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# Inhibition by Antidepressant Drugs of Cyclic AMP Response Element-Binding Protein/Cyclic AMP Response Element-Directed Gene Transcription

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## SUMMARY

Clinical observations agree that antidepressant drugs are effective only after a lag phase of 1–3 weeks. This delay could be explained at the molecular level by an action on gene transcription. Transcription of many genes is directed by the cAMP/$Ca^{2+}$-responsive element (CRE) and its cognate transcription factor CRE-binding protein (CREB). Membrane depolarization and cAMP induce the phosphorylation of CREB at Ser-119 and thereby stimulate the transcriptional activity of CREB. The effect of antidepressant drugs on CREB/CRE-directed gene transcription was investigated using transient transfections of reporter fusion genes in HIT and PC-12 cells. Clomipramine, imipramine, fluoxetine, doxepin, desipramine, amitriptyline, maprotiline, mianserin, and trazodone inhibited CRE-directed gene transcription that was stimulated by membrane depolarization, with $IC_{50}$ values between 70 nM and 1.73 μM. Desipramine had no effect on transcription after stimulation by cAMP but blocked the synergistic effect of cAMP and membrane depolarization to the level of stimulation by cAMP alone. Upon membrane depolarization, desipramine reduced the phosphorylation of CREB at Ser-119 and also blocked the depolarization-induced increase in the intracellular free $Ca^{2+}$ concentration in HIT cells. Thus, by interfering with the depolarization-induced activation of the transcription factor CREB, antidepressant drugs can inhibit CRE-directed gene transcription, which could underlie the pharmacological effects of these clinically important drugs.

Antidepressant drugs have been in clinical use since the 1950s. Although it is generally agreed that they are effective, their mechanism of action is not understood. Primary attention has focused on their ability to increase the concentration of biogenic amines in synaptic clefts through inhibition of amine transporters or amine degradation. It was suggested that this corrects the amine deficiency thought to underlie depression (1). However, studies have not consistently demonstrated a lack of biogenic amines or their metabolites in body fluids of depressed patients (1). Moreover, there is a clear discrepancy between the rapid effect of antidepressants in increasing synaptic concentrations of biogenic amines and the lack of immediate clinical efficacy of antidepressant treatment. Antidepressant therapy is known to be associated with a lag phase of 1–3 weeks before the onset of beneficial effects. The clinical course of the antidepressant effect is consistent with slow-onset adaptive changes that can be viewed as drug-induced neural plasticity and may be based, at the molecular level, on drug-induced changes in gene expression. Recently, diverse effects of antidepressant drugs on gene expression have been described, although their functional significance is unclear (2–5). Changes in gene expression were suggested to be the biological basis of the detrimental effect of psychosocial stressors on the course of depressive illnesses (6). Antidepressant drugs might therefore balance changes in gene expression that are pathogenic in depression.

A well characterized pathway that transduces environmental signals to gene transcription uses the CRE, a DNA motif found in promoters of many genes. The CRE was initially identified by its ability to confer stimulation by cAMP on gene transcription (7). Recently it was recognized that membrane depolarization also can stimulate CRE-directed transcription (8). Membrane depolarization was shown to activate CRE-mediated gene transcription via $Ca^{2+}$ influx through L-type VDCC (8, 9). The increase in $[Ca^{2+}]_i$ leads, through activation of CaM kinases, to phosphorylation of the CRE-binding transcription factor CREB at the same residue, Ser-119 (in CREB-327), that is phosphorylated after stimu-

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**ABBREVIATIONS:** CRE, cAMP response element; CREB, cAMP response element-binding protein; CaM kinase, $Ca^{2+}$/calmodulin-dependent protein kinase; VDCC, voltage-dependent $Ca^{2+}$ channel(s); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TBST, Tris-buffered saline/Tween; $[Ca^{2+}]_i$, intracellular free $Ca^{2+}$ concentration.

lation by cAMP (10). Phosphorylation of CREB-327 at Ser-119 is necessary for its transcriptional activation (9, 10). CRE-directed gene transcription is further regulated by the $Ca^{2+}$/calmodulin-dependent protein phosphatase calcineurin, which removes an inhibitory phosphorylation (11). The following experiments investigate the effects of antidepressant drugs on CRE-directed gene transcription.

## Materials and Methods

**Cell culture.** HIT-T15 cells, derived from a hamster insulinoma (12), were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 5% horse serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. PC-12 cells, derived from a rat adrenal pheochromocytoma (13), were obtained from DSM (Braunschweig, Germany). They were grown in RPMI 1640 medium supplemented with 10% horse serum, 5% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, on tissue culture dishes (Falcon) that had been coated with collagen.

**Transfection.** Cells were transfected with the indicator plasmid 4xSomCRET81Luc (14). 4xSomCRET81Luc contains four copies of the rat somatostatin gene CRE (5′-GATCCTCCTTGGCTGACGTCAGAGAGAGAGTA-3′). HIT cells were transfected with 2 μg of 4xSomCRET81Luc/6-cm dish. When indicated, 2 μg of the expression plasmid for CaM kinase IV (amino acids 1–313) (15) were co-transfected in each 6-cm dish. These co-transfections were done with a constant DNA concentration, which was maintained by adding Bluescript (Stratagene, La Jolla, CA). Cells were trypsinized and transfected in suspension by the DEAE-dextran method, as described previously (16). PC-12 cells were transfected with 10 μg of DNA/6-cm dish, using the calcium phosphate precipitation method. This was followed 5 hr later by glycerol shock. Cells were collected 48 hr after transfection. Antidepressant drugs were added 7 hr before harvest, and cells were stimulated with high KCl levels (elevation of the KCl concentration of the medium from 5 mM to 45 mM), or forskolin 6 hr before harvest. Cell extract preparation and the luciferase assay were performed as described previously (16). Concentration-response curves were analyzed by fitting the data with a nonlinear least-squares routine.

**Immunoblots.** After treatment with desipramine for 4 hr and membrane depolarization for 3 hr, HIT cells were lysed (50 mM Tris·HCl, pH 7.0, 1% sodium dodecyl sulfate, 2% 2-mercaptoethanol), samples containing 20 μg of protein were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes, and membranes were rinsed in TBST (10 mM Tris·HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20), incubated for 1 hr with 10% nonfat dry milk dissolved in TBST, and then incubated for 2 hr with polyclonal anti-phospho-CREB antibodies (0.2 μg/ml, in TBST) obtained from M. Greenberg (Program in Neuroscience, Harvard Medical School, Boston, MA) (17). The IgG fraction of the antiserum had been collected by Protein A-Sepharose chromatography and further purified by peptide affinity chromatography (17). The specificity of the anti-phospho-CREB antibodies was demonstrated previously (17) and was confirmed by preabsorption of the antibody with the peptide. Antibody-antigen complexes were detected with enhanced chemiluminescence reagents (Amersham). After autoradiography, films were evaluated by densitometry (ScanPack; Biometra).

**Measurement of $[Ca^{2+}]_i$.** For $[Ca^{2+}]_i$ measurements, cells were subcultured on quartz glass coverslips and were used on the second or third day. HIT cells were loaded with 5 μM fura-2/acetoxymethyl ester for 30 min at 37°, in medium containing 120 mM NaCl, 4.7 mM KCl, 1.2 mM $KH_2PO_4$, 1.2 mM $MgSO_4$, 0.42 mM $CaCl_2$, 10 mM glucose, 20 mM HEPES, 2% (w/v) bovine serum albumin, and 0.1% (w/v) pluronic acid, pH 7.4 (gassed with 100% $O_2$). After loading, the coverslips were washed, mounted in a temperature-controlled perfusion chamber (Intracel, Royston Herts, UK) at 37°, and placed on the stage of a Zeiss Axiovert IM 135 microscope equipped with a 40× Achrostigmat oil-immersion objective. The chamber was perfused at 0.75 ml/min with the same buffer as used for loading, except that bovine serum albumin was present at 0.1% (w/v) and pluronic acid was omitted. Coverslips that were not used immediately after dye loading were kept at room temperature until use, to minimize leakage of fura-2. $Ca^{2+}$ measurements were made in cells of average size and healthy appearance (round shape, with no membrane blebs). Fura-2 fluorescence from single cells was recorded with a dual-excitation spectrofluorometer system (Deltascan 4000; Photon Technology Instruments, Wedel, Germany). $[Ca^{2+}]_i$ values were calculated according to the formula (18)

$$[Ca^{2+}]_i = K_d \times (R - R_{min})/(R_{max} - R) \times B,$$

where $K_d$ = 225 nM (18) and $R_{max}$, $R_{min}$, and $B$ are constants that were determined in the perfusion chamber with solutions containing fura-2 free acid (1 μM) and various concentrations of free $Ca^{2+}$ (data not shown). All records were corrected for autofluorescence of unloaded cells at each wavelength before the ratio was used. Fura-2-loaded cells were stimulated two times by membrane depolarization (45 mM KCl). Thirty minutes after the first stimulation, the cells were again stimulated by KCl either in the presence or in the absence of drug. Drugs were added 25 min before the second stimulation.

**Materials.** Moclobemide was provided by Hoffman-La Roche (Grenzach Whylen, Germany) and fluoxetine hydrochloride by Lilly (Bad Homburg, Germany). Forskolin and the hydrochloride salts of desipramine, imipramine, doxepin, amitriptyline, trazodone, maprotiline, mianserin, and clomipramine were purchased from Sigma, and fura-2/acetoxymethyl ester was from Molecular Probes. Stock solutions were prepared in water, with the exception of forskolin (10 mM in dimethylsulfoxide).

## Results

**Effects of antidepressant drugs on CRE-directed gene transcription.** To investigate the effects of antidepressant drugs on CRE-directed gene transcription, a transient transfection assay was used (9). Cells were transfected with a DNA construct in which the expression of a reporter gene is controlled by the CRE. The construct contains four copies of the rat somatostatin gene CRE in front of the truncated viral thymidine kinase promoter and the luciferase reporter gene (Fig. 1). The somatostatin gene CRE contains the CRE consensus octamer sequence TGACGTCA and is well known as a high affinity CREB binding site that mediates responsiveness to both cAMP and $Ca^{2+}$ (14, 19). This construct was transiently transfected into HIT cells, an electrically excitable cell line in which CRE-mediated gene transcription has been characterized previously (9, 11, 14). The CRE did not confer basal transcriptional activity (data not shown). Membrane depolarization by 45 mM KCl stimulated CRE-mediated gene transcription 3.5–5-fold (Figs. 1 and 2), as described before (9). Treatment of cells with desipramine alone led to a slight increase in CRE-mediated transcription. Transcriptional activity was 122.1 ± 7.1, 148.7 ± 3.0, 137.0 ± 6.5, 165.9 ± 7.0, and 150.8 ± 2.7% of controls in the presence of desipramine at 0.1, 0.3, 1.0, 3.0, and 30.0 μM, respectively. This slight increase by desipramine of CRE-mediated transcription was, however, not consistently observed (Fig. 2A and data not shown). The stimulatory effect of KCl on CRE-mediated gene transcription was greatly reduced in the presence of desipramine (Fig. 1). The inhibition by desipramine was concentration dependent, with an $IC_{50}$ of

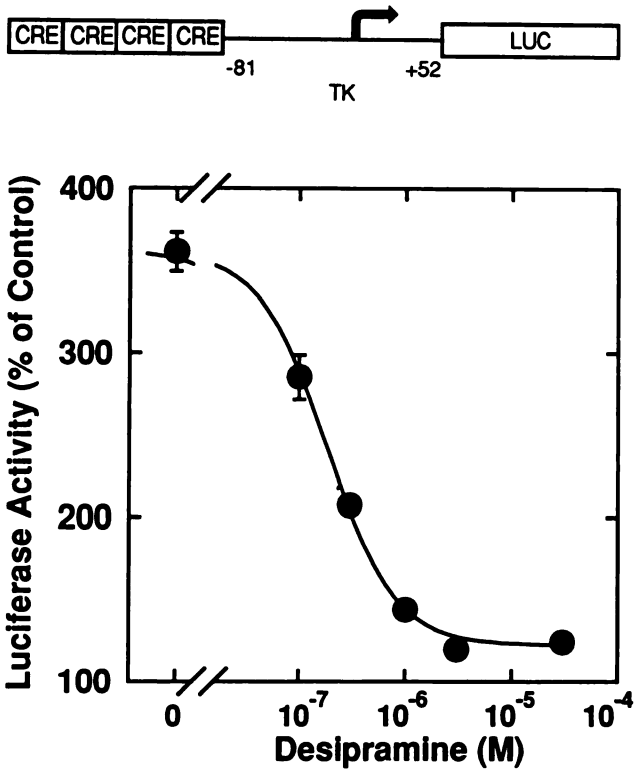


**Fig. 1.** Inhibition by desipramine of CRE-directed gene transcription. HIT cells were transfected with the indicated plasmid (4xSomCRET81Luc). CRE-directed gene transcription was stimulated with 45 mM KCl. Luciferase activity is expressed as a percentage of the control that received the same desipramine concentration but was not stimulated with KCl. Values are means ± standard errors of four experiments, each done in duplicate. *TK*, thymidine kinase promoter. *LUC*, coding sequence of the firefly luciferase gene.

180 nM. At a concentration of 3 μM, desipramine was maximally effective (93% inhibition).

Both cAMP and $Ca^{2+}$ signaling pathways activate CREB/CRE-mediated gene transcription. To further characterize the effect of desipramine on CRE-directed gene transcription, the question of whether desipramine also interferes with cAMP-induced gene transcription was investigated. The adenylate cyclase activator forskolin (3 μM) stimulated CRE-directed transcription 148-fold (Fig. 2A). Treatment of cells with 3 μM desipramine had no effect on forskolin-induced transcription, whereas in parallel control groups transcription stimulated by membrane depolarization was inhibited (Fig. 2A). The lack of inhibition by desipramine of forskolin-induced transcription was not due to stimulus strength, because the effects of lower concentrations of forskolin were also not changed by desipramine (data not shown). Thus, desipramine does not inhibit forskolin-induced CRE-directed gene transcription but selectively interferes with the stimulation by membrane depolarization.

cAMP and $Ca^{2+}$ have been shown by several studies to synergistically stimulate CRE-directed gene transcription (8, 9, 20). Whereas CRE-directed gene transcription was stimulated 3.6-fold by KCl and 99-fold by forskolin, the combination of the two induced a 346-fold increase in gene transcription (Fig. 2B). This synergism was inhibited by desipramine to the level reached with forskolin alone (Fig. 2B). Thus, desipramine inhibits both the effect of membrane depolariza-

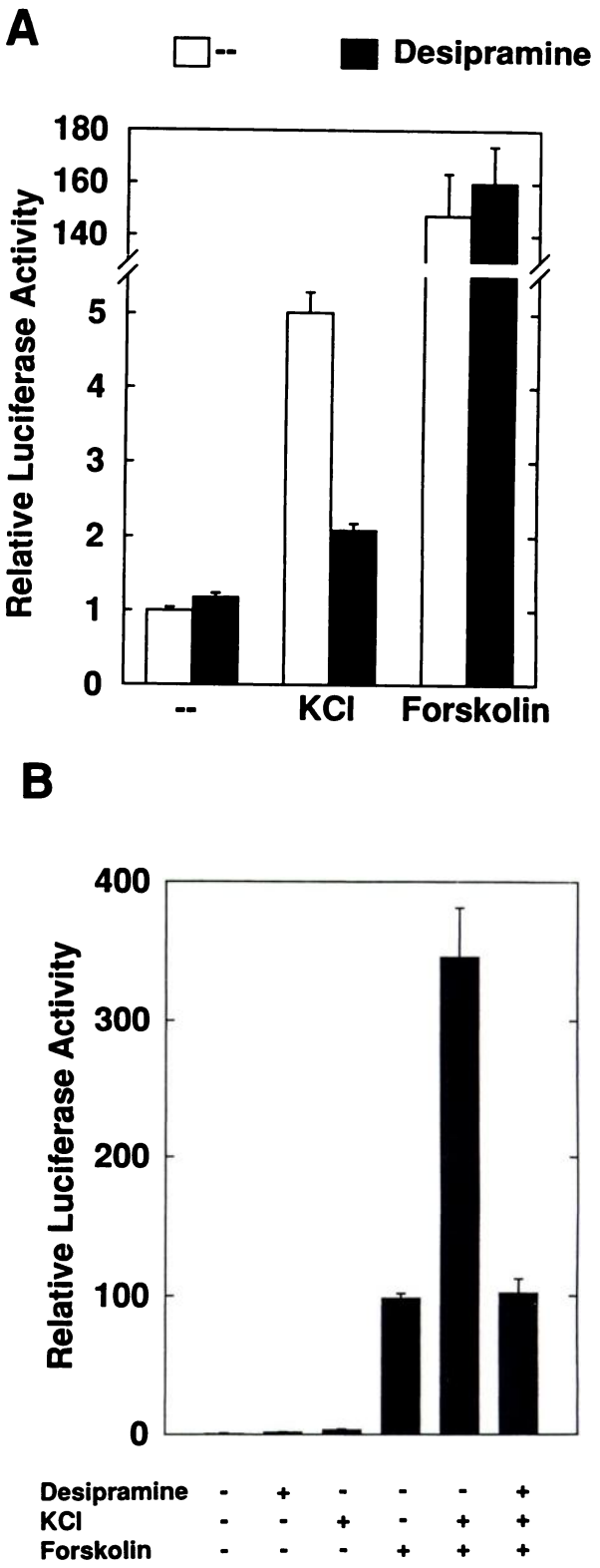


**Fig. 2.** A, Effect of desipramine on CRE-directed gene transcription stimulated by membrane depolarization or forskolin. B, Inhibition by desipramine of the synergistic effect of forskolin and membrane depolarization on CRE-directed gene transcription. HIT cells were transfected with 4xSomCRET81Luc. KCl was used at 45 mM, forskolin at 3 μM, and desipramine at 3 μM. The relative luciferase activity after desipramine treatment alone was 1.5 ± 0.3 (not statistically significant). Luciferase activity is expressed relative to the mean value, in each experiment, of the activity measured in controls (5 mM KCl, no drug). Values are means ± standard errors of three experiments, each done in duplicate.

tion alone and the potentiation by membrane depolarization of the effect of cAMP.

To investigate whether the ability to inhibit CRE-directed transcription is a common feature of antidepressant drugs, several structurally divergent compounds were used (Table 1). Some of the compounds tested, when given alone at concentrations up to 30 μM, slightly increased basal CRE-directed gene transcription, which did not exceed 10% of the stimulation elicited by 45 mM KCl (data not shown). However, tricyclic antidepressant drugs (desipramine, imipramine, clomipramine, amitriptyline, and doxepin) and atypical antidepressant drugs (maprotiline, mianserin, trazodone, and fluoxetine) inhibited depolarization-induced CRE-directed gene transcription in a concentration-dependent manner, with complete inhibition being the maximal effect. The deduced $IC_{50}$ values differed and are given in Table 1. The monoamine oxidase inhibitor moclobemide had no effect (Table 1). Other psychotropic drugs, such as cocaine, morphine, and diazepam, did not block depolarization-induced transcription (Table 1). Fluspirilene (10 μM) inhibited CRE-directed transcription stimulated by KCl (data not shown). Fluspirilene has high affinity for $Ca^{2+}$ channels, and the $Ca^{2+}$ channel-blocking activity of neuroleptic drugs has been related to the antidepressant activities of these drugs (21, 22).

**Inhibition by desipramine of the phosphorylation of the transcription factor CREB.** Activation of CREB/CRE-directed gene transcription by membrane depolarization depends on the phosphorylation of CREB at Ser-119 (in CREB-327, corresponding to Ser-133 in CREB-341) (9, 10). The phosphorylation of CREB at Ser-119 was investigated in immunoblots of HIT cell lysates using antibodies that are specific for CREB phosphorylated at Ser-119 (17). After treatment of HIT cells with KCl (45 mM), a band of 43 kDa comigrating with CREB, as stained by another anti-CREB antiserum (data not shown), and a band of 38 kDa were increased in intensity (Fig. 3). The 38-kDa protein could correspond to activating transcription factor-1, which is highly homologous to CREB and seems to cross-react with the anti-phospho-CREB antiserum (17). In addition, a nonspecific, higher molecular weight band was stained in all lanes, irrespective of the treatment. Whereas desipramine (3 μM) alone had no effect, after membrane depolarization it

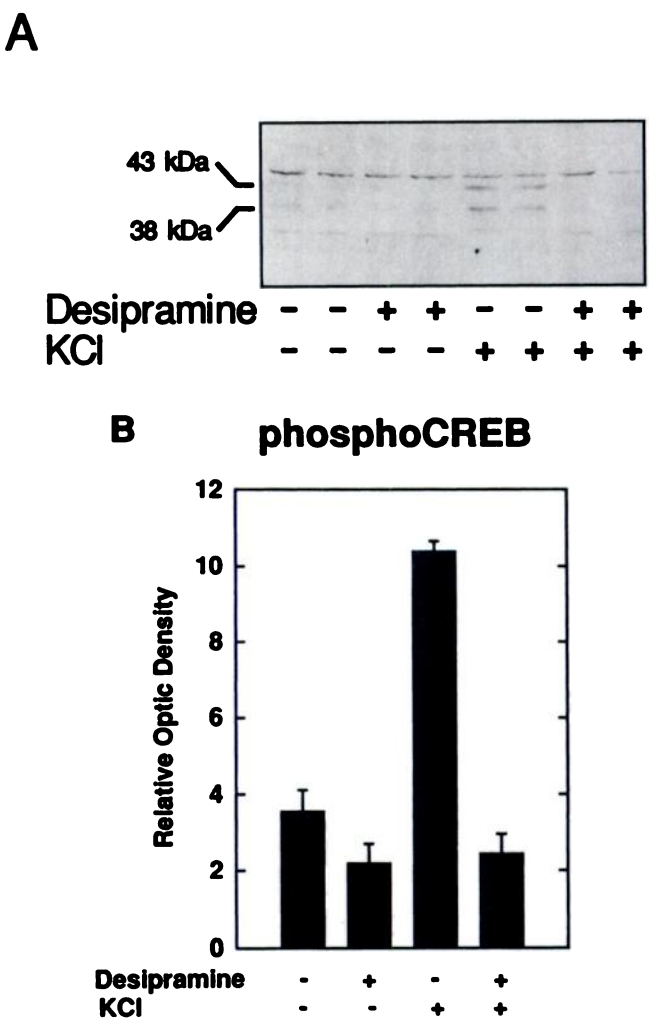


**Fig. 3.** Desipramine inhibition of CREB phosphorylation at Ser-119 induced by membrane depolarization. The phosphorylation of CREB at Ser-119 was investigated in immunoblots of HIT cell lysates. HIT cells were treated with desipramine (3 μM) and KCl (45 mM) as indicated. The blots were incubated with anti-phospho-CREB, an antibody that recognizes CREB phosphorylated at Ser-119 but fails to recognize CREB not phosphorylated at Ser-119. A, Immunoblot from a typical experiment. B, Relative absorbance of the 43-kDa band corresponding to phospho-CREB, expressed in arbitrary units. Values are means ± standard errors of three independent experiments.

TABLE 1

**Potency of antidepressant drugs in the inhibition of CRE-directed gene transcription stimulated by KCl**

Values are means ± standard errors from three separate experiments.

| Antidepressant drug | $IC_{50}$ |
|---|---|
| | nM |
| Clomipramine | 70 ± 20 |
| Imipramine | 110 ± 60 |
| Fluoxetine | 120 ± 30 |
| Doxepin | 160 ± 90 |
| Desipramine | 180 ± 10 |
| Amitriptyline | 260 ± 10 |
| Maprotiline | 900 ± 200 |
| Mianserin | 1,470 ± 300 |
| Trazodone | 1,730 ± 1,230 |
| Moclobemide | >30,000 |
| Morphine | >10,000 |
| Cocaine | >10,000 |
| Diazepam | >10,000 |

reduced the intensities of the bands corresponding to phospho-CREB and the phosphorylated 38-kDa protein (Fig. 3). Thus, desipramine inhibits the membrane depolarization-induced phosphorylation of CREB at Ser-119.

**Blockade of $Ca^{2+}$ influx by desipramine and other antidepressants.** The phosphorylation and activation of CREB by membrane depolarization rely on $Ca^{2+}$ influx through L-type VDCC (9). Therefore, the effect of desipramine on the depolarization-induced increase in $[Ca^{2+}]_i$ was investigated. In single-cell measurements of $[Ca^{2+}]_i$ in HIT cells loaded with fura-2, $[Ca^{2+}]_i$ was increased from basal values of 142 ± 4 nM ($n$ = 32) by 169 ± 9 nM ($n$ = 32) at its peak after membrane depolarization by 45 mM KCl. Reexposure of the cells to 45 mM KCl after 30 min elicited a nearly identical response (Fig. 4B). In the presence of desipramine, however, the depolarization-induced increase in $[Ca^{2+}]_i$ was inhibited (Fig. 4A). Desipramine reduced the depolarization-induced increase in $[Ca^{2+}]_i$ in a concentra-

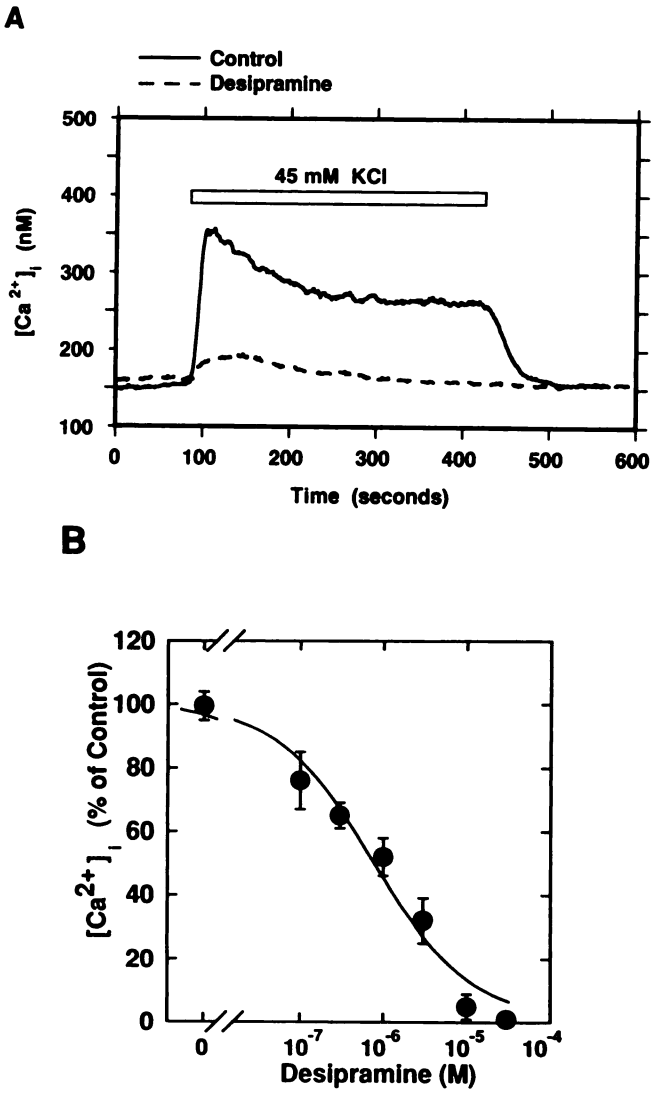


**Fig. 4.** Effect of desipramine on $[Ca^{2+}]_i$ elevated by membrane depolarization in HIT cells. Fura-2-loaded cells were stimulated two times by membrane depolarization (45 mM KCl). Thirty minutes after the first stimulation, the cells were again stimulated by KCl, either in the presence or in the absence of drug. Desipramine was added 25 min before the second stimulation. A, Representative measurement of changes in $[Ca^{2+}]_i$ in a single HIT cell during the first stimulation (*Control*) and during the second stimulation in the presence of desipramine (10 $\mu$M). B, Concentration-response curve for the effect of desipramine on depolarization-induced increases in $[Ca^{2+}]_i$ in single HIT cells. The integral of the increase in $[Ca^{2+}]_i$ induced by 45 mM KCl in the second stimulation is expressed as a percentage of the increase in $[Ca^{2+}]_i$ induced by KCl in the first stimulation of the same cell (control). Values are means ± standard errors of measurements of four to eight cells.

tion-dependent manner (Fig. 4B). Complete inhibition was observed with 10 $\mu$M desipramine, and the $IC_{50}$ was 0.75 $\mu$M desipramine. Clomipramine, fluoxetine, and mianserine also inhibited depolarization-induced increases in $[Ca^{2+}]_i$, with $IC_{50}$ values of 0.65 $\mu$M, 1.36 $\mu$M, and 16.9 $\mu$M, respectively. Moclobemide at concentrations of up to 100 $\mu$M had no effect. Thus, the rank orders of potency for inhibition of depolarization-induced transcription and $Ca^{2+}$ influx are similar.

**Evidence that desipramine does not change CRE-directed transcription after stimulation by CaM kinase IV.** Previous studies suggested that the effects of membrane depolarization and $Ca^{2+}$ influx on CREB/CRE-directed gene transcription are likely mediated by activation of CaM kinases (8–10). Consistent with this view, overexpression of a constitutively active form of CaM kinase IV has been found to activate CREB in three different cell lines (15). An expression vector for the constitutively active form of CaM kinase IV was used in this study to test the effect of desipramine on CRE-directed transcription after stimulation through a CaM kinase in the absence of $Ca^{2+}$ influx through VDCC. Overexpression of CaM kinase IV in HIT cells increased CRE-directed transcription to 482 ± 24% of controls (six experiments). Desipramine did not change this transcriptional response. In the presence of desipramine (3 $\mu$M), overexpression of CaM kinase IV increased CRE-directed transcription to 428 ± 60% of controls (six experiments; not significantly different).

**Inhibition by desipramine of CREB/CRE-directed gene transcription in PC-12 cells.** The clonal rat pheochromocytoma PC-12 cell line has become a widely used preparation for various model studies on neurons (13). To test whether desipramine inhibits CRE-directed gene transcription also in these cells, 4xSomCRET81Luc was transiently transfected into PC-12 cells. Desipramine (3 $\mu$M) had no effect on basal CRE-directed gene transcription (data not shown). In the presence of forskolin (3 $\mu$M), membrane depolarization by 45 mM KCl elevated luciferase expression from 100.0 ± 28.0 to 410.0 ± 15.6% of controls. Desipramine (3 $\mu$M) inhibited the effect of membrane depolarization by 60% (increase by KCl to 224.3 ± 22.1% of controls in three independent experiments). Thus, desipramine inhibits CRE-directed gene transcription stimulated by membrane depolarization in HIT and PC-12 cells.

## Discussion

This study investigates a molecular mechanism by which antidepressant drugs can modulate gene transcription. It is shown that desipramine and several other tricyclic or atypical antidepressant drugs, but not moclobemide, inhibit CREB/CRE-directed transcription after stimulation by membrane depolarization. Consistent with the current concept that membrane depolarization stimulates CRE-directed transcription through phosphorylation and thus activation of CREB (10), desipramine inhibited the phosphorylation of CREB. The underlying mechanism is probably the observed inhibition of depolarization-induced $Ca^{2+}$ influx into the cells. The rank orders of potency for inhibition of depolarization-induced transcription and $Ca^{2+}$ influx were similar. The increase in $[Ca^{2+}]_i$ is thought to activate CaM kinases (9, 10) and, consistent with the proposed mechanism, desipramine did not inhibit CRE-directed transcription when stimulated by constitutively active CaM kinase IV, i.e., in the absence of $Ca^{2+}$ influx through VDCC. Because the stimulation of CRE-directed gene transcription by membrane depolarization can be blocked by verapamil, nifedipine, and diltiazem in HIT cells (9), the transcriptionally relevant $Ca^{2+}$ may enter the cells through L-type VDCC. Antidepressants have been reported previously to inhibit depolarization-induced $Ca^{2+}$ influx into neuronal cells (23) or synaptosomes (24), to inhibit L-type $Ca^{2+}$ currents in cardiac myocytes (25), a neuroblastoma cell line (26), and primary neuronal cells (27), and to compete for (−)-desmethoxyverapamil binding to L-type VDCC in brain membranes (28). In some of these studies

high concentrations of antidepressant drugs were used. However, the rank order of affinities for the (−)-desmethoxyverapamil binding site in rat cerebral cortex (clomipramine > imipramine > amitriptyline > doxepin > desipramine > mianserin) (28) is very similar to the rank orders of potency we found in this study for inhibition of both depolarization-induced $Ca^{2+}$ influx and CREB/CRE-directed transcription. Futhermore, the reported affinity of desipramine for the L-type VDCC in rat forebrain membranes (0.98 $\mu$M) (28) closely resembles the $IC_{50}$ for inhibition of depolarization-induced increases in $[Ca^{2+}]_i$ found in this study (0.75 $\mu$M). The concentrations needed by us to inhibit $Ca^{2+}$ influx were, however, somewhat higher than those needed to inhibit depolarization-induced gene transcription ($IC_{50}$ for desipramine, 0.18 $\mu$M). This could be due to different experimental procedures for measurement of $[Ca^{2+}]_i$ and gene transcription or, alternatively, to the dependence of gene transcription on high $Ca^{2+}$ concentrations. CaM kinases that phosphorylate CREB at Ser-119 are activated only by high $Ca^{2+}$ concentrations (29). A limited decrease of $[Ca^{2+}]_i$ might therefore lower CREB phosphorylation and gene transcription significantly. The concentrations of antidepressant drugs that inhibited CRE-directed gene transcription in this study are about 2–40-fold higher than those that block amine reuptake (30) but fall within the range of therapeutic serum concentrations or the even higher brain concentrations in depressed patients (31). Although blockade of VDCC *in vivo* by antihypertensive calcium channel blockers like nifedipine or by antidepressant drugs seems to be of little significance for the endocrine system, the effect of antidepressants found in this study might be relevant for the brain. HIT cells express the neuronal isoform of the pore-forming $\alpha_1$ subunit of VDCC (32), and inhibition of CREB/CRE-directed gene transcription stimulated by membrane depolarization was observed in this study also in PC-12 cells, which are widely used for various model studies on neurons (13). L-type VDCC are found in the perikarya of neurons, close to the nucleus (33). Opening of these channels induces CRE-directed gene transcription in cortical neurons (34). Thus, in the brain, blockade of L-type VDCC by antidepressant drugs could result in inhibition of CRE-mediated gene transcription.

In the brain, antidepressant drugs could influence CRE-directed gene transcription not only through inhibition of depolarization-induced phosphorylation and activation of CREB. CREB is regulated by cAMP as well as by $Ca^{2+}$ (7–11), and monoamine reuptake inhibition by antidepressant drugs is assumed to change $\beta$-adrenergic receptor number, receptor-associated adenylate cyclase activity, and cAMP formation. Although potentiation of monoaminergic neurotransmission, with enhanced cAMP formation, may be an early event, prolonged administration of tricyclic or atypical antidepressant drugs has been shown to reduce the number of $\beta$-adrenergic receptors and the responsiveness of brain adenylate cyclase to norepinephrine, leading to decreased cAMP formation (35). This decrease in cAMP formation and the inhibition by antidepressants of depolarization-induced increases in $[Ca^{2+}]_i$ would synergize to inhibit CREB/CRE-directed gene transcription. Because repeated administration of monoamine oxidase inhibitors also decreases $\beta$-adrenergic receptor number (35), moclobemide could inhibit transcriptional activation of CREs through this pathway. This study shows that antidepressants can inhibit CREB/CRE-directed transcription after stimulation by membrane depolarization in cultured cells. The questions, however, of whether treatment with antidepressant drugs influences the regulation by $Ca^{2+}$ and/or cAMP of CREB/CRE-directed transcription *in vivo* at critical sites within the central nervous system and whether this contributes to the therapeutic effects of antidepressants remain unclear. These questions can be approached using transgenic animal models.

In a physiological context, CREB and CRE were shown to be involved in stress-induced gene transcription in the hypothalamus (36) and to be necessary for neuronal plasticity and long term memory formation from mollusks to mammals (37). CREB/CRE-directed gene transcription is therefore a candidate to mediate the long term effect of environmental stressors in depression (6). Assuming such a model, tricyclic and atypical antidepressants could elicit therapeutic effects by normalizing increased transcription through the CRE. Support for a link between the antidepressant effect and blockade of $Ca^{2+}$ channels, resulting possibly in inhibition of CRE-directed gene transcription, is provided by reports on the antidepressant properties of $Ca^{2+}$ channel blockers of the dihydropyridine, phenylalkylamine, and benzothiazepine classes in animals and humans (38). Although this model is largely hypothetical, it is of note that a number of neurochemical effects observed with chronic antidepressant treatment could possibly be explained by an inhibition of CRE-directed gene transcription. Examples include $\beta_1$-adrenergic receptors, tyrosine hydroxylase, and corticotropin-releasing factor, which contain CREs in their promoters (20, 39, 40) and show reduced expression after antidepressant treatment (2, 3, 5). While this manuscript was under review, Thompson *et al.* (41) demonstrated that the mechansims underlying $Ca^{2+}$-induced, CREB/CRE-mediated gene transcription are perhaps more complicated than previously thought. Whereas those authors provided evidence that supports the notion that CREB is directly phosphorylated on Ser-119 by a $Ca^{2+}$-activated kinase after membrane depolarization, they suggested that a second, protein kinase A-dependent, event is necessary for $Ca^{2+}$-activated CREB/CRE gene transcription. By inhibiting depolarization-induced phosphorylation of CREB on Ser-119, antidepressant drugs block a mechanism that is required but not sufficient for $Ca^{2+}$-activated, CREB/CRE-directed gene transcription.

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