not directly contribute any current across the membrane. However, in many tissues, this exchange, at least under certain conditions, is not one for one. Rather, more Na^+ ions are extruded than K^+ ions brought in. The result is a net movement of positive ions outward to produce an electrogenic current (1). A simple equation for membrane potential is: $E_m = E_{ec} + I_p R_m$.

The electrogenic current, I_p, across the resistance of the membrane, R_m, creates an electrogenic potential, $I_p R_m$. Both E_{ec} and $I_p R_m$ contribute to the negativity on the inner side of the membrane.

The coupling ratio of the electrogenic pump has been established in red blood cells as 3Na:2K (1). Estimates in several other tissues are somewhat variable (1) but generally approximate the 3:2 ratio. An estimate of a 3:2 coupling ratio has also been made for the smooth muscle of the taenia coli (3). It is important to recognize that, within an individual tissue, the coupling ratio may change with the experimental conditions. Thomas has calculated that, with a 3:2 ratio, the pump cannot contribute more than 10 mV to the resting membrane potential under steady state conditions in frog muscle.

Fortunately, much is known about the Na^+, K^+ pump. Its enzymatic correlate is the membrane bound Na^+, K^+-ATPase (1, 4). The pump's activity is a function of the intracellular concentration of Na^+ and the extracellular concentration of K^+(1, 4, 5). Rubidium substitutes well for K^+ but caesium is a poor substitute (6). Because the pump is energy dependent, its activity is sensitive to temperature and metabolic inhibitors (1, 5). The cardiac glycosides are specific inhibitors of the pump (1, 4) and there is considerable evidence to support the hypothesis that the Na^+, K^+-ATPase is the receptor for cardiac glycosides (4, 7). The cardiac glycoside most frequently used to study the Na^+, K^+ pump is ouabain. There is

specific, high affinity binding of ^{3}H-ouabain to plasma membranes of many cells, including smooth muscle (8, 9). This binding of ouabain is Na^+- and ATP-dependent and is antagonized by K^+ (8, 9).

METHODS OF DETECTING ELECTROGENIC ACTIVITY

It is possible to study the electrogenic contribution of the pump to membrane potential (E_m) by measuring E_m and simultaneously stimulating or inhibiting the pump. Recognizing that the pump in a cell may not have the ability to be electrogenic at all times, there are two questions one may ask: 1. Is the pump in a given cell type capable of being electrogenic? 2. Is the pump electrogenic under a specific set of circumstances, for example "at rest" or during spontaneous activity.

The capability of a cell to initiate electrogenic pumping is generally determined by Na^+ loading, that is, by allowing the cell to accumulate $[Na]_i$ (1, 5). This procedure is effective because the electrogenicity of the pump is a function of $[Na]_i$; and $[K]_o$ (10). In other words, the faster the pump is running, the more electrogenic it may become. There are several ways by which Na loading may be accomplished, such as decreasing $[K]_o$, lowering the temperature, or exposing the cell to a pump inhibitor. Na loading by decreasing $[K]_o$ will be taken as an example. While measuring E_m, generally with a microelectrode, the normal bathing medium is replaced with K-free medium. In the absence of $[K]_o$ the pump is inactivated. As a result, Na^+ gradually accumulates intracellularly and the cell gradually undergoes a marked depolarization. Readmitting K^+ to the bathing medium in either normal or excess concentrations at a time when $[Na]_i$ is high causes a sudden and marked stimulation of the pump. If, under these conditions, the cell rapidly and transiently hyperpolarizes, beyond the steady state level, one can assume that the pump is electrogenic. This test is particularly convincing if the hyperpolarization exceeds the calculated steady state E_K, the potassium equilibrium potential.

The Na^+-loading type of experiment can be illustrated with results from Daniel's laboratory (5) obtained in the rat myometrium. The mean E_m of cells bathed in normal Krebs solution for 40 min at 37°C was –46 mV. Replacement of the solution with K-free solution for 18 hr at 4°C caused E_m to decrease to –15 mV. This low E_m was maintained in K-free solution at 37°C. Subsequently, 2 min after readmission of 4.6 mM K^+, the E_m reached –69 mV, which was 23 mV greater than the resting E_m (–46 mV) and 5 mV greater than the calculated E_K (–64 mV).

Two approaches have been used to estimate the contribution of electrogenic pumping to resting membrane potential. One of these involves determination of electrochemical potential by use of the Goldman equation and

subtracting it from the resting potential (1). This approach has been used by Casteels to estimate the contribution of electrogenic pumping to the "resting" potential of the taenia coli of the guinea pig (3, 11). By measuring intracellular ion concentrations and calculating permeabilities to the ions, Casteels estimated that the electrochemical potential in the taenia coli is –37 mV. Based on this calculation electrogenic pumping would contribute 15–20 mV to the total resting membrane potential. The large electrogenic potential could be due to the high membrane resistance, estimated to be 25 K Ω cm^2 (3).

The second method to determine whether there is an electrogenic component to resting membrane potential involves measuring E_m during inhibition of the pump (1, 12). The inhibition is usually achieved by decreasing extracellular potassium ($[K]_o$) or by adding a specific inhibitor, such as ouabain, to the bathing medium. There are several problems in estimating electrogenic activity by pump inhibition, including (*a*) a decrease in E_m due to gradual loss of $[K]_i$, (*b*) the possible accumulation of K^+ on the outside of the cell membrane, (*c*) changes in P_{Na} and P_K secondary to the depolarization itself, and (*d*) opposition to depolarization by changes in chloride gradient, secondary to altered movement of positive ions (1).

Inhibiting the pump by decreasing $[K]_o$ creates an additional complication. A reduction in $[K]_o$ has a direct and immediate effect on the electrochemical potential which, from the Goldman equation (see above), can be seen to be in the direction of hyperpolarization. This will tend to offset or even conceal the depolarization due to any decrease in electrogenic pumping. Thus, a decrease in $[K]_o$ may cause either a depolarization or a hyperpolarization depending upon (*a*) whether or not the pump is electrogenic, (*b*) the relationship between the change in $[K]_o$ and the half-optimum $[K]_o$ for pump activity, (*c*) the permeability of the membrane to K^+, and (*d*) the preexisting K^+ gradient.

These problems make quantification of the electrogenic contribution difficult by simple inhibition of the pump. However, it is generally accepted that depolarizations following within a few minutes of pump inhibition, before marked changes in ion distribution due to diffusion can occur, indicate that there is an electrogenic contribution to the resting potential (1).

EVIDENCE FOR ELECTROGENIC PUMP ACTIVITY IN VARIOUS TYPES OF SMOOTH MUSCLE

Evidence from Na-Loading Experiments

In 1969, Daniel and his colleagues carried out Na-loading experiments to establish the existence of an electrogenic Na pump in rat myometrium (5, 13). Some of the data from their study have been summarized above. The investigators demonstrated that the hyperpolarization that occurred when

the tissue was reexposed to K^+ was inhibited by low temperature or ouabain. Furthermore, the hyperpolarization was greater when the tissue was incubated in Cl^--deficient solution. This was presumably due to a short circuiting effect of passive Cl^- movement during activation of the pump.

Electrogenic pumping by myometrial cells may be a species dependent phenomenon. Kao & Nishisyama produced Na- loading in rabbit uterus by cold storage (14). In the cold, E_m was about –40 mV and, upon rewarming, increased only modestly to –50 mV. The potential value achieved on rewarming was always less negative than E_K, leading the authors to conclude that the pump in that tissue is not electrogenic.

Na-loading experiments have demonstrated electrogenic pumping in the smooth muscle cells of the guinea pig taenia coli (15, 16). Reexposure of Na-loaded taenia to K^+ leads to a hyperpolarization which is temporarily more negative than E_K (16). This hyperpolarization occurs when potassium conductance (g_K) is actually decreased. The fact that a decrease in g_K would decrease the electrochemical potential adds further support for the role of electrogenic current to the hyperpolarization. Rb^+, but not Cs^+, could substitute for K^+ in initiating the hyperpolarization (15).

Similar types of experiments have indicated the capability for electrogenic pumping in longitudinal muscle of the guinea pig ileum (10, 17), the rabbit ear artery (18), the rabbit main pulmonary artery (19), and the guinea pig portal vein (20). Na loading and reexposure to K^+ leads to a rapid hyperpolarization in the guinea pig ureter (21). Unfortunately, the magnitude of hyperpolarization in the ureter was not given by the author. Nevertheless, the rapidity of the hyperpolarization (maximum within 1 min) is highly indicative of electrogenic activity of the pump.

Evidence for an Electrogenic Contribution to Resting Membrane Potential

Casteels and associates (3, 11) have estimated the contribution of the electrochemical component of E_m in the taenia coli of the guinea pig. Subtracting this value from the resting E_m they have estimated that –15 to –20 mV of the resting E_m is due to an electrogenic component of the pump. The current contributed by the pump was estimated to be $-0.8\ \mu A \cdot cm^{-2}$. Using an estimate of $25\ k\Omega \cdot cm^2$ for membrane resistance in the taenia coli, Droogmans & Casteels (22) calculated an electrogenic component of –20 mV across the resistance. These authors also determined that the concentration of $[K^+]_o$ for half maximum activation of the pump is 4.7 mM. This value is greater than has been found in biochemical studies on Na^+, K^+-ATPase, a discrepancy possibly due to competition for the pump between extracellular K^+ and Na^+ in intact tissues (22).

Placing the taenia coli in K-free solution or the addition of ouabain (1.7×10^{-6}M) produces a depolarization of 10 mV within 20 min (12, 15). This

is further evidence for an electrogenic contribution to the pump. However, for reasons already discussed, one should not assume from this that the magnitude of the electrogenic component of resting E_m is 10 mV.

Bolton (10) has presented evidence for electrogenic pumping in longitudinal muscle of the guinea pig ileum. The tissues were kept for a time at room temperature. This would induce Na loading by the inhibition of the pump at low temperature. The tissues were then brought to 35°C, which temporarily initiates hyperactivity of the pump of the Na-loaded tissues. After 15–30 min at 35°C, the tissues depolarized a mean of 21 mV in response to removing $[K]_o$. At this time $[Na]_i$ was probably still abnormally high because after 1 hr at 35°C, the removal of $[K]_o$ caused a mean depolarization of only 13 mV. Assuming that 1 hr is adequate for the pump to restore the normal ionic gradients, the results at 1 hr probably constitute relatively steady state conditions and indicate the presence of an electrogenic component to resting E_m. This conclusion is strongly supported by the rapidity of the depolarization which was nearly maximal within 2 min. After 1 hr at 35°C, ouabain (1.7×10^{-6}M) produced a depolarization of 8 mV. The lesser effect of ouabain than of K-free solution may reflect the fact that this concentration of ouabain is less then the optimal concentration for inhibition of the pump, in the ileum, which may be about 10^{-4}M (10).

Using the Goldman equation, Casteels has estimated the electrochemical component of resting E_m in smooth muscle cells of the guinea pig vas deferens to be –30 mV (22). Mean values for resting E_m from several sources in the literature range from –57 to –61 mV [see reference (23) for sources]. The difference between the calculated electrochemical potential and the resting E_m is therefore approximately 30 mV, a value which is very large and probably represents an overestimation of the contribution of the electrogenic pump. As discussed in the following paragraph, the contribution of the electrogenic pump may be closer to 10 mV.

Three different procedures inhibit electrogenic pumping and depolarize by 8 to 10 mV the smooth muscle cells of the guinea pig vas deferens. The procedures are (*a*) chronic interruption of the innervation (23, 24; see, also section on supersensitivity), (*b*) ouabain, 10^{-5}M (25), and (*c*) reduction of $[K]_o$ to one half normal (25). It should be noted that 10^{-5}M ouabain produces 80–90% of the maximum inhibition of Na^+, K^+-ATPase in broken cell preparations of the vas deferens (24). The depolarizing effect of ouabain is rapid, developing over a period of 1–2 min (25). One would not expect very great changes in intracellular ion contents in that period of time, especially in a quiescent preparation such as the vas deferens. Furthermore, the depolarization produced by ouabain was not accompanied by any measurable changes in electrotonic potentials (25). Thus there must not have been any marked changes in membrane conductance. The importance of these points is that they minimize the errors in quantifying electrogenicity

by pump inhibition. The depolarizations induced by denervation, ouabain, and reduced $[K]_o$ are similar in magnitude but are not additive (24). It is, therefore, quite possible that the depolarization of 8 to 10 mV is a rather close approximation of the contribution of an electrogenic pump to the resting E_m in this tissue.

With the vas deferens there again appears to be a species difference. Results from our laboratory indicate that there is no electrogenic pump contribution to the resting E_m in the rat vas deferens. Ouabain does not cause any depolarization in concentrations up to 10^{-4}M (25) even though it is an effective inhibitor of Na^+, K^+-ATPase in broken cell preparations (26). Decreasing $[K]_o$ to one half the normal concentration also caused no depolarization in the rat vas deferens (25). Greater decreases in $[K]_o$ (one fourth normal, one eighth normal, or zero) caused marked *hyperpolarizations* (25). Na-loading experiments have not been reported in the rat vas deferens and, therefore, it is not known whether the pump in that tissue lacks electrogenic capability or simply is neutral under resting conditions.

Casteels et al (19) have calculated the electrochemical potential in the rabbit main pulmonary artery, by use of the Goldman equation, to be –31 mV, a value 25 mV less negative than the measured E_m. On the other hand, exposure to K-free solution or ouabain, 2×10^{-6}M, produces a rapid depolarization of approximately 7 mV which is nearly maximal within 10 min (19). The authors concluded that electrogenic activity of the pump does contribute to the membrane potential of the main pulmonary artery but less than the –25 mV inferred from the Goldman equation.

The above data obtained from the guinea pig vas deferens and the rabbit main pulmonary artery suggest that the application of the Goldman equation yields an underestimation of the electrochemical potential and therefore an overestimation of the contribution of an electrogenic component of the Na^+, K^+ pump. The reason(s) may be unavoidable errors in measuring or estimating the intracellular concentrations of, and permeabilities to, ions and/or unequal intracellular distribution of ions not reflected in measurements of total intracellular ion concentrations.

Evidence is available to support the contribution of an electrogenic Na^+, K^+-pump to E_m in a variety of other vascular smooth muscle preparations. Figure 4 in the paper by Matthews & Sutter (27) shows a depolarization of 5–10 mV in the rabbit anterior mesenteric vein within 5 min of exposure to ouabain, 10^{-5}M. This depolarization progressively increased with time. Both ouabain (1.7×10^{-5}M) and K-free solution produced marked depolarizations of the guinea pig portal vein within 15 min (20). Kuriyama et al (20) estimated that the electrogenic pump contributes 10–20 mV to the resting E_m of the portal vein. In the rabbit ear artery, either ouabain (10^{-5}M) or K-free solution produces rapid depolarizations with little effect on the permeability to K (18). An optimum concentration of

ouabain (3×10^{-6}M) produces a mean depolarization of approximately 6 mV in the rabbit saphenous artery (28). This depolarization develops in less than 10 min and, thereafter, the E_m remains quite constant in the continued presence of ouabain for 1 to 2 hr (P. W. Abel and W. W. Fleming, unpublished observations). An analysis of Na^+ and K^+ transport in dog carotid artery (29) indicates that, with $[K]_o$ less than 30 mM, the pump is electrogenic with a net outward movement of positive ions (Na^+) and that when $[K]_o$ is above 30 mM there is a net inward movement of positive ions (K^+).

Tension Development and the Electrogenic Pump

It is well known that vasodilation and vasoconstriction are associated with hyperkalemia and hypokalemia respectively (30, 31). Until the role of an electrogenic pump was appreciated, this relationship was puzzling. If we assume that vasoconstriction is a function of depolarization and vasodilation is a function of hyperpolarization, the Nernst equation, which relates the electrochemical potential to $[K]_o$, predicts the opposite relationship between $[K]_o$ and constriction or dilation. This interesting topic has been reviewed by Haddy (30) and Anderson (31). The changes in resistance of vascular beds, as $[K]_o$ is altered, are not prevented by denervation and are therefore not the result of transmitter release. However, the responses are antagonized by ouabain. When these facts are added to the extensive evidence of an electrogenic pump obtained from experiments with vascular smooth muscle in vitro [see, for example, ref. (18)], the conclusion is reached that the effects of changing $[K]_o$ in vivo are manifest primarily by increases and decreases in the electrogenic component of the Na^+, K^+ pump. Indeed, a mathematical model incorporating an electrogenic pump predicts the actual in vivo responses (31). It would seem therefore, that the pump in vascular smooth muscle is electrogenic at physiological $[K]_o$ but that the optimum $[K]_o$ for electrogenicity is greater than physiologic $[K]_o$. Thus, an increase in $[K]_o$ further stimulates the electrogenic pump, leading to hyperpolarization, while a decrease in $[K]_o$ inhibits the pump leading to a depolarization.

Hendrickx & Casteel (18) have demonstrated that either K-free solution or ouabain (10^{-5}M) causes rapid depolarization with little change in permeability to K in the isolated rabbit ear artery. The depolarization was closely followed by tension development. The authors concluded that tension development with K-free solution or ouabain was a simple consequence of depolarization with a threshold change of about 10 mV.

The immediate effect of ouabain (1.7×10^{-5}M) on the guinea pig taenia coli is depolarization accompanied by increased spike frequency and tension development (32). Subsequently there is a delayed relaxation perhaps due to accumulation of $[Na]_i$ (32).

The Electrogenic Pump and Slow Waves in Intestinal Smooth Muscle

Smooth muscle of the intestine of several mammalian species displays gradual fluctuations in E_m called slow waves (33). Prosser (33) distinguishes between slow waves and graded prepotentials observed in some preparations. There is extensive evidence that the slow waves, but *not* the prepotentials, are a function of spontaneous changes in electrogenic pumping (33–36). The slow waves are sensitive to ouabain, K-free solution, and low temperature. The amplitude of the slow waves is greatest at a time when Na^+ pumping is maximal and the slow waves are not associated with changes in membrane resistance. Voltage clamp experiments demonstrate current pulses which have a frequency similar to slow waves and are inhibited by ouabain.

In experiments on longitudinal smooth muscle of rabbit jejunum, El-Sharkawy & Daniel (37) have confirmed the existence of an electrogenic pump. However, these authors have used a modified Goldman equation to estimate the contribution of the electrogenic pump to be not more than 10 mV. They conclude that the electrogenic pump cannot fully account for the rhythmic changes in E_m which averaged 18 mV. Daniel & Sarna (38) have concluded that initiation of slow waves (which they term *electrical control waves*) is related to an increase in conductance, probably to Na^+. The latter authors agree that the Na pump is involved in electrical control waves but suggest that its role is probably secondary to the conductance change. The literature on slow waves is extensive. Variations in the part of the gastrointestinal tract studied, the species used, and the electrophysiological procedures employed have resulted in conflicting results concerning the importance of electrogenic pumping to slow waves. For further references and a discussion of the difficulties in interpreting the results, see (33) and (38).

THE ELECTROGENIC PUMP AND THE RELEASE OF NEUROTRANSMITTERS

The study of electrogenicity in smooth muscle obviously depends on measuring changes in E_m as the pump is acutely inhibited or stimulated. The smooth muscles investigated are innervated by autonomic nerves, and the transmitters from these nerves frequently produce changes in E_m in the smooth muscle cells. Consequently, changes in the spontaneous release of transmitters during stimulation or inhibition of the pump would complicate the interpretation of any changes in E_m which occur.

Ouabain, 10^{-4}M, has been shown to increase approximately sixfold the spontaneous (i.e. unstimulated) release of norepinephrine over a 40 min period (39). The mechanism is presumed to involve Na^+ loading in the

nerves secondary to inhibition of the pump (39). The high $[Na]_i$ would enhance Na^+-Ca^{2+} exchange, increase $[Ca]_i$, and thereby enhance spontaneous release. The Na^+ gradient is also important for neuronal uptake of norepinephrine. Thus, it is not surprising that both K-free solutions and ouabain (10^{-3}M) inhibit neuronal uptake of norepinephrine (40), presumably by increasing $[Na]_i$ and thus reducing the Na gradient across the membrane.

Either ouabain or K-free solution can be expected, therefore, to increase the concentration of norepinephrine in the synaptic cleft by increasing spontaneous release and by inhibition of reuptake (39, 40). Both effects are presumably a function of rising $[Na]_i$, and the rate of rise of transmitter concentration in the biophase should be a function of the rate of increase in $[Na]_i$. Resting Na^+ conductance is quite low in quiescent nerve but increases explosively in the presence of an action potential (41). Therefore, as long as the nerves were not stimulated in an isolated preparation, $[Na]_i$ should rise rather slowly in the presence of pump inhibition simply because Na^+ enters the cell slowly. Indeed, the inhibition by ouabain of ^3H norepinephrine uptake in rat heart slices does not reach its maximum for approximately 30 min (40), and the efflux of ^3H norepinephrine from the rat tail artery does not begin to increase until after 15 min of exposure to K-free solution and is still increasing after 25 min (42). Consequently, the increase in the biophase concentration of transmitters may not be a serious problem during the first 5–10 min of exposure to ouabain or K-free solution, a time period in which pump inhibition often causes depolarization of effector cells consequent to loss of electrogenicity.

Moderate depolarization of smooth muscle cells makes them more sensitive to stimulant drugs (see below). Ouabain, by partially depolarizing smooth muscle cells which have an electrogenic pump, potentiates various other drugs which cause contraction (25). Karaki et al (43) used the potentiation by ouabain of the stimulant effect of norepinephrine on the rabbit aorta to differentiate between effects of ouabain on the smooth muscle versus effects on endogenous transmitter levels. In normal pieces of aorta, potentiation by ouabain was concentration dependent over a range of 5×10^{-8}M to 10^{-3}M. In arteries with the adventitia (and, therefore, the adrenergic nerves) removed, the optimum concentration was about 10^{-6}M and the potentiation fell off sharply at higher concentrations. The potentiating effect of ouabain, 10^{-6}M, was no less in the acutely denervated arteries than in control arteries. These data suggest that, in the rabbit aorta, the potentiating effect of ouabain, 10^{-6}M, is related to an effect on the smooth muscle cells and that the effect of ouabain on the nerves (increased transmitter release and decreased uptake) occurs only at higher concentrations (10^{-5} to 10^{-3}M).

The contractile effects of digitoxigenin (10^{-5}M) have been investigated in veins from dogs (44). Digitoxigenin caused a rapid, short-lasting contraction followed by a slowly developing contraction , reaching maximum after 30–40 min in the portal vein. Only the delayed contraction was observed in saphenous, external jugular and femoral veins. The slow contraction, but *not* the rapid contraction, was antagonized by α-adrenoceptor antagonists or pretreatment with reserpine. Again, there appears to be a separation in time between the rapidly developing effects of cardiac glycosides on the smooth muscle and a delayed indirect effect via the adrenergic nerves.

Ouabain causes a moderate increase in the release of epinephrine from guinea pig vasa deferentia which have been preloaded with the amine, and Katsuragi & Suzuki concluded that they were measuring release from extraneuronal sites dependent upon the Na^+, K^+ pump (45, 46). However, most of their experiments were carried out with no attempt to control neuronal uptake and, in the few experiments in which the vasa deferentia were chronically denervated, no data, such as the percent reduction in endogenous norepinephrine, were presented to establish the success of the denervation. Furthermore, pump inhibition leads to depolarization of the vas deferens of the guinea pig (25). Katsuragi & Suzuki did not consider that extraneuronal transport may be affected by membrane potential rather than being directly dependent upon the pump. Stronger evidence is needed to support the idea that there is any direct dependence of extraneuronal transport mechanisms on the Na^+, K^+ pump.

Inhibition of the Na^+, K^+ pump can increase the release of transmitter from cholinergic nerves. This has been shown, for example, by measuring acetylcholine release from Auerbach's plexus (47). Removal of $[K]_o$ increased both the spontaneous and stimulus-induced release. Readmission of K^+ promptly reduced the release of transmitter.

THE ELECTROGENIC PUMP AND THE RESPONSE OF SMOOTH MUSCLE TO DRUGS

Bülbring (48) was probably the first investigator to recognize the importance of electrogenic pumping in smooth muscle and its response to pharmacologic agents, especially the relationship between the depolarizing effect of a cardiac glycoside, strophanthin, and inhibition of the pump. Evidence at that time also indicated the possibility that the hyperpolarizing effect of epinephrine in some smooth muscles was due to acceleration of electrogenic pumping. However, subsequent investigations in intestinal smooth muscle (49) and myometrium (50) have shown that enhanced electrogenic activity does not contribute to the hyperpolarizing effect of epinephrine.

During the stimulatory action of acetylcholine or carbachol on the guinea pig ileum, there is an enhancement of electrogenic pumping (51). This effect is particularly noticeable as a hyperpolarization immediately after removal of the drug. Bolton (51) concluded that the cholinomimetics were stimulating the pump indirectly by altering the Na^+ and K^+ gradients in a direction favoring pump activity.

Cardiac glycosides have been shown to potentiate the stimulatory effects of other drugs on smooth muscles. For example, acetylstrophanthidin (2×10^{-6}M – 2×10^{-5}M) caused potentiation of contractions of the lateral saphenous vein of the dog in response to norepinephrine, 5-hydroxytryptamine, acetylcholine, and barium (52). This potentiation was apparent within 5 min of exposure to the glycoside. Ozawa & Katsuragi (53) showed that ouabain (1.7×10^{-6}M) potentiated adrenomimetics, cholinomimetics, histamine, 5-hydroxytryptamine, and angiotensin in the vas deferens.

Work in our laboratory and others has demonstrated several important factors relative to this potentiation in the guinea pig vas deferens. 1. This smooth muscle is generally activated by action potentials, the threshold for which is 20–25 mV positive to the resting E_m (54). 2. Norepinephrine causes depolarization and the generation of action potentials (55). 3. Ouabain (10^{-5}M) or low $[K]_o$ (3 mM, that is, one half the normal concentration in Krebs solution) reduces membrane potential 8–10 mV and shifts to the left, by a factor of three, the dose-response curves of methoxamine (an α agonist which is not a substrate for neuronal uptake) and histamine (25). 4. The potentiation is not related to release of endogenous transmitter, because it is not prevented by destruction of the nerves with 6-hydroxydopamine (25). 5. The depolarization with ouabain develops rapidly, is maintained for up to 2 hr and occurs without measurable changes in membrane conductance (25). 6. A high $[K]_o$ (12 mM, or twice normal) causes a similar degree of depolarization and a similar shift of the dose-response curve of methoxamine (25). These findings suggest that any procedure which narrows the separation of the resting E_m and the E_m at which action potentials are elicited will increase the sensitivity of the guinea pig vas deferens to stimulant drugs. Ouabain and low $[K]_o$ produce the necessary depolarization by inhibition of an electrogenic pump (see above; 25) and high $[K]_o$ produces it by decreasing the electrochemical potential.

Further support for this analysis comes from experiments with the rat vas deferens (25). In that tissue, three times normal $[K]_o$ depolarizes the smooth muscle cells by mean of 8 mV and increases the sensitivity to methoxamine by a factor of three. Doubling the normal $[K]_o$ produced only a slight depolarization (mean of 2.2 mV) and no significant change in sensitivity. Ouabain (10^{-5}M to 10^{-4}M) and one half normal $[K]_o$ had no significant effect on either E_m or sensitivity. The absence of a depolarizing effect by

ouabain and low $[K]_o$ in the rat vas deferens has been judged to be due to the lack of an electrogenic component of the pump contributing to resting E_m (see above; 25, 26). Thus, the results obtained from the rat vas deferens support the conclusion that potentiation of stimulant drugs by ouabain and changes in $[K]_o$ are secondary to change in E_m.

Murthy et al (56) demonstrated the potentiation of contractions induced by acetylcholine in the rabbit myometrium by ouabain, 5×10^{-7}M, but claimed a temporal dissociation between the potentiating effect and the binding of ouabain to Na^+, K^+-ATPase. The potentiating effect appeared within 10 min and disappeared within 10 min after removal of the ouabain. However, the inhibition by ouabain (5×10^{-7}M) of Na^+, K^+-ATPase in the membrane component of subcellular fractions was only slightly reduced 10 min after removal of the drug. Furthermore, the inhibitory effect of ouabain (5×10^{-7}M) on ion recovery in Na-loaded tissues required more than 10 min to appear. The authors concluded that ouabain's potentiating action is independent of its inhibition of Na^+, K^+-ATPase (56). Akera & Brody (4) disagree with the conclusion of Murthy et al (56). Akera & Brody (4) emphasize that, "force of contraction and enzyme activity were not studied in the same tissue preparation and that many factors influence the binding." They state that ouabain would be expected to have a later onset of action in a tissue that is not contracting.

POSTJUNCTIONAL SUPERSENSITIVITY IN SMOOTH MUSCLE AND THE ELECTROGENIC PUMP

Postjunctional supersensitivity is a phenomenon whereby a variety of types of excitable cells become more sensitive to stimulant drugs during chronic suppression of the normal physiological stimuli. Research prior to 1976 on this subject has been extensively reviewed (57, 58). Space allows only the following brief summary of the background information on this topic. The reader is referred to the above reviews for references and more detailed information.

Postjunctional supersensitivity is generally produced by chronically depressing or interrupting the activation of the effector cells by their normal stimulatory innervation. This may be achieved by a variety of experimental procedures, including surgical or chemical denervation, decentralization (surgical preganglionic denervation), depletion of the transmitters, chronic ganglionic blockade or blockade of the receptors for the transmitter. Such procedures have been shown to induce supersensitivity in skeletal muscle, cardiac muscle, smooth muscle, central neurons, exocrine glands, and the pineal gland. Postjunctional supersensitivity may represent a compensatory, or homeostatic, mechanism common to most, or perhaps all, excitable

cells and may contribute to pathological problems as well as such pharmacological phenomena as tolerance and withdrawal syndromes.

In smooth muscle, postjunctional supersensitivity has two consistent characteristics. 1. The supersensitivity appears only after a delay and is not maximal for several days to several weeks. The duration of the onset is a characteristic of the muscle under investigation and not of the procedure used, as long as the procedure produces marked suppression of transmission and the suppression is maintained. 2. The increase in sensitivity is nonspecific. That is, the sensitivity is increased to a variety of agonists, often including ions, which act independently of the transmitter. The phenomenon is demonstrated as a shift to the left of the dose-response curve for each agonist, in most instances without a change in maximum response.

Several different changes in effector cells have been associated with the development of postjunctional supersensitivity (58). These include (*a*) an increased density of receptors, (*b*) a partial depolarization, (*c*) changes in calcium binding and/or permeability, (*d*) an enhanced activity of adenylate cyclase. Several of these cellular changes can contribute to supersensitivity in a given type of effector cell. For example, changes *a, b,* and *c* all occur in denervated skeletal muscle. Furthermore, which cellular mechanism(s) is (are) more important for supersensitivity varies from one type of effector cell to another.

Evidence presently available indicates that two of these mechanisms, a partial depolarization (58–60) and altered calcium binding and/or permeability (61), are of particular importance in postjunctional supersensitivity in smooth muscle. It should be noted that postganglionic denervation of the vas deferens causes an increased maximum response to agonists which is associated with improved electrical coupling among the smooth muscle cells (62, 63). This last change is separable from, and independent of, postjunctional supersensitivity.

Of primary relevance to this review is the contribution of a partial membrane depolarization to postjunctional supersensitivity in smooth muscle. The first hint of a role of membrane potential in supersensitivity in a smooth muscle preparation was obtained in experiments with the guinea pig ileum (64). Chronic treatment with ganglionic blocking agents was used to induce postjunctional supersensitivity. Dose-response curves of several stimulant agonists were determined in isolated pieces of ileum. In normal Krebs solution, pieces of ileum from pretreated guinea pigs were supersensitive in comparison to pieces of ileum from control guinea pigs. If the tissues were studied in a solution which caused complete depolarization, responses could still be obtained but there was no measurable difference in sensitivity between the ileal pieces from treated and control animals. It was therefore suggested that the demonstration of postjunctional supersensitivity in that tissue required some factor related to membrane potential (64).

This was followed by more definitive evidence. Denervation or decentralization of the guinea pig vas deferens produces nonspecific
(65). Both procedures also produced a depolarization of 8–10 mV in the smooth muscle cells (23). The supersensitivity and the partial depolarization both appear on the fourth day after decentralization and are maintained for at least two weeks thereafter (23). This decreased negativity of the resting E_m occurs without any change in the threshold E_m at which action potentials are generated (63). As a result, resting E_m is moved closer to threshold E_m.

Procedures which acutely inhibit the Na^+, K^+ pump (one half normal $[K]_o$; ouabain, 10^{-5}M) cause a similar depolarization of the control vas deferens and make it nonspecifically
potentiating effect of ouabain in the guinea pig vas deferens has also been reported by Ozawa & Katsuragi (53). The effects of ouabain or low $[K]_o$ on sensitivity are not due to release of endogenous transmitter (25). The effects of low $[K]_o$ or ouabain are quite different in the 7-day denervated vasa deferentia (24). As indicated above, 7 days after denervation, the vas deferens is supersensitive and depolarized 8–10 mV. Under these conditions, ouabain, 10^{-5}M, has no effect on E_m and one-half normal $[K]_o$ actually causes a hyperpolarization. As discussed earlier in this review, there is strong evidence that electrogenic pumping does contribute to resting E_m in the guinea pig vas deferens. The facts that (*a*) chronic denervation produces a depolarization similar in magnitude to that produced acutely by ouabain or low $[K]_o$, (*b*) ouabain does *not* depolarize the chronically denervated vas deferens, and (*c*) low $[K]_o$ *hyperpolarizes* the chronically denervated vas deferens indicate that chronic denervation leads to almost complete loss of electrogenic pumping.

The results of biochemical experiments support this conclusion (24). Na^+, K^+-ATPase activity of the guinea pig vas deferens is decreased by chronic denervation, decentralization, or reserpine pretreatment, each of which induces postjunctional supersensitivity. The time course for the reduction in enzyme activity was similar to that for the partial depolarization and appearance of supersensitivity. It was further shown that the reduced enzyme activity was not due to reduced affinity of the enzyme for ATP, Na^+, K^+, or its inhibitor, ouabain. However, V_{max} was significantly decreased. It is therefore proposed that chronic interruption of the innervation of the smooth muscle of the guinea pig vas deferens causes a reduction in Na^+, K^+-ATPase activity which is particularly apparent as a decline in electrogenicity. This decrease in electrogenic pumping results in a partial depolarization and increased sensitivity.

Electrophysiologic experiments have yielded results indicating that similar events contribute to postjunctional supersensitivity in the rabbit saphenous artery. Pretreatment of rabbits with reserpine, 0.3 mg/kg/day for 1

or 3 days caused depletion of norepinephrine by more than 95% (60). Such treatment for 3 days produced nonspecific supersensitivity and a mean depolarization of 4 mV in the arterial smooth muscle cells in contrast to pretreatment with reserpine for 1 day which caused no change in either E_m or sensitivity (60, 66, 67).

Ouabain, 3 X 10^{-6}M, caused rapid depolarizations of 5–6 mV and a nonspecific increase in sensitivity in cells of control saphenous arteries (60, 67). These effects of ouabain were not due to release of endogenous norepinephrine or changes in neuronal uptake, since the effects of ouabain were not altered by short-term (1-day) depletion of the transmitter with reserpine or by inhibition of uptake by desipramine (28, 67). However, in arteries from rabbits pretreated with reserpine for 3 days, ouabain had no effect on membrane potential (28). Therefore, electrogenic pumping may contribute approximately 4–6 mV to the normal resting E_m and supersensitivity coincides with a marked reduction in the electrogenic component of E_m (60). It should be noted, however, that the depolarization of 4 mV of smooth muscle cells of the saphenous artery by chronic pretreatment of rabbits with reserpine is not adequate to account completely for the magnitude of supersensitivity observed. It has been suggested that a second contributing factor is a change in permeability to, or binding of, calcium (60).

Aprigliano & Hermsmeyer have reported an association between supersensitivity and partial depolarization in the rat portal vein (68). Pretreatment with 6-hydroxydopamine caused destruction of adrenergic nerves. There was an increase in sensitivity to both Ba^{2+} and norepinephrine which was maximal 3 days after treatment with 6-hydroxydopamine. There was also a depolarization which was maximal (mean change of 3.8 mV) 3 days after treatment. It is noteworthy that supersensitivity is associated with a depolarization of approximately 4 mV in both the rabbit saphenous artery (67) and the rat portal vein (68).

HYPERTENSION AND THE ELECTROGENIC PUMP

Na-loading experiments have established that the caudal artery of the rat is electrogenic (69). Lowering of temperature from 36°C to 16°C or exposure to K-free solution causes immediate depolarization of caudal arteries, indicating that an electrogenic pump contributes directly to the resting E_m (69). Comparison of caudal arteries from normal (KNR) and spontaneously hypertensive rats (SHR) by Hermsmeyer revealed some interesting differences (69, 70). The resting E_m value was similar in the two groups, about –55 mV. However, in the arteries from SHR, the electrogenic pump contributes a larger portion and the electrochemcial gradients contribute a smaller portion of the resting E_m. These results are consistent with the

report by Friedman & Friedman that P_{Na} and P_K are increased in caudal arteries from SHR and that this increased permeability is compensated for by enhanced Na^+ pumping (71).

Caudal arteries from SHR are more sensitive to norepinephrine than arteries from normal rats (69). Hermsmeyer (69, 70) has suggested that norepinephrine, by increasing permeability and passive diffusion of ions, which short circuits the pump, would produce a greater depolarization in those arteries in which the electrogenic pump contributes a greater portion of the resting E_m. In turn, the hypertension could be partly the consequence of greater sensitivity of vessels to endogenous norepinephrine.

Results obtained by Friedman & Friedman (71) indicated that similar changes may occur in caudal arteries from DOCA-hypertensive rats. That is, both the loss of K^+ (by passive diffusion) from arteries perfused by K-free solution and the gain of K^+ (by active transport) upon reexposure to normal solution were more rapid in arteries from DOCA-hypertensive rats than from normal rats. However, a report from Haddy's laboratory (72) reached the opposite conclusion. These investigators determined ouabain-sensitive uptake of ^{86}Rb as a measure of activity of the Na^+, K^+ pump. Since ouabain-sensitive uptake of ^{86}Rb was decreased in caudal arteries of rats made hypertensive with DOCA, the authors concluded that the pump is suppressed in volume-expanded hypertension. The difference in results obtained by the two groups may be related to the interval of time that elapsed between removal of the arteries and the measurements of ion movement (72).

There is, in fact, extensive evidence that depression of the Na^+, K^+ pump does contribute to hypertension resulting from volume expansion. A decreased activity of the electrogenic pump would partially depolarize the vascular smooth muscle cells (72). The evidence has been thoroughly reviewed by Haddy et al (73) and extensive discussion is beyond the scope of a review of this length. Briefly, some of the key points from the review by Haddy et al (73) are as follows.

Decreased activity of the Na^+, K^+ pump has been observed in blood vessels of several different models of volume-expanded hypertension. There may be a circulating substance in the blood which inhibits the pump. Inhibition of the pump would lead to partial depolarization. Such a change in E_m would be important because small decreases in E_m are associated with large increases in tension. In addition, partial depolarization would result in nonspecific (postjunctional) supersensitivity to endogenous constrictor substances (see section on supersensitivity). Finally, essential hypertension may well be a volume-expanded phenomenon and there is evidence that Na^+, K^+ pump activity is lower in patients with essential hypertension than in control patients.

CONCLUSIONS

By actively transporting more Na^+ ions out of the cells than K^+ ions in, the Na^+, K^+ pump can directly contribute to the membrane potential in a variety of smooth muscle cells. As a result, changes in pump activity can rapidly change membrane potential. Because the pump exchanges Na^+ inside the cell for K^+ outside the cell, the pump activity and, likewise, its electrogenic contribution, are readily modified by changes in the extracellular K^+ concentration. Cardiac glycosides, such as ouabain, are specific inhibitors of the pump and, therefore, cause moderate and rapid depolarizations of many smooth muscle cells.

In some cells, such as vascular smooth muscle in which small changes in membrane potential cause marked changes in tension, alterations in extracellular potassium or exposure to cardiac glycosides can cause marked changes in tension. Furthermore, low extracellular potassium or ouabain, by causing a partial depolarization, can potentiate other stimulant drugs.

Inhibition of the Na^+, K^+ pump can also alter the concentration of endogenous neurotransmitters in the biophase by increasing spontaneous release and, in the case of norepinephrine, inhibiting neuronal uptake. This effect appears to occur much more slowly than the effects of inhibiting the electrogenic pump in smooth muscle and to require larger concentrations of ouabain. Nevertheless, an increased concentration of endogenous transmitter in the biophase may complicate some experiments aimed at investigating pump inhibition in smooth muscle. This potential problem can be avoided by acute depletion of transmitter or acute destruction of the nerves.

Postjunctional supersensitivity, the enhanced nonspecific sensitivity which occurs in smooth muscles and other excitable cells after chronic suppression of the stimulatory innervation, has been shown to be associated with a partial depolarization in several types of smooth muscle. This depolarization is due to a decrease in electrogenic pumping and coincides with a decrease in Na^+, K^+ ATPase activity in subcellular fractions. In the vas deferens of the guinea pig, the partial depolarization results in supersensitivity by moving the resting potential closer to the potential at which action potentials are elicited.

Electrogenic pumping contributes a greater portion of the resting membrane potential in arterial smooth muscle of spontaneously hypertensive rats. The smooth muscle is also supersensitive to norepinephrine. It has been suggested that the catecholamine, by increasing permeability to ions, short circuits the pump and thus causes greater depolarization in the muscle of spontaneously hypertensive rats than in the muscle of normal rats. In contrast, volume expanded hypertension, and possibly human essential hypertension, is associated with depressed pump activity. It has been sug-

gested that decreased electrogenic pumping causes partial depolarization, increased tone, and supersensitivity to endogenous vasoconstrictors.

Literature Cited

1. Thomas, R. C. 1972. Electrogenic sodium pump in nerve and muscle cells. *Physiol. Rev.* 52:563–94
2. Casteels, R. 1970. The relation between the membrane potential and the ion distribution in smooth muscle cells. In *Smooth Muscle,* ed E. Bülbring, A. F. Brading, A. W. Jones, T. Tomita, pp. 70–99. Baltimore: Williams & Wilkins. 676 pp.
3. Casteels, R., Droogmans, G., Hendrickx, H. 1973. Active ion transport and resting potential in smooth muscle cells. *Phil. Trans. R. Soc. London Ser. B* 265:47–56
4. Akera, T., Brody, T. M. 1977. The role of Na^+, K^+-ATPase in the inotropic action of digitalis. *Pharmacol. Rev.* 29:187–220
5. Taylor, G. S., Paton, D. M., Daniel, E. E. 1969. Evidence for an electrogenic sodium pump in smooth muscle. *Life Sci.* 8:769–73
6. Taylor, G. S., Paton, D. M., Daniel, E. E. 1971. Effect of rubidium and caesium on electrogenic sodium pumping in rat myometrium. *Comp. Biochem. Physiol. A* 38:251–64
7. Akera, T. 1977. Membrane adenosine triphosphatase: A digitalis receptor? *Science* 198:569–74
8. Murthy, R. V., Kidwai, A. M., Daniel, E. E. 1972. The uptake of cardiac glycosides by rabbit and rat myometrium. *J. Pharmacol. Exp. Ther.* 182:166–78
9. Murthy, R. V., Kidwai, A. M., Daniel, E. E. 1974. Subcellular distribution of cardiac glycosides in rabbit and rat myometrium and their binding to isolated plasma membrane. *J. Pharmacol. Exp. Ther.* 188:582–95
10. Bolton, T. B. 1973. Effects of electrogenic sodium pumping on the membrane potential of longitudinal smooth muscle from terminal ileum of guinea-pig. *J. Physiol. London* 228:693–712
11. Casteels, R. 1969. Calculation of the membrane potential in smooth muscle cells of the guinea-pig taenia coli by the Goldman equation. *J. Physiol. London* 205:193–208
12. Casteels, R., Droogmans, G., Hendrickx, H. 1971. Membrane potential of smooth muscle cells in K-free solution. *J. Physiol. London* 217:281–95
13. Taylor, G. S., Paton, D. M., Daniel, E. E. 1970. Characteristics of electrogenic sodium pumping in rat myometrium. *J. Gen. Physiol.* 56:360–75
14. Kao, C. Y., Nishiyama, A. 1969. Ion concentrations and membrane potentials of myometrium during recovery from cold. *Am. J. Physiol.* 217:525–31
15. Tomita, T., Yamamoto, T. 1971. Effects of removing external potassium on the smooth muscle of guinea-pig taenia coli. *J. Physiol. London* 212:851–68
16. Casteels, R., Droogmans, G., Hendrickx, H. 1971. Electrogenic sodium pump in smooth muscle cells of the guinea-pig's taenia coli. *J. Physiol. London* 217:297–313
17. Bolton, T. B. 1971. Electrophysiologic evidence of an electrogenic sodium pump in the longitudinal muscle of the guinea-pig ileum and its involvement in the response to acetylcholine. *J. Physiol. London* 218(1):58–59P (Abstr.)
18. Hendrickx, H., Casteels, R. 1974. Electrogenic sodium pump in arterial smooth muscle cells. *Pfleugers Arch.* 346:299–306
19. Casteels, R., Kitamura, K., Kuriyama, H., Suzuki, H. 1977. The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol. London* 271:41–61
20. Kuriyama, H., Ohshima, K., Sakamoto, Y. 1971. The membrane properties of the smooth muscle of the guinea-pig portal vein in isotonic and hypertonic solutions. *J. Physiol. London* 217:179–99
21. Shuba, M. F. 1977. The effect of sodium-free and potassium-free solutions, ionic current inhibitors and ouabain on electrophysiological properties of smooth muscle of guinea-pig ureter. *J. Physiol. London* 264:837–51
22. Droogmans, G., Casteels, R. 1976. Membrane potential and ion transport in smooth muscle cells. In *Physiology of Smooth Muscle,* ed. E. Bülbring, M. F. Shuba, pp. 11–18. New York: Raven. 438 pp.
23. Fleming, W. W., Westfall, D. P. 1975. Altered resting membrane potential in the supersensitive vas deferens of the guinea pig. *J. Pharmacol. Exp. Ther.* 192:381–89

24. Gerthoffer, W. T., Fedan, J. S., Westfall, D. P., Goto, K., Fleming, W. W. 1979. Involvement of the sodium-potassium pump in the mechanism of postjunctional supersensitivity of the vas deferens of the guinea pig. *J. Pharmacol. Exp. Ther.* 210:27–36
25. Urquilla, P. R., Westfall, D. P., Goto, K., Fleming, W. W. 1978. The effects of ouabain and alterations in potassium concentration on the sensitivity to drugs and the membrane potential of the smooth muscle of the guinea-pig and rat vas deferens. *J. Pharmacol. Exp. Ther.* 207:347–55
26. Fedan, J. S., Westfall, D. P., Fleming, W. W. 1978. Species differences in sodium-potassium adenosine triphosphatase activity in the smooth muscle of the g inea-pig and rat vas deferens. *J. Pharmacol. Exp. Ther.* 207:356–63
27. Matthews, E. K., Sutter, M. C. 1967. Ouabain-induced changes in the contractile and electrical activity, potassium content and response to drugs, of smooth muscle cells. *Can. J. Physiol. Pharmacol.* 45:509–20
28. Abel, P. W., Urquilla, P. R., Fleming, W. W. 1978. Postjunctional supersensitivity and the effect of ouabain on the rabbit saphenous artery. *Pharmacologist* 20(3):227 (Abstr.)
29. Siegel, G., Roedel, H., Nolte, J., Hofer, H. W., Bertsche, O. 1976. Ionic composition and ion exchange in vascular smooth muscle. See Ref. 22, pp. 19–39
30. Haddy, F. J. 1975. Potassium and blood vessels. *Life Sci.* 16:1489–98
31. Anderson, D. K. 1976. Cell potential and the sodium-potassium pump in vascular smooth muscle. *Proc. Fed. Am. Soc. Exp. Biol.* 35:1294–97
32. Bose, D. 1974. Mechanism of inhibition of smooth muscle tension in guinea-pig taenia coli by ouabain. *Can. J. Physiol. Pharmacol.* 52:898–901
33. Prosser, C. L. 1978. Rhythmic potentials in intestinal muscle. *Proc. Fed. Am. Soc. Exp. Biol.* 37:2153–57
34. Job, D. D. 1969. Ionic basis of intestinal electrical activity. *Am. J. Physiol.* 217:1534–41
35. Liu, J., Prosser, C. L., Job, D. D. 1969. Ionic dependence of slow waves and spikes in intestinal muscle. *Am. J. Physiol.* 217:1542–47
36. Connor, J. A., Prosser, C. L., Weems, W. A. 1974. A study of pacemaker activity in intestinal smooth muscle. *J. Physiol. London* 240:671–701
37. El-Sharkawy, T. Y., Daniel, E. E. 1975. Electrogenic sodium pumping in rabbit small intestinal smooth muscle. *Am. J. Physiol.* 229:1277–86
38. Daniel, E. E., Sarna, S. 1978. The generation and conduction of activity in smooth muscle. *Ann. Rev. Pharmacol. Toxicol.* 18:145–66
39. Weiner, N., Lee, F. L. 1975. The role of calcium in norepinephrine release and synthesis. *In Chemical Tools in Catecholamine Research* 11, ed. O. Almgren, A. Carlsson, J. Engel, pp. 61–71. New York: Am. Elsevier. 310 pp.
40. Bogdanski, D. F., Brodie, B. B. 1969. The effects of inorganic ions on the storage and uptake of H^3-norepinephrine by rat heart slices. *J. Pharmacol. Exp. Ther.* 165:181–89
41. Hodgkin, A. L. 1958. The Croonian Lecture: Ionic movements and electrical activity in giant nerve fibers. *Proc. R. Soc. London Ser. B.* 148:1–37
42. Bonaccorsi, A., Hermsmeyer, K., Smith, C. B., Bohr, D. F. 1977. Norepinephrine release in isolated arteries induced by K-free solution. *Am. J. Physiol.* 232:H140–45
43. Karaki, H., Ozaki, H., Urakawa, N. 1978. Effects of ouabain and potassium-free solution on the contraction of isolated blood vessels. *Eur. J. Pharmacol.* 48:439–43
44. Tsuru, H., Shigei, T. 1976. Participation of catecholamine in the digitoxigenin-induced contraction of isolated dog veins. *Jpn. J. Pharmacol.* 26:120–22
45. Katsuragi, T., Suzuki, T. 1976. Ouabain-induced release of extraneuronal catecholamine in the isolated guinea-pig vas deferens. *Experientia* 32:727–28
46. Katsuragi, T., Suzuki, T. 1977. Effects of calcium-removal and sodium-potassium adenosine triphosphatase inhibition on the release of epinephrine from the extraneuronal site. *Life Sci.* 21: 137–44
47. Vizi, E. S. 1977. Termination of transmitter release by stimulation of sodium-potassium activated ATPase. *J. Physiol. London* 267:261–80
48. Bülbring, E. 1962. Electrical activity in intestinal smooth muscle. *Physiol. Rev.* 42: Suppl. 5, pp. 160–74
49. El-Sharkawy, T. Y., Daniel, E. E. 1976. The electrophysiological basis of the motor inhibitory effect of adrenaline on rabbit small intestinal smooth muscle. *Can. J. Physiol. Pharmacol.* 54:446–56
50. Marshall, J. M. 1977. Modulation of smooth muscle activity by catecholamines. *Proc. Fed. Am. Soc. Exp. Biol.* 36:2450–55

51. Bolton, T. B. 1973. The role of electrogenic sodium pumping in the response of smooth muscle to acetylcholine. *J. Physiol. London* 228:713–31
52. Brender, D., Strong, C. G., Shepherd, J. T. 1970. Effects of acetylstrophanthidin on isolated veins of the dog. *Circ. Res.* 26:647–55
53. Ozawa, H., Katsuragi, T. 1974. Ouabain-induced potentiation on the contractions of the guinea-pig vas deferens. *Eur. J. Pharmacol.* 25:147–54
54. Goto, K., Millecchia, L. L., Westfall, D. P., Fleming, W. W. 1977. A comparison of the electrical properties and morphological characteristics of the smooth muscle of the rat and guinea-pig vas deferens. *Pfleugers, Arch.* 368:253–261
55. Magaribuchi, T., Ito, Y., Kuriyama, H. 1971. Effects of catecholamines on the guinea-pig vas deferens in various ionic environments. *Jpn. J. Physiol.* 21:691–708
56. Murthy, R. V., Kidwai, A. M., Daniel, E. E. 1974. Dissociation of contractile effect and binding and inhibition of Na^+-K^+-adenosine triphosphatase by cardiac glycosides in rabbit myometrium. *J. Pharmacol. Exp. Ther.* 188: 575–81
57. Fleming, W. W., McPhillips, J. J., Westfall, D. P. 1973. Postjunctional supersensitivity of excitable tissues to drugs. *Rev. Physiol. Biochem. Exp. Pharmacol.* 68:55–119
58. Fleming, W. W. 1976. Variable sensitivity of excitable cells: possible mechanisms and biological significance. *Rev. Neurosci.* 2:43–90
59. Fleming, W. W. 1978. The trophic influence of autonomic nerves on electrical properties of the cell membrane in smooth muscle. *Life. Sci.* 22:1223–28
60. Fleming, W. W., Abel, P. W., Urquilla, P. R., Westfall, D. P. 1979. Electrophysiologic correlations with supersensitivity in vascular smooth muscle; a mini-review. In *Proc. 3rd Int. Symp. Vascular Neuroeffector Mechanisms*, ed. P. Vanhoutte. In press
61. Carrier, O. 1975. Role of calcium in postjunctional supersensitivity. *Proc. Fed. Am. Soc. Exp. Biol.* 34:1975–80
62. Westfall, D. P., Millecchia, L. L., Lee, T. J.-F., Corey, S. P., Smith, D. J., Fleming, W. W. 1977. Effects of denervation and reserpine on nexuses in the rat vas deferens. *Eur. J. Pharmacol.* 41:239–42
63. Goto, K., Westfall, D. P., Fleming, W. W. 1978. Denervation-induced changes in electrophysiologic parameters of the smooth muscle of the guinea-pig and rat vas deferens. *J. Pharmacol. Exp. Ther.* 204:325–33
64. Fleming, W. W. 1968. Nonspecific supersensitivity of the guinea-pig ileum produced by chronic ganglion blockade. *J. Pharmacol. Exp. Ther.* 162:277–85
65. Westfall, D. P. 1970. Nonspecific supersensitivity of the guinea-pig vas deferens produced by decentralization and reserpine treatment. *Br. J. Pharmacol.* 39:110–20
66. Abel, P. W., Urquilla, P. R., Fleming, W. W. 1976. Reserpine-induced supersensitivity in the rabbit saphenous artery. *Pharmacologist* 18(2):141 (Abstr.)
67. Abel, P. W., Urquilla, P. R., Fleming, W. W. 1977. A possible role of resting membrane potential in postjunctional supersensitivity in the rabbit saphenous artery. *Proc. Fed. Am. Soc. Exp. Biol.* 36(3):1028 (Abstr.)
68. Aprigliano, O., Hermsmeyer, K. 1977. Trophic influence of the sympathetic nervous system on the rat portal vein. *Circ. Res.* 41:198–206
69. Hermsmeyer, K. 1976. Electrogenesis of increased norepinephrine sensitivity of arterial vascular muscle in hypertension. *Circ. Res.* 38:362–67
70. Hermsmeyer, K. 1976. Cellular basis for increased sensitivity of vascular smooth muscle in spontaneously hypertensive rats. *Cir. Res.* 38: Suppl. 11, pp. 1153–57
71. Friedman, S. M., Friedman, C. L. 1976. Cell permeability, sodium transport, and the hypertensive process in the rat. *Circ. Res.* 39:433–41
72. Pamnani, M. B., Clough, D. L., Haddy, F. J. 1978. Altered activity of the sodium-potassium pump in arteries of rats with steroid hypertension. *Clin. Sci. Mol. Med.* 55:415–35
73. Haddy, F., Pamnani, M., Clough, D. 1978. The sodium-potassium pump in volume expanded hypertension. *Clin. Exp. Hyperten.* 1:295–336