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THE EFFECT OF QUINIDINE ON MEMBRANE ELECTRICAL ACTIVITY IN FROG AURICULAR FIBRES STUDIED BY CURRENT AND VOLTAGE CLAMP

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1 The action of quinidine sulphate 50 μM has been investigated on frog auricular trabeculae transmembrane currents recorded with a double sucrose gap apparatus. Results were obtained either in current or in voltage clamp conditions.

2 Quinidine modified the time course of repetitive activity elicited by long lasting depolarizing currents and reduced the range of current over which repetitive activity could be triggered, eventually abolishing repetitive responses altogether.

3 Several authors have emphasized the limitations of the voltage clamp method. Taking into account these limitations, the numerical values of the parameters obtained in the present work must not be considered as exact values but may be interpreted as indicators of the variations of the parameters.

4 The results are in agreement with previous findings that the main features of the action of quinidine are to produce (a) a reduced maximum rate of depolarization (MRD), (b) a reduced total amplitude of action potential, (c) a flattening of the plateau of the action potential, (d) a slight prolongation of the tail of the action potential, (e) an increased effective refractory period without greatly prolonging action potential duration, (f) no change of resting potential and of 50% repolarization time.

5 The analysis of ionic conductances has provided explanations for the above effects.

6 Quinidine reduced the reactivation kinetics of the sodium inward current, and decreased sodium conductance and the steady state of activation. These effects account for (a) and (b).

7 Quinidine increased the activation and inactivation time constants of sodium conductances, which account in part for (e).

8 Quinidine delayed reactivation of slow inward current, reduced calcium conductance, and decreased the steady state of activation of calcium conductance. These effects could account for (c).

9 The amplitudes of the two components of the delayed conductances responsible for repolarization were decreased by quinidine, and the time constant of activation for the faster of the two was slowed. These effects could account for (d) and in part for (e).

Introduction

Microelectrode intracellular recordings have permitted a decisive advance in the electrophysiological study of the mode of action of antiarrhythmic drugs. It has been demonstrated that quinidine does not alter the resting potential (Vaughan Williams, 1958); that the intracellular concentrations of potassium and sodium remain unchanged during the action of quinidine (Goodford & Vaughan Williams, 1962); that this drug strongly decreases the maximum rate of rise (Weidmann, 1955) and that it lengthens the effective refractory period even if the action potential duration remains the same (Vaughan Williams, 1961). From the above evidence, Vaughan Williams (1958) concluded that quinidine has a direct effect on the cardiac membrane. Therefore it was of interest to study the action of quinidine with the double sucrose gap technique which permits the measurement of the transmembrane ionic conductances responsible for cardiac electrical activity. This technique has previously been applied to frog auricular trabeculae by Rougier, Ildefonse & Gargouïl (1966), to the rat ventricular myocardium by Besseau & Gargouïl (1969) and to monkey intraventricular fibres by Walden, Kréher, Aka & Tricoche (1973).

Owing to the fact that a certain loss of voltage control exists when voltage clamping the preparation (Johnson & Lieberman, 1971; Tarr & Trank, 1974; Léoty & Poindessault, 1974; Ramón, Anderson, Joyner & Moore, 1975), the results obtained from the experiments described here must be considered as

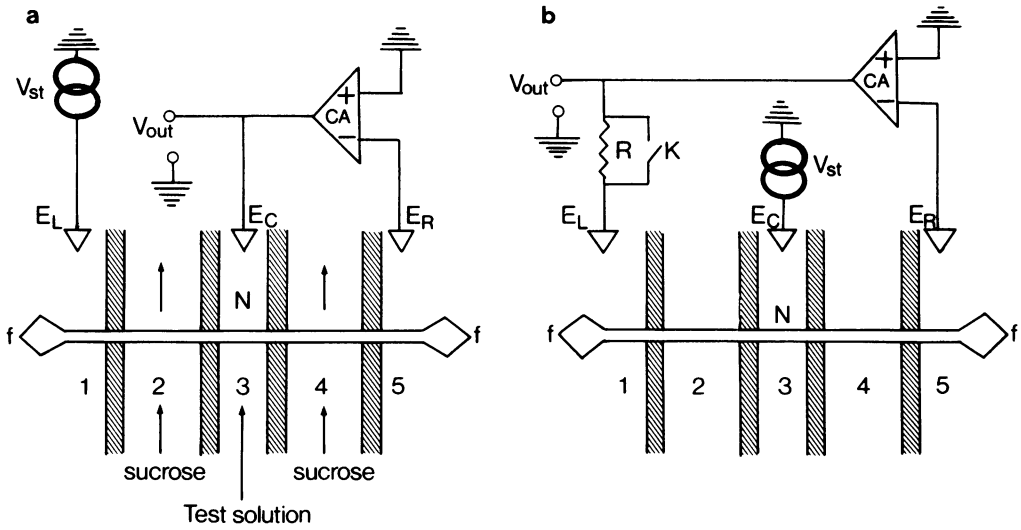

Figure 1 Arrangement for current and voltage clamp recordings. (a) Stimulation and recording diagram in current clamp conditions. E_R is the reference electrode used to hold the intracellular potential of the artificial node N; E_L is the left electrode or electrode for control of the transmembrane current in the artificial node N. (b) Stimulation and recording diagram in voltage clamp conditions. E_R has the same role as in (a); E_c becomes the electrode for control of the transmembrane potential in the artificial node; E_L becomes the electrode measuring the transmembrane current in the artificial node. The different compartments are bathed as follows: 1 and 5 by Ringer solution, 2 and 4 by sucrose solution which is used to insulate the central artificial node from compartments 1 and 5 (insulation being also provided by means of partitions made of mixed vaseline and paraffin between each compartment); 3 or test-compartment by Ringer solution or modified Ringer solution (single or with the studied drug added). *ff* is the cardiac auricular preparation transversely arranged on the different compartments; V_{st} is the stimulating signal, V_{out} is the recorded signal and CA is the control amplifier.

qualitative results which reveal only the direction of variation of the different parameters during quinidine's action. Nevertheless it must be pointed out that the limitations of the voltage clamp experiments are largely dependent on the type of double sucrose gap device used and from recent work by de Hemptinne (unpublished observations) that the partitions with vaseline seals lead to less important errors than a double sucrose gap technique using fluid partitions. So the technique used in the present work can be considered at present as a relatively efficient one.

In this work, the phenomenological equations proposed by Hodgkin & Huxley (1952c) will be used to describe the effects of quinidine on the transmembrane conductances and their parameters.

Methods

The experiments were performed on auricular trabeculae of the frog (*Rana esculenta*) about 100 to 250 μm in diameter and about 3 to 4 mm in length. The chamber was similar to that described by Rougier, Vassort & Stämpfli (1968). The sucrose compartments were 250 μm wide and the central 'node' was 150 μm wide. The separation of solutions was made with vaseline seals. Vaseline was added to paraffin wax in such a proportion as to obtain the required viscosity at 18°C to build strong enough seals. All the electrodes were calomel half cells. Their impedance was essentially resistive (less than 50 Ω) and the junction potential difference between any of them was less than 1 mV.

The electronic circuitry

Referring to Figure 1, the voltage sensing electrode (E_R) and the current passing one (E_L) were placed in the outer (respectively right and left) compartments of the chamber. The electrode in the central compartment is placed downstream, 1 cm distant from the central node. The central compartment widens rapidly on both sides of the central node in order to lower the bath resistance. Assuming that the vaseline seals and the sucrose fluxes provide a perfect electrical isolation between the compartments i.e. no current can

flow between two adjoining compartments outside the preparation, the operation of the electronic set up may be described as follows:

Current clamp conditions (Figure 1a). A stimulating voltage (V_{st}) is applied to the current pool electrode (E_L) and is converted in the intracellular longitudinal resistance between the current pool and the test node into a stimulating current. This current flows across the preparation and the central node towards electrode E_C since the inverting input of the control amplifier (CA) draws a negligible current (10^{-12} A). The potential recorded at the output of the CA is that lying between E_C and E_R. The CA clamps E_R at the earth potential since its non-inverting input is grounded. As practically no current flows in the intracellular longitudinal resistance between the central node and the voltage pool (E_R), the inside of the preparation in the central node is clamped at earth potential. Thus, the measured potential (V_{out}) represents the voltage across the preparation in the central node.

Voltage clamp conditions (Figure 1b). The aim of this circuit is to maintain a known and fixed potential across the preparation in the test node. As in the preceding description, the inside of the preparation is clamped at earth potential. The stimulating or clamp voltage is applied to E_C, so the voltage across the preparation would be that potential (V_{st}). The current required to clamp the inside of the preparation at earth potential is delivered through the current pool electrode E_L by CA. It develops the output potential (V_{out}) on the intracellular longitudinal resistance between the current pool and the test node. This current is also the current flowing across the preparation in the test node since the inverting input of the CA draws a negligible current (10^{-12} A). So the output voltage (V_{out}) of the CA is directly proportional to the current flowing in that condition out of the preparation in the test node i.e. the 'membrane' current. The longitudinal intracellular resistance between the current pool (E_L) and the test node is calculated from the variation in V_{out} when a known resistance (R) is added in series with E_L whereas the stimulation V_{st}, and thus the current, remain the same.

Solutions and experimental conditions

Quinidine sulphate (Merck) or dihydroquinidine gluconate (Houdé) 50 μM was added to a Ringer solution, which had the following composition (mM): NaCl 110, KCl 2.5, $CaCl_2$ 1.8 and Tris(hydroxymethyl) aminomethane 10. The pH was adjusted to 7.8 with HCl. Two permeability inhibitors were used in an attempt to obtain the net membrane currents: tetrodotoxin (TTX), considered as a specific inhibitor of the fast sodium inward current, was used at 0.1 μg/ml. Manganese chloride, 2 mM, which is considered to inhibit the slow inward current, was also used. The use of manganese did not always produce a total suppression of the slow inward current and results of these experiments were discarded. Such inhibitors have been successfully used by Rougier, Vassort, Garnier, Gargouïl & Coraboeuf (1969). The experiments were performed at a temperature near 18°C. The rate of stimulation was adjusted so that no new stimulus was delivered before complete repolarization (in current clamp conditions) or before complete inactivation or deactivation of the currents (in voltage clamp conditions) had occurred.

Experimental protocols

Experiments carried out in current clamp conditions. The preparation was stimulated by constant currents so as to obtain an action potential (AP) whose amplitude, duration and rate of rise were used to decide if the experiment was worth pursuing. The stability of the AP or repetitive activity (RA) was then checked. The test compartment was bathed with a Ringer solution added with the drug under study and recordings were subsequently made at different times. After that, the preparation was again bathed with Ringer solution (to study the reversibility of the drug effect). The depolarizing stimulating currents were displayed on the oscilloscope screen as variations of potential, so it was then necessary, in voltage clamp conditions, to measure the internal longitudinal resistance of the preparation in order to calculate in amperes the amplitude of the depolarizing currents used for stimulation (see appendix). For AP time course studies, the rate of stimulation, chosen in order to obtain a constant AP time course, was approximately one stimulus every 10 seconds.

Experiments carried out in voltage clamp conditions. The state of the preparation bathed in a Ringer solution was first checked by observation of the AP time course. Then the preparation was placed in voltage clamp conditions. The type of study to be tried was decided from observation of the time course and amplitude of the studied current and from the determination of its reversal potential.

Studies concerning the inward currents. The preparation was held at a potential allowing the current to be fully activated when the preparation was stimulated with a suitable value of depolarizing pulse. The test compartment was then bathed with a Ringer solution with added TTX (if slow inward current was studied) or with $MnCl_2$ (if fast inward current was studied) or with a modified Ringer solution allowing only the calcium inward current to develop. By using $MnCl_2$ it was possible partly to reduce the artifact appearing on the rectangular time course of the imposed potential due to the presence of the slow

inward calcium-sodium current. The recordings obtained in such conditions were taken as the reference ones. Then the internal longitudinal resistance of the preparation was measured. The test compartment was then bathed with the same solution as above but with drug added and the same recordings and measurements were made after the drug effect was obtained. It was possible to use this general procedure to obtain the current-voltage curves, the reactivation curves and the inactivation curves. Nevertheless, current-voltage curves as current time courses used to calculate the conductances time courses were also obtained by the subtraction methods described below. The subtractions were made over the range of clamp potentials allowing inward currents to develop, these inward currents being the fast sodium current and the slow calcium-sodium one. In some experiments a series of four solutions were used: (1) a solution inhibiting both inward currents; (2) a solution inhibiting only one inward current; (3) the effect of quinidine on one inward current; (4) a solution inhibiting both inward currents. Subtraction of the current in (1) from (2) gave the control or reference size of the current under study while subtraction of the current in (4) from (3) gave its size in the presence of quinidine. This type of protocol allowed relatively easy subtractions but its major disadvantage was the long time needed to perform it, such a long time decreasing the probability of success in the experiments. In most experiments a series of three solutions were used: (1) a solution allowing only the studied current to develop; (2) the effect of quinidine on this current; (3) a solution inhibiting the inward currents. Subtraction of the current in (3) from (1) gave the control or reference size of the current under study while subtraction of the current in (3) from (2) gave its size in the presence of quinidine. This type of protocol is simpler and needed less time to perform but it needed very good stability of the preparation during the series of three solutions.

Studies concerning the delayed outward currents. The test compartment was bathed with a Ringer solution with TTX and $MnCl_2$ added. When the inward currents were entirely inhibited the reversal potential of each outward current component was determined. Recordings of the reference current were made in this first medium and then the test compartment was bathed with a Ringer solution with TTX, $MnCl_2$ and quinidine added. During the action of quinidine the same recordings as above were made. Particular care was taken concerning the stimulation rhythm because it was essential before applying another stimulation to wait for a complete deactivation of the delayed currents (see the observations of de Hemptinne, 1971).

Nomenclature

TRC is the triggering range of currents, that is the range of current by which a repetitive activity can be triggered. V (mV) is the variation of the membrane potential from the resting potential $E_m = -70$ to -75 mV (Rougier *et al.*, 1968) being taken as zero. Depolarizations are positive values of V and negative values are hyperpolarizations. V_{Na}, V_{Ca}, V_{x1}, V_{x2} are respectively the reversal potentials for sodium and calcium inward currents, and for the fast and slow components of the delayed outward currents. τ_{re1} and τ_{re2} are the reactivation time constants of a given inward current (respectively for the fast component and the slow one). I_{Na}, I_{Ca}, I_{Ca-Na}, $I_t = \Sigma I = i_{x1} + i_{x2}$ are respectively the fast sodium current, the slow calcium, the slow calcium-sodium and the total delayed outward currents. g_{Na}, g_{Ca}, $\Sigma g = g_{x1} + g_{x2}$ are respectively the conductances calculated from the same currents as above. The nomenclature used for i_{x1} and i_{x2} corresponds to systems 1 and 2 of Brown & Noble (1969a, b) and is identical to that used by Besseau (1972), as regards i_{x1}, i_{x2}, g_{x1} and g_{x2}. i'_{x1} and i'_{x2} are the values of the tail currents determined at the origin on semi-logarithmic plots (i'_{x1} for the fast component and i'_{x2} for the slow component of the delayed current).

Conductance parameters for g_{Na}. m_∞ is the steady state of activation function; 'a' is the exponent of the activation variable m; g'_{Na} is a quantity proportional to the steady state of the activation (the exponent of m being known); $1/\tau_m$ is the reciprocal activation time constant; $1/\tau_h$ is the reciprocal inactivation time constant; α_m and β_m are the rate constants of the activation system; h_∞ is the steady state of inactivation function.

Conductance parameters for g_{Ca}. In order to be homogeneous with the nomenclature used by Reuter (1974) the symbol *m* is replaced by *d* and the symbol *h* is replaced by *f*; consequently, the parameters are d_∞, f_∞, 'a', g'_{Ca}, $1/\tau_d$, $1/\tau_f$, α_d and β_d.

Conductance parameters for g_{x1} *and* g_{x2}. $n_{\infty 1}$ and $n_{\infty 2}$ are the steady states of the activation functions; $1/\tau_{n1}$ and $1/\tau_{n2}$ are reciprocal activation time constants; α_{n1}, β_{n1} and α_{n2}, β_{n2} are the rate constants.

Calculations

The details of the different calculations are explained in the appendix. The statistical significance of differences between means was calculated from Student's *t* test.

Results

Current clamp conditions

Action potential time course. Figure 2(a) shows an AP obtained in a Ringer solution, with an amplitude of

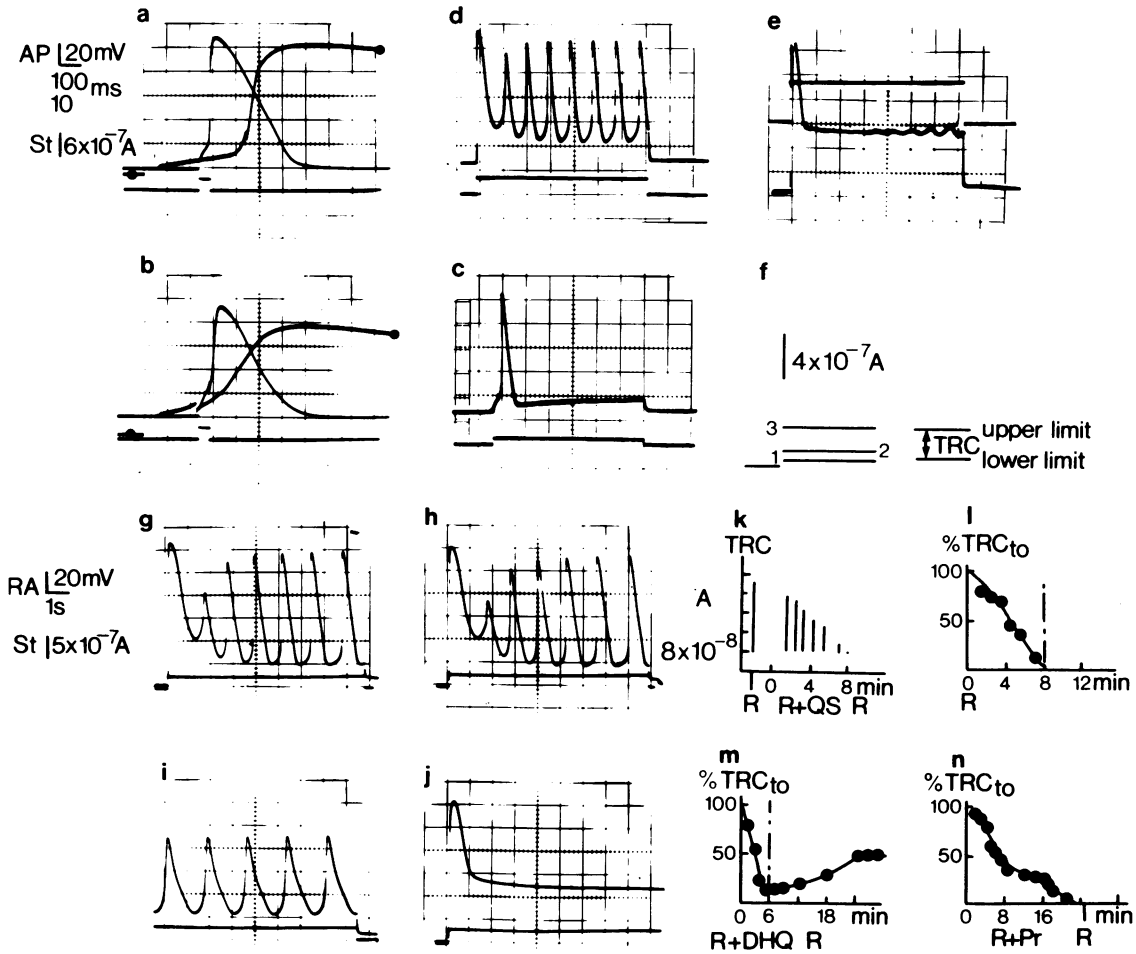

Figure 2 The effect of quinidine sulphate 50 μM on the action potential time course, the repetitive activity and the triggering range of current (TRC) of this repetitive activity. (a & b) Modification of the action potential in presence of quinidine: (a) in a Ringer solution; (b) in a Ringer solution + quinidine after 24 min of drug action. The upper trace is the action potential at a sweep speed of 100 ms/division, the middle trace is the depolarization phase of the action potential at a sweep speed of 10 ms/division; the lower trace is the stimulating current. (c, d, e and f) Method of obtaining the triggering range of current (TRC): (c) potential response (upper trace) to a depolarizing current, whose amplitude is labelled 1 in (f) is only a single spike, so the amplitude of stimulation labelled 1 is lower than the lower limit of the TRC; (d) the potential response (upper trace) to a depolarizing current, whose amplitude is labelled 2 in (f) is repetitive activity, so amplitude of stimulating current labelled 2 is within the TRC; (e) the potential response (lower trace) to a depolarizing current, whose amplitude is labelled 3 in (f) is a single spike and a few oscillations at the end of the pulse, so amplitude of stimulation labelled 3 is higher than the upper limit of the TRC; (f) the TRC corresponds to values of depolarizing currents which elicited repetitive activity. (g, h, i and j) Modification of the time course of the induced repetitive activity in the presence of quinidine. Upper trace is the potential response and lower trace is the depolarizing current the amplitude of which is the same for the four figures: (g) in Ringer solution; (h) after 4 min of quinidine action; (i) after 8 min of drug action; (j) after 10 min of drug action, repetitive activity has ceased. (k, l, m and n) Modification of the TRC in presence of antiarrhythmic agents: (k) decrease of the amplitude of TRC in presence of quinidine sulphate 50 μM (QS) applied to the preparation from 0 to 8 min; the preparation was then bathed with a Ringer solution (R); (l) same experiment but the result is expressed in percentage of the TRC obtained in a Ringer medium (=100% TRC); after 8 min the preparation is again bathed in a Ringer solution; (m) decrease of the TRC in presence of dihydroquinidine 5 μM (DHQ) applied to the preparation from 0 to 6 min; then the preparation was again bathed with Ringer solution; (n) decrease of the TRC in presence of procainamide 100 μM (Pr) applied to the preparation from 0 to 25 min; after 25 min the preparation was again bathed with Ringer solution.

110 mV and maximum rate of rise of about 13.5 V/second. This low rate of rise underlines the difference between intracellular microelectrode recordings and extracellular recordings. The time for 95% of full repolarization was about 380 milliseconds. The AP time course was altered by the action of quinidine sulphate 50 μM (Figure 2b) which also induced a depression of the plateau. There was a slowing of the final repolarization phase of the AP, this slowing becoming observable after 24 min of quinidine's action; the time needed for 95% full repolarization was now 400 milliseconds. The effects of quinidine on frog atrial AP are summarized in Table 1. Quinidine induced no change in resting membrane potential: in

Table 1 Effect of quinidine sulphate 50 μM on frog atrial action potential

	No. of fibres	*AP amplitude*	*Time to 50% repolarization*	*Time to 90% repolarization*	*Time to 95% repolarization*	*Maximum rate of rise*
		(mV)	*(ms)*	*(ms)*	*(ms)*	*(V/s)*
Control	21	114 ∓ 1	214 ∓ 4	351 ∓ 8	390 ∓ 5	18.6 ∓ 1.2
Quinidine sulphate 50 μM	21	99 ∓ 1	204 ∓ 4	352 ∓ 7	411 ∓ 5	6.9 ∓ 0.6
Difference from control		−15	−10	+1	+21	−11.7
Statistical significance of differences from control		$P<0.001$	NS	NS	$P<0.01$	$P<0.001$

NS = not significant.

Table 2 Modification by quinidine sulphate 50 μM of the time course of repetitive activity corresponding to the experiment shown in Figure 2g, h and i

Parameters	*Control*	*Quinidine sulphate (50 μM)*	
		4 min of action	*8 min of action*
Average spike amplitude (mV) (measured from maximum diastolic repolarization level)	97	96	68
Average spike repolarization time (ms)	780	860	1230
Average spike frequency (Hz)	0.7	0.7	0.5
Average spike depolarization rate (V/s)	1	1	0.5
Average diastolic depolarization rate (V/s)	0.03	0.02	*
Difference between the potential at rest and the maximum diastolic repolarization potential during repetitive activity (mV)	17	18	21

* Not measurable.

31 experiments during the action of quinidine, the mean difference in resting membrane polarization from control values was less than 2 mV.

Induced repetitive activity. By means of weak and long-lasting depolarizing currents under current clamp conditions, it was possible to trigger repetitive activity in frog auricular fibres as in Purkinje fibres (Hauswirth, Noble & Tsien, 1969). This activity was maintained throughout the period of the depolarization (Figure 2d).

The time course of the rhythmic activity obtained in Ringer medium (Figure 2g) was modified by the action of quinidine sulphate 50 μM (Figure 2h and i). Such modifications observed in the experiment shown in Figure 2(g, h and i) are summarized in Table 2. Quinidine decreased the spike amplitude, the spike frequency, the spike depolarization rate, the diastolic depolarization rate and increased the spike repolarization time and the difference between the potential at rest and the maximum diastolic repolarization potential during repetitive activity. After 10 min of action of quinidine, in the case of the results shown in Figure 2(g, h and i) and in Table 2, all repetitive activity ceased. Table 3 shows the modification of the repetitive activity time course after 8 min of action of quinidine in 11 experiments. As regards the diastolic depolarization rate the results correspond to 4 min of action of quinidine because after 4 min the time course of the diastolic depolarization is modified in such a way that it becomes impossible to measure its slope (as in Figure 2i).

It was interesting to study the range of current which can trigger repetitive activity, the triggering range of current (TRC). The preparation was depolarized by long lasting currents (see Figure 2f). The value 1 of the depolarizing current (in Figure 2f) which corresponds to the response shown in Figure 2(c), was not strong enough to elicit repetitive activity, only a single spike being observed. If the amplitude of the depolarizing pulse was increased it became possible to elicit repetitive activity and the lowest value of depolarizing pulse which allowed repetitive activity to develop was the lower limit of the TRC. Then the amplitude of the depolarizing pulse was again increased. Value 2 (in Figure 2f) is the value of depolarization which allowed repetitive activity in Figure 2(d) to develop. Then if the amplitude of this depolarizing pulse was again increased, until repetitive activity was no longer able to develop (Figure 2e), the corresponding value of the depolarizing pulse was higher than the upper limit of the TRC (Figure 2f).

In Figure 2(k) the TRC is indicated first in the reference medium (R) and during the action of quinidine (R + QS) from zero to 8 min of action of quinidine. This drug decreased the TRC by a gradual decrease of the upper limit of the TRC; after 8 min of action there was no longer a value of depolarizing current which was able to elicit repetitive activity, so the TRC was zero. Another expression of the same result is shown in Figure 2(l) where the TRC obtained in Ringer solution was taken as 100% and where the percentage of this reference TRC, obtained during the action of quinidine, was plotted against the time of action of the drug. The use of a less concentrated solution of quinidine sulphate (5 μM) or of dihydroquinidine (5 μM) such as in Figure 2(m), showed the same results but here the phenomenon was

Table 3 Effect of quinidine sulphate 50 μM on the time course of repetitive activity in frog atrium

	No. of fibres	*Spike amplitude*	*Spike repolarization time*	*Spike frequency*	*Spike depolarization rate*	*Diastolic depolarization rate*	*Difference between potential at rest and maximum diastolic potential*
		(mV)	*(ms)*	*(Hz)*	*(V/s)*	*(V/s)*	*mV)*
Control	11	96 ±3	749 ±10	0.85 ±0.04	1.19 ±0.07	0.050 ±0.004	15.2 ±0.4
Quinidine sulphate (50 μM)	11	67 ±2	1036 ±23	0.62 ±0.04	0.60 ±0.05	0.033 ±0.003	18.1 ±0.5
Difference from control		−29	+287	−0.23	−0.59	−0.017	+2.9
Statistical significance of differences from control (*P* value)		<0.001	<0.001	<0.001	<0.001	<0.005	<0.001

partially reversible. For these two quinidine compounds reversibility was slower than the onset of the effect.

The result obtained with quinidine was not unique. For instance procainamide (100 μM) also induced a decrease and an annulment of the TRC (Figure 2n). In contrast, when using a cardiotonic drug such as adrenaline the TRC remained nearly unchanged or was increased. On preparations showing an appreciable TRC in the reference medium, the increase of the TRC was between 0% and 20% (6 preparations) while on preparations showing a weak TRC in the reference medium, the increase was from 50 to 300% (5 preparations).

Voltage clamp conditions

The fast initial sodium current I_{Na} or fast sodium inward current

Current-voltage relationship. The result shown in Figure 3(a) was obtained by the subtraction method using three series of records (see methods section). For the determination of the reversal potential of I_{Na}, two procedures were used. The first used the current-voltage curves obtained by the subtraction method. In this case the reversal potential value was the value of potential at the intersection of the current line with the voltage axis (an important advantage of the subtraction method is that it avoids errors due to leakage current). A second determination used was based on the double pulse method. Here the inward current under study was activated. The amplitude of the stimulating pulse was chosen in such a way that it allowed the maximum amplitude of the inward current to be obtained. The duration of the stimulus was first chosen so that the inward current inactivated completely before the end of the depolarizing pulse V_1. The duration of V_1 was reduced till its end coincided with the time to peak of the inward current. In these conditions the inward current was activated till its maximum amplitude and then there was a tail current which represented the current deactivation. Then a second pulse V_2 following immediately the end of pulse V_1 was applied to the preparation and the value of depolarization of V_2 that allowed the tail current to be flat (that is the value of V_2 for which the tail current was neither inward nor outward) was the value of the reversal potential for the studied inward current activated by V_1. These two methods gave similar results. In Figure 3(a) the peak amplitude of the sodium current taken as reference b, and of the current modified by quinidine a, have been plotted as a function of the voltage to which the preparation was clamped. The curve b was obtained after about 15 to 20 min of quinidine action. The values of I_{Na} on lines a and b are the mean values observed from 12 experiments, the vertical bars represent one standard error of the mean.

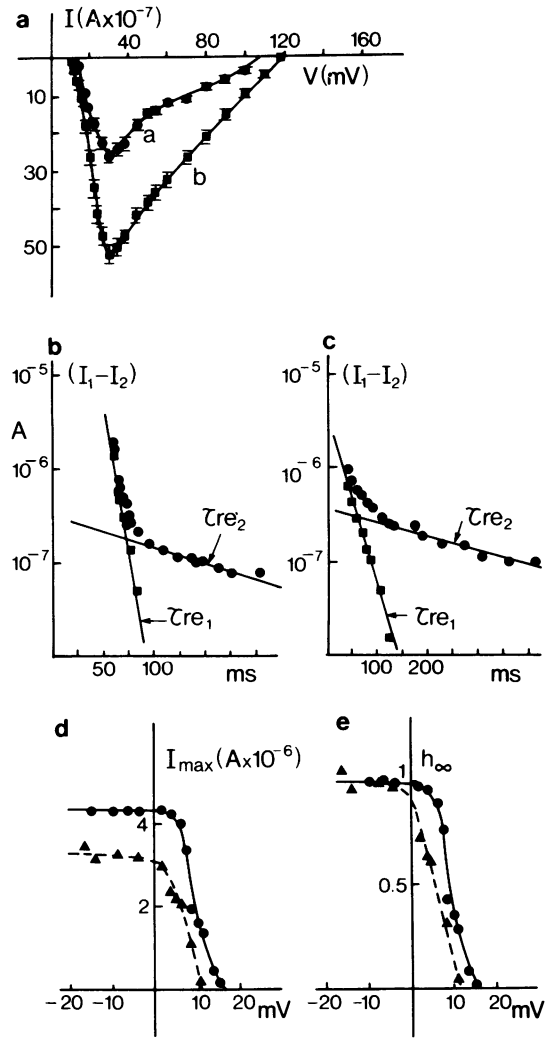

Figure 3 Modification of the fast sodium inward current by quinidine sulphate 50 μM. (a) Current-voltage relation of the sodium inward current; curve b is the reference sodium current, curve a is the sodium current obtained after quinidine action; curves a and b pass through points which are the mean amplitude values of sodium current obtained from 12 experiments. Vertical bars are one standard error of the mean. (b and c) The reactivation kinetics of the sodium inward current: (b) results obtained in a Ringer + manganese medium; there are two reactivation components with time constants τ_{re1} for the fast component and τ_{re2} for the slow component; (c) results obtained after the action of quinidine, the reactivation kinetics are decreased. (d and e) Inactivation curves of the sodium inward current: (d) I_{max} is the maximum of I_{Na} obtained either in the reference medium (continuous line) or after quinidine action (dotted line) plotted as a function of the potential to which preparation was clamped; (e) values of the steady state of the inactivation variable either in the reference medium (continuous line) or during quinidine's action (dotted line).

Quinidine decreased both the maximum amplitude of I_{Na} and the actual amplitude in response to any given clamp potential. For a depolarizing potential of 30 mV the decrease of the amplitude of I_{Na} was about 50%. The potential at which the maximum amplitude of I_{Na} was observed was not significantly altered (= +30 mV). All the experiments showed a shift of the reversal potential in the hyperpolarizing direction. The reversal potential determined in the reference medium was +118 ±1 mV and after the action of quinidine it became +109 ±1 mV ($P < 0.001$, 12 preparations) (Figure 3a). The activation threshold was not in general modified or it underwent a maximum increase of only about 2 mV.

The reactivation kinetics of I_{Na}. The current studied was that obtained in a Ringer + $MnCl_2$ (2 mM) solution. The experimental procedure was based on the double step technique of Hodgkin & Huxley (1952b). Provided two pulses were sufficiently separated in time, two depolarizing pulses identical in amplitude and duration resulted in two currents of maximum amplitude. At the beginning of the experimental procedure the two pulses were applied close together. Then the second depolarization was progressively delayed until two identical currents of maximum amplitude were observed. The interval between the pulses (Δt) then measured the time for complete reactivation, I_1 being the amplitude of the current elicited by the first pulse and I_2 the amplitude of the current elicited by the second pulse.

The graph $(I_1-I_2)=f(t)$ on semi-logarithmic coordinates allowed the determination of the reactivation time constants $\tau_{re1} = 6.5$ ms and $\tau_{re2} = 125$ ms in the case of the experiment shown in Figure 3(b). Quinidine clearly increased τ_{re1} and τ_{re2} which became 23 ms and 295 ms respectively (Figure 3c). The results of several experiments are summarized in Table 4.

The sodium conductance g_{Na}. The general procedure has been described in the methods section. The first experiments were done by the subtraction method needing four series of records. The action of quinidine was always studied after 15 to 20 min of action. The duration of the stimulating pulses eliciting the sodium current was sufficient in each experiment to allow in every case a complete inactivation of the current before the end of the pulse. The records made in the first solution (see methods section) had to be done quickly because of an incomplete restoration of the sodium current after inhibition by TTX if this drug had acted for too long. Most of the experiments were carried out with a series of three solutions (see methods section) which were (1) Ringer + $MnCl_2$; (2) Ringer + $MnCl_2$ + quinidine; (3) Ringer + $MnCl_2$ + TTX. In both methods the preparation was stimulated by means of rectangular depolarizing pulses the amplitude of which was increased by steps of 2 mV for the low values of depolarization and then by steps of 4 mV and then of 10 mV towards a value of about 10 mV lower than the reversal potential of I_{Na}. During the experiment the reversal potential was determined by the double pulse method. After the experiment the reversal potential was checked on the current-voltage curve. The sodium conductance was calculated according to the well known following formula (Hodgkin & Huxley, 1952a):

$$I_{Na} = g_{Na}(V - V_{Na})$$

where V is the potential imposed on the preparation and V_{Na} the equilibrium potential for Na^+ ions (measured). Calculations were made by the method proposed by Jacquenod (see appendix). Those calculations allowed the following parameters to be obtained: g'_{Na}, m_∞, $1/\tau_h$, $1/\tau_m$, α_m and β_m. The curves shown are drawn by hand through the experimental points.

The results shown in Figure 4 are those corresponding to one experiment carried out on a single preparation and with a protocol using three series of records. These results are similar to those obtained in

Table 4 Effect of quinidine sulphate 50 µM on the reactivation time constants of I_{Na}

	No. of fibres	*Control (§)*	*Quinidine sulphate (†)*	*Difference from control*	*Statistical significance of differences from control (P)*
τ_{re1} ms	15 (§) 14(†)	7.2 ∓ 0.3	22.7 ∓ 0.9	+ 15.5	<0.001
τ_{re2} ms	14	105 ∓ 6	234 ∓ 15	+129	<0.001

(§) and (†): among all the experiments 15 of them (§) allowed the determination of the reactivation time constants in the control medium and after the action of quinidine the determination of the reactivation time constants could be made in 14 (†) of these experiments.

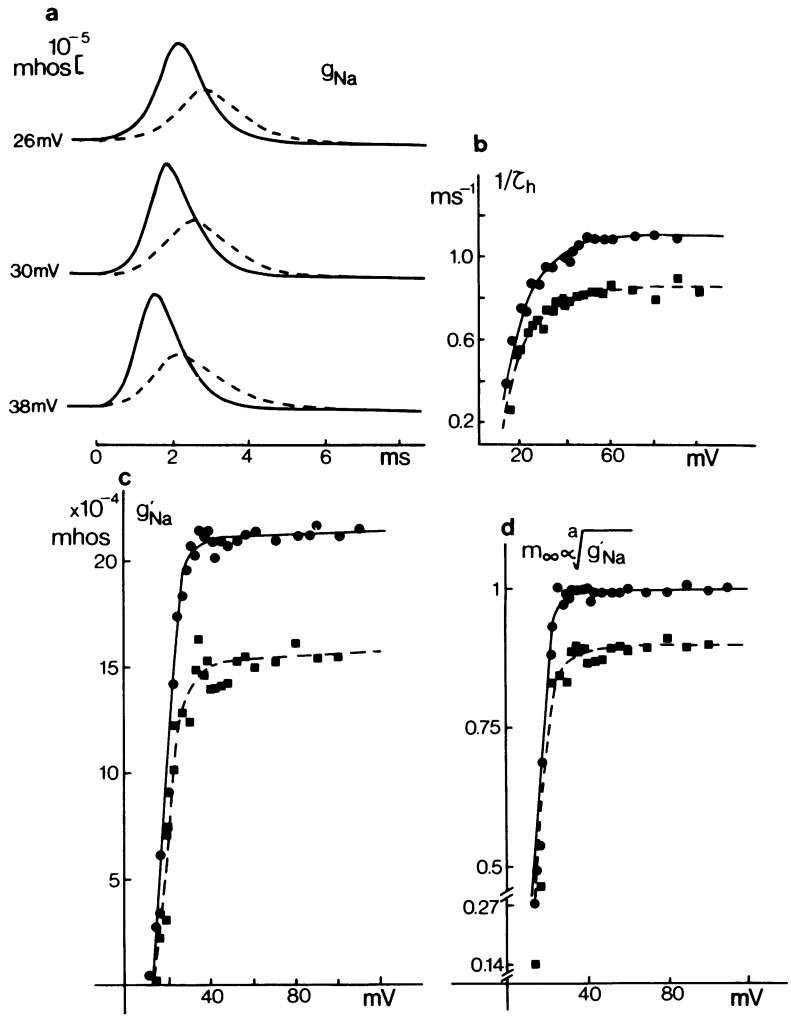

Figure 4 Modification of the conductance g_{Na} and its related parameters by quinidine sulphate 50 μM. (a) Time course of sodium conductance in a preparation clamped at +26, +30 and +38 mV. Continuous lines: g_{Na} taken as reference; dotted lines: g_{Na} modified by quinidine. Time 0 is time from which the depolarizing pulse is applied to the preparation. The continuous and interrupted lines are curves drawn through a great number of experimental points. (b) The reciprocal inactivation time constant expressed as a function of potential to which the preparation was clamped. Continuous line through (●) are reference values; dotted lines through (■) are values obtained during quinidine's action. (c) g'_{Na} is expressed as a function of the potential to which the preparation was clamped. Continuous line through (●): reference values; dotted lines through (■): decrease of g'_{Na} during quinidine's action. (d) Steady state of activation expressed as a function of the potential to which the preparation was clamped. Continuous line through (●): reference values; dotted line through (■): values obtained during the action of quinidine (on the vertical scale, value 1 corresponds to the maximum value obtained for the reference values).

4 experiments carried out with a protocol using four series of records and those obtained in 5 other experiments carried out with a protocol using three series of records. The tracings modified by quinidine were recorded after 15 to 20 min of drug action.

The time course of g_{Na} *tracings.* In Figure 4(a) the tracings of the sodium conductance plotted from a large number of experimental points are presented for three consecutive depolarizing potentials: 26 mV, 30 mV and 38 mV. The dotted lines obtained during

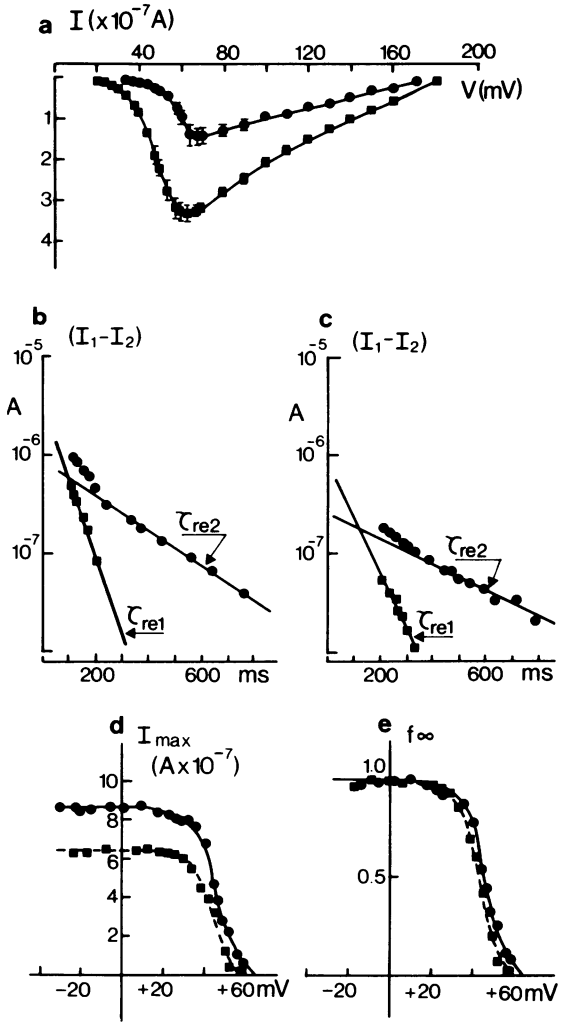

Figure 5 Modification of the slow inward calcium current and of the slow inward calcium-sodium current by quinidine sulphate 50 μM. (a) Current-voltage relationship of the calcium inward current I_{Ca}. The lower curve is the reference curve, the upper curve is the current obtained during quinidine's action. The two curves pass through points which are the mean values of the amplitude of the calcium current obtained from 10 experiments. Vertical bars show standard error of the mean. (b and c) Reactivation kinetics of the slow calcium-sodium inward current: (b) results obtained in a Ringer+TTX medium. There are two reactivation components whose time constants are τ_{re1} for the fast one and τ_{re2} for the slow one. (c) Results obtained during the action of quinidine added to a Ringer+TTX medium. There is an increase of the time constants of the two reactivation components. (d and e) The inactivation of the slow inward current: (d) I_{max} is the maximum of I_{Ca} obtained either in the reference medium (continuous line) or during quinidine's action (dotted line) plotted as a function of the potential to which the preparation was clamped. (e) Values of the steady state inactivation variable either in the reference medium (continuous line) or during the action of quinidine (dotted line).

the action of quinidine sulphate 50 μM show a decrease in the maximum amplitude of g_{Na}, an increase of the time to maximum g_{Na}, and an increase of the time needed for its complete inactivation. These results were observed for all potentials imposed on the preparation that is from +14 to +100 mV.

g'_{Na} as a function of the imposed potential. The method for obtaining the value of g'_{Na} is described in the appendix. In the case of the results shown in Figure 4 the exponent of the activation variable m was 3 for all the imposed potentials (see appendix, equation 2). Figure 4(c) shows that quinidine produced a clear decrease of the g'_{Na} values for the whole range of imposed potentials. Therefore this graph indicates a possible effect on steady state activation (m_∞).

m_∞-potential curve. Figure 4(d) shows that steady state activation was clearly decreased in the presence of quinidine. The value of the maximum steady state (that is the plateau curve) reached by m_∞ was decreased. There was no significant shift of the curve along the x-axis.

Results concerning $1/\tau_m$, α_m and β_m. In the case of I_{Na}, for reasons given in the Introduction and in the Discussion, it was not possible to be confident that the voltage control for currents of fast kinetics was satisfactory, particularly during the beginning of the pulse. Consequently quantitative estimates for $1/\tau_m$, α_m and β_m are not given here. However, it is possible to say, on a purely comparative basis, that there was a decrease of $1/\tau_m$ for all the applied potentials in the presence of quinidine, a decrease of the values of α_m and a non-significant change of the values of β_m.

Curve $1/\tau_h$-potential. Figure 4(b) shows that quinidine induced a decrease in $1/\tau_h$ for all the potentials applied to the preparation.

Inactivation of the sodium current. The results shown in Figure 3(d) and (e) were obtained from a different preparation from that of Figure 4. The results in Figure 3(d) and (e) are similar to those obtained under the same conditions in 8 other preparations. The experimental procedure was directly derived from that used by Hodgkin & Huxley (1952b). Figure 3(e) shows that the $h_\infty = f(V)$ curve was shifted towards the hyperpolarizing potentials in the presence of quinidine. The potential allowing the complete

inactivation of I_{Na} was shifted by 4 to 5 mV in the hyperpolarizing direction. A similar shift of the inactivation curve in the same direction is seen in Figure 3(d). Moreover a decrease of the steady state of I_{max} (upper part of the curve) in the presence of quinidine can be observed.

The calcium current and the calcium-sodium current

Current-voltage relation of the calcium current I_{Ca} (Figure 5a). To obtain the current-voltage relation of I_{Ca} the general protocol was similar to that used for I_{Na} but the media used were different. Results shown in Figure 5(a) were obtained by the subtraction method using three series of records (see methods section), the three media being successively (1) Ringer solution without Na^+; (2) Ringer without Na^+ + quinidine and (3) Ringer without $Na^+ + MnCl_2$. It was not possible to determine the reversal potential of I_{Ca} during the experiments, by the double pulse method. Because of the slow kinetics of this current, the outward current often began to be partially activated at the end of the first pulse V_1, so that it was impossible to obtain a flat tail of current during the second pulse V_2. Thus the reversal potential of I_{Ca} was only determined from the current-voltage curves.

The continuous curve drawn in Figure 5(a) was obtained after about 15 to 20 min of action of quinidine sulphate 50 µM. The values of I_{Ca} are the mean values observed from 10 experiments. The vertical bars represent the standard error of the mean. The maximum amplitude of I_{Ca} was decreased (upper curve), and for a 70 mV amplitude stimulus applied to the preparation the decrease was about 57%. The potential at maximum amplitude was shifted slightly positively (about 4 mV); the reversal potential was shifted in the hyperpolarizing direction (about 10 mV). The reversal potential determined in the reference medium was $+181 \pm 2$ mV, and after the action of quinidine it was $+171 \pm 2$ mV ($P < 0.005$, 10 preparations). The potential threshold of I_{Ca} was often higher in the presence of quinidine.

Reactivation kinetics of the slow inward calcium-sodium current. The current studied was that obtained in Ringer + TTX. The experimental method was the same as that used for I_{Na}. The pulses used to elicit the slow inward current were longer than for I_{Na} and lasted long enough to allow a complete inactivation of this slow current. The experiment shown in Figure 5(b) and (c) indicates that quinidine increased the reactivation time constants. Thus in the reference medium the reactivation time constants were $\tau_{re1} = 59$ ms and $\tau_{re2} = 256$ ms; after about 20 min of quinidine action these values became respectively 80 and 345 milliseconds. From a summary of all experiments of this type it was possible to establish that quinidine decreased the reactivation kinetics of the slow inward current (see Table 5).

The calcium conductance g_{Ca}. Here, it should be recalled that the results of Besseau (1972) obtained on frog auricular preparations showed that the Hodgkin-Huxley model can be successfully applied to the calcium conductance, the g_{Ca} time courses calculated from the Hodgkin and Huxley parameters allowing a good fit to the experimental points.

As for I_{Na} two experimental procedures were tried in order to obtain a relatively 'pure' I_{Ca} and to calculate the calcium conductance. The first experiments were carried out with a subtraction method using four series of records after testing the state of the preparation (see methods section). A series of records was made in each medium. The amplitude of the depolarizing pulses was increased by steps of 4 mV and then of 10 mV in order to have enough points to draw the m_∞-potential curve. Particularly because of the time needed to do such experiments and because of the difficulty of recovery of I_{Ca} after the first solution, a second type of experiment with a subtraction method using three series of records was preferred (same solutions used as for the inward calcium current). An example of the results obtained with such a method is shown in Figure 6(a–d). These

Table 5 Effect of quinidine sulphate 50 µM on the reactivation time constants of the calcium-sodium inward current of slow kinetics

	No. of fibres	*Control*	*Quinidine sulphate*	*Difference from control*	*Statistical significance of difference from control (P)*
τ_{re1} ms	14	94 ∓ 7	127 ∓ 9	+ 33	<0.01
τ_{re2} ms	14	305 ∓ 11	412 ∓ 15	+107	<0.001

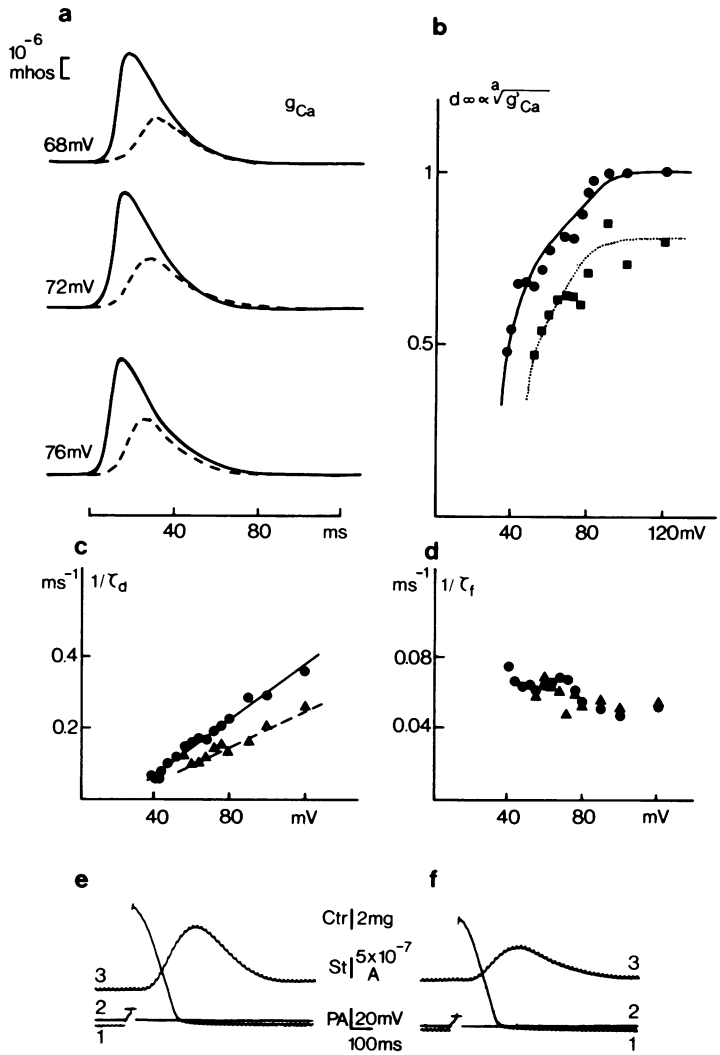

Figure 6 Modification of the calcium conductance and of its related parameters by the action of quinidine sulphate 50 μM. (a) Calcium conductance time course for 68, 72 and 76 mV depolarizations imposed on the preparation. Continuous line: g_{Ca} reference time course; dotted line: g_{Ca} time course obtained after action of quinidine. The continuous and interrupted lines are curves drawn through a great number of experimental points. (b) Steady state of activation expressed as a function of the potential to which the preparation was clamped. Continuous line through filled circles: values of d_∞ taken as reference; dotted line through filled squares: values obtained during quinidine's action (on vertical scale 1 corresponds to the maximum value of d_∞ obtained in the reference medium). (c) Values of $1/\tau_d$ as a function of the potential to which the preparation was clamped. Continuous line through (●): reference values; dotted line through (▲): values obtained during quinidine's action. (d) Values of $1/\tau_f$ as a function of the potential to which the preparation was clamped: (●): reference values; (▲): values obtained during the action of quinidine. (e and f) The action of quinidine on the mechanical activity of frog cardiac auricular trabeculae: (e) simultaneous recordings of the action potential (trace 1) elicited by a depolarizing current (trace 2) and of the contraction (trace 3) recorded with a RCA 5734 transducer in the reference medium: (f) the same recordings made after 25 min of action of quinidine. Notice the decrease in the peak amplitude (PA) of contraction.

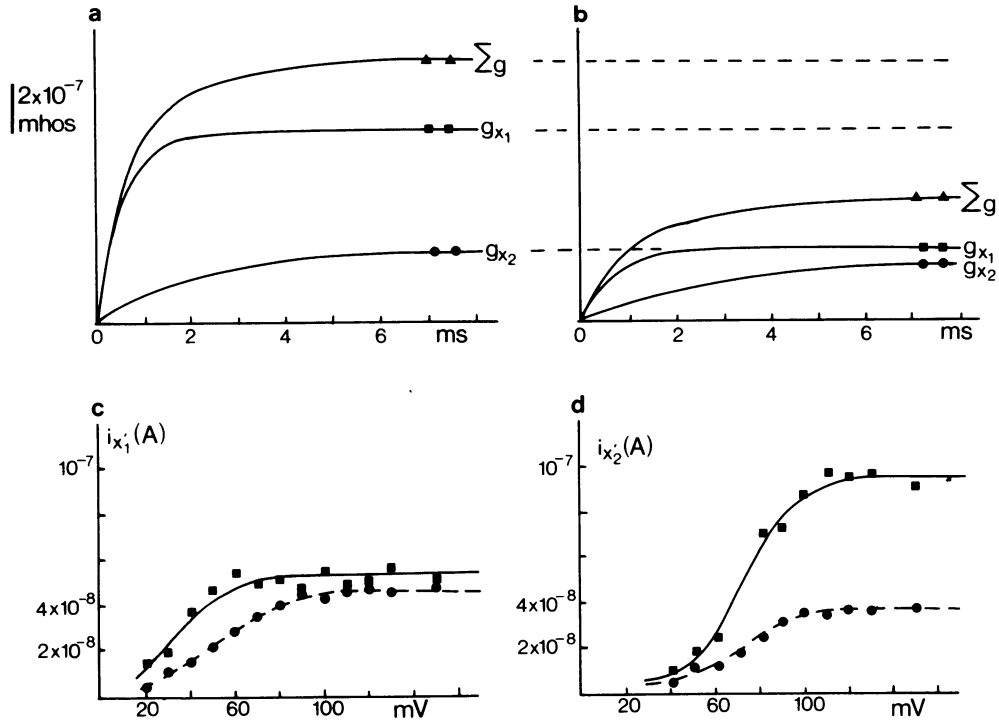

Figure 7 The delayed conductances and their activation curves. (a and b) The delayed conductances time courses: (a) in reference medium, g_{x1} is the fast component labelled with two filled squares; g_{x2} is the slow component labelled with two filled circles: Σg is the sum of g_{x1} and g_{x2} labelled with two triangles; (b) g_{x1}, g_{x2} and Σg time courses after quinidine's action. Notice the strong decrease of the amplitude of g_{x1}. (c and d) The activation curves of the fast component (c) and of the slow component (d) of the total delayed outward current. Continuous lines through (■): reference values; dotted lines through (●): values obtained during quinidine's action.

results are similar to those obtained in 3 experiments carried out with a protocol using four series of records and those obtained in 5 other experiments carried out with a protocol using three series of records. The calculations of the parameters concerning g_{Ca} were similar to those carried out for g_{Na}. The determination of the exponent 'a' of the activation variable d led to higher values than for g_{Na}; the values encountered for 'a' ranged from 6 to 14.

g_{Ca} time course. Figure 6(a) shows that quinidine sulphate 50 μM decreased the maximum amplitude of g_{Ca}, increased the time to reach maximum, while it did not seem to modify significantly the time needed for the complete inactivation of g_{Ca}.

d_∞-potential curve. In Figure 6(b) a clear decrease of the steady state of the activation in the presence of quinidine is shown. This result applies to all potentials applied to the preparation. The threshold of d_∞ was higher, the shift being about 8 to 10 mV towards more positive potentials.

$1/\tau_d$-potential curve. Quinidine induced a decrease of the values of $1/\tau_d$, that is, an increase of the activation time constant (Figure 6c).

The rate constants α_d and β_d. A clear decrease of the values of α_d was observed in the presence of quinidine; the greater the imposed potential, the greater the decrease. There was also a decrease of the values of β_d, this decrease being less than for α_d and not always significant.

$1/\tau_f$-potential curve. Quinidine did not significantly alter the values of $1/\tau_f$ (Figure 6d).

Inactivation of the inward calcium-sodium current of slow kinetics. The current was studied in a

Ringer + TTX medium. In frog auricular trabeculae, the calcium component seems to be greater than the sodium component (Besseau, 1971), both constituting the slow current. The results shown in Figure 5(d) and (e) were obtained from a different preparation from that of Figure 6(a–d). The results reported in Figure 5(d) and (e) are similar to those obtained under the same conditions in 7 other preparations. Figure 5(d) shows that quinidine induced a decrease of the steady state of I_{max} (plateau of the curve). In addition there was a slight shift of the curve towards hyperpolarizing potentials. Figure 5(e) indicates that quinidine did not modify the inactivation curve, there being only a slight and insignificant shift of the curve towards hyperpolarizing potentials.

The delayed system ($i_{x1} + i_{x2}$) and the corresponding conductances ($g_{x1} + g_{x2}$). The analysis of the total delayed current recorded on the auricular trabeculae of frog showed the existence of two components i_{x1} and i_{x2}, the first having fast activation kinetics and the second slow activation kinetics (Brown & Noble, 1969a, b). The reversal potential of the first component seemed to vary between +5 (or less) and +15 mV with regards to the holding potential taken as zero. The reversal potential of the second component was often between +30 and +40 mV or more. In some preparations only one component of the delayed outward current was found. The effect of quinidine was only studied on preparations showing two components of this current. To obtain the results, the method indicated in the appendix is directly derived from that of Brown & Noble (1969a, b) and Noble & Tsien (1969a, b).

The current traces obtained in a Ringer + TTX + $MnCl_2$ medium appeared to be the sum of two exponential functions. The measurement of the two reversal potentials V_{x1} and V_{x2} of the two components gave the value of the two conductances $g_{x1} = i_{x1}/(V - V_{x1})$ and $g_{x2} = i_{x2}/(V - V_{x2})$. The activation time constants of the fast and slow systems were obtained from the following relations: $\alpha_{n1} = n_\infty 1/\tau_{n1}$; $\alpha_{n2} = n_\infty 2/\tau_{n2}$; $\beta_{n1} = (1 - n_{\infty 1})/\tau_{n1}$; $\beta_{n2} = (1 - n_{\infty 2})/\tau_{n2}$. The values of g_{x1} and g_{x2} determined at time zero on semi-logarithmic plots express the steady state activation of each conductance component. The activation curves of the delayed systems were obtained from the two components of the tail currents when these two components were plotted on semi-logarithmic scales. Then it was possible to determine the ordinates at time zero: i'_{x1} for the fast component i_{x1}, and i'_{x2} for the slow component i_{x2}. These ordinates at the origin would be representative of the initial state of the deactivation state n_∞ (at the steady state) of the current components obtained during the preceding stimulus (Noble & Tsien, 1969a).

The results shown in Figures 7(a) and (b) and 8(a–d) were obtained in the same preparation and are representative of those obtained in 8 other preparations. The results indicated in Figure 7(c) and (d) were obtained in different preparations and are similar to those obtained from 7 other preparations.

The conductances g_{x1} and g_{x2}. Quinidine induced an important decrease of the amplitude of the g_{x1} component and a weak decrease of the amplitude of the g_{x2} component, leading to an important decrease of their algebraic sum Σg. This effect was the same for all the potentials applied to the preparation (Figure 7a and b).

The activation time constants of g_{x1} and g_{x2} components. Quinidine decreased $1/\tau_{n1}$ particularly for the potential values higher than +70 mV. The values of $1/\tau_{n2}$ were slightly decreased over the whole range of potentials applied (Figure 8a and b).

The activation rate constants of the g_{x1} and g_{x2} components. Quinidine clearly decreased the values of α_{n1} and α_{n2} but did not significantly alter the values of β_{n1} and β_{n2} (Figure 8c and d).

The activation curves of the i_{x1} and i_{x2} components of the delayed outward current. Quinidine decreased the values of i'_{x1}. The half activation potential was increased about 15 mV. There was a very clear decrease of the values of i'_{x2} while the half activation potential was not significantly modified (Figure 7c and d).

The reversal potential of i_{x1} and i_{x2}. From 14 experiments it can be said that in the reference medium the reversal potential of i_{x1} component was $+13 \pm 1$ mV and that of the i_{x2} component $+39 \pm 1$ mV. Quinidine decreased slightly the value of i_{x1} reversal potential which became $+10 \pm 1$ mV ($P < 0.05$) and the value of i_{x2} reversal potential was decreased to $+34 \pm 1$ mV ($P < 0.005$).

Discussion

Two types of results have been obtained: those obtained in current clamp conditions and in voltage clamp conditions. Results obtained in voltage clamp conditions allowed a fairly good understanding of those obtained in current clamp conditions and of results obtained with microelectrode recordings by other authors.

Serious strictures have been made concerning the validity of voltage clamping by the double sucrose gap technique (Johnson & Lieberman, 1971). In addition to errors recalled by Besseau (1972) the presence of an external series resistance (Rougier *et al.*, 1968) must be mentioned, the notable role of which has been

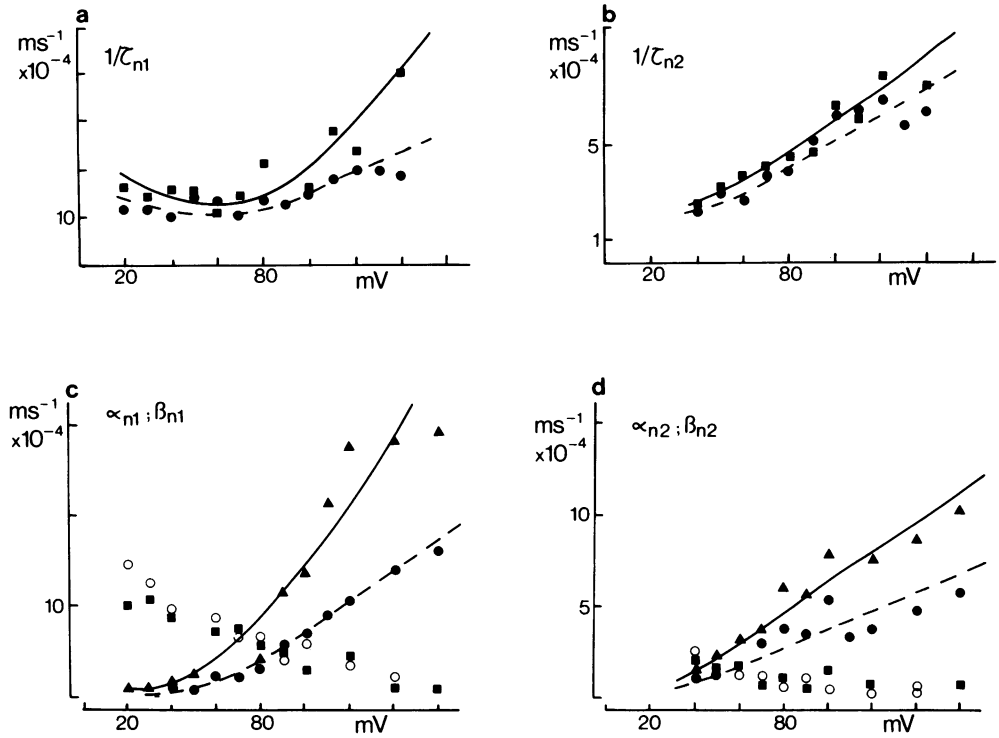

Figure 8 The activation parameters of the delayed conductances g_{x1} and g_{x2}. (a and b) The reciprocal activation time constant of the delayed conductance component: $1/\tau_{n1}$ for the fast component in (a) and $1/\tau_{n2}$ for the slow component in (b). Continuous line through (■): reference values; dotted line through (●): values obtained during action of quinidine sulphate 50 μM. (c and d) The activation rate constants of the fast delayed conductance α_{n1} and β_{n1} (c) and of the slow delayed conductance α_{n2} and β_{n2} (d). Symbols: (▲) and continuous line are values of α_{n1} or α_{n2} in the reference medium; (●) are values of α_{n1} or α_{n2} after quinidine's action; (○) are the values of β_{n1} or β_{n2} in the reference medium and (■) are values of β_{n1} or β_{n2} during quinidine's action.

emphasized by Léoty & Poindessault (1974) and by Poindessault, Duval & Léoty (1975). In view of this it must be recognized that the control of the potential of the membrane in the central compartment of the preparation is suspect. It is necessary to point out that I have not made a quantitative analysis but have tried to identify the components of the ionic conductances which might be affected by the action of quinidine. The results must be discussed from a strictly qualitative viewpoint and the major interest of the results comes from the comparisons which could be made between reference results and those obtained during the action of quinidine.

The above considerations and criticisms apply particularly to experiments performed with double sucrose gap devices with a large central compartment and without vaseline seals. Recent work by de Hemptinne (unpublished observations) leads to the conclusion that a double sucrose gap technique using partitions of vaseline is better as regards the errors made with a double sucrose gap technique with no physical partitions between solutions (this remark being valid because of the same test compartment width used, as in the present work, that is 150 μm, but with the restriction that de Hemptinne used an electronic circuitry allowing a compensating procedure of the series resistance in the central compartment).

Before discussing the results it should be recalled that in the frog auricular preparation a voltage and time-dependent inward current of fast kinetics carried by sodium ions is mainly responsible for the depolarizing phase of the AP (Rougier *et al.*, 1969). A time and voltage-dependent inward current of slow kinetics carried both by sodium and calcium ions is mainly responsible for the final depolarization phase and the plateau phase of the AP (Rougier *et al.*, 1969). The calcium component is of greater amplitude than the sodium component (Besseau, 1971). The

repolarization phase of the AP can be explained by the existence of a delayed rectification (Noble, 1962, 1966; McAllister & Noble, 1966, 1967; Rougier *et al.*, 1968); potassium ions would constitute a part of this current which has very slow kinetics (Besseau, 1972).

The decrease of the amplitude of I_{Na} as of g_{Na} in the presence of quinidine simply explains the decrease of the amplitude of the AP. The reactivation time constants of I_{Na} were increased by quinidine; thus this drug lengthened the time required for the membrane to return to its fully activated state. This delay of reactivation explains the lengthening of the effective refractory period observed with quinidine by Vaughan Williams (1958) in the absence of any prolongation of the AP duration. The decrease of m_∞ expresses a reduced total capacity of the cardiac membrane to carry sodium ions. The increase of τ_m, expressing an increased delay of the g_{Na} activation, accounts for the decrease of the rate of rise of the AP and would result in a slower spread of intracellular current responsible for propagation of impulses through the myocardium. The reduced values of α_m in presence of quinidine help to understand the slowing down of the activation process. The increase of τ_h, expressing a delay in the inactivation of g_{Na}, accounts for the increase observed in the total time needed for the inactivation of g_{Na}. This result partly explains the increase of reactivation time constants of I_{Na}. The shift of the h_∞-potential curve in the hyperpolarizing direction would cause a decrease of the fraction of the sodium carrying system in non-inactivated state. Quinidine would thus maintain a partial inactivation of this system.

The decrease of the amplitude of the slow inward calcium current can explain the negative inotropic effect of quinidine observed in Figure 6(e) and (f), because according to Léoty, Raymond & Gargouïl (1970) the mechanical response of the frog auricular fibre depends, for its 'phasic' component, on the entry of calcium from the external medium. The decrease of the amplitude of the contraction is in agreement with the depressant effect on myocardial contractility recalled by Thorpe (1973) and established by Vaughan Williams (1958), Angelakos & Hastings (1960) and Parmley & Braunwald (1967). The delayed reactivation of the total (calcium + sodium) slow inward current could account for the failure of responses to a high stimulus frequency in the presence of quinidine, when the action potentials would normally have developed a high level of activation of calcium-sodium current. Therefore quinidine increases the time needed for a total reavailability of the membrane for the transport of the fast and slow inward currents. This effect would both contribute to an increase of the relative refractory period, and would oppose the maintenance of responses to an ectopic pacemaker.

The decrease of the maximum amplitude of g_{Ca} can partially explain the depression of the plateau of the AP and of the spike amplitude during repetitive activity, both observed in presence of quinidine. The decrease of d_∞ would express, in Hodgkin–Huxley terms, a decrease of the proportion of the activating molecules for calcium transport in the α state. Although the increase of the activation time constant of g_{Ca} could not account for the slowing of MRD, it could nevertheless explain the gradual 'rounding' of the final phase of depolarization of the AP, i.e. in Hodgkin–Huxley terms, α_d is decreased.

The decrease of the amplitude of g_{x1} partially explains the lengthening of the final phase of the AP observed in the presence of quinidine. The increase of τ_{n1} could contribute to the increase of the maximum repolarization time of the spikes during repetitive activity observed in presence of quinidine. The effects of this drug on g_{x2} amplitude however were small; moreover in view of the much slower activation kinetics of g_{x2}, it is doubtful whether a modification of this system could contribute to the action of quinidine on the time course of the AP. However, the increase in the half-activation potential for i_{x1} indicates that higher depolarizations would be required to activate the latter system, and this delay of i_{x1} activation may be considered partly responsible for the delayed repolarization.

The results obtained in the present work are clearly useful for a better understanding of the modifications induced by quinidine on both the AP and the repetitive activity. Concerning the AP the present results are in general agreement with the finding of a decrease of the rate of rise produced by quinidine observed by Weidmann (1955) on Purkinje fibres of sheep and calf; they also confirm those obtained by Vaughan Williams (1973) using intracellular microelectrodes impaled in rabbit auricular muscle. Since the rate of rise of the AP affects the conduction velocity (Trautwein, 1963), the decrease of the rate of rise of the AP by quinidine partially explains the slowing of conduction observed with quinidine (Vaughan Williams, 1958; Szekeres & Papp, 1971). Quinidine induced a lengthening of the final repolarization phase of the AP. The maximum value of the plasma concentration of quinidine observed clinically is near 10 μM, a concentration at which there is only a little change in AP duration. For example, a concentration of 9 μM quinidine induced only a small lengthening of the 'tail of the repolarization phase' of the AP (Vaughan Williams, 1958). In fact, on the frog auricular preparation, the concentration required to alter significantly the conductance parameter was 50 μM, so the lengthening of the final repolarization phase of the AP observed with 50 μM quinidine can hardly explain the lengthening of the effective refractory period but this last effect has already been explained by the increase of the reactivation time constants of both fast and slow inward currents. So it becomes possible to understand why a lengthening of the effective refractory period can be observed even

when the AP duration remains unchanged (Vaughan Williams, 1973).

The repetitive activity elicited by depolarizing currents in frog auricular preparations depends on the deactivation of a delayed outward system with fast kinetics (Brown & Noble, 1969a) and on the activation of a slow inward system (Lenfant, Mironneau & Aka, 1972). The decrease of the spike amplitude during repetitive activity can be partly explained by the decrease of the values of d_∞ and therefore the decrease of the maximum amplitude of g_{Ca}. The inactivation time constant of g_{Ca} seems not to be modified, so one can assign the increase of the maximum repolarization time of the spikes to the increase of the activation time constant of the g_{x1} component of the delayed conductance. The decrease of the spike depolarization rate can be partially explained by the increase of the activation time constant τ_d of g_{Ca}. The decrease of the diastolic depolarization slope is perhaps better understood if one takes into account the decrease of the amplitude of g_{x1}. Component g_{x1} being less activated, the conductance is therefore smaller during activation but also during deactivation, so that it would result in a longer time to reach the activation threshold of the slow inward system. The increase of each spike duration and the decrease of the diastolic depolarization slope explain the decrease of the firing frequency of repetitive activity. Eventually after quinidine has acted for sufficient time and all the effects reported above are sufficiently established a total abolition of repetitive activity is to be expected. It would then be interesting to study the effects of new drugs on the triggering range of current (TRC). It could test whether a drug has or has not the ability to reduce or depress repetitive activity, experimentally triggered by a depolarizing current. This procedure might prove useful as a screening test for drugs able to suppress ectopic pacemakers or extrasystoles.

In spite of the limitations of the method used in the present work, reproducible variations of the apparent values of the parameters have been observed on frog auricular preparations in the presence of quinidine. Such results may be useful in elucidating the mode of action of drugs. It is noteworthy that results obtained in voltage clamp conditions aid clearly in the interpretation of results obtained in current clamp conditions. It should also be pointed out that the conclusions drawn from these experiments with the double sucrose gap technique are in excellent agreement with results obtained previously with intracellular microelectrodes. It seems reasonable therefore to suggest that similar or related types of study may be of use in the attempt to understand better the mode of action of cardioactive drugs, and particularly of antiarrhythmic agents, on the electrical properties of cardiac membranes.

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Appendix

Sodium (as calcium) conductance

The sodium current was obtained by subtraction of a tracing obtained in a medium allowing the development of I_{Na} from a tracing obtained in a medium where I_{Na} was inhibited (Figure 9). The calculations made, assumed that the conductances can be described reasonably well by the Hodgkin and Huxley equations proposed for the giant axon of the squid (Besseau, 1972). The method that has been used and the corresponding programmes have been elaborated by J.C. Jacquenod (Assistant, Laboratoire de Physiologie Animale, Faculté des Sciences de l'Université de Poitiers, France: method and results to be published).

It was possible to calculate the conductance g_{Na} from the reversal potential of I_{Na} measured during the experiment and the imposed potential. In Figure 9(b), the sodium conductance obtained for a depolarizing pulse of 40 mV is drawn as a function of time (the beginning of the pulse being time zero). The relation between the current and the corresponding conductance given by Hodgkin & Huxley (1952a) is:

$$g_{Na} = I_{Na}/(V - V_{Na}) \quad (1)$$

where I_{Na} is the amplitude of the current, V the imposed potential and V_{Na} the equilibrium potential for Na ions. Hodgkin & Huxley (1952c) made the assumption that g_{Na} was a function depending on two variables: m, the activation variable and h, the inactivation variable:

$$g_{Na} = m^a \cdot h^i \cdot \overline{g_{Na}} \quad (2)$$

where $\overline{g_{Na}}$ is a constant and is the absolute maximum value that can be reached by g_{Na}. The exponent i of h is assumed to be 1. m and h variables yield to the following differential equations:

$$dm/dt = \alpha_m (1-m) - \beta_m \cdot m \quad (3)$$

$$dh/dt = \alpha_h (1-h) - \beta_h \cdot h \quad (4)$$

where m represents the proportion of activating molecules in the α state, (1-m) the proportion of activating molecules in the β state, h the proportion of inactivating molecules in the α state and (1-h) the proportion of inactivating molecules in the β state. m and h are dimensionless variables which can vary between 0 and 1 (Hodgkin & Huxley, 1952c). The solutions of equations (3) and (4) which satisfy the

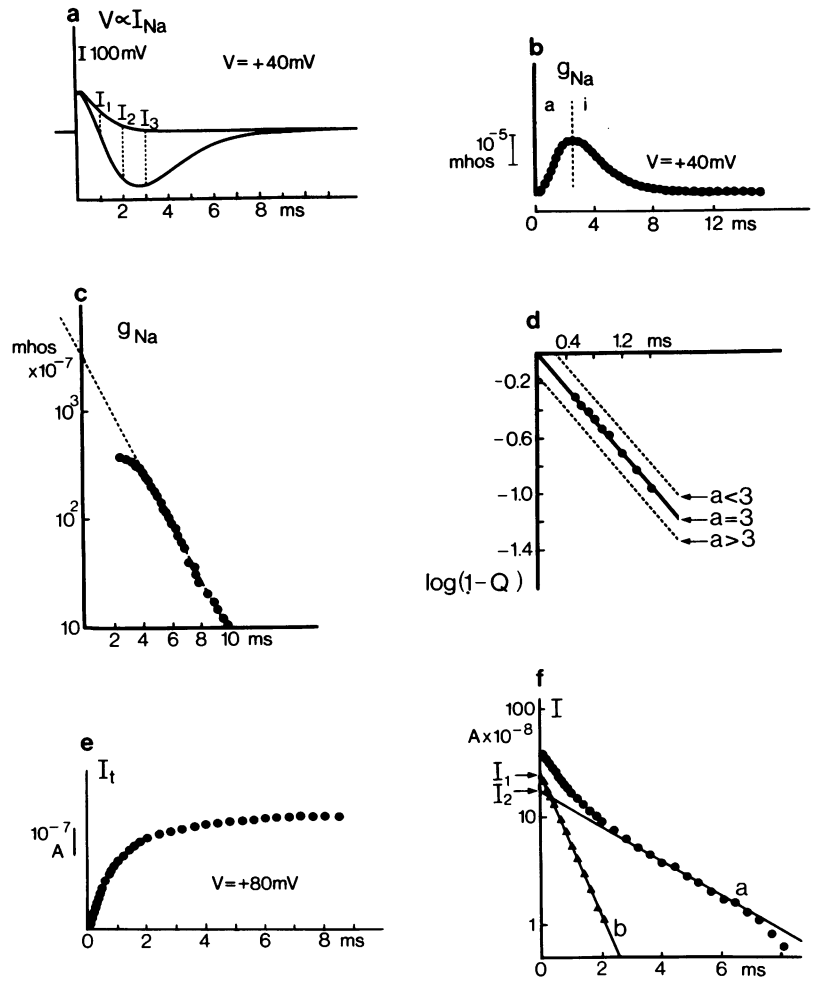

Figure 9 Calculation method used for ionic conductances and related parameters. (a) To obtain the sodium current values two current tracings obtained for a 40 mV depolarizing pulse were superimposed. The horizontal line on the left of the y-axis is the zero value of the current. Time zero corresponds to the beginning of the depolarizing pulse. Values I_1, I_2, $I_3 \ldots I_n \ldots$ are obtained from the subtraction of the two superimposed current curves (upper curve being obtained when the current is inhibited). (b) g_{Na} time course. The values calculated for g_{Na} are plotted against time; time zero is the beginning of the stimulating pulse; a='activation phase', i='inactivation phase'. (c) Determination of g'_{Na} and $1/\tau_h$. As explained in the text the values of g_{Na} during the inactivation phase (b) are plotted in (c) on a semi-logarithmic scale. A linear regression on the aligned points enables the values of $-1/\tau_h$ to be obtained. The value of the ordinate at the origin gives g'_{Na}. (d) Determination of exponent 'a' of the activation variable m. Values of 'a' higher than 3 and values of 'a' below 3 lead to lines which do not pass through the origin. In (d) only a=3 allows a straight line to pass through the origin; therefore the value a=3 was taken for the following calculations concerning this experiment. The results shown in (d) come from those of (a), (b) and (c). (e) The values of the total delayed outward current I_t obtained for a depolarizing pulse of +80 mV are plotted against time. Time zero corresponds to the beginning of the stimulating pulse. (f) The values of current (I) obtained by subtraction of each of the experiment values from the maximum value of I_t (e) are plotted against time. Two linear regressions allow the determination of I_1 and I_2 and the slopes $-1/\tau_{n1}$ and $-1/\tau_{n2}$ of the two individualized components.

following conditions $m=m_0$ and $h=h_0$ at $t=0$, are:

$$m = m_\infty - (m_\infty - m_0)\exp(-t/\tau_m) \quad (5)$$

where $m_\infty = \alpha_m/(\alpha_m+\beta_m)$ and $\tau_m = 1/(\alpha_m+\beta_m)$ = activation time constant and

$$h = h_\infty - (h_\infty - h_0)\exp(-t/\tau_h) \quad (6)$$

where $h_\infty = \alpha_h/(\alpha_h+\beta_h)$ and $\tau_h = 1/(\alpha_h+\beta_h)$ = inactivation time constant.

At rest, that is at time $t=0$ and before ($t=0$ is the beginning of the depolarizing pulse), g_{Na} is very small and it can be considered negligible if it is compared with the g_{Na} value reached during a large depolarization (so $m_0=0$). Then we have:

$$m = m_\infty - m_\infty \exp(-t/\tau_m) = m_\infty(1-\exp(-t/\tau_m))$$

At time $t=\infty$, inactivation can be considered nearly complete and g_{Na} is negligible, so h_∞ is taken as zero; thus, $h = h_0 \cdot \exp(-t/\tau_h)$ and equation (2) becomes:

$$g_{Na} = \overline{g_{Na}} \cdot [m_\infty(1-\exp(-t/\tau_m))]^a \cdot \cdot [h_0 \cdot \exp(-t/\tau_h)]^i \quad (7)$$

Taking

$$g'_{Na} = \overline{g_{Na}} \cdot m_\infty^a \cdot h_0^i \quad (8)$$

the relation (7) therefore becomes:

$$g_{Na} = g'_{Na}(1-\exp(-t/\tau_m))^a \cdot \exp(-t/\tau_h) \quad (9)$$

g'_{Na} represents the value that g_{Na} would be able to reach if inactivation would be maintained at its resting level h_0 (in absence of inactivation). After the maximum value of g_{Na}, $\exp(-t/\tau_m)$ tends towards 0 and that carries $1-\exp(-t/\tau_m)$ near 1, so g_{Na} becomes:

$$g_{Na} = g'_{Na} \cdot \exp(-t/\tau_h) \quad (10)$$

With equation (10) it became easy to determine the values of τ_h and g'_{Na} by plotting the values of g_{Na} during the inactivation phase of g_{Na} on semi-logarithmic paper (Figure 9c). The slope of the straight line determined by means of a linear regression is $-1/\tau_h$ and the value of the ordinate at the origin gives g'_{Na}. By doing such calculations for all the depolarization values applied to the preparation during the experiment it was possible to draw the curves $g'_{Na}=f(V)$ and $1/\tau_h=f(V)$. Since g'_{Na} is known it became possible to obtain a number proportional to m_∞ from equation (8) where g_{Na} is a constant. During all the experiments h_0 was a constant because between depolarizing pulses the resting potential was constant. Taking $\overline{g_{Na}} \cdot h_0 = K$, then $m_\infty^a = g'_{Na}/K$, $m_\infty \alpha \sqrt[a]{g'_{Na}}$. Therefore, it was possible to draw the curve $m_\infty = f(V)$. 'a' and 'i' are the exponents of the activation and inactivation variables. The experiments showed that 'i' had the value 1. It was necessary to determine the values of 'a' which could account for the activation function. From equation (9) can be obtained:

$$-t/\tau_m = \log[1-(g_{Na}/g'_{Na} \cdot \exp(t/\tau_h))^{1/a}]$$

Taking $Q = (g_{Na}/g'_{Na} \cdot \exp(t/\tau_h))^{a-1}$ the relation becomes:

$$(-1/\tau_m) \cdot t = \log(1-Q) \quad (11)$$

which is an equation of a straight line passing through the origin,

where $$\tau_m = -t/\log(1-Q) \quad (12)$$

So if 'a' is accurately determined the curve $\log(1-Q)=f(t)$ is a straight line crossing the ordinate at the origin as can be seen for 'a'=3 in the case of the experimental points shown in Figure 9d.

More, equation (12) allows the calculation of τ_m and then the curve $1/\tau_m=f(V)$ can be constructed. If the values of α_m and β_m are needed they can be calculated from $\alpha_m = m_\infty/\tau_m$ and $\beta_m = (1-m_\infty)/\tau_m$.

Outward current and delayed conductance

The outward current was assumed to be the sum of two exponential functions of time as did Noble & Tsien (1969a) for their i_{x1} and i_{x2} currents elicited in the plateau range of potentials in cardiac Purkinje fibres. The currents recorded here were identical with the first and second components of current studied by Brown & Noble (1969a) in frog atrial muscle. The equations used were derived from those used by Hodgkin & Huxley (1952c) for their study of g_K on nerve. The total outward current was recorded for various values of depolarizing pulses. An example of total outward current obtained with a +80 mV pulse is drawn against time in Figure 9(e) (similarly the tail currents obtained at the end of a stimulating pulse were drawn). For each value of time, subtraction of the experimental values of current from the maximum value of I_t (the value at the end of the pulse) gives the values of current I which were plotted on a semi-logarithmic scale as in Figure 9(f). A straight line (a) passing through experimental points and determined by a linear regression, allowed to determine the time constant τ_{n2} of the delayed system of slow kinetics and to determine the ordinate at the origin I_2. Then the values of the points situated on the straight line (a) were subtracted from the corresponding experimental values. This led to another series of points and another linear regression was used to determine the position of a second straight line (b) crossing the y-axis at the value I_1. From straight line (b) it was possible to determine the time constant τ_{n1} of the delayed system of fast kinetics. With the reversal potential of the i_{x1} and i_{x2} components measured during the experiment it was easy to calculate the g_{x1} and g_{x2} conductance values from the following equations:

$$g_{x1} = i_{x1}/(V-V_{x1}) \quad \text{and} \quad g_{x2} = i_{x2}/(V-V_{x2}) \quad (13)$$

where V_{x1} and V_{x2} are the reversal potentials of i_{x1} and i_{x2}, and V the imposed potential. The determination of the activation time constants τ_{n1} and τ_{n2} of the two conductances allowed the curves $1/\tau_{n1}=f(V)$ and $1/\tau_{n2}=f(V)$ to be drawn.

Now considering one of the two delayed systems g_{x1} or g_{x2} (that is in general g_x) we made the assumption that:

$$g_x = \overline{g_x} \cdot n^b \qquad (14)$$

and

$$dn/dt = \alpha_n(1-n)-\beta_n \cdot n \qquad (15)$$

where $\overline{g_x}$ is a constant, b has the value 1 (a value of one fits the experimental points well), n is the activation variable and it varies between 0 and 1. n would represent the proportion of particles (molecules) in state α and $(1-n)$ would represent the proportion of particles in a β state.

During a depolarizing pulse time varies from t_0 to t_∞ (time at the end of the pulse) and n varies from n_0 to n_∞. The solution of equation (15) is:

$$n = n_\infty-(n_\infty-n_0)\exp(-t/\tau_n) \qquad (16)$$

where $n_\infty = \alpha_n/(\alpha_n+\beta_n)$ and $\tau_n = 1/(\alpha_n+\beta_n)$ and $\alpha_n = n_\infty/\tau_n$ and $\beta_n = (1-n_\infty)/\tau_n$.

The n_∞ values used to determine α_n and β_n are determined from the maximal values of the g_{x1} and g_{x2} conductances determined on semi-logarithmic paper (ordinates at the origin).

The activation curves $i'_{x1}=f(V)$ and $i'_{x2}=f(V)$ were obtained from tail current recordings. The tail currents were plotted on semi-logarithmic scales in a similar manner to Figure 9(f). The i'_{x1} and i'_{x2} values correspond to values determined like I_1 and I_2 (Figure 9f) but obtained from the tail currents (deactivation currents). The ordinates at the origin (i'_{x1} and i'_{x2}) would be representative of the initial state of the deactivation of the delayed current and would be proportional to the activation state n_∞ (at the steady state) of the current components obtained during the preceding stimulus (Noble & Tsien, 1969a).

References

ANGELAKOS, E.T. & HASTINGS, E.P. (1960). The influence of quinidine and procaine amide on myocardial contractility *in vivo*. *Amer. J. Cardiol.*, **5**, 791–798.

BESSEAU, A. (1971). Analyse, selon le modèle de Hodgkin–Huxley, des conductances de la membrane myocardique de grenouille (*Rana esculenta*). *Thèse de Doctorat ès-Sciences, Poitiers* (CNRS: A.O. 5839).

BESSEAU, A. (1972). Analyse, selon le modèle de Hodgkin–Huxley, des conductances membranaires du myocarde de grenouille (*Rana esculenta*). *J. Physiol., Paris,* **64**, 647–670.

BESSEAU, A. & GARGOUÏL, Y.M. (1969). Ionic currents in rat ventricular heart fibres: voltage clamp experiments using double sucrose gap technique. *J. Physiol., Lond.*, **204**, 95–96P.

BROWN, H.F. & NOBLE, S.J. (1969a). Membrane currents underlying delayed rectification and pacemaker activity in frog atrial muscle. *J. Physiol., Lond.*, **204**, 717–736.

BROWN, H.F. & NOBLE, S.J. (1969b). A quantitative analysis of the slow component of delayed rectification in frog atrium. *J. Physiol., Lond.*, **204**, 737–747.

GOODFORD, P.J. & VAUGHAN WILLIAMS, E.M. (1962). Intracellular Na and K concentrations of rabbit atria, in relation to the action of quinidine. *J. Physiol., Lond.*, **160**, 483–493.

HAUSWIRTH, O., NOBLE, D. & TSIEN, R.W. (1969). The mechanism of oscillatory activity at low membrane potentials in cardiac Purkinje fibres. *J. Physiol., Lond.*, **200**, 255–265.

HEMPTINNE, A. de (1971). The frequency dependence of outward current in frog auricular fibres. An experimental and theoretical study. *Pflügers Arch.*, **329**, 332–340.

HODGKIN, A.L. & HUXLEY, A.F. (1952a). The components of membrane conductance in the giant axon of *Loligo*. *J. Physiol., Lond.*, **116**, 473–496.

HODGKIN, A.L. & HUXLEY, A.F. (1952b). The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol., Lond.*, **116**, 497–506.

HODGKIN, A.L. & HUXLEY, A.F. (1952c). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol., Lond.*, **117**, 500–544.

JOHNSON, E.A. & LIEBERMAN, M. (1971). Heart: excitation and contraction. *Ann. Rev. Physiol.*, **33**, 479–532.

LENFANT, J., MIRONNEAU, J. & AKA, J.K. (1972). Activité répétitive de la fibre sino-auriculaire de grenouille, analyse des courants membranaires responsables de l'automatisme cardiaque. *J. Physiol., Paris,* **64**, 5–18.

LÉOTY, C. & POINDESSAULT, J.P. (1974). Effects and compensation of the series resistance in voltage-clamp experiments using double surcrose-gap technique. *J. Physiol., Lond.*, **239**, 108–109P.

LÉOTY, C., RAYMOND, G. & GARGOUÏL, Y.M. (1970). Tension phasique et tonique de la fibre myocardique de grenouille et less courants ioniques transmembranaires; étude en voltage imposé et par microphotométrie. *C. R. Acad. Sci., Paris,* **271**, 1545–1548.

McALLISTER, R.E. & NOBLE, D. (1966). The time and voltage dependence of the slow outward current in cardiac Purkinje fibres. *J. Physiol., Lond.*, **186**, 632–662.

McALLISTER, R.E. & NOBLE, D. (1967). The effect of subthreshold potentials on the membrane current in cardiac Purkinje fibres. *J. Physiol., Lond.*, **190**, 381–387.

NOBLE, D. (1962). A modification of the Hodgkin–Huxley equations applicable to Purkinje fibre action and pacemaker potential. *J. Physiol., Lond.*, **160**, 317–352.

NOBLE, D. (1966). Application of Hodgkin–Huxley equations to excitable tissues. *Physiol. Rev.*, **46**, 1–50.

NOBLE, D. & TSIEN, R.W. (1969a). Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. *J. Physiol., Lond.*, **200**, 205–231.

NOBLE, D. & TSIEN, R.W. (1969b). Reconstruction of the repolarization process in cardiac Purkinje fibres based on voltage clamp measurements of membrane currents. *J. Physiol., Lond.*, **200**, 233–254.

PARMLEY, W.W. & BRAUNWALD, E. (1967). Comparative myocardial depressant and antiarrhythmic properties of *d*-propranolol, *dl*-propranolol and quinidine. *J. Pharmac. exp. Ther.*, **158**, 11–21.

POINDESSAULT, J.P., DUVAL, A. & LÉOTY, C. (1975). Voltage clamp with double sucrose gap technique: external series resistance compensation. *Biophys. J.* (in press).

RAMÓN, F., ANDERSON, N., JOYNER, R.W. & MOORE, J.W. (1975). Axon voltage clamp simulations. IV. A multicellular preparation. *Biophys. J.*, **15**, 55–69.

REUTER, H. (1974). Localization of beta-adrenergic receptors, and effects of noradrenaline and cyclic nucleotides on action potentials, ionic currents and tension in mammalian cardiac muscle. *J. Physiol., Lond.*, **242**, 429–451.

ROUGIER, O., ILDEFONSE, M. & GARGOUÏL, Y.M. (1966). Application de la technique du double 'sucrose gap' à l'étude électrophysiologique du muscle cardiaque. *C. R. Acad. Sci., Paris*, **263**, 1482–1485.

ROUGIER, O., VASSORT, G. & STÄMPFLI, R. (1968). Voltage clamp experiments on frog atrial heart muscle fibres with the sucrose gap technique. *Pflügers Arch.*, **301**, 91–108.

ROUGIER, O., VASSORT, G., GARNIER, D., GARGOUÏL, Y.M. & CORABOEUF, E. (1969). Existence and role of a slow inward current during the frog atrial action potential. *Pflügers Arch.*, **308**, 91–110.

SZEKERES, L. & PAPP, J.Gy (1971). In *Experimental Cardiac Arrhythmias and Antiarrhythmic Drugs*. Budapest: Akadémiai Kiadó.

TARR, M. & TRANK, J.W. (1974). An assessment of the double sucrose gap voltage clamp technique as applied to frog atrial muscle. *Biophys. J.*, **14**, 627–643.

THORPE, W.R. (1973). Some effects of caffeine and quinidine on sarcoplasmic reticulum of skeletal and cardiac muscle. *Can. J. Physiol. Pharmac.*, **51**, 499–503.

TRAUTWEIN, W. (1963). Generation and conduction of impulse in the heart as affected by drugs. *Pharmacol. Rev.*, **15**, 277–332.

VAUGHAN WILLIAMS, E.M. (1958). The mode of action of quinidine in isolated rabbit atria interpreted from intracellular records. *Br. J. Pharmac. Chemother.*, **13**, 276–287.

VAUGHAN WILLIAMS, E.M. (1961). The action of quinidine, acetylcholine, and anaphylaxis interpreted from simultaneous records of contractions and intracellular potentials in the heart. In *The Scientific Basis of Medicine, Annual Review*, **19**, 302–323. Bristol: Western Printing Services Ltd.

VAUGHAN WILLIAMS, E.M. (1973). Progrès dans la connaissance des nouveaux médicaments antidisrythmiques. *Ann. Cardiol. Angéiol.*, **22**, 1–12.

WALDEN, M., KRÉHER, P., AKA, K.J. & TRICOCHE, R. (1973). Activité électrique et courants ioniques transmembranaires de la fibre myocardique de singe (famille des Cercopithécidés). *J. Physiol., Paris*, **66**, 455–472.

WEIDMANN, S. (1955). Effects of calcium ions and local anesthetics on electrical properties of Purkinje fibres. *J. Physiol., Lond.*, **129**, 568–582.

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