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Reduction of spike frequency adaptation and blockade of M-current in rat CA1 pyramidal neurones by linopirdine (DuP 996), a neurotransmitter release enhancer

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1 Linopirdine (DuP 996) has been shown to enhance depolarization-induced release of several neurotransmitters in the CNS through a mechanism which may involve K^+ channel blockade. The electrophysiological effects of linopirdine were therefore investigated directly, by use of conventional voltage recording and single electrode voltage-clamp.

2 Linopirdine (10 μM) reduced spike frequency adaptation (SFA) in rat hippocampal CA1 pyramidal neurones *in vitro*. The reduction of SFA comprised an increase in number of spikes and a reduction in inter-spike intervals after the first, but with no effect on time to first spike. Linopirdine also caused a voltage-dependent depolarization of resting membrane potential (RMP).

3 M-current (I_M), a current known to underlie SFA and to set RMP, was blocked by linopirdine in a reversible, concentration-dependent manner ($IC_{50}=8.5$ μM). This block was not reversed by atropine (10 μM).

4 Linopirdine did not affect I_Q, the slow after-hyperpolarization following a spike train, or spike duration.

5 Linopirdine may represent a novel class of K^+ blocker with relative selectivity for the M-current. This block of I_M is consistent with the suggestion from a previous study that linopirdine may affect a tetraethylammonium-sensitive channel, and it could be speculated that I_M blockade may be involved with the enhancement of neurotransmitter release by linopirdine.

Keywords: Linopirdine; DuP 996; acetylcholine; neurotransmitter release; K^+ current; M-current; electrophysiology; voltage-clamp; spike frequency adaptation; CA1 hippocampal neurone

Introduction

Linopirdine (DuP 996; 3,3-*bis*(4-pyridinylmethyl)-1-phenylindolin-2-one) has been shown to enhance the release of acetylcholine (ACh) from rat brain slices *in vitro* in response to a pulse of high K^+ (Nickolson *et al.*, 1990), without affecting basal release. Linopirdine also increases ACh release *in vivo*, as demonstrated by microdialysis studies in freely moving rats (Marynowski *et al.*, 1993; Smith *et al.*, 1993). In addition to ACh, linopirdine has been shown to enhance the *in vitro* release of dopamine and 5-hydroxytryptamine (5-HT), and, to a lesser extent, γ-aminobutyric acid and glutamate (Nickolson *et al.*, 1990). The enhanced release of one or more neurotransmitters may account for the activity linopirdine displays in behavioural models of learning and memory (Cook *et al.*, 1990).

Despite this interesting pharmacological profile of linopirdine, and despite the potential use of this compound for studying the presynaptic processes involved in release, the mechanism by which it enhances stimulus-evoked release of neurotransmitters remains poorly defined. Recent studies utilizing hippocampal synaptosomes (Vickroy, 1993) or cerebral cortical slices (Maciag *et al.*, 1994) have suggested that linopirdine-induced enhancement of central ACh release may involve K^+ channel blockade. Maciag *et al.* (1994) found that the potassium concentration-response curve for release enhancement was shifted to the left by linopirdine, suggesting an enhancement of neuronal excitability. It could therefore be speculated that linopirdine may block K^+ conductance and thus raise the level of cell excitability. To study this possibility further, we have investigated the electrophysiological effects of linopirdine on rat CA1 hippocampal neurones *in vitro*. Since K^+ currents play a major role in determining neuronal firing patterns and the shape of the action potential (AP), these neuronal properties were initially examined. Based on the results of these initial studies, and on the suggestion by Maciag *et al.* (1994) that linopirdine may affect a channel sensitive to tetraethylammonium (TEA), the effects of linopirdine on the M-current (I_M; Brown & Adams, 1980) were then studied in hippocampal CA1 neurones, by use of the single electrode voltage-clamp technique. Portions of this work have previously been published in a preliminary form (Lampe & Brown, 1991; Aiken & Brown, 1994).

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Methods

Slice preparation

Pathogen-free male CD rats from Charles River (Wilmington, M.A., U.S.A.), weighing 100-200 g, were anaesthetized with either halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) or Metofane (2,2-dichloro-1,1-difluoroethylmethyl ether). After decapitation, the brain was rapidly excised and submerged in an ice-cold oxygenated physiological solution while one hippocampus was removed. Transverse slices (400 μm thick) were prepared on a McIlwain tissue chopper (Mickle Laboratory Engineering Co., Gomshall, Surrey), and transferred to a Perspex holding chamber (Medical Systems Corp., Greenvale, N.Y., U.S.A.) filled with an oxygenated physiological solution (room temperature, 23°C). The physiological solution for both dissection and recording was of the following composition (mM): NaCl 127.0, $NaHCO_3$ 26.0, KCl 3.0, $CaCl_2$ 2.5, NaH_2PO_4 1.25, $MgSO_4$ 1.0 and glucose 10.0, gassed with 5% carbon dioxide in oxygen (pH 7.35).

Recording

Hippocampal slices were placed on a nylon mesh in a submersion-type recording chamber (Medical Systems), and pinned to the Sylgard base. Oxygenated physiological solution (35°C in spike frequency adaption experiments, 23°C otherwise) was superfused at 1–3 ml min^{-1}. A pellet of Ag/AgCl in the solution provided a reference ground.

Microelectrodes were pulled from borosilicate glass (1.2 mm/0.68 mm outer/inner diameter; A-M Systems, Inc., Everett, W.A., U.S.A.) with a Sutter P-80/PC electrode puller (Sutter Instruments, Novato, C.A., U.S.A.). Resistances were 60–100 MΩ when filled with 4 M potassium acetate (neuronal firing experiments) or 50–80 MΩ when filled with 3 M KCl (voltage-clamp recordings). Cell recordings were obtained by means of an Axoclamp-2A amplifier (Axon Instruments, Inc., Foster City, C.A., U.S.A.). A chloride-coated silver wire formed the bridge from electrode to headstage.

Impalements were obtained in 'bridge' mode. The following criteria were used to decide the suitability of a CA1 pyramidal neurone for recording: resting membrane potential (RMP) negative of −55 mV, input resistance >20 MΩ (as measured at RMP by a 500 pA hyperpolarizing pulse), and APs that overshot 0 mV. In general, RMPs were negative of −60 mV and input resistances were >70 MΩ. Having obtained a stable recording, TTX (500 nM) was added to eliminate Na^+ current (except in neuronal firing experiments). Cells were then voltage clamped in discontinuous mode, using a 2.0 kHz switching frequency (30% duty cycle). This relatively low rate gave satisfatory voltage control for the slow kinetics of the currents under study. The monitor output was observed on an oscilloscope to ensure adequate 'settling' of the clamp, and cells were not used if the settling characteristics of the monitor output were not adequate when the cell was depolarized to −30 mV. In between voltage-clamp protocols, RMP was monitored by chart recorder.

Specific K^+ currents were recorded by means of pCLAMP software (version 6.0.1, Axon Instruments), and each episode obtained represented the mean of 6–8 individual runs. Except where stated, application time for drugs was 30 min. Spike frequency adaptation (SFA) was examined by applying depolarizing pulses in 'bridge' mode, digitizing the data and storing it on video tape for off-line analysis.

Statistics

In SFA experiments, an analysis of covariance with control response as the covariate and drug as the class variable was used to determine whether linopirdine exerted a significant effect on the number of spikes or time to first spike over a range of stimulus current intensities, and on inter-spike interval over the first five spike intervals. In SFA and I_M experiments, Student's two-tailed t test for paired data was used to compare mean values for the amplitude of the slow after-hyperpolarization and I_M amplitude and time constant, before and after linopirdine administration. Student's two-tailed t test for paired data was also used to compare the effect of linopirdine on I_M amplitude before and after administration of atropine, and the changes in spike duration in response to linopirdine and TEA. A P value <0.05 was considered to indicate a significant difference. Data values are expressed as mean ± s.e.mean.

Drugs

Linopirdine (free base) was synthesized at The DuPont Merck Pharmaceutical Company (Wilmington, D.E., U.S.A.). A stock solution in 0.1 N HCl was prepared immediately before use and added directly to the superfusing solution. TTX was obtained from Calbiochem (La Jolla, C.A., U.S.A.). Other chemicals were from J.T. Baker, Inc. (Phillipsburg, N.J., U.S.A.) or EM Science (Gibbstown, N.J., U.S.A.).

Results

Effect of linopirdine on spike frequency adaptation

Since AP arrival at the presynaptic terminal evokes neurotransmitter release, and since linopirdine is known to enhance depolarization-induced transmitter release (Nickolson *et al.*, 1990), its effects on repetitive firing behaviour were initally examined. Under control conditions, injection of depolarizing current resulted in initial discharge of APs followed by a period of quiescence (spike frequency adaptation; accommodation; Figure 1a). In the presence of linopirdine (10 μM) however, neuronal firing continued throughout the depolarizing current pulse (Figure 1b). Subsequent administration of atropine (5 μM) had no effect on linopirdine-induced reduction in SFA.

Specific K^+ currents are believed to underlie various portions of SFA (for review, see Storm, 1990). To gain insight into which of these currents may be affected by linopirdine, a more detailed analysis of its effects on the number of spikes, the time required for firing of the first spike, inter-spike intervals, and amplitude of the cumulative slow after-hyperpolarization (sAHP) subsequent to depolarizing current injection was performed, in 9 CA1 pyramidal neurones.

Increasing the strength of the depolarizing current over the range of 200–600 pA increased the number of APs in the initial discharge (Figure 2a), without preventing the subsequent slowing and cessation of firing. Linopirdine increased the mean number of spikes elicited by each of the current intensities examined (Figure 2a). Increasing the depolarizing current intensity reduced the delay before the firing of the first spike (Figure 2b). The effect of linopirdine on this parameter was highly variable: in 4 of 9 cells, linopirdine reduced the time to first spike; in 1 of 9 cells, linopirdine increased time to first spike; and in 4 of 9 cells there was no effect. The averaged response (Figure 2b), therefore, indicated no significant change.

At all stimulus intensities, inter-spike intervals became progressively longer with each successive AP, as shown in

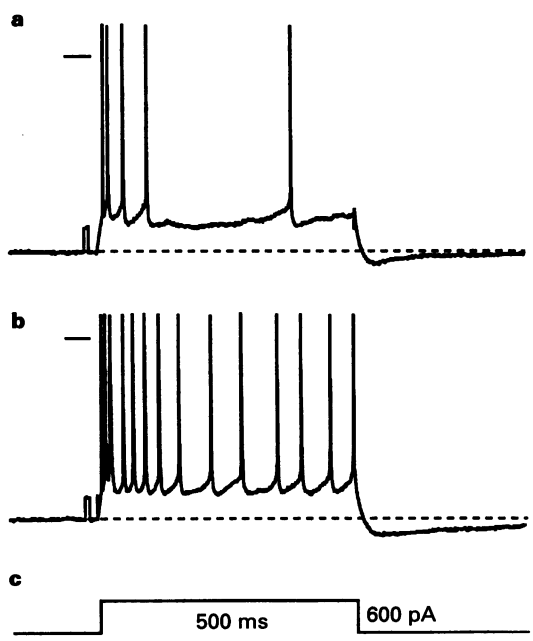

Figure 1 Response of a CA1 pyramidal cell to a pulse of depolarizing current (600 pA). (a) Spike frequency adaptation (SFA) under control conditions. The horizontal bar represents 0 mV. The broken line represents RMP. A short 10 mV calibration pulse is seen prior to the depolarization. (b) Linopirdine (10 μM) reduces SFA. (c) The current pulse applied.

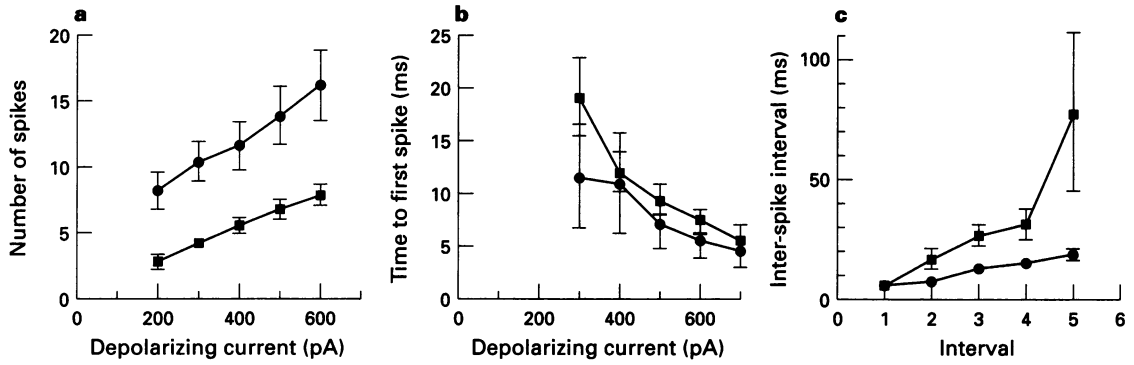

Figure 2 Analyses of parameters relating to spike frequency adaptation. All points represent the mean ± s.e.mean of data from 9 neurones. (a) Number of spikes recorded during 500 ms pulses of varying amplitude, before (■) and after linopirdine (10 μM; ●). (b) Time to first spike, as a function of depolarizing current amplitude. (c) Inter-spike interval using a 500 pA pulse, plotted as a function of the interval number. See text for statistical analysis.

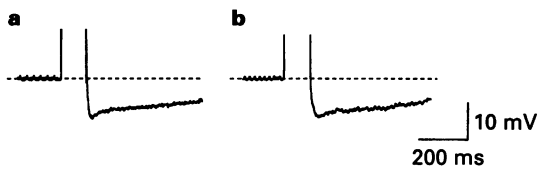

Figure 3 The slow after-hyperpolarization (sAHP) following a 500 ms depolarizing current pulse of 800 pA before (a) and of 400 pA after (b) linopirdine (10 μM). Each pulse elicited a train of 9 action potentials. The broken line represents RMP (−63 mV).

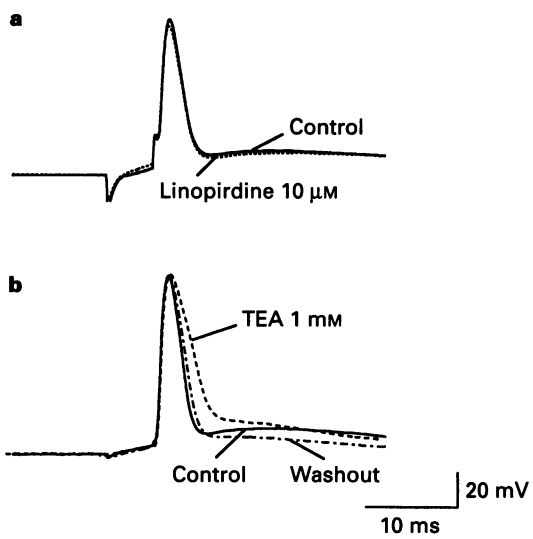

Figure 4 (a) Effect of linopirdine (10 μM) on a single AP elicited by a 5 ms depolarizing pulse (RMP = −74 mV). (b) Effect of tetraethylammonium (TEA, 1 mM) on a single AP elicited by a 5 ms depolarizing pulse in a different cell (RMP = −72 mV).

Figure 2c for 600pA. This pattern of progressive prolongation of inter-spike intervals was maintained in the presence of linopirdine, although at significantly shorter intervals than in the pretreatment controls (except for the first, Figure 2c).

The sAHP that occurred at the offset of long depolarizing current pulses was not abolished by linopirdine. In fact, the sAHP often appeared larger in the presence of linopirdine (see Figure 1). Because the amplitude of the cumulative sAHP is dependent on the number of APs which precede it (Madison & Nicoll, 1984; Lancaster & Adams, 1986), a comparison of sAHP amplitudes in the absence and presence of linopirdine was made where there were equal spike numbers. A representative example of the sAHP following a pulse which elicited 9 APs both before (800 pA/500 ms) and 30 min after (400 pA/500 ms) linopirdine (10 μM) is illustrated in Figure 3. The results indicate that, when controlled for spike number, linopirdine had no significant effect on the peak amplitude of the cumulative sAHP (7.4 ± 0.9 mV for control and 6.6 ± 0.9 mV after linopirdine; $n = 14$). RMP in these cells was also not significantly altered by linopirdine (−71.7 ± 1.4 mV for control and −71.5 ± 2.1 mV after linopirdine; $n = 9$). Thus, analysis of SFA data indicated that linopirdine had little or no effect on either the kinetically rapid (time to first spike/I_A, I_D; first inter-spike interval/I_C) or the kinetically slow (sAHP/I_{AHP}) components. Linopirdine primarily affected a kinetically intermediate component of SFA.

Effect of linopirdine on action potential duration

To examine further whether linopirdine affected the rapid components of neuronal excitability, its effects on AP duration were examined. Single spikes were evoked by injection of a 5 ms depolarizing current pulse at just above threshold intensity (usually 200–800 pA) and spike duration was determined as the time from onset to 75% repolarization of the AP. Mean spike duration (at 23°C) was 3.8 ± 0.18 ms in untreated neurones ($n = 8$) and 3.8 ± 0.16 ms after a 30 min exposure to linopirdine (10 μM). Input resistance, as determined from the voltage response to a 500 pA hyperpolarizing pulse at RMP, was not significantly altered by linopirdine in these experiments (72.4 ± 5.3 MΩ in control, 70.4 ± 7.2 MΩ with linopirdine; $n = 7$).

In contrast to linopirdine, TEA (1 mM) significantly increased spike duration, from 3.3 ± 0.08 ms in untreated neurones ($n = 6$) to 5.0 ± 0.35 ms, an increase of 50.1 ± 8.4% ($P < 0.01$). The TEA-induced prolongation of AP duration was readily reversible upon washout. Typical APs from a neurone treated with linopirdine and one treated with TEA are shown in Figure 4. These data suggest that I_A and I_C, the currents mainly responsible for regulating spike repolarization (Warman *et al.*, 1994), were not affected by linopirdine at this concentration (10 μM). Thus, I_M, the current believed to underlie the kinetically intermediate component of SFA (Storm, 1990), was chosen for further study.

Effect of linopirdine on I_M

I_M was recorded in voltage-clamped pyramidal neurones by stepping to −40 mV or −30 mV for 1 s from a holding potential of −70 mV, repolarizing by 20 mV for 1 s, and then returning the cell to −40 mV or −30 mV for an additional 1 s. I_M is inactive at −70 mV (Brown *et al.*, 1989), and is

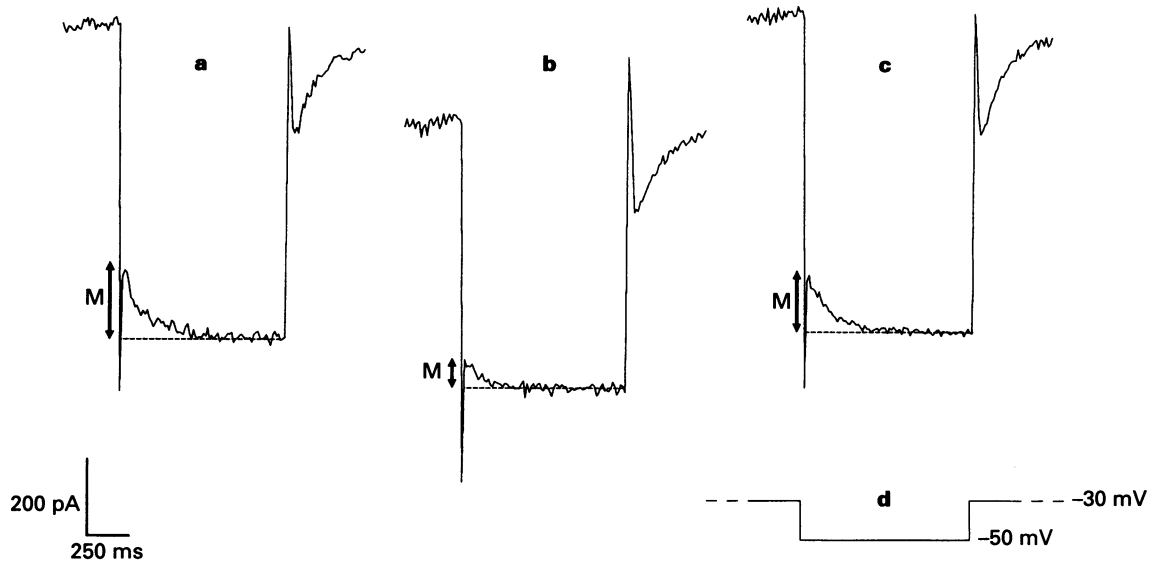

Figure 5 Effect of linopirdine (10 μM) on I_M, recorded by single electrode voltage-clamp. The arrows labelled 'M' represent the current measured as I_M. (a) Control Note the reduction in I_M with linopirdine (b), which is reversed on washout (c), and also note the downward 'shift' of the current recording in (b), which represents a reduction in the current required to clamp the cell at the depolarized potential. (d) Voltage protocol used (holding potential −70 mV).

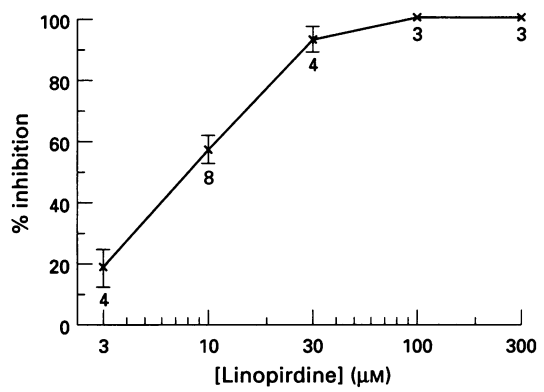

Figure 6 Concentration-response curve for the inhibition of I_M by linopirdine. The number of determinations is indicated below each point.

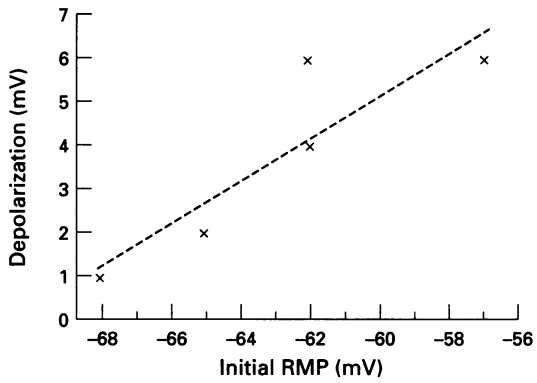

Figure 7 The relationship between degree of depolarization c linopirdine and initial RMP. Data points are shown fr individual cells treated with linopirdine (10 μM). Only results cells where a reliable RMP record was obtained are plotted straight line drawn has correlation coefficient 0.8907 (least sc analysis).

activated by the depolarization to −40 mV or −30 mV. When the cell is repolarized by 20 mV (*i.e.* to −60 mV or −50 mV, respectively), I_M deactivated in a mono-exponential fashion, and can be seen as the slow component of the response following the 20 mV repolarization step (Figure 5a). The amplitude of I_M was measured by extrapolating this curve (Clampfit, Axon Instruments) to meet the onset of the repolarization step, as indicated by the arrows. Time constants for current deactivation were calculated from these curves and the values (typically 120–190 ms) compared well with other reported values (*e.g.* 118–176 ms in guinea-pig CA1 pyramidal cells at 23°C; Halliwell & Adams, 1982). The voltage-dependency of this current was also consistent with literature reports, and the current was inhibited by carbachol (50 μM; data not shown). Based upon these observations, this current was assumed to be I_M.

Figure 5b shows a representative example of the effect of 10 μM linopirdine on I_M. In this particular cell, linopirdine reduced I_M by 60.2%, without significantly affecting its decay constant or voltage-dependence. In 8 neurones, linopirdine (10 μM) reduced I_M from 212 ± 36 pA to 86 ± 10 pA ($P < 0.01$), a reduction equivalent to 56.2 ± 4.6% of control ($P < 0.01$). The effect of linopirdine on I_M was reversible upon washout (Figure 5c), typically taking 45–60 min. Figure 6 show concentration-response relationship for the effect of pirdine (3–300 μM) on I_M. Complete block of I_M was se 100 μM and 300 μM linopirdine (and at 30 μM in 2 of 4 An IC_{50} value of 8.5 μM was calculated for I_M inhib However, linopirdine had no significant effect on the constant of I_M deactivation (167 ± 4 ms in control, 16(with 10 μM linopirdine; $n = 8$).

In some cells used for I_M recording, a depolarizatic RMP was seen with 10 μM linopirdine, which was reversit washing. However, it was noted that the amount of dep ization in a particular cell seemed to be related to its i RMP (Figure 7), suggesting that the depolarization was d block of a current that activated at membrane pote slightly positive of normal RMP (possibly I_M), rather block of a 'leak' conductance active at RMP (Benson e 1988).

It was of interest to know whether the effect of linopi on I_M was mediated in any way via muscarinic reporters, these receptors are responsible for regulation of the cu

under physiological circumstances (Brown & Adams, 1980). Cells were treated with linopirdine (10 μM, 30 min) and atropine (10 μM) was then applied. After 20 min exposure to atropine in the presence of linopirdine, I_M was not significantly changed compared to recordings with linopirdine alone (Figure 8). However, when the linopirdine and atropine were washed out, I_M returned almost to control levels (data not shown), demonstrating that it was not irreversibly blocked or run down in these cells. These results suggest that the action of linopirdine in blocking I_M was not dependent on muscarinic receptors.

Effect of linopirdine on other currents

The mixed Na^+/K^+ current, I_Q, was examined in the same neurones treated with linopirdine, by stepping from a −60 mV holding potential to −90 mV for 1 s. I_Q was not affected by linopirdine (Figure 9). This lack of effect on the Na^+/K^+ conductance, coupled with linopirdine's inability to block the sAHP (Figure 3) or alter spike duration (Figure 4a), indicates a relative selectivity for the block of I_M by linopirdine.

Discussion

The objective of the present investigation was to identify the electrophysiological actions of linopirdine, a compound that has been shown to enhance the release of ACh and other neurotransmitters in the CNS (Nickolson *et al.*, 1990). Our primary findings, in rat CA1 hippocampal neurones, were that linopirdine: (1) altered neuronal firing patterns as evidenced by an inhibition of SFA; (2) caused a small depolarization of RMP in cells with RMPs positive to −68 mV; and (3) potently and reversibly blocked the M-current, I_M. Since the functional role of I_M includes involvement in SFA (Madison & Nicoll, 1984) and 'clamping' of the membrane potential at rest to resist depolarizing input (Brown, 1988), the block of I_M observed with linopirdine could explain each of the first two primary electrophysiological effects noted above.

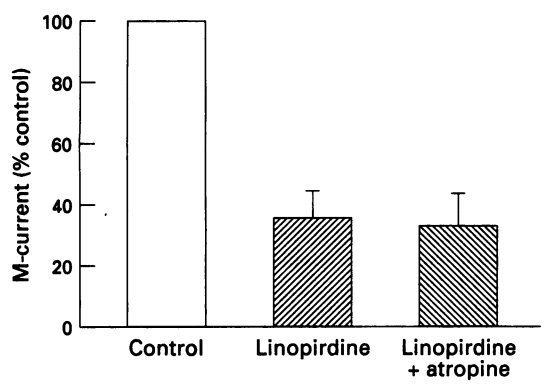

Figure 8 Block of I_M by linopirdine (10 μM) is not reversed by atropine (10 μM). Means ± s.e.mean are shown, $n = 3$.

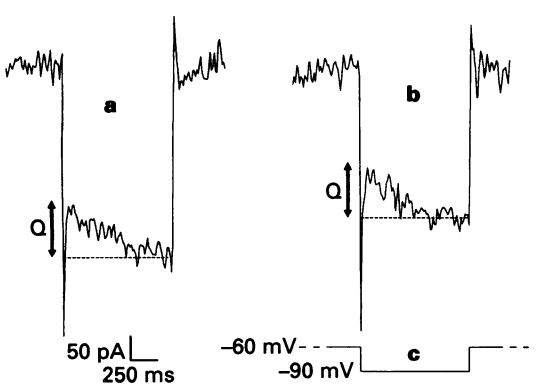

Figure 9 (a) Typical I_Q recorded from a CA1 pyramidal cell before and after (b) treatment with linopirdine (10 μM). (c) The voltage protocol used.

Repetitive discharge of CA1 pyramidal cells is governed by several K^+ currents, including I_A (Nakajima *et al.*, 1986), I_D (Storm, 1988), I_C (Lancaster & Nicoll, 1987), I_{AHP} (Madison & Nicoll, 1984) and I_M (Brown *et al.*, 1982). It is unlikely that reduction of SFA by linopirdine was primarily mediated by block of I_A, I_D or I_C, since time to first spike (Figure 2b), the first inter-spike interval (Figure 2c) and AP duration (Figure 4a) were not significantly affected. It is more likely that linopirdine reduced SFA by attenuating a more slowly activating K^+ conductance (I_{AHP}, I_K, I_M), since late inter-spike intervals were more affected by the drug than early ones (Figure 2c).

The Ca^{2+}-activated K^+ conductance, I_{AHP}, would be expected to be more developed late in a train of APs. However, measurements of after-hyperpolarizations indicated no significant reduction after application of linopirdine. An action of linopirdine on I_K remains a possibility, although the exact physiological function of I_K in CA1 neurones remains uncertain (Storm, 1990; Warman *et al.*, 1994). Frey *et al.* (1991) reported that linopirdine did block I_K and I_A in cultured neocortical neurones, but at much higher concentrations than those used in the present study (IC_{50}s ≈ 300 μM). Thus, while actions on other K^+ currents have not yet been totally excluded, the attenuation of I_M by linopirdine offers the most likely explanation for the drug's observed effects on SFA. Furthermore, the lack of effect of linopirdine (10 μM) on the sAHP (Figure 3) or on the mixed Na^+/K^+ current, I_Q (Figure 9), coupled with the indirect evidence that it does not block I_C or I_A at this concentration (Figure 2, Figure 4a) suggests that the drug is not merely a non-selective cation channel blocker but has relative specificity for I_M. The finding that linopirdine blocks I_M substantiates the report by Maciag *et al.* (1994) that a TEA-sensitive channel may be involved in the action of linopirdine, since I_M is known to be TEA-sensitive (Storm, 1989).

Another electrophysiological effect of linopirdine, membrane depolarization (Figure 7), also can be explained by I_M inhibition. Since input resistance at RMP was not decreased by linopirdine, it is unlikely that an outward current active at RMP was being blocked (*e.g.* the K^+ 'leak' conductance described by Benson *et al.*, 1988).

The discovery of a compound which blocks I_M with reasonable potency has importance beyond understanding the actions of linopirdine. Thus far, the only compounds that have been shown to block I_M in mammalian neurones are either receptor agonists (*e.g.* muscarine, 5-HT, luteinizing hormone-releasing hormone, bradykinin), non-specific channel blockers (*e.g.* TEA, Ba^{2+}) or compounds with mixed pharmacological actions (*e.g.* caffeine; Pfaffinger *et al.*, 1988; Schäfer *et al.*, 1991; for review, see Brown, 1988). Such compounds are of only limited use in electrophysiological experiments aimed at isolating I_M, since their effects on I_M cannot be separated from other effects mediated via their own receptors or via block of other channels. In the case of receptor agonists, block of I_M is likely to be a secondary action. Even muscarinic agonists such as carbachol, after which I_M was named, block other K^+ conductances (*e.g.* I_{AHP}; Benardo & Prince, 1982) and activate intracellular second messengers, at lower concentrations than those required to block I_M (Dutar & Nicoll, 1988). If results of further studies support the selectivity of action suggested here, linopirdine may represent the first generation of compounds that selectively and potently block I_M.

The block of I_M by linopirdine was not reversed by atropine (Figure 8), suggesting that the mechanism of block does not involve the activation of muscarinic receptors. A direct action of linopirdine at this receptor was considered unlikely in any case, since Nickolson *et al.* (1990) reported that linopirdine at

this concentration (10 μM) caused only approximately 5% displacement of [^{3}H]-3-quinuclidinyl benzilate from its binding site on rat brain membranes. This binding study makes it doubtful that the negative effect of atropine in the present work was due simply to the fact that the linopirdine (added first) was bound so tightly to muscarinic receptors that it could not be displaced by the atropine. The inability of atropine to reverse the linopirdine-induced block of I_M also excludes an indirect action of linopirdine, on other neurones in the slice, which could be transmitted to the impaled neurone via muscarinic receptors. Although the interaction of linopirdine with other receptor sites cannot be ruled out, the presence of TTX in the recording medium makes it very unlikely that the block of I_M by linopirdine was due to effects on neurones other than the one from which recording was made. Apart from the fact that linopirdine does not block I_M via the muscarinic receptor, our knowledge of the subcellular site of action of this drug is uncertain. The drug could be a direct channel blocker, an agonist at other transmitter receptors known to couple to I_M, or it could interact with a G-protein or second messenger system. Further studies are required to identify the site of action, for instance using the single channel recording technique for I_M developed by Stansfeld *et al.* (1993).

In conclusion, linopirdine, a putative cognitive enhancer, has been shown to reduce spike frequency adaptation and to block M-current in CA1 pyramidal neurones of rat hippocampus. The block of I_M may underlie the effect on SFA. It may be speculated that the block of I_M may contribute to the release-enhancing effects of this drug, especially since Maciag *et al.* (1994) have presented evidence that a TEA-sensitive conductance may be involved in the mechanism of action of linopirdine.

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