PRIORITY COMMUNICATION

The Stress-Induced Cytokine Interleukin-6 Decreases the Inhibition/Excitation Ratio in the Rat Temporal Cortex via Trans-Signaling

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Background: Although it is known that stress elevates the levels of pro-inflammatory cytokines and promotes hyper-excitable central conditions, a causal relationship between these two factors has not yet been identified. Recent studies suggest that increases in interleukin 6 (IL-6) levels are specifically associated with stress. We hypothesized that IL-6 acutely and directly induces cortical hyper-excitability by altering the balance between synaptic excitation and inhibition.

Methods: We used patch-clamp to determine the effects of exogenous or endogenous IL-6 on electrically evoked postsynaptic currents on a cortical rat slice preparation. We used control subjects or animals systemically injected with lipopolysaccharide or subjected to electrical foot-shock as rat models of stress.

Results: In control animals, IL-6 did not affect excitatory postsynaptic currents but selectively and reversibly reduced the amplitude of inhibitory postsynaptic currents with a postsynaptic effect. The IL-6-induced inhibitory postsynaptic currents decrease was inhibited by drugs interfering with receptor trafficking and/or internalization, including wortmannin, Brefeldin A, 2-Br-hexadecanoic acid, or dynamin peptide inhibitor. In both animal models, stress-induced decrease in synaptic inhibition/excitation ratio was prevented by prior intraventricular injection of an analog of the endogenous IL-6 trans-signaling blocker gp130.

Conclusions: Our results suggest that stress-induced IL-6 shifts the balance between synaptic inhibition and excitation in favor of the latter, possibly by decreasing the density of functional γ-aminobutyric acid A receptors, accelerating their removal and/or decreasing their insertion rate from/to the plasma membrane. We speculate that this mechanism could contribute to stress-induced detrimental long-term increases in central excitability present in a variety of neurological and psychiatric conditions.

Key Words: 2-Br-hexadecanoic acid, postsynaptic, Brefeldin A, dynamin inhibitory peptide, foot-shock, γ-aminobutyric acid (GABA), interleukin-6 (IL-6), lipopolysaccharide (LPS), PI3K/AKT, patch-clamp, stress, gp130, rat, temporal cortex, trans-signaling, wortmannin

Temporary stress-induced increase in excitability is a symptom common to many psychiatric conditions, such as schizophrenic psychoses (1,2), posttraumatic stress disorder (3), anxiety (4), depression (1), and autistic spectrum disorders (ASD) (5–7), but also to neurological conditions, including epilepsy (8,9) and tinnitus (10). Such increase in excitability can lead to impaired behavior (abnormal startle response, irritability, aggression), abnormal perception (hyperacusis, hypersensitivity to touch, sensory-induced seizures), and/or altered emotion (paranoia, delusions, emotional arousal).

A plethora of factors (adenosine triphosphate depletion, infection, trauma, chronic fatigue, acute stress) potentially triggering hyper-excitable neurological or psychiatric conditions are also known to elevate the synthesis and release of cytokines (11–14). Pro-inflammatory cytokines—including interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α)—are known to affect the brain at the behavioral, morphological, and functional level, inducing for example, sickness behavior (15), neurogenesis (16), and synaptic plasticity (17).

Recent studies have indicated a specific role of IL-6 in stress-related pathophysiology. For example, messenger RNA levels of IL-6, synthesized in pyramidal neurons of the cortex (18) and of the hippocampus (19), are greatly increased by psychological stress (20), whereas abnormal increases in systemic levels of IL-6 follow the administration of a standardized social stress test in nonpsychiatric patients with a history of childhood maltreatment but not in control subjects (21). Furthermore, noninflammatory stressors selectively activate IL-6-producing vasopressin-positive neurons of the paraventricular and supraoptic nuclei of the hypothalamus, which in turn release systemic IL-6 (22).

Although neuronal membranes do not seem to display IL-6 receptors (23), they possess gp130 receptors—which upon stimulation by the complex formed by IL-6 and its soluble receptor "shed" by non-neuronal brain cells initiate the Janus kinase/signal transducer and activator of transcription/extracellular signal-regulated kinase/phosphatidyl inositol 3 kinase (PI_3K) signal transduction, a process referred to as "trans-signaling pathway" (24,25). Importantly, an abnormally high level of IL-6 has the potential to induce *status epilepticus*, probably by decreasing the expression of the β_{2-3} and γ_2 subunits of γ-aminobutyric acid-A receptors ($GABA_A$Rs) in several brain regions, including the temporal cortex (26). A temporary or long-term hyper-excitability of the temporal cortex is hy-

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pothesized to underlie the onset of tinnitus and hyperacusia, positively correlated with increased levels of IL-6 (27). An exquisite vulnerability of the temporal lobe to damage by diverse stressors might contribute to its relevance to schizophrenic psychoses, autism, and epilepsy (28).

The causal relationship linking the increase in IL-6 levels and central hyper-excitability is yet poorly understood. We considered the possibility that IL-6 increases neuronal excitability by a direct action at the synaptic level. To test this hypothesis we determined the effects of the exogenous application of IL-6 as well as the IL-6-dependence of the balance between inhibitory and excitatory synaptic transmission on two types of stress, on a rat temporal cortex preparation. We found that acute administration of IL-6, lipopolysaccharide (LPS) systemic injection, or foot-shock (FS) shifts the balance between excitation and inhibition in favor of the former. The pharmacological sensitivity of the IL-6 modulation of GABAergic responses is consistent with an increased, possibly ligand-dependent, internalization of $GABA_A$Rs from the neuronal membrane.

Materials and Methods

Preparation

We used a temporal cortex slice preparation similar to a previously described one (Supplement 1) (29). 6,7-Dinitroquinoxaline-2,3-dione (DNQX) (10 μmol/L), kynurenate (2 mmol/L) were used in a series of experiments for blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor- and *N*-methyl-D-aspartate receptor-mediated currents. Gabazine (20 μmol/L) was used to block $GABA_A$R-mediated currents.

Drugs and Solutions

Recombinant rat IL-6 (R&D Systems, Minneapolis, Minnesota) was activated with phosphate-buffered saline at a concentration of .5 μg/1 mL and was used at 10 ng/mL, in the range of central (up to 2 ng/mL) and systemic (up to 15 ng/mL) concentrations measured after immune challenge (30,31). The blocker of the IL-6 trans-pathway (32), soluble glycoprotein 130Fc (sgp130Fc), was produced in the laboratory of S.R.-J. at the Department of Biochemistry at the Christian Albrecht Universitat (Kiel, Germany). Lipopolysaccharide (serotype 0127:B8) was purchased from Sigma (St. Louis, Missouri). All other drugs were purchased from Sigma or Tocris (Ellisville, Missouri). After recording an initial baseline for 10–15 min, drugs were bath-applied for 10 min or longer, until reaching a stable condition (as defined in Statistical Analysis). For slice incubation with wortmannin (200 nmol/L), the drug was dissolved in ethanol (final dilution 1/2000). Brefeldin A (400 nmol/L), similar to 2-Br-hexadecanoic acid (20 μmol/L) was also dissolved in ethanol (final dilution 1/500). The dynamin inhibitory peptide (P4) (20 μmol/L) was dissolved as described previously (33).

Electrophysiology

Electrophysiological methods are discussed in detail in Supplement 1. Electrically evoked postsynaptic currents were measured by delivering one or two electric stimuli (90–180 μsec, 10–50 μA) 100 msec apart, every 10 sec, with an isolation unit, through a glass stimulation monopolar electrode filled with artificial cerebrospinal fluid at approximately 100–200 μm from the recorded neuron. Synaptic responses were monitored at different stimulation intensities before baseline recording. Detection threshold was set at approximately 150% of the SD of the noise (typical noise approximately 4–5 pA, threshold approximately 7–8 pA). A −2 mV 100-msec-long voltage pulse was applied at the beginning of every episode to monitor the quality of the recording. Access resistance (10–20 MΩ) was monitored throughout the experiment. Recordings displaying >20% change in input or access resistance were discarded from the analysis. All signals were filtered at 2 kHz and sampled at 10 kHz. Reversal potential for postsynaptic currents were evaluated, determining current-voltage (I-V) relationships for the evoked postsynaptic current (peak amplitude of 10 events at each holding potential V_h in the range from $V_h = -90$ mV up to $V_h = +60$ mV). Evoked inhibitory postsynaptic currents (IPSCs) reversed polarity close to the theoretical reversal potential of −65 mV (−64 ± 2 mV, $n = 3$), whereas evoked excitatory postsynaptic currents (EPSCs) reversed at $V_{exc} = 10.5 \pm 3$ mV ($n = 3$). All experiments were performed at room temperature (22°C).

Surgery

Rats were anesthetized with isoflurane, and body temperature and respiration were maintained at physiological levels. The surgical site was shaved and sterilized, and subjects were positioned in a stereotaxic (David Kopf Instruments, Tujunga, California) instrument so that the frontal and parietal bones of the skull were parallel to the surgical platform. Rats received stereotaxic implantation with bilateral guide cannulae in the lateral ventricles under aseptic conditions, as follows. Skull screw anchors were fixed to the skull, and cannulae (23-gauge stainless steel) were implanted (coordinates: from bregma P-1.0 mm, L-1.3 mm; from cortical surface −3.5 mm) and secured in place with dental acrylic. A dummy cannula was inserted in the guide cannulae to prevent occlusion and infection. Rats received intraperitoneal (IP) injection with aspirin (150.0 mg/kg) after surgery and then again 7–8 hours later to aid with postoperative discomfort and were allowed 72 hours to recover. Accurate placement of the cannulae was confirmed by allowing 2 μL of sterile saline to flow via gravity into the lateral ventricles. If cannulae placement could not be confirmed, the animal was excluded from the study. All procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Animal Studies

LPS Injections. On test day, rats were injected (CMA/100 microinjector, 10 μL Hamilton syringe) intracerebro-ventricularly (ICV) with sterile saline containing .1% bovine serum albumin (BSA) (vehicle) or 100 ng sgp130Fc dissolved in 2 μL vehicle. One hour later, rats received IP injection, with sterile saline (.3 mL) or a dose of LPS (.33 mg/kg body weight) eliciting a pro-inflammatory cytokine response in the brain (34) and were decapitated by a guillotine 8 hours after treatment for obtaining brain slices (29).

Footshock. Rats were placed in an FS chamber (30 cm × 10 cm). A 1.6-mA FS lasting 5 sec was administered every 4 min for a total session duration of 64 min. Immediately after treatment, rats were sacrificed, and brains slices were obtained. One hour before being subjected to the FS procedure, animals received injection with the microinjector through the cannulae with either .1% BSA (vehicle) or 100 ng sgp130Fc dissolved in 2 μL vehicle.

Statistical Analysis

Statistical methods are described in the online Analysis section in Supplement 1. Data comparisons were reported as different only if $p < .05$. Single, double, and triple asterisks (*, **, and ***) indicate $p < .05$, $p < .02$, or $p < .01$, respectively, unless otherwise indicated.

Results

IL-6 Selectively Decreases GABAergic Postsynaptic Currents

IL-6 application (10 ng/mL or 45 nmol/L) did not change the mean evoked excitatory postsynaptic currents (eEPSC) amplitude (time course in Figure 1A) (Figure 1B: mean eEPSC amplitude in control or after IL-6). Neither paired pulse ratio (PPR) (Figure 1C) nor