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The synaptic and nonsynaptic glycine transporter type-1 inhibitors Org-24461 and NFPS alter single neuron firing rate in the rat dorsal raphe nucleus Further evidence for a glutamatergic–serotonergic interaction and its role in antipsychotic action

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Abstract

Single neuron firing rate was recorded from dorsal raphe nucleus of anesthetized rats. The firing rate of raphe neurons varied from 4 to 8 discharge per second before drug administration and this neuronal activity was decreased by L-701,324 (2 mg/kg i.v. injection), a competitive antagonist of glycine$_B$ binding site of *N*-methyl-D-aspartate (NMDA) receptors. The glycine transporter type-1 (GlyT1) antagonists Org-24461 (10 mg/kg i.v.) and NFPS (3 mg/kg i.v.) reversed the inhibitory effect of L-701,324 on single neuron activity recorded from dorsal raphe nucleus of the rat. Org-24461 and NFPS both tended to increase the raphe neuronal firing rate also when given alone but their effect was not significant. This finding serves further evidence that glutamate released from axon terminals of the cortico-striatal projection neurons stimulates serotonergic neurons in the raphe nuclei and this effect is mediated at least in part by postsynaptic NMDA receptors. Thus, GlyT1 inhibitors are able to reverse the hypofunctional state of NMDA receptors, suggesting that these drugs may have beneficial therapeutic effects in neurological and psychiatric disorders characterized with impaired NMDA receptor-mediated transmission.

Keywords: Glycine transporter inhibitors; Serotonergic neurons; Raphe nuclei; NMDA receptors; Org-24461; NFPS

1. Introduction

The dorsal raphe nucleus (DRN) in the brainstem contains at least 50% of all serotonergic neurons of the central nervous system (Wiklund and Bjorklund, 1980). These neurons are medium sized cells with spiny dendritic arborization, which establish local neuronal network with their dendrodendritic connections and recurrent axon collaterals. Beside serotonergic projection neurons, DRN also contains GABAergic interneurons as well as numerous noradrenergic and glutamatergic axon terminals. Morphological and functional observations confirmed the existence of a glutamatergic pathway arising from the median prefrontal cortex (Behzadi et al., 1990; Hajos et al., 1998) whereas serotonergic neurons of DRN form extended projection to the cerebral cortex (Kidd et al., 1991; Sesack et al., 1989). Thus, the raphe-cortical serotonergic and the cortico-raphe glutamatergic projections establish a long-axon neuronal loop between these two brain structures. Interaction between glutamatergic axon terminals and serotonergic neurons forms an excitatory-inhibitory connection by which incoming excitatory signals are converted into inhibitory output projecting back to cerebral cortex where stimulation was generated from.

The primary target of the cortical glutamatergic neurons within the raphe nuclei is the serotonergic projection neurons

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(Tao et al., 1996; Harsing et al., 2004). The excitatory glutamatergic influence in DRN may be mediated by ionotropic glutamate receptors expressed in postsynaptic membrane of serotonergic neurons. In isolated raphe nuclei slices, activation of *N*-methyl-D-aspartate (NMDA) receptors evokes serotonin release (Harsing et al., 2004). The NMDA receptor requires glycine as cotransmitter for its activation (Johnson and Ascher, 1987). At synaptic level, glutamate is released from glutamatergic axon terminals and glycine is released from astroglial cells. Being released into the synaptic cleft, they diffuse to the vicinity of postsynaptic NMDA receptors (Zafra et al., 1997). Operation of glycine transporter type-1 (GlyT1) expressed in astocytes assures to set glycine concentrations at NMDA receptor below saturating levels (Bergeron et al., 1998). Inhibition of GlyT1 enforces NMDA receptor-mediated functions, particularly in conditions when glycine_B binding site at NR1 subunit is blocked by selective inhibitors (Danysz and Parson, 1998). The aim of the present study was to investigate whether the GlyT1 inhibitors Org-24461 and NFPS (Fig. 1) are able to facilitate glutamate-mediated excitation of serotonergic projection neurons in rat DRN.

2. Experimental procedure

2.1. Animals and drugs

Male Wistar rats weighing 250–300 g were obtained from Charles River Hungary. The animals were housed up to five to a cage in a temperature- and humidity-controlled animal facility on a 12-h light:12-h dark cycle (6.00 a.m. on; 6.00 p.m. off) with food and water available ad libitum. The animals were allowed at least 1 week of habituation to their housing prior to experimentation.

NFPS, *N*[3-(4′-fluorophenyl)-3-(4′phenylphenoxy)-propyl]sarcosine (Herdon et al., 2001) and Org-24461, *R*,*S*-(±)*N*-methyl-*N*-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine (Brown et al., 2001) were synthesized by Dr. Peter Matyus, Department of Organic Chemistry, Semmelweis University, Budapest, Hungary. Org-24461 (50 mg/ml) and NFPS (10 mg/ml) were dissolved in dimethyl sulfoxide (DMSO), briefly sonicated and kept in a water bath at 37 °C for ca. 10 min. Immediately before administration; they were diluted in a double volume of saline. L-701,324 was purchased from Tocris Bioscience (Bristol, UK) and dissolved in 25% polyethylene glycol (PEG)-300 and 75% saline. Intravenous administration of drug vehicle alone showed no change in single-unit activity. Urethane was purchased from Reanal (Budapest, Hungary). All other chemicals were of analytical grade.

2.2. Recording single neuron activity from rat DRN

Rats were anesthetized with urethane (1 g/kg i.p.). A polyethylene cannula was inserted into the left femoral vein. Then the head of the animals was fixed, the skull was opened on a 6 mm × 6 mm square around the lambda point using a dental drill and the dura was cut. The opening was sealed with bone wax, and the animal was put aside for at least 30 min. For recording, the rat was mounted in a stereotaxic frame (Narishige SR-6N) and a wolfram microelectrode (World Precision Instruments, WPI TM33B20) was advanced into the area of DRN (AP: 8; L: 0; and V: 5.8–6.5 mm, Paxinos and Watson, 1998) by a single axis micromanipulator (Narishige SM15). Extracellular single-unit activity was recorded and amplified, amplified gain: 1000×; low-pass filter at 0.1 kHz, high-pass filter 5 kHz. The amplified biological signals were registered in a Tektronix oscilloscope and sampled by a PC-based computer using Neurosys 1.1.0.357 software (Experimetria, Hungary). As soon as the firing in DRN reached a stabile rate, control activity was recorded for 10 min. Then, with uninterrupted recording, L-701,324 was injected i.v. in a dose of 2 mg/kg and activity was recorded for another 10-min period. GlyT1 inhibitors were added i.v. (Org-24461 10 mg/kg, NFPS 3 mg/kg) and neuronal single-unit activity was further recorded for a 10-min period.

To verify the position of the microelectrode, dc current (1 mA for 15 s) was delivered through the recording electrode when all recording was completed. The animals received then an overdose of urethane and were perfused transcardially with saline followed by buffered formalin. Brain sections (70 μm) containing the raphe nuclei were cut using a freezing microtome and the sections were washed, dehydrated with alcohol and Nissl staining was used for evaluation. A further proof that activity of neurons from a correct location was recorded was provided by changes in neuronal activity after L-701,324 administration (see below).

2.3. Evaluation of the records and statistical analysis

For evaluation, the records were played back and a threshold level was set so that all extracellular spikes, but no noise, crossed it. Crossings were counted as events, from which neuronal firing rate was calculated by the software. For data analysis, firing rates were collected for 250 s before injection of L-701,324 (control period) and for 250 and 500 s periods before and after the administration of the GlyT1 inhibitors. All group data were presented as mean ± S.D. and one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) tests were used to compare differences between group mean data with control group mean. Differences between control and experimental responses with $P < 0.05$ were considered significant, *n* indicate the number of experiments.

3. Results

Our preliminary experiments indicated that single neuron firing registered from rat DRN was due to activation of

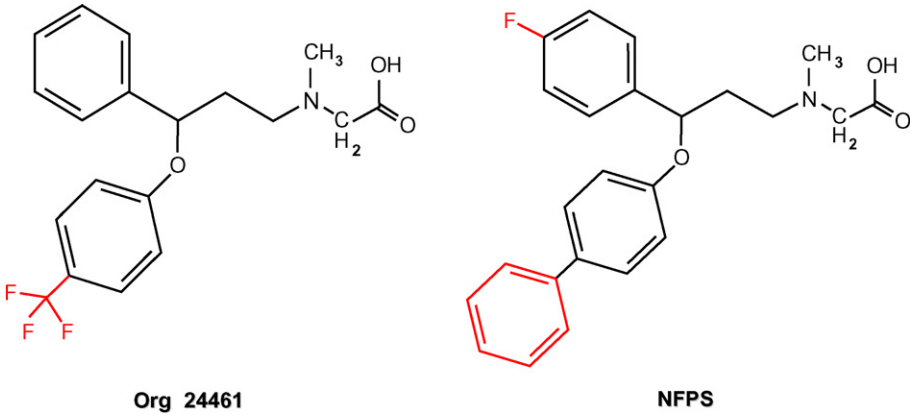

Fig. 1. The chemical structures of the glycine transporter type-1 inhibitors Org-24461 and NFPS.