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Research report

Adenosine signaling in reserpine-induced depression in rats

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HIGHLIGHTS

- Reserpine produces symptoms of major depression in humans and rats.
- This pathology usually is attributed to the depletion of brain monoamines, although empirical support remains equivocal.
- We examined the potential contribution of brain adenosine signaling as an alternative in five experiments in rats.
- Reserpine produced a profound increase in floating time in a forced swim test.
- Swim deficits were reversed by the nonselective adenosine receptor antagonist caffeine, a selective A_2 antagonist, and a highly selective A_{2A} antagonist.

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ABSTRACT

A single, 6 mg/kg intraperitoneal injection of reserpine increased floating time during forced swim testing 24 h after administration in rats in five experiments. Although such behavioral depression traditionally is attributed to drug-induced depletion of brain monoamines, we examined the potential contribution of adenosine signaling, which is plausibly activated by reserpine treatment and contributes to behavioral depression in other paradigms. Whereas peripheral administration of the highly selective A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (0.5, 1.0, or 5.0 mg/kg i.p.) 15 min before swim testing failed to improve performance in reserpine-treated rats, swim deficits were completely reversed by 7 mg/kg of the nonselective receptor antagonist caffeine. Performance deficits were also reversed by the nonselective A_2 antagonist 3,7-dimethylxanthine (0, 0.5, 1.0 mg/kg i.p.), and the highly selective A_{2A} receptor antagonist (CSC: 8-(3chlorostyral)caffeine) (0.01, 0.1, or 1.0 mg/kg i.p.) in a dose-dependent manner. The highly selective A_{2B} antagonist alloxazine had no beneficial effect on swim performance at any dose under study (0.1, 1.0, and 5.0 mg/kg i.p.).

1. Introduction

Reserpine is an alkaloid extract from the root of a climbing shrub (*Rauwolfia serpentina*) that is indigenous to India. The compound served as a traditional tranquilizing agent in the Orient, before being introduced in the United States in the early 1950s as a treatment for hypertension [12,13,33,69,70]. The extract reduces both cardiac output and peripheral vascular resistance by depleting stores of biogenic amines in the central and autonomic nervous systems. Specifically, reserpine binds irreversibly to the vesicular monoamine transporter types 1 and 2 to interfere with vesicular storage of monoamines [57,74]. Transmitter concentrations increase in the cytoplasm as a consequence, where molecules are metabolized by intraneuronal monoamine oxidase or diffuse into the synaptic cleft. The end result is that little or no active transmitter is released at the synapse at the time of depolarization [13,65].

The historic importance of reserpine is more related to its unwanted side effects than to its efficacy as an antihypertensive or tranquilizing agent. Unfortunately, a significant portion of the population undergoing reserpine treatment for hypertension developed symptoms of major depression [66]. These symptoms were severe enough to require antidepressant treatment and, at times, hospitalization. This observation, along with the findings a few years later that monoamine oxidase inhibitors and tricyclic antidepressants enhance brain biogenic amines, served as the empirical cornerstone of catecholamine [72,73], and later, monoamine theories of depression [1,8].

Despite the promise of the original catecholamine theory, the empirical evidence linking the biogenic amines and major depression is less than convincing [16,63]. As an alternative, we

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focus on the potential contribution of a brain-signaling pathway involving purine nucleoside adenosine in this paper. Adenosine signaling is implicated in a number of animal models of depression, including learned helplessness [53], behavioral despair [18,22,23], cytokine-induced depression [2,39,40,51,64], and exertion of effort [49,58,59,61,71]. This regulatory mechanism links neural excitability to energy state and is actively engaged by challenges to metabolic homeostasis [30,48,55,62,87,92,95,97]. Adenosine is extruded into extracellular space or hydrolyzed from extracellular nucleotides whenever the rate of adenosine triphosphate (ATP) hydrolysis exceeds the synthesis rate [48,55,99]. Such an imbalance of the energy supply–demand ratio can result from excessive neural activation or from a shortage in brain glucose or oxygen. The extracellular nucleoside binds to specific adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3), which are widely distributed on pre- and post-synaptic membranes and in the brain micro-vascular bed [14,44,60,67]. Adenosine interacts with a number of cellular effector systems via these receptors to modulate membrane excitability and transmitter release, thereby changing metabolic demand in the target neuron [5,6,56].

Adenosine A_1 and A_{2A} receptors also occur in heteromeric complexes that have an important regulatory influence on dopamine signaling in the striatum [63]. Activation of the adenosine component of these complexes increases uncertainty about appropriate courses of action [100], shifts choices to less effortful alternatives [49], and uncouples motivation from ongoing behavior [63]. This effect of adenosine in the striatum has been variously characterized as anergia [49,61], fatigue [49,58,61,63], or conservation-withdrawal [50,53], all of which are central to major depression.

An increase in adenosine signaling is a plausible outcome of reserpine treatment, particularly the type that leads to symptoms of major depression. This outcome is reliably produced by a large dose of reserpine that rapidly depletes brain monoamines. Indeed, it is the rapid depletion of monoamines, rather than their absolute concentration, that seems to be the critical factor [3,65,66]. The unregulated release of transmitter under these conditions could enhance adenosine regulation as a compensatory measure [48,55,87], leading to the depression-like performance deficits that occur in other animal models.

The present experiments assessed the ability of nonselective (A_1/A_2), or highly selective A_1, A_2, A_{2A}, or A_{2B} adenosine receptor antagonists to reverse evidence of reserpine-induced depression in rats. If enhanced adenosine signaling contributes importantly to reserpine-induced depression, then pharmacological blockade of adenosine receptors should substantially improve performance. We indexed behavioral depression using performance in a forced swim task 24 h after an injection of a large, 6 mg/kg dose of reserpine in all of these experiments. Adenosine receptor antagonists were administered shortly before testing.

2. General method

2.1. Subjects

Male Sprague–Dawley rats (280–356 g) from a breeding colony in the Psychology Department at the University of California Los Angeles were housed in individual cages with free access to food and water in a vivarium room maintained on 12:12 h light–dark cycle for 1 week prior to experimentation. Experiments occurred in the light portion of the cycle. All rats were handled daily. All procedures described in this article were pre-approved by the UCLA IACUC.

2.2. Swim test

Swim testing occurred in a water tank consisting of a Plexiglas cylinder 65 cm in height and 30 cm in diameter, which was filled to a height of 30 cm with water at approximately 23 °C. Three overhead 25-watt red fluorescent lights provided constant illumination during swim testing. We equipped each rat with a floatation device consisting of a curved piece of Styrofoam, which we attached to a rat's back via a Velcro harness. We monitored behavior in the swim tank for 15 min using a Vicon (model VC2150) low-illumination camera and later scored videotapes for the duration of two types of activity: (1) *struggling*, which is defined as vigorous movement of all paws with the forepaws breaking the surface of the water; and (2) *floating*, which is defined as the animal remaining motionless with no limb movement.

2.3. Drug administration

Reserpine (methyl reserpate 3,4,5-trimethoxybenzoic acid ester; Sigma Chemical Co) was dissolved in 10% dimethyl sulfoxide (DMSO; Fisher Scientific) in physiological saline at a concentration of 6 mg/ml and administered at a dose of 6 mg/kg. This dose was selected based on pilot studies demonstrating depressive effects.

Adenosine receptor antagonists caffeine (nonselective A_1/A_2 antagonist; Sigma), 8-cyclopentyl-1,3-Dipropylxanthine (CPDPX: a highly selective A_1 receptor antagonist; Sigma), and 3,7-dimethyl-1-propargylxanthine (DMPX: highly selective A_2 antagonist; Sigma) were dissolved in 10% ethyl alcohol, and then placed into a uniform suspension with 40% propylene glycol and 50% saline. The adenosine receptor antagonists 8-(3chlorostyryl)caffeine (CSC: a highly selective A_{2A} receptor antagonist; Sigma) and alloxazine (AX: a highly selective A_{2B} antagonist; Sigma) were dissolved in 10% DMSO/90% saline. All drugs were administered via intraperitoneal (i.p.) injection at a volume of 1 ml/kg body weight.

2.4. Procedure

The same general design and procedure were used in all of the following experiments. Rats were injected with 6 mg/kg reserpine or vehicle and then assessed for swim performance 24 h later. Adenosine receptor antagonists or vehicles were administered 15 min prior to swim testing in an attempt to reverse behavioral impairment.

2.5. Experiment 1: effect of the nonselective antagonist caffeine

This experiment assessed the efficacy of the high-affinity, nonselective (A_1/A_2) adenosine receptor antagonist caffeine at reversing swim deficits 24 h after reserpine treatment. The caffeine doses used in this experiment were based on earlier research on the dose-dependent reversal of behavioral depression in the learned helplessness paradigm [43].

We randomly assigned rats to one of five groups of eight rats each. Two groups received an i.p. injection of DMSO vehicle (DMSO), and three groups (Res) received an i.p. injection of 6 mg/kg of reserpine. Twenty-four hours later, one of the DMSO-treated groups received an i.p. injection of caffeine vehicle (cVeh: 10% alcohol, 40% propylene glycol, and 50% water). The other DMSO-treated group (DMSO + Caf[7.0]) received a 7 mg/kg i.p. injection of caffeine to assess any untoward effects of the drug during later swim testing. The three reserpine-treated groups received an i.p. injection of 0, 3, or 7 mg/kg of caffeine (Groups Res + 0.0, Res + 3.0, and Res + 7.0, respectively). We tested all rats in the swim apparatus 15 min later.