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EFFECTS OF HARMALA ALKALOIDS ON TRANSMEMBRANE POTENTIALS OF GUINEA-PIG PAPILLARY MUSCLES

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1 The significance of the presence of a methoxy group in the 7 position of the indole nucleus of harmala alkaloids in terms of their effects on the action potential of cardiac muscle was analysed. Guinea-pig papillary muscles were superfused with Tyrode solution at 30°C and exposed to harmine or its analogue harmane, in which the methoxy group has been removed from the molecule.

2 Harmine 2×10^{-5} M enhanced the amplitude of the action potential (AAP) of normal fibres and of slow responses elicited by noradrenaline in fibres depolarized by 21.6 mM K^+-Tyrode. The effect of harmine on AAP occurred in the absence of any change in membrane resting potential or maximum velocity of the upstroke and it was abolished by propranol. Harmane 2×10^{-5} M did not have any effect on normal action potentials and slow responses.

3 Higher concentrations of the two analogues depressed both AAP and the maximum velocity of the upstroke of the action potential, without affecting the duration of the action potential.

4 It is concluded that: (a) removal of the methoxy group from harmine (1) abolishes the catechol-mediated stimulatory effect of a low concentration of the drug on the slow component of the upstroke of the action potential, and (2) does not modify the depressant effect of a high concentration of the drug. (b) The two analogues, harmine and harmane, do not affect the duration of the action potential of ventricular muscle.

Introduction

The harmala alkaloids, harmaline and harmine, share a dual effect on the upstroke of the action potential of atrial muscle. The two analogues depress the fast component of the upstroke, but they also enhance the slow component responsible for the last part of the upstroke and the beginning of repolarization (Carpentier & Narvarte, 1975; Carpentier, 1980a; Carpentier, 1981). Thus, the effect of these harmala alkaloids on the amplitude of the atrial action potential is determined by these opposite actions on the two components of the upstroke, and it is dose- and rate-dependent (Carpentier & Narvarte, 1975; Carpentier, 1981). The presence of a methoxy group in the indole nucleus of indole-derivatives has been found to enhance the depressant effect on the automaticity of the sinus node (Zetler, Lenschow & Prenger-Berninghoff, 1968). It was therefore important to investigate whether harmaline and harmine owe their actions on the two components of the action potential to this methoxy group, which is not present in the analogue harmane (Figure 1). On the other hand, harmaline and harmine prolong the duration of the action potential of guinea-pig atrial muscle (Carpentier, 1980a) but it has been observed that harmine does not decrease the speed of repolarization of the papillary muscle (Brasch, Iven & Zetler, 1977). Therefore, it seemed appropriate to analyse the effects of harmane and harmine on the transmembrane potentials of guinea-pig papillary muscles, in order to: (1) elucidate the significance of the presence of the methoxy group in terms of the actions of harmala alkaloids on the amplitude of the action potential; (2) analyse the effect of harmala alkaloids on the repolarizing phase of the action potential.

Methods

Guinea-pigs were killed by a blow on the head, the thorax opened and the heart removed and immersed in a room-temperature oxygenated and buffered Tyrode solution (see below for composition). A left ventricular papillary muscle was then isolated and set up horizontally in a tissue bath holding 2 ml of Tyrode solution flowing at the rate of 5 ml/min. The composition of the Tyrode solution was, unless otherwise specified, (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: 5% CO_2 and the pH was 7.4. The tissue

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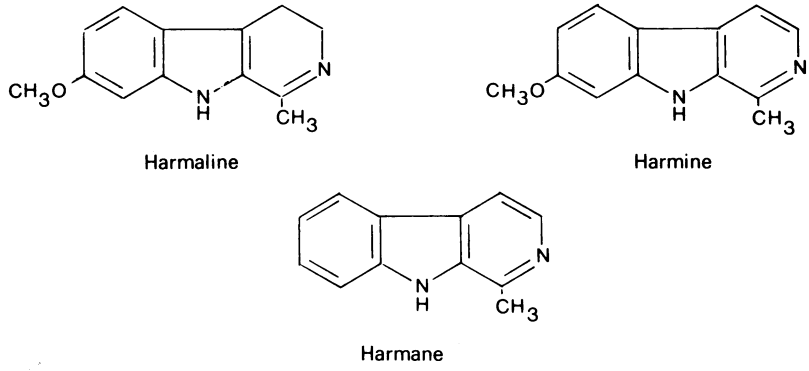

Figure 1 Chemical structure of harmala alkaloids. Note the absence of the methoxy group (CH_3O) in the analogue, harmane.

bath was surrounded by a thermostatically controlled water bath which maintained the temperature of the Tyrode solution constant at 30°C. The preparations were driven at a constant rate by a Grass stimulator model S88 through a Grass SIU5 isolation unit and a pair of silver electrodes in contact with one end of the muscle. Unless otherwise specified, the duration of the stimulus was 1 ms, the intensity 20% suprathreshold and the frequency 60 Hz.

Transmembrane potentials of the contractile fibres were recorded with microelectrodes of the Ling Gerard type filled with 3 M KCl. The recording system consisted of a WH 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 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 preparations were equilibrated in Tyrode solution for 90 min before any experimental procedure. A muscle fibre was then impaled and transmembrane potentials were recorded just before (control), during exposure to harmala alkaloids and after withdrawal of the drug (recovery). Two analogues were used: harmine (harmine hydrochloride) and harmane (harmane hydrochloride), both from Sigma Chemical Co. Immediately after the control procedure, harmine or harmane was added to the Tyrode solution and the preparation was superfused for 30 min with the physiological solution now containing a given final concentration of one of the two compounds, while monitoring transmembrane potentials. Recovery traces were recorded 30 min after withdrawal of the drug. In some experiments, after completing the control procedures in Tyrode solution the preparation was superfused with a modified Tyrode solution in which the concentration of K^+ was increased to 21.6 mM and noradrenaline ((−)-arterenol bitartrate, Sigma Chemical Co.) was added. The preparation was again allowed to equilibrate, control potentials were recorded and the procedures described above for superfusion with harmine or harmane and recovery were carried out. Upon completion of recovery procedures, the preparation was exposed to verapamil (Isoptin, Knoll Pharmaceutical Co.) and transmembrane potentials were recorded again during and after superfusion with the drug.

The following parameters were measured for each experimental condition: membrane resting potential (MRP), total amplitude of the action potential (AAP) and its overshoot (OS), maximum velocity of the upstroke (dV/dt), duration of the action potential (APD) measured at 20%, 50% and 80% of repolarization. The mean values (± s.e.) were calculated for the membrane potentials recorded during each experimental condition (control–harmala alkaloid–recovery) in each series of single impalements in 6 muscles. The statistical treatment was carried out using the Student's t test for paired data, with significance set at a P value less than 0.01.

Results

Figure 2a shows the effect of harmine 2×10^{-5} M on six papillary muscles. The overshoot (OS) of the action potential was increased by 7.5 ± 1.7 mV during exposure to the drug while the membrane resting potential (MRP) remained unmodified. The resulting enhancement of the amplitude of the action potential (AAP) occurred in the absence of any change

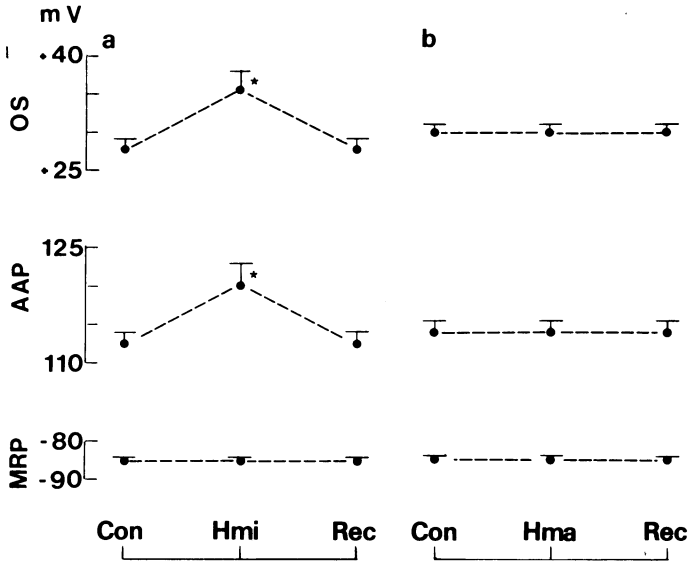

Figure 2 Effects of harmine (a) and harmane (b) 2×10^{-5} M on membrane potentials of papillary muscles. Each value is the mean of values obtained from 6 fibres, each one from a different muscle; vertical lines show s.e.mean. Con = control values; Hmi and Hma = values obtained at the end of a 30 min exposure to harmine or harmane, respectively; Rec = recovery values obtained 30 min after withdrawal of the drug. MRP = membrane resting potential; AAP = amplitude of the action potential; OS = overshoot of the action potential.
*Significantly different from control and recovery.

of the maximum velocity of the upstroke (195 ± 7.5 V/s). Figure 2b shows that harmane in the same concentration did not affect the transmembrane potentials of six ventricular fibres: MRP, OS and AAP remained unchanged throughout control, exposure to the drug and recovery.

The absence of any effect of harmane on AAP suggested that the removal of the methoxy group from the molecule resulted either in complete abolition of the stimulatory effect or at least in a reduced activity of the drug. In an effort to clarify this, the concentration of the two analogues was doubled (4×10^{-5} M) in a new series of experiments. The mean values of transmembrane potentials obtained from 6 preparations superfused with harmine are shown in Figure 3a: the MRP, the AAP and the maximum velocity of the upstroke (dV/dt) were not affected by the drug. However, even though there was no significant difference between control value for dV/dt and the value obtained with harmine, the latter was slightly lower than control and recovery values. This was because in 3 out of the 6 preparations dV/dt was slightly depressed. Harmane in the same concentration did not affect MRP, AAP and dV/dt (Figure 3b). Again, a slight depressant effect on dV/dt was evident in 2 of 6 preparations. The duration of the action potential was not affected by this concentration of the two analogues.

High concentrations of the drug (8×10^{-5} M) were required to depress the action potential to a significant extent. In 12 preparations, 6 exposed to harmine and the others to harmane, the AAP and the dV/dt were always clearly depressed within the first 30 min of superfusion with the drug, while MRP was not affected. The duration of the action potential (APD) was not modified: Figure 4 makes clear that even this highest concentration of the two drugs did not modify APD measured at 20%, 50% and 80% of repolarization.

The stimulatory effect of harmala alkaloids on AAP of atrial muscle is due to a catechol-mediated enhancement of the slow component of the upstroke (see Discussion). It was therefore reasonable to assume that the effect of harmine on AAP of ventricular muscle would be abolished by a β-adrenoceptor blocking agent. This was verified in four preparations superfused with Tyrode containing propranolol (±-propranolol, Sigma Chemical Co.) 2×10^{-6} M. Propranolol did not affect MRP (-84.5 ± 0.4 mV), AAP (117.3 ± 0.8 mV) and dV/dt (196.4 ± 4.5 V/s), which also remained unmodified throughout the exposure to harmine 2×10^{-5} M and the recovery in the presence of propranolol.

If harmine 2×10^{-5} M enhances the AAP of ventricular muscle through a stimulation of the slow component of the upstroke, it should also enhance the amplitude of the action potentials of fibres in which the fast system has been inactivated and the

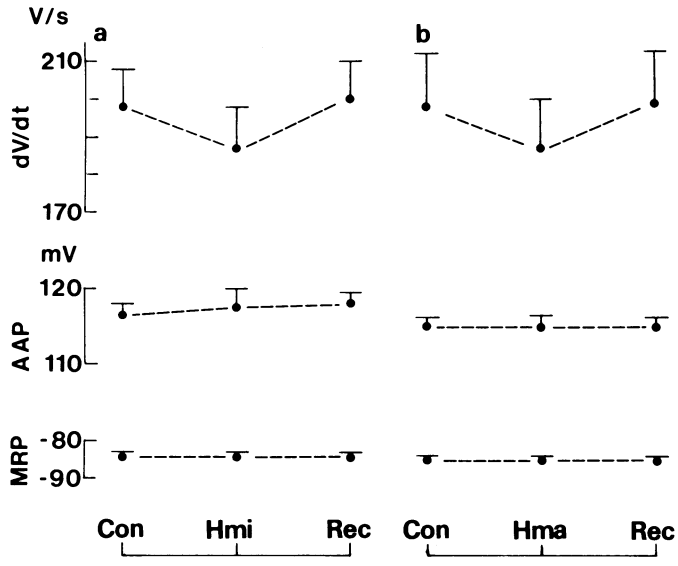

Figure 3 Effects of harmine (a) and harmane (b) 4×10^{-5} M on membrane potentials of papillary muscles. Each value is the mean of values obtained from 6 fibres, each one from a different muscle; vertical lines show s.e.mean. dV/dt = maximum velocity of the upstroke. Other abbreviations are as in Figure 2.

upstroke is determined only by the slow component (slow responses). The following results show that this is true. Six preparations were superfused with 21.6 mM K^+-Tyrode. As expected, the MRP fell to a low value (-56.3 ± 0.9 mV) and the fibres became unexcitable even when driven at 6/min with pulses that were 6 V in amplitude and 5 ms in duration. The preparations were then superfused with the high K^+-Tyrode now containing noradrenaline 3×10^{-6} M. Noradrenaline restored rapidly action potentials that increased progressively in size until responses of constant amplitude with a well developed OS (23.7 ± 3.3 mV) were obtained, while the MRP remained unchanged. Under the influence of harmine 2×10^{-5} M, the OS of these slow responses increased by 8.8 ± 2.1 mV within 30 min. The effect was reversible: 30 min after withdrawal of the drug the OS (25.0 ± 3.0 mV) was not different from control.

The lack of effect of harmane 2×10^{-5} M on AAP of fibres superfused with 5.4 mM K^+-Tyrode suggested that the stimulatory effect on slow responses would also be markedly reduced. This was found to be true: harmane did not enhance significantly the OS of slow responses. In six preparations the OS was 25.8 ± 2.3 mV during control in high K^+-Tyrode plus noradrenaline and 28.0 ± 2.1 mV at the end of 30 min of exposure to the drug.

In all 12 experiments in which the preparations were superfused with high K^+-Tyrode plus noradrenaline, once the recovery traces were obtained, the muscles were exposed to verapamil 1 mg/l. This was done as a control procedure to verify that the responses could be abolished by the suppressive action of the drug on the slowly activated current that determines the upstroke in fibres in which the fast system has been inactivated. The responses were always suppressed by verapamil and all preparations became unexcitable. Upon removal of verapamil the responses recovered slowly, as expected in view of the prolonged effect of verapamil on the slow current (Shigenobu, Schneider & Sperelakis, 1974).

Discussion

The upstroke of the action potential of rat and guinea-pig cardiac muscle has two components: the initial 'fast component' is due to an increase in the sodium conductance g_{Na+} and it is followed by a 'slow component' determined by the Ca^{2+}-dependent slowly activated current I_{si} (Coraboeuf & Vassort, 1968). The harmala alkaloids, harmaline and harmine, share a dual effect on the upstroke of the action potential of rat and guinea-pig atrial muscle. The two analogues depress the g_{Na+}-dependent fast depolarization (Carpentier & Narvarte, 1975; Iven & Zetler, 1974) and enhance the slow depolarization determined by I_{si} (Carpentier, Narvarte & Sanhueza, 1977; Carpentier, 1980a). The results presented here demonstrate that harmine exerts the same dual effect on guinea-pig ventricular muscle. Harmine

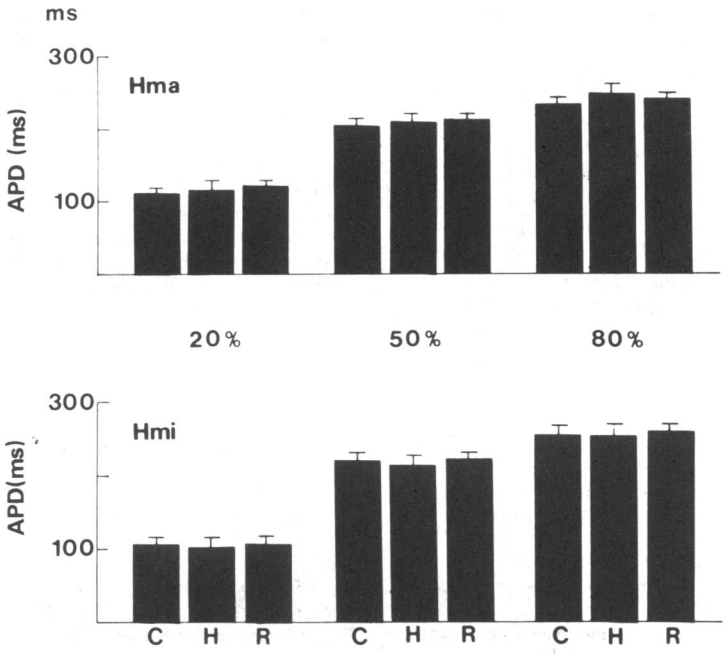

Figure 4 Effects of harmine (Hmi) and harmane (Hma) 8×10^{-5} M on the duration of the action potential (APD) measured at 20%, 50% and 80% of repolarization. Each column is the mean of values obtained from 6 experiments during control (C), at the end of a 30 min exposure to the drug (H), and 30 min after withdrawal of the drug (R). Vertical lines show s.e.mean.

2×10^{-5} M enhanced the amplitude of normal action potentials as well as the amplitude of responses restored by noradrenaline in K-depolarized fibres, in which the fast g_{Na} component of the upstroke has been inactivated. The upstroke of such responses has only the slow component determined by I_{si} and it should be suppressed, as verified in our experiments, by verapamil (Shigenobu, Schneider & Sperelakis, 1974). The stimulatory effect of harmine described here mimics the effect of an increase in the concentration of catecholamines and also the catechol-mediated effect of harmaline on rat and guinea-pig atrial muscle (Carpentier *et al.*, 1977; Carpentier, 1980a) and it is blocked by propranolol.

Our results demonstrate that the stimulatory effect of harmine on the amplitude of normal ventricular action potentials is absent when the methoxy group is removed from the molecule. The first noticeable effect of harmane, observed in two of the fibres exposed to 4×10^{-5} M, was already depressant. A further increase in the concentration of harmane or harmine (8×10^{-5} M) clearly depressed both the maximum velocity of the upstroke and the amplitude of the action potential. This result is in agreement with those obtained with harmaline and harmine in atrial muscle (Iven & Zetler, 1974; Carpentier & Narvarte, 1975; Carpentier, 1980a). The lack of stimulant action by harmane suggested that the stimulatory effect on the slow component of the upstroke was greatly reduced, it not abolished, by the removal of the methoxy group from the molecule. The results obtained with fibres depolarized by high $[K]_o$ indicate that this is true: the amplitude of the responses elicited by noradrenaline was clearly enhanced by harmine while it was little affected by harmane.

The two harmala alkaloids tested in the experiments presented here did not prolong the duration of the action potential (APD) of ventricular muscle. Harmine in concentrations between 1 and 8×10^{-5} M always prolongs APD measured at 20%, 50% and 80% of repolarization in guinea-pig atrial fibres driven at different rates (Iven & Zetler, 1974; Carpentier, 1980a,b). Harmaline 8×10^{-5} M also increases APD at all three levels of repolarization in rat atrial beating spontaneously or driven at a constant rate (Carpentier & Narvarte, 1975). The present results confirm previous observations indicating that harmine does not affect APD of ventricular muscle (Brasch *et al.*, 1977). This applies also to harmane and may be true for harmala alkaloids in general.

In summary: (1) removal of the methoxy group from the indole nucleus of harmine abolished the stimulatory effect of a low concentration of the drug

(2×10^{-5} M) on the amplitude of the action potential (AAP) of guinea-pig papillary muscle. The AAP was not modified by this low concentration of the analogue, harmane and only the depressant effect of a higher concentration of the drug was evident. The lack of enhancement of AAP by harmane can be explained by the fact that the removal of the methoxy group reduced markedly the stimulant action of the drug on the slow component of the upstroke. In other words, the introduction of the methoxy group enhances the catechol-mediated effect of harmala alkaloids. The depressant effect of a higher concentration of the drug persisted. (2) Contrary to what occurs in atrial muscle, harmine or harmane in concentrations as high as 8×10^{-5} M did not diminish the velocity of repolarization of ventricular muscle.

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