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Differential effect of imipramine and related compounds on Mg^{2+} efflux from rat erythrocytes

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Abstract

The effect of imipramine on Mg^{2+} efflux in NaCl medium (Na^+/Mg^{2+} antiport), on Mg^{2+} efflux in choline·Cl medium (choline/Mg^{2+} antiport) and on Mg^{2+} efflux in sucrose medium (Cl^--coupled Mg^{2+} efflux) was investigated in rat erythrocytes. In non-Mg^{2+}-loaded rat erythrocytes, imipramine stimulated Na^+/Mg^{2+} antiport but inhibited choline/Mg^{2+} antiport and Cl^--coupled Mg^{2+} efflux. The same effect could be obtained by several other compounds structurally related to imipramine. These drugs contain a cyclic hydrophobic ring structure to which a four-membered secondary or tertiary amine side chain is attached. At a physiological pH, the amine side chain expresses a cationic choline-like structure. The inhibitory effect on choline/Mg^{2+} antiport is lost when the amine side chain is modified or abandoned, pointing to competition of the choline-like side chain with choline or another cation at the unspecific choline antiporter or at the Cl^--coupled Mg^{2+} efflux. Other related drugs either stimulated Na^+/Mg^{2+} antiport and choline/Mg^{2+} antiport, or they were ineffective. For stimulation of Na^+/Mg^{2+} antiport and choline/Mg^{2+} antiport, there is no specific common structural motif of the drugs tested. The effects of imipramine on Na^+/Mg^{2+} antiport and choline/Mg^{2+} antiport are not mediated by PKCα but are caused by a direct reaction of imipramine with these transporters. By increasing the intracellular Mg^{2+} concentration, the stimulation of Na^+/Mg^{2+} antiport at a physiological intracellular Mg^{2+} concentration changed to an inhibition of Na^+/Mg^{2+} antiport. This effect can be explained by the hypothesis that Mg^{2+} loading induced an allosteric transition of the Mg^{2+}/Mg^{2+} exchanger with low Na^+/Mg^{2+} antiport capacity to the Na^+/Mg^{2+} antiporter with high Na^+/Mg^{2+} antiport capacity. Both forms of the Mg^{2+} exchanger may be differently affected by imipramine.

Keywords: Na^+/Mg^{2+} antiport; Choline/Mg^{2+} antiport; Rat erythrocyte; Imipramine; Structural requirements

1. Introduction

To date, there is no specific inhibitor available for characterizing Na^+/Mg^{2+} antiport and Na^+-independent Mg^{2+} efflux systems. Subsequent to the finding of Feray and Garay [1] that the neuropharmacologic drug imipramine inhibits Na^+/Mg^{2+} antiport in Mg^{2+}-loaded human erythrocytes, this substance has been used among others as an unspecific inhibitor of Na^+/Mg^{2+} antiport. So far, imipramine has proven to be an effective inhibitor of Mg^{2+} efflux in the following Mg^{2+}-loaded cells: rat erythrocytes [2], cardiomyocytes [3,4], collagenase-dispersed isolated rat hepatocytes [5], rat liver plasma membranes [6], vascular smooth muscle cells [7], and rat renal epithelial cells (NRK-52E) [8].

On the other hand, imipramine has also inhibited Na^+/Mg^{2+} antiport in some non-Mg^{2+}-loaded cell types. These were ferret erythrocytes [9], Ehrlich ascites tumor cells [10], lymphocytes [11], HL-60 promyelocytic leukemia cells [12], and MDCK cells after treatment with angiotensin II [13].

During our investigation of the properties of Na^+/Mg^{2+} antiport and choline/Mg^{2+} antiport in non-Mg^{2+}-loaded

Abbreviations: BIM I, bisindolylmaleimide I; CAM II-K, calmodulin-dependent kinase II; HC-3, hemicholinum-3; PK, protein kinase; PKC, protein kinase C; PL, phospholipid; PLD, phospholipase D; PMA, phorbol-12-myristate-13-acetate; PTK, protein tyrosine kinase; TCA, trichloroacetic acid

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erythrocytes [14,15], we were surprised to detect that in these cells imipramine stimulated Na^+/Mg^{2+} antiport but inhibited choline/Mg^{2+} antiport. Mg^{2+} loading of cells could change the properties of the Na^+/Mg^{2+} antiporter, as seen by its drastic activation. This was explained by an allosteric transition of the Mg^{2+}/Mg^{2+} exchanger [16]. Therefore, we investigated the effect of imipramine on the various Mg^{2+} efflux systems in non-Mg^{2+}-loaded and Mg^{2+}-loaded rat erythrocytes. Because Mg^{2+} efflux can be mediated (a) by Na^+/Mg^{2+} antiport that operates in NaCl medium, (b) by choline/Mg^{2+} antiport that is active in choline · Cl as well as in KCl medium and (c) by Cl^--coupled Mg^{2+} efflux in sucrose medium [17,18], the effect of imipramine on Mg^{2+} efflux was tested in these media.

2. Materials and methods

2.1. Materials

Nembutal® (pentobarbital sodium) was obtained from Abott (North Chicago, IL, USA). The following substances, including imipramine hydrochloride, were obtained from SIGMA®, Taufkirchen, Germany: amitriptyline hydrochloride, amoxapine, carbamazepine, chlorpromazine hydrochloride, cyproheptadine hydrochloride, desipramine hydrochloride, diclofenac sodium salt, diphenhydramine hydrochloride, fluvoxamine maleate, hemicholinum-3, lidocaine, phenylbutazone, staurosporine, trazodone hydrochloride, trifluoperazine dihydrochloride. BIM I, Ro 318425 and U73122 were obtained from CALBIOCHEM®. All other chemicals were purchased at the highest purity available from Merck®, Darmstadt, Germany. Filtered, de-ionized and virtually Mg^{2+}-free water with a resistance of 15–18 MΩ/cm was used for the solutions.

2.2. Preparation of red blood cells

Red cells were prepared as described earlier [15]. In brief, blood (6–8 ml) was always obtained from only one anesthetized male Sprague–Dawley rat (50 mg/kg Nembutal® i.p.), weighing 350–450 g. The abdominal vein was catheterized with a heparinized syringe. Portions of the blood were transferred to heparinized tubes, diluted 1:3–1:5 with NaCl medium consisting of 10 mmol · l^{-1} NaCl, 5 mmol · l^{-1} D-glucose and 10 mmol · l^{-1} HEPES–Tris, pH 7.4. The cell suspension was centrifuged at 1000×*g* for 10 min at 24 °C. The plasma and the buffy coat containing the white cells were aspirated and discarded. The sedimented red cells were washed twice at 24 °C in NaCl medium. Finally, the red cells were resuspended and incubated with gentle shaking as a 10% (v/v) suspension in one of the following media, each containing 5 mmol · l^{-1} D-glucose and 10 mmol · l^{-1} HEPES–Tris, pH 7.4: (a) 150 mmol · l^{-1} NaCl (NaCl medium), (b) 150 mmol · l^{-1} choline · Cl (choline · Cl medium) and (c) 150 mmol · l^{-1} KCl (KCl medium), (d) 300 mmol · l^{-1} sucrose (sucrose medium).

To minimize hemolysis, the cells were handled with utmost caution, temperature was kept at 24 °C, and centrifugation was carried out at 1000×*g*. Usually, hemolysis ranged between 0.5% and 1.5%, and when more than 2%, the red cells were not used. Paired experiments were always done.

2.3. Mg^{2+} loading of red blood cells

The cells were loaded with Mg^{2+} by incubating a 10% (v/v) cell suspension for 30 min at 37 °C in Mg^{2+} loading medium containing (in mmol · l^{-1}) 140 KCl, 50 sucrose, 5 D-glucose and 10 HEPES–Tris, pH 7.4, 12 $MgCl_2$ and 6 μmol · l^{-1} A 23187 (dissolved in dimethyl sulfoxide). After loading, the ionophore was removed by incubating the cells four times in ionophore-free Mg^{2+} loading medium plus 1% bovine serum albumin for 10 min at 37 °C. Thereafter, the erythrocytes were washed two times in cold NaCl medium.

2.4. Mg^{2+} efflux

At the beginning and at various time intervals, 1-ml aliquots of the cell suspensions were centrifuged at 1000×*g* for 10 min. To determine Mg^{2+}, the supernatant was diluted with TCA. The final concentration of TCA was 5% (w/v), containing 0.1% (w/v) La_2O_3 and 0.16% (v/v) HCl. Mg^{2+} was measured in triplicates by means of atomic absorption spectrometry (Perkin Elmer, 2380). Mg^{2+} efflux was calculated from the increase in extracellular Mg^{2+} concentration during the time interval, and was related to the original cell volume measured by hematocrit. Hematocrit and hemolysis were measured in each sample. Mg^{2+} efflux was corrected for hemolysis. For this purpose, Mg^{2+} was extracted from the sedimented erythrocytes with 5% (v/v) TCA and was measured after appropriate dilution of the extract with TCA and La_2O_3–HCl as described above.

2.5. Statistical analysis

Data were expressed as means±S.E., and statistical significances were determined by Student's paired and two tailed *t*-test. A value of $P<0.05$ was considered significant.

3. Results and discussion

3.1. Effects of imipramine in non-Mg^{2+}-loaded rat erythrocytes

The time course of the imipramine effect on Na^+/Mg^{2+} antiport (in NaCl medium) and on choline/Mg^{2+} antiport (in choline · Cl medium) was investigated in a first series of paired experiments. In order to keep hemolysis low, a dose of only 100 μmol · l^{-1} imipramine was applied, while other

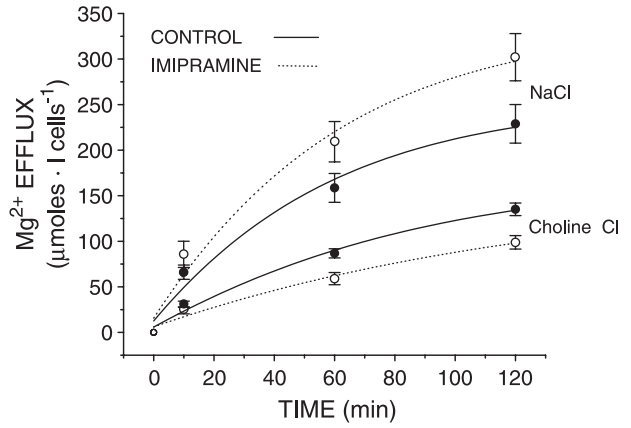


Fig. 1. Time course of the imipramine effect (100 µmol·l^{-1}) on Mg^{2+} efflux in NaCl medium and choline·Cl medium of non-Mg^{2+}-loaded rat erythrocytes. Means±S.E. of paired experiments, n=5. At t=60 and 120 min, the imipramine effects in NaCl or choline·Cl were significant when compared to the control.

authors used 200 µmol·l^{-1} [6], or 500 µmol·l^{-1} [10,11,14] of the drug. Fig. 1 shows that at 60- and 120-min imipramine stimulated Na^+/Mg^{2+} antiport by about 30% but inhibited choline/Mg^{2+} antiport by about 30%.

The different reactions of Na^+/Mg^{2+} antiport and of choline/Mg^{2+} antiport support our previous conclusion that Na^+/Mg^{2+} antiport and choline/Mg^{2+} antiport may be different transporters [15]. However, the surprising stimulation of Na^+/Mg^{2+} antiport by imipramine is in contrast to the behaviour of Na^+/Mg^{2+} antiport in other cell types, where the transporter was inhibited by imipramine [1–13].

In a next series of paired experiments, the effect of imipramine on Na^+-independent Mg^{2+} efflux systems was measured. Mg^{2+} efflux in choline·Cl medium was included as a reference and for comparison with Fig. 1. The results are presented in Fig. 2. Imipramine inhibited Mg^{2+} efflux in choline·Cl medium by 27%, and in other series of experiments by 39% and 40% (Table 2, Table 4). In KCl medium imipramine inhibited Mg^{2+} efflux by 49%. Since the choline

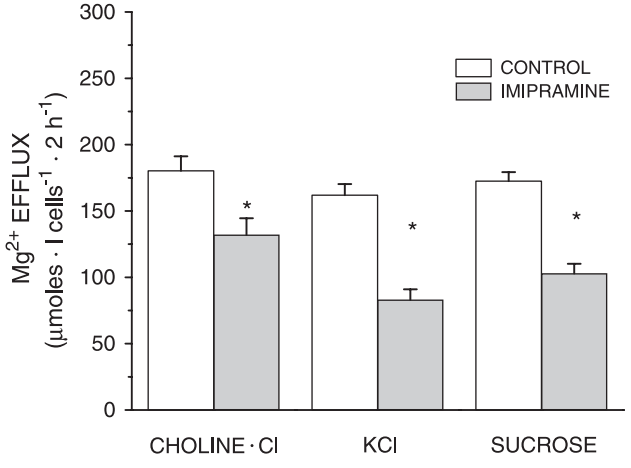


Fig. 2. Effect of imipramine (100 µmol·l^{-1}) on Mg^{2+} efflux from non-Mg^{2+}-loaded rat erythrocytes in choline·Cl, KCl and sucrose medium, t=120 min. Means±S.E. of paired experiments, n=4–5. *P<0.05.

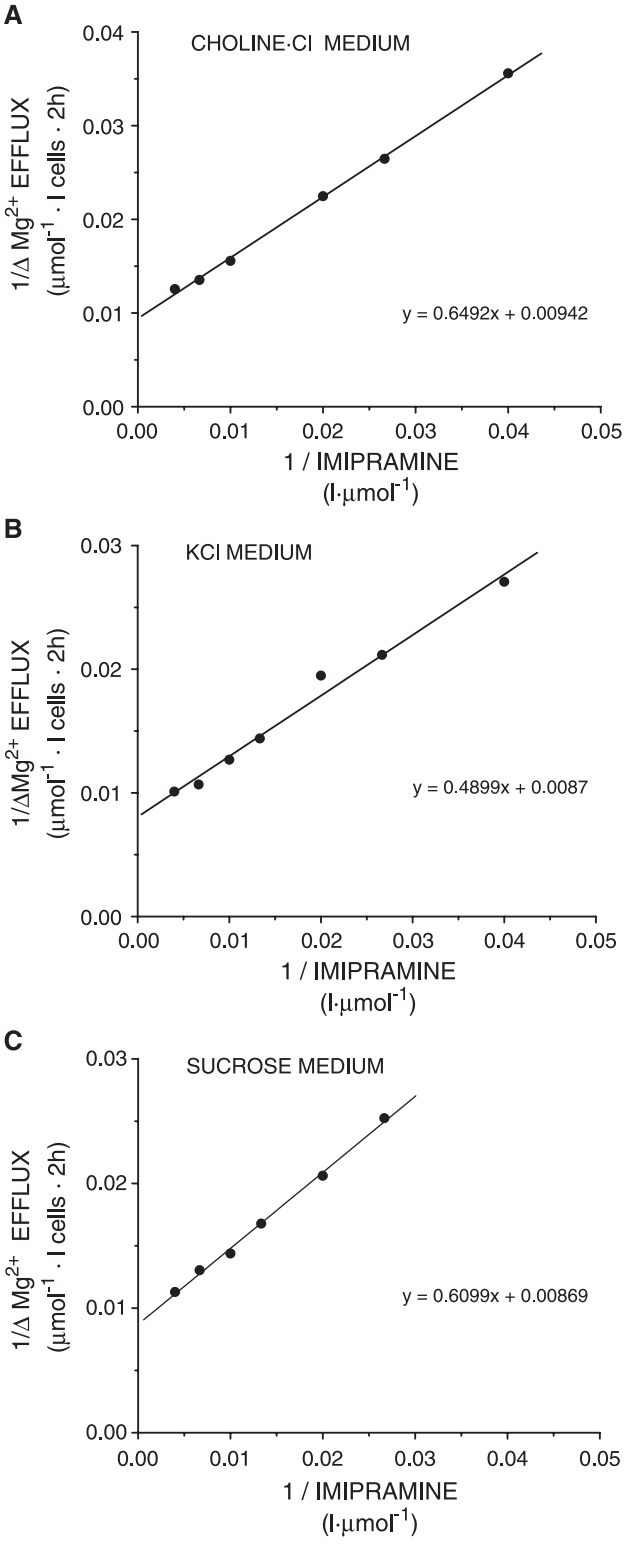


Fig. 3. Lineweaver–Burk plot for determination of the IC_{50} values for imipramine inhibition of Mg^{2+} efflux from non-Mg^{2+} -loaded rat erythrocytes in choline·Cl medium (A), in KCl medium (B) and in sucrose medium (C), t=120 min. The linear regression used for calculating the IC_{50} is given in the inset. The IC_{50} values were 69 µmol·l^{-1} in choline·Cl medium, 56 µmol·l^{-1} in KCl medium and 70 µmol·l^{-1} in sucrose medium. ΔMg^{2+} efflux is the difference of Mg^{2+} efflux without and with imipramine. For details, see Ref. [47]. Means of four to five paired experiments.

exchanger is rather unspecific, it also accepts K^+ instead of choline so that Mg^{2+} efflux in KCl medium may reflect K^+/Mg^{2+} antiport via the unspecific choline exchanger [16]. In sucrose medium, Mg^{2+} efflux accompanied by Cl^- for charge compensation was reduced by 40% by imipramine.

For further characterization, Mg^{2+} efflux was measured as a function of imipramine concentration in the different media. As shown in Fig. 3A–C, the Lineweaver–Burk plots yielded an IC_{50} value of 69 $\mu mol \cdot l^{-1}$ for imipramine in choline · Cl medium, 56 $\mu mol \cdot l^{-1}$ in KCl medium and 70 $\mu mol \cdot l^{-1}$ in sucrose medium.

The stimulatory effect of imipramine on Na^+/Mg^{2+} antiport is a real effect and is not mimicked by an increased Mg^{2+} leakage. First, in choline · Cl, in KCl and in sucrose medium, Mg^{2+} efflux was inhibited by imipramine, which is not congruent with increased Mg^{2+} leakage. Second, at the relative low concentration of imipramine used in our experiments, hemolysis was not different from controls and was always lower than 1%. Third, in Mg^{2+}-loaded human erythrocytes suspended in NaCl medium, a leakage of Mg^{2+} was only observed at much higher imipramine concentrations of 1 $mmol \cdot l^{-1}$ [1]. Also, the missing effect of imipramine on K^+ efflux in NaCl and choline · Cl medium as shown in Table 1 contradicts Mg^{2+} leakage.

The effect of imipramine on K^+ efflux in sucrose medium has not been included in Table 1 because at low ionic strength, various transport systems are activated such as that for glutamine, glutamic acid, lactic acid, histidine, taurine, glycine, serine, choline, carnitine, and K^+ [19,20].

3.2. Structural requirements for the effect of imipramine

Imipramine and other tricyclic antidepressives have been previously demonstrated to produce a blockade of α-adrenergic receptors [21], β-adrenergic receptors [22,23], cholinergic-muscarinergic receptors [24,25], nicotine receptors [26], $P2X_2$ receptors [27,28], and $5\text{-}HT_3$ receptors [29]. Furthermore, these drugs inhibited neuronal [30,31] and myocardial [32] Na^+ currents and different types of K^+ channels [33–35]. Insofar, a stimulation Na^+/Mg^{2+} antiport in non-Mg^{2+}-loaded rat erythrocytes deviates from the usual inhibitory action of imipramine on receptors and channels.

In order to see whether the unexpected stimulation of Na^+/Mg^{2+} antiport by imipramine is specific for imipramine, we tested the effect of related compounds. The results are documented in Table 2. Here, the structural formula and the change of Na^+/Mg^{2+} antiport as well as of choline/Mg^{2+} antiport relative to untreated controls are documented. The compounds were divided into three groups according to their effects. Group I compounds stimulated Na^+/Mg^{2+} antiport but inhibited choline/Mg^{2+} antiport. Thus, the stimulation of Na^+/Mg^{2+} antiport is not a curious effect of imipramine but is shared by several structurally related compounds. Group II compounds stimulated Na^+/Mg^{2+} antiport as well as choline/Mg^{2+} antiport. Group III compounds had a small or no significant effect on both Mg^{2+} transporters.

Table 1
Effect of imipramine (70 $\mu mol \cdot l^{-1}$) on K^+ efflux ($mmol \cdot l^{-1}$ cells · $2\ h^{-1}$) from non-Mg^{2+}-loaded rat erythrocytes incubated in NaCl medium and choline · Cl medium

Substance	NaCl medium	Choline · Cl medium
Control	4.04±0.08	2.64±0.08
Imipramine	4.00±0.06	2.85±0.11

Paired experiments; means±S.E., n=6.

Evidently, the drug effects on Mg^{2+} efflux were unrelated to the clinical effects of the drugs. Group I incorporates the antidepressives imipramine, amitriptyline and desipramine, the selective choline uptake inhibitor hemicholinum-3, the neuroleptic chlorpromazine and the histamine H_1 receptor as well as the $5\text{-}HT_2/5\text{-}HT_1$ receptor antagonist cyproheptadine. Group II contains the neuroleptic trifluoperazine and the serotonin uptake inhibitor fluvoxamine as well as the antidepressive trazodone. Group III contains the H_1 receptor antagonist diphenhydramine, the antidepressive amoxapine, the anticonvulsant carbamazepine, the anti-inflammatory diclofenac as well as phenylbutazone, the local anaesthetic lidocaine and the muscle relaxant decamethonium.

In contrast to the clinical actions, the drug effect on Mg^{2+} efflux can be related, at least in part, to the chemical structure. All drugs contain a cyclic hydrophobic ring structure to which a side chain is attached.

Due to the cyclic hydrophobic ring structure, group I drugs are intercalated into the hydrocarbon phase of the membrane bilayer [36,37] according to their octanol/water partition coefficients [38,39]. Within the inner leaflet of the membrane, their cationic hydrophilic secondary/tertiary amine side chain interacts with the negatively charged head groups of PL [37]. In erythrocytes these PLs are particularly phosphatidylserine and phosphatidylinosites [40]. Generally, membrane-bound proteins (receptors, channels, enzymes) are surrounded by negatively charged PL. The action of group I drugs depends at least on their interaction with PLs and membrane-bound proteins, as was directly shown for the inhibition of PKC by chlorpromazine. Chlorpromazine competes with PL and prevents the activation of PKC by PL [41].

Besides their reaction with phospholipids, group I drugs could also react with Mg^{2+} efflux by other mechanisms. The side chain of group I drugs has a choline-like structure, which at a physiological pH value is positively charged. When the amine side chain is eliminated, elongated or otherwise modified (group II and group III), the inhibition of the choline antiporter was lost. This indicates competition of the choline-like amine side chain with choline or another cation with the unspecific choline/Mg^{2+} antiporter.

In experiments with a cloned and mutated $P2X_2$ receptor/channel expressed in *Xenopus* oocytes it was found that imipramine interacting with Asn^{333} and Thr^{330} blocked the channel, whereas the interaction with Asp^{315} attenuated the block [27]. Also, in case of the Na^+ channel, a reaction of

Table 2
Effect of various drugs on Mg^{2+} efflux from non-Mg^{2+}-loaded rat erythrocytes in NaCl and choline · Cl medium

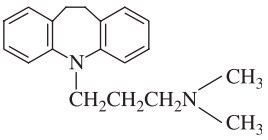
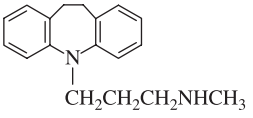
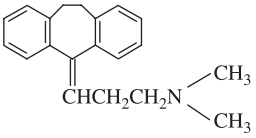
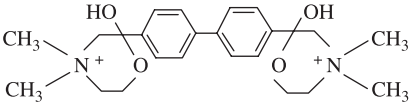
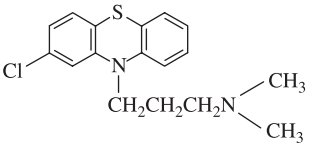
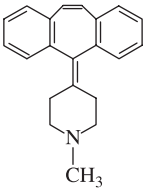
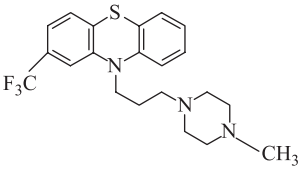
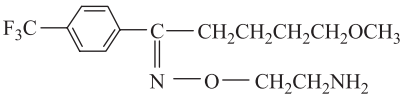
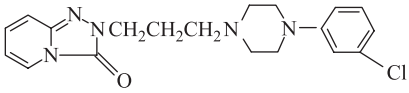
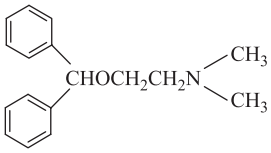
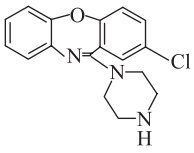
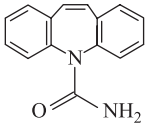
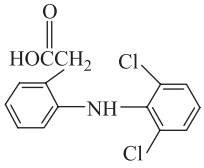
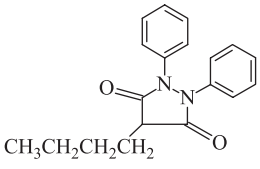
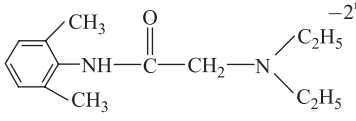
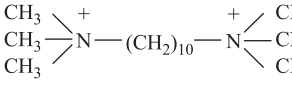
Drug	Structure	NaCl	Choline · Cl
Group I			
Imipramine		+32*	−39*
Desipramine		+23*	−44*
Amitriptyline		+21*	−47*
Hemicholinum-3		+26*	−32*
Chlorpromazine		+12*	−8*
Cyproheptadine		+9*	−21*
Group II			
Trifluoperazine		+12*	+18*
Fluvoxamine		+12*	+16*

Table 2 (*continued*)

Drug	Structure	NaCl	Choline · Cl
Group II			
Trazodone		+5*	+7*
Group III			
Diphenhydramine		+7*	$-1^{n.s.}$
Amoxapine		+5*	$-4^{n.s.}$
Carbamazepine		$0^{n.s.}$	$-3^{n.s.}$
Diclofenac		$-1^{n.s.}$	$-2^{n.s.}$
Phenylbutazone		$-2^{n.s.}$	$+3^{n.s.}$
Lidocaine		$-2^{n.s.}$	$0^{n.s.}$
Decamethonium		$+2^{n.s.}$	$-1^{n.s.}$

Values are expressed as a change in percentage of control. In NaCl medium, Mg^{2+} efflux of control was 228.9±21.2 μmol l^{-1} cells 2 h^{-1}, in choline · Cl medium, Mg^{2+} efflux of control was 134.7±5.8 μmol l^{-1} cells 2 h^{-1}. Drug concentration in μmol · l^{-1} usually was 100 with exception of 50 for trifluoperazine, and 4600 for hemicholinum-3. Paired experiments, means of n=4. *P<0.05; n.s., not significant.

the phenyl rings of imipramine with aromatic amino acids of the channel has been discussed [30].

The stimulation of Mg^{2+} efflux in NaCl by group I drugs or stimulation of Mg^{2+} efflux in NaCl and choline·Cl medium by group II drugs as well as the minor or negligible effect of group III drugs is more difficult to explain. According to their cyclic hydrophobic ring structure (exception decamethonium), the drugs are also intercalated into the membrane bilayer. However, there seems to be no apparent common motif in the structure of these compounds to explain the drug effect.

3.3. Exclusion of protein kinase in the action of imipramine on Mg^{2+} efflux

In a previous study we found that the Na^+/Mg^{2+} antiport of non-Mg^{2+}-loaded rat erythrocytes could be activated by PKCα [42]. Furthermore, evidence has been presented that in cultured rat hippocampal cells 10^{-4} to 10^{-5} mol·l^{-1} desipramine stimulated the release of the neurotransmitter glutamate, probably by activating PKC [43]. Also, the administration of tricyclic antidepressives stimulated PLC [44] and PLD [45] in neuronal tissue. These findings were the reason for us to test the hypothesis whether the stimulation of Na^+/Mg^{2+} antiport by imipramine in non-Mg^{2+}-loaded rat erythrocytes may be due to the stimulation of PKCα. As can be seen from line 3 of Table 3, PMA as an activator of PKC increased Na^+/Mg^{2+} antiport to the same degree as imipramine (line 2 of Table 3). The effects of PMA and imipramine behaved additively (line 4 of Table 3). The PMA concentration used was 1 μmol·l^{-1}, which induced maximum stimulation of Mg^{2+} efflux via PKC in rat erythrocytes. In these cells the K_m value for PMA stimulation of Mg^{2+} efflux amounted to 51 nmol·l^{-1} [42]. Therefore, the activation of Na^+/Mg^{2+} antiport by imipramine must be an additional effect independent of PKC. This conclusion is supported by the finding that the specific PKC inhibitors BIM I, Ro 318425 (line 5,6 of Table 3) and the phospholipase C inhibitor U73122 (line 7 of Table 3) did not significantly affect the stimulation of Na^+/Mg^{2+} antiport by imipramine. Staurosporine reduced Mg^{2+} efflux by 29 μmol·l^{-1} cells 2 h^{-1} (line 8 of Table 3), which is about the same amount by which staurosporine reduced Mg^{2+} efflux from imipramine-stimulated cells (line 9 of Table 3, compare: line 2 minus line 8). This finding confirms our previous findings [42] and could be interpreted as evidence for the presence of a PK other than PKC, which stimulates Na^+/Mg^{2+} antiport and is inhibited by staurosporine. This unidentified PK is not inhibited by imipramine.

Table 3
Effect of various protein kinase effectors and imipramine on Mg^{2+} efflux from non-Mg^{2+}-loaded rat erythrocytes in NaCl medium

No	Group	NaCl medium	ΔControl
1	Control	232.0±6.11	0
2	Imipramine	305.3±0.01*	73
3	PMA	307.0±0.05*	75
4	Imipramine+PMA	387.3±0.01*	155
5	Imipramine+BIM I	300.5±0.01*	69
6	Imipramine+Ro 318425	296.9±0.01*	65
7	Imipramine+U73122	311.8±0.01*	80
8	Staurosporine	203.2±0.02*	−29
9	Imipramine+Staurosporine	272.5±0.04*	41

Values are shown in μmol·l^{-1} cells·2 h^{-1}. The concentrations in μmol·l^{-1} were: imipramine 100, PMA 1, BIM I 0.5, Ro 318425 0.5, U73122, staurosporine 1. ΔControl, Mg^{2+} efflux in the presence of effector minus Mg^{2+} efflux in their absence. Means±S.E., n=4.

* P<0.05.

Table 4
Effect of the protein kinase inhibitor staurosporine and of imipramine on Mg^{2+} efflux from non-Mg^{2+}-loaded rat erythrocytes incubated in choline·Cl medium

No.	Group	Choline·Cl medium	ΔControl
1	Control	123.0±9.7	–
2	Imipramine	74.4±10.0*	−48.6
3	Staurosporine	98.8±6.4*	−24.2
4	Imipramine+Staurosporine	57.8±15.7*	−65.2

Values are shown in μmol·l^{-1} 2 h^{-1}. The concentrations in μmol·l^{-1} were: imipramine 100, staurosporine 0.5. ΔControl, Mg^{2+} efflux in the presence of inhibitor minus Mg^{2+} efflux in their absence. Means±S.E., n=4.

* P<0.05.

As the choline/Mg^{2+} antiporter could be stimulated by PKC [42], we investigated the possible role of PKs in the inhibition of choline/Mg^{2+} antiport by imipramine. From line 2 of Table 4 it can be depicted that imipramine inhibited choline/Mg^{2+} antiport in accordance with the results presented in Figs. 1–3. Furthermore, as with Na^+/Mg^{2+} antiport, staurosporine inhibited choline/Mg^{2+} antiport (line 3 of Table 4). Thus, the unidentified PK stimulating Na^+/Mg^{2+} antiport can also stimulate choline/Mg^{2+} antiport and is inhibited by staurosporine. The inhibition of choline/Mg^{2+} antiport by staurosporine and imipramine was additive. Because a maximum PKC inhibitory dose of staurosporine was used, this result indicates that the inhibition of choline/Mg^{2+} antiport by imipramine is an additional effect independent of PK. Therefore, imipramine must directly inhibit the choline/Mg^{2+} antiporter. Exclusion of PKC and the direct reaction of imipramine with the Na^+/Mg^{2+} and the choline/Mg^{2+} antiporters are supported by further arguments: First, in the inhibition of purified brain PKC, chlorpromazine was about 10 times more potent than imipramine [41]. However, as shown in Table 2, chlorpromazine had a much smaller effect on Mg^{2+} efflux than imipramine. Second, for the inhibition of purified skin PKC, 10 to 30 times higher doses of imipramine were needed than for the inhibition of Mg^{2+} efflux. Dependent on experimental conditions, the IC_{50} value for the inhibition of skin PKC by imipramine was 0.6 or 2.5 mmol·l^{-1} [46] whereas the IC_{50} value of the imipramine inhibition of choline/Mg^{2+} antiport and of Na^+/Mg^{2+} antiport amounted to 69 μmol·l^{-1} (Fig. 3A) and 25 μmol·l^{-1} [1], respectively. Taken together, cumulative evidence favors the hypothesis that imipramine must directly affect Na^+/Mg^{2+} and choline/Mg^{2+} antiport.

Perhaps, the reason for the reported contradictory results on the action of imipramine on PKC is that the stimulatory effect of imipramine on PKC was deduced indirectly from experiments with cellular systems [43], whereas the inhibitory effect was found directly with the partially purified enzyme [41,46].

3.4. Effect of imipramine on Mg^{2+}-loaded erythrocytes

Due to the fact that in previous studies with human erythrocytes imipramine was found to inhibit Na^+/Mg^{2+} antiport [1–8] when the cells were loaded with Mg^{2+}, we tested the effect of imipramine on Mg^{2+} efflux on Na^+/Mg^{2+} antiport as a function of intracellular Mg^{2+}, which was produced by loading the cells with different concentrations of Mg^{2+}. Fig. 4A shows a sigmoidal kinetic, indicating allosteric activation of Na^+/Mg^{2+} antiport by intracellular Mg^{2+}, as discussed earlier in detail [16].

Moreover, Fig. 4A shows the inhibition of Na^+/Mg^{2+} antiport by imipramine with increasing intracellular Mg^{2+} content. Since the effect is not clearly seen in Fig. 4A at a physiological Mg^{2+} content, the effect of imipramine on Na^+/Mg^{2+} antiport was plotted as percentage change (Δpercent) of controls without imipramine at different intracellular Mg^{2+} contents, as shown in Fig. 4B. Here, it can be seen that at a physiological Mg^{2+} concentration imipramine stimulated Na^+/Mg^{2+} antiport by 30%, as is also shown in Fig. 1. Upon Mg^{2+} loading at an intracellular Mg^{2+} content above 3 mmol·l^{-1} cells, imipramine increasingly inhibited Na^+/Mg^{2+} antiport, and approached maximal inhibition at an Mg^{2+} concentration above 10 mmol·l^{-1} cells.

The effect of other antidepressant and neuroleptic drugs on Mg^{2+} efflux of Mg^{2+}-loaded erythrocytes was not studied because this has already been done by other authors [1]. Imipramine, amitriptyline and desipramine inhibited Mg^{2+} efflux from Mg^{2+}-loaded human erythrocytes in NaCl medium. The IC_{50} values of these drugs in the inhibition of Na^+/Mg^{2+} antiport in Mg^{2+}-loaded human erythrocytes amounted to 25 μmol·l^{-1} for imipramine, 50 μmol·l^{-1} for desipramine and 60 μmol·l^{-1} for amitriptyline [1], which is in the same range as we have found for the inhibition of Mg^{2+} efflux in non-Mg^{2+}-loaded rat erythrocytes by imipramine in KCl, choline Cl and sucrose medium (see Fig. 3).

As reported earlier, in Mg^{2+}-loaded rat erythrocytes, imipramine, amitriptyline and desipramine had about the same inhibitory effect on Na^+/Mg^{2+} antiport, whereas chlorpromazine inhibited Na^+/Mg^{2+} antiport to a minor degree [1]. Furthermore, in Mg^{2+}-loaded erythrocytes trazodone had a similar effect as chlorpromazine [1].

However, in our study with non-Mg^{2+}-loaded rat erythrocytes, trazodone had a small stimulatory effect on Na^+/Mg^{2+} and choline/Mg^{2+} antiport (Table 2).

These controversial effects in non-Mg^{2+}-loaded and Mg^{2+}-loaded erythrocytes can be explained as follows. As discussed earlier in detail [16], Mg^{2+}-loading transferred the Mg^{2+}/Mg^{2+} exchanger of non-Mg^{2+}-loaded erythrocytes with low Na^+/Mg^{2+} antiport capacity to the Na^+/Mg^{2+} antiporter with high Na^+/Mg^{2+} antiport capacity. These two forms of the exchanger in non-Mg^{2+}-loaded and Mg^{2+}-loaded erythrocytes may be differently affected by imipramine. The finding that imipramine inhibited Na^+/Mg^{2+} antiport in some non-Mg^{2+}-loaded cell types (see Section 1) may indicate the existence of isoforms of the Na^+/Mg^{2+} antiporter in various cell types that are inhibited by imipramine without Mg^{2+}-loading. For a definite explanation, the structure of the Na^+/Mg^{2+} antiporter and its possible cell-specific isoforms must be characterized.

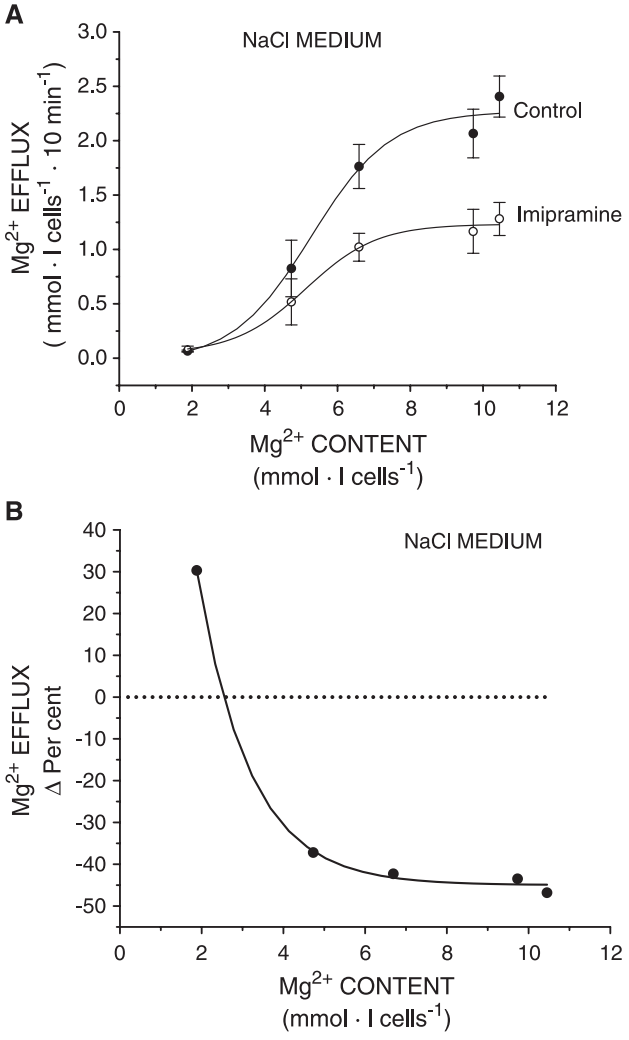


Fig. 4. Effect of imipramine (100 μmol·l^{-1}) on Mg^{2+} efflux in NaCl medium at different intracellular Mg^{2+} concentrations obtained by Mg^{2+} loading. (A) Effect of imipramine on Mg^{2+} efflux at various intracellular Mg^{2+} concentrations. Means±S.E. of paired experiments, n=6–8. The effect of imipramine was significant (P<0.05) at all investigated intracellular concentrations of Mg^{2+}. (B) Relative effect of imipramine as a function of intracellular Mg^{2+} concentration. Mg^{2+} efflux was given as percentage change (Δpercent) from control without imipramine at each Mg^{2+} concentration.

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