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EFFECT OF METHUENINE ON THE FAST INWARD SODIUM CURRENT IN FROG ATRIAL FIBRES

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ABSTRACT The effect of methuenine (a synthesized molecule derived from the ervatamine molecule; a natural α acyl alkaloid) on action potential and fast inward Na current of frog atrial fibres was studied using the double sucrose gap technique. The drug 13 μ*M* blocked the action potential without altering the resting membrane potential. Methuenine inhibited the fast Na conductance without changing the selectivity of the Na channel. The kinetic parameters of the Na current were not markedly shifted. The steady state inactivation curve was only slightly shifted towards more negative membrane potentials. The inhibition of the Na conductance by methuenine was frequency dependent. The data suggest that methuenine

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effect on Na current are slightly different from the effect of ervatamine. Methuenine might be effective in the control of cardiac arrhythmias since the drug mainly decreased the excitability of depolarized fibres.

KEY WORDS frog heart; voltage clamp; ionic current; methuenine

Methuenine (Figure 2 A) is synthesized[1] from ervatamine. Ervatamine is known to decrease the maximal Na conductance ($\overline{G_{Na}}$) in a frequency dependent manner either on the giant axon of the cockroach [2], the squid [3-5], neuroblastoma cells [8] or frog atrial fibres [7]. Very little is known about the pharmacology of methuenine. In the squid giant axon, methuenine decreased $\overline{G_{Na}}$ [8,9]. The aim of the present paper was to extend the analysis of the action of methuenine to cardiac Na ionic current by means of the double sucrose gap technique [10].

MATERIAL AND METHODS

Current and voltage clamp experiments were performed at 5–8°C on fine atrial fibres (75 150 µm in diameter; 2–4 mm in length) isolated from heart of *Rana esculenta*. The double sucrose gap technique with vaseline seals was used [7,10,11]. The pH of the Ringer solution was maintained at 7.3 with HEPES or MOPS buffer (5 m*M*). In some experiments, tetrodotoxin (TTX, 0.57 µ*M*) a specific inhibitor of the fast Na conductance and manganese ($MnCl_2$, 5 m*M*), an inhibitor of the slow Ca and Na conductance were added to the Ringer solution. Methuenine was readily soluble in water. Preparations were stimulated with square waves pulses.

RESULTS

Action potential (AP) The effect of methuenine on the AP was a decrease in fast initial depolarizing phase associated with a reduction of V_{max}, a decrease in plateau amplitude and a lengthening of the AP duration (Fig 1). Methuenine did not noticeably alter the membrane resting potential. The reduction of V_{max} was 23% and 40% when the drug concentration was increased from 13 to 20 µ*M* in the control solution respectively. The effects of methuenine on the AP were reversible.

Fast inward Na current (I_{Na}) The addition of methuenine (10 µ*M*) to the control solution decreased the amplitude of the peak inward current (Fig 2 B). The inhibition reached 77% of the control value within 2 min. The inhibition of I_{Na} occurred without noticeable alteration of the time to peak value of the current as well as of the time constant of the inactivation phase of the current. Current-voltage relationships plotted in Fig 2 C show 4 important features: 1) The magnitude of I_{Na} was reduced when the drug concentration increased. 2) The minimum of the curve was not appreciably shifted as the drug inhibition increased. 3) The difference in the slope of the positive region of the I–V curves drawn for each solution suggests that methuenine decreased the maximum conductance $\overline{G_{Na}}$. 4) The apparent reversal potential for Na^+

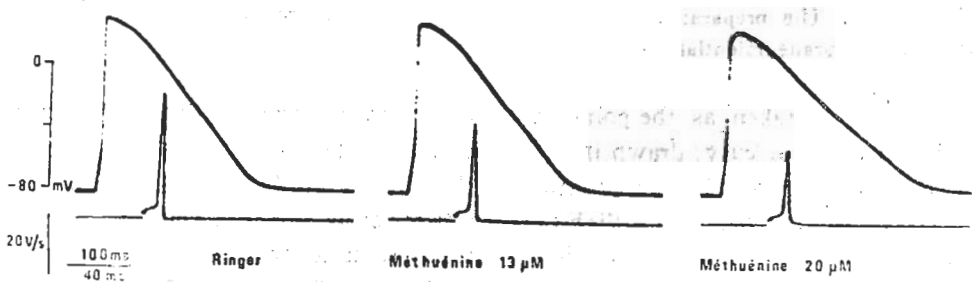

Fig 1. Alteration of frog atrial resting membrane and action potential (upper trace) and V_{max} (lower trace) after 3 min of methuenine treatment.

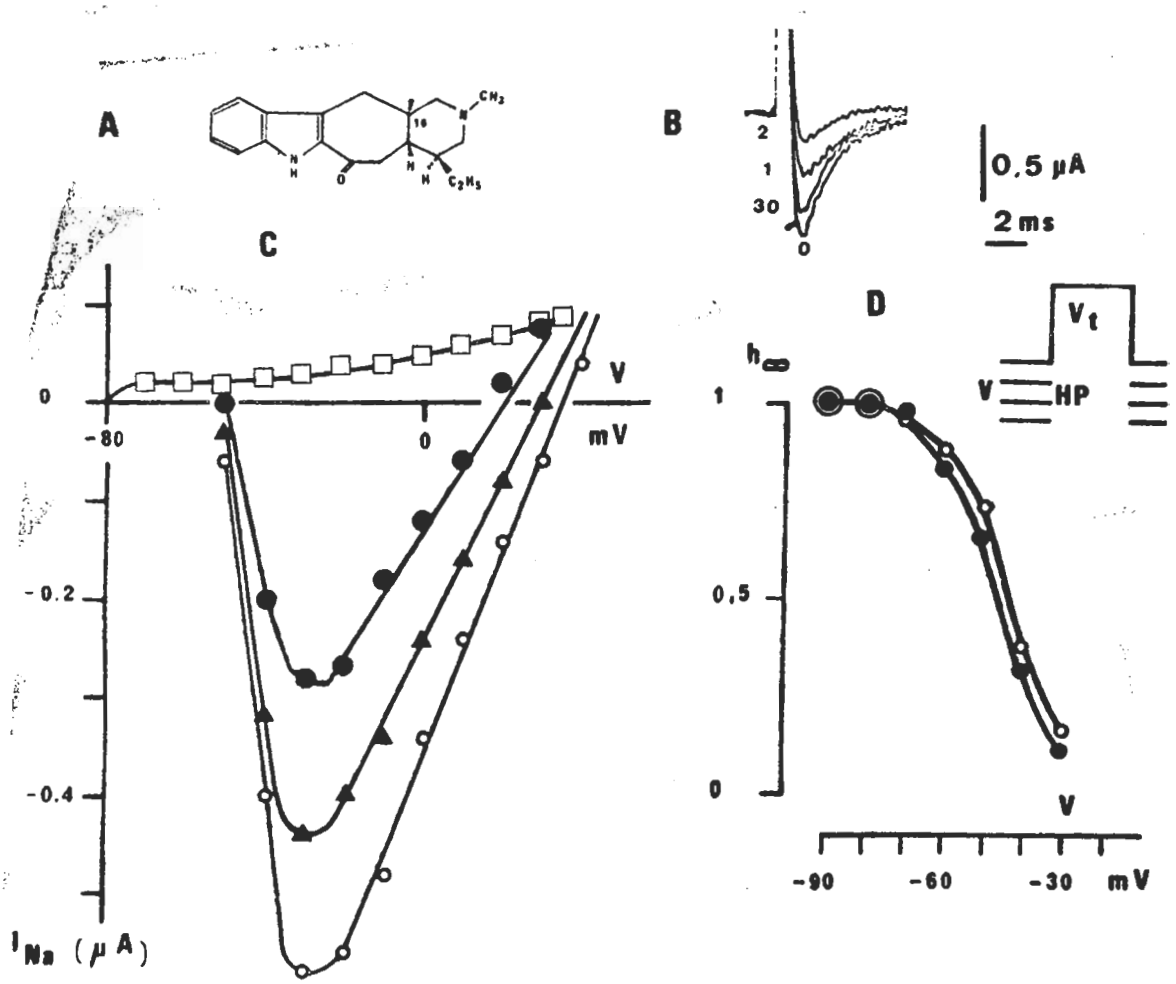

Fig 2. A–Chemical structure of methuenine molecule. In this molecule, the CH_3COO group at C_{16} of ervatamine molecule was replaced by H. B–Superimposed records of the peak Na current recorded in Ringer solution (0) and after 30 s, 1 and 2 min of methuenine (10 μ*M*) treatment at a frequency of 1 Hz. C–Current-voltage relationships of the peak inward Na current plotted in Ringer solution (○); (▲) methuenine (15 μ*M*) and (●) (30 μ*M*) containing solution. The curve indicated by open squares was recorded after addition of TTX (0.57 μ*M*) to the drug containing solution. D–Effect of methuenine (20 μ*M*; ●) on the steady state inactivation of the Na channel (h_∞) as a function of the membrane potential (V). A test pulse of constant value ($V_t = 80$ mV; 10 ms) was applied and the holding potential (HP) was changed 1 min before each record. The preparation was driven at 0.2 Hz. The ordinate: the relative magnitude of I_{Na}; the abscissa: membrane potential changes.

was not changed (E_{rev}; taken as the point at which I–V curves cross the curve drawn in the presence of TTX (0.57 μ*M*) in the control solution[11]). Methuenine (20 μ*M*) slightly shifted the steady state inactivation curve (h_∞–V) of the Na carrying system towards more negative membrane potentials (Fig 2 D).

Dose–response relation Fig 3 shows the doseresponse curve for the effect of methuenine on Na conductance obtained either by measuring the decrease of V_{max} or the inhibition of I_{Na} as a function of drug concentration. The half maximal response was reached at a drug concentration of about 20 μ*M*. The apparent dissociation constant of the reaction drug Na channel was $K_d = 17 \pm 0.4$ μ*M* (average of 6 experi-

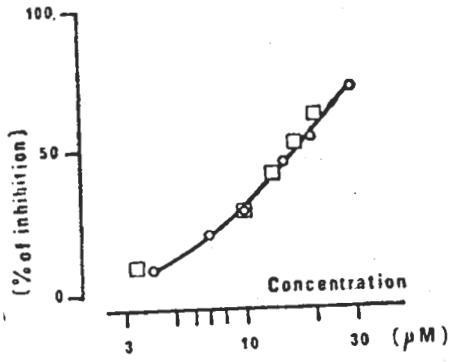

Fig 3. Log concentration–response of the effect of methuenine (abscissa) on the fast inward current amplitude (○) and V_{max} (□) (ordinate). HP was −80 mV and the stimulation rate was 0.2 Hz.

ments). The Hill plot of the data ((log % inhibition/(100 − % inhibition) vs log methuenine concentration) gave a stoichiometric parameter value $n = 1.7 \pm 0.4$ (average of 6 experiments). This suggests a relation between more than one molecule of drug and one Na channel.

Frequency–dependence of the block Fig 4 A illustrates the behaviour of the peak inward current partially inhibited after drug (3.4 μM) treatment as a function of the stimulation rate. Increasing the frequency of the clamp increased the inhibition of I_{Na} while the same increase only moderately decreased the current recorded in the absence of the drug. The effect of the frequency of stimulation are also a function of the membrane potential. Fig 4 B shows that the sudden application of a train of 20 identical pulses at a rate of 2 Hz produced an extra-block of the current recorded in the presence of the drug. The amplitude of this extra-block was a function of the membrane potential. It was larger when the membrane was more depolarized (Holding Potential HP = −70 mV) than when the membrane was hyperpolarized (HP = −90 mV). The same experiment performed in the absence of drug only slightly alter the amplitude of I_{Na}. The recovery of I_{Na} from the extra-block measured at low rate (0.2 Hz) as a function of the membrane potential did not show any significant difference; it was almost completely achieved in about 10–15 s whatever the membrane potential investigated.

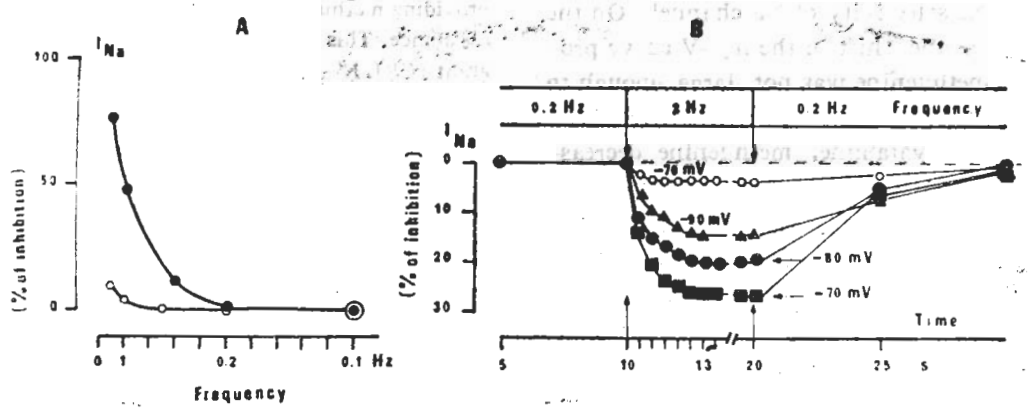

Fig 4. A–Effect of increasing the rate of stimulation on the peak inward current elicited by a 70 mV (10 ms duration) depolarizing clamp step starting from a −80 mV HP in a fibre bathing in Ringer solution before (○) and after 3 min methuenine treatment (3.4 μM) (●). I_{Na} was recorded after 1 min of stimulation at each rate; each; sequence was followed by a sequence where the fibre was driven for 2 min at 6/min. I_{Na} is expressed in % ($I_{Na} = 0$ when the stimulation rate remains without effect). B–Effect of a repetitive train of 20 identical pulses application at 2 Hz on I_{Na} elicited by a 80 mV (10 ms duration) depolarizing clamp step (V) applied from different levels of holding potential (HP) during (filled symbols) and before (open symbols) methuenine (20 μM) treatment. The HP value was changed 2 min before each train pulse application. After each train of pulses, the preparation was driven at 0.2 Hz during 5 min at the same HP value was changed 2 min before the application of each train of pulses. After each sequence of train of pulses, the preparation recovered at 0.2 Hz during 3 min. I_{Na} (ordinate) is expressed in % ($I_{Na} = 0$ % when the stimulation rate was 0.2 Hz) as a function of the duration (abscissa).

DISCUSSION

The most predominent effect of methuenine reported in the present study on voltage clamped frog atrial fibres was a reduction in peak Na current. Although there was some uncertainty concerning the applicability of the Hodgkin Huxley[12] theory to cardiac tissues, the similarities between Na current of squid axon and frog atrial fibres are sufficient to justify the use of this theory in the interpretation of the results. Thus, it is generally accepted that, in cardiac tissue, as in the nerve membrane, the Na conductance is the product of the maximum conductance $\overline{G_{Na}}$ and 2 kinetic factors m and h; m governs the activation process and h governs the inactivation process[12]. The present experiments show that methuenine did not modify the kinetic factors of the Na current. The reduction of I_{Na} brought about by the drug did not seem to be related to an alteration of the Na equilibrium potential since methuenine did not alter E_{rev}. This absence of effect on E_{rev} also suggests that the drug did not alter the selectivity of Na channel. On the other hand, the shift in the h_∞ –V curve produced by methuenine was not large enough to account for the I_{Na} reduction. Thus, as previously found for ervatamine, methuenine decreased the Na current by decreasing the maximal Na conductance ($\overline{G_{Na}}$). Methuenine inhibited also I_{Na} in a frequency-dependent manner indicating that the blocking effect of the drug depend upon how often Na channel are opened and suggests that the drug has to penetrate into the channel before being effective. The frequency-dependent block produced by methuenine increased at more depolarized membrane potentials and decreased at more negative membrane potentials. According to Schwarz *et al* [13] hyperpolarizing membrane potentials (–90 mV) removed the Na channel inactivation, ie, opened more h gates and favored the unbinding of the drug. This also suggests that the inhibitory form of the drug is charged. The observation that methuenine left Na channel with the same kinetic at low and high frequency whatever the membrane potential suggests that the drug did not bind firmly to its site of action. In the present study, methuenine effect on I_{Na} are slightly different from the effect of ervatamine. The inhibition of I_{Na} by methuenine is faster; methuenine shifts the h_∞ –V curve while ervatamine did not; the stoichiometry of the relation drug–Na channel is 1.7 for methuenine instead of a 1:1 relation for ervatamine. Thus, the suppression of the CH_3COO group from the ervatamine molecule slightly alter the pharmacological properties of the native molecule and, as present results show, seems to increase the ability of the new molecule to inhibit the Na channel.

Our results lead to the conclusion that methuenine exert a marked inhibitory effect on the Na conductance and therefore a greater conduction blocking effect when the drug acts on partially depolarized fibres. This could give potent anti-arrhythmic effect for this drug.

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Methuenine 对蛙心房纤维的快钠内流的作用

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提要　应用双蔗糖间隙技术研究 methuenine 对蛙心房纤维动作电位和快钠内流的作用。药物浓度为13 μM时阻滞动作电位而不改变静息膜电位。Methuenine 抑制快钠传导但不改变钠通道的选择性。钠电流的动力学参数没有明显变化。稳态无动作曲线稍有移动，更趋于负性膜电位。Methuenine 抑制的钠传道有频率依赖性。本结果提示 methuenine 作用于钠电流是与 ervatamine 稍有不同，Methuenine 可以有效地控制心律失常，因为此药主要降低去极化纤维的兴奋性。

关键词　蛙心；　电压钳制；　离子流；　methuenine

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