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Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (I_f) in guinea-pig dissociated sinoatrial node cells

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1 ZENECA ZD7288 (4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride) is a sinoatrial node (SAN) modulating agent which produces a selective slowing of the heart rate. Its effects have been studied in single, freshly dissociated guinea-pig SAN cells, by standard patch clamp procedures.

2 Whole-cell inward currents were evoked by hyperpolarizing voltage clamp steps from a holding potential of −40 mV. ZD7288 inhibited the hyperpolarization activated cationic current (I_f) in a concentration-dependent manner. The 'selective bradycardic agents' alinidine and UL-FS 49 (zatebradine) both also inhibited I_f.

3 The activation of I_f was investigated by measuring tail current amplitudes at +20 mV after hyperpolarizing steps to different potentials to activate the current. The reduction in I_f resulted from both a shift in the I_f current activation curve in the negative direction on the voltage axis, and also a reduction in the activation curve amplitude.

4 ZD7288 did not affect the ion selectivity of the I_f channel, since the tail current reversal potential was unchanged in the presence of the drug.

5 With ZD7288 the inhibition of I_f was not use-dependent, whereas UL-FS 49 displayed use-dependence in the block of the I_f current.

6 Whereas ZD7288 had no significant effect on the delayed rectifier current (I_k) in these cells, both alinidine and UL-FS 49 significantly reduced I_k at the same concentrations which reduced I_f.

7 The data show that ZD7288 reduces I_f by affecting the activation characteristics of the I_f current; this inhibition may account for this agent's selective bradycardic properties.

Keywords: Sinoatrial node; hyperpolarization activated current; I_f; specific bradycardic agent; ZD7288; alinidine; zatebradine; patch clamp; UL-FS 49

Introduction

In the presence of a restricted coronary blood supply to the heart, an increase in beating rate is an important contributory factor in myocardial ischaemia (Guth *et al.*, 1987a). The use of β-adrenoceptor antagonists in coronary angina is based on their ability to attenuate increases in heart rate by reducing the effects of sympathetic activity (Guth *et al.*, 1987b). However, the non-cardiac actions of these agents have disadvantages, and drugs have been identified recently which have the ability to reduce heart rate while allowing the occurrence of responses to exercise, without the non-cardiac side effects of β-adrenoceptor blockade. Alinidine and UL-FS 49, which have been termed 'specific bradycardic agents' (Kobinger *et al.*, 1979; Kobinger & Lillie, 1984; Lillie & Kobinger, 1986), are examples of this type of drug. By improving blood flow and reducing the metabolic demand of the heart, these agents should have beneficial actions in the treatment of myocardial ischaemia.

ZENECA ZD7288 (4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride; Figure 1; known as ICI D7288 in previous publications) is a new heart rate modulating agent (Hargreaves *et al.*, 1992). It slows the beating rate of the guinea-pig right atrium *in vitro* without affecting the force of contraction of the paced left atrium (Marshall *et al.*, 1992; 1993), and in the anaesthetized dog, reduces the beating rate without directly affecting inotropy (Rouse & Johnson, 1992). Its effects on the action potentials of guinea-pig sinoatrial node (SAN) cells in isolated intact tissues have been described, in comparison with the effects of the known bradycardic agents alinidine and UL-FS 49 (Briggs & Heapy, 1992). This paper describes the effects of ZD7288 on whole-cell currents of isolated single SAN cells and compares them with the effects of alinidine and UL-FS 49. Some of these data have been published in abstract form (BoSmith *et al.*, 1992).

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Methods

Sinoatrial node cell dissociation

Single SAN cells were dissociated from guinea-pig nodal tissues by the method of Denyer & Brown (1990a) with minor modifications. The procedure was as follows. Two or three guinea-pigs (Duncan Hartley) each weighing 250–300 g were killed by cervical dislocation. The hearts were removed

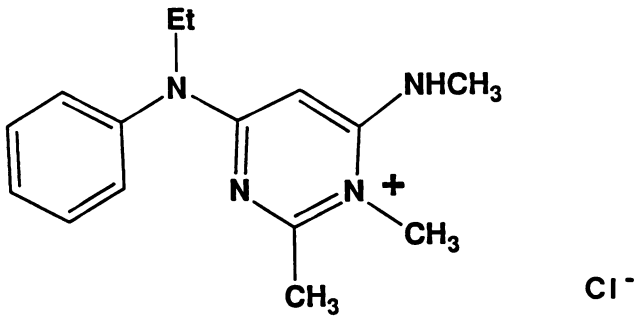

Figure 1 Chemical structure of ZD7288.

and placed in oxygenated Tyrode solution at 33–37°C. Each node was dissected out, cut into four strips (0.5–1.0 mm wide), and placed in oxygenated Ca^{2+}-free Tyrode solution containing 50 μM ethyleneglycol-*bis*-(β-amino-ethyl ether)-*N,N'*-tetra-acetic acid (EGTA) for 5 min after which the tissue was washed in Ca^{2+}-free Tyrode solution without EGTA for a further 2 min. The tissue strips were incubated in oxygenated low Ca^{2+} Tyrode solution containing 20 mM taurine, 169 units ml^{-1} collagenase (Worthington), 16 units ml^{-1} elastase (Sigma Type IV), 30 units ml^{-1} hyaluronidase (Sigma Type I-S) and 2.5 mg ml^{-1} bovine serum albumin (Miles Diagnostics) at 37°C for 25–35 min. After this time, the strips were placed in a 'KB' recovery solution (Isenberg & Klöckner, 1982) with the addition of 5 mM D-fructose-1,6-diphosphate (Sigma) for 60 min at 4°C. The tissue was gently teased apart with fine forceps in 2.5 ml of 'KB' solution in a petri-dish to release single cells. Aliquots of the suspension of SAN cells were plated onto 35 mm plastic petri-dishes (Falcon 3080, Becton Dickinson), which were stored at 4°C for 20 min to allow the cells to settle. Petri-dishes containing dissociated SAN cells were then transferred to an Open Perfusion Micro Incubator (PDMI-2; Medical Systems Corp.), mounted on the stage of an inverted microscope (Nikon Diaphot).

Cells were superfused with a Ca^{2+} containing Tyrode solution, at 1 to 2.5 ml min^{-1}, either by a gravity-fed or peristaltic pump perfusion systems. Excess solution was continuously aspirated from the side of the petri-dish opposite to the inflow tube. The solution temperature in the centre of the petri-dish was maintained at 34–36°C. The volume of the fluid in the dish was 2 ml.

Solutions and drugs

The solutions used had the following compositions (in mM): Tyrode solution: NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, N-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) 5 and glucose 10; pH 7.2 with NaOH. Low Ca^{2+} Tyrode solution: NaCl 140, KCl 5.4, $CaCl_2$ 0.06, $MgCl_2$ 3.5, taurine 20, HEPES 5 and glucose 10; pH 7.2 with NaOH. Ca^{2+}-free Tyrode solution: NaCl 140, KCl 5.4, $MgCl_2$ 3.5, taurine 20, HEPES 5, glucose 10 and EGTA 0.05; pH 7.2 with NaOH. 'KB' solution: KCl 70, adenosine triphosphate (ATP; dipotassium salt) 5, $MgSO_4$ 5, potassium glutamic acid 5, taurine 20, trisphosphocreatine 5, succinic acid 5, KH_2PO_4 20, glucose 10 and HEPES 5; pH 7.0 with KOH. Electrode solution: KCl 140, $MgCl_2$ 3, ATP 3, guanosine triphosphate (GTP; sodium salt) 0.4, trisphosphocreatine 5 and HEPES 11; pH 7.2 with KOH.

In some instances when I_f tail currents were recorded, the Tyrode solution also contained 2 mM $BaCl_2$ (Sigma), 1 mM $NiCl_2$ (Sigma) and 0.3 μM tetrodotoxin (Sigma) in order to block any contaminating outward K^+ (delayed rectifier), Ca^{2+} and Na^+ currents respectively (Denyer & Brown, 1990a; Frace *et al.*, 1992).

ZD7288, UL-FS 49 (1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylimino]propyl]-2H-3-benzazepin-2-on hydrochloride) and alinidine (2-[N-allyl-N-(2,6-dichlorophenyl)-amino]-2-imidazoline hydrochloride) were all synthesized at ZENECA Pharmaceuticals. ZD7288, UL-FS 49 and alinidine were all made up as stock solutions of 1 mM in Tyrode solution, and were appropriately diluted to give final concentrations of 0.1–10 μM. All water used to prepare solutions was of reagent grade from a Millipore water purification system.

Electrical recording and analysis

Whole-cell patch clamp recordings under voltage and current clamp were performed by standard procedures (Hamill *et al.*, 1981). Recording electrodes were pulled from borosilicate glass (GC120-10; Clark Electromedical Instruments) with a Sutter P-87 electrode puller. When filled with the internal recording solution, they had resistances of 1–6 MΩ. Currents were recorded with an EPC-7 (List Electronics) or Axopatch 1D (Axon Instruments) patch clamp amplifier employing capacitance and series resistance compensation, and voltage clamp steps were controlled and applied using a Tandon PCA/12 PC microcomputer and pClamp 5.5.1 software via a TL-1 DMA Interface A-D/D-A Converter (Axon Instruments). The currents were low-pass filtered by a 3 or 4-pole Bessel filter with a cut-off frequency (3 dB down) of 3 or 2 kHz respectively. Current or voltage data were stored on hard or floppy disc for later analysis and/or recorded simultaneously onto magnetic tape (Racal 4DS), digital audio tape using a DTR1200 digital tape recorder (Biologic) or video tape using a TEAC XR-310 cassette data recorder. Current amplitudes were measured with pClamp 5.5.1 software (Axon Instruments), and hard copies produced with a Hewlett Packard 7475A digital plotter or Laserjet IIID printer. Voltage recordings of cell action potentials were obtained under current clamp conditions, and plotted on an Astromed Dash IV thermal array recorder.

Under whole-cell recording conditions, peak membrane currents for I_f and I_k (delayed rectifier) were measured at the end of the voltage clamp pulses, at 5 min intervals until stable amplitudes were established. Consistent current amplitude readings were required before the addition of any drugs. In the presence of the drugs, data were considered acceptable if at least 3 consecutive stable current amplitudes were measured, typically after some 15–40 min of drug application. The drug effects were thus always assessed after attainment of a steady state of inhibition.

All data in text and tables are presented as mean values ± standard error. The statistical significance of differences between experimental groups were assessed by Student's *t* test for unpaired data.

Results

Inhibition of I_f *by ZD7288*

When the single, dissociated SAN cells were whole-cell voltage clamped at − 40 mV and hyperpolarizing step voltage pulses applied, a time-dependent, inward current was evoked at voltages more hyperpolarized than − 60 mV (Figure 2). This hyperpolarization activated current was recognized as being the cationic I_f current observed in single SAN cells by other workers (Denyer & Brown, 1990a; DiFrancesco, 1987). The principal effect of ZD7288 was to inhibit this current, in a concentration-dependent manner. Concentrations of 0.1, 0.3 and 1.0 μM ZD7288 inhibited the I_f current measured at − 120 mV by 44 ± 4% ($n = 15$), 65 ± 4% ($n = 14$) and 78 ± 4% ($n = 9$) respectively (e.g. Figure 2). The current-voltage curves for I_f in the absence and presence of 0.3 μM ZD7288 revealed that the current was inhibited over the range of voltages − 60 to − 120 mV (Figure 2b). We found that it was essential to establish stable current amplitudes in response to the voltage steps before drug administration in all experiments, because over the initial period of recording after establishing the whole-cell configuration, run-down of I_f was often seen. It would therefore have been difficult to differentiate any drug effects from spontaneous changes. In the majority of cells the evoked current at − 120 mV was found to stabilize, and all the data used were from cells in which the amplitudes of the evoked currents reached stability before and during drug application. The time course of the inhibition of the I_f current by ZD7288 was slow; equilibration of the drug effect required some 35 min of drug application. This meant that a stable whole-cell configuration had to be maintained for 1 h or more in order to ascertain the effects of a single concentration of drug on I_f and I_k.

Although a comprehensive study was not attempted, in three cells where action potentials were examined as well as membrane currents, the reduction of I_f by 0.1 or 1 μM

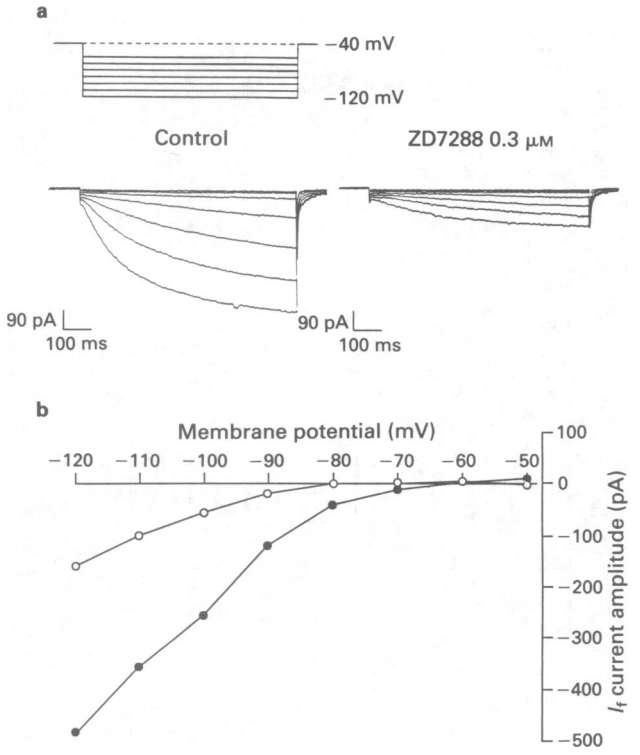

Figure 2 Inhibition of the whole-cell hyperpolarization activated current, I_f, by 0.3 μM ZD7288 in a SAN cell. The cell was voltage-clamped at a holding potential of − 40 mV and stepped to − 120 mV in 10 mV intervals. The current was measured at the end of the clamp pulses, and the percentage inhibition was calculated from the currents evoked at − 120 mV. (a) Superimposed current records showing inhibition by 0.3 μM ZD7288; 62% inhibition. (b) Current-voltage relationship of the I_f currents in (a) in the absence (●) and presence (○) of 0.3 μM ZD7288.

ZD7288 was accompanied by a slowing of the spontaneous action potential firing rate and decreased rate of diastolic depolarization (Figure 3). In one cell, 0.1 μM ZD7288 increased mean cycle length (CL) from 580 to 624 ms (+ 8%), and in two others, 1 μM ZD7288 increased CL from 524 to 780 ms (+ 48%) and from 430 to 890 ms (+ 107%) respectively. These drug concentrations had similar effects on the firing rate and the rate of diastolic depolarization of cells in guinea-pig isolated SAN tissue (Briggs & Heapy, 1992).

The reduction in I_f by ZD7288 resulted from both a concentration-dependent shift in the current activation curve in a negative direction on the voltage axis, and also a reduction in the activation curve amplitude. Activation curves for

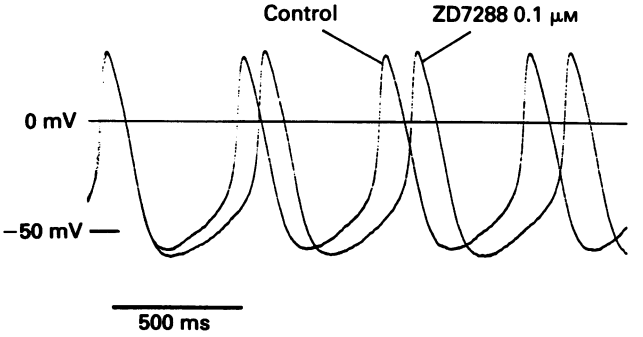

Figure 3 Action potentials recorded from a single isolated SAN cell in current-clamp mode in the absence and presence of 0.1 μM ZD7288. Waveforms are superimposed to show the reduction, by ZD7288, of diastolic depolarization rate and the slowing of action potential generation.

I_f were constructed by measuring the peak tail current amplitudes at + 20 mV from various test potentials in the absence and presence of the drug (Figure 4). In the absence of ZD7288 the mean half-maximal activation potential was − 98.4 ± 0.8 mV ($n = 32$). ZD7288 (0.1 μM) shifted the I_f half-maximal activation by − 6.1 ± 1.1 mV ($n = 8$) and reduced the activation curve maximum by 35 ± 3% ($n = 8$); 0.3 μM produced a shift of − 16.2 ± 1.8 mV ($n = 5$) in the half-maximal activation, and reduced the maximum by 52 ± 6% ($n = 5$).

The reversal potential for I_f was determined by constructing fully activated current-voltage relationships (data not shown). This involved hyperpolarizing the cell to − 120 mV (to activate I_f) and then returning to more positive voltages (− 80 mV to + 30 mV) in 10 mV increments. The resulting tail current amplitudes gave a mean control reversal potential of − 30.6 ± 1.0 mV ($n = 16$). Similar values have been reported for I_f under similar ionic conditions (DiFrancesco, 1987; van Ginneken & Giles, 1991; Frace *et al.*, 1992). ZD7288 (0.3 μM) reduced the slope of the current-voltage relationship, but did not alter the reversal potential for I_f (− 32.3 ± 3.9 mV; $n = 3$; $P > 0.5$), indicating that the drug does not alter the I_f channel selectivity properties.

The inhibitory effect on I_f of ZD7288 was compared with those of alinidine and UL-FS 49. Alinidine (10 μM) and UL-FS 49 (0.3 μM) also decreased I_f by 51 ± 5% ($n = 8$) and 37 ± 4% ($n = 11$) respectively (Figure 5).

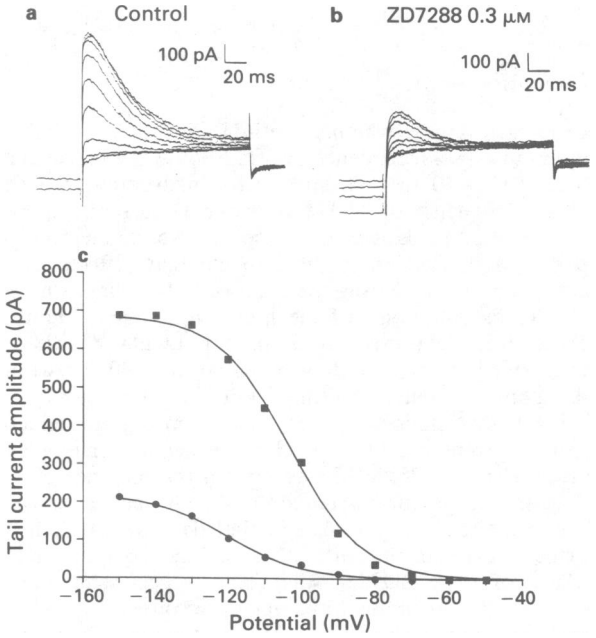

Figure 4 Action of ZD7288 on the I_f current activation characteristics. The SAN cell was voltage clamped at − 40 mV and I_f activated by successive hyperpolarizing steps (− 70 mV to − 150 mV shown) in 10 mV intervals. Each hyperpolarizing step was followed by a depolarizing step to + 20 mV to elicit I_f tail currents and then returned to the holding potential. (a) Superimposed control I_f tail currents recorded at + 20 mV. (b) I_f tail currents recorded from the same SAN cell in the presence of 0.3 μM ZD7288. (c) Activation curves for I_f in control conditions (■) and in the presence of 0.3 μM ZD7288 (●), obtained by plotting peak I_f tail current amplitudes against the potentials (− 50 to − 150 mV) of test hyperpolarization. The activation curves (continuous lines) were obtained by least squares fitting of the data with a Boltzmann equation ($I = \{1 + \exp[(V_t - V_{0.5})K^{-1}]\}^{-1}$ where I = peak tail current amplitude; V_t = hyperpolarizing test voltage; $V_{0.5}$ = half-maximal activation voltage; K = slope factor). The half-maximal activation potential was − 103 mV in the control and − 120 mV in the presence of 0.3 μM ZD7288. The amplitude of the activation curve was reduced by 69% in the presence of the drug.

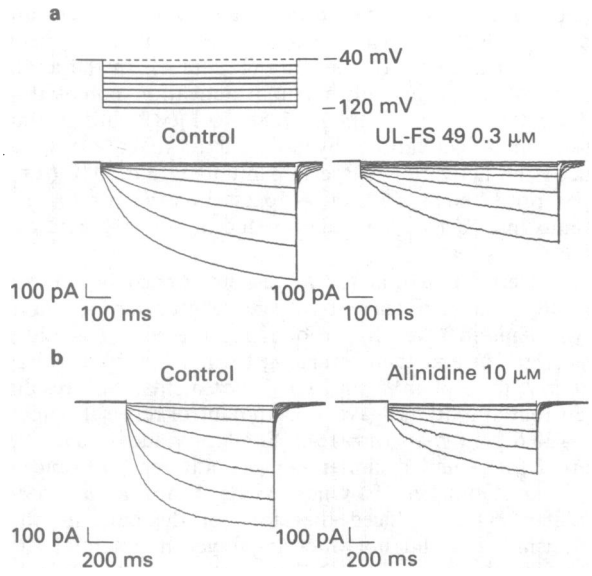

Figure 5 Effect of UL-FS 49 and alinidine on I_f currents in two separate SAN cells. The cells were clamped at a holding potential of − 40 mV and stepped to − 120 mV in 10 mV intervals. The first step in record (b) was to − 60 mV. Percentage inhibition of I_f was measured at − 120 mV. (a) Superimposed current records showing inhibition of I_f by 0.3 μM UL-FS 49; 29% inhibition. (b) Current records showing inhibition by 10 μM alinidine; 43% inhibition. Compare these inhibitions with the effect of 0.3 μM ZD7288 shown in Figure 2.

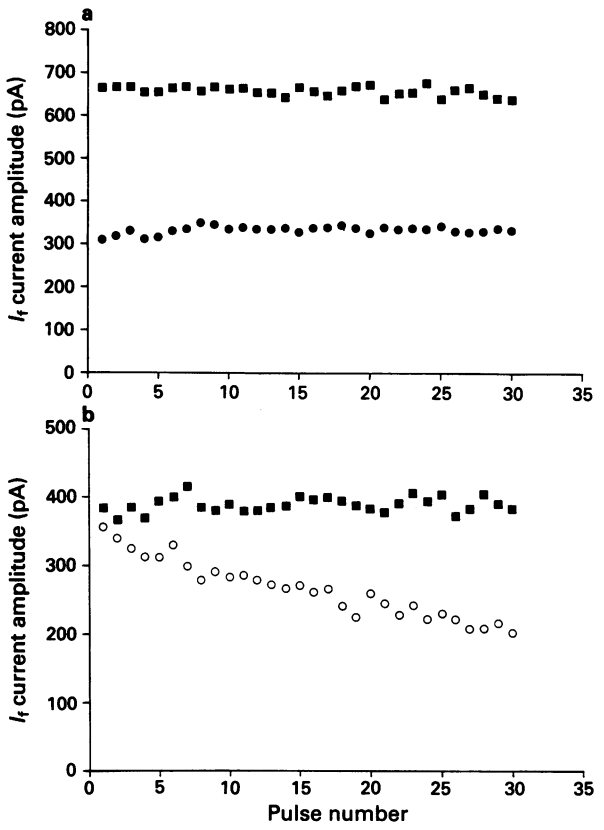

Figure 6 Use-dependence of I_f blockade: 30 voltage clamp pulses from − 40 mV to a potential of − 120 mV were applied as a train at a rate of 0.2 Hz. The amplitude of the I_f current is plotted. (a) Lack of use-dependence of ZD7288. I_f current amplitude in drug-free Tyrode solution (■) and after 35 min exposure to 1 μM ZD7288 (●). (b) Use-dependence of UL-FS 49. I_f current amplitude in drug-free Tyrode solution (■) and after 35 min exposure to 1 μM UL-FS 49 (○).

Examination of use-dependence

In order to examine whether the inhibition of I_f by ZD7288 showed any use-dependence, a train of 30 voltage clamp pulses from − 40 to − 120 mV, 1.8 s in duration, were applied at a frequency of 0.2 Hz, to evoke 30 current responses. The train of 30 pulses was only applied after stable I_f current responses had been established, to eliminate effects due to possible run-down. During the train of pulses there was little change in the amplitude of the I_f current evoked (Figure 6). The cell was then exposed to 0.3 or 1.0 μM ZD7288 for 35 min, and kept at a holding potential of − 40 mV without evoking any currents. Another train of 30 voltage clamp pulses was then imposed. There was an immediate reduction of the I_f current amplitude evoked during the first pulse of the train (e.g. to 47% of the control in the presence of 1 μM ZD7288), with no further reduction during the successive 29 pulses in the train (Figure 6a). Similar data were obtained in a further 3 experiments with ZD7288. This indicates that the blockade of I_f by ZD7288 was not use-dependent, and did not require the I_f channel to be open to produce its inhibitory effect. When the experiments were repeated with 1 μM UL-FS 49 ($n = 3$), the amplitude of the evoked current from the first pulse of the train after addition of the drug was the same as in the control conditions, but successive pulses in the train elicited progressively less current until the final pulse in the train activated only 54% of the amplitude of I_f evoked during the first pulse of the train (Figure 6b). This clearly illustrates the use-dependent nature of the inhibition of I_f by UL-FS 49 in SAN cells.

Selectivity of ZD7288

Effects of ZD7288 on other currents tested were absent or not significant at the concentrations which markedly decreased I_f. The inward rectifier current (I_{k1}) was observed in some cells as an instantaneous component of current evoked during hyperpolarization, which was distinguishable from I_f in these cells by the slower development of the latter. This instantaneous current was not reduced significantly (− 4.1 ± 6.1%; $n = 4$) by ZD7288 (1 μM) even when I_f was inhibited profoundly in the same cells (− 90 ± 3%; e.g. Figure 7).

Calcium current (I_{Ca}) was observed as an inward current during depolarizing clamp steps from a holding potential of − 60 mv which was used in a few experiments. In most of these cells, this current showed run-down which prevented studies of the effect of ZD7288, but in two cells it was possible to demonstrate that the compound (0.1 or 1.0 μM) affected I_{Ca} much less than I_f measured in the same cell: I_{Ca} was reduced by 18% and 14% while I_f was reduced by 66% and 82% respectively. This inward current rapidly inactivated and an outward current developed during the clamp pulse. In most cells, which were held at − 40 mV, the initial inward component was not seen, presumably because of inactivation or rapid run-down, and the delayed outward rectifier component (I_k) was seen alone. ZD7288 (0.1, 0.3 and 1.0 μM) did not significantly affect this outward current: changes of − 1 ± 6% ($n = 10$), − 12 ± 8% ($n = 8$) and + 9 ± 15% ($n = 3$) were observed at these concentrations respectively (Figure 8).

In contrast to ZD7288, alinidine (10 μM) and UL-FS 49 (0.3 μM) caused reductions in the delayed rectifier (I_k) of 45 ± 11% ($n = 6$) and 33 ± 7% ($n = 11$) respectively. The reductions in I_k by alinidine and UL-FS 49 were significantly greater than the effects of ZD7288 at 0.1 μM, which had a similar inhibitory effect on I_f. For the effects on I_k of alinidine compared with ZD7288, $P < 0.01$, and for UL-FS 49 compared with ZD7288, $P < 0.005$, by Student's t test. The inhibitory effects of alinidine and UL-FS 49 on I_k current-voltage relationships are illustrated in Figure 8.

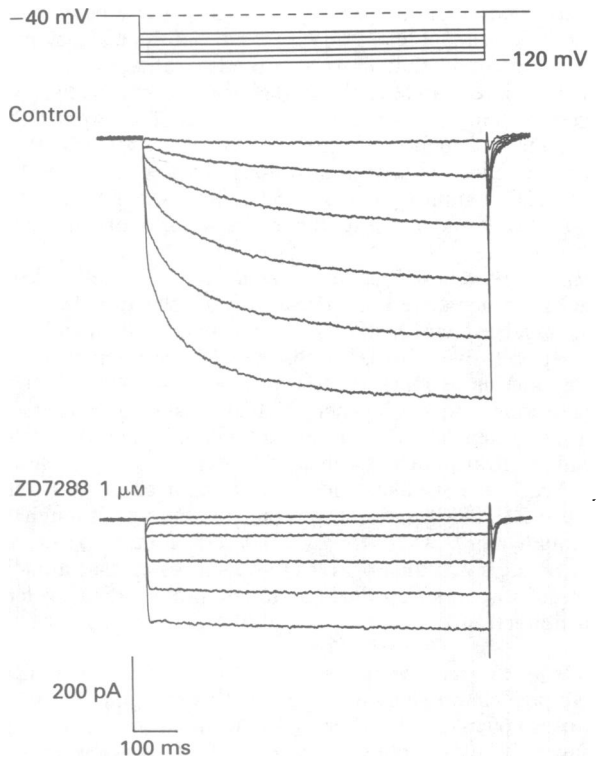

Figure 7 Lack of effect of 1 μM ZD7288 on the inward rectifier, I_{k1}, in a SAN cell with prominent I_{k1} and I_f currents together. The cell was clamped at a holding potential of − 40 mV and stepped to − 70, − 80, − 90, − 100, − 110 and − 120 mV. The amplitude of the I_{k1} (instantaneous) current remained unchanged in the presence of 1 μM ZD7288, whilst the time-dependent I_f current was inhibited by 91% when measured at − 120 mV.

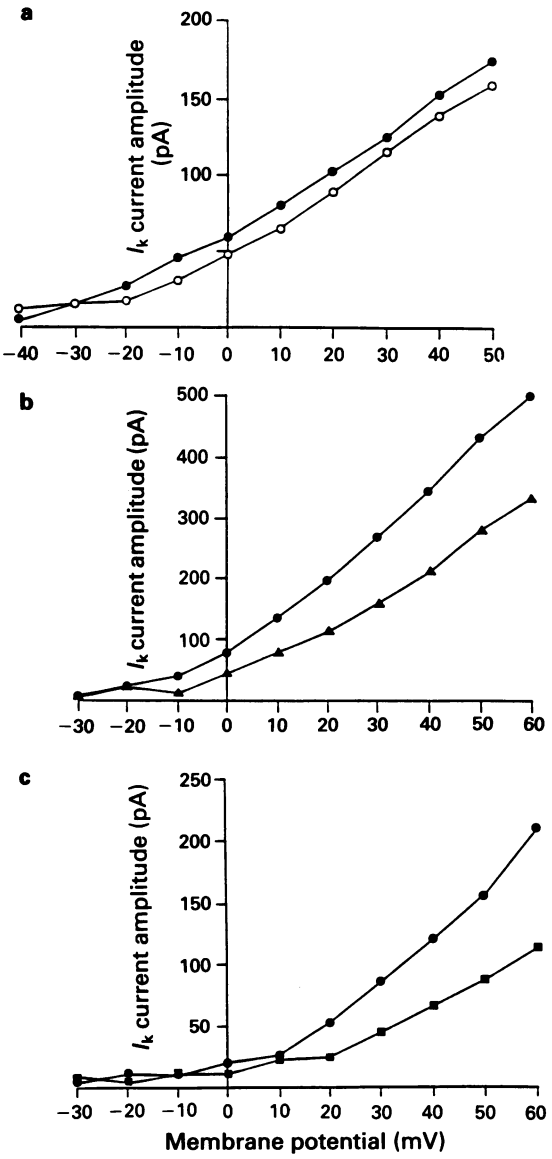

Figure 8 Effect of ZD7288, UL-FS 49 and alinidine on delayed outward (I_k) currents. Current-voltage relationships of peak currents are shown. (a) Lack of effect of ZD7288 on I_k currents elicited from a cell clamped at a holding potential of − 60 mV and stepped to − 40 mV to + 50 mV; (●) control; (○) 0.3 μM ZD7288. (b) Inhibition by UL-FS 49 of I_k currents elicited from a cell clamped at a holding potential of − 40 mV and stepped to − 30 mV to + 60 mV; (●) control; (▲) 0.3 μM UL-FS 49. (c) Inhibition by alinidine of I_k currents elicited from a cell clamped at a holding potential of − 40 mV and stepped to − 30 mV to + 60 mV; (●) control; (■) 10 μM alinidine.

Discussion

Inhibition of I_f *by ZD7288*

The principal effect of ZD7288 in this study was on the hyperpolarization-activated current (I_f) of the SAN cells. The effects on this current were seen at concentrations which reduced the rate of diastolic depolarization recorded with intracellular microelectrodes in cells in isolated SAN tissue preparations (Briggs & Heapy, 1992) and in isolated cells in the present series of experiments. Noma *et al.* (1983) and Hagiwara & Irisawa (1989) have questioned whether I_f has a role in pacemaking. However, many workers have concluded that I_f is indeed functional in pacemaking in the SAN, though other currents are also involved (DiFrancesco *et al.*, 1986; Oei *et al.*, 1989; Denyer & Brown, 1990a,b; DiFrancesco, 1991; van Ginneken & Giles, 1991). It seems reasonable to conclude therefore that the inhibitory action of ZD7288 on the pacemaker activity of the sinoatrial node is probably mediated by its selective reduction of the I_f current of the pacemaker cells.

The prolonged time to reach equilibrium for the inhibition of I_f by ZD7288, even in single cells, suggests that the compound may have an intracellular site of action, to which it penetrates only slowly, or that it causes slow changes in some factor which modulates the I_f current. In this it appears to resemble UL-FS 49 (Van Bogaert *et al.*, 1990; Van Bogaert, 1992). The ZD7288 molecule has a positive charge which is likely to be distributed between the different nitrogens of the structure; its quaternary properties would hinder penetration into cells, but the distribution of the charge may render it less impermeable than would a non-distributed charge.

The present data clearly show that the reduction in I_f at a given membrane potential by ZD7288 is not due to any changes in the ionic selectivity of the I_f channel, since the I_f reversal potential remained unchanged in the presence of the drug. The I_f current-voltage curve did however have a reduced slope in the presence of ZD7288, showing that the drug had reduced the I_f conductance. The effects of the drug on the activation characteristics of I_f suggest that ZD7288 reduces the current in a number of ways. The reduction in the activation curve amplitude (maximum evokable current; I_{max}) may reflect either a reduction in the channel conductance or a reduced probability of opening. The negative shift of the I_f activation curve, which results in the activation of a reduced fraction of I_{max} at a given potential, may indicate an action by the drug on the voltage sensor mechanism of the channel.

There is some controversy about the effects of alinidine

on the activation characteristics of I_f; Snyders & Van Bogaert (1987) found a negative shift of the activation curve and also a reduction of the fully activated I_f in Purkinje fibres, but in SAN cells, DiFrancesco (1987) found little change in I_f at -98 mV with 50 μM alinidine. UL-FS 49 was found not to alter the I_f half-maximal activation potential (E_y) in sheep Purkinje fibres, even when I_{max} was reduced by more than 70% (Van Bogaert *et al.*, 1990). In contrast, acetylcholine (ACh) is reported to shift the I_f activation curve without reducing the maximum evokable I_f current (DiFrancesco & Tromba, 1988a). ACh acts by reduction of basal adenylate cyclase activity via a G protein link (DiFrancesco & Tromba, 1988b). It is not yet known whether the selective bradycardic drugs also affect the gating of I_f directly or by an action on the G-protein and cyclic nucleotide-modulated mechanism. More detailed studies of single channel kinetics and of possible interactions with G-protein modulation of the channels might help to elucidate the mechanism further.

The lack of use-dependence in the blockade of I_f by ZD7288 illustrates a further difference between this compound and UL-FS 49. Use-dependent blockade of I_f by UL-FS 49 has been shown in sheep Purkinje fibres (Van Bogaert & Goethals, 1987; Van Bogaert *et al.*, 1990), and the experiments reported in the present paper clearly demonstrate that this compound blocks I_f in SAN cells in a similar manner. The absence of use-dependence in the action of ZD7288 on I_f could indicate an affinity of the drug for the closed or resting state of the channel, resulting in the development of block even in the absence of activation, whereas UL-FS 49 shows little or no inhibition of the current in the absence of activation. This may mean that UL-FS 49 can only block the I_f channel in its open state. The action of alinidine on I_f resembles that of ZD7288, since it has also been reported not to show any use-dependence in its blockade of I_f in Purkinje fibres (Van Bogaert & Goethals, 1987); however, its effects on SAN cells remain to be investigated.

It has previously been noted that I_f run-down is seen in many cells, and makes accurate quantitative comparison between current-voltage relations unreliable (DiFrancesco *et al.*, 1986). The long durations of the recordings which were necessary in the present experiments made it essential that the current amplitudes were stable before the drugs were applied, since the tendency to run down would otherwise have given misleading results. Doerr & Trautwein (1990) failed to find any inhibition of I_f in guinea-pig SAN cells by UL-FS 49. This is in striking contrast to our data; the explanation for this discrepancy is not clear, although these authors commented that it was difficult to separate the effects of the drug from those of run-down of I_f in their experiments. The short durations of the drug applications used by these authors may explain some of the differences in degree of effect; if stability of the responses was not attained before the drug was applied, the small drug effect likely to be obtained during a short period would be difficult to separate from the run-down. In our experiments both ZD7288 and UL-FS 49 required prolonged applications before equilibration was attained, but the effects were clear, using the protocol described.

Selectivity of action of ZD7288

Effects on the delayed rectifier current, I_k In inhibiting I_f, ZD7288 resembles alinidine and UL-FS 49. However, ZD7288 appears to have a greater degree of selectivity for I_f than the other agents, in that they can have inhibitory effects on the delayed rectifier current I_k in addition to their actions on I_f. These data provide a probable explanation for the effects of the compounds on the action potential waveforms of sinoatrial cells *in situ*: ZD7288 resembles the other compounds in reducing the rate of diastolic depolarization of the cells, but causes less prolongation of the action potential duration than alinidine or UL-FS 49 (Briggs & Heapy, 1992). Snyders & Van Bogaert (1987) and Satoh & Hashimoto (1986) also found that I_k was reduced by alinidine, in sheep Purkinje fibres and rabbit SAN cells respectively. Van Bogaert & Goethals (1987) and Doerr & Trautwein (1990) also found a reduction of outward current by UL-FS 49, but only at relatively high concentrations. This contrasts with its effect in the guinea-pig SAN cells in our experiments, in which both I_f and I_k were affected at similar concentrations.

Effects on the inward rectifier current, I_{kl} The cells used in these experiments were almost always of the spindle shaped type, regarded as being primary/transitional nodal cells (Denyer & Brown, 1990a). Cells with less prominent nuclear bulges and more marked striations were also found in the preparations, but they generally had smaller I_f currents (if any) and often had a large instantaneous hyperpolarization activated inward current, which we interpret as I_{k1}. A few of these cells were studied, and no significant effect was found of 1 μM ZD7288 on this instantaneous current, though the accompanying I_f was strongly inhibited (Figure 7). In contrast, Snyders & Van Bogaert (1987) suggested that alinidine did reduce I_{k1} in sheep Purkinje fibres, though their evidence was indirect.

Effects on the calcium current, I_{Ca} In some cells, depolarizing voltage clamp steps were imposed from a holding potential of -60 mV, which caused an initial inward current (thought to be I_{Ca}) followed by a slowly developing outward current (I_k). Denyer & Brown (1990a) reported that this inward current was stable for a period, after which it rapidly ran down. However, in our experiments, in most of the cells in which it was seen, this current disappeared rapidly during the early stages of recording, thus preventing studies of the drug effects. In two cells, which did have stable inward currents, there were only slight reductions by ZD7288 at concentrations which markedly inhibited I_f. We cannot exclude the possibility that a proportion of the apparent reduction of I_{Ca} was in fact due to run-down, but this possibility does not invalidate the relative lack of effect on I_{Ca}, and may even cause an overestimation of any effect. We have not investigated the actions of the other agents using this protocol, but Doerr & Trautwein (1990) found that the most prominent effect of UL-FS 49 was a use-dependent block of L-type calcium channels. However, Tytgat *et al.* (1990) found that UL-FS 49 was not a specific L- or T-type calcium channel blocker, as it acts only at much higher concentrations on Ca channels than on pacemaker current. It is thus not clear whether ZD7288 is significantly different from UL-FS 49 with respect to its relative lack of effect on the calcium current. In current-clamp recordings, no slowing by ZD7288 of the later part of diastolic depolarization or of the upstroke was observed, further suggesting that no inhibition of I_{Ca} had occurred.

The selective action of ZD7288 on the hyperpolarization-activated current produces a limited slowing of heart rate, since this current is only one of several components which contribute to the pacemaker activity of the sinoatrial node cells (DiFrancesco, 1991; van Ginneken & Giles, 1991). It is difficult to quantify whether the inhibitory effect of ZD7288 on I_f is sufficient to account fully for its action on heart rate *in vivo*, since the contribution of I_f to pacemaker activity *in vivo* is unknown. However, since the effect of I_f inhibition in single cells is a slowing of beating rate similar to that seen in isolated SAN tissues *in vitro*, and *in vivo*, and no other action of the compound has been observed which could have this effect, it seems reasonable to conclude that these actions are related. If the actions of ZD7288 are shown finally to be as selective as they appear from the present series of experiments, the effects of this compound on the diastolic depolarization provide further evidence that I_f is important in the modulation of pacemaker function in the guinea-pig SAN.

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