Archival Report

Biological Psychiatry

The Novel Metabotropic Glutamate Receptor 2 Positive Allosteric Modulator, AZD8529, Decreases Nicotine Self-Administration and Relapse in Squirrel Monkeys

Zuzana Justinova, Leigh V. Panlilio, Maria E. Secci, Godfrey H. Redhi, Charles W. Schindler, Alan J. Cross, Ladislav Mrzljak, Amy Medd, Yavin Shaham, and Steven R. Goldberg

ABSTRACT

BACKGROUND: Based on rodent studies, group II metabotropic glutamate receptors (mGluR2 and mGluR3) were suggested as targets for addiction treatment. However, LY379268 and other group II agonists do not discriminate between the mainly presynaptic inhibitory mGluR2 (the proposed treatment target) and mGluR3. These agonists also produce tolerance over repeated administration and are no longer considered for addiction treatment. Here, we determined the effects of AZD8529, a selective positive allosteric modulator of mGluR2, on abuse-related effects of nicotine in squirrel monkeys and rats.

METHODS: We first assessed modulation of mGluR2 function by AZD8529 using functional in vitro assays in membranes prepared from a cell line expressing human mGluR2 and in primate brain slices. We then determined AZD8529 (.03–10 mg/kg, intramuscular injection) effects on intravenous nicotine self-administration and reinstatement of nicotine seeking induced by nicotine priming or nicotine-associated cues. We also determined AZD8529 effects on food self-administration in monkeys and nicotine-induced dopamine release in accumbens shell in rats.

RESULTS: AZD8529 potentiated agonist-induced activation of mGluR2 in the membrane-binding assay and in primate cortex, hippocampus, and striatum. In monkeys, AZD8529 decreased nicotine self-administration at doses (.3–3 mg/kg) that did not affect food self-administration. AZD8529 also reduced nicotine priming- and cue-induced reinstatement of nicotine seeking after extinction of the drug-reinforced responding. In rats, AZD8529 decreased nicotine-induced accumbens dopamine release.

CONCLUSIONS: These results provide evidence for efficacy of positive allosteric modulators of mGluR2 in nonhuman primate models of nicotine reinforcement and relapse. This drug class should be considered for nicotine addiction treatment.

Keywords: Allosteric modulation, Glutamate, Nicotine, Relapse, Self-administration, Smoking cessation

http://dx.doi.org/10.1016/j.biopsych.2015.01.014

Tobacco smoking, the leading cause of preventable death, is primarily driven by nicotine (1,2). Several medications currently are available for smoking cessation (varenicline, bupropion, nicotine replacement), but high relapse rates are observed during and after treatment (3–5). Novel treatments to prevent relapse are needed. In rodents, manipulations of metabotropic glutamate receptors (mGluRs) decrease nicotine-induced potentiation of brain stimulation reward and nicotine withdrawal symptoms (6). Based on these and related findings, group II mGluRs have been considered potential targets for nicotine addiction treatment (7). Group II mGluRs consist of mGluR2 and mGluR3 subtypes (8). The mGluR2s are expressed primarily on presynaptic glutamate neurons, and their activation leads to decreased evoked glutamate release (8,9). The mGluR3s are expressed on postsynaptic and presynaptic neurons and on glia (8,10). The prototype drug used to assess the function of group II mGluRs is LY379268, an orthosteric agonist that binds to both mGluR2s and mGluR3s (8,11).

In rats, systemic injections of LY379268 or related mGluR2/mGluR3 agonists decrease discriminative cue-induced reinstatement of cocaine seeking and context-induced reinstatement of heroin seeking (12,13), discrete cue-induced reinstatement of heroin seeking (14), cocaine priming-induced reinstatement (15), spontaneous recovery of alcohol seeking (16), cue-induced cocaine seeking (17), and discrete cue-induced and drug priming-induced reinstatement of methamphetamine seeking (18). LY379268 injections also decrease cocaine priming-induced reinstatement in squirrel monkeys (19). Finally, LY379268 injections decrease nicotine self-administration and discrete cue-induced reinstatement of nicotine seeking (20).

SEE COMMENTARY ON PAGE 436

However, from a medication development perspective, there are limitations with LY379268 and related orthosteric agonists. LY379268 has low bioavailability (21), and tolerance develops to its effects (20). Additionally, LY379268 activates the mGluR3 subtype whose physiologic functions are unknown (8). These limitations led to development of selective positive allosteric modulators (PAMs) of mGluR2 (22–24). The PAMs of mGluR2 bind to an allosteric site of the receptor and facilitate agonist-mediated receptor activity (25,26).

Jin *et al.* (27) reported that a selective PAM of mGluR2, BINA, decreases cocaine self-administration and cue-induced reinstatement. They also reported that a BINA analogue with superior pharmacokinetic properties and brain penetration decreases nicotine self-administration in rats (21). Based on these studies, we used our squirrel monkey model (28,29) to determine the effects of AZD8529, a selective PAM for mGluR2 (30), on nicotine self-administration and relapse to nicotine seeking, as assessed in the reinstatement procedure (31). We also provide results from in vivo and in vitro assays on the selectivity of AZD8529 to mGluR2 and results on the drug's effect on nicotine-induced dopamine release in nucleus accumbens shell.

METHODS AND MATERIALS

Subjects

For the autoradiography experiment, we used three male 5- to 6-year-old cynomolgus monkeys (*Macaca fasciculari*; Covance, Inc, Denver, Pennsylvania). The AstraZeneca (Wilmington, Delaware) animal care and use committee approved the experiment, and procedures were performed in accordance with the AstraZeneca Global Research and Development animal care standards.

For the behavioral experiment, we used 9- to 13-year-old male squirrel monkeys (*Saimiri sciurea*), weighing 750–1050 g. The monkeys had been trained to self-administer nicotine or food before the study and had no self-administration history with other drugs. We implanted intravenous catheters as previously described (32). The monkeys wore nylon-mesh jackets to protect these catheters. Each weekday, we flushed the catheters, refilled them with saline, and sealed them with obturators. For microdialysis, we used male Sprague Dawley rats (300–350 g; Charles River Laboratories, Inc, Wilmington, Massachusetts). Squirrel monkeys were housed individually, and rats were group-housed at the National Institute on Drug Abuse Intramural Research Program facility (regular 12-hour light/dark cycle). The National Institute on Drug Abuse Intramural Research Program animal care and use committee approved the experiments, which were carried out in accordance with the 2003 National Research Council Guidelines.

Functional mGluR2 Assays

Receptor Selectivity Assay. To determine the selectivity of AZD8529 within the mGluR family, we used fluorescence-based assays (33,34) and HEK 293 cell lines expressing human mGluR constructs. The cell lines expressed chimeric fusion constructs hmGluR2/hCaR*, hmGluR1/hCaR*, hmGluR3/hCaR*, hmGluR4/hCaR*, hmGluR5/hCaR*, hmGluR6/hCaR*, hmGluR7/hCaR*, and hmGluR8/hCaR*, each including the extracellular domain and transmembrane domain of human mGluR and the intracellular domain of the human calcium receptor fused to the promiscuous chimeric protein Gqi5 as described previously (35).

Receptor Screening. We evaluated AZD8529 at 10 μmol/L for off-target effects using radioligand binding assays (MDS Pharma Services, Bothell, Washington) based on published methods. We ran reference standards for each assay. We determined inhibitory concentration of 50% (IC_{50}) values using nonlinear, least squares regression analysis of the Data Analysis Toolbox (MDL Information Systems, formerly of San Leandro, California).

[^{35}S]GTPγS Binding Human mGlu2-CHO Membranes. We used membranes prepared from a Chinese hamster ovary (CHO) cell line expressing the human mGluR2 and performed the assay in a scintillation proximity assay format. We grew CHO cells expressing the human mGluR2 to ~80% confluence, washed the cells in ice-cold phosphate-buffered saline, and stored them frozen until membrane preparation. Assay buffer contained .05 mol/L *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, .10 mol/L sodium chloride, and .01 mol/L magnesium chloride, pH 7.4 plus 100 μmol/L dithiothreitol and 3 μmol/L guanosine diphosphate (GDP). We started the assay by adding a mixture of wheat germ agglutinin scintillation proximity assay beads (.75 mg/mL) and membranes (6 μg/mL) in assay buffer containing AZD8529 or vehicle. After 15-min incubation, we added a solution containing the [^{35}S]GTPγS and L-glutamate (final concentrations 100 pmol/L [^{35}S]GTPγS and 0–100 μmol/L glutamate). After incubation at room temperature (60 min), we centrifuged the assay plates and read them on the TopCount scintillation counter (Perkin Elmer, Waltham, Massachusetts). We determined [^{35}S]GTPγS binding in the absence of glutamate and in the presence of 100-μmol/L glutamate as 0% and 100% levels, respectively. We estimated the modulator activity of AZD8529 on mGluR2 activation from the concentration response curves of AZD8529 fitted with a four-parameter logistic equation to calculate the apparent potency (EC_{50}) and maximal efficacy (E_{max}).

[^{35}S]GTPγS Autoradiography in Cynomolgus Monkey Brain Slices. We anesthetized the monkey with sodium pentobarbital (100 mg/kg), perfused it with saline, and then removed the brain and froze it in cooled isopentane. We cut 20-μm striatum and hippocampus sections on a cryostat, mounted the sections on glass slides, and stored them at −80°C until use. We warmed the sections to room temperature in a vacuum chamber over 3 hours on the day of the experiment. We incubated the sections in 50 mmol/L Tris HCl, 3 mmol/L magnesium chloride, .2 mmol/L ethylene glycol tetraacetic acid, 100 mmol/L sodium chloride, and 0.2 mmol/L dithiothreitol (Tris assay buffer [TAB]), pH 7.4 at 25°C for 10 min. We then incubated the slides in TAB containing 2 mmol/L GDP for 15 min at 25°C. We placed the slides in one of the following four conditions for 2 hours at 25°C: basal, TAB + 2 mmol/L GDP + .04 nmol/L [^{35}S]GTPγS; agonist alone, TAB + 2 mmol/L GDP + .04 nmol/L [^{35}S]