brane currents in the rabbit atrioventricular node cell. Pflügers Arch. *393* (1982) 15–22.
33 Matsuda, H., Noma, A., Kurachi, Y., and Irisawa, H., Transient depolarization and spontaneous voltage fluctuations in isolated single cells from guinea pig ventricles. Circ. Res. *51* (1982) 142–151.
34 Matsuda, H., and Noma, A., Isolation of calcium channel current and its sensitivity to monovalent cations in internally dialysed ventricular cells of guinea pig. J. Physiol. *357* (1984) 553–573.
35 Maylie, J., and Morad, M., Ionic currents responsible for generation of pacemaker current in rabbit sino-atrial node. J. Physiol. *355* (1984) 215–235.
36 Mitra, R., and Morad, M., Evidence for two types of calcium channels in guinea-pig ventricular myocytes. J. gen. Physiol. *86* (1985) 22–23a.
37 Nakamura, S., Hama, K., Asai, J., and Irisawa, H., Observations on the fine structure of nodal, Purkinje and working myocardial cells isolated from rabbit hearts. Arch. hist. jap. *49* (1986) 105–116.
38 Nakayama, T., Kurachi, Y., Noma, A., and Irisawa, H., Action potential and membrane currents of single pacemaker cells of the rabbit heart. Pflügers Arch. *402* (1984) 248–257.
39 Nakayama, T., and Irisawa, H., Transient outward current carried by potassium and sodium in quiescent atrioventricular node of rabbits. Circ. Res. *57* (1985) 65–73.
40 Nathan, R. D., Two electrophysiologically distinct types of cultured pacemaker cells from rabbit sinoatrial node. Am. J. Physiol. *250* (1986) (Heart Circ. Physiol. 19) H325–329.
41 Nilius, B., Hess, P., Lansman, J. B., and Tsien, R. W., A novel type of cardiac calcium channel in ventricular cells. Nature *316* (1985) 443–446.
42 Nilius, B., Possible functional significance of a novel type of cardiac Ca channel. Biochim. biophys. Acta (1986) in press.
43 Noble, D., The surprising heart: A review of recent progress in cardiac electrophysiology. J. Physiol. *353* (1984) 1–50.
44 Noble, D., and Noble, S. J., A model of sino-atrial node electrical activity based on a modification of DiFrancesco-Noble (1984) equations. Proc. R. Soc. B *222* (1984) 295–304.
45 Noma, A., ATP regulated $K^+$ channels in cardiac muscle. Nature *305* (1983) 147–148.
46 Noma, A., and Irisawa, H., Membrane currents in the rabbit sinoatrial node cell as studied by the double microelectrode method. Pflügers Arch. *364* (1976a) 45–52.
47 Noma, A., and Irisawa, H., A time and voltage dependent potassium current in the rabbit sinoatrial node cell. Pflügers Arch. 366 (1976b) 251–258.
48 Noma, A., Kotake, H., and Irisawa, H., Slow inward current and its role mediating the chronotropic effect of epinephrine in the rabbit sinoatrial node. Pflügers Arch. *388* (1980) 1–9.
49 Noma, A., Morad, M., and Irisawa, H., Does the 'pacemaker current' generate the diastolic depolarization in rabbit S-A node cells? Pflügers Arch. *397* (1983) 190–194.
50 Noma, A., Nakayama, T., Kurachi, Y., and Irisawa, H., Resting K conductances in pacemaker and non-pacemaker heart cells of the rabbit. Jap. J. Physiol. *34* (1984) 245–254.
51 Nowycky, M. C., Fox, A. P., and Tsien, R. W., Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature *316* (1985) 440–443.
52 Reuter, H., Kokubun, S., and Prod'hom, B., Properties and modulation of cardiac calcium channels. J. exp. Biol. (1987) in press.
53 Sackmann, B., Noma, A., and Trautwein, W., Acetylcholine activation of single muscarinic $K^+$ channels in isolated pacemaker cells of mammalian heart. Nature *303* (1983) 250–253.
54 Shibasaki, T., and Irisawa, H., Single delayed rectifier K channels in pacemaker cells of rabbit heart. Proc. int. Union physiol. Sci. *16* (1986) P266.07.
55 Soejima, M., and Noma, A., Regulation of the K channel by the muscarinic receptor in the rabbit atrial cells. Pflügers Arch. *400* (1984) 424–431.
56 Toda, N., and West, T. C., Interactions of K, Na and vagal stimulation in S-A node of the rabbit. Am. J. Physiol. *212* (1967) 416–423.
57 Yanagihara, K., and Irisawa, H., Inward current activated during hyperpolarization in the rabbit sinoatrial node cell. Pflügers Arch. *385* (1980a) 11–19.
58 Yanagihara, K., and Irisawa, H., Potassium current during the pacemaker depolarization in rabbit sinoatrial node cell. Pflügers Arch. *388* (1980b) 255–260.
59 Yanagihara, K., Noma, A., and Irisawa, H., Reconstruction of sinoatrial node pacemaker potential based on the voltage clamp experiments. Jap. J. Physiol. *30* (1980) 841–857.

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## Contribution of the $Na^+/K^+$-pump to the membrane potential

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*Summary.* The inward movement of sodium ions and the outward movement of potassium ions are passive and the reverse movements against the electrochemical gradients require the activity of a metabolism-driven $Na^+/K^+$-pump. The activity of the $Na^+/K^+$-pump influences the membrane potential directly and indirectly. Thus, the maintenance of a normal electrical function requires that the $Na^+/K^+$-pump maintain normal ionic concentrations within the cell. The activity of the $Na^+/K^+$-pump also influences the membrane potential directly by generating an outward sodium current that is larger when the $Na^+/K^+$-pump activity is greater. The activity of the $Na^+/K^+$-pump is regulated by several factors including the intracellular sodium concentration and the neuromediators norepinephrine and acetylcholine. The inhibition of the $Na^+/K^+$-pump can lead indirectly to the development of inward currents that may cause repetitive activity. Therefore, the $Na^+/K^+$-pump modifies the membrane potential in different ways both under normal and abnormal conditions and influences in an essential way many cardiac functions, including automaticity, conduction and contraction.
*Key words.* Active transport of ions; cardiac tissues; electroneutral and electrogenic $Na^+/K^+$ pump; control of $Na^+/K^+$-pump; normal and abnormal electrical events.

### *The 'necessity' of a sodium-potassium pump*

The concentration of sodium is far greater outside than inside the cardiac cells whereas the opposite verifies for potassium ions. In addition, the inside of the cells is negative at rest. This creates an inwardly directed electrochemical gradient for the sodium ions. At rest, the sodium conductance is relatively small and so is the background sodium current. However, the large sodium gradient is exploited in several ways. Because of the inwardly directed electrochemical gradient, it is only necessary to open the fast sodium channels to initiate a fast inward current through the cell membrane

which is responsible for the upstroke[17] in many cardiac cells. The sodium gradient across the cell membrane is also used for extrusion of calcium and hydrogen ions from the cell through the $Na^+/Ca^{++}$[10,38] and $Na^+/H^+$ exchange[13,38], respectively, as well as for the co-transport of other ions and substances. Therefore, the entry of sodium into the cell subserves several essential functions but over any length of time the influx must be matched by an equal sodium efflux.

As for the potassium ions, there is a small outwardly directed electrochemical gradient at rest (as the resting potential is less negative than the potassium equilibrium potential) and such gradient increases as the cells depolarize during the action potential. The increased electrochemical gradient for potassium during activity facilitates the potassium efflux and repolarization.

If the influx of sodium and the efflux of potassium are passive (down their electrochemical gradient), the efflux of sodium and the re-uptake of potassium necessitates an active transport by the $Na^+/K^+$-pump ('the sodium pump'), requiring metabolic energy for its operation. If the active transport of $Na^+$ and $K^+$ fails to match the passive movements of these ions over a length of time, the membrane potential (as well as other cell functions) can not be maintained. Therefore, the function of the $Na^+/K^+$-pump contributes powerfully to the maintenance of the membrane potential as well as to the ionic homeostasis of the cell.

*Electroneutral versus electrogenic sodium pump and membrane potential*

The fact that the extrusion of sodium ions by the $Na^+/K^+$-pump is linked with the uptake of potassium ions raises the question as to whether the $Na^+/K^+$-pump is electroneutral or electrogenic. An electroneutral $Na^+/K^+$-pump would influence the membrane potential indirectly but quite substantially. At rest, the membrane is not totally impermeable to sodium ions and in addition these ions enter the cell in a larger amount during activity and through the various other routes mentioned above. This means that the resting potential has to be maintained through an active extrusion of sodium by the pump. That the $Na^+/K^+$-pump does not determine the resting potential is shown by the fact that the potential is maintained (*actually increases*[57]) when the extracellular sodium is substituted by an impermeant cation: in the absence of external sodium, the intracellular sodium should decrease[34] and this would eventually lead to the cessation of the $Na^+/K^+$-pump activity. However, that the $Na^+/K^+$-pump is needed for the maintenance of the resting potential is clearly indicated by the slow decline of the resting potential when the sodium $Na^+/K^+$-pump is inhibited (e.g., cardiac steroids[61]). An electroneutral $Na^+/K^+$ pump would indirectly control also the action potential by regulating (directly or indirectly) the intracellular concentrations of several ions ($Na^+$, $K^+$, $Ca^{++}$, $H^+$, etc.) which, in turn, would modify concentration gradients and membrane conductances. Thus, the $Na^+/K^+$-pump conditions the maintenance of the membrane potential by maintaining the appropriate concentrations of several ions across the cell membrane.

However, an electrogenic $Na^+/K^+$-pump would in addition influence the membrane potential directly. If a fraction of the sodium extruded is uncoupled to the potassium taken up (as in many other tissues[49]), the $Na^+/K^+$-pump would create an outward sodium current that would make the membrane potential more negative not only at rest but also during activity.

There are several findings indicating that the $Na^+/K^+$-pump is electrogenic also in cardiac tissues. Thus, cooling cardiac tissues below 20 °C decreases the resting potential more than it would be expected from a diffusion potential[14]. This potential drop is reduced in the presence of a metabolic inhibitor or anoxia, suggesting that the fall in resting potential is due to the decreased activity of an electrogenic $Na^+/K^+$-pump[14]. The contribution of the $Na^+/K^+$-pump to the resting potential appears to be less for a moderate decrease in temperature (to 27 °C), possibly because in resting fibers the intracellular sodium activity decreases[1,12] and therefore the activity of the $Na^+/K^+$-pump is possibly already reduced. Still, the administration of strophanthidin quickly decreases the resting potential of quiescent Purkinje fibers and may induce the onset of spontaneous discharge[61], although it is not clear whether this is the result of the inhibition of an electroneutral or electrogenic sodium pump.

That the $Na^+/K^+$-pump can be electrogenic is supported also by the fact that the rewarming of cooled cardiac tissues leads to an increase in resting potential above the control (precooling) value[24,26,42,48]. The hyperpolarization is absent if during hypothermia Na is substituted by Li or if cardiac steroids were administered[26]. The low temperature acts by inhibiting the sodium pump and therefore causing an accumulation of sodium: when the temperature is restored to normal, the activity of the $Na^+/K^+$-pump is transiently enhanced above the control value. This is supported by the fact that if the $Na^+/K^+$-pump is inhibited by exposure to zero $[K]_o$, a similar hyperpolarization occurs on restoration of normal $[K]_o$[40,41]. However, the hyperpolarization due to the reactivation of the $Na^+/K^+$-pump does not necessarily mean that the $Na^+/K^+$-pump is electrogenic, since the hyperpolarization could be brought about by an increase in the potassium equilibrium potential consequent to temporary depletion of K in restricted spaces.

In order to distinguish between these two possibilities, Glitsch et al.[24] decreased the $[K]_o$ to various values: the hyperpolarization after hypothermia was maximal at moderate $[K]_o$ and it was greater than that produced by a potassium-free solution at normal temperature (before or after the hypothermia). These findings support the existence of an electrogenic sodium pump rather than the occurrence of potassium depletion. In another approach, the effect of a potassium-free solution was tested in Purkinje fibers depolarized at the plateau: at this low level of potential, exposure to zero $[K]_o$ causes a further depolarization and on return to normal $[K]_o$ there is a transient hyperpolarization, which is abolished by cardiac steroids[22]. The hyperpolarization can not be due to a depletion of potassium (which would have caused depolarization, not hyperpolarization) and was attributed to an electrogenic sodium extrusion. In active fibers, an increase of the maximum diastolic potential and shortening of the action potential also follows a brief exposure to zero $[K]_o$: since a decrease in extracellular potassium increases the duration of the action potential[51,65], the observed shortening is compatible with the activation of an electrogenic $Na^+/K^+$-pump but not with the depletion of potassium in narrow spaces[23]. Similarly, the exposure of a spontaneously active fiber to zero $[K]_o$ is followed by a period of hyperpolarization and quiescence: since lowering $[K]_o$ enhances pacemaker activity[51], the temporary suppression and hyperpolarization are best explained by the activation of an electrogenic Na extrusion[23]. Also, it has been shown that adding sodium to a sodium-free solution induces a hyperpolarization which is abolished by procedures inhibiting the sodium pump activity[67].

When the $Na^+/K^+$-pump is inhibited (e.g., by cardiac glycosides[46]), the $K^+$ uptake decreases[43] whereas the intracellular sodium activity[35,47] increases and so does calcium activity through the Na/Ca exchange[10,38]. An accumulation of calcium in turn can increase potassium conductance[28] which could contribute to the hyperpolarization, the shortening of the action potential and the temporary suppression of spontaneous activity after the exposure to zero $[K]_o$. This point was tested by briefly (60 s) exposing Purkinje fibers to zero $[K]_o$ under different conditions[4]. The K-free solution initially

increased the maximum diastolic potential before decreasing it; at the same time, the contractile force increased, as would be expected from sodium and therefore calcium accumulation. Restoring $[K]_o$ to normal resulted in a quick hyperpolarization which subsided in about 3 min. When a potassium-free calcium-free solution was tested, the hyperpolarizations during and after the exposure were little affected but the contractile force did not increase and actually fell. The perfusion of zero $[Ca]_o$ alone decreased the contractile force sooner and to a larger extent but failed to alter the maximum diastolic potential. Lithium does not exchange with Ca (as Na does) but it can substitute for K in the activation of the $Na^+/K^+$-pump[18]. Therefore, substituting NaCl for LiCl in the zero K-Ca solution should allow a smaller accumulation of Na and a greater accumulation of calcium than in the absence of lithium. The exposure to the lithium solution still caused hyperpolarization but the hyperpolarization on return to Tyrode solution was markedly reduced or abolished. Finally, tetrodotoxin reduces sodium influx and in its presence the hyperpolarization was reduced[4]. The results suggest that the changes in membrane potential that follow the exposure to zero K under the conditions tested are mostly due to the activation of an electrogenic sodium extrusion rather than an accumulation of calcium. However, an accumulation of calcium can modify the effects of an electrogenic sodium pump (see below).

*Changes in the activity of the electrogenic sodium pump and membrane potential*

The physiological stimulus determining the activity of the $Na^+/K^+$ is the intracellular sodium activity[3,6]. Therefore, if the intracellular sodium were to increase due to a temporary decrease in pump activity (hypothermia, zero $[K]_o$), the activity of the $Na^+/K^+$ subsequently increases in relation to the sodium load[26,40-42]. If the $Na^+/K^+$ pump is electrogenic, the pump current ought to increase accordingly if the Na/K coupling remains the same as suggested by several findings[19,21,25].

Under physiological conditions, an increase in rate of discharge should increase cellular sodium, since the number of action potentials increases in the unit of time. Actually, the 'physiological' rate of discharge of different cardiac pacemaker fibers differs, since the rate of the sinus node is faster than the rate of either the atrio-ventricular node or the Purkinje fibers (when these tissues are allowed to discharge spontaneously). Since under normal conditions the sinus node discharges all cardiac tissues at its own rate, the subsidiary pacemakers are actually overdriven under normal conditions. In other words, the intracellular sodium concentration of Purkinje fibers is higher when driven by the sinus node than when spontaneously active (as in complete atrio-ventricular block). In fact, the intracellular sodium activity has been shown to increase as the rate of discharge increases[12]. As a consequence, the pump current should be greater in overdriven Purkinje fibers than in spontaneously active fibers. On that basis, one would expect that the membrane potential should be more negative when the Purkinje fibers are overdriven. While the negativity induced by the pump may amount to only a few millivolts[52], still this change may have marked consequences on the function of subsidiary pacemakers[53].

The contribution of the electrogenic sodium pump to the membrane potential of Purkinje fibers can be made evident by driving spontaneous fibers at a rate similar to that of the sinus node. The sudden increase in rate is associated with an initial decline of the maximum diastolic potential which is related to a transient accumulation of potassium outside the cell membrane[32,52,58]. However, as the overdrive is continued the maximum diastolic potential becomes more negative than the pre-drive value. When the overdrive is terminated, there is a period of inhibition ('overdrive suppression') which is due to the fact that diastolic depolarization remains negative to the threshold and its slope is markedly reduced. Eventually the diastolic depolarization decays sufficiently for the spontaneous activity to resume gradually. The maximum diastolic potential of the resumed beats declines and the spontaneous rate increases gradually to the pre-drive value. The duration of the suppression is a function of the rate and of the duration of overdrive: the longer or faster drives are followed (within limits) by a more pronounced inhibition[52,58]. Hyperpolarization during and after overdrive has been demonstrated also in other cardiac tissues[15,37,53,54] although the mechanism is not necessarily identical in each case.

One particular instance of overdrive suppression appears to be the ventricular standstill that is present at the beginning of vagal stimulation. Several lines of evidence[53,54,58,62] indicate that the standstill is not the result of direct vagal inhibition of the ventricular Purkinje fiber automaticity but rather of the overdrive suppression exerted by the sinus node upon the idioventricular pacemakers. The vagal stimulation reveals such an overdrive suppression by inhibiting the discharge of the sinus node. In fact, the ventricular standstill decreases progressively and disappears during the administration of ouabain as the sodium pump is progressively inhibited[60].

*Relation of overdrive suppression to the sodium pump*

That the reduced slope of diastolic depolarization after overdrive is mostly related to an electrogenic extrusion of sodium seems to be demonstrated by several findings. Thus, the hyperpolarization induced by overdrive is reduced or abolished in the presence of the metabolic inhibitors dinitrophenol[52], iodoacetic acid and antimycin[5]. Also, the hyperpolarization is reduced by the administration of strophanthidin[8]. A decrease in membrane conductance in the presence of a lower $[K]_o$ (2.7 mM) increases the amount of the overdrive hyperpolarization[52].

The effects of short overdrives are modified by $[Ca]_o$ in that the hyperpolarization and the suppression are more marked in the presence of high and are reduced in the presence of low $[Ca]_o$[39]. Some of the effects are related to changes in the threshold[64] but a change in potassium conductance brought about by calcium accumulation appears to contribute to the membrane changes. With prolonged overdrives, the difference in hyperpolarization in high and low $[Ca]_o$ disappears. However, it should be pointed out that the metabolic inhibitors and strophanthidin increase cellular calcium and yet the hyperpolarization and suppression are reduced or abolished. This stresses the importance of the electrogenic sodium extrusion versus calcium accumulation in the events associated with overdrive.

*The relation between sodium and pump current*

The pump current has been measured by voltage clamping under conditions that lead to sodium overload. In one approach, the cells were exposed to zero $[K]_o$ for a brief period of time[2,19-21]. Exposure to $K^+$-free solution induces membrane depolarization which is followed in the absence of voltage clamp by a transient hyperpolarization and in the presence of voltage clamp by a outward current. That the outward current is related to the activity of the electrogenic sodium pump is supported by the finding that strophanthidin abolished it. The pump current is a function of the amount of sodium loading and decays exponentially after different sodium loads[21]. An outward current is recorded also after a series of clamp steps which displaces the mem-

brane potential from the resting to the plateau levels[11]. A time- and voltage-independent outward pump current has been also shown by the use of ouabain[29].

The relation of the $Na+/K^+$-pump current to intracellular sodium activity was demonstrated by recording at the same time these two parameters, together with tension[20]. The preparation was bathed in a solution where $K^+$ was substituted by $Rb^+$: removing the external $Rb^+$ resulted in an increase in intracellular sodium activity, in twitch and tonic tension while the membrane current became more inward. Adding 4 mM $Rb^+$ (or $K^+$) decreased $a^i_{Na}$ and the tension toward control value: at the same time there was a transient outward current which declined exponentially with an half time similar to that of $a^i_{Na}$. Similar results have been reported also by Glitsch and Pusch[25]. These findings support the concepts that the reactivation of the pump produces an electrogenic sodium extrusion and that the fraction of sodium extruded electrogenically is constant (as the coupling ratio does not vary).

*The control of the sodium pump activity*

*The enhancement of the sodium pump activity*

As already mentioned, a physiological regulator of the sodium pump activity is the intracellular sodium concentration[3,6] and this would appear to be a suitable means of maintaining cellular sodium homeostasis. Overdrive is one condition that increases sodium influx and therefore stimulates the activity of the sodium pump. However, there are situations in which it might be convenient to modulate the activity of the pump without increasing the intracellular sodium (or increasing it less). One such situation may be present when catecholamines are acting on the beta receptor. In fact, catecholamines have been shown to decrease intracellular sodium activity, an effect reduced by strophanthidin[36]. Also, norepinephrine increases the inward potassium movements and the potassium content in Purkinje fibers[56] and in the sinus node[9] and this increase is abolished by beta blockade[7]. In agreement with these findings, norepinephrine increases the hyperpolarization associated wiht overdrive[8,57], although it usually shortens the suppression[53]. The stimulation of the sodium pump by catecholamines may be useful in that catecholamines increase the rate of discharge (which increases both cellular sodium[12] and calcium[33]) and increase cellular calcium independently through the increase in the slow inward current. An excessive increase of calcium due to a combination of these factors might more easily lead to calcium overload which is arrhythmogenic in more than one way (see below). In addition, the hyperpolarization induced by catecholamines enhances the availability of the fast sodium channel[63] and this (together with the increase in sodium gradient) increases conduction velocity.

*The inhibition of the sodium pump activity*

Depending on the concentration, catecholamines have different effects on the automaticity of Purkinje fibers in that norepinephrine increases spontaneous discharge at high concentrations and decreases at low concentrations[44]. Experiments with radio-potassium have shown that low concentrations of catecholamines reduce the uptake of potassium[45] (in contrast to the large concentrations). Thus, it might appear that the activity of the pump is depressed by low concentrations of norepinephrine, an effect blocked by phentolamine[45].

Catecholamines often have effects opposite to those of acetylcholine (e.g., on sinus node automaticity) and therefore it is possible that acetylcholine (ACh) might depress the function of the pump. This was tested by administering ACh to sheep Purkinje fibers while recording at the same time action potential, force and intracellular sodium activity[27]. The results show that ACh increases intracellular sodium activity and force and in spontaneous fibers the rate of discharge. The increase in intracellular sodium is prevented by atropine (showing the involvement of a muscarinic receptor) and by strophanthidin (as the pump is already inhibited prior to ACh administration), but it may be increased with lower concentrations of strophanthidin when the inhibitory action of acetylcholine can summate with that of strophanthidin[27]. In the same tissue, acetylcholine induces overdrive excitation (the induction of repetitive activity by a fast drive[2a]), a finding consistent with the inhibition of the sodium pump.

As is well known, the inhibition of the sodium pump is brought about by large concentrations of cardiac steroids[46], which in fact abolish the overdrive suppression at the beginning of vagal stimulation[60]. The accumulation of sodium and therefore of calcium with the inhibition of the pump has another consequence on the electrical properties of cardiac cells, namely, an increase in intercellular resistance[66]. This uncoupling contributes to the depression of conduction velocity and therefore facilitates re-entry rhythms.

*Electrophysiological consequences of the pump inhibition*

The inhibition of the sodium pump can affect the membrane potential directly (by eliminating the pump current as discussed above) and indirectly by the way of Na/Ca exchange. The inhibition of the $Na^+/K^+$ pump leads to an increase in cellular sodium[35,47] and therefore[10,38] of cellular calcium. If the stage of calcium overload is attained, an oscillatory release of calcium results in the generation of an oscillatory transient inward current[30,31]. The oscillatory current causes an oscillatory potential which may attain the threshold and initiate repetitive discharge.

The administration of caffeine eliminates the oscillatory potential[16] and the oscillatory current[59] but increases an inward tail current which may prevent the full repolarization of the action potential and induce spontaneous activity at a depolarized level[16]. Alternatively, calcium overload may induce a slow afterdepolarization at the resting level, which may initiate slow discharge[50,55].

Therefore, the inhibition of the activity of the sodium pump not only results in the elimination of the overdrive suppression but also in the induction of events leading to overdrive excitation. The elimination of the outward pump current is direct whereas the induction of transient inward currents is the result of the calcium overload consequent to the depression of the $Na^+/K^+$ pump. It should be emphasized that the calcium overload can occur independently of an increased cellular sodium. In fact, it can occur when the intracellular sodium is decreased (e.g., by norepinephrine or high $[Ca]_o$) provided that cellular calcium is increased through other mechanisms (see Vassalle[55]).

*Conclusions*

The relation between the sodium pump and membrane potential is a complex one. This is so because the pump extrudes $Na^+$ and takes up $K^+$ but the entry of sodium is dependent of several routes associated with different functions. Because of this, a change in pump activity affects not only the intracellular sodium activity but also that of other ions. The consequent changes in concentration gradients and conductances are bound to modify the membrane potential in different ways. In addition to the regulation the transmembrane gradient of several ions, the sodium pump can also affect the membrane potential directly through the electrogenic extrusion of sodium. This action has several important electrophysiological consequences: it 1) helps shorten the action potential at higher rates of discharge and therefore shorten the refractory period and allows the cells to discharge at the

required rate; 2) increases the take-off potential of Purkinje fibers thereby increasing the availability of the fast sodium channel and conduction velocity; 3) insures that the subsidiary pacemakers are kept inhibited when not needed (overdrive suppression) and therefore contributes to the electrical stability of the heart by facilitating the dominance of the sinus node. Because of these actions, the depression of the pump function results in changes in automaticity, conduction and contraction of cardiac tissues. As typically exemplified by the action of large concentrations of digitalis, the inhibition of the pump action causes not only a depression of conduction and the removal of the overdrive suppression, but also induces calcium overload which in itself modifies in more than one way the membrane potential and leads to abnormal discharge.

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1 Abete, P., and Vassalle, M., Strophanthidin inotropy, overdrive and intracellular sodium activity. Proc. int. Union physiol. Sci. *26* (1986) 351.

2 Akasu, T., Ohta, Y., and Koketsu, K., The effect of adrenaline on the electrogenic $N^+$ pump in cardiac muscle cells. Experientia *34* (1978) 488–490.

2a Apfel, H., and Vassalle, M., Fedn Proc. *46* (1987) 366.

3 Baker, P.F., Blaustein, M.P., Keynes, R.D., Manil, J., Shaw, T.I., and Steinhardt, R.A., The ouabain-sensitive fluxes of sodium and potassium in squid giant axons. J. Physiol. London *200* (1969) 459–496.

4 Bernabei, R., and Vassalle, M., The inotropic effects of strophanthidin in Purkinje fibers and the sodium pump. Circulation *69* (1984) 618–631.

5 Bhattacharyya, M., and Vassalle, M., Metabolism-dependence of overdrive-induced hyperpolarization. Archs int. Pharmacodyn. Thér. *246* (1980) 28–37.

6 Brinley, F.J. Jr, and Mullins, L.J., Sodium fluxes in internally dialyzed squid axons. J. gen. Physiol. *52* (1968) 181–211.

7 Borasio, P.G., and Vassalle, M., Dibutyryl cyclic AMP and potassium transport in cardiac Purkinje fibers. Am. J. Physiol. *226* (1974) 1232–1237.

8 Carpentier, R., and Vassalle, M., Enchancement and inhibition of a frequency-activated electrogenic sodium pump in cardiac Purkinje fibers, pp. 81–98. Eds F.F. Kao, K. Koizumi and M. Vassalle. Aulo Gaggi, Bologna 1971.

9 Carpentier, R., and Vassalle, M., Actions and interactions of norepinephrine and acetylcholine on sinus node potassium movements. Archs Sci. biol. *61* (1977) 75–88.

10 Chapman, R.A., Control of cardiac contractility at the cellular level. Am. J. Physiol. *245* (1983) H535–H552.

11 Cohen, I., Falk, R., and Kline, R., Membrane currents following activity in canine cardiac Purkinje fibers. Biophys. J. *33* (1981) 281–288.

12 Cohen, C.J., Fozzard, H.A., and Sheu, S.-S., Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. Circ. Res. *50* (1982) 651–662.

13 Deitmer, J.W., and Ellis, D., Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. J. Physiol. *304* (1980) 371–488.

14 Délèze, J., Possible reasons for the drop of resting potential of mammalian heart preparations during hypothermia. Circ. Res. *8* (1960) 553–557.

15 Diacono, J., Suggestive evidence for the activation of an electrogenic sodium pump in stimulated rat atria: apparent discrepancy between the pump inhibition and the positive inotropic response induced by ouabain. J. molec. cell. Cardiol. *11* (1979) 5–30.

16 Di Gennaro, M., and Vassalle, M., Relationship between caffeine effects and calcium in canine cardiac Purkinje fibers. Am. J. Physiol. *28* (1985) H520–H533.

17 Draper, M.H., and Weidmann, S., Cardiac resting and action potentials recorded with an intracellular electrode. J. Physiol. London *115* (1951) 74–94.

18 Eisner, D.A., and Lederer, W.J., The role of the sodium pump in the effects of potassium-depleted solutions on mammalian cardiac muscle. J. Physiol. London *294* (1979) 279–301.

19 Eisner, D.A., and Lederer, W.J., Characterization of the electrogenic sodium pump in cardiac Purkinje fibres. J. Physiol. London *303* (1980) 441–474.

20 Eisner, D.A., and Lederer, W.J., The relationship between sodium pump activity and twitch tension in cardiac Purkinje fibres. J. Physiol. London *303* (1980) 475–494.

21 Gadsby, D.C., Activation of electrogenic $Na^+/K^+$ exchange by extracellular $K^+$ in canine cardiac Purkinje fibers. Proc. natn. Acad. Sci. USA *77* (1980) 4035–4039.

22 Gadsby, D.C., and Cranefield, P.F., Direct measurement of changes in sodium pump current in canine cardiac Purkinje fibers. Proc. natn. Acad. Sci. USA *76* (1979) 1783–1787.

23 Gadsby, D.C., and Cranefield, P.F., Electrogenic sodium extrusion in cardiac Purkinje fibers. J. gen. Physiol. *73* (1979) 819–837.

24 Glitsch, H.G., Grabowski, W., and Thielen, J., Activation of the electrogenic sodium pump in guinea-pig atria by external potassium ions. J. Physiol. London *276* (1978) 515–524.

25 Glitsch, H.G., and Pusch, H., Correlation between changes in membrane potential and intracellular sodium activity during K activated response in sheep Purkinje fibres. Pflügers Arch. *384* (1980) 189–191.

26 Hiraoka, M., and Hecht, H.H., Recovery from hypothermia in cardiac Purkinje fibers: considerations for an electrogenic mechanism. Pflügers Arch. *339* (1973) 25–36.

27 Iacono, G., and Vassalle, M., Acetylcholine increases intracellular sodium activity in sheep Purkinje fibers. (Abstract) Circulation *74* (1986) II-256.

28 Isenberg, G., Cardiac Purkinje fibres. $[Ca^{2+}]_i$ controls steady state potassium conductance. Pflügers Arch. *371* (1977) 71–76.

29 Isenberg, G., and Trautwein, W., The effect of dihydro-ouabain and lithium-ions on the outward current in cardiac Purkinje fibers. Evidence for electrogenicity of active transport. Pflügers Arch. *350* (1974) 41–54.

30 Kass, R.S., Lederer, W.J., Tsien, R.W., and Weingart, R., Role of calcium ions in transient inward currents and after contractions induced by strophanthidin in cardiac Purkinje fibres. J. Physiol. London *281* (1978) 187–208.

31 Kass, R.S., Tsien, R.W., and Weingart, R., Ionic basis of transient inward current induced by strophanthidin in cardiac Purkinje fibres. J. Physiol. London *281* (1978) 209–226.

32 Kunze, D.L., Rate-dependent changes in extracellular potassium in the rabbit atrium. Circ. Res. *41* (1977) 122–127.

33 Lado, M.G., Sheu, S.-S., and Fozzard, H.A., Changes in intracellular $Ca^{2+}$ activity with stimulation in sheep cardiac Purkinje strands. Am. J. Physiol. *243* (1982) H133–H137.

34 Lee, C.O., Abete, P., Pecker, M., Sonn, J.K., and Vassalle, M., Strophanthidin inotropy: Role of intracellular sodium ion activity and sodium-calcium exchange. J. molec. cell. Cardiol. *17* (1985) 1043–1053.

35 Lee, C.O., and Dagostino, M., Effect of strophanthidin on intracellular Na ion activity and twitch tension of constantly driven canine cardiac Purkinje fibers. Biophys. J. *40* (1982) 185–198.

36 Lee, C.O., and Vassalle, M., Modulation of intracellular $Na^+$ activity and cardiac force by norepinephrine and $Ca^{2+}$. Am. J. Physiol. *244* (1983) C110–C114.

37 Lu, H.-H., Lange, G., and Brooks, C.McC., Factors controlling pacemaker action in cells of the sinoatrial node. Circ. Res. *17* (1965) 460–471.

38 Mullins, L.J., The generation of electric currents in cardiac fibers by Na/Ca exchange. Am. J. Physiol. *236* (1979) C103–C110.

39 Musso, E., and Vassalle, M., The role of calcium in overdrive suppression of canine cardiac Purkinje fibers. Circ. Res. *51* (1982) 167–180.

40 Noma, A., and Irisawa, H., Electrogenic sodium pump in rabbit sinoatrial node cell. Pflügers Arch. *351* (1974) 177–182.

41 Noma, A., and Irisawa, H., Contribution of an electrogenic sodium pump to the membrane potential in rabbit sinoatrial node cells. Pflügers Arch. *358* (1975) 289–301.

42 Page, E., and Storm, S.R., Cat heart muscle in vitro. VIII. Active transport of sodium in papillary muscles. J. gen. Physiol. *48* (1965) 957–972.

43 Polimeni, P.I., and Vassalle, M., On the mechanism of ouabain toxicity in Purkinje and ventricular muscle fibers at rest and during activity. Am. J. Cardiol. *27* (1971) 622–629.

44 Posner, P., Farrer, E., and Lambert, C.R., Inhibitory effects of catecholamines in canine cardiac Purkinje fibers. Am. J. Physiol. *231* (1976) 1415–1420.

45 Posner, P., and Vassalle, M., The inhibitory action of norepinephrine on potassium uptake in cardiac Purkinje fibers. T.I.I. J. Life Sci. *1* (1971) 67–78.

46 Schatzmann, H.-J., Herzglykoside als Hemmstoffe für den aktiven Kalium- und Natriumtransport durch die Erythrocytenmembran. Helv. physiol. pharmac. Acta *11* (1953) 346–354.
47 Sheu, S.S., and Fozzard, H.A., Transmembrane $Na^+$ and $Ca^{2+}$ electrochemical gradients in cardiac muscle and their relationship to force development. J. gen. Physiol. *80* (1982) 325–351.
48 Tamai, T., and Kagiyama, S., Studies of cat heart muscle during recovery after prolonged hypothermia. Hyperpolarization of cell membranes and its dependence of the sodium pump with electrogenic characteristics. Circ. Res. *22* (1968) 423–433.
49 Thomas, R., Electrogenic sodium pump in nerve and muscle cells. Physiol. Rev. *52* (1972) 563–594.
50 Valenzuela, F., and Vassalle, M., Overdrive excitation and cellular calcium load in canine cardiac Purkinje fibers. J. Electrocardiol. *18* (1985) 21–34.
51 Vassalle, M., Cardiac pacemaker potentials at different extra- and intracellular K concentrations. Am. J. Physiol. *208* (1965) 770–775.
52 Vassalle, M., Electrogenic suppression of automaticity in sheep and dog Purkinje fibers. Circ. Res. *27* (1970) 361–377.
53 Vassalle, M., The relationship among cardiac pacemakers. Circ. Res. *41* (1977) 269–277.
54 Vassalle, M., The role of the electrogenic sodium pump in controlling excitability in nerve and cardiac fibers, in: Current Topics in Membranes and Transport. 16, Electrogenic Ion Pumps, pp. 467–483. Ed. C.L. Slayman. Academic Press, New York 1982.
55 Vassalle, M., Overdrive excitation: the onset of spontaneous activity following a fast drive, in: Cardiac Electrophysiology and Arrhythmias, pp. 97–107. Eds D.P. Zipes and J. Jalife. Grune and Stratton, New York 1985.
56 Vassalle, M., and Barnabei, O., Norepinephrine and potassium fluxes in cardiac Purkinje fibers. Pflügers Arch. *322* (1971) 287–303.
57 Vassalle, M., and Carpentier, R., Hyperpolarizing and depolarizing effects of norepinephrine in cardiac Purkinje fibers, in: Research in Physiology, pp. 373–388. Eds F.F. Kao, K. Koizumi and M. Vassalle. Aulo Gaggi, Bologna 1982.
58 Vassalle, M., Caress, D.L., Slovin, A.J., and Stuckey, J.H., On the cause of ventricular asystole during vagal stimulation. Circ. Res. *20* (1967) 228–241.
59 Vassalle, M., and Di Gennaro, M., Caffeine actions on currents induced by calcium-overload in Purkinje fibers. Eur. J. Pharmac. *106* (1984) 121–131.
60 Vassalle, M., Greenspan, K., and Hoffman, B.F., An analysis of arrhythmias induced by ouabain in intact dogs. Circ. Res. *13* (1963) 132–148.
61 Vassalle, M., and Musso, E., On the mechanism underlying digitalis toxicity in cardiac Purkinje fibers, in: Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 9, The Sarcolemma, pp. 355–376. Eds P.-E. Roy and N.S. Dhalla. University Press, Baltimore 1976.
62 Vassalle, M., Vagnini, F.J., Gourin, A., and Stuckey, J.H., Suppression and initiation of idioventricular automaticity during vagal stimulation. Am. J. Physiol. *212* (1967) 1–7.
63 Weidmann, S., The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. J. Physiol. London *127* (1955) 213–224.
64 Weidmann, S., Effect of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. J. Physiol. London *129* (1955) 568–582.
65 Weidmann, S., Elektrophysiologie der Herzmuskelfaser. Huber, Bern 1956.
66 Weingart, R., The actions of ouabain on intercellular coupling and conduction velocity in mammalian ventricular muscle. J. Physiol. London *264* (1977) 341–365.
67 Wiggins, J.R., and Cranefield, P.F., Effect of membrane potential and electrical activity of adding sodium to sodium-depleted cardiac Purkinje fibers. J. gen. Physiol. *64* (1974) 473–493.

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## Sodium/calcium exchange in ventricular muscle

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*Summary.* Ventricular cells possess two Ca extrusion mechanisms, a Na/Ca exchange system and a Ca pump. Reversing the exchanger by extracellular Na removal causes $[Na]_i$ to decrease, and the cells take up mmolar quantities of calcium. Since $[Ca]_i$ shows only a marginal increase the calcium load must be buffered. The capacity of the SR is limited so the mitochondria probably buffer a large part of this load. However, when Ca uptake into the mitochondria is blocked, the gain in Ca is still mmolar and the increase in $[Ca]_i$ still marginal, suggesting an additional buffering site.
Measurements of the Na/Ca stoichiometry on sarcolemmal vesicles gave a value of 3, but in ventricle values of around 2.5 or 3 are found. Reasons for this are discussed, as are the differences amongst the different methods of Ca measurement.
The interaction of the sarcolemmal Ca pump and the exchanger are considered and it is suggested they could interact via $[Na]_i$. At rest both systems could remove Ca from the cell but on a large perturbation the Na/Ca exchange would be the more important of the two.
*Key words.* Ferret heart; Na/Ca exchange; Ca buffering; Ca homeostasis.

### *Introduction*

The inflowing calcium current is one of the sequence of events leading to contraction in heart muscle[45] and a rough calculation shows that unless some mechanism or mechanisms are present to expel Ca from the cell, the cytoplasmic Ca concentration ($[Ca]_i$) would, in a few minutes reach mmolar values. To remove Ca from the cytoplasm three mechanisms exist and these are shown diagrammatically in figure 1. There is a Na/Ca exchange system and a Ca pump, both of which can efflux Ca from the cell. Ca buffer systems also exist and while they can prevent a rise in cytoplasmic Ca concentration, to maintain Ca homeostasis this buffered Ca must eventually be actively expelled from the cell (reviewed in Carafoli[9]). In this article we will look at the evidence for a Na/Ca exchange system, describe possible sites for Ca buffering and discuss the role of the exchanger and the pump. We will not consider the role of the Na/Ca exchange system during the action potential for this is covered in the article by D. Noble.

### *Na/Ca exchange system*

To investigate the Na/Ca exchange system a rapid perfusion chamber, shown diagrammatically in figure 2, was used. Ferret trabeculae, around 250 µm in diameter are fixed at one end to a small support and tied at the other end by a wire loop