# The electrogenic Na-Ca exchange and the cardiac electrical activity. I–Simulation on Purkinje fibre action potential.

R Fischmeister, G Vassort

# The electrogenic Na-Ca exchange and the cardiac electrical activity. I — Simulation on Purkinje fibre action potential*

**R. FISCHMEISTER and G. VASSORT**

*Laboratoire de Physiologie Cellulaire Cardiaque (FRA 51 INSERM), Laboratoire de Physiologie Cellulaire associé au C.N.R.S. (LA 89-3), Bât. 443, Université de Paris-Sud, 91405 Orsay Cedex***.*

SUMMARY :

1° The effects of the current ($i_{ex}$) generated by the Na-Ca exchange mechanism have been investigated on the electrical activity of a cardiac cell using the model of MC ALLISTER *et al.* (1975) for the Purkinje fibre action potential (AP).

2° The reversal potential and the steady-state value of $i_{ex}$ were described by the same equations as for the squid axon (MULLINS, 1976). The maximal intensity and the time constant of $i_{ex}$ were extrapolated from experiments using frog hearts.

3° A compact program written with the Adams method in Fortran IV (PLANT, 1979) allowed a minicomputer to be used.

4° The addition of $i_{ex}$ to the previous model induced a prolongation of the AP and a reduction of the duration of the diastolic phase.

5° Increasing the maximal steady-state amplitude of $i_{ex}$ may lead to early after depolarizations and oscillatory behaviour. These effects can be prevented by adequate changes in the amplitude of some potassium outward currents.

6° It is concluded that : (i) alterations of the Na-Ca exchange, *e.g.*, by cellular Na loading, should induce variations of both AP repolarization and diastolic phase durations and, consequently, alter the beating rate ; (ii) $i_{ex}$ could interfer with the outward currents whose characteristics should be reconsidered.

*Key-words :* Computer simulation. Cardiac action potential. Electrogenic Na-Ca exchange. Repetitive activity.

## INTRODUCTION

In most excitable cells, a leaky entry of Ca ions occurs (because of the large driving force for these ions) together with a large transient influx due to the increase in Ca conductance which is partly responsible for the development of the action potential (AP). In order to prevent cellular overloading, an extrusion of Ca ions should take place. Two mechanisms have been described on cardiac cellular membrane : a Ca-ATPase (CARONI and CARAFOLI, 1980) and a Na-Ca exchange (REUTER and SEITZ, 1968). The latter mechanism should have a particular importance, because it is sensitive not only to the concentration gradients of both Na and Ca ions, but also to the membrane potential. Depending on these three parameters, the Na-Ca exchange can move Ca either outwardly or inwardly. A model mechanism for the Na-Ca transport was proposed (MULLINS, 1977). In the frog heart, it was shown that the Na-Ca exchange participates in the control of tonic tension (BENNINGER *et al.*, 1976 ; HORACKOVA and VASSORT, 1979) and relaxation (ROULET *et al.*, 1979). Furthermore, it was suggested that, in the heart, the countertransport of probably 4 Na ions for 1 Ca ion generates a current (HORACKOVA and VASSORT, 1979 ; see also MULLINS, 1979). The current generated will tend to reduce the difference between the equilibrium potential of the Na-Ca exchange and the membrane potential. It can be inferred that such a mechanism may alter the action potential time course and the repetitive activity if this current is not negligible. This possibility was examined using a mathematical model of cardiac electrical activity. A preliminary report has been presented (VASSORT and FISCHMEISTER, 1980).

## THEORY

The equations described by MC ALLISTER *et al.* (1975) for the reconstruction of the (automatic) Purkinje action potential were the basis of our mathematical model. To add the current $i_{ex}$, generated by the Na-Ca exchange, the following hypotheses were set forth :

1° The carrier coupling ratio, x, was assumed to be constant at a value of 4 (MULLINS, 1976). This induces a reversal potential of the exchange

$$E_{ex} = 2 E_{Na} - E_{Ca} . \qquad (1)$$

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** Reprint address : Laboratoire de Physiologie Cellulaire Cardiaque, Université de Paris Sud, Bât. 443, F-91405 Orsay Cedex.

Taking $E_{Na} = +40$ mV and $E_{Ca} = +130$ mV (with $Ca_o$ and $Ca_i$ equal respectively to 1.8 and $10^{-4}$ mM), $E_{ex} = -50$ mV at maximal diastolic potential ($E_{mdp} = -87.5$ mV in our model).

2° The steady-state value of this carrier-mediated current is given by the relation (MULLINS, 1976)

$$\bar{i}_{ex} = k \sinh [(E_m - E_{ex}) F/RT] \quad (2)$$

where $E_m$ is the membrane potential, F, R and T have their usual meanings and k is a scaling factor. No precise maximal amplitude of $i_{ex}$ can be given, because this current occurs simultaneously with other currents, and no specific inhibitor of the Na-Ca exchange exists as yet. An approximate value allowing us to determine the range of k was given by the amplitude of the outward current generated on top of the others at a given potential on switching from high to low (110 to 22 mM) Na solutions. For a 60 mV depolarization from the resting potential, the extracurrent could be up to 5 $\mu A.cm^{-2}$ on the frog heart (VASSORT and FISCHMEISTER, unpublished). Since a 5 fold reduction of the external Na concentration shifts $E_{ex}$ to a value which is 81 mV more negative (from approximately $-50$ mV to $-130$ mV at resting potential), it can be calculated from Eq. 2 that this current is roughly 25 fold the one generated on applying the 60 mV depolarization in the normal Ringer solution.

3° $i_{ex}$ is supposed to follow a first order differential equation

$$\frac{di_{ex}}{dt} = \frac{1}{\tau_{ex}} (\bar{i}_{ex} - i_{ex}) . \quad (3)$$

4° The time constant of the exchange, $\tau_{ex}$, was set at a constant value of 35 ms. This value is derived from the time constant of relaxation of the frog heart ; this time constant was shown to be dependent on the Na-Ca exchange (ROULET *et al.*, 1979) and extrapolated to 37 °C using an experimental $Q_{10}$ of 2.8 (VENTURA-CLAPIER, VASSORT and FISCHMEISTER, unpublished). For sake of simplicity and in the absence of further indications, the carrier coupling ratio, the scaling factor and the time constant were assumed not to vary with the membrane potential.

5° Ca influx and efflux have been shown to occur at rest (WINEGRAD and SHANES, 1962 ; NIEDERGERKE *et al.*, 1969). Since the time independent component of the slow inward current, $i_{si}$, in MC ALLISTER *et al.* (1975), is not significant at $E_{mdp}$, a Ca background conductance, $g_{Ca,b}$, was added to the model. This conductance is responsible for a Ca background inward current, $i_{Ca,b}$, assuming that the efflux of Ca ions via the Na-Ca exchange just compensates for the leaky Ca current at $E_{mdp}$. Then,

$$i_{Ca,b} = g_{Ca,b}(E_m - E_{Ca}) \quad (4)$$

and

$$i_{Ca,b}(E_{mdp}) = \bar{i}_{ex}(E_{mdp}). \quad (5)$$

The Na background conductance ($g_{Na,b}$) of the model of MC ALLISTER *et al.* was reduced in order to achieve the same amplitude of inward background currents at $E_{mdp}$ (e.g., with a scaling factor $k = 0.6$, $g_{Na,b}$ and $g_{Ca,b}$ equal 0.0877 and 0.0051 mmho.cm$^2$ respectively).

6° The internal Ca concentration was supposed to vary as in BEELER and REUTER's model (1977) of ventricular action potential taking into account uptake and release by the sarcoplasmic reticulum (internal stores). We included in their equation both Ca background and Na-Ca exchange currents

$$\frac{d[Ca_i]}{dt} = -10^{-7}(i_{Ca} + i_{Ca,b} - i_{ex}) + 0.07(10^{-7} - [Ca_i]) . \quad (6)$$

The differential equations (3) and (6), added to the set of ten differential equations given by MC ALLISTER *et al.* (1975), were integrated using a variable-step Adams algorithm with a second order predictor and a third order corrector. This method is based on the use of an interpolating polynomial as an approximation of the derivative. As opposed to the more classical Runge-Kutta method, the Adams method permits the building of a compact program, written in Fortran IV (PLANT, 1979), which can be easily used on our small computer (Intertechnique, Plurimat S). The maximum accepted magnitude of the relative local truncation error for each of the integrated components was set at $5 \times 10^{-3}$ in the program. The truncation error estimate was used to control the step size of the integration procedure. The method was tested on the Na activation parameter, m, which shows no oscillation during the AP ascending phase. The maximal value of the upstroke velocity was 477 $V.s^{-1}$ at $-9.8$ mV ; this value is similar to that obtained by MC ALLISTER *et al.* (1975). The graphic output was displayed either on a 16 K octets numerical visual memory or on an Ifelec printer. All the computed action potentials were initiated by a sudden depolarization to $-50$ mV from a steady pre-potential of $-.80$ mV. The execution time necessary for the reconstruction of a 600 ms action potential was roughly 20 min.

## RESULTS

A computed action potential, with assumption 6 added to the model of MC ALLISTER *et al.*, is shown in Fig. 1 (thick trace). The addition of the current generated by the Na-Ca exchange, with the above assumptions, does not alter the spike nor the plateau amplitude.

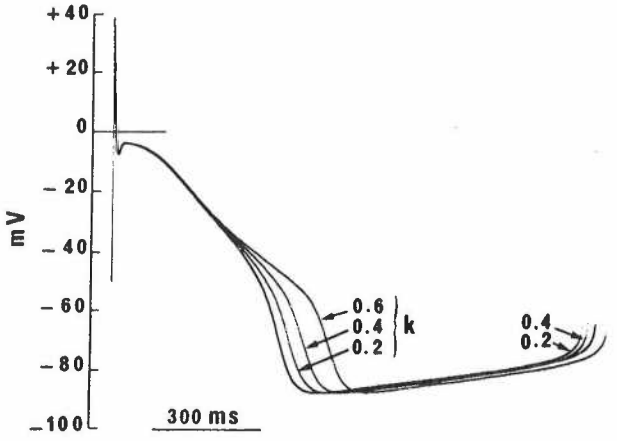


FIG. 1. — *Computed Purkinje action potentials modified from Mc ALLISTER et al. (1975).*

The addition of a current generated by the Na-Ca exchange mechanism prolongs the action potential and decreases the diastolic phase duration. The maximal steady-state amplitude of this current was determined by the scaling factor k from 0 to 0.6 (for further details see text).

However, according to its amplitude, fixed by a chosen scaling factor, $i_{ex}$ delays the repolarization without changing its maximal rate (Fig. 1). For all values of k, the exchange current accelerates the rate of diastolic depolarization so that its duration is reduced. Nevertheless, the beating rate can be reduced because of the lengthening of the action potential.

In Fig. 2, an action potential, computed with a given $i_{ex}$ (k = 0.6) is reported together with the variations of different Ca parameters. The large increase in the Ca concentration during the AP induces a large change in the reversal potential of the Na-Ca exchange, $E_{ex}$, so that $i_{ex}$ remains always inward for a Purkinje AP which has a low plateau amplitude. The Ca concentration recovers its initial value, $10^{-7}$ M, at the end of the AP. This is partly due to the large $i_{ex}$ which develops as a consequence of the increase in $Ca_i$ and the low plateau amplitude. After about 200 ms, the Ca current through the Ca conductance decreases to about 6 $\mu A.cm^{-2}$ and remains nearly constant for a further 200 ms. During that period, $i_{ex}$ increases by 1 $\mu A.cm^{-2}$. This increase of $i_{ex}$ is a significant value which antagonises the developing outward K currents and, thus, induces AP prolongation. For an action potential with a more positive plateau amplitude, consequent to less outward currents, $i_{ex}$ can be reversed. Thus, the Ca efflux would be suppressed, and an influx of Ca will occur via the Na-Ca exchange together with an outward $i_{ex}$.

During the slow diastolic depolarization, $Ca_i$ increases slightly because of the Ca leak current and the Ca conductance ; the latter appears partially activated even at these negative potentials. Consequently, $i_{ex}$ increases ; the slow diastolic depolarization accelerates ; and Ca extrusion is enhanced.

The model of Mc ALLISTER *et al.* (1975) fits the Purkinje action potential. Due to the types of alterations that the addition of a Na-Ca exchange current induces on the computed AP (as shown in Fig. 1), we investigated the influence of some changes in the potassium currents. Fig. 3 illustrates that a very similar action potential and frequency of beating can be obtained after adding $i_{ex}$ if appropriate changes in $\bar{i}_{x1}$ and $\bar{i}_{K2}$ are made.

Increasing the value of the steady state current, $\bar{i}_{ex}$, results in an oscillatory behaviour. This is shown in Fig. 4. With a scaling factor of 0.7, a hump is elicited in the repolarization phase of the AP. This is a threshold value, since for k = 0.701, an early after depolarization is observed. With k = 0.8, the early after

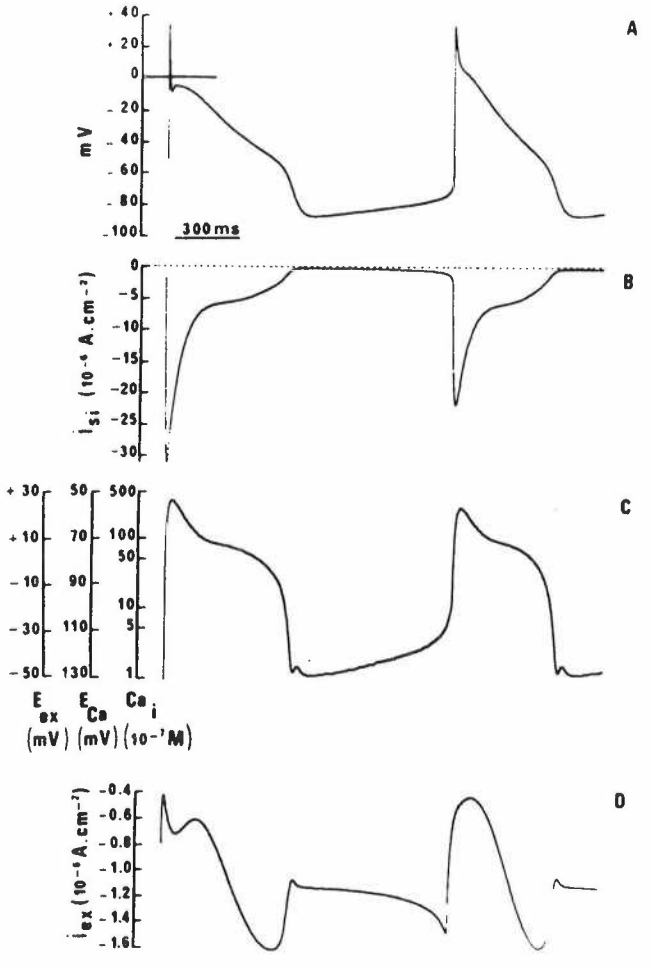


FIG. 2. — *Computed simultaneous variations of several parameters involved in the electrical activity of cardiac Purkinje fibres.*

A Na-Ca exchange current, $i_{ex}$, with a scaling factor k = 0.6, is added to the model of Mc ALLISTER *et al.* (for further details see text).

A : action potential ; B : slow inward current ; C : $Ca_i$, $E_{Ca}$, $E_{ex}$ ; D : $i_{ex}$.

depolarization does not fully repolarize and a second slow response is elicited. Larger values of k induce multiple re-excitations which finally reach a stationnary value at around − 35 mV.

## DISCUSSION

In the present paper, we used the model of MC ALLISTER *et al.* (1975) to simulate cardiac Purkinje action potentials. A set of equations, describing the electrogenic behaviour of the Na-Ca exchange, was added to this model. The first evidence suggesting that the Na-Ca exchange mechanism is electrogenic was obtained in the squid axon (MULLINS and BRINLEY, 1975). In the heart, this mechanism has also been shown to be voltage dependent and electrogenic (frog heart : HORACKOVA and VASSORT, 1979). Some recent studies in mammalian heart reported that 3 or more Na ions are exchanged for 1 Ca ion (PITTS, 1979 ; MCGUIGAN *et al.*, 1980).

In the frog heart, it was demonstrated that Ca fluxes through the Na-Ca exchange have a significant role during the build up and recovery of contraction (HORACKOVA and VASSORT, 1979 ; ROULET *et al.*, 1979) ; thus, its time course should be in the same range as those of other systems involved in the control of the AP. From the experiments on relaxation, we chose a time constant of 35 ms. However, increasing (decreasing) $\tau_{ex}$ has nearly the same effect as decreasing (increasing) k.

Although we are aware that numerous parameters (*e.g.*, ionic conductances, ionic depletion and accumulation, Na-K pump...) are involved in the control of AP duration and frequency of beating (BOYETT and JEWELL, 1980), the above calculations suggest that $i_{ex}$, the current generated by the Na-Ca exchange mechanism, should also be taken into account. Furthermore, $i_{ex}$ may also intervene in the alterations in AP duration (and frequency of beating) which are induced by cardiac glycosides or imposed pacing rate.

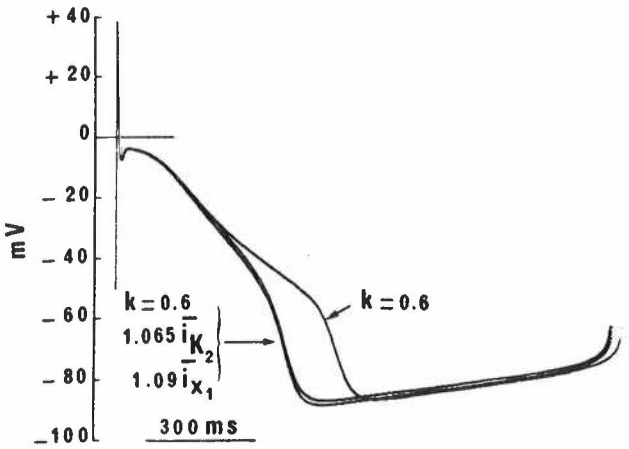


FIG. 3. — *Computed Purkinje action potentials.*

The effects on AP duration and beating rate of a given exchange current, whose maximal steady state amplitude is determined by the scaling factor k = 0.6, can be prevented by adequately changing the steady-state amplitudes of $i_{K2}$ and $i_{x1}$.

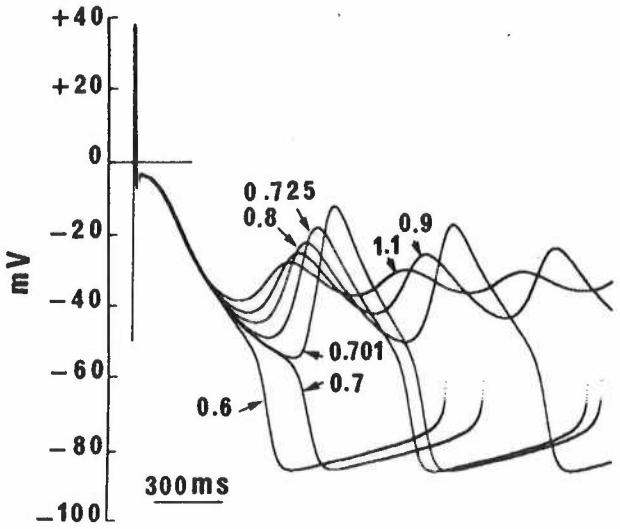


FIG. 4. — *Effects of increasing the steady-state value of the Na-Ca exchange current on computed Purkinje action potentials.*

On the hump which occurs for k = 0.7 can develop an early after depolarization (k = 0.701, k = 0.725) a transitory oscillation (k = 0.8) or multiple re-excitations (k ≥ 0.9).

It was suggested that alterations in the pace maker current $i_{K2}$ and in the outward current $i_{x1}$ can trigger premature re-excitations (COULOMBE *et al.*, 1980) or oscillatory activity (HAUSWIRTH *et al.*, 1969). Similar observations are obtained by the addition of an inward current due to the Na-Ca exchange mechanism. Humps and re-excitations described here resemble those observed in Purkinje fibres submitted to long-lasting periods of anoxia (TRAUTWEIN *et al.*, 1954) or acidosis (CORABOEUF *et al.*, 1980), both conditions would be expected to change $Na_i$ and the Na-Ca exchange. Thus, together with $i_{x1}$ and $i_{K2}$, the current generated by the Na-Ca exchange likely plays a role in the genesis of cardiac arythmias.

Finally, in the absence of a specific inhibitor of the Na-Ca exchange, the characteristics of $i_{ex}$ are difficult to define, since this current overlaps with the other currents involved in the AP. For the same reason, depending on the relative amplitudes of the currents, most of the characteristics of the ionic currents might have to be reconsidered.

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