European Journal of Pharmacology 728 (2014) 100–106

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Risperidone inhibits voltage-gated sodium channels

Jan M. Brauner [a,b], Sabine Hessler [b], Teja W. Groemer [a], Christian Alzheimer [b], Tobias Huth [b,*]

[a] Department of Psychiatry and Psychotherapy, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[b] Institute of Physiology and Pathophysiology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Universitätsstr. 17, 91054 Erlangen, Germany

ARTICLE INFO

Article history:
Received 11 October 2013
Received in revised form
26 December 2013
Accepted 26 January 2014
Available online 5 February 2014

Keywords:
Risperidone
Sodium channel
$Na_V1.6$
Use dependence
Antipsychotic drug
Schizophrenia
Chemical compounds studied in this article:
Risperidone (PubChem CID: 5073)

ABSTRACT

In contrast to several other antipsychotic drugs, the effects of the atypical antipsychotic risperidone on voltage-gated sodium channels have not been characterized yet, despite its wide clinical use. Here we performed whole-cell voltage-clamp recordings to analyze the effects of risperidone on voltage-dependent sodium currents of N1E-115 mouse neuroblastoma cells carried by either endogenous sodium channels or transfected $Na_V1.6$ channels. Risperidone inhibited both endogenous and $Na_V1.6$-mediated sodium currents at concentrations that are expected around active synaptic release sites owing to its strong accumulation in synaptic vesicles. When determined for pharmacologically isolated $Na_V1.6$, risperidone inhibited peak inward currents with an IC_{50} of 49 μM. Channel block occurred in a state-dependent fashion with risperidone displaying a fourfold higher affinity for the inactivated state than for the resting state. As a consequence of the low state dependence, risperidone produced only a small, but significant leftward shift of the steady-state inactivation curve and it required concentrations ≥ 30 μM to significantly slow the time course of recovery from inactivation. Risperidone (10 μM) gave rise to a pronounced use-dependent block when sodium currents were elicited by trains of brief voltage pulses at higher frequencies. Our data suggest that, compared to other antipsychotic drugs as well as to local anesthetics and sodium channel-targeting anticonvulsants, risperidone displays an unusual blocking profile where a rather low degree of state dependence is associated with a prominent use-dependent block.

1. Introduction

Risperidone (RIS), a benzisoxazole compound, is a clinically important atypical antipsychotic drug with a characteristic binding profile at dopamine D2 receptors and, in contrast to typical antipsychotic drugs (APD), to $5HT_{2A}$ receptors (Schotte et al., 1996).

Besides transmitter receptors, RIS, like other APD, also targets ion channels. For example, RIS might prolong the QT interval by inhibiting the cardiac potassium channel hERG (Crumb et al., 2006). More recently, inhibition of neuronal voltage-gated sodium channels (Na_V) by APD has garnered considerable attention, as it might represent a novel mechanism of action. Being weak bases, APD get trapped in acidic synaptic vesicles and accumulate at intravesicular concentrations 50–120 times higher than their mean therapeutic plasma concentrations (Tischbirek et al., 2012). When APD are released by synaptic vesicle exocytosis, the synaptic cleft is exposed to high concentrations of APD and further exocytosis is inhibited by the block of presynaptic Na_V in a use-dependent fashion (Tischbirek et al., 2012; Yang and Wang, 2005). Whereas a direct interaction with Na_V has been proven for some APD (Lenkey et al., 2010; Bolotina et al., 1992; Ito et al., 1997; Ogata et al., 1990; Wakamori et al., 1989) it is still unknown whether RIS, one of the most prescribed APD worldwide (Aparasu and Bhatara, 2006; Schwabe and Paffrath, 2012), also employs this mechanism. Here, we characterized the effects of RIS on neuronal Na_V and found that, at clinically relevant concentrations, the drug inhibited sodium currents in a strongly use-dependent fashion. Unlike typical APD or "classical" Na_V blockers like local anesthetics or anticonvulsants such as phenytoin, RIS exhibits little state dependence suggesting an unusual blocking profile.

2. Material and methods

2.1. Cell culture

N1E-115 mouse neuroblastoma cells as previously described (Huth et al., 2008) were cultured at 37 °C in 5% CO_2 in DMEM medium

* Corresponding author. Tel.: +49 9131 8522495; fax: +49 9131 8522497.
E-mail address: Tobias.Huth@fau.de (T. Huth).

http://dx.doi.org/10.1016/j.ejphar.2014.01.062
0014-2999

(Gibco, Karlsruhe, Germany) with 5 g/l glucose, supplemented with 10% fetal bovine serum (Biochrom, Berlin, Germany) and 1% penicillin/streptomycin (Biochrom, Berlin, Germany).

2.2. Plasmid and transfection

mNa$_V$1.6 in pCDNA3 was a gift from E. Leipold and S. Heinemann (Leipold et al., 2006). mNa$_V$1.6r, a tetrodotoxin (TTX)-resistant mutant (Y371S) of mNa$_V$1.6, was created with the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies, Stratagene, USA) (Herzog et al., 2003; Wittmack et al., 2005).

N1E-115 cells were trypsinized and plated in 3.5 cm dishes (Corning, Lowell, MA, USA). The following day, the cells were transfected with 0.9 μg of Na$_V$1.6r and 0.4 μg of pEGFP-C1 (Mountain View, CA, USA) with the use of Nanofectin (PAA, Pasching, Austria), according to the manufacturer's protocol. Transfected cells were recorded 2 days after transfection.

2.3. Electrophysiology

Transfected N1E-115 cells were identified by green fluorescence. TTX (1 μM, Biotrend AG, Wangen, Switzerland) was used to isolate Na$_V$1.6r-mediated currents from TTX-sensitive endogenous sodium currents (Fig. 1A and B). Current signals were recorded in whole-cell voltage-clamp mode at room temperature (21 ± 1 °C) using a Multiclamp 700B amplifier in conjunction with a Digidata1322A and pClamp10 software (Molecular Devices, Sunnyvale, CA). Recordings were sampled at 100 kHz (20 kHz for use dependence experiments) and filtered with a 6 kHz Bessel filter. P/5 leak correction was applied to activation protocols and P/4 leak correction to recovery protocols.

Patch electrodes were made from borosilicate glass (Harvard Apparatus, Edenbrigde, Kent, UK) using a DMZ-Universal Puller (Zeitz-Instruments, Munich, Germany). Pipette resistance in the bath solution was 1.5–2.1 MΩ. Series resistance in whole-cell mode was < 6 MΩ before compensation (≥ 75%). Pipette solution was composed of (in mM) 125 CsCl, 5 NaCl, 2 Mg_2ATP, 10 Hepes free acid and 5 EGTA adjusted to pH 7.2 with CsOH. Bath solution contained (in mM): 145 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 D-Glucose, 10 Hepes free acid and 10 TEA adjusted to pH 7.4 with NaOH. Risperidone (RIS, Sigma-Aldrich, Deisenhofen, Germany) was dissolved in DMSO (Sigma-Aldrich, Deisenhofen, Germany) and added to the bath solution. The total concentration of DMSO in the bath solution was 0.03%, 0.1%, 0.3%, and 1% for 3 μM, 10 μM, 30 μM and 100 μM RIS, respectively. To exclude possible DMSO effects, DMSO in the respective concentration was also added to the control solutions. RIS solution or control solution was applied using a rapid, gravity-driven Y-tube system. The experimental protocol was as follows: recording started 4 min after whole-cell access. A sequence of voltage protocols was recorded in bath solution. Then RIS solution was applied using a rapid, gravity-driven Y-tube system. 5 min after perfusion, the inhibition had reached its maximal amplitude (data not shown) and the same sequence of voltage protocols was recorded again. To exclude possible effects not attributable to RIS, control experiments (equal DMSO concentration) were performed following the same procedure. Each cell was subjected to just one concentration of RIS.

2.4. Data analysis

From the activation protocols, the whole-cell sodium conductance G was calculated for every command potential V according to the following equation:

$$G = \frac{I}{V - E_{rev}}.$$

The equilibrium potential E_{rev} for sodium was 85.3 mV under our experimental conditions. G was normalized and fitted with a Boltzmann function of the form

$$G_{norm} = 1/\left[1+\exp\left(-\frac{V-V_{mid}}{k}\right)\right].$$

For dose–response relationships, we used the peak inward current of I/V-curves. The normalized current was fitted with a logistic relation:

$$I_{norm} = 1/\left[1+\left(\frac{[RIS]}{IC_{50}}\right)^{p}\right].$$

Steady-state inactivation was analyzed by normalizing the sodium current to the maximum peak current of the protocol (100% availability). The dependence on the pre-pulse potential V was fitted by a Boltzmann function

$$I_{norm} = 1/\left[1+\exp\left(\frac{V-V_{mid}}{k}\right)\right].$$

For the calculation of the dissociation constant K_i of RIS from inactivated channels, we used the equation of Bean et al. (1983) for 1:1 binding stoichiometry:

$$\Delta V = k \ln\left[\left(1+\frac{[RIS]}{k_r}\right)\Big/\left(1+\frac{[RIS]}{k_i}\right)\right],$$

where ΔV is the drug-induced shift in the steady-state inactivation curve, and k_r and k_i the dissociation constants for the resting and inactivated channel states, respectively.

Recovery from inactivation was analyzed as follows: for every inter-pulse interval Δt, the peak current of the second (test) pulse was divided by the current of the first (conditioning) pulse. For control experiments, the data points were fitted by a mono-exponential function of the form

$$I_{norm} = 1-\exp\left(\frac{-\Delta t}{\tau}\right).$$

However, in the presence of ≥ 30 μM RIS, the data points were more adequately fitted with the sum of two exponentials using the following equation:

$$I_{norm} = 1 - A_{fast}\exp\left(\frac{-\Delta t}{\tau_{fast}}\right) - A_{slow}\exp\left(\frac{-\Delta t}{\tau_{slow}}\right),$$

where A_{fast} and A_{slow} are the percentages of fast and slow recovering currents, respectively, and τ_{fast} and τ_{slow} are the respective time constants.

All curve fitting was performed in Origin 9pro (OriginLab Corporation, Northampton, MA, USA) using the Levenberg–Marquardt algorithm.

2.5. Data and statistical analysis

Data are given as mean ± S.E.M. In order to exclude possible effects of DMSO, we chose not to use paired statistics (before vs. during application of RIS), but to test instead the effect of RIS vs. the effect of DMSO alone (unpaired statistics). Shift of half-maximal activation and inactivation was analyzed as follows. The voltage shift V_{mid} of control vs. DMSO treatment was calculated for the control group. The shift of control vs. RIS treatment was