Pflügers Arch – Eur J Physiol (1997) 435:99–106

ORIGINAL ARTICLE

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Action of the hyperpolarization-activated current (I_h) blocker ZD 7288 in hippocampal CA1 neurons

Received: 14 February 1997 / Received after revision: 4 July 1997 / Accepted: 21 July 1997

Abstract The effects of ZD 7288, a "bradycardic" agent, in young rat hippocampal slices in vitro were studied. ZD 7288 (1–1000 μM) reduced the hyperpolarization-activated current (I_h) in CA1 pyramidal neurons by a voltage-independent blocking mechanism. Under current-clamp conditions, the bradycardic agent (10 μM) caused membrane hyperpolarization (by 5.9 ± 0.5 mV) and a reduction of membrane conductance (by 17.9 ± 4.1%). These data are consistent with the block of an inward current which is active at rest. The drug-induced hyperpolarization depressed the cell's excitability by increasing the threshold current necessary to induce firing. When the drug-induced hyperpolarization was compensated for by injection of a tonic depolarizing current, ZD 7288 caused a reduction of the inhibitory post-synaptic potential (IPSP) in EPSP-IPSP sequences. Since Cs^+, another known blocker of I_h, is able to reverse long-term depression (LTD) of the CA3-CA1 synapse in hippocampal slices, we tested the effect of ZD 7288 on synaptic transmission. We found that ZD 7288 did not significantly modify LTD, suggesting that Cs^+-induced inhibition of LTD maintenance is not directly related to block of I_h.

Key words Hyperpolarization-activated current (I_h) Hippocampal slices · CA1 pyramidal neurons · Bradycardic agents · ZD 7288

Introduction

A hyperpolarization-activated Na^+ and K^+ current (termed I_q/I_h) has been demonstrated in a variety of nerve cells ([1, 3, 27, 29]; reviewed in [26]), as well as in cardiac pacemaker myocytes (current I_f) where its activity was first described (for review see [11]). The basic properties of this current are common to different preparations: I_f/I_h is activated upon hyperpolarization below –40/–50 mV with slow activation kinetics and no inactivation; it is carried by both Na^+ and K^+ ions and has a reversal potential of about –10/–20 mV [9, 13, 23]; it is blocked by extracellular Cs^+ but is insensitive to Ba^{2+} and Cd^{2+} [10, 13, 16]. Since it is an inward current activated by hyperpolarization, I_h produces a Cs^+-dependent slow depolarizing "sag" during prolonged injection of hyperpolarizing current [21]. In some of the tissues examined, this current is responsible for the depolarizing phase of rhythmic-oscillatory activity [6, 24] and its modulation by neurotransmitters plays a key role in controlling heart rate [5, 14] and thalamic firing [24, 27]. In the normally silent hippocampal pyramidal CA1 neurons, I_h is active at rest and contributes a moderate depolarization to the resting membrane potential [21]. Although the I_h contribution, as inferred by Cs^+ block experiments, appears to be small (some 4 mV), I_h strongly affects the excitability of CA1 neurons. The functional relevance of I_h in the hippocampus has been confirmed by the finding that the current is modulated by acetylcholine and noradrenaline [8, 28].

Since the hippocampal I_h shares several properties with the cardiac I_f, we have characterized the action on I_h of ZD 7288 [4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride], a "bradycardic" agent reported to exert a specific blocking action on the cardiac I_f and to slow, by this action, the rate of cardiac pacemaker activity [4]. ZD 7288 has also been shown to reduce I_h in substantia nigra pars compacta neurons [17].

We found that: (1) I_h of CA1 pyramidal neurons is blocked by micromolar concentrations of ZD 7288 in a voltage-independent way; (2) ZD 7288 abolishes the inhibitory phase (IPSP) of the EPSP-IPSP sequence, suggesting that I_h of inhibitory interneurons is involved in this process; (3) the I_h blocking agent does not affect induction and maintenance of long-term depression (LTD), thus excluding a direct correlation between I_h block and LTD (this possibility had been previously suggested based on the action of Cs^+, another blocker of I_h [20]).

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Materials and methods

Slice preparation and solutions

Hippocampal transverse slices were obtained from young male Wistar rats (16–25 day old, Charles River) as described elsewhere [21]. Briefly, rats were anaesthetized with ether and killed by decapitation. The brain was rapidly dissected out in ice-cold solution containing (mM): NaCl 120; KCl 3.1; $MgCl_2$ 4; $CaCl_2$ 1; KH_2PO_4 1.25; $NaHCO_3$ 26; glucose 10. Slices of 300 μm were cut by a vibratome (Tpi Vibratome 1000) and stored in the same saline solution as above, and single slices were transferred to the recording chamber when required. The slice was held by a nylon mash, fully submerged and superfused continuously with an artificial cerebrospinal fluid composed of (mM): NaCl 120; KCl 3.1; $MgCl_2$ 1; $CaCl_2$ 2; KH_2PO_4 1.25; $NaHCO_3$ 26; glucose 10. All solutions were equilibrated with 95% O_2-5% CO_2 to a pH of 7.4. Recordings were performed at room temperature. ZD 7288 was kindly provided by Zeneca Pharmaceuticals, Macclesfield, UK (Dr. I. Briggs).

Electrophysiological recordings

Whole-cell recordings from single CA1 pyramidal neurones were performed at room temperature with the patch-clamp technique in the whole-cell configuration. Seals were obtained using the blind technique [2]. The pipette was filled with (mM): K-gluconate 130; NaCl 10; $MgCl_2$ 1; adenosine-triphosphate (ATP) 2; guanosine triphosphate (GTP) 0.5; *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES) 0.5; ethylenebis(oxonitrilo)tetra-acetic acid (EGTA) 10; to a final pH of 7.2 (resistance 3–8 MΩ). Cells were identified as pyramidal neurons [22] by visual localization of the stratum pyramidale and routine observation of their firing pattern in response to a depolarizing pulse (100 pA for 400 ms).

In some experiments, bipolar concentric electrodes were positioned in the stratum radiatum to stimulate afferent fibres (Schaffer collaterals) and evoke post-synaptic potentials (15–40 μA) or action potentials (35–80 μA) in CA1 pyramidal cells.

The I_h current was measured by applying hyperpolarizing steps of variable duration from a holding potential of –40 mV to the activation range (–100 to –120 mV). Pilot experiments indicated that the whole-cell recording yielded stable I_h traces for sufficiently long times with minimal run-down (6.0 ± 3.3%, n = 5, after 14 min recording), suggesting that dilution of intracellular substances necessary for I_h development was negligible under our experimental conditions.

Extracellular field EPSPs (fEPSPs) in the stratum radiatum were recorded using electrodes filled with the extracellular solution (3–8 MΩ). The initial slope of the fEPSP (fEPSP slope) was used as a measure of synaptic activity. Data are presented as mean ± SEM. The data presented in this work were obtained from 44 slices.

Results

Effect of ZD 7288 on I_h

The resting membrane potential of neurons was about –59 mV (–58.83 ± 0.45 mV, n = 37). As shown in Fig. 1a, hyperpolarizing steps from a holding potential of –35 mV to variable potentials (–40 to –120 mV) elicited a time- and voltage-dependent relaxation (current I_h) [16, 21]. We tested the possible effect of the bradycardic agent ZD 7288 on I_h by superfusing slices with different concentrations of the drug. In the presence of ZD 7288 (10 μM) I_h was partly blocked, as shown in Fig. 1b.

The component blocked by ZD 7288 at different potentials and the resulting current/voltage (*I*/*V*) relation

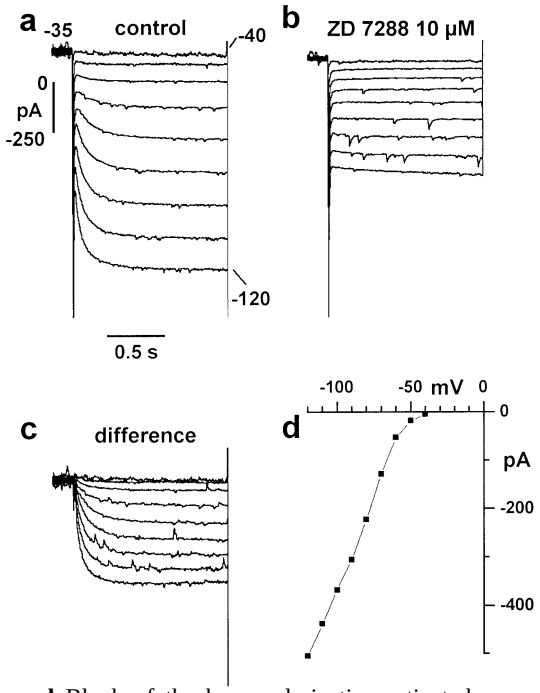

Fig. 1 a–d Block of the hyperpolarization-activated current (I_h) by ZD 7288. **a** Current traces recorded during voltage steps from a holding potential of –35 mV to the range –40/–120 mV, in 10 mV steps; **b** current traces recorded with the same voltage protocol as in **a** in the presence of 10 μM ZD 7288; **c** ZD 7288-sensitive current traces and **d** corresponding current/voltage (*I*/*V*) relation, obtained by plotting steady-state currents

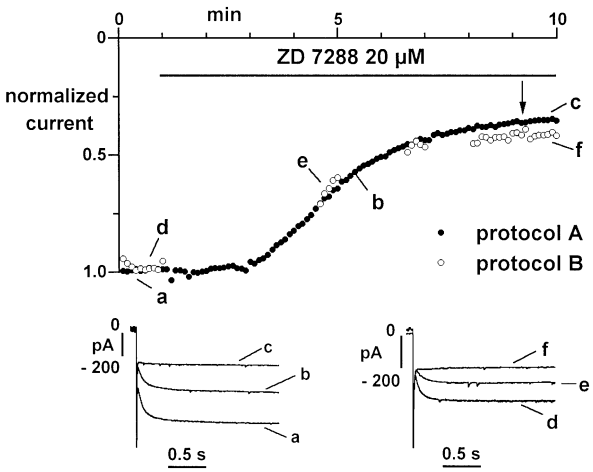

Fig. 2 Time course of I_h amplitude during ZD 7288 application (*bar*) and lack of use dependence of block. *Top*: plot of steady-state current during steps to –100 mV, from the holding potential of –40 mV after leakage subtraction. The step was applied every 6 s for the whole duration of the experiment (*filled circles, protocol A*), whereas in another slice it was interrupted for various periods during the exposure to the drug (*open circles, protocol B*). Both curves are normalized to the maximal current amplitude recorded prior to drug perfusion (A: –720 pA; B: –410 pA). The current reduction at the time marked by an *arrow* (8 min after beginning of drug perfusion, mean of 3 traces) was 63.6% (A) and 58.3% (B). *Bottom*: original traces recorded at times labelled *a*, *b*, *c* (protocol A, *left*) or *d*, *e*, *f* (protocol B, *right*)

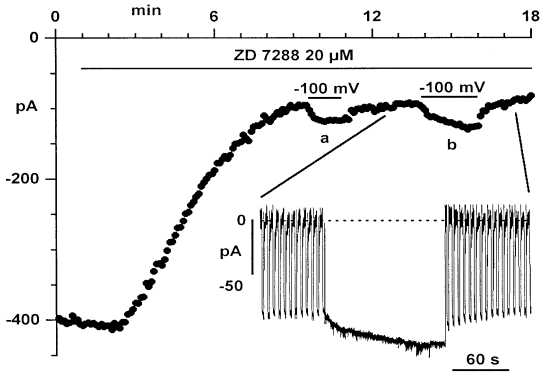

Fig. 3 Hyperpolarization-induced unblock of I_h. Time course of I_h activated by 2-s hyperpolarizing steps from –40 to –100 mV applied every 6 s during ZD 7288 perfusion (20 µM, *bar*). Records were corrected for linear capacitative and leak components. Following full block development, long (1–2 min) hyperpolarizations to –100 mV were applied as indicated by *bars* (*a* and *b*), during which times the current increased slowly. The *inset* shows current traces recorded during protocol b. Data points during prolonged hyperpolarizations represent the I_h amplitude at 6-s intervals. The fractional block by ZD 7288 was 75.9% after an 8.6-min perfusion of the drug. Prolonged hyperpolarizations reduced block by 5.9% (*a*) and 10.2% (*b*), as referred to the control I_h amplitude

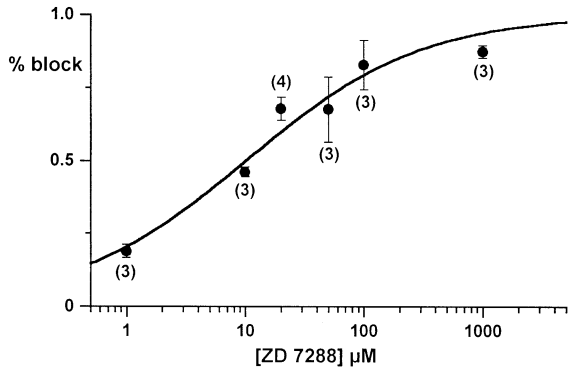

Fig. 4 Dose/response relation for the block by ZD 7288 of I_h from n = 18 cells. Data points were fitted to the Hill equation: $I_{h,b}/I_h = [1 + (k'/C)^n]$, where I_h and $I_{h,b}$ are fully activated current before and after block, respectively, C is the concentration of ZD 7288, and n is the Hill coefficient. Plotted are mean ± SEM values. Maximal fractional block was set to 1. Best fitting values of apparent dissociation constant (k', µM) and Hill factor were 10.5 and 0.6 respectively

are shown in Fig. 1c, d. From the *I*/*V* relation of Fig. 1d, I_h appears to be activated from a threshold near –50 mV, in agreement with previous indications [20].

Properties of block of I_h by ZD 7288 and the dose/response relation

In order to investigate the time course of the block and the dose/response relation, we measured the block induced by different concentrations of ZD 7288 on I_h at a fixed voltage of –100 mV. Hyperpolarizing steps were applied from a holding of –40 mV to –100 mV for 2 s at a rate of 1/6 Hz. Drug perfusion was started after at least 2 min of stable recording. ZD 7288 blocked I_h in a time- and concentration-dependent manner. There was a delay of approximately 2 min from the drug application to the beginning of block development, which was complete in some 10–15 min (Fig. 2). Only limited recovery was visible even after more than 30 min of drug wash-out at all concentrations used (not shown).

Other blockers of I_h, such as the "bradycardic agent" UL-FS 49, act as "open channel" blockers and thus exert a "use-dependent" blockade [12, 25, 30]. We checked for the presence of use dependence in the I_h block by ZD 7288, with the protocol shown in Fig. 2. If the protocol of repetitive (1/6 Hz) activation of I_h by a hyperpolarizing pulse to –100 mV was arrested (open circles, protocol B) and the membrane potential was held at –40 mV, a voltage at which I_h channels are closed [21], ZD 7288 was still effective at blocking I_h. The rate of block development was evaluated by activating the current for short periods of time around the 4th, 6th and 8th minute following drug superfusion. Comparison with the standard protocol (filled circles, protocol A) showed that the block proceeded at a similar rate in the two cases: the current reduction around the 8th minute of drug perfusion with protocol B was 59.2%, which compares with the 65.1% reduction obtained with protocol A; in another experiment we obtained similar results (reduction after 8 min of 75.3% with protocol B as compared to 72.3% with protocol A). These data suggest that ZD 7288 blocks open and closed I_h channels equally well.

We further checked for the presence of a voltage dependence of channel block/unblock by use of the protocol shown in Fig. 3.

Here, a train of 2-s voltage-clamp hyperpolarizing steps to –100 mV was interrupted by long (1–2 min) steps to the same voltage in an attempt to verify if, as reported elsewhere for the block of neuronal h-channels [17] and cardiac f-channels [12] by bradycardic agents, negative voltage also affects the degree of I_h block by ZD 7288 in CA1 cells. The plot in Fig. 3 shows the time course of I_h (at –100 mV) during superfusion of ZD 7288 and repeated applications of prolonged –100 mV steps (1–2 min, bars). As also apparent in the inset of Fig. 3, showing details of current changes during the second prolonged hyperpolarization (b), I_h clearly increased during prolonged hyperpolarizations, and fully recovered to its original size when the normal activating/deactivating protocol was resumed. This is consistent with a partial block relief associated with prolonged membrane hyperpolarization. In four cells, I_h recovered an average 10.8 ± 1.3% of its pre-block amplitude during 2 min hyperpolarizing steps to –100 mV.

Steady-state block of I_h obtained at various ZD 7288 concentrations and estimated with protocols such as the one in Fig. 2 was used to construct the dose/response relation in Fig. 4. Curve fitting by the Hill equation yielded values of 10.5 µM for the half-maximal effective dose (IC_{50}) and 0.59 for the Hill coefficient (n).

Effect of ZD 7288 on membrane properties

Since I_h controls the resting membrane potential and conductance of hippocampal CA1 neurons [21], we studied possible effects of the bradycardic agent on these properties under current-clamp conditions. The membrane conductance was monitored by injecting a hyperpolarizing current pulse every 4 s (–75 pA for 300 ms) (Fig. 5a).

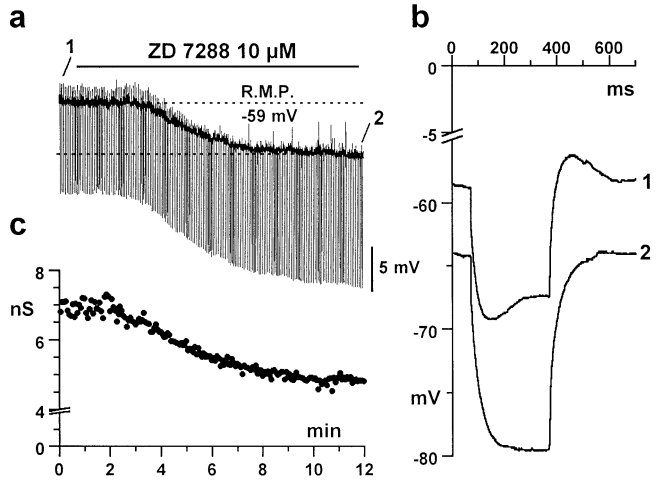

Fig. 5 a–c Effect of ZD 7288 (10 µM) on the membrane conductance and resting potential of a CA1 neuron. **a** Recording of the membrane potential during drug perfusion. Hyperpolarizing current steps (–75 pA for 300 ms) were injected every 4 s to monitor membrane conductance; **b** membrane voltage displacement due to current injection as recorded at the times *1* and *2* indicated in **a**; sag and depolarizing rebound disappear in the presence of ZD 7288; **c** time course of membrane conductance, measured as the ratio between current injected and maximum voltage displacement

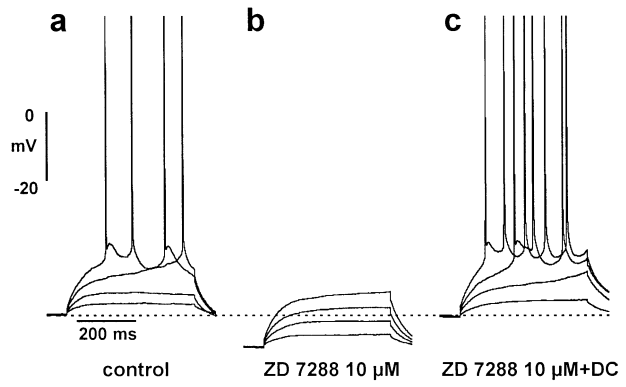

Fig. 6 a–c Action of ZD 7288 on the firing response elicited by depolarizing current (20–80 pA) in a CA1 pyramidal cell. **a** Under control conditions, a 60 pA step was required to evoke a single action potential, whereas an 80 pA step caused repetitive firing; **b** perfusion with ZD 7288 led to a hyperpolarization of resting potential (8 mV) and abolished the action potential; **c** when the drug-induced hyperpolarization was compensated for by the injection of tonic current (30 pA), the firing response was re-established. Action potentials truncated at 30 mV

Under control conditions, the membrane hyperpolarization due to tonic current injection was followed by a slow depolarizing "sag" generated by I_h activation at hyperpolarized potentials (Fig. 5b, trace 1). Following the hyperpolarizing pulse, a depolarizing rebound was also visible due to deactivation of I_h (Fig. 5b, trace 1). After a 1 min period of stable recording, during which the resting membrane potential was –59 mV, perfusion with ZD 7288 (10 µM) hyperpolarized the membrane by about 5 mV. ZD 7288 simultaneously reduced the membrane conductance by 30% (Fig. 5c). In similar experiments, ZD 7288 caused a hyperpolarization of 5.93 ± 0.49 mV (n = 7) and a reduction of membrane conductance of 17.88 ± 4.07% (n = 5).

If measured from brief (50 ms) current steps applied from reference potentials in the range –62/–58 mV, we obtained an increase in input resistance of 24.6 ± 6.0% and an increase in the membrane time constant of 28.0 ± 6.7% (n = 7).

Both the resting potential hyperpolarization and the reduction of membrane conductance are in agreement with the block of an inward current tonically active at rest. Accordingly, block of I_h by ZD 7288 was accompanied by abolition of the sag during hyperpolarizing current injection and of the rebound depolarization (Fig. 5b, trace 2).

Effect of ZD 7288 on CA1 neuron excitability

To investigate possible effects of ZD 7288 on the firing activity of CA1 neurons, we stimulated cells by injecting 450-ms depolarizing current steps of variable intensity (20–80 pA) under control conditions and in the presence of the drug.

In the example of Fig. 6, a current step of 60 pA or larger was required to elicit firing activity under control conditions in a CA1 neuron with resting potential of –62 mV (Fig. 6a). After 8 min of perfusion with ZD 7288 (10 µM) the cell resting voltage had hyperpolarized by 8 mV and injection of current steps up to 80 pA could not generate action potentials (Fig. 6b). The cell's excitability was resumed (Fig. 6c) by restoring the original resting membrane potential with the injection of tonic current (30 pA). In fact, as apparent from the increased rate of spike generation, excitability was higher than under control conditions due to the lower membrane conductance associated with I_h blockade. The ZD 7288-induced decrease in conductance can be appreciated by the larger voltage displacement associated with current injection in Fig. 6c. Similar results were obtained from two more neurons.

The relevance of the activation of I_h at rest to the cell's frequency response was further investigated by injecting prolonged (1.5 s) depolarizing current steps in the presence and in the absence of ZD 7288 (10 µM). In the example of Fig. 7a,b, the activity recorded from a CA1 neuron in response to depolarizing currents of variable size, applied from a holding potential of –60 mV, is

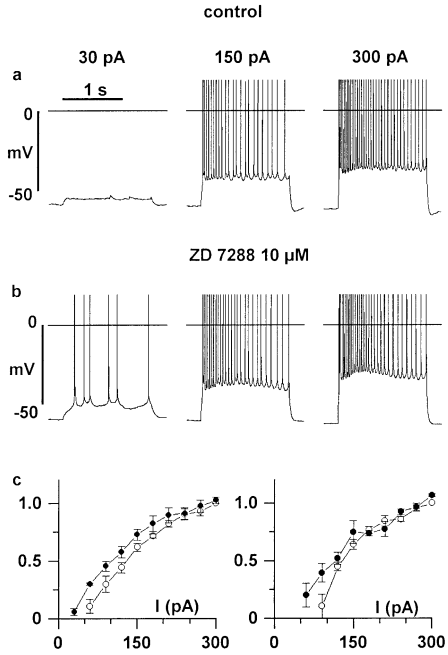

Fig. 7 **a–c** Modification of the firing frequency by ZD 7288. **a**, **b** Activity recorded in one CA1 neuron upon step current injections of 30 (*left*), 150 (*middle*) and 300 (*right*) pA under control conditions (**a**) and during perfusion of ZD 7288 (10 µM, 12 min, **b**). The reference potential was –60 mV in both cases. The ZD-7288-induced hyperpolarization was compensated for by constant current feed. Action potentials truncated at +20 mV. **c** Frequency/current (*f*/*I*) curves under control conditions (*open circles*) and in the presence of the drug (*filled circles*). Data are averaged from $n = 3$ neurons and represent the frequency (reciprocal of interspike interval) after the first (*left*) and 15th (*right*) spike normalized to maximal frequency

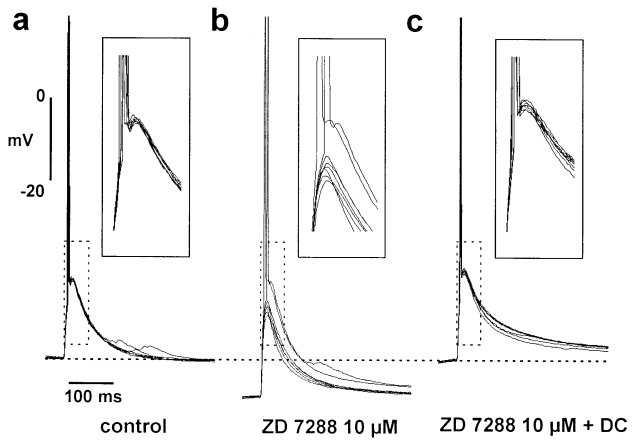

Fig. 8 **a–c** Action of ZD 7288 on the firing response elicited by extracellular stimulation of afferent fibres. **a** Under control conditions, each stimulation (50 µA) successfully resulted in a single action potential generation, superimposed on the excitatory postsynaptic potential; **b** ZD 7288 caused membrane hyperpolarization and depressed the firing response to the same stimulus strength; **c** after compensation of membrane hyperpolarization by current injection, responsiveness returned to that seen under control conditions. In each case, 8 records are plotted (the first 8 over 16 in **b**; all records truncated at +20 mV). The *insets* show part of the records on expanded time and voltage scales

shown. Higher frequency responses are apparent in the presence of ZD 7288 (see also Fig. 6 above).

Investigation over a fuller range of injected current levels yielded the frequency/current (*f*/*I*) curves plotted in Fig. 7c. These were obtained by normalizing frequencies measured during current injection at the first (left, "early" times) and the 15th interspike interval (right, "late" times). Perfusion with ZD 7288 was characterized by a shift of the "early" *f*/*I* curve to the left, corresponding to an increased discharge rate at all, except the highest current strengths. On the other hand, the "late" *f*/*I* curve was essentially unmodified by ZD 7288, and displayed only a moderate frequency increase at the lowest current strength used (90 pA). These data are in agreement with an increased membrane input resistance in the presence of ZD 7288.

The reduction of excitability due to the hyperpolarization of CA1 pyramidal cells in the presence of ZD 7288 may also affect signal propagation through the hippocampal circuitry. We tested this hypothesis by recording the activity of the same CA1 neuron as in Fig. 6 in response to stimulation of afferent fibres (Schaffer collaterals) with the stimulus intensity set to just above firing threshold under control conditions, such that each stimulus resulted in one action potential (50 µA, Fig. 8a, eight stimuli shown). After perfusion of ZD 7288 (10 µM), only 5 of 16 stimuli were effective at eliciting one action potential (Fig. 8b). A normal response (eight of eight stimuli) was resumed upon restoring the resting voltage by current injection. In three neurons, the firing probability in response to just suprathreshold activation of the Schaffer collateral was 0.95 ± 0.05 under control conditions, 0.15 ± 0.10 in the presence of ZD 7288 (10 µM), and 0.96 ± 0.04 during injection of tonic current to restore the resting potential.

Effect of ZD 7288 on the EPSP-IPSP sequence

To investigate whether ZD 7288 could affect, through I_h blockade, the response of CA1 neurons to synaptic input, we investigated the effect of ZD 7288 on the sequence generated by subthreshold activation of the afferent fibres by an extracellular stimulating electrode. Under control conditions, stimulation of Schaffer collaterals yielded a typical EPSP-IPSP sequence, characterized by an early EPSP, due to the opening of glutamatergic receptors, followed by an IPSP, due to the subsequent activation of GABA-ergic (where GABA is γ-aminobutyric acid) inhibitory interneurons activated by Schaffer collaterals or by CA1 pyramidal cells themselves (Fig. 9, control trace). Superfusion of ZD 7288 led to hyperpolarization of the membrane potential, which was compensated for by injection of a tonic current (18 pA); under these conditions, the sequence generated by stimulation of Schaffer collaterals consisted only of a depolarizing phase (Fig. 9 top trace); subtraction of the signal in the presence of the drug from the control signal indicated that ZD 7288 strongly reduced the IPSP (Fig. 9, bottom

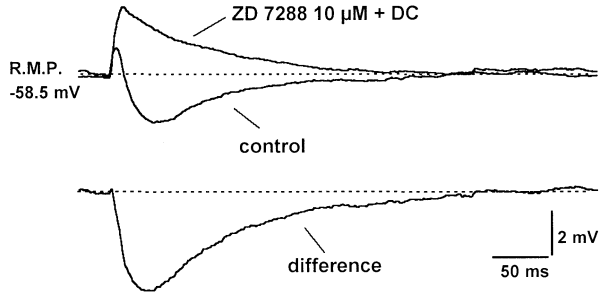

Fig. 9 Reduction of the inhibitory post-synaptic potential by ZD 7288. Under control conditions (*top*) the EPSP, recorded from a CA1 neuron during afferent pathway stimulation, was followed by the IPSP in a normal EPSP-IPSP sequence. ZD 7288 (10 μM) produced a hyperpolarization of approximately 5.5 mV, which was compensated for by the injection of a tonic depolarizing current (18 pA). In the presence of the drug, the IPSP was abolished, as apparent by the *difference trace* shown below. Every trace represents the mean of 3 consecutive recordings

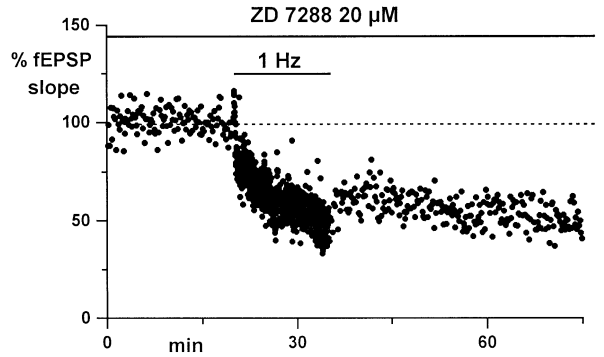

Fig. 10 Lack of effect of ZD 7288 (20 μM) on long-term depression (*LTD*) of synaptic transmission. In the presence of the bradycardic agent, the LTD-inducing protocol (1 Hz stimulation of Schaffer collaterals for 15 min) caused, in this preparation, a depression of 48.6% of the fEPSP slope, as measured 40 min after LTD induction. In $n = 4$ experiments, the depression measured after 40 min was 32.48 ± 7.03%

trace). A similar action was observed in two more CA1 neurons. These data suggest that a major component of the inhibitory input to CA1 neurons is controlled by an I_h-dependent mechanism.

Lack of effect of ZD 7288 on LTD

We used extracellular recordings to check if ZD 7288 modifies synaptic plasticity. Our aim was to identify any changes in synaptic plasticity directly linked to I_h block in hippocampal slices and particularly to use ZD 7288 as a tool to investigate the possible involvement of I_h in LTD. Previous experiments have shown that Cs^+ can reverse LTD and elicit spontaneous activity in the absence of external stimulation of the Schaffer collaterals [20]. We used the blocking action of ZD 7288 on I_h to test if this effect is related to block of I_h or to other non-selective actions of Cs^+.

In the continuous presence of ZD 7288 (20 μM), test pulses were delivered at 0.1 Hz for 10 min or longer, until a stable fEPSP was recorded. The protocol of induction of LTD was then delivered by stimulating afferent fibres at 1 Hz for 15 min [15, 20] (Fig. 10).

We obtained a stable LTD of synaptic transmission (32.48 ± 7.03% of the fEPSP slope recorded in the presence of ZD 7288 20 μM, $n = 4$). Furthermore, spontaneous activity was not observed in the presence of the bradycardic agent during or after the LTD-inducing 1 Hz stimulation protocol ($n = 4$). Thus, I_h block does not directly lead to abolition of LTD maintenance, nor to rhythmic, interictal-like spontaneous activity.

Discussion

This study shows that (the hyperpolarization-activated cation current (I_h)) of hippocampal CA1 neurons is blocked by ZD 7288 and that the block is not voltage dependent. The agent was developed as a "bradycardic" substance able to slow cardiac rate by specific block of the cardiac "pacemaker" (I_f) current [4]. Thus, our data confirm previous suggestions that the cardiac f-channel and the neuronal hyperpolarization-activated h-channel may belong to the same family [21, 26].

The time course of the block onset by ZD 7288 was noticeably slower than that obtained with Cs^+ [17]. In hippocampal slices, we found that the block induced by ZD 7288 at the highest concentration used (1000 μM) develops with a time constant of 66.61 ± 5.79 s ($n = 3$), whereas the Cs^+-induced I_h block has been reported to be completed within 20 s [20]. This could be due to a different mechanism of block. Indeed, whereas it is known that Cs^+ blocks the h-channel from the external side [10], the slow time course of I_h block by ZD 7288 is compatible with an intracellular blocking mechanism. In agreement with our results, ZD 7288 has a lipophilic structure and has been proposed to behave as a "lipophilic quaternary cation", which needs to cross the membrane to exert its action from the internal side [17]. The different side of action of the two blockers could also account for the different behaviour during wash-out: if on the one hand the block by Cs^+ can be rapidly relieved [20], the action of ZD 7288 was not reversible, at least in brain slices ([17] and this work).

Further support of an intracellular site of action of ZD 7288 comes from the evidence that prolonged hyperpolarization relieves block (Fig. 3). Since ZD 7288 is a quaternary cation, this behaviour is consistent with the presence of a blocking site located inside the channel pore which is accessible from the channel cytoplasmic side. A similar hyperpolarization-dependent unblock has been reported for the I_f current block by UL-FS 49 in cardiac cells [12] and for the I_h block by ZD 7288 in substantia nigra neurons [17]. Unlike UL-FS 49, which is known to block preferentially open f-channels in cardiac preparations and in the thalamus [12, 25, 30], ZD 7288 did not however appear to need open h-channels to exert its blocking action (Fig. 2).

Since I_h is activated at rest, its block by ZD 7288 causes hyperpolarization of the membrane and a decrease of the resting membrane conductance. The hyperpolarizing shift caused by ZD 7288 (10 μM) was 5.93 ± 0.49 mV (n = 4). Although this concentration is able to block only about 50% of the current at steady-state (see Fig. 4), the hyperpolarization observed in the presence of the bradycardic agent is slightly greater than that observed during perfusion with Cs^+ (2 mM) [21] This may be due to a higher specificity of I_h block by ZD 7288. Also, the Cs^+-induced hyperpolarization may be partly antagonized by a secondary, depolarizing action that develops more slowly and is attributable to extracellular K^+ changes caused by Cs^+ interaction with glia-mediated K^+ buffering [18]. ZD 7288 strongly depressed the excitability of CA1 pyramidal neurons by reducing, with respect to control conditions, their ability to respond to afferent stimulation and to generate action potentials upon external stimulation.

This effect was not due to altered conditions in impulse generation and conduction, since the cell's excitability could be restored by compensating the ZD 7288-induced hyperpolarization by injection of depolarizing current. The presence of ZD-7288 led to a shift of the frequency/current (f/I) relation to the left, indicating a lowered firing threshold (Fig. 7c, left). This is compatible with an increased membrane input resistance due to block of a current flowing at rest. Modification of firing rate was lost at late times during current injection (Fig. 7c, right), in agreement with the loss of the contribution by I_h due to time-dependent inactivation at depolarized voltages.

These observations imply that the depolarizing contribution of I_h to the resting potential plays an important role in controlling the excitability of hippocampal CA1 neurons. An important consequence of this finding is that I_h may be relevant to signal transmission between CA3 and CA1 neurons and thus to the flow of information in the hippocampus. It may be worth noticing in this respect that I_h has been found to be modulated by neurotransmitters in hippocampal CA1 neurons and inhibitory interneurons [8, 19, 28].

ZD 7288 strongly reduced the IPSP of the EPSP-IPSP sequence. This suggests an inhibitory action of the drug on inhibitory GABA-ergic interneurons which, once activated by Schaffer collaterals or by CA1 neurons themselves, are responsible for the IPSP. This agrees with evidence indicating that the IPSP phase of EPSP-IPSP sequences is abolished following block of GABA-ergic receptors [7]. A possible contribution to the IPSP phase of I_h deactivation in the CA1 neuron itself, such as it occurs in the Cd-insensitive (medium duration) afterhyperpolarization following a burst [21], can be discarded based on the limited size (2.92 ± 0.62 mV) and duration (19.2 ± 2.2 ms, n = 6) of the depolarizing (EPSP) phase. If I_h is expressed in CA1 inhibitory interneurons, as has been recently reported [19], its block by ZD 7288 could hyperpolarize their resting voltage level and result in a depressed activity and a reduced release of GABA. A similar effect on the EPSP-IPSP sequence, also attributable to I_h block, has been reported to occur in the presence of Cs^+ [18].

The dose required for effective block of I_h in hippocampal slices was greater than that for I_f block in isolated cardiac sinoatrial node cells [4]. This may reflect a less efficient diffusion of this strongly lipophilic substance through the layers of the hippocampal slice rather than an actual difference in the binding affinity to the blocking site. Alternatively, a different binding affinity could result from structural differences between cardiac and neuronal isoforms of hyperpolarization-activated channels.

In contrast to Cs^+ [20], ZD 7288 was ineffective on LTD maintenance. In the presence of ZD 7288 (20 μM), the fEPSP slope was reduced by 32.5 ± 7%, a depression similar to that observed when LTD protocols were applied in the absence of the drug (26.7 ± 3.3%, n = 8; not significantly different at $P > 0.05$ according to independent t-test). The lack of action of ZD 7288 on LTD maintenance implies that simple block of I_h is not sufficient to affect LTD, as was proposed as a possible interpretation of the action of Cs^+ on LTD maintenance [21].

Also in contrast to the action of Cs^+ under similar conditions [20], no spontaneous activity was observed when the LTD-inducing protocol was applied in the presence of ZD 7288. Removal of LTD maintenance and induction of spontaneous rhythmic activity by Cs^+ in hippocampal slices therefore appear to be unrelated to the blocking action on I_h (see also [18]).

Acknowledgements This work was supported by the C.N.R. (CT04.95.02241 to D.D.). We wish to thank A. Ferroni and D. Janigro for discussion.

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