Inhibition of the cardiac L-type calcium channel current by antidepressant drugs

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Abbreviations: EGTA – ethylene glycol-bis(β-amino-ethyl ether) N,N,N',N'-tetraacetic acid;
HEPES – N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]; IBMX – isobutylmethyl-
xanthine; I_{Ca} – calcium current; TTX – tetrodotoxin;

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ABSTRACT

Antidepressants inhibit many membrane receptors and ionic channels, including the L-type calcium channel. Here we investigated the inhibition of calcium current (I_{Ca}) by antidepressants in enzymatically isolated rat ventricular myocytes using whole-cell patch-clamp. The molecular mechanism of inhibition was studied by comparing the voltage- and state-dependence of antidepressant inhibition of I_{Ca} to the respective properties of calcium antagonists, and by studying the effect of BayK 8644 or diltiazem on the inhibitory potency of the antidepressants. All selected antidepressants inhibited calcium currents reversibly and concentration-dependently. At a stimulation frequency of 0.33 Hz, the antidepressants imipramine, clomipramine, desipramine, amitriptyline, maprotiline, citalopram, and dibenzepin blocked I_{Ca} with IC_{50} values of 8.3, 11.6, 11.7, 23.2, 31.0, 64.5 and 364 $\mu\text{mol/L}$. The antidepressant drugs shifted steady-state inactivation curves of I_{Ca} to negative voltages. The extent of the shift was similar to that induced by diltiazem or verapamil, but significantly smaller than that induced by felodipine. The use-dependent component of the antidepressant-induced block was similar to that of diltiazem, and significantly more and less, respectively, than those of felodipine and verapamil. In the presence of Bay K 8644, antidepressants were more effective in inhibiting I_{Ca} . However, the inhibitory effect of antidepressants was also augmented by diltiazem, suggesting that these drugs do not compete with diltiazem for a single binding site. These data suggest that antidepressants exert their inhibitory action on cardiac L-type calcium channels by a specific interaction at a receptor site similar to, but distinct from, the benzothiazepine site.

INTRODUCTION

Antidepressants of different chemical structure and mechanism of action are used in the therapy of major depression (DeVane, 1998). Due to their unspecific mode of action, they produce many side effects, cardiotoxicity being among the most serious ones (Glassman, 1998). Tricyclic antidepressants are known to inhibit calcium currents in heart myocytes (Isenberg and Tamargo, 1985; Delpon et al., 1991) as well as in neurons (Ogata *et al.*, 1989; Choi *et al.*, 1992). They also modulate binding of dihydropyridines as well as that of diltiazem and gallopamil to the calcium channel (Schaeffer *et al.*, 1991; Rehavi *et al.*, 1993), and calcium antagonists of the verapamil type were shown to affect binding of ^3H -imipramine (Rehavi *et al.*, 1988). However, no attempt was made to specify the mechanism of the interaction between the antidepressant drugs and the L-type calcium channel. Antidepressants affect diverse membrane receptors and transport systems (Hall and Ogren, 1981; White et al., 1990; Habuchi et al., 1991; Rana et al., 1993; Woollorton and Mathie, 1993), as do many other amphiphilic molecules, including calcium antagonists, in the high (micromolar) concentration range (*see e.g.*, Hargreaves et al., 1996; Rauer and Grissmer, 1996). Thus, understanding the degree of specificity of these drugs and the mechanisms involved in the drug-receptor interaction is of general importance.

In the present work, we study the mechanism of the inhibitory effects of a series of antidepressants on L-type calcium channels. We have employed seven antidepressants (Figure 1): imipramine and its closely related derivatives clomipramine and desipramine; the less structurally related tri- and tetracyclic drugs amitriptyline, maprotiline, and dibenzepin; and the very different citalopram (a selective serotonin reuptake inhibitor), to test whether the effects of antidepressants on the L-type calcium current (I_{Ca}) are determined by their specific molecular characteristics. All these drugs are widely used in the therapy of major depression. We show that all the studied antidepressants inhibit L-type calcium channel current

specifically and in a use- and voltage-dependent manner. Our results suggest that the antidepressants exert their inhibitory action at a binding site that has similar properties to the benzothiazepine (BTZ) receptor of the L-type calcium channel but is distinct from it.

METHODS

Isolation of myocytes. Ventricular myocytes were isolated from male Wistar rats (200-250 g) as previously described (Zahradník and Palade, 1993). Cell isolation routinely yielded 50-70% of rod-shaped, viable, calcium-tolerant cells. The experimental protocol was approved by the State Veterinary and Food Administration of the Slovak Republic and all animal procedures complied with the appropriate animal care regulations.

Calcium current measurements. The L-type calcium currents (I_{Ca}) were recorded using standard whole-cell patch clamp (Hamill *et al.*, 1981). The myocytes were placed into the perfusion channel (volume 50 μ l) allowing rapid (<1 sec) and complete exchange of external solutions. Sodium current was inhibited using -50 mV holding potential and 20 μ mol/L tetrodotoxin (TTX) in the external solution, and potassium currents were blocked by replacing K^+ with Cs^+ in both external and internal solution. The standard pulse protocol for recording calcium currents consisted of a continuous train of 70 ms long voltage pulses elicited every 3 sec from a holding potential of -50 mV. Calcium channels were kept in phosphorylated state by including 50 μ mol/L cAMP to the internal solution, and 10 μ mol/L isobutylmethylxanthine (IBMX, a membrane-permeant phosphodiesterase inhibitor) into the external solution. These conditions were chosen to standardize cell status by reducing the variability of calcium currents due to variable phosphorylation level of calcium channels in isolated myocytes (Zahradník and Palade, 1993), to reduce calcium current rundown (Ono and Fozzard, 1992) and to eliminate the consequences of eventual interactions of the studied drugs with the phosphorylation/dephosphorylation pathways. Experiments were carried out at 20-24°C.

Solutions for patch-clamp recordings. The external solution contained (in mmol/L): 135 NaCl, 5.4 CsCl, 10 N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), 5 $MgCl_2$, 0.33 NaH_2PO_4 , 1 $CaCl_2$, 0.02 TTX, 0.01 IBMX, pH 7.3. This solution was also used as the

vehicle for the drugs. The internal solution contained (in mmol/L): 135 CsCH₃SO₃, 10 CsCl, 10 HEPES, 1 EGTA, 3 MgSO₄, 3 ATP, 0.050 cAMP, pH 7.3.

Data analysis. Whole-cell currents were measured with the EPC-7 patch-clamp amplifier (List Medical Electronic, Germany), filtered at 2 kHz and digitized at 5 kHz by the Labmaster interface (Scientific Solutions, USA) using pClamp (Ver. 5.5.1, Axon Instruments, USA). Series resistance (≤ 2.5 M Ω) was compensated electronically by $\geq 50\%$. The cell capacitance was canceled in part electronically, and in part with an on-line subtraction procedure. The results are expressed as mean $\pm$ standard error. The values of fitted parameters are given with the standard errors of the fit. Tests of statistical significance were carried out using the Student's t-test. Multiple comparisons were made by one-way ANOVA, using Bonferroni correction for post-tests. All analyses were performed in Origin (Ver. 7.0, OriginLab, USA)

Structural analysis. Calculations of lipophilicity were performed and the structures of the drugs were optimized (MM2 force field) using the program Molgen (Baricic and Mackov, 1995).

pKa measurements. The pKa values of desipramine, imipramine, and clomipramine were determined by titration of 5 mM solution of the hydrochlorides with 1 M NaOH in unbuffered Tyrode solution.

Drugs. TTX and IBMX were from Sigma (St. Louis, MO). The drugs were from the following sources: amitriptyline (Léčiva, Prague, Czech Republic), citalopram (courtesy of Lundbeck, Copenhagen, Denmark), clomipramine and maprotiline (Ciba-Geigy, Basel, Switzerland), ($\pm$)verapamil, imipramine and desipramine (Sigma, St. Louis, MO), dibenzepin (Sandoz, Basel, Switzerland), ($\pm$)Bay K 8644 (Calbiochem, Lucerne, Switzerland), D-*cis*-diltiazem (Lachema, Brno, Czech Republic), felodipine (courtesy of Astra Hässle, Mölndal, Sweden). All other chemicals were of analytical grade. All solutions were made using double distilled water. Drug solutions were prepared by dilution in the extracellular solution at the

beginning of each experiment. Stock solutions of dihydropyridines were made in ethanol (the maximum final ethanol concentration of 0.01% was without effect on calcium currents).

RESULTS

The effect of the antidepressants on the peak amplitude of calcium current (I_{Ca}) was investigated using the standard pulse protocol. In the typical experiment of Figure 2A, application of progressively higher concentrations of desipramine led to a concentration-dependent inhibition of I_{Ca} . The IC_{50} for desipramine in this particular experiment was 11.0 μ mol/L. The inhibition of I_{Ca} proceeded rapidly, and was reversible. All antidepressants tested in this study provided qualitatively similar results. Although inhibition by the antidepressants required higher drug concentrations than inhibition by the calcium antagonists felodipine, verapamil, and diltiazem (Zahradníková et al., 2007), their concentration dependence was of the same form as that of the calcium antagonists and full inhibition could be achieved at a sufficiently high drug concentration. The concentration dependence of antidepressant action on calcium currents is illustrated in Figure 3. The fast and concentration-dependent inhibitory action of antidepressants suggests that their effect might be caused by interaction with a specific receptor site on the L-type calcium channel.

State-dependence of the inhibitory action.

Inhibition of I_{Ca} by Ca antagonists is known to be accompanied by a shift of the Ca current inactivation (I_{Ca} availability) curve to negative voltages (Sanguinetti and Kass, 1984; Uehara and Hume, 1985), resulting in increase of drug potency with membrane depolarization. To assess the effect of antidepressants on I_{Ca} availability, we measured I_{Ca} in response to test voltage pulses to +10 mV, applied every 10 seconds from a holding potential of -50 mV. The test pulses were preceded by 5 s prepulses to a set of inactivating voltage levels (ranging between -80 to -10 mV) separated from the test pulse by a 15 ms sojourn at -50 mV. Typical currents recorded with this protocol in the absence and presence of 20 μ mol/L amitriptyline are shown in Figure 2B. Peak amplitudes of currents were normalized relative to the maximal amplitude in each experiment (Figure 2C), and the data were fitted with the

Boltzmann equation with a midpoint $V_{0.5}$ and a slope parameter k . Antidepressants evoked a negative shift of $V_{0.5}$ of the I_{Ca} availability curve, reversible on washout of the drugs. The values of the shifts in $V_{0.5}$ and k for the antidepressants as well as for the representatives of the three types of calcium antagonists (the dihydropyridine felodipine, the phenylalkylamine verapamil, and the benzothiazepine diltiazem) are given in the left columns of Table 1. The shift of I_{Ca} availability curve was statistically significant for all antidepressants, and not significantly different from the shift induced by diltiazem and verapamil, while the dihydropyridine antagonist felodipine induced a significantly larger shift.

The antidepressant-evoked inhibition could be relieved by negative membrane potentials. Successive application of 10 and 50 $\mu\text{mol/L}$ desipramine (Figure 4A) led to almost full inhibition of I_{Ca} with the standard stimulation protocol from the holding potential of -50 mV. A 30-s rest period at -80 mV relieved 86% of the inhibition, which was restored fully and rapidly after resuming stimulation. All tested antidepressants provided similar results. These results further support the idea of a selective and state-dependent interaction of the antidepressants with the L-type calcium channel.

The extent of I_{Ca} inhibition depended on the frequency of depolarizing pulses, in a way similar to use-dependent action of calcium antagonists such as verapamil and diltiazem (Lee and Tsien, 1983; Sanguinetti and Kass, 1984; Uehara and Hume, 1985). As illustrated in Figure 4B, the degree of tonic inhibition evoked by application of 10 $\mu\text{mol/L}$ clomipramine was 33.5%. Stimulation at a reduced frequency of 0.1 Hz resulted in a slight increase in the inhibition to 36.1%. Increase of the stimulation frequency to 1 Hz led to further inhibition to 55.7%. Reduction of the stimulation rate back to 0.1 Hz led to full relief of the inhibition evoked by increased stimulation frequency. The cumulative inactivation of I_{Ca} in the absence of a drug was less than 10% with 1-Hz stimulation (data not shown). Qualitatively similar frequency dependence was observed for all tested antidepressants. ANOVA analysis of the

extent of use-dependence, estimated as in Uehara and Hume (1985) and summarized in the right columns of Table 1, revealed that there was no significant difference between the antidepressants and diltiazem, while verapamil and felodipine were significantly more and less use-dependent, respectively, than either diltiazem or any of the antidepressants. These results suggest a strong similarity between the mode of action of diltiazem and of the studied antidepressants.

Interaction between antidepressants and Bay K 8644.

The properties of the antidepressant-binding site on the calcium channel were further investigated by studying the inhibitory effects of antidepressants on calcium currents pre-stimulated by Bay K 8644. Figure 5A shows typical responses of the L-type calcium channel, measured as I_{Ca} , to application of antidepressants (desipramine in the illustrated case) in the continuing presence of 300 nmol/L Bay K 8644. It can be seen that in the presence of Bay K 8644 desipramine induced a more pronounced decrease in I_{Ca} than in the absence of the agonist. In the illustrated experiment, the IC_{50} for desipramine in the presence of Bay K 8644 was 7.3 μ mol/L. The results of these experiments are compared in Table 2 (middle columns) to those performed in the absence of Bay K 8644 (Table 2, leftmost columns), together with the data of Zahradníková *et al.* (2007) that were obtained under the same conditions for the three types of calcium channel antagonists. The differences between the effect of BayK 8644 on the inhibitory potency of antidepressants and calcium antagonists were tested at concentrations of drugs that induced approximately 50% inhibition in the absence of Bay K 8644. The apparent value of IC_{50} of individual antidepressants was decreased by 45-75%, while IC_{50} of diltiazem was decreased by ~50% and the values of IC_{50} of verapamil and felodipine were increased 3x and 15x, respectively, in the presence of BayK 8644. The effect of BayK 8644 on the potency of antidepressants was not significantly different from its effect on the potency of diltiazem, while there was a statistically significant difference between the

effect of BayK 8644 on the potency of verapamil and felodipine on one hand, and on the potency of antidepressants on the other hand.

Interaction between antidepressants and diltiazem.

The previously shown results demonstrate a high similarity in the mode of action of diltiazem and the studied antidepressants. Therefore, the possibility that antidepressants interact with the BTZ receptor site on the calcium channel was investigated by comparing the extent of antidepressant-evoked I_{Ca} inhibition in the absence and presence of diltiazem. If diltiazem and antidepressants competed for a common binding site on the calcium channel, the presence of diltiazem would lead to a decrease of the apparent affinity of the channel to the antidepressants, i.e., to an increase in their IC_{50} . Concentrations of diltiazem that induced at least 50% inhibition were chosen, so that at least a 100% increase of the apparent IC_{50} for a competing drug should be observed. Figure 5B shows a typical example of the time course of I_{Ca} during such an experiment. The data for five of the antidepressants - desipramine, amitriptyline, maprotiline, citalopram, and dibenzepin - are summarized in Table 2 (rightmost columns). It is clear from Figure 5B and Table 2 that the effect observed is opposite to that expected for a competitive interaction: the antidepressant-evoked inhibition of I_{Ca} is more pronounced in the presence than in the absence of diltiazem. In the illustrated experiment, 20 μ mol/L amitriptyline induced 60% inhibition of I_{Ca} , while in the absence of diltiazem the same concentration induced 47% inhibition on average. The higher decrease in I_{Ca} was not caused by rundown, as after removal of amitriptyline and in the continuing presence of diltiazem, the current returned to 90% of the value before amitriptyline application, and after removing diltiazem block by 1 min lasting hyperpolarization to -80 mV, I_{Ca} increased to 110% of pre-drug control. In none of the experiments did diltiazem induce a decrease in the inhibitory potency of antidepressants. The increase in potency of antidepressants in the presence of diltiazem was significant for all antidepressants tested, when the apparent IC_{50}

values were compared. In the presence of diltiazem, the apparent IC_{50} of desipramine and amitriptyline was decreased by 25-35%, and that of maprotiline, citalopram, and dibenzepin was decreased by >50% (Table 2). These results strongly suggest that the antidepressants do not act at the same binding site as diltiazem does.

DISCUSSION

The principal finding of this work is that antidepressants inhibit cardiac calcium current by binding to a specific receptor on the L-type calcium channel. From the electrophysiological viewpoint, these drugs in every aspect resemble calcium antagonists. The shape of the concentration dependence and the rates of the onset and washout of inhibition at concentrations of the antidepressants close to their IC_{50} are similar to those of calcium antagonists (Uehara and Hume, 1985; Mery et al., 1996; Zahradnikova et al., 2007). The inhibitory action of the antidepressants is dependent on the conformational state of the channel in a fashion comparable to non-dihydropyridine calcium antagonists, as demonstrated by the shift in the Ca channel availability curve.

The above-mentioned properties are shared with all types of Ca antagonists - dihydropyridines, phenylalkylamines, and benzothiazepines (*see* McDonald *et al.*, 1994) for a review). The level of use-dependence of the investigated antidepressants is intermediate, as is the case with diltiazem, i.e., they are significantly less use-dependent than verapamil, but significantly more so than the dihydropyridine felodipine (see Table 1 and McDonald *et al.*, 1994).

The study of the effect of Bay K 8644 on the potency of the inhibitors showed direct evidence for interaction of antidepressants with a receptor allosterically coupled to the dihydropyridine binding site. Antidepressants share the property with diltiazem of strongly enhanced inhibitory potency in the presence of Bay K 8644. These observations are in accordance with the previously observed antidepressant-induced increase of the Hill slope and binding affinity of the dihydropyridine felodipine to the calcium channel (Minarovic *et al.*, 1996), what is typical for the benzothiazepines (Minarovic and Meszaros, 1998); *see also* Striessnig *et al.* (1986), but they contrast with the results of Murphy *et al.* (1983), who found

no change in binding of [³H]nitrendipine to smooth muscle membranes after application of clomipramine and nortriptyline at 25 μmol/L concentrations.

Our direct measurements of the action of antidepressants on I_{Ca} in cardiac myocytes in the presence of diltiazem show that these drugs do not directly compete with diltiazem, as opposed to the observation in neuronal membrane preparations (Schaeffer *et al.*, 1991). The inability to observe competition between drugs in electrophysiological experiments is not inherent to the method used, since direct competition between drugs that act at the dihydropyridine receptor, i.e., Bay K 8644 and felodipine, could be convincingly demonstrated using the same protocols (Zahradnikova *et al.*, 2007). Therefore, the antidepressant drugs most probably act at a site distinct from the benzothiazepine receptor.

To give molecular interpretation to our findings we extended the guarded modulated receptor model of the L-type calcium channel (Berjukow *et al.*, 1999) to incorporate the simultaneous action of an agonist and a non-DHP antagonist. These authors have shown the importance of both, the preferential drug binding to inactivated channels and the slow rate of drug unbinding from non-inactivated channels in the phenomenon of use-dependence (Figure 6A). In the depolarized state, drugs have high affinity to the channel, because of the high rate of drug binding in the open state and of drug trapping in the inactivated state. Unbinding of the drug occurs mainly upon recovery from inactivation. In polarized cells, occupation of the antidepressant site hinders unbinding of the drug from the DHP or BTZ site (Figure 6B, C), and *vice versa*, thus effectively increasing drug affinity and accounting for positive cooperativity. In other words, the rate constants from the ternary complexes are decreased in the closed states of the channel. In depolarized cells, when the channels reside in the open state(s), the interaction of antidepressants and the BTZ or DHP drugs with their respective binding sites is sterically hindered. As a result, the association rate constants of the ternary complex receptor–antidepressant–BTZ or DHP drug are lower than the rate constants of

binary drug–channel complexes in the open state(s) of the channel (Figure 6B, C), effectively decreasing drug affinity and accounting for negative cooperativity. It is conceivable that steady-state inhibition by antidepressants (moderately use-dependent compounds) is more dependent on the interaction of the drugs with the closed and recovering channel than with the open channels, and thus positive cooperativity prevails during steady-state stimulation.

We have shown here that all of the investigated antidepressants block the L-type Ca currents in a concentration-dependent manner. The potency of the drugs ranged over 2 orders of magnitude. Because of the voltage- and use-dependence of antidepressant action (Table 1), their concentration dependence will be affected by changes in the holding potential, test pulse duration, and stimulation frequency. Additionally, the level of calcium channel phosphorylation may affect binding of the antidepressants, in analogy to the observed effects of β -adrenergic stimulation on inhibition of calcium current by the dihydropyridines (Legssyer et al., 1997). Our estimates of drug potency are in good agreement with the single concentration data for imipramine obtained by others (Isenberg and Tamargo, 1985; Delpón et al., 1991; Choi *et al.*, 1992), when differences in stimulation frequency and holding potential are taken into account. The potency of citalopram to inhibit barium currents in cultured rat cardiomyocytes was reported the same but the potency of amitriptyline was reported to be ~ 3 times lower than in this study (Hamplova-Peichlova *et al.*, 2002). This difference might have been due to differences in experimental conditions (cultured vs. acutely isolated cells), differences in the phosphorylation status of the cells, or due to the use of Ba^{2+} as the charge carrier in their experiments.

The inhibitory potency of the studied antidepressants was not clearly related to their chemical structure, i.e., it did not decrease with increasing structural disparity from the most potent imipramine. The least related drug, citalopram, was more than 6 times more potent than the tricyclic dibenzepin. The relationship between IC_{50} and drug lipophilicity is also not

direct: The three structurally similar drugs, desipramine, imipramine, and clomipramine, have similar potency (IC_{50} of 11.7, 8.1, and 11.6 μM , respectively), but their experimentally determined logP at pH 7.4 varies from 1.5 (desipramine) through 2.5 (imipramine) to 3.3 (clomipramine) (Hansch *et al.*, 1987). On the other hand, the calculated lipophilicities of the lipophilic core of these drugs are less different (3.73, 3.73, and 4.25, respectively). We have determined that the pK_a values of these three drugs under the conditions of our experiments are 9.0, 7.8, and 6.7, respectively. This means that while more than 95% of desipramine is ionized at pH=7.4, almost 75% of imipramine and less than 50% of clomipramine is ionized under the same conditions. Therefore, one could speculate that for drugs with similar structure, the lipophilicity of the drug core is determining their IC_{50} , i.e., both charged and uncharged forms of the drugs are equally effective. The distance between the carbon joining the lipophilic ring structure with the alkylamine moiety, and the partially ionized amine nitrogen is similar in all drugs (5.02 ± 0.01 nm) except the least potent dibenzepin (3.82 nm). On the basis of these data, we suggest that the drugs interact with a binding site that is within the membrane, but close enough to the external side of the membrane to allow interaction of both charged and uncharged drugs having at least 2 carbons between the amine/ammonium group and the lipophilic ring.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1: **The chemical structures of the antidepressants used in this study.**

Figure 2: **Concentration- and voltage dependence of antidepressant action.** **A** – Onset, concentration dependence and washout of the inhibition of calcium current by antidepressants. The myocyte was first exposed to control solution. During the periods indicated by horizontal lines, the myocyte was exposed to progressively higher concentrations of desipramine, applied by fast perfusion. Then the myocyte was perfused with drug-free solution again. The values of I_{Ca} are plotted as a function of time. In the top panel, current traces recorded at the times indicated (solid symbols in the lower panel) are shown. **B** – Voltage dependence of I_{Ca} inhibition by antidepressants. Currents were evoked by depolarizations to 0 mV, preceded by 5 sec prepulses to the indicated potentials in the absence (left) and presence (right) of 20 μ M amitriptyline. The inhibition of I_{Ca} by the drugs is more apparent after larger depolarizing prepulses. **C** – The effect of antidepressants on the I_{Ca} availability curve. The peak currents from panel B were normalized and plotted as a function of voltage. In the presence of 20 μ M amitriptyline (open symbols), the I_{Ca} availability curve is shifted to the left with respect to the control curve (solid symbols).

Figure 3: **Concentration dependence of the studied antidepressants.** The peak currents from experiments such as those in Figure 2A were normalized and plotted as a function of drug concentration. Different symbols represent different antidepressants: solid open squares, circles and triangles stand for imipramine, desipramine and clomipramine, respectively; open squares, circles and triangles stand for amitriptyline, maprotiline and citalopram, respectively; and the semi-solid squares stand for dibenzepin. Solid lines represent the theoretical concentration dependences, parameters of which are given in Table 2. The Hill coefficients were not significantly different from $n_H = 1$ nor from each other.

Figure 4: The action of antidepressants is dependent on the state of the calcium channel.

A - Relief of inhibition by hyperpolarization. The response of I_{Ca} to two progressively higher concentrations of desipramine is shown. Transient hyperpolarization of the membrane (thick line) led to relief of inhibition, which was restored after resuming stimulation. Top panel shows current traces recorded at times indicated (solid symbols). **B** - The effect of stimulation frequency on the extent of inhibition. After stabilization of I_{Ca} , stimulation was interrupted and the myocyte was exposed to 10 $\mu\text{mol/L}$ clomipramine until the end of experiment (upper line). After 60 s stimulation was resumed with a frequency of 0.1 Hz (lower line). Increase of stimulation to 1 Hz led to faster and deeper inhibition, which was fully reversible. In the top panel, current traces corresponding to the indicated time points (solid symbols) are shown.

Figure 5: Antidepressant action in the presence of calcium channel drugs. A - During continuous stimulation the myocyte was exposed to 300 nmol/L Bay K 8644 solution and then, in the continuing presence of Bay K 8644, to progressively higher concentrations of desipramine. Then the myocyte was perfused with antidepressant-free, Bay K 8644-containing solution again. The values of I_{Ca} are plotted as a function of time. In the top panel, current traces recorded at the times indicated (solid symbols in the lower panel) are shown. **B** - During continuous stimulation the myocyte was exposed to 1.5 $\mu\text{mol/L}$ diltiazem and then, in the continuing presence of diltiazem, to 10 $\mu\text{mol/L}$ desipramine. The black bar denotes a 60 s period of hyperpolarization to -80 mV. The values of peak I_{Ca} are plotted as a function of time. In the top panel, current traces recorded at the times indicated (solid symbols in the lower panel) are shown.

Figure 6. A model of antidepressant binding to the L-type calcium channel.

A – The guarded modulated receptor model of Berjukow et al. (1999) for interaction of BTZ drugs with the L-type calcium channel is adapted to depict the interaction of antidepressants with the channel. In the closed and inactivated states, binding/unbinding of the drug is

hindered. Binding occurs predominantly in the open state of the channel (depolarized cells), while unbinding is predominant during recovery from inactivation (polarized cells). B – The presence of BayK 8644 decreases the rate of antidepressant binding to the open state as well as the rate of antidepressant unbinding from the channel recovering from inactivation. Because the antidepressants are only moderately use-dependent, the effect of BayK 8544 on drug unbinding predominates (grey square). C – The presence of diltiazem decreases the rate of antidepressant binding to the open state as well as the rate of antidepressant unbinding from the channel recovering from inactivation. Because the antidepressants are only moderately use-dependent, the effect of diltiazem on drug unbinding predominates (grey square).

Table 1: State-dependence of antidepressant effects.

Drug	concentration [$\mu\text{mol/L}$]	$\Delta V_{0.5}$ [mV]	Δk [mV]	n	use-dependence [%]	n
imipramine	10	$-7.4 \pm 0.9^{**}$	0.5 ± 0.3	3	41 ± 3	3
clomipramine	20	$-4.1 \pm 0.9^{**}$	0.5 ± 0.2	5	33 ± 3	3
desipramine	20	$-3.9 \pm 0.9^{**}$	0.5 ± 0.2	3	37 ± 3	3
amitriptyline	20	$-3.5 \pm 0.4^{**}$	0.5 ± 0.3	3	39 ± 2	3
maprotiline	20	$-2.4 \pm 0.4^{**}$	0.3 ± 0.3	3	29 ± 1	3
citalopram	50	$-3.5 \pm 0.5^{**}$	0.2 ± 0.1	6	39 ± 3	3
dibenzepin	500	$-6.4 \pm 1.2^{**}$	$1.4 \pm 0.4^{**}$	4	32 ± 3	3
felodipine	0.01	$-15.3 \pm 2.0^{**,\dagger\dagger}$	$4.8 \pm 0.2^{**,\dagger\dagger}$	3	$8 \pm 3^{\dagger\dagger}$	4
verapamil	0.5	$-4.3 \pm 0.3^{**}$	0.6 ± 0.2	4	$87 \pm 5^{\ddagger\dagger}$	3
diltiazem	1	$-4.9 \pm 1.0^{**}$	0.3 ± 0.2	5	47 ± 8	4

The values are given as mean $\pm$ standard error.

n - number of experiments

The average values of inactivation curve parameters under control conditions were:

$$V_{0.5} = -25.4 \pm 0.8 \text{ mV}; k = 3.9 \pm 0.1 \text{ mV} (n = 21).$$

$^{**} p < 0.01$; $^* p < 0.05$ (paired t-test)

†† Significantly less than other drugs (ANOVA; $p < 0.05$)

‡† Significantly more than other drugs (ANOVA; $p < 0.05$)

Table 2: The effect of Bay K 8644 or diltiazem on parameters of the concentration dependence of I_{Ca} inhibition by antidepressants and Ca antagonists

Drug	Control			in 300 nmol/L BayK 8644		in 1.5 μ mol/L diltiazem	
	IC_{50} [μ mol/L]	n_H	n	IC_{50} [μ mol/L]	n	IC_{50} [μ mol/L]	n
imipramine	8.1 ± 0.6	1.02 ± 0.03	11	$5.38 \pm 0.42^*$	4	n.d.	
clomipramine	11.6 ± 0.5	1.09 ± 0.06	22	$6.81 \pm 0.73^{**}$	5	n.d.	
desipramine	11.7 ± 0.6	1.11 ± 0.07	10	$6.93 \pm 0.37^{**}$	3	$9.30 \pm 0.79^*$	4
amitriptyline	23.2 ± 1.2	1.12 ± 0.08	11	$11.95 \pm 0.64^{**}$	6	$15.7 \pm 4.5^*$	4
maprotiline	31.0 ± 2.8	1.06 ± 0.07	8	$18.0 \pm 1.8^{**}$	3	$15.1 \pm 2.1^{**}$	3
citalopram	64.5 ± 3.3	1.08 ± 0.05	7	$22.0 \pm 1.3^{**}$	5	$32.0 \pm 4.7^{**}$	5
dibenzepin	364 ± 12	1.14 ± 0.08	15	$87 \pm 11^{**}$	4	$91 \pm 31^{**}$	3
felodipine ^a	0.011 ± 0.003	1.02 ± 0.10		$0.165 \pm 0.026^{\dagger\dagger}$		n.d.	
verapamil ^a	0.246 ± 0.013	1.02 ± 0.04		$0.737 \pm 0.071^{\dagger\dagger}$		n.d.	
diltiazem ^a	0.512 ± 0.041	1.07 ± 0.07		$0.227 \pm 0.014^{**}$		N/A	

The values are given as mean $\pm$ standard error.

IC_{50} – 50% inhibitory concentration

n_H - Hill slope

n - number of experiments

^adata from Zahradníková et al. (2007)

significantly less than in control: * $p < 0.05$, ** $p < 0.01$

significantly more than in control: $\dagger\dagger$ $p < 0.01$

n.d. – not determined

N/A – not applicable

Figure 1

Figure 2

Figure 3

Figure 4

A

B

Figure 5

Figure 6