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Neutralization of endogenous digitalis-like compounds alters catecholamines metabolism in the brain and elicits anti-depressive behavior

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Abstract

Depressive disorders are among the world's greatest public health problems. Na^+, K^+-ATPase is the established receptor for the steroidal digitalis-like compounds (DLC). Alteration in brain Na^+, K^+-ATPase and DLC have been detected in depressive disorders raising the hypothesis of their involvement in these pathology. The present study was designed to further elaborate this hypothesis by investigating the behavioral and biochemical consequences of neutralization in brain DLC activity attained by anti-ouabain antibodies administrations, in normal Sprague–Dawley (SD) and in the Flinders Sensitive Line (FSL) of genetically depressed rats. Chronic i.c.v. administration of anti-ouabain antibodies to FSL rats elicited anti-depressive behavior. Administration of anti-ouabain antibodies intracerebroventricularly (i.c.v.) to SD rats significantly changed the levels of catecholamines and their metabolites in the hippocampus, ventral tegmentum and nucleus accumbence. These results are in accordance with the notion that endogenous DLC may be involved in the manifestation of depressive disorders and suggests that alteration in their levels may be of significant therapeutic value.

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1. Introduction

Depressive disorders, including major depression, dysthymia and bipolar disorder (BD) are a group of diseases characterized by a dejected mood and a despondent lack of activity that require medical treatment (Kessler, 2003). The treatment of depressive disorders is limited owing to the lack of appropriate drugs and the side effects of the existing ones. Thus the need for new therapeutics for the treatment of the various depressive disorders is evident.

The sodium and potassium-activated adenosine triphosphatase (Na^+, K^+-ATPase) is a key enzyme present in the plasma membrane of all mammalian cells (for review see, Blanco and Mercer, 1998; Scheiner-Bobis, 2002). Since Na^+, K^+-ATPase was discovered, it has been established that plant steroids, collectively termed digitalis or cardiac glycosides, bind to a specific site on the enzyme and that this binding results in the inhibition of ATP hydrolysis and ion transport (Kelly and Smith, 1996). In the past decades digitalis and digitalis-like compounds (DLC) have been identified in human tissue. These steroids are synthesized in and released from the adrenal gland (Hamlyn et al., 2003; Laredo et al., 1994; Lichtstein et al., 1998) and are considered a novel family of hormones involved in the regulation of salt homeostasis and blood pressure (for review see, Blaustein et al., 2006; Nesher et al., 2007). Previous studies suggested that brain Na^+, K^+-ATPase/DLC system activity is involved in the etiology of depressive disorders (Coppen et al., 1966; El-Mallakh and Wyatt, 1995; Looney and el-Mallakh, 1997; Nurnberger et al., 1982; Shaw, 1966). We have previously shown that DLC levels in the brain of BD patients were significantly higher than in normal individuals (Goldstein et al., 2006) and that the neutralization of rat brain DLC by acute intracerebroventricular (i.c.v.) injection of anti-ouabain antibodies, elicits an anti-depressive effect (Goldstein et al., 2006). We have also recently demonstrated a genetic polymorphism in Na^+, K^+-ATPase α isoforms associated with BD (Goldstein et al., 2009).

All available antidepressant medications are based on the monoamine theory (Schildkraut et al., 1965). In the cerebrospinal fluid (CSF), dopamine (DA) levels were reported to be higher in depressed patients than in healthy individuals (Gjerris et al., 1987). Noradrenalin (NE) levels in the CSF of depressed patients were not significantly different from those in healthy volunteers (Gjerris et al., 1987; Roy et al., 1986), although plasma NE levels have been reported to be higher in depressed patients (Rothschild et al., 1987). In the serotonergic system, the levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) were reported to be lower in the CSF of patients with depression (Gjerris et al., 1987). Although the hippocampus and frontal cortex are undoubtedly involved in depression and in its treatment, it is unlikely that these regions account for all the symptoms of the disorder. In recent years, the nucleus accumbens (NA) and its dopaminergic inputs from the ventral tegmental area (VTA) of the midbrain, identified as one of the most important anatomical substrates for natural rewards such as food, sex and social interaction, were found to be impaired in depression (Nestler and Carlezon, 2006). An additional brain region, the locus coeruleus (LC), associated with depression, contains a large group of NE neurons innervating almost the entire brain (Foote et al., 1983). More recent evidence showed that the LC displays some of the highest antidepressant binding site densities in the brain (Richards et al., 1992). Most antidepressants improve monoaminergic neurotransmission directly or indirectly, but their pharmacological action is not immediate and the therapeutic mechanism is not clear (Berton et al., 2006).

In the present study, the hypothesis that brain endogenous DLC are involved in depression was further tested by studying the effect of a neutralization in brain DLC on behavior in chronic experiments in Flinders Sensitive Line (FSL) rats, a genetic model of depression (Overstreet et al., 2005). The neutralization in DLC was achieved by continuous i.c.v. administration of anti-ouabain antibodies into the lateral ventricle. Additionally, since the chatecholaminergic system is implicated in depressive disorders, the consequence of this perturbation on catecholamines and their metabolite levels in brain regions thought to be associated with depression was measured.

2. Experimental procedures

2.1. Animals

Male FSL (250–300 g) and SD rats (100–200 g) were maintained under conditions of constant temperature (25 °C) and humidity (50%), in a 12:12 h light/dark cycle and with free access to food and water. All animal procedures were approved by The Hebrew University-Hadassah Medical School and the Bar-Ilan University and Animal Care Committees and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Partial purification and concentration of rabbit anti-ouabain antibodies

Anti-ouabain antibodies were prepared in rabbits by injection of ouabain-BSA conjugate as described previously, with minor modifications (Goldstein et al., 2006; Lichtstein et al., 1998). Protein A Sepharose CL-4B beads (Pharmacia Biotech, Uppsala, Sweden) and columns were prepared according to the manufacturer's instructions. A total 2.5 ml of rabbit plasma containing ouabain antibodies was loaded onto each column. Unbound immunoglobulin was eluted with phosphate-buffere-saline (PBS). Bound immunoglobulin was then eluted with glycine buffer (0.2 M glycine, 0.2 M NaCl, pH 3.0) into tubes containing 50 μl of 1 M Na_2HPO_4. The presence and quantification of ouabain antibodies in the eluant was determined by ELISA, as described below. The partially purified antibody was concentrated by centrifugation (5000 *g*, 15 min) using Centricon (Millipore/Amicon, MA, USA) centrifugal filters (3000 NMWL membrane) at 10 mg IgG per ml. The concentrated anti-ouabain antibodies and control rabbit IgG (Sigma Aldrich, Israel) were dialyzed overnight against saline in a cellulose tubular dialysis membrane (6000–50,000 cutoff, nominal pore size ~0.002 μm, Spectrapor Medical Inc., Los Angeles, CA, USA), which was pre-incubated in boiling water containing 1 mM EDTA, 2% Na_2CO_3 for 20 min.

The antibodies used in this study are highly specific for ouabain and cross-react only with ouabagenin (53%), strophantidine (16.5), digoxin (0.76%) and bufalin (0.6%). Other steroids, including cholesterol, testosterone, progesterone, corticosterone, 17-hydroxy pregnenolone and 21-deoxycortisol do not cross-react with the antibodies, even at 10 μM (Lichtstein et al., 1998).

2.3. Absorption of endogenous ouabain from CSF by anti-ouabain antibodies

The ability of anti-ouabain antibodies to absorb endogenous ouabain was determined using pooled human CSF. The fluid was obtained from five patients suffering from various neurological diseases hospitalized at Hadassah Medical Center, Jerusalem. Samples of