Pharmacology, Biochemistry and Behavior 124 (2014) 48–57

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of the δ opioid agonist AZD2327 upon operant behaviors and assessment of its potential for abuse

T.J. Hudzik [a,*], M.R. Pietras [b], R. Caccese [b], K.H. Bui [c], F. Yocca [a], C.A. Paronis [e], M.D.B.Swedberg [d]

[a] Departments of Neuroscience, AstraZeneca R & D Wilmington, United States
[b] Safety Assessment, AstraZeneca R & D Wilmington, United States
[c] DMPK, AstraZeneca R & D Wilmington, United States
[d] AstraZeneca R & D Sodertalje, Sweden
[e] McLean Hospital, Harvard University Medical School, Cambridge, MA, United States

ARTICLE INFO

Article history:
Received 19 March 2014
Received in revised form 7 May 2014
Accepted 14 May 2014
Available online 22 May 2014

Keywords:
AZD2327
Abuse potential
Operant behavior

ABSTRACT

AZD2327 is a brain-penetrant agonist at δ opioid receptors which has antidepressant and anxiolytic properties in a wide array of animal models. As part of the preclinical safety pharmacology assessment, a number of studies were conducted in order to characterize its behavioral effects and its potential for abuse, in order to enable testing in humans. AZD2327 produced only modest effects when tested in a multiple fixed-ratio differential reinforcement of low rate schedule in rats, and did not enhance the rate-suppressing effects of ethanol in the procedure. In a suppressed responding test, AZD2327 only reduced rates of unpunished responding. In drug discrimination studies, AZD2327 produced partial or no generalization from known drugs of abuse. In primates trained to self-administer cocaine, substitution with AZD2327 did not result in appreciable self-administration of AZD2327, indicating that it does not behave as a positive reinforcer under the present conditions. Following termination of repeated administration of AZD2327, no signs of physical dependence (withdrawal) were noted. Overall, the data suggest that AZD2327 does not possess a high potential for abuse, and appears to have only subtle behavioral effects as measured by operant behaviors.

1. Introduction

δ opioid receptors were the first cloned among the 3 opioid receptor subtypes (Kieffer et al., 1992), but has been less well-characterized than the mu opioid receptor, which mediates the analgesia, sedation and abuse liability associated with morphine-like drugs. It has also been poorly characterized relative to the kappa opioid receptor, which mediates visceral analgesia and clearly plays a role in affective regulation as agonists are highly dysphorigenic and antagonists appear to possess properties consistent with antidepressant activity (Lutz and Kieffer, 2013). Study of selective δ-opioid agonists, such as SNC-80 (4-[(*R*)-[(2*S*,5*R*)-4-allyl-2,5-dimethylpiperazin-1-yl](3-methoxyphenyl)methyl]-*N*,*N*-diethylbenzamide) or BW-373U86 (4-[(R)-[(2S,5R)-2,5-dimethyl-4-prop-2-enylpiperazin-1-yl]-(3-hydroxyphenyl)methyl]-N,N-diethylbenzamide) has begun to elucidate the role that the receptor plays in the central nervous system as well as in the periphery. Distribution of the receptor in peripheral ganglia suggests a role in sensory and nociceptive signaling (Pradhan et al., 2010), which is further reinforced by demonstration of its presence throughout the central nervous system pathways known to regulate pain perception (Pradhan and Clarke, 2005). A host of functional and pharmacological studies have confirmed the role of the receptor in analgesia (Pradhan and Clark, 2005; Gaveriaux-Ruff and Kieffer, 2011; Saitoh et al., 2011). In addition to nociceptive regulation, agonists of the δ opioid receptor have been shown to present potentially novel approaches for a number of therapeutic indications, including neuroprotection (Zhang et al., 2002) cardioprotection (Patel et al., 2002), Parkinson's disease (Hudzik et al., 2000; Hille et al., 2001) and psychiatric indications, including anxiety and depression (Hudzik et al., 2011; Jutkiewicz et al., 2006; Filliol et al., 2000).

AZD2327 is a high affinity, high selectivity agonist of δ opioid receptor (Hudzik et al., 2011). It has been shown to be active in a wide variety of animal models predictive of activity in anxiety and depression from such standard models such as the conflict test and learned helplessness (Hudzik et al., 2011) to some novel work in a prenatal stress model utilizing an elevated plus maze (Hudzik et al. unpublished observations). Given a potential therapeutic activity in both anxiety and in depression, specialized utility in anxious depression or in post-traumatic stress disorder is possible.

While the effects of the drug in animal models predictive of activity in psychiatric illness have already been documented (Hudzik et al., 2011) a broader examination of the pharmacology was conducted in

* Corresponding author at: Department of Preclinical Safety, AbbVie, 1 N Waukegan Rd, North Chicago, IL 60064, United States. Tel.: +1 847 937 3626; fax: +1 847 938 3076.
E-mail address: Thomas.Hudzik@Abbvie.com (T.J. Hudzik).

http://dx.doi.org/10.1016/j.pbb.2014.05.009
0091-3057/

order to support the clinical work that was conducted with AZD2327. The pharmacological characterization was conducted in the rat by using a multiple Fixed-Ratio 10 Differential Reinforcement of Low Rates 15-s schedule of food presentation (Mult FR-10 DRL-15"). The schedule was chosen to characterize behavioral effects because it is highly sensitive to pharmacologic manipulation, due to a range of baseline rates of responding engendered (high numbers of responses per min in the FR, and low responses per min in the DRL (Branch, 1984)). Further characterization of the behavioral pharmacology of AZD2327 in species other than rodent was also conducted in order to assess species generality of its effects. While the homology of the receptor is extremely high among species, the degree to which it may modulate circuitry may differ, and to this end, AZD2327 was tested in a primate conflict test. Finally, as part of the safety package of studies, alcohol interaction, and full abuse potential assessment was conducted.

2. Methods

Overview of studies

2.1. Schedule-controlled behavior and interaction with ethanol: Mult FR10-DRL15"

2.1.1. Subjects

Ten male Long–Evans rats weighing 325–350 g at the start of training were maintained at 90% of free-feeding weight and individually housed under a standard 12-h light/dark cycle.

2.1.2. Apparatus

During the experimental sessions, the animals were placed into standard 2-lever Med Associates operant chambers, with only 1 of the 2 levers extended into the chamber during each of the 2 components of the session, with a different lever corresponding to each component.

2.1.3. Procedure

The method follows that of Hudzik and Slifer (1992), with the exception that cumulative dosing was not employed in the present study. In the fixed ratio component, each 10th lever press resulted in the delivery of a single 45-mg food pellet (Noyes, Inc). In the DRL component, each response that was preceded by a minimum of 15 s of no responding resulted in food pellet delivery. Responses preceded by shorter intervals than 15 s or longer than 1 min (limited hold of 45 s) resulted in no food pellet delivery, and a 5-s time-out period in which house lights were extinguished and the lever was retracted. The lever (left or right) that was assigned to each associated component was randomized among the animals. Each FR component lasted for 2 min and each DRL component for 5 min, and sessions consisted of 6 cycles of FR and DRL components. Sessions always began with a 5 min acclimation period, followed by a fixed ratio component. Once responding stabilized under the schedule (defined as no greater than 15% variation in rates of responding in each of the 2 components for 5 consecutive days) drug testing began.

2.1.4. Drugs

Animals were dosed by gavage in a volume of 2 mL/kg body weight. Ethanol was diluted with sterile water, and AZD2327 was dissolved in sterile water to which was added a few drops of 85% lactic acid (pH = 3–4) to promote dissolution. Both AZD2327 and ethanol were administered 15 min prior to placement into the operant chambers.

2.1.5. Data analysis

Rates of responding in each component (responses/s) were measured, and in the DRL component, accuracy (% reinforced responses) and average inter-response time (IRT), were also measured. Frequency histograms were additionally plotted for IRTs. ANOVA was used to determine the main effects of drug dose.

2.2. Punished responding in the squirrel monkey

2.2.1. Subject

Four adult, male, squirrel monkeys (*Saimiri sciureus*) weighing 0.8 to 1.0 kg were maintained on a 12 h light/dark cycle (lights on from 6:45 AM to 6:45 PM). Experimental sessions were conducted 5 days/week during the light phase. Between sessions the monkeys were housed in individual cages in a climate controlled vivarium with unrestricted access to water. Monkeys were fed 4 to 6 chow pellets (High Protein Diet, Purina Mills Inc.) and fresh fruit daily to maintain bodyweights at 90% to 100% of free-feeding weight. Four monkeys were trained under the behavioral procedures described below and had previous experience with benzodiazepines and other psychoactive compounds. A fifth monkey was experimentally naïve and was included in experiments to determine plasma levels of AZD2327.

2.2.2. Apparatus

During experimental sessions, monkeys were seated in Plexiglas chairs enclosed in ventilated, sound-attenuating chambers provided with white noise to mask extraneous sounds. While seated, monkeys faced a panel equipped with colored lights, one response lever, and a food-pellet dispenser. Each press of a lever with a force greater than 0.2 N produced an audible click and was recorded as a response. Before each session, a shaved portion of each monkey's tail was coated with electrode paste and placed under brass electrodes for delivery of brief, low-intensity shock stimuli (200 ms, 1.0 mA).

2.2.3. Procedure

Monkeys were trained to respond under a multiple fixed-ratio schedule of food reinforcement. In the presence of white (non-punished component) or red (punished component) stimulus lights, completion of 15 lever press responses resulted in the delivery of a 190 mg banana-flavored food pellet, followed by a 10-s timeout. When the red light was on, a second schedule was superimposed under which completion of every 25th response produced a brief electric shock to the monkey's tail. Daily sessions began with a 10-min timeout period, during which the chamber was dark and responding had no consequences, followed by a 3-min presentation of the non-punished component, then a 60-s timeout period, then a 3-min presentation of the punished component. This cycle of long timeout, non-punished component, short timeout, and punished component was presented 3 or 4 times during a 51-min or 68-min session, respectively.

2.2.4. Drugs

The effects of 0.0003 to 3.0 mg/kg, im AZD2327 and 0.3 to 10.0 mg/kg lorazepam were determined by using cumulative dosing procedures, and the behavioral effects of orally administered AZD2327 (0.3 mg/kg) were determined following a single injection immediately prior to the onset of the behavioral session. AZD2327 (free base) was dissolved to a concentration of 3 mg/mL in sterile water with a few drops of 85% lactic acid and was further diluted in sterile water. Lorazepam was dissolved to a concentration of 15 mg/mL in a solution of 20% ethanol, 20% Alkamuls and 60% saline, and was further diluted with saline. All doses are expressed as the weight of the free base. Cumulative dosing procedures were used to determine the effects of up to 4 i.m. doses of drug in a single session. Briefly, injections were given at the start of each 10 min timeout period (17-min inter-injection interval) such that the total dose of the drug was increased by one-half a log unit. Single dosing procedure was used to determine the effects of p.o. AZD2327 and for determining plasma levels of AZD2327 following im injection. For behavioral studies, a single p.o. injection was administered immediately before the start of the first 10 min timeout period. Drug effects were determined once or twice per week.