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EFFECTS OF HARMINE ON TRANSMEMBRANE POTENTIALS OF GUINEA-PIG ATRIAL MUSCLE

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1 The effects of harmine 8.3×10^{-5} M on membrane potentials of guinea-pig atrial muscle were analyzed and compared with those of harmaline. Transmembrane potentials of contractile fibres were measured during exposure to the drug at 30°C.

2 In preparations superfused with 5.4 mmol K^+-Tyrode, harmine produced a progressive reduction in the amplitude of the action potential (AP), in the absence of any change in resting potential (RP) and a prolongation of the duration of the action potential (APD).

3 Harmaline produced an enhancement of AP; RP was not affected and APD was prolonged.

4 The amplitude of slow responses elicited by noradrenaline in 16.2 mmol K^+-Tyrode was enhanced by harmine.

5 It is proposed that dehydrogenation of harmaline to harmine reverses the initial stimulatory action of harmaline on AP because the depressant action on the fast component of the upstroke prevails over the stimulatory effect on the slow component.

Introduction

Harmaline enhances transiently the amplitude of the rat atrial action potential through a stimulation of the slow component of the upstroke (Carpentier, Narvarte & Sanhueza, 1977). This effect contributes to the positive inotropic action of harmaline (Carpentier & Diaz, 1977). Harmine depresses the force of contraction of guinea-pig atrial muscle beating spontaneously or driven at a relatively high rate (Zetler, Lenschow & Prenger-Berninghoff, 1968). The effects of harmine on transmembrane potentials are still far from clear. It has been shown that after 60 min of exposure to harmine the amplitude of the action potential is reduced (Iven & Zetler, 1974), a late effect that occurs also with harmaline (Carpentier & Narvarte, 1975). It is not known whether harmaline shares with harmine the short lasting stimulatory effect on the amplitude of the action potential, determined by the transient enhancement of the slow component of the upstroke. In the present work an attempt is made to clarify the influence of dehydrogenation of harmaline into harmine on the effect of the drug on the amplitude of the action potential.

Methods

Guinea-pigs were killed by a blow on the head and the heart was quickly removed. A strip of the left atrium was superfused in a tissue bath holding 5 ml of Tyrode solution flowing at the rate of 5 ml/min at 30°C. The composition of the Tyrode solution was (mM): NaCl 137, KCl 5.4, $CaCl_2$ 2.7, $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.45 and glucose 5.55. The solution was bubbled with 95% O_2 and 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 Tyrode solution constant within 0.5°C. The preparations were driven at a constant rate (120/min, 2 Hz) by a Grass Stimulator Model S4 through a Stimulus Isolation Unit (SIU) and a pair of silver electrodes in close proximity to the muscle. Transmembrane potentials of the contractile fibres were recorded with microelectrodes of the Ling Gerard type filled with 3 M KCl (Ling & Gerard, 1949). The recording apparatus consisted of a W-H Instrument Company Electrometer Amplifier Model A-35C, a Tektronix 5A20N differential amplifier and a Tektronix 5103N/D13 Dual Beam Storage Oscilloscope. The tracings were photographed with a Grass C4 camera.

The experimental procedure was to allow the preparations to equilibrate in Tyrode solution for 60 min and then record the membrane potentials by impaling different fibres. After completing this control procedure, one fibre was impaled before the superfusion

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with the drug was started. The membrane potentials were then monitored for at least 5 min, to be sure that the fibre was properly impaled and in a steady state, and the membrane potentials of this single cell were measured just before and during the first 10 min of exposure to the drug. Two drugs, harmine hydrochloride and harmaline hydrochloride (Sigma Chemical Co.) were used, added to the Tyrode solution in a final concentration of 8.3×10^{-5} M. After the first 10 min of superfusion with the drug, membrane potentials were again successively recorded from different fibres during the 50 remaining minutes 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. The mean values $\pm$ 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 by Student's t test.

Results

Figure 1(a) shows the effect of harmine 8.3×10^{-5} M on six atrial strips driven at 120/min: the amplitude of the action potential (AP) was depressed by harmine in the absence of any change in resting potential (RP). The duration of the action potential (APD) was prolonged. The effect was entirely reversible. Figure 2(a) shows typical traces from one experiment. The reduction in the amplitude of the action potential was a progressive phenomenon so that no enhancement was evident at any time during the exposure to harmine: (1) membrane potentials of a single fibre were recorded during the first 10 min of superfusion with the drug (see Methods) and no increase in the amplitude of the action potential was observed in any of the six experiments; (2) the control value of the overshoot of the action potential was 27.2 ± 0.3 mV ($n = 89$ impalements). It was already decreased to 21.5 ± 0.7 mV between 11 and 30 min of exposure to harmine ($n = 44$) and more so during the remaining 30 min (18.5 ± 0.4 mV, $n = 40$).

The effects of harmine described above differ from those of harmaline acting on rat atrial contractile fibres: the amplitude of the action potential increases within 60 min of exposure to harmaline 8.3×10^{-5} M (Carpentier & Narvarte, 1975; Carpentier *et al.*, 1977). Thus, preparations driven at 120/min were exposed to harmaline 8.3×10^{-5} M during 60 min to verify the stimulatory effect of the drug on guinea-pig atrial muscle. Figure 1(b) shows that the amplitude of the action potential increased during exposure to harmaline in the absence of any change in resting potential. The duration of the action potential was prolonged. All changes were entirely reversible. Figure 2(b) shows typical traces from one experiment.

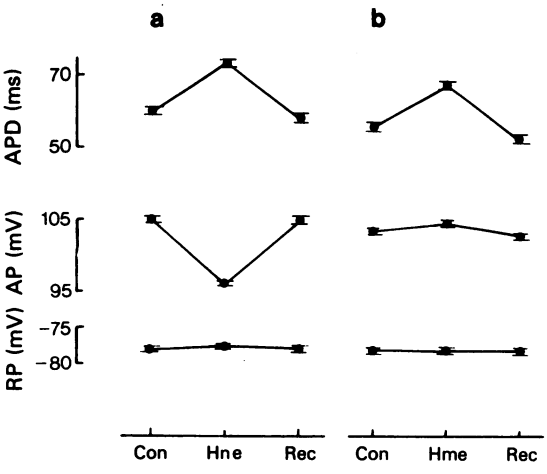

Figure 1 Effects of (a) harmine (Hne) and (b) harmaline (Hme) on transmembrane potentials of left atrial contractile fibres driven at 120/min. Each value is the mean for 84 to 89 impalements in six preparations (a) and for 76 to 83 impalements in six preparations (b); vertical lines show s.e. mean. Values collected during: control period in Tyrode (Con), exposure to the drug (Hne or Hme), and recovery in Tyrode (Rec). RP = resting potential; AP = amplitude of the action potential; APD = duration of the action potential measured at 50% of repolarization. Values for AP and APD during exposures to the drugs are all significantly different from their respective control and recovery values ($P < 0.005$).

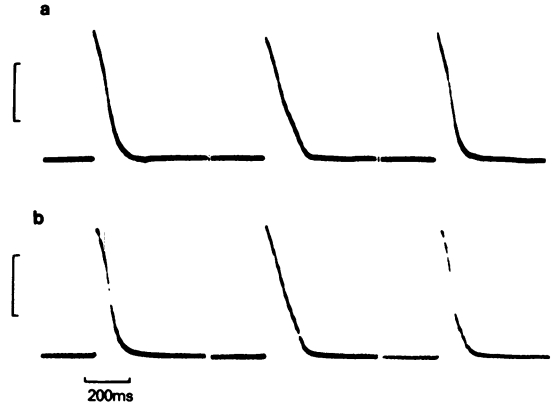

Figure 2 Effects of harmine (a) and harmaline (b) on membrane potentials of atrial contractile fibres. First traces: control in Tyrode; middle traces: 30 min after the beginning of superfusion with the drug; last traces: recovery in Tyrode. Voltage (0 to -50 mV) and time (200 ms) calibrations are the vertical and horizontal lines, respectively.

Slow responses induced by noradrenaline in preparations depolarized by high extracellular K^+ concentration are determined by the slowly activated current (I_s) responsible also for the slow component of the upstroke of the action potential of atrial fibres superfused with 5.4 mmol K^+-Tyrode (Coraboeuf & Vassort, 1968; Pappano, 1970). Harmaline enhances the amplitude of such slow responses and experimental evidence indicates that this effect is due to a stimulation of I_s (Carpentier *et al.*, 1977). The results shown in Figure 1 suggested that the stimulatory action on the slow component of the action potential might be abolished by dehydrogenation of harmaline into harmine. To investigate this possibility, membrane potentials were recorded from a single fibre in each of 6 preparations made unexcitable by superfusion with 16.2 mmol K^+-Tyrode that depolarized the fibres to a level of resting potential of -50.8 ± 1.0 mV. Noradrenaline 3.1×10^{-7} M elicited slow action potentials that increased progressively in size to reach a constant amplitude (58.3 ± 2.9 mV), in the absence of any change in resting potential. Under the influence of harmine this amplitude increased to 65.5 ± 3.0 mV within 15 min. In other words, the overshoot increased by 7.2 ± 1.6 mV ($P < 0.005$).

Discussion

Dehydrogenation of harmaline resulted in a reversal of the initial stimulatory effect of the drug on the amplitude of the action potential. Harmaline 8.3×10^{-5} M produces a transient enhancement of the amplitude of the action potential of rat atrial fibres in the absence of any change in resting potential (Carpentier & Narvarte, 1975). This is due to a transient catechol-mediated stimulation of the slowly activated current (I_s) responsible for the slow component of the upstroke (Carpentier *et al.*, 1977). The current results show that harmaline exerts the same stimulatory effect on the amplitude of the action potential of guinea-pig atrium, which is in agreement with the fact that I_s is responsible for the last part of the upstroke and beginning of repolarization of the cardiac muscle action potential both in the rat and the guinea-pig (Coraboeuf & Vassort, 1968).

The explanation for the fact that dehydrogenation of harmaline into harmine results in the reversal of its initial stimulatory effect on the amplitude of the action potential could be either of the following: (1) harmine does not stimulate the slow component and therefore the amplitude of the action potential is not only not enhanced but in fact diminished because of the depressant effect of the drug on the fast component of the upstroke. (2) Harmine does stimulate the slow component but the depressant action on the fast component prevails and therefore the net result of these two effects acting in opposite directions on the action potentials is a reduction in its amplitude.

Harmine, in the concentration used, shares with harmaline a direct depressant action on the fast component of the upstroke: it decreases the maximum velocity of the upstroke of the action potential in the absence of any change in resting potential (Iven & Zetler, 1974). The present results show that harmine in the same concentration also stimulates slow action potentials, which is not surprising as harmine has catechol-mediated effects on the guinea-pig atrium (Zetler, 1974). Thus, one would expect the slow component of the upstroke to be enhanced and the amplitude of the action potential to be increased (Smejkal, Mironneau, Ojeda & Gargouil, 1970), unless the direct depressant action of harmine on the fast component of the upstroke prevails.

In conclusion, the absence of a transient stimulatory effect of harmine on the amplitude of the action potential may be explained by the fact that the stimulatory effect on the slow component is overcome by the direct depressant effect on the fast component of the upstroke. The amplitude of action potentials determined exclusively by a slow component (slow responses) is enhanced by harmine.

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