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THE EFFECT OF HARMINE ON THE ACTION POTENTIAL OF THE GUINEA-PIG ATRIAL MUSCLE DEPENDS ON THE EXTERNAL CALCIUM CONCENTRATION

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1 The influence of the external calcium concentration on the effect of harmine 2×10^{-5} M upon the guinea-pig atrial muscle was analysed. Transmembrane potentials of contractile fibres were measured during exposure to the drug at 30°C.

2 In preparations superfused with 1.35 mM Ca^{2+}-Tyrode solution and driven at 60/min (1 Hz) harmine depressed the amplitude of the action potential (AP) and the maximum velocity of the upstroke (dV/dt). The resting potential was not affected. Harmine depressed similarly the dV/dt of fibres superfused with 2.7 mM Ca^{2+}-Tyrode solution but the AP was slightly enhanced.

3 Harmine diminished both the AP and the dV/dt of fibres superfused with 2.7 mM Ca^{2+}-Tyrode solution and driven at a fast rate (180/min, 3 Hz). Increased external calcium concentration (5.4 mM) annulled the depressant effect on AP while the action on dV/dt persisted.

4 It is concluded that the effect of harmine on the AP depends on the external calcium concentration. Increase $[Ca^{2+}]_o$ reverses the depressant effect of harmine because it annuls the effect of the drug on the slow component of the upstroke. The action on the initial fast component of the rising phase of the action potential persists.

Introduction

The harmala alkaloids, harmaline and harmine, prolong the duration of the action potential (APD) of the guinea-pig atrial muscle (Carpentier, 1980a). It has been shown recently that increases in $[Ca^{2+}]_o$ annul the APD-prolonging effect of harmine (Carpentier, 1980b). On the other hand, dehydrogenation of harmaline into harmine reverses the stimulatory effect of high concentrations of the drug (8.3×10^{-5} M) on the amplitude of the action potential (AP) of atria driven at 120 min (2 Hz). However, the AP of action potentials determined exclusively by the Ca^{2+}-dependent slowly activated current I_{si} (slow responses) is enhanced both by harmaline and harmine (Carpentier, 1980a). The upstroke of the action potential of guinea-pig cardiac muscle has an initial 'fast component' determined by the fast sodium current I_{Na+} followed by a 'slow component' determined by I_{si} (Coraboeuf & Vassort, 1968). These considerations outline the aim of the present experiments, namely the investigation of the relationship between $[Ca^{2+}]_o$ and the effect of harmine on AP of guinea-pig atrial muscle.

Methods

Guinea-pigs were killed by a blow on the head and the heart was quickly removed. A small strip of the left atrium was isolated and superfused in a tissue bath holding 5 ml of Tyrode solution flowing at 5 ml/min at 30°C. The composition of the Tyrode solution was (mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.35, 2.70 or 5.40 (see Results for concentration used in each series), $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.45 and glucose 5.55. The solution was bubbled with 95% O_2: 5% CO_2 and the pH was 7.4. The tissue bath was surrounded by a thermostatically controlled water bath which maintained the temperature of the Tyrode solution constant within 0.5°C. The preparations were driven at a constant rate by a Grass stimulator model S88 through a SIU5 and a pair of silver electrodes in close proximity to the fibres. The duration of the stimulus was 1 ms and the intensity 20% suprathreshold.

Transmembrane potentials of the contractile fibres were recorded with microelectrodes of the Ling-Gerard type (Ling & Gerard, 1949) filled with 3M KCl. The recording apparatus consisted of a WH

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Instrument Company electrometer amplifier model A-35C, a Tektronix 5A26 dual differential amplifier and a Tektronix 5113 dual beam storage oscilloscope. Transmembrane potentials were displayed on one channel of the dual beam oscilloscope. The maximum rate of rise of the action potential (dV/dt) was determined by electronic differentiation using a Tektronix AM-501 operational amplifier with an RC circuit with a time constant of 50 μs. The amplitude of the differentiated trace displayed on the second channel of the oscilloscope during the upstroke of the action potential gave a precise measurement of dV/dt and was linear within the range 10–500 V/s. The traces were photographed with a Grass C4R camera.

The experimental procedure was to allow the preparations to equilibrate in Tyrode solution for 60 min and record the membrane potentials by impaling different contractile fibres. After completing this control procedure, the preparation was exposed to Tyrode containing harmine (harmine HCl, Sigma Chemical Co.) 2×10^{-5} M. Membrane potentials were again successively recorded from different cells during the last 45 min of a 60 min exposure to the drug. Recovery impalements were performed at the end of each experiment after an equilibration period of 60 min in Tyrode solution. Twelve to fifteen different fibres were impaled during each experimental period (Control — Harmine — Recovery). The mean values ± the standard errors (s.e.) were calculated for the membrane potentials in each experimental condition and the statistical treatment of the data was carried out using Student's t test. The statistical significance was set at P values less than 0.01.

Results

The effect of harmine 2×10^{-5} M on the amplitude on the action potential (AP) of preparations driven at 60/min (1 Hz) is shown in Figure 1. Panel (b) (1.35 mM Ca^{2+}-Tyrode) shows that harmine produced a reversible depression of both the amplitude of the action potential (AP) and the maximum velocity of the upstroke (dV/dt) in the absence of any change in resting potential (RP). When the $[Ca^{2+}]_o$ was increased to 2.7 mM (Panel a) dV/dt was similarly depressed by harmine, again in the absence of any change in RP. Interestingly, AP was not depressed: in each of the six atria the AP increased slightly in spite of the fact that dV/dt was simultaneously depressed.

A depressant effect of harmine on AP of preparations superfused with 2.7 mM Ca^{2+}-Tyrode became evident when the frequency of drive was increased from 60/min (Figure 1 a) to 180/min (3 Hz) (Figure 2 a). Figure 2 (b) shows that the depressant effect of harmine on AP was annulled by the increase in $[Ca^{2+}]_o$ even though the action on dV/dt persisted.

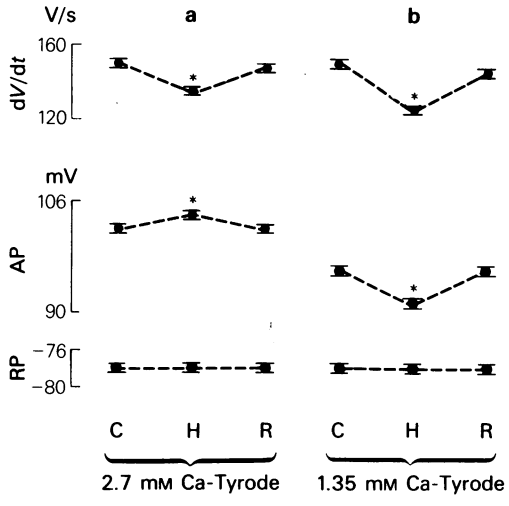

Figure 1 Effects of harmine 2×10^{-5} M on transmembrane potentials of atrial fibres driven at 60/min (1 Hz). C = control values; H = values obtained during exposure to harmine; R = recovery values. Each value is the mean for 64 to 73 impalements in six preparations; bars show s.e.mean; RP = resting potential; AP = amplitude of the action potential; dV/dt = maximum rate of rise of the action potential. *Significantly different from control and recovery.

The values of transmembrane potentials obtained during recovery period were not different from control values in the two series in which the preparations were driven at 60 min (1 Hz). This applies also to RP and AP of preparations driven at 180 min (3 Hz). However, recovery of the rate of rise of the upstroke at fast drive was only partial.

To analyse the action of harmine on each of the two components of the upstroke of the action potential, 4 atrial strips driven at 180/min (3 Hz) were superfused with 5.4 mM Ca^{2+}-Tyrode solution in which the concentration of K^+ was increased to 10.8 mM. As expected, the RP fell and AP became smaller. The two components of the upstroke then became clearly identifiable and accurately measurable: the fast component could be measured from the level of the resting potential and the slow component from the end of the fast component to the peak of the action potential. Table 1 shows that under the influence of harmine the amplitude of the fast component of the upstroke decreased whereas the slow component increased in size. As a result, the total amplitude of the action potential remained unchanged.

Discussion

The effect of harmine on the AP of (a) fibres driven at 60/min in the presence of 1.35 mM Ca^{2+}, and (b)

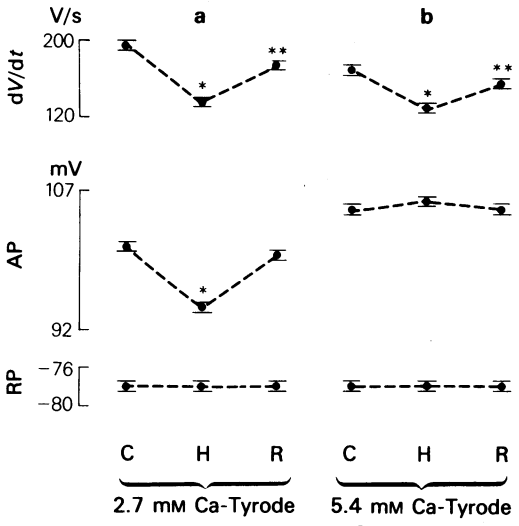

Figure 2 Effects of harmine 2×10^{-5} M on transmembrane potentials of atrial fibres driven at 180/min (3 Hz).
Each value is the mean for 69 to 86 impalements in six preparations; bars indicate s.e.mean. Abbreviations as in Figure 1. *Significantly different from control and recovery. **Significantly different from control and harmine.

fibres driven at 180/mm in 2.7 mM Ca^{2+} clearly indicates that both the 'slow' and the 'fast' components of the upstroke were depressed: (1) the maximum velocity of the upstroke was diminished, indicating that the fast sodium system was depressed. (2) the peak of the action potential was shifted to less positive values i.e. the overshoot of the action potential became smaller. The degree of positivity attained at the peak of the action potential is determined by two factors: (a) Once I_{si} is activated at a level of potential of some −40 mV, the level of voltage the membrane potential will reach at the peak of the upstroke is determined by the magnitude of the increase of the membrane conductance gs (Antoni & Delius, 1965; Coraboeuf & Vassort, 1968). (b) The magnitude of the voltage-dependent turnoff of the potassium conductance due to anomalous rectification. Obviously, if (a) and (b) remain constant the peak of the action potential and the size of the overshoot should not change even if the fast system is depressed.

Interestingly, Figures 1 and 2 clearly show that the depressant effect of harmine on the AP of fibres driven at slow and fast rate can be completely reversed by doubling the $[Ca^{2+}]_o$. At both slow and fast rate the fast component was similarly depressed at the two $[Ca^{2+}]_o$ and yet the AP was not diminished when the $[Ca^{2+}]_o$ was doubled. This can be explained only by an increase in size of the slow component of the upstroke, concomitant with the depression of the fast component. The enlargement of the slow component can be explained as follows: (1) the inhibition of the fast component resulted in the slow component appearing to take off from a more negative potential. This causes an enlargement of the slow component with no change in the amplitude of the action potential (see Table 1). (2) 'slow responses' determined exclusively by I_{si} in depolarized atrial fibres driven at a very low rate are enhanced both by harmine and its analogue harmaline through a catechol-mediated effect (Carpentier, Narvarte and Sanhueza, 1977; Carpentier, 1980a). Noradrenaline increases I_{si} and shifts the threshold for this current to more negative values (Vassort, Rougier, Garnier, Sauviat, Coraboeuf & Gargouil, 1969; Reuter, 1974) and increases the overshoot of the cardiac muscle action potential (Pappano, 1970; Smejkal, Mironneau, Ojeda & Gargouil, 1970). I_{si} and the slow component of the upstroke are highly sensitive to changes in $[Ca^{2+}]_o$. A reduction in the extracellular concentration of the cation depresses I_{si}, the slow component of the upstroke and the overshoot and makes them more vulnerable (Niedergerke & Orkand, 1966; Beeler & Reuter, 1970; Robinson & Sleator, 1977). Thus, it is not surprising that the depressant effect of

Table 1 Effects of harmine on the two components of the upstroke of the action potential of fibres superfused with high K^+-Tyrode

	Resting Potential (mV)	*Amplitude of action potential* *Total* (mV)	*Fast comp.* (mV)	*Slow comp.* (mV)
Control	67.2 ± 0.4	84.9 ± 0.9	71.8 ± 1.6	13.1 ± 1.1
Harmine	67.4 ± 0.4	83.8 ± 1.1	64.0 ± 2.3*	19.8 ± 1.4*
Recovery	67.7 ± 0.5	86.0 ± 1.4	73.9 ± 2.3	12.1 ± 1.0

Control (a), Harmine (b) and Recovery (c) = values obtained: (a) during superfusion with 10.8 mM K^+-Tyrode; (b) within the last 45 min of a 60 min exposure to harmine (2×10^{-5} M) and (c) 60 min after withdrawal of the drug. Fast and Slow comp are the amplitudes of the fast and slow components of the upstroke measured as described in the text. Each value is the mean (± s.e.) for 39–46 impalements in four preparations.
*Significantly different from Control and Recovery

harmine on the overshoot occurred at a lower $[Ca^{2+}]_o$ when the rate of drive was lower. Moreover, harmine enhanced slightly the size of the overshoot when both slow drive and high $[Ca^{2+}]_o$ were present, most probably as the result of the indirect stimulatory effect of harmine on the slow component (see above).

Our results indicate that the effects of harmine on the fast as well as on the slow channel are rate-dependent. In preparations superfused with 2.7 mM-Ca^{2+} and driven at a slow rate, dV/dt was depressed by 11% and AP was slightly enhanced. When driven fast, the depression of dV/dt was larger (31%) and AP was diminished.

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