Archival Report

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Antidepressants Rescue Stress-Induced Disruption of Synaptic Plasticity via Serotonin Transporter–Independent Inhibition of L-Type Calcium Channels

Claus Normann, Sibylle Frase, Verena Haug, Gregor von Wolff, Kristin Clark, Patrick Münzer, Alexandra Dorner, Jonas Scholliers, Max Horn, Tanja Vo Van, Gabriel Seifert, Tsvetan Serchov, Knut Biber, Christoph Nissen, Norbert Klugbauer, and Josef Bischofberger

ABSTRACT

BACKGROUND: Long-term synaptic plasticity is a basic ability of the brain to dynamically adapt to external stimuli and regulate synaptic strength and ultimately network function. It is dysregulated by behavioral stress in animal models of depression and in humans with major depressive disorder. Antidepressants have been shown to restore disrupted synaptic plasticity in both animal models and humans; however, the underlying mechanism is unclear.

METHODS: We examined modulation of synaptic plasticity by selective serotonin reuptake inhibitors (SSRIs) in hippocampal brain slices from wild-type rats and serotonin transporter (SERT) knockout mice. Recombinant voltage-gated calcium (Ca^{2+}) channels in heterologous expression systems were used to determine the modulation of Ca^{2+} channels by SSRIs. We tested the behavioral effects of SSRIs in the chronic behavioral despair model of depression both in the presence and in the absence of SERT.

RESULTS: SSRIs selectively inhibited hippocampal long-term depression. The inhibition of long-term depression by SSRIs was mediated by a direct block of voltage-activated L-type Ca^{2+} channels and was independent of SERT. Furthermore, SSRIs protected both wild-type and SERT knockout mice from behavioral despair induced by chronic stress. Finally, long-term depression was facilitated in animals subjected to the behavioral despair model, which was prevented by SSRI treatment.

CONCLUSIONS: These results showed that antidepressants protected synaptic plasticity and neuronal circuitry from the effects of stress via a modulation of Ca^{2+} channels and synaptic plasticity independent of SERT. Thus, L-type Ca^{2+} channels might constitute an important signaling hub for stress response and for pathophysiology and treatment of depression.

Keywords: Antidepressants, Calcium channels, Long-term synaptic plasticity, Major depression, SSRI, Stress

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Major depression is a devastating disorder that leads to enormous amounts of individual suffering, high treatment costs, and severe loss of productivity (1). In the United States, more than 10% of individuals older than 12 years of age take antidepressant medication (2). Despite its vast health impact, neither the pathophysiology of depression nor the mechanism of action of antidepressants is completely understood (3). Most antidepressants target the serotonin transporter (SERT), reducing the reuptake of serotonin, or 5-hydroxytryptamine (5-HT), into presynaptic structures and thereby increasing the availability of 5-HT in the synaptic cleft. For many years, the neurobiological basis of depression has been linked to this supposed mechanism of action of antidepressants. This resulted in the monoamine hypothesis of depression, which proposes that depletion of 5-HT and other monoamines underlies the pathophysiology of depression (4). However, evidence showing either that a monoamine deficiency causes depression or that increased extracellular serotonin concentrations alleviate depressed mood is not compelling (5,6).

In recent years, evidence has accumulated that neuronal plasticity, i.e., the ability of the brain to dynamically adapt to external stimuli, is dysregulated in depression and preserved by antidepressants. Specifically, research on the pathophysiology of depression has focused on long-term synaptic plasticity. This ubiquitous form of functional plasticity regulates the strength of synaptic transmission in response to neuronal activity. Long-term potentiation (LTP) increases, whereas long-term depression (LTD) decreases, synaptic transmission for hours in brain slices or even for weeks and months in living animals (7–9). In both LTP and LTD, postsynaptic calcium (Ca^{2+}) transients are decisive key signals for the induction of

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plasticity. In late phases of synaptic plasticity, changes in synaptic efficacy are consolidated via gene transcription, protein synthesis, and synaptogenesis (10,11). Long-term synaptic plasticity is believed to be the molecular correlate of learning and memory, consistent with the notion that emotional and other forms of learning might be disturbed in depression (12,13).

Research in preclinical models and humans suggests a dysregulation of synaptic plasticity in depression (14,15). In the chronic mild stress animal model of depression, we have shown a facilitation of LTD that could be prevented by an antidepressant (16). Various correlates of synaptic plasticity have been altered in humans with depression (17–19). Antidepressants have been shown to modulate synaptic plasticity in both animal models (16,20–25) and humans (17,26).

An important factor that links clinical depression to synaptic plasticity is the modulating effect of stress. Psychosocial stress from life events is among the most potent triggers of depressive episodes, leading to increased activity of the hypothalamic-pituitary-adrenal axis with a return to normal activity levels on treatment or remission (27–30). Almost all animal models of depression depend on exposure to behavioral stress (31). Rapid and moderate increases of steroid hormones seem to promote synaptic transmission and plasticity and produce an adaptive stress response, whereas both understimulation and excessive or chronic stress have been consistently shown to impair hippocampal LTP and to facilitate LTD, resulting in bell-shaped modulation of hippocampal plasticity by stress (9,16,32–37).

METHODS AND MATERIALS

Animals

For electrophysiological and behavioral experiments, we used juvenile (2–3 weeks old) or adult (10–14 weeks old) Wistar rats and SERT knockout (KO) mice of both sexes.

Nonneuronal Cell Cultures

L-type $Ca_v1.2b$ Ca^{2+} channels were stably expressed in human embryo kidney 293 cells. N-type $Ca_v2.2$ Ca^{2+} channels were stably expressed in Chinese hamster ovary cells and were originally obtained from B. Fakler, Institute of Physiology II, University of Freiburg.

Electrophysiology

Details on preparation of hippocampal brain slices and dissociated cells and for whole-cell current-clamp (for LTD and LTP measurements) and voltage-clamp (for Ca^{2+} current experiments) recordings are provided in the Supplement.

Behavioral Experiments

All behavioral experiments were performed with adult mice. For the chronic behavioral despair (CBD) model, the mice were forced to swim for 10 minutes daily in the afternoon for 5 consecutive days and then on day 32. From day 6 to day 31, the animals received intraperitoneal injections with either 0.3 mL saline or 30 mg/kg body weight fluvoxamine dissolved in 0.3 mL saline. Procedures for the forced swim test (FST), open field test, and tail suspension test are described in the Supplement.

Data Analysis and Chemicals

Details regarding data analysis, statistics, and chemicals are included in the Supplement.

RESULTS

To study the modulation of hippocampal synaptic plasticity by selective serotonin reuptake inhibitors (SSRIs), we first induced a spike timing-dependent form of LTD in rat hippocampal brain slices by asynchronous pairing of postsynaptic action potentials in CA1 neurons with excitatory postsynaptic potentials (EPSPs) induced by electrical stimulation of the Schaffer collateral pathway (Figure 1A) (8,9). This protocol resulted in a sustained reduction of the EPSP amplitude to 56.7 ± 5.2% of the baseline ($p < .001$, $n = 20$) (Figure 1B). In the presence of the SSRI fluvoxamine (5 μmol/L) in the bath solution, no significant LTD could be induced (Figure 1C, D). Fluvoxamine had no effect on LTD when applied intracellularly through the recording pipette (data not shown). A dose-response curve revealed a half maximum effect of fluvoxamine on LTD at 0.2 μmol/L (Figure 1E). All the currently marketed SSRIs significantly reduced the amount of LTD (Figure 1F, Supplemental Table S1). The concentrations used in these experiments correspond to the brain concentrations measured by magnetic resonance spectroscopy in patients treated with therapeutic doses of SSRIs. These studies revealed a brain-to-plasma ratio of 10–20:1, resulting in fluvoxamine and fluoxetine brain concentrations of 10 to 13 μmol/L (38,39). SSRIs had no significant effect on LTP, basal neurotransmission, action potential firing, EPSP amplitude, or passive membrane properties (Supplemental Figure S2, Supplemental Tables S2 and S3). These data suggest that SSRIs selectively inhibit hippocampal LTD.

In previous experiments, we have shown that LTD depends both on postsynaptic activation of metabotropic glutamate receptors and on Ca^{2+} influx into postsynaptic neurons through high-voltage–activated (HVA) Ca^{2+} channels. Further downstream, protein kinase C and protein interacting with C-kinase 1 serve as coincidence detectors for asynchronous presynaptic and postsynaptic activity—ultimately resulting in internalization of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors and depression of synaptic transmission (9).

HVA Ca^{2+} channels are inhibited by activation of 5-HT_{1A} receptors via a G protein–coupled pathway (8), thereby reducing LTD induction. Consistent with this notion, irreversible activation of the G protein–coupled pathway in the postsynaptic neuron by replacement of guanosine triphosphate with the nonhydrolyzable analogue GTPγS in the intracellular solution blocked LTD induction (97.8 ± 4.7% of baseline, $p > .5$, $n = 13$) (Figure 2A). As shown previously, bath application of 5-HT completely blocked LTD induction (1 μmol/L, 105.1 ± 2.7% of baseline, $p > .5$, $n = 10$) (Figure 2B). This effect was abolished by the coapplication of the 5-HT_{1A} antagonist WAY 100635 (5 μmol/L, 41.9 ± 2.5% of baseline, $p < .001$, $n = 7$) (Figure 2C), leading to normal LTD induction similar to control. These results so far suggest that the