Freund's adjuvant (Sigma Chemical) was injected into the dorsal neck muscle and foot pads. Each rabbit used in the study was initially sensitized with 3 ml of the antigen emulsion. Two weeks later, rabbits were given a booster injection (1–1.5 ml), in the neck muscle that contained BSA and Freund's incomplete adjuvant (1:1 emulsion). On the 20th day following initial BSA exposure, rabbit serum samples were collected for BSA antibody determination using ELISA techniques.

Twenty-eight days following initial immunization, acute local inflammatory responses were stimulated by intra-articular administration of BSA antigen. Antigen was injected into a patellofemoral joint in a vehicle (0.5 ml) containing 20% aqueous hydroxypropyl-cyclodextrin (HPCD) (Pharmos Corp., Alachua, Florida) Groups of rabbits (n = 7) were given one of the following treatments: 30% BSA, 30% BSA with 0.1% LE or 30% BSA with 0.1% dexamethasone, HPCD vehicle. Three days after intra-articular-arterial injection, animals were examined and sacrificed. Synovial fluid was removed for white blood cell evaluation, using 50–50 field counting methods, and joint were fixed in 10% neutral formalin. Synovial tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin or trichrome for fibrin and fibrinoid. Histologic sections were examined for inflammatory alterations including: synovial cell hyperplasia, inflammatory, infiltration and fibrin deposits.

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The new antiarrhythmic substance AWD 23-111 inhibits the delayed rectifier potassium current (IK) in guinea pig ventricular myocytes

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The effects of *N*-(dicyclohexyl-carbamoylmethyl)-*N*-(3-diethylamino-propyl)-4-nitro-benzamide hydrochloride (AWD 23-111), a novel antiarrhythmic compound, were studied in isolated cardiomyocytes of guinea pigs. Using whole-cell configuration of the patch-clamp technique AWD 23-111 was tested for its ability to block the delayed rectifier potassium channel (IK). In guinea pig ventricular myocytes the current is composed of two components: IK_r, a rapidly activating current and IK_s, a slowly activating component which were discriminated by their different activation and deactivation behaviour. In this preparation AWD 23-111 displayed concentration dependent inhibitory effects on IK_r as well as on IK_s in the tested concentration range between 1 and 100 μmol/l. This blocking effect was independent of the stimulation frequency (0.2, 1 and 2 Hz). There was no influence of AWD 23-111 on the amplitude of L-type calcium whole-cell currents. The compound significantly prolonged action potential duration (APD) at a stimulation frequency of 2 Hz (1 and 10 μmol/l). At 0.2 Hz there was no effect on APD. Our results suggest that AWD 23-111 blocks both components of IK without a reverse use-dependent effect on APD which limits the therapeutic potential of most other class III agents.

1. Introduction

N-(Dicyclohexyl-carbamoylmethyl)-*N*-(3-diethylamino-propyl)-4-nitro-benzamide hydrochloride (AWD 23-111) is a newly synthesised aminocarbonacide amide derivative structurally related to detajmium, a compound with sodium channel antagonistic properties [1]. In preliminary experiments it was shown that AWD 23-111 exhibits antiarrhythmic actions. AWD 23-11 prolongs the action potential duration (APD) and the effective refractory period (ERP) in papillary muscles of guinea pigs dose dependently between 0.3 and 1 μmol/l [2]. In conscious dogs the drug increases the cardiac repolarisation time in AV-node and ventricular tissue [3]. These effects were frequency independent. Because of its action potential prolonging characteristics AWD 23-111 has been classified as a novel class III antiarrhythmic substance according to Vaughan Williams [4].

Antiarrhythmic actions that selectivity prolong APD and ERP are known to suppress or prevent atrial and ventricular arrhythmia in animal models and in humans [5–7]. APD prolongation by antiarrhythmic drugs is a result of either an increase in inward currents or a decrease in outward currents. Several types of outward currents have been identified in mammalian tissues including time independent currents such as IK_1, ICl [8] and IK_p [9], inactivating currents such as I_{to} [10] and the delayed rectifier

potassium current (IK). The antiarrhythmic effect of class III substances has been related in most cases to the ability of blocking IK [11]. Antiarrhythmics with selective action toward potassium outward current have been developed to overcome the proarrhythmogenic risks that have been related to the application of class I antiarrhythmics which act on the sodium channel as well as K^+-channels and in some cases Ca^{2+}-channels. The block of Na^+-channels may facilitate the development of serious arrhythmias such as ventricular fibrillation [12]. This class I drugs exert marked use-dependence, i.e. the block is pronounced at high beat frequencies. Frequency dependent effects of class III antiarrhythmics have been described, too. Hondegham and Snyders [13] have suggested that the therapeutic potential of currently available class III compounds is limited by a diminished ability to prolong repolarisation at high heart rates. That would reduce the ability to terminate ventricular tachycardias. It has been shown that the block of K^+-channels by class III antiarrhythmics is reduced with an increased frequency of channel activation. This behaviour is the opposite of the conventional use-dependence displayed by class I compounds and has therefore been referred to as "reverse-use-dependence". The attenuation of the APD prolonging effect at higher beating frequencies decreases drug efficacy during ventricular tachyarrhythmia. In addition, torsades-de-pointes tends to occur when APD is markedly prolonged by blocking potassium outward current, particularly at slow heart rates [14, 12].

The delayed rectifier potassium current (IK) in guinea pigs is composed of two distinct components, IK_r and IK_s. They were distinguished based on their different kinetics, pharmacology, voltage dependence, and rectification properties [15, 16]. IK_r activates rapidly, rectifies inwardly and is sensitive to La^{3+}, whereas IK_s displays slow activating kinetics and slight outward rectification at positive test potentials. Several pharmacological agents that block IK act preferentially on IK_r and display often "reverse-use-dependence" during increased heart rate or β-adrenergic tone [17]. The open state of IK_s accumulates during rapid stimulation because of incomplete deactivation, therefore contributing progressively to shorten APD [18]. Compounds that are able to block also IK_s should therefore have an improved potency to prolong APD at rapid heart rate. At present, although side effects of diuretic agents like indapamide or triamterene to block cardiac IK_s are described [19, 20], antiarrhythmic substances selective for IK_s, like clofilium [16], are rarely known.

The purpose of the study was to examine whether the APD prolonging effect of AWD 23-111 is due to the alteration of time and voltage dependence of the outward current IK and the slow calcium inward current ICa.

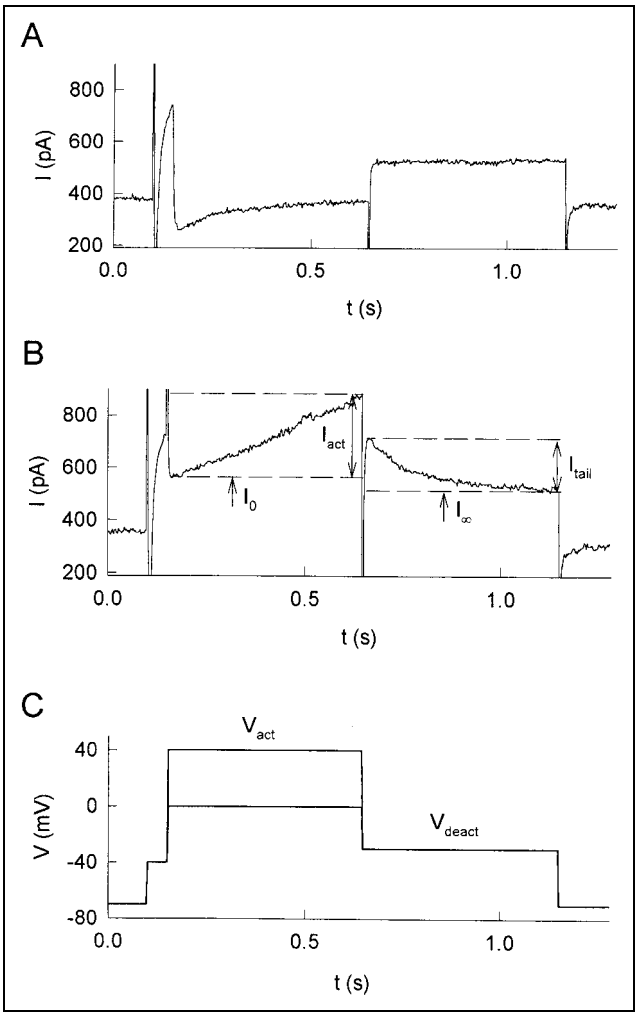

Fig. 1: Recording of delayed rectifier potassium currents of one cell during depolarising steps of 500 ms to potentials of 0 (**A**) and +40 mV (**B**) and subsequent repolarisation to −30 mV. INa was inactivated by a 50 ms prepulse going from the holding potential (−70 mV) to −40 mV. Currents were recorded in control solution 2 (buffered saline, 1 mmol/l Ca^{2+}, 0.5 mmol/l $CdCl_2$) in order to block L-type Ca^{2+} current. The levels for the current before activating $IK(I_0)$, for the amplitude of activating IK, (I_{act}), the amplitude of the deactivating current (I_{tail}) and the plateau current after deactivation of $IK(I_\infty)$ are indicated. **C**: Stimulation protocol

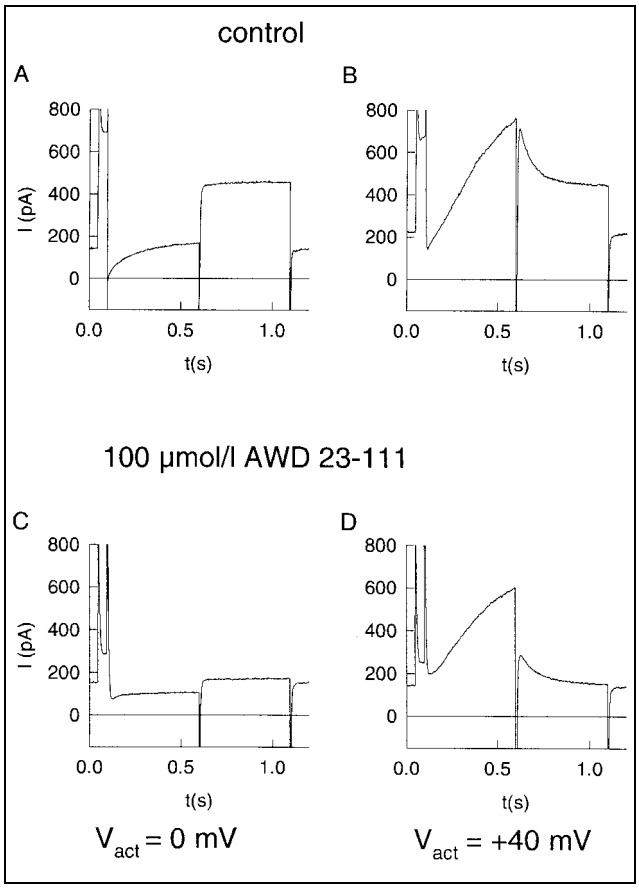

Fig. 2: Example of the blocking effect of AWD 23-111 on the delayed rectifier current (IK) during 500 ms depolarising voltage steps to 0 (left) and +40 mV (right) according to the recording conditions in Fig. 1. Upper panel: control conditions. Lower panel: After application of 100 µmol/l AWD 23-111

2. Investigations and results

2.1. Voltage clamp studies

2.1.1. Block of time dependent IK

Whole cell currents of IK ($IK_r + IK_s$) were evoked by applying depolarising steps between −30 mV and +40 mV. Fig. 1 shows an example of a membrane current elicited by a 500 ms voltage clamp step after a prepulse to −40 mV to block INa. Fig. 1C describes the stimulation protocol. All measurements were done in the presence of 0.5 mmol/l Cd^{2+} for blocking ICa. Fig. 1A reveals the trace of a time dependent outward current during depolarisation to the activation potential V_{act} of 0 mV. During repolarisation to the deactivation potential of −30 mV no deactivating current was detected. In the same cell a slowly increasing outward current developed during a voltage-clamp step to +40 mV (Fig. 1B). On repolarisation to −30 mV a decaying outward current was observed. This tail current (I_{tail}) has considered to display the deactivation of the delayed rectifier [16]. We tried to differentiate between the effects of AWD 23-111 on the two components of IK, IK_r and IK_s, by their different activation and deactivation behaviour. Despite of the fact that the activation curve of IK_r is shifted to more positive potentials by Cd^{2+} [21], it can be assumed that the deactivating current measured after depolarisation to test potentials more positive to 0 mV predominantly represents the slowly activating IK (IK_s). The activating current up to test potentials of 0 mV represents the rapid activating IK (IK_r) because of the following reasons: Tail currents could only be registered after depolarising pulses more positive than +10 mV, the range for activation of the slow component of the outward current. The tail currents measured after depolarising steps to +10 mV up to +40 mV followed by repolarisation to −30 mV could be fitted successfully by a monoexponential equation leading to the assumption that the tail currents contained only one component. At test potential to 0 mV a rapid activating current is clearly to be recognised, but no tail current was detectable following repolarisation to −30 mV.

Fig. 2 shows an example of membrane currents obtained from a cell during control conditions and after superfusion with 100 µmol/l AWD 23-111. The drug reduced the amplitudes of the activating currents (I_{act}) at depolarising steps to 0 mV and to +40 mV. The amplitude of the deactivating current following the repolarising step from +40 to −30 is also diminished. So it can be assumed that IK_r as well as IK_s were inhibited by the drug. But this blocking effect does not seem to be exclusively directed toward IK because the plateau outward current I_∞ is also reduced by the drug, a phenomenon which has not been further investigated in this study.

The voltage dependence of activating and tail currents is demonstrated in Fig. 3. During control conditions the activating current (I_{act}) shows a biphasic dependence on the activating potential. I_{act} increases with rising activation potentials showing an inward rectification up to +20 mV. The lack of inward rectification of activating currents at potentials ≥20 mV is consisted with the predominance of IK_s in this range of V_{act}. The hump in the I–V-relationship between 0 and +20 mV of V_{act} is diminished by the drug indicating that IK_r is blocked in this voltage range. The deactivating current of IK (I_{tail}) is significantly blocked by 10 µmol/l AWD 23-111 in the voltage range of V_{act} between +20 mV and +40 mV. The voltage dependence of the blocking effect becomes more clear in Fig. 3B where

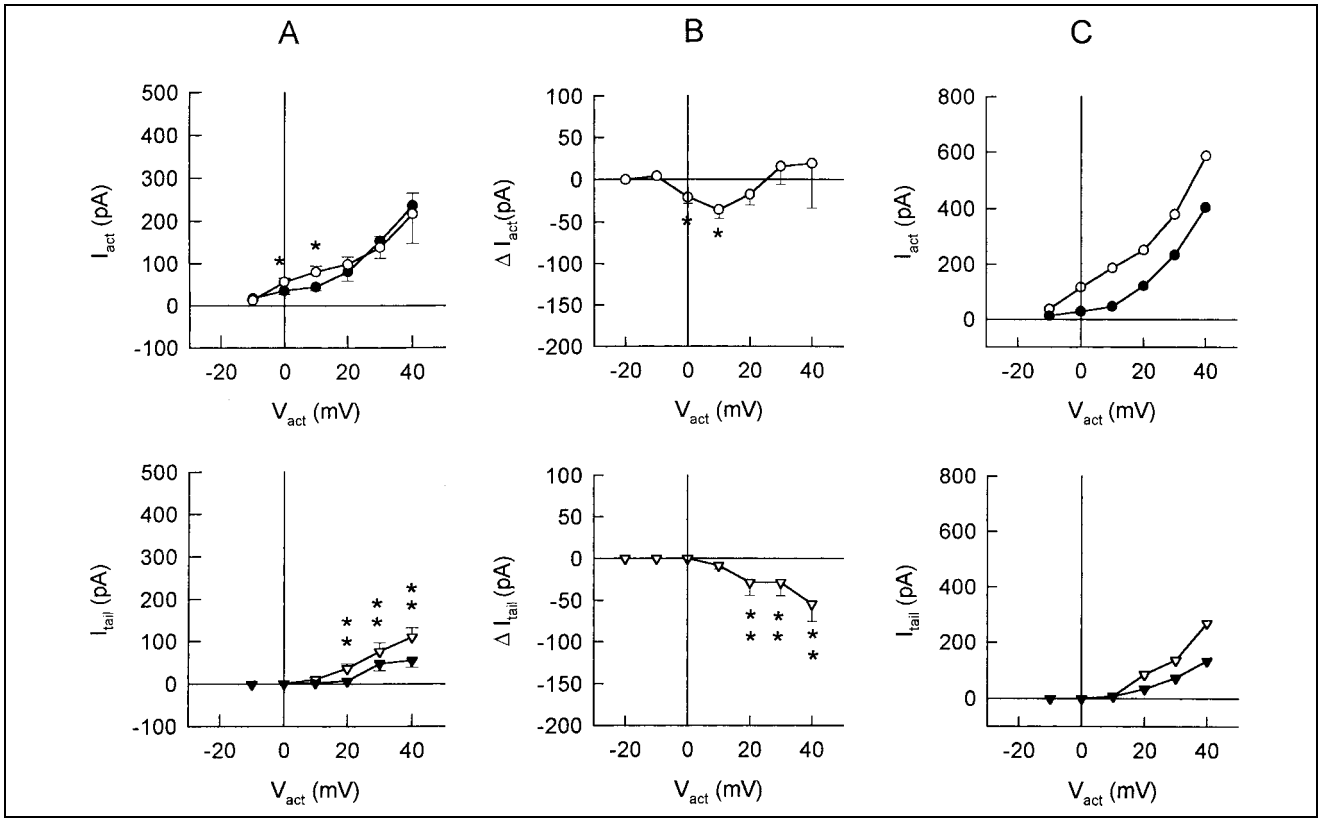

Fig. 3: **A**: I–V relations for the current amplitudes (I_{act} and I_{tail}) as explained in Fig. 1. Empty symbols: control conditions, filled symbols: Currents after application of 10 µmol/l AWD 23-111. Values are mean ±S.E.M.; * $p < 0.05$, ** $p < 0.01$, $n = 6$. **B**: Differences of the currents I_{act} (○) and I_{tail} (▽) before (control conditions) and after superfusion with 10 µmol AWD 23-111 have been calculated according to: $\Delta I_{act} = I_{act}$(10 µmol/l AWD 23-111) − I_{act} control and $\Delta I_{tail} = I_{tail}$(10 µmol/l AWD 23-111) − I_{tail} (control). The data are taken from the same experiment as plotted in Fig. 3A. **C**: I–V relation for a single measurement as described in Fig. 3A after superfusion with 100 µmol/l AWD 23-111, recording conditions as described in Fig. 1

the differences between the amplitudes of I_{act} during superfusion with 10 μmol/l AWD 23-111 and under control conditions (ΔI_{act}) are shown. The ΔI_{act} is a measure for the drug sensitive activating current. This current disappeared at $V_{act} > +20$ mV pointing to a marked inward rectification of the drug sensitive current. This indicates the inhibition of IK_r by the drug. The drug sensitive ΔI_{tail}, the differences between I_{tail} during superfusion with 10 μmol/l AWD 23-111 and under control conditions, shows a positive dependence on V_{act} and does not saturate i.e. the block of I_{tail} is higher at positive test potentials. Because this voltage dependence is a property of IK_s it can be concluded that not only IK_r but also IK_s is blocked by AWD 23-111. Fig. 3C shows an example (single measurement) of I_{act} and I_{tail} under conditions equal to Fig. 3A but with the application of 100 μmol/l AWD 23-111. At this high dose not only I_{tail} but also I_{act} is blocked in the voltage range between 0 and +40 mV. The dose-effect-relation of AWD 23-111 on I_{tail} is displayed in Fig. 4. The half maximum blocking concentration can be estimated to about 1 μmol/l AWD 23-111.

2.1.2. Rate-dependent effects on IK

For all experiments concerning rate dependent effects of AWD 23-111, 250 ms lasting depolarising pulses were applied from a holding potential of −40 mV. An example of the dependence of the current amplitude on the stimulation time is given in Fig. 5 for the activating current elicited by depolarisation to 0 mV at a stimulation frequency of 2 Hz. With this protocol no deactivating current was seen. Deactivating currents were measured using the same protocol but applying test pulses to +40 mV. As shown in Fig. 5 the amplitude of I_{act} is dependent on the number of depolarsing pulses applied reaching a steady state ($I_{act,c}$) whereas $I_{act,t}$ represents the time dependent part amplitude of I_{act} until reaching the steady state. The amplitudes of I_{act} and I_{tail} were measured as shown in Fig. 1. The time dependence of the amplitudes were approximated according to

$$I_{act}(t) = I_{act,t} \cdot \exp(-t/\tau_{act}) + I_{act,c} \quad (1)$$

for the activating current (solid line in Fig. 5) and according to

$$I_{tail}(t) = I_{tail,t} \cdot \exp(-t/\tau_{tail}) + I_{tail,c} \quad (2)$$

for the deactivating current (not shown). The time constants of the stimulation-dependent decay of I_{act} and I_{tail} were indicated as τ_{act} and τ_{tail} with mean values for the reciprocal time constants of $1/\tau_{act} = 0.30 \pm 0.03\ s^{-1}$ and $1/\tau_{tail} = 0.31 \pm 0.05\ s^{-1}$. The time constants were independent of the stimulation frequency and the applied dose of AWD 23-111 (0, 1 and 3 μmol/l).

The dependence on stimulation frequency and on AWD concentration of the stimulation time independent current amplitudes $I_{act,c}$ and $I_{tail,c}$ are shown in Fig. 6. The activating current ($I_{act,c}$) after depolarisation to 0 mV (Fig. 6A) and +40 mV (Fig. 6B) and the deactivating current during repolarisation from +40 mV to −40 mV ($I_{tail,c}$, Fig. 6C) were measured at different stimulation frequencies (0.2, 1.0 and 2.0 Hz). In each stimulation frequency group $I_{act,c}$ and $I_{tail,c}$ were significantly blocked in a dose dependent manner compared to the respective control group. $I_{act,c}$ and $I_{tail,c}$ of each dose group were independent of the stimulation frequency.

2.1.3. Effect on ICa

Fig. 7a shows a single measurement of an I–V-curve of ICa under control conditions and after superfusion with 3 μmol AWD 23-111. There was neither an effect on the slope conductance nor on the voltage or half maximal activation. As displayed in Fig. 7b there is a significant difference between the amplitude of ICa at a stimulation frequency of 0.2 and 2 Hz but there is no influence of AWD 23-111 at both frequencies.

2.2. AP studies

The average APD_{80} of cells bathed in control solution was 242 ± 17 ms when stimulated with 0.2 Hz and 212 ± 34 when stimulated with 2 Hz.

The effects produced by AWD 23-111 on the AP configuration of isolated ventricular myocytes were variable and inconstant. Fig. 8 demonstrates the effect of AWD 23-111

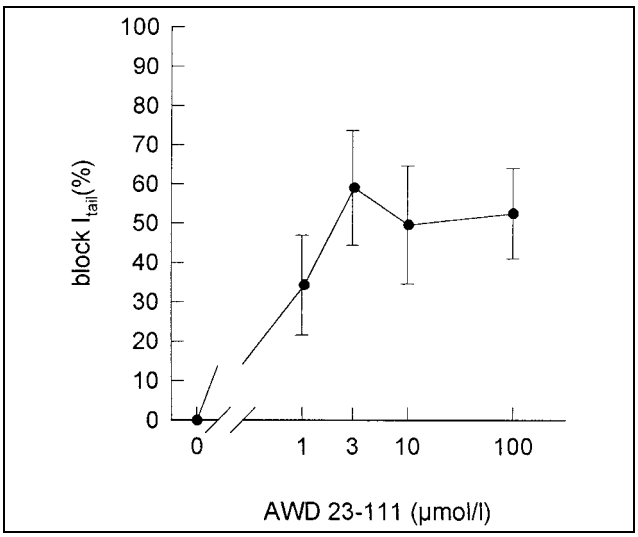

Fig. 4: Concentration-dependence of the blocking effect of AWD 23-111 on I_{tail}. n = 6–11, recording conditions as described in Fig. 1B

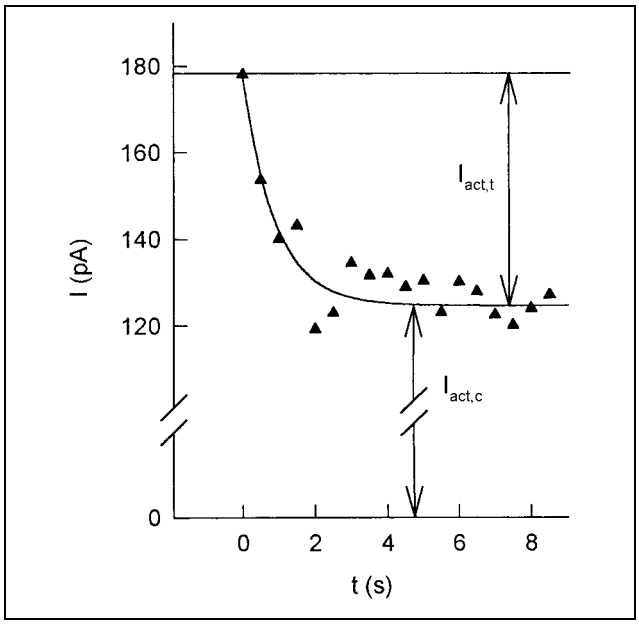

Fig. 5: Stimulation-dependence of the amplitude of the activating current at a stimulation frequency of 2 Hz (example). Recording conditions: 250 ms lasting depolarising steps were applied from a holding potential of −40 mV to $V_{act} = 0$ mV. The time dependence of the amplitude of I_{act} (▲) was approximated according to eq. (1) where $I_{act,t}$ represents the time dependent part amplitude of I_{act} until reaching the steady state current ($I_{act,c}$). The time constant of the stimulation dependent decay of I_{act} was indicated as τ_{act}. In this example $I_{act,t} = 52.6$ pA, $I_{act,c} = 124.6$ pA and $\tau_{act} = 0.9$ s

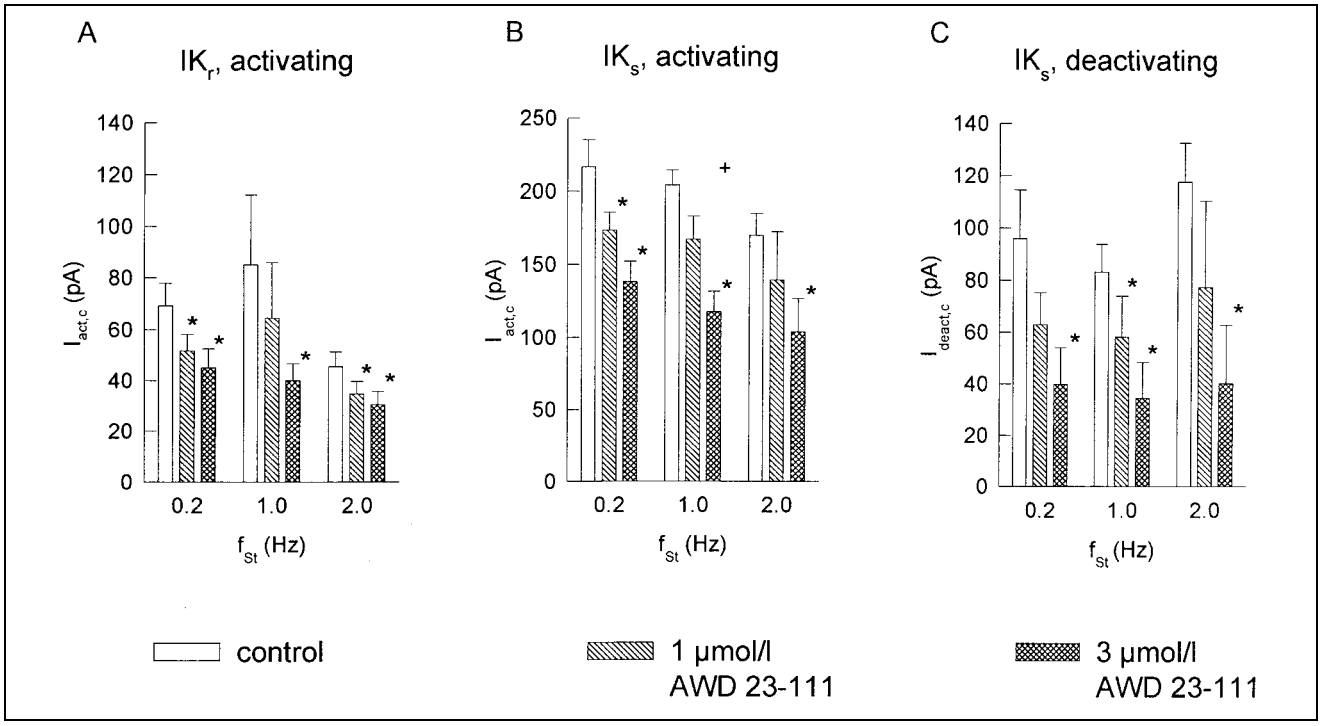

Fig. 6: Effect of AWD 23-111 on the stimulation independent amplitude (explanation see Fig. 5) of the potassium outward current after 250 ms lasting depolarisation either from **A**) −40 mV to 0 mV (IK$_r$, activating); **B**) −40 mV to +40 mV (IK$_s$, activating) and **C**) the deactivating current amplitude after depolarisation from −40 mV to +40 mV and subsequent repolarisation to −40 mV (IK$_s$, deactivating). * $p < 0.05$, $n = 6–35$

on APD_{80} at 0.2 and 2 Hz with drug concentrations of 1, 10 and 100 µmol/l. A significant AP prolonging effect of AWD 23-111 was only detected at concentrations of 1 and 10 µmol/l at the stimulation frequency of 2 Hz. At 100 µmol/l and 0.2 Hz AWD 23-111 was without effects.

3. Discussion

In the present study we have shown that AWD 23-111, a new antiarrhythmic compound [2, 3, 22] decreased potassium outward currents in isolated guinea pig ventricular myocytes. The delayed rectifier potassium current in these cells consists of a fast and a slowly activating component. With prolonged pulse duration IK_s contributes increasingly to the aggregate IK. Whereas IK_r is fully activated after 200 ms, IK_s needs several seconds for complete activation. In the present study IK was elicited either by 500 ms lasting depolarising pulses to test the voltage dependence or 250 ms depolarising pulses for testing the dependence on stimulation frequency. Voltage clamp studies in guinea pig demonstrated that the ratio of IK_r to IK_s is larger than 1 during the first 50 to 100 ms of an activating clamp step and declines as the clamp step is prolonged. During the plateau phase of a normal action potential the magnitude of the two components is roughly the same [23, 17, 16]. IK_r displays strong inward rectification properties at activation potentials more positive then +10 mV so IK_r comprises less to the whole IK above this test potentials. It is described for guinea pig ventricular myocard cells that the La^{3+}-insensitive component of IK (IK_r) comprises about one third to the activating outward current after a 500 ms lasting depolarising step to +50 mV. [24]. In the present work the two components of IK were discriminated only by their different activating and deactivating behaviour and by their different voltage dependence. The application of selective blockers of IK_r, like E-4031 or $LaCl_3$, has been avoided not only because it has been argued that these blockers may influence the deactivation behaviour of IK_s [25] but also to prevent possible interferences with AWD 23-111. The only blocking substance we used was $CdCl_2$ to inhibit ICa. The I–V-relation (Fig. 3) showed that at activating potentials up to +10 mV (500 ms depolarisation) the outward current developed without exhibiting a tail current following repolarisation. This is in opposite to other IK_r described in the literature that show a prominent deactivating tail current also after depolarisation to +10 mV and following repolarisation to −40 mV [21, 26]. This may be due to the strong acceleration of the deactivation of IK_r by Cd^{2+} as described for the IK in cat ventricular myocytes [21] or a blocking effect of the relatively high Cd^{2+} concentration (0.5 mol/l) we used for inhibition of ICa. Tail currents we could only measure at test potentials more positive than +10 mV where IK_s starts to activate and IK_r shows a strong inward rectification. So we can assume that under the described conditions I_{act} at $V_{act} = 0$ mV represents IK_r and the current during repolarisation from +40 to −30 mV represents mainly the deactivating IK_s.

The voltage dependence of the effects of AWD 23-111 was assessed by analysing the I–V relationship. It was shown that I_{act} is only blocked significantly between $V_{act} = -10$ and +10 mV by the drug at 10 µmol/l, whereas tail currents were increasingly blocked with more positive activation potentials. There is an apparent dissociation between drug effects on the current flowing during test depolarisation and the tail currents elicited by repolarisation. AWD 23-111 is able to block I_{tail} without producing corresponding changes in the time dependent outward current at positive test potentials (Fig. 3A). Only at high doses (100 µmol AWD 23-111) a considerable block of the outward current at positive test potentials is obvious (Fig. 3C). Similar behaviour is described for encainide and flecainide being able to block I_{tail} without altering the outward current at positive test potentials in cat ventricular myocytes. Because of the enhanced suppression of I_{tail} at more positive test potentials the authors suggested a vol-

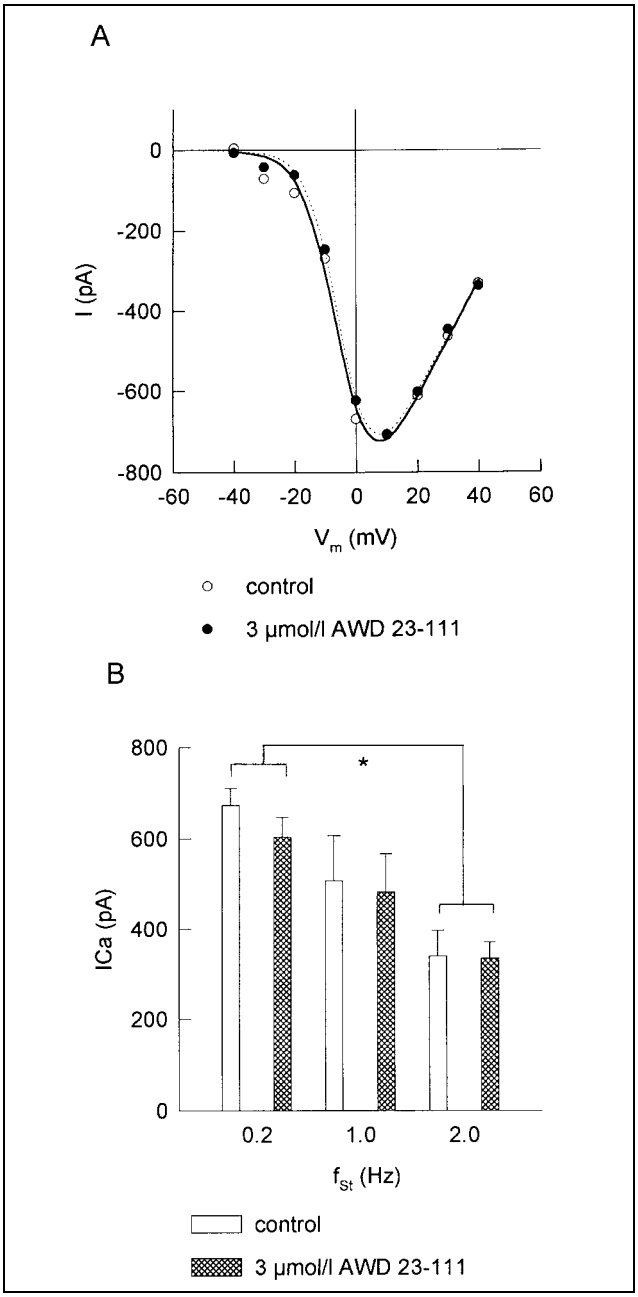

Fig. 7: **A**: I–V relationship of a single measurement of I_{Ca} under control conditions and after superfusion with 3 µmol AWD 23-111. I_{Ca} was elicited from a holding potential of −40 mV to variable test potentials between −30 mV and +40 mV in 10 mV steps with a test plus duration of 250 ms and a stimulation frequency of 0.2 Hz. **B**: Effect of 3 µmol/l AWD 23-111 on the amplitude of I_{Ca}. Application of test pulses from −40 mV to +40 mV with stimulation frequencies between 0.2 and 2 Hz. * $p < 0.05$, $n = 5–10$

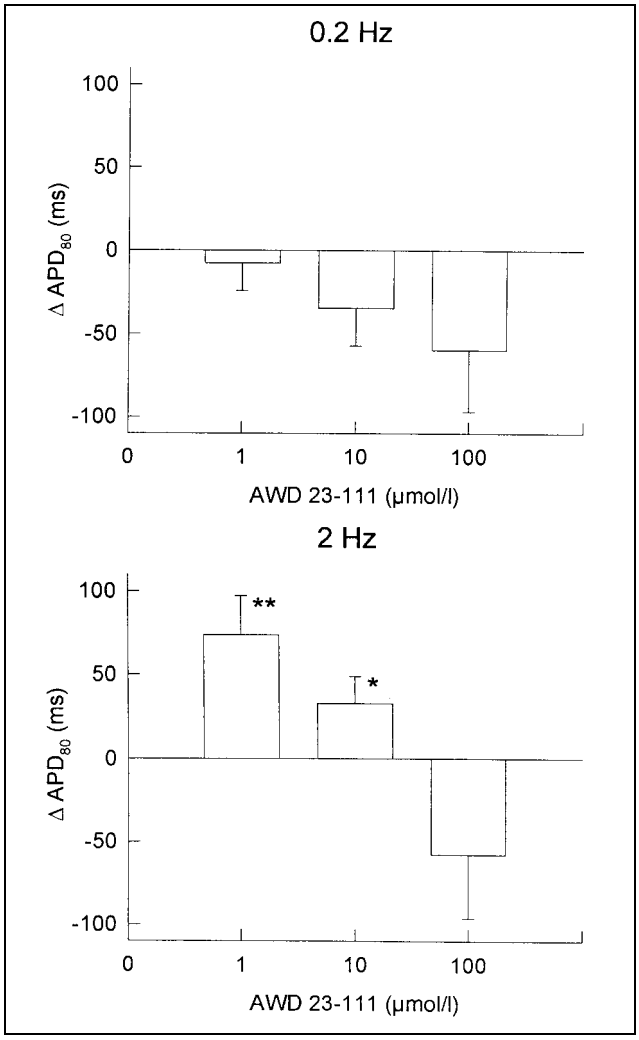

Fig. 8: Changes of APD_{80} after application of AWD-23-111. Measurements were obtained at stimulation frequencies of 0.2 and 2 Hz. Significant concentration dependent changes were marked by ** $p > 0.01$ or * $p < 0.05$, $n = 5$

tage dependent block of IK [27]. This may also be true for AWD 23-111. Hence, our findings suggest that AWD 23-111 is able to block the two components of the IK. That means the action of the drug is not specifically directed toward one component of IK but seems to be more pronounced at negative potentials. The blocking effect on IK is dose dependent (Figs. 4 and 6), reaching a maximum blocking effect of 50% for I_{tail} independent of the stimulation frequency with an IC_{50} of 1 µmol/l.

The time dependence of the blocking effect of AWD 23-111 has been evaluated by comparing the drug effects at stimulation frequencies between 0.2 and 2 Hz. The blocking effect of AWD 23-111 toward IK_r (I_{act}, $V_{act} = 0$ mV) or IK_s (I_{tail}, $V_{act} = +40$ mV) is not dependent on stimulation frequency. So there is no hint for a use dependence of the drug action on IK. The often observed decrease in efficacy of class III agents in prolonging the action potential at higher frequencies is described as "reverse-use dependence" [13]. This behaviour is also described for quinidine, a class I reagent with significant blocking effects on IK [28, 29]. It is suggested that other repolarising currents like the caffeine sensitive outward current [30], contribute increasingly to the repolarisation of the action potential at faster stimulation rates [28]. During rapid stimulation the open state of the slowly activating IK_s accumulates contributing more to the repolarisation process. Because most of the class III agents are shown to act specifically on IK_r, the blocking effect declines with increasing heart rate. A blocking agent whose action is not specifically directed toward IK_r but also IK_s could be effective at higher stimulation rates, too. It is suggested that a compound that affects IK_s might be devoid of reverse use-dependence [18]. But until now, the specifity of substances that have been described to act on IK_s remains to be proved [31].

Our finding that the blocking effect of AWD 23-11 is independent of the stimulation frequency is consistent with Poppe and Bartsch [2], who found a prolongation of APD_{90} and ERP in papillary muscle of guinea pigs at doses between 0.3 and 10 µmol/l acting with slower (1 Hz) and faster (3 Hz) stimulation in similar potency. In

conscious chronically instrumented beagles after myocardial infarction AWD 23-111 prolonged significantly the paced QT-interval, AV-node ERP and ventricular ERP under infusion of AWD 23-111 ($160\ \mu g/kg) \cdot min^{-1}$). The incidence of polymorphic ventricular tachycardias (PVT) with specific features of torsade-des-pointes in anaesthetised rabbits under α_1-adrenoceptor stimulation was low (1 out of 8 animals) [3]. The frequency independent action of AWD 23-111 may minimise the risk of the occurrence of torsades-de-pointes-like arrhythmia described for class III antiarrhythmic compounds [12]. It could therefore be expected that the drug will remain effective also in tachyarrhythmia. Class III antiarrhythmic agents are defined as drugs that act primarily by prolonging the APD by reducing the repolarising current. We also tested the effect of AWD 23-111 on the AP of guinea pig ventricular cells. Instead of the expected marked increase of APD_{80} due to the inhibition of IK we found very variable effects of the drug on the action potential of single myocard cells. Statistically significant APD prolonging effects of AWD 23-111 were obvious only at a pacing frequency of 2 Hz with a drug concentration of 1 and 10 μmol/l. Moreover, in some cells AP shortenings were measured. These in part contradicting effects might be explained by the heterogeneous cell suspension used. We did not separate endocard from epicard and it is known that they differ concerning their ion channel occurrence [32]. Stark et al. [22] who recently investigated the effects of AWD 23-111 on spontaneously beating guinea pig hearts found a concentration dependent marked slowing of the intraventricular and His-bundle conduction. These effects were combined also with a concentration dependent significant increase in atrial and ventricular effective refractory period. These findings could indicate a sodium antagonistic effect which they have characterised by a very slow association and dissociation kinetic to the channel. Furthermore, the drug produced a prolongation of the repolarisation period (JT-interval) without reverse use-dependence. Stark et al. [22] argued that the main cause for the depressant effect of AWD 23-111 on AV-conduction may be a direct calcium antagonistic activity. But an effect of this drug on ICa was not confirmed in ventricular myocard cells, so the depression of sinus rhythm and slowing of AV-conduction [22] might be mainly due to the potassium antagonistic effect of AWD 23-111.

The combination of the rate dependent block of INa observed by Stark et al. [22] and the rate independent block of IK demonstrated in the present study, may produce the variable effects of AWD 23-111 on APD_{80} in single ventricular myocard cells during different pacing rates. While an IK block usually prolongs the AP, an INa block with slow binding kinetics should be expected to decrease AP duration at higher pacing rates. This is demonstrated by Follmer et al. [27] for recainam, a INa blocking compound without IK blocking properties that shortens APD. The effects on AP duration are dependent on kinetics of block and unblock the sodium channel determining whether the substances shorten or lengthen the APD. But the drug effect on APD also depends on the type of cardiac tissue studied. Detajmium, a compound structurally related to AWD 23-111, is reported to shorten APD in dog Purkinje fibres with no effect on ventricular muscle fibres [1]. Actions on potassium channels were not considered in this work.

In conclusion we demonstrated in this study that a potassium antagonistic effect of AWD 23-111 exists which was already supposed by Stark et al. [22]. It may be responsible for the prolongation of the repolarisation period. The depressive effects on spontaneous sinus rate, which was observed in the isolated guinea pig heart may also be due to the potassium antagonistic effect of AWD 23-111 because prolongation of the repolarisation period reduces the firing rate of these cells [33, 34]. A substance acting not only on IK_r but also on IK_s is expected to retain its antiarrhythmic activity also at risen heart rates [13] and elevated β-receptor stimulation. These conditions increase the relative contribution of IK_s to the net repolarising current [17] that diminishes the relative blocking effects of pure IK_r blockers on outward current [35]. However, the efficacy of such a drug is determined by the density of IK_r and IK_s channels in the human heart [36]: The majority of human atrial cells possess a slowly developing outward current that is thought to contribute to the repolarisation period of the AP. It seems to be a composite of a rapidly activating (IK_r) and a slowly activating (IK_s) component [37]. DNA encoding IsK protein that evokes a very slowly activating K-conductance similar to IK_s is also found in the human heart [32]. Recently, Li et al. [38] have demonstrated that IK is present in human ventricular cells and that the current can be dissociated into components corresponding to IK_r and IK_s.

The potential electrophysiological effects of AWD 23-111 in human heart cells remain to be proven.

4. Experimental

4.1. Single myocytes

Ventricular myocytes were isolated from the heart of guinea pigs (250–400 g) as described by Markwardt and Nilius [39]. In brief: The animals were killed by cervical dislocation. The hearts were quickly excised and mounted on a Langendorff apparatus. After a 5 min perfusion at 37 °C with nominally Ca^{2+}-free buffer solution (composition: (mmol/l) 135 NaCl, 5.4 KCl, 4 $MgCl_2$, 10 Hepes, 15 glucose, pH 7.2 (NaOH)) a perfusion followed with the same solution but containing collagenase (Sigma type I, 25 mg/100 ml), protease from *Aspergillus oryzae* (Sigma, 2.5 mg/100 ml) and bovine serum albumine, essentially free fatty-acid free (Sigma, 100 mg/100 ml). All solutions were gassed with O_2. Usually after 7 to 10 min the perfusion pressure declined and the ventricles were cut off. The digested ventricle tissue was taken into an incubation-medium of following composition: (mmol/l): 140 K-glutamate, 10 Hepes, 5 EGTA, 2 glucose, pH 7.2, adjusted with KOH, minced to pieces and triturated briefly. The cell suspension was washed three times by centrifugation and resuspension in incubation-medium. Finally, the cells were stored in MEM-medium and used between 1 and 7 h later.

4.1.1. Extracellular and drug solutions

Control solution 1: (mmol/l) 140 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, pH 7.4, adjusted with NaOH
Control solution 2: (mmol/l) 140 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, 0.5 $CdCl_2$, pH 7.4, adjusted with NaOH
Drug solution: It was prepared from a stock solution of 1 mmol/l AWD 23-111 by filution with control solution 1 or 2 to obtain the desired final drug concentration.

4.1.2. Intracellular solution

The intracellular pipette solution contained: (mmol/l): 65 KCl, 65 K-aspartate, 0.5 $MgCl_2$, 10 Hepes, 10 EGTA, 10 glucose, 5 Mg-ATP 0.1 cAMP, pH 7.2 adjusted with KOH.
The same intracellular solution was applied for ICa- and AP-measurements but without containing cAMP.

4.2. Electrophysiological methods

An aliquot of dissociated cells was placed in a 0.5 ml chamber mounted on the stage of an inverted microscope. The chamber was equipped with a double glass bottom to thermostate the chamber by a water circuit to 34 °C. The cells were allowed to adhere at the glass bottom and were then superfused continuously with control solution 1 preheated at 34 °C. All currents were recorded by a standard voltage clamp technique [40]: A patch-clamp amplifier (EPC 7, List, Darmstadt) was used. Voltage-clamp commando pulses were generated by a digital-analogue converter con-

trolled by a software package. The software for recording and analysing membrane currents was developed in our department. Membrane currents were filtered at 2 kHz and stored on disc for subsequent analysis.
Only rod shaped cells with clear cross striation and a membrane potential more negative than −65 mV were selected.
To study outward potassium currents, pipettes with resistances between 2 and 4 mΩ when filled with intracellular solution were used. The whole-cell configuration was established while superfusing the cells with control solution 1. Then, this solution was exchanged by control solution 2 containing 0.5 mmol/l $CdCl_2$ to block the L-type Ca^{2+}-current. According to the stimulation protocol (Fig. 1) Na^+- and T-type Ca^{2+}-currents were inactivated by an prepulse from the holding potential (−70 mV) to −40 mV. Potassium outward currents were elicited as shown in Fig. 1. After assessment of membrane currents in the absence of drug (3 min), the perfusate was changed to one containing the various concentrations of AWD 23-111 and data were collected continuously. Frequency dependent effects of AWD 23-111 on IK were tested by applying 250 ms lasting depolarising pulses from a holding potential of −40 mV to 0 mV or to +40 mV at stimulation frequencies of 0.2, 1 and 2 Hz.
Ca^{2+}-currents were recorded in a similar way but using control solution 1 and the intracellular solution but without cAMP. The L-type Ca^{2+}-current was elicited by depolarising steps from a holding potential of −40 mV to test potentials between −30 and +40 mV at a stimulation frequency of 0.5 Hz for the registration of the I–V-relationship. To test the frequency-dependent effects of AWD 23-111 on ICa 250 ms lasting test potentials to +40 mV were applied at stimulation frequencies of 0.2, 1 and 2 Hz starting from a holding potential of −40 mV.
Action potentials were recorded in the current clamp mode. The cells were superfused by control solution 1 and the intracellular solution contained no cAMP as described above. APs were evoked by 20 ms lasting suprathreshold depolarising currents at stimulus frequencies between 0.2 and 2 Hz during control and after superfusion with AWD 23-111.

4.3. Statistics

Data were expressed as mean ±SEM. Student's paired t-test was used to compare two means. Comparisons between multiple means were performed by two-way ANOVA followed by subsequent pairwise t-test if the null hypothesis could be rejected. A two-tailed probability was considered to be statistically significant. Null hypothesis was rejected at the $p < 0.05$ level.

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Can diastereoisomerism of alkoxyphenylcarbamates influence their local anesthetic activity?

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The surface local anesthetic activity (LAA) in the homologous series of racemic (±)-*cis*- and (±)-*trans*-*N*,*N*-dimethyl-2-(2-alkoxyphenylcarbamoyloxy)cyclopentylmethylamonium chlorides was evaluated. The potency was expressed in rabbits as efficiency indices (EI) in comparison to the standard drug cocaine. All tested racemic mixtures of the phenylcarbamates were local anesthetically active and their potency increased with the size of alkoxysubstitution from the propyloxy- to the hexyloxyderivative and then decreased abruptly (cut-off effect). When different mixtures of both diastereoisomers were applied the synergistic effect – i.e. increase of the LAA of one diastereomer when adding the other – was observed. It seems that an optimal racemic ratio of the compounds could increase their local anesthetic efficiency.