Br. J. Pharmac. (1980) **71**, 41–49

EFFECTS OF ERVATAMINE CHLORHYDRATE ON CARDIAC MEMBRANE CURRENTS IN FROG ATRIAL FIBRES

MARTIN-PIERRE SAUVIAT

Laboratoire de Physiologie Comparée et de Physiologie Cellulaire associé au CNRS, Université de Paris XI, 91405, ORSAY, Cedex, France

1 The effects of a new alkaloid, ervatamine, on transmembrane currents of frog atrial fibres were studied by the double sucrose gap voltage clamp technique.

2 Ervatamine (2.8×10^{-4} M) blocked the action potential without altering the resting membrane potential.

3 The alkaloid depressed the peak I_{Na}. The dissociation constant for the blocking effect of ervatamine on $g_{Na\,fast}$ was 2.35×10^{-5} M with a one to one relationship between the drug molecule and the Na channel. Ervatamine did not alter the apparent equilibrium potential for Na, as well as the activation and inactivation parameters of $g_{Na\,fast}$. This suggests that the alkaloid inhibitory effect on g_{Na} can be attributed to a reduction in $\bar{g}_{Na}$.

4 Ervatamine prolonged the rate of reactivation of the Na system. It inhibited g_{Na} in a frequency-dependent manner; this indicates that the alkaloid acts on open Na channels i.e. that the drug has to enter the channel or cross the membrane to produce the block.

5 Ervatamine inhibited $I_{Na\,slow}$ which occurs in Ca-free, tetrodotoxin-containing solutions and moderately decreased I_{Ca} which occurs in Na-free solutions. The drug increased the background K current (I_{K_1}) and did not alter the time-dependent K current (I_{x_1}).

6 The present study shows that ervatamine is a good inhibitor of both fast and slow g_{Na}. This drug also shares some common electrophysiological properties with antiarrhythmic drugs namely: the frequency-dependent inhibition of the fast g_{Na} and the ability to slow the reactivation of the Na carrying system.

Introduction

Ervatamine (Figure 2e) is an α acyl-indolic alkaloid first isolated by Knox & Slobbe (1971) from an Australian tree, *Ervatamia orientalis*, and then synthesized by Husson, Bannai, Mompon, Freire & Reis, (1978). Very little is known about the pharmacological properties of the alkaloid. In the isolated giant axon of the cockroach (Sauviat & Pichon, 1977) and in the squid giant axon (Pichon & Sauviat, 1978a, b), the main effect of ervatamine is to block Na conductance. The inhibition developed by the alkaloid does not affect the activation (m) or inactivation (h) gating parameters of the Na conductance. The block occurs in a frequency-dependent manner. Unlike tetrodotoxin (TTX), ervatamine is more effective from the axoplasmic side of the membrane. The effects of the drug are similar to those of local anaesthetics. The aim of the present paper was to extend the analysis of the action of ervatamine to cardiac ionic currents, by means of the double sucrose gap technique applied to frog atrial fibres (Rougier, Vassort & Stämpfli, 1968). This preparation has a fast, TTX-sensitive, Na conductance and a slow, TTX-insensitive, Na conductance that develops in the absence of Ca ions (Rougier, Vassort, Garnier, Gargouïl & Coraboeuf, 1969; Garnier, Rougier, Gargouïl & Coraboeuf, 1969). In the present paper, ervatamine was found to block both types of Na conductance, whereas the slow Ca conductance was little altered. A preliminary report on part of this work has already been published (Sauviat, 1977).

Methods

Current and voltage clamp experiments were performed at 12 to 18°C on fine atrial trabeculae (75 to 150 μm in diameter, 2 to 4 mm in length) isolated from hearts of *Rana esculenta*. The double sucrose

0007-1188/80/130041-09 $01.00

gap technique with vaseline seals was used (Rougier *et al.*, 1968). In some experiments an iso-osmolar concentration of mannitol was substituted for sucrose and gave identical or better results (see below). The composition of the Ringer solutions was (mM): NaCl 110.5, KCl 2.5, $CaCl_2$ 1.8, the pH was maintained at 7.6 either with $NaHCO_3$, 2.4 mM or Tris-HCl buffer, 5 mM. Ca-free solutions were made by omitting $CaCl_2$ from the standard Ringer solution. This gave Ca concentrations lower than 10^{-6} M as measured by a photometric method. In Na-free solutions, NaCl was replaced by mannitol. In some experiments, TTX (5.7×10^{-7} M), a specific inhibitor of the fast Na conductance, and manganese ($MnCl_2$, 5 mM), an inhibitor of the slow Ca and Na conductance, were added to the Ringer solution. Ervatamine chlorhydrate was readily soluble in water. Preparations were stimulated with square wave pulses at a rate of 0.1 Hz. Dose-response curves were calculated by the following modified Langmuir equation (Yeh & Narahashi, 1976):

$$Y = Y_{max}(X)^n/(K_d + (X)^n) \quad (1)$$

where Y is the percentage inhibition of ionic current, X the concentration of ervatamine, n the stoichiometric parameter, and K_d the dissociation constant, Y_{max} being taken as 100%.

Limitation of the method

The double sucrose gap technique as applied to frog atrial trabeculae is subject to some uncertainties, mainly attributable to the complexity of the cell membrane geometry of a multicellular preparation. The existence of a series resistance (R_s) introduces a spatial and temporal non-uniformity of the membrane potential applied to the fibre during the flow of the fast inward Na current. The drop in voltage resulting from this non-uniformity always introduces some distortions in the amplitude and the time course of the current. The quality of the clamp control was checked during the flow of the fast I_{Na}, in the present experimental conditions, by means of a microelectrode system independent of the voltage clamp system itself. The membrane potential (V_m) was measured with high resistance microelectrodes (50 to 60 MΩ; tip potential ≤ 5 mV) impaled in the middle part of the fibre lying in the test compartment. Figure 1a shows that V_m deviated from the clamp potential (V_c) by 6, 14 and 9 mV, respectively; these values are close to those recorded with a similar sucrose gap method using vaseline seals by Benninger, Einwächter, Haas & Kern (1976), de Hemptine (1976) and Horackova, Shrier & Vassort (1977). The results obtained here show, that in a good preparation, the voltage drop

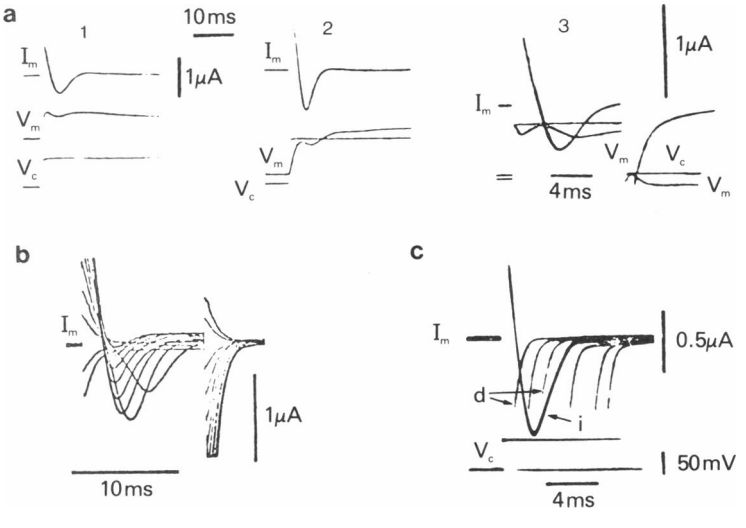

Figure 1 (a) Control of the membrane potential using the double sucrose gap technique during the flow of the fast inward Na current elicited in Ringer solution by a 30 mV (1), 46 mV (2) and 53 mV (3) depolarizing command potential (V_c). V_m: transmembrane potential recorded by intracellular microelectrode system; I_m: membrane current. V_m was very close to the holding potential value (60 mV in (1); 70 mV (2) and (3)). (1, 2 and 3 different fibres). (b) Family of fast inward currents elicited in Mn-containing (5 mM) Ringer solution by depolarizing pulse (V_c) from 10 to 120 mV. (c) Deactivation test of the fast inward current recorded in Mn-containing (5 mM) Ringer solution with a 65 mV depolarizing clamp pulse (V_c). The time course of deactivation (traces marked d) is obviously faster than that of inactivation (trace i). Downward deflections correspond to inward current.

across R_s during the flow of the maximum fast I_{Na} did not exceed 15 mV.

Experimental procedure

All trabeculae were submitted to the following experimental procedure. Action potentials were first recorded across each sucrose gap. The fibre was used for further investigations when the action potential (AP) amplitudes exceeded 90 mV, indicating a short-circuiting factor of 0.8 or more for both gaps. In voltage clamp conditions, the family of peak I_{Na} (which generally develops between 20 to 120 mV) was elicited in order to detect eventual notches or double peaks which develop particularly on the falling phase of the current in badly or poorly clamped fibres. This generally occurs in the negative slope region of the I.V. curves. According to Johnson & Lieberman (1971), the steepness of the negative slope region and the non-linearity of the positive slope region of the I.V. curve are indications of the spatial non-uniformity of the control of the membrane potential. Therefore a check was made that the membrane potential at which $I_{Na\ fast}$ reached its maximum was at least 40 mV less positive than the resting potential and that the I.V. curve was almost linear in the positive slope region. I also checked that the time to peak of I_{Na} ranged from about 7 ms for a depolarizing step of 30 mV to about 3 ms or less at 80 or 100 mV. The inactivation time constant of the current τ_h was about 1 ms (Figure 4b). The test of deactivation of $I_{Na\ fast}$ (Figure 1c) was used routinely (de Hemptine, 1976). This consists of fully activating $I_{Na\ fast}$ and resetting the membrane potential at its resting level at a time when the Na channel is still far from being inactivated. If at that time, the membrane is properly under control, the deactivation of the Na channel (closure of m gate) must be faster than the inactivation process (closure of h gate). These conditions ensured that only reasonably well clamped fibres were used.

Current measurements

The fast inward Na current ($I_{Na\ fast}$) and the outward K currents were measured as net inward or outward currents respectively if not otherwise specified (Chesnais, Coraboeuf, Sauviat & Vassas, 1975). The slow inward current (I_{slow}) recorded in TTX-containing Ringer solution and the slow inward Ca current (I_{Ca}) recorded in Na-free solution were measured according to the method previously described by Horackova & Vassort (1976). The slow inward Na current ($I_{Na\ slow}$) recorded in TTX-containing, Ca-free solution was measured as net inward current, the method used for measuring I_{slow} being not easily applicable to currents like $I_{Na\ slow}$ with a very slow inactivation process. In current–voltage relationships (I.V. curves), outward currents correspond to positive currents, and positive potentials to depolarizations from the resting potential.

Results

Action potential

The effect of ervatamine chlorhydrate (2.8×10^{-4} M) on the AP was a decrease in the fast initial depolarizing phase and in the plateau amplitude, with a shortening of the AP duration (Figure 2a). Ervatamine did not change the resting membrane potential. The effects of ervatamine were reversible.

Fast inward Na current ($I_{Na\ fast}$)

In order to study $I_{Na\ fast}$, voltage clamp experiments were carried out either in the absence or in the presence of Mn ions known to inhibit I_{slow} (Coraboeuf & Vassort, 1968) and also only slightly to alter the magnitude of $I_{Na\ fast}$ (Rougier *et al.*, 1969). The addition of ervatamine (2.8×10^{-4} M) to the control solution completely blocked $I_{Na\ fast}$ within 4 min (Figure 2b); the decrease of the peak current occurred without noticeable alteration of the current time course. The effects of the alkaloid on $I_{Na\ fast}$ were independent of Mn ions. After the alkaloid effect on the peak current was complete (Figure 2c), further addition of TTX (5.7×10^{-7} M concentration, known to inhibit completely $I_{Na\ fast}$ in less than 30 s) to the ervatamine-containing solution did not exert any detectable effects. This indicates that ervatamine had entirely blocked $I_{Na\ fast}$. The inhibitory effect of ervatamine was partially reversible; the maximum peak inward current recovered to $77.5 \pm 21.1\%$ ($n = 8$) of the initial control value after 10 min. The effects of lower concentrations of ervatamine were investigated (Figure 2d). The value of the maximum inward currents was reduced by 15% and 55% in the presence of ervatamine 2.8×10^{-6} M and 2.8×10^{-5} M respectively. Ervatamine did not alter the membrane potential at which $I_{Na\ fast}$ reached its maximum value or the apparent Na equilibrium potential (i.e. the membrane potential at which the current curve crosses the abscissa). For each drug concentration, the current ratios (ervatamine-treated/control) did not change over the entire range of membrane potentials investigated.

Dose-response relationship The dose-response curve for the inhibitory effect of ervatamine on sodium conductance is shown in Figure 3. The Hill plot of the data [(log % inhibition)/(100 − % inhibition) vs log ervatamine concentration] gave a stoichiometric parameter value close to unity ($n = 1.15 \pm 0.22$), suggesting a one to one relationship between the ervatamine

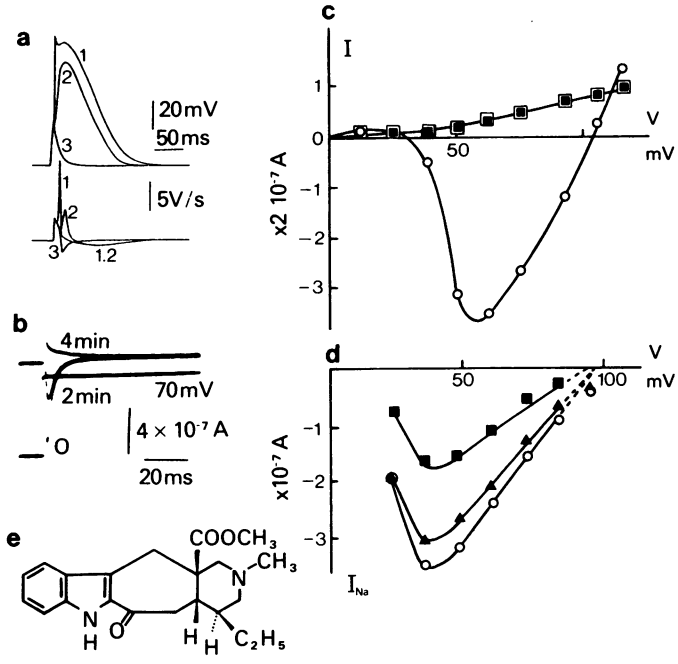

Figure 2 (a) Effects of ervatamine (2.8×10^{-4} M) on the action potential (upper traces) and dV/dt_{max} (lower traces) recorded in Ringer solution (1) and during ervatamine treatment: after 2 min 30 s in (2) and 4 min in (3). (b) Superimposed records of the peak Na current recorded in Mn-containing (5 mM) Ringer solution (0) and after 2 and 4 min of ervatamine (2.8×10^{-4} M) treatment. (c) Current-voltage relationships of the peak inward Na current in (○) Mn-containing (5 mM) Ringer solution; (■) ervatamine (2.8×10^{-4} M) containing control solution. The curve indicated by open squares was recorded after addition of tetrodotoxin (5.7×10^{-7} M) to the ervatamine-containing solution. (d) Effects of low ervatamine concentrations: (▲) 2.8×10^{-6} M and (■) 2.8×10^{-5} M on the fast Na current recorded in Mn-containing (5 mM) Ringer solution (○). I.V. curves (plotted on the same preparation) were obtained by substracting the peak current magnitude measured in the tested solutions from the current magnitude measured at the peak time after ervatamine (2.8×10^{-4} M) treatment. (e) Chemical structure of ervatamine.

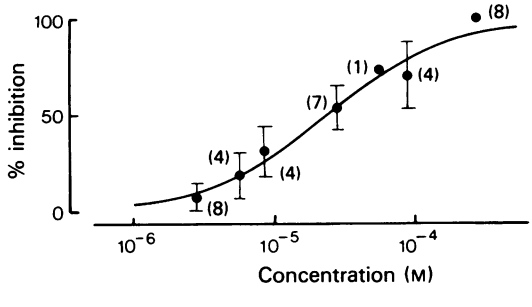

Figure 3 Log concentration-response relationships of the effects of ervatamine (abscissa scale) on the fast Na current amplitude (ordinates). The theoretical concentration-response curve which fits the experimental data was drawn according to equation (1) with $n = 1$ and $K_d = 2.35 \times 10^{-5}$ M. The number beside each point represents the number of experiments performed; vertical lines represent s.d.

molecule and the Na channel. The apparent dissociation constant was estimated to be: $K_d = 2.35 \pm 0.66 \times 10^{-5}$ M. n and K_d are average values ± standard deviation calculated from seven fibres.

Sodium activation and inactivation The effects of ervatamine on the activation (m) and inactivation (h) parameters controlling the Na conductance in the Hodgkin–Huxley theory (1952) were determined by using an ervatamine concentration of 2.8×10^{-5} M which inhibits $I_{Na\,fast}$ by about 50%. The time required for the peak current to reach one half of its maximum value ($T_{1/2}$) was measured as a function of the membrane potential and used as an index of the activation parameter (m) (Takata, Moore, Kao & Fuhrman, 1966). Figure 4(a) shows that the value of $T_{1/2}$ was not modified by ervatamine. Similarly, the voltage-dependence of the time constant, τ_h, associated with the falling phase of $I_{Na\,fast}$ was not altered by erva-

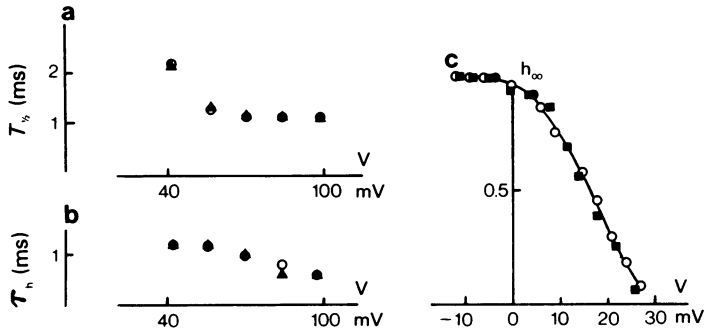

Figure 4 Effects of ervatamine (2.8×10^{-5} M) on the activation and inactivation parameters of the fast Na conductance. (a) Comparison of the time needed for $I_{Na\,fast}$ to reach one half of its peak value ($T_{1/2}$) in Mn-containing (5 mM) Ringer solution (○) and after ervatamine treatment (▲). (b) Comparison of the inactivation time constant of $I_{Na\,fast}$ (τ_h) measured (using the semi-logarithmic plot method) in Mn-containing (5 mM) Ringer solution (○) and after ervatamine treatment (▲) (a and b same preparation). (c) Curve for the steady state Na inactivation h_∞ in Mn-containing (5 mM) Ringer solution (○) and after ervatamine treatment (■). The ordinate scale gives the relative magnitude of $I_{Na\,fast}$ which develops with a 48 mV depolarizing test pulse. Abscissa scale: membrane potential during the conditioning pulses.

tamine (Figure 4b). Figure 4(c) shows that the steady state inactivation curve (h_∞) versus the membrane potential was not changed by ervatamine.

Sodium reactivation The rate of the Na system reactivation (removal of inactivation) was measured by use of a double pulse arrangement. Ervatamine (2.8×10^{-5} M) decreased the rate of reactivation of $I_{Na\,fast}$ (Figure 5a). The reactivation time constant (τ_{re}), measured by the method described by Haas, Kern, Einwächter & Tarr (1971), was increased by the alkaloid. In Figure 5(a) τ_{re} was 51.5 ms before and 121 ms during drug application.

Frequency-dependence Figure 5(b) shows that the degree of inhibition of $I_{Na\,fast}$ by ervatamine was dependent on the frequency of stimulation. In the presence of ervatamine (2.8×10^{-5} M), the peak current was markedly decreased when the rate of stimulation increased, whereas, in the absence of ervatamine, only a small dependence of $I_{Na\,fast}$ on the stimulation rate was observed.

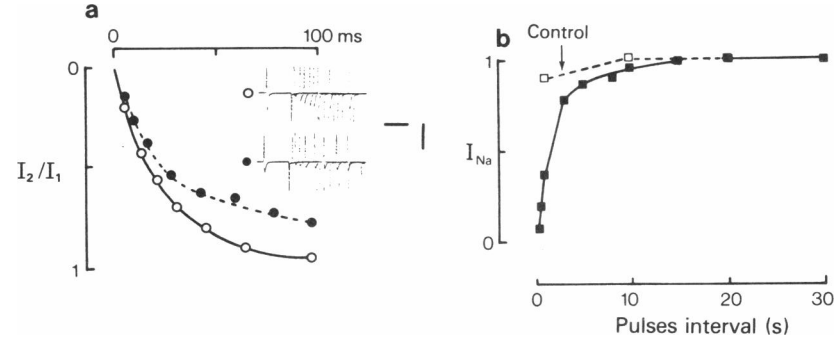

Figure 5 (a) Time course for recovery of the peak I_{Na} from inactivation in Mn-containing (5 mM) Ringer solution (○) and after ervatamine (2.8×10^{-5} M) treatment (10 min) (●). The inset illustrates the method and shows currents recorded in both conditions. Two successive depolarizations of equal amplitude and duration (60 mV from the holding potential of −60 mV; 40 ms) were imposed on the membrane. I_1 and I_2 were respectively the peak current associated with the first and the second depolarization. The ratio I_2/I_1 was plotted as a function of the time interval. Horizontal scale: 20 ms; vertical scale 10^{-7} A. (b) Effect of increasing the rate of stimulation on the peak I_{Na} elicited by a 50 mV (40 ms duration) depolarizing clamp step in a fibre bathing in a Mn-containing (5 mM) Ringer solution before (□) and after 4 min ervatamine (2.8×10^{-5} M) treatment (■). I_{Na} was recorded after 1 min of stimulation at each rate; each sequence was followed by a sequence where the fibre was driven for 2 min at 6 pulses per minute. I_{Na} is expressed in relative units ($I_{Na} = 1$ when the stimulation rate remains without effect).

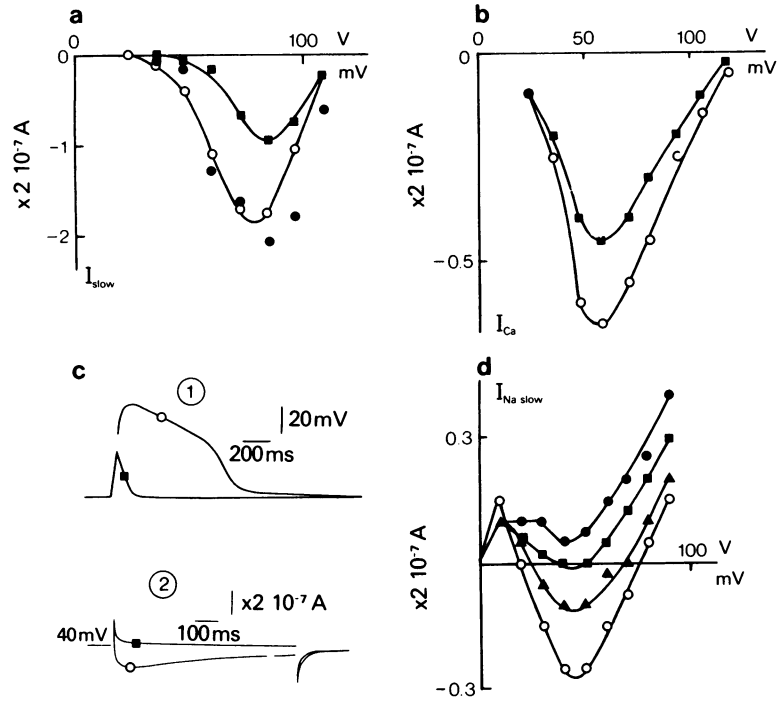

Figure 6 Effects of ervatamine (2.8×10^{-4} M) on slow conductance. (a) I.V. curves of the slow Ca–Na current recorded in tetrodotoxin (TTX)-containing Ringer solution before (○) and during ervatamine treatment (■) and 10 min after removal of ervatamine from the TTX-containing solution (●). (b) I.V. curves of I_{Ca} measured in TTX-containing Na-free (mannitol) solution before (○) and after ervatamine application (■). (c1) Long lasting action potential recorded in TTX-containing Ca-free solution after recovery from ervatamine application (○) and during further alkaloid treatment (●). (c2) Superimposed records of $I_{Na\ slow}$ elicited by a 40 mV depolarizing pulse in a TTX-containing, Ca-free solution before (○) and after ervatamine application (●). (d) Effects of low ervatamine concentrations ((▲) 2.8×10^{-6} M); (■) 5.6×10^{-5} M; (●) 2.8×10^{-5} M) on the I.V. curve of $I_{Na\ slow}$ recorded in TTX-containing, Ca-free solution (○). ((a), (c) same fibre; different fibres in (a), (b) and (d)).

Slow inward Ca–Na and Ca currents (I_{slow} and I_{Ca})

The addition of ervatamine (2.8×10^{-4} M) to the TTX-containing Ringer solution decreased the magnitude of I_{slow} (Figure 6a). This decrease is more marked in the negative slope region of the I.V. curve than in the positive one; the current ratio (ervatamine treated/control) was depressed by 80% for a pulse of 60 mV whereas it was depressed by only 30% for a pulse of 96 mV. In Na-free, TTX-containing solution, ervatamine (2.8×10^{-4} M) decreases the magnitude of I_{Ca} by about 30% (Figure 6b). This effect was of the same magnitude whatever the amplitude of the depolarizing steps. The membrane potential at which I_{Ca} reached its maximum value (about 60 mV) was not shifted by the drug application as well as the apparent equilibrium potential for Ca ions. The ervatamine effect on the currents were reversible either in the presence or in the absence of Na ions.

Slow inward Na current ($I_{Na\ slow}$)

Figure 6(c2) shows a typical record of $I_{Na\ slow}$ which develops in TTX-containing, Ca-free solution; the current inactivated very slowly since it remains inward for more than 700 ms after the onset of the pulse. Addition of ervatamine (2.8×10^{-4} M) completely and reversibly suppressed $I_{Na\ slow}$. In the absence of or after recovery from the drug effect, long lasting action potentials could be initiated under current clamp conditions. These AP were markedly shortened by ervatamine (Figure 6c1). Further addition of Mn ions (3 mM) to the ervatamine-treated fibres no longer altered AP duration or current amplitude, indicating that ervatamine (2.8×10^{-4} M) completely inhibits $g_{Na\ slow}$. I.V. curves plotted in Figure 6(d) illustrated the effects of lower ervatamine concentrations on $I_{Na\ slow}$.

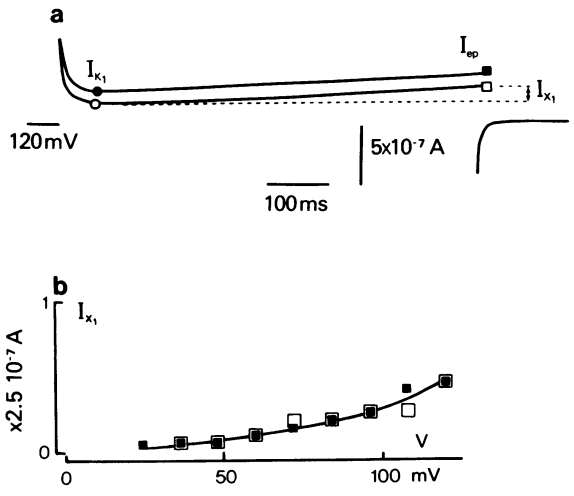

Figure 7 Effects of ervatamine (2.8×10^{-4} M) (filled symbols) on the outward current measured in tetrodotoxin (TTX) and Mn (8 mM)-containing Ringer solution (open symbols). (a) Superimposed record of the outward current: I_{K_1}, time-independent background current measured about 30 ms after the onset of the depolarizing pulses (○, ●); I_{ep}, outward current measured at the end of the depolarizing pulses (□, ■). I_{x_1}, time-dependent outward current measured by substracting I_{ep} from I_{K_1}. (b) I.V. curves for I_{x_1} measured in the absence (□) and in the presence of ervatamine (■).

Background and delayed K currents

The effects of ervatamine on the outward K currents were analysed in the absence of inward currents i.e. in a TTX and Mn (8 mM)-containing, Ringer solution. Figure 7(a) shows that both the magnitude of the time-independent outward background current, I_{K1}, and the magnitude of the outward K current measured at the end of depolarizing pulse (called I_{ep} for simplification) were increased after ervatamine (2.8×10^{-4} M) treatment. The difference, $I_{K_1} - I_{ep}$, represents the time-dependent outward current (termed I_{x_1} by Noble & Tsien (1969) and I_1 by Ojeda & Rougier (1974)). I.V. curves of Figure 7(b) show that ervatamine did not affect I_{x_1}.

Discussion

Since most of the results described here concern the inhibitory effect of ervatamine on the fast Na current, the question of the validity of the technique used to measure this current needs some comment. The influence of series resistances (R_s) has been studied by voltage clamp simulations in multicellular preparations (Ramon, Anderson, Joyner & Moore, 1975). The flow of the membrane current through R_s alters the voltage change across the membrane. The present results (Figure 1a) demonstrate that, in these experimental conditions, the change in V_m is indeed altered during the flow of $I_{Na\,fast}$ compared with V_c, but the maximum difference between V_m and V_c is hardly greater than 10 to 15 mV for depolarizations of 40 to 50 mV giving rise to maximal I_{Na}. Calculations by Ramon *et al.*, (1975, see Figure 2) clearly show that, due to R_s the $I_{Na\,fast}$ curve is shifted to the left along the voltage axis. Since this shift in amplitude depends on the magnitude of the current through R_s, it must decrease when g_{Na} decreases. For this reason, in a fibre with R_s large enough to induce a marked shift of the I.V. curve towards negative potentials when g_{Na} is large, a progressive inhibition of g_{Na} should progressively 'reset' the minimum of the I.V. curve towards positive potentials as a function of inhibition. The fact that this was not observed either with ervatamine (Figure 2d) or TTX (not illustrated), strongly suggests that the effect of R_s on the I.V. curve remains of moderate amplitude even at the largest values of g_{Na}, thus excluding major loss of control in our experimental conditions. Such a conclusion is particularly applicable to the slow inward current which had a much smaller amplitude.

Fast Na conductance

The main result described in the present paper is that ervatamine is a strong inhibitor not only of fast Na conductance but also of slow Na conductance. It is to my knowledge, the first example of a substance inhibiting these two different systems simultaneously. Inhibition of the fast Na channel has already been described in the giant axon of cockroach (Sauviat & Pichon, 1977) and in the squid giant axon (Pichon & Sauviat, 1978a, b). It is generally accepted that in cardiac tissue, as in the nerve, g_{Na} is governed by the product of a constant value, the maximum conductance $\bar{g}_{Na}$ and two kinetic factors m and h; m governs the activation process and h the inactivation process (Hodgkin & Huxley, 1952). The present experiments show that ervatamine does not modify the kinetic factors, since neither $T_{1/2}$ (Figure 4a) nor τ_h (Figure 4b) is changed by the drug and since the curve $h_\infty - E_m$ also remains unaltered (Figure 4c). Furthermore, the reduction in $I_{Na\,fast}$ brought about by ervatamine does not seem to be related to an alteration of the Na equilibrium potential (Figure 2d); ervatamine also reduces the maximum inward current without any appreciable shift of the membrane potential at which it occurs. These results show that the alkaloid only decreases $\bar{g}_{Na}$, an effect which is similar to that of TTX. However, some differences exist between the inhibitory effects of ervatamine and TTX on the fast Na channel; at the frequency used in the present work,

ervatamine (2.8×10^{-4} M) required 4 min or more to suppress $I_{Na\,fast}$ (Figure 2) instead of 30 s for TTX (5.7×10^{-7} M). The relatively long time required by the alkaloid to inhibit g_{Na} strongly suggests that ervatamine has to enter the membrane and diffuse towards its internal side before being effective on the fast Na channel. Moreover, ervatamine also inhibits $I_{Na\,fast}$ in a frequency-dependent manner (Figure 5b); this indicates that the blocking effect of the drug depends upon how often the Na channels are opened. This suggests that the drug has to penetrate into the channel before being effective. A similar conclusion has also been drawn from experiments performed in the squid giant axon (Pichon & Sauviat, 1978a, b). In this tissue, as in the frog atrial fibre, m and h gating mechanisms are not altered by ervatamine; the stoichiometric parameters have about the same value and the inhibition of g_{Na} occurs in a frequency-dependent manner. This last property is generally shared by local anaesthetics and antiarrhythmic drugs (Weidmann, 1955; Gettes & Reuter 1974; Hille, 1977). Ervatamine does not modify the inactivation time constant τ_h but increases the time constant of reactivation of the Na system τ_{re}. According to Haas *et al.* (1971), in frog atrial tissue τ_{re} is much longer than τ_h; a situation which is quite different from that observed in the squid giant axon membrane where τ_h and τ_{re} are similar.

Slow conductance

In Ca-free media, ervatamine also inhibits slow TTX-insensitive Na conductance (Figure 6c and d). Since the reduction in current amplitude was not associated with a shift in the membrane potential at which the maximum of the I.V. curves occurs (Figure 6d), it may be assumed that the alkaloid decreases $\bar{g}_{Na\,slow}$; a situation similar to that already described for the reduction in $g_{Na\,fast}$. It is of interest to see that both slow and fast sodium conductance are inhibited by the same drug in spite of their different pharmacological properties i.e. sensitivity to TTX, Mn (Rougier *et al.*, 1969; Garnier *et al.*, 1969) or to Li and Mg ions (Chesnais *et al.*, 1975) and kinetic behaviour (the inactivation phase of $g_{Na\,slow}$ is about 100 times slower than that of the fast conductance). In the present work, ervatamine (2.8×10^{-4} M) inhibited g_{Ca} only moderately (Figure 6b). This inhibition also occurred without any detectable shift of the membrane potential at which the current reached its maximum value and without appreciable change of the Ca apparent equilibrium potential. In contrast the inhibition of the slow Ca–Na conductance by ervatamine presents a marked potential-dependence (Figure 6a), the current being more inhibited for small than large depolarizing clamp steps. Ervatamine is without effect on the time-dependent outward K current I_{x_1} responsible for the delayed rectification (Figure 7). This current corresponds to the one termed I_1, (Ojeda & Rougier, 1974) and I_{slow} (Brown, Clark & Noble, 1976). Because of its activation time constant it may be considered as being not appreciably altered by accumulation-depletion phenomena (Brown *et al.*, 1976).

Conclusion

Ervatamine is a strong inhibitor of both fast and slow g_{Na} in frog atrial fibres whereas its inhibitory effect on g_{Ca} is much less marked; it also alters the background K conductance. Ervatamine shares some electrophysiological properties with antiarrhythmic drugs, namely the frequency-dependent inhibition of the fast g_{Na} and the ability to slow the reactivation of the Na carrying system. This drug appears to be a good tool for the analysis of the duality of Na and Ca ions in the so-called slow channel.

I am indebted to Dr H. P. Husson (ICNS GIF/YVETTE France) for kindly providing ervatamine chlorhydrate and to Professor E. Coraboeuf for a critical reading of the manuscript. I also thank Mr M. Suchaud for electronic engineering maintenance, Mr J. Pochard for photographic assistance, Mrs P. Richer and J. Tansini for secretarial assistance.

References

BENNINGER, C., EINWÄCHTER, H.M., HAAS, H.G. & KERN, R. (1976). Calcium–sodium antagonism on the frog's heart: a voltage clamp study. *J. Physiol.*, **259**, 617–645.

BROWN, H.F., CLARK, A. & NOBLE, S.J. (1976). Analysis of pacemaker and repolarization currents in frog atrial muscle. *J. Physiol.*, **258**, 547–577.

CHESNAIS, J.M., CORABOEUF, E., SAUVIAT, M-P. & VASSAS, J.M. (1975). Sensitivity to H, Li and Mg ions of the slow inward sodium current in frog atrial fibres. *J. mol. cell. Cardiol.*, **7**, 627–642.

CORABOEUF, E. & VASSORT, G. (1968). Effects of some inhibitors of ionic permeabilities on ventricular action potential and contraction of rat and guinea-pig hearts. *J. Electrocardiol.*, **1**, 19–30.

De HEMPTINE, A. (1976). Voltage clamp analysis in isolated cardiac fibres as performed with two different perfusion chambers for double sucrose gap. *Pflügers Arch.*, **363**, 87–95.

GARNIER, D., ROUGIER, O., GARGOUÏL, Y.M. & CORABOEUF, E. (1969). Analyse électrophysiologique du plateau des réponses myocardiques mise en évidence d'un courant entrant lent en absence d'ions divalents. *Pflügers Arch.*, **313**, 321–342.

GETTES, L.S. & REUTER, H. (1974). Slow recovery from

inactivation of inward currents in mammalian myocardial fibres. *J. Physiol.*, **240**, 703–724.

HAAS, H.G., KERN, R., EINWÄCHTER, H.M. & TARR, M. (1971). Kinetics of Na inactivation in frog atria. *Pflügers Arch.*, **323**, 141–157.

HILLE, B. (1977). Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. gen. Physiol.*, **69**, 497–515.

HODGKIN, A.L. & HUXLEY, A.F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.*, **117**, 500–536.

HORACKOVA, M. & VASSORT, G. (1976). Calcium conductance in relation to contractility in frog myocardium. *J. Physiol.*, **259**, 597–616.

HORACKOVA, M., SHRIER, A. & VASSORT, G. (1977). Control of membrane potential in double sucrose gap voltage clamp technique (Letter to the editor). *Circulation Res.*, **40**, 603.

HUSSON, H.P., BANNAI, K., FREIRE, R., MOMPON, B. & REIS, F.A.M. (1978). Synthèses totales d'alcaloïdes α-acylindoliques. (±) Oxo-6 silicine et (±) epi-16 20, N (a) methylervatamine. *Tetrahed.*, **34**, 1363–1368.

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

KNOX, J.R. & SLOBBE, J. (1971). Three novel alkaloids from Ervatamia orientalis, *Tetrahed. Letters*, **24**, 2149–2151.

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

OJEDA, C. & ROUGIER, O. (1974). Kinetic analysis of the delayed outward currents in frog atrium. Existence of two types of preparations. *J. Physiol.*, **239**, 51–73.

PICHON, Y. & SAUVIAT, M-P. (1978a). Effects of ervatamine on the sodium current in squid giant axons. *J. Physiol.*, **280**, 29–30p.

PICHON, Y. & SAUVIAT, M-P. (1978b). Inhibition de la conductance sodique de l'axone géant de calmar par l'ervatamine. *J. Physiol., Paris*, **74**, 43A.

RAMON, F., ANDERSON, N., JOYNER, R.W. & MOORE, J.W. (1975). Axon voltage clamp simulations IV. A multicellular preparation. *Biophys. J.*, **15**, 55–70.

ROUGIER, O., VASSORT, G. & STÄMPFLI, R. (1968). Voltage clamp experiments on frog atrial heart muscle 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.

SAUVIAT, M-P. (1977). Reversible blockage of the fast sodium conductance in frog atrial fibres by ervatamine. *XXVIIth Int. Congress Physiol. Sci.*, **13**, 663.

SAUVIAT, M-P. & PICHON, Y. (1977). Effects of ervatamine chlorhydrate on the axonal membrane of the cockroach. *XXVIIth Int. Congress Physiol. Sci.*, **13**, 663.

TAKATA, M., MOORE, J.W., KAO, C.Y. & FUHRMAN, F.A. (1966). Blockage of sodium conductance increase in lobster giant axon by tarichatoxin (tetrodotoxin). *J. gen. Physiol.*, **49**, 977–988.

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

YEH, J.Z. & NARAHASHI, T. (1976). Mechanism of action of quinidine on squid axon membranes. *J. Pharmac. exp. Ther.*, **196**, 62–70.

(Received July 23, 1979.
Revised February 29, 1980.)