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The pharmacological stimulation of NMDA receptors via co-agonist site: an fMRI study in the rat brain

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Abstract

D-Serine has been proposed as an endogenous modulator at the co-agonist glycine-binding site of *N*-methyl-D-aspartate (NMDA) receptors. There is still some debate as to whether this site is saturated in vivo, but it seems likely that this depends on regional differences in local glycine or D-serine concentrations. In order to identify areas where the co-agonist site was not fully activated in vivo, we studied the effect of intraperitoneal D-serine administration in the rat brain using functional magnetic resonance imaging (fMRI). Using contrast agent injection, the variations in the relative cerebral blood volume (CBV_{rel}) in several regions of interest were evaluated. D-Serine (50 mg/kg) elicited a significant statistical increase in the CBV_{rel} in the hippocampus. This effect was inhibited by the specific full antagonist of the co-agonist glycine site L-701,324 indicating that the hippocampal activation occurred through the binding of the agonist D-serine to the glycine-binding site of NMDA receptors. This result demonstrates that in the hippocampus, the co-agonist sites of NMDA receptors are not endogenously saturated under our experimental conditions, suggesting an important role of D-serine in the modulation of receptor function in the hippocampus.

Keywords: D-Serine; NMDA; Glutamate receptor; Glycine site; Rat brain; fMRI; Contrast agent

Glutamate is the primary neurotransmitter mediating fast excitatory neurotransmission in the central nervous system (CNS) via ligand-gated cation channels [9]. Among the ionotropic glutamate receptor subtypes, the *N*-methyl-D-aspartate (NMDA) receptor plays important roles in brain function and neurotoxicity. The opening of the NMDA receptor channel requires not only the binding of glutamate but also the activation of its co-agonist glycine-binding site by D-serine or glycine [19,38]. Since the site has a rather high affinity for its ligands and both glycine and D-serine are abundant in the CNS, it has been suggested that the site is fully activated under physiological conditions [24]. However, several studies have indicated that this may not always be the case: Experiments in superfused hippocampal slices showed that an increase in glycine concentration in the medium can enhance the NMDA receptor function [3,41]. Other studies demonstrated an increase in the receptor function through co-agonist supplementation both in vivo and in vitro (for review see [7]). These findings, suggesting incomplete occupancy of the glycine-binding site, are in line with the observation that site agonists also have clinical effects when administered to patients [10,15,16,39].

Functional magnetic resonance imaging (fMRI) has proven its utility for the non-invasive mapping of brain function with high temporal and spatial resolution [2]. fMRI signals rely on the intact neurovascular coupling by which neuronal activity is translated into altered local perfusion rates. In fact, relative perfusion increases exceed the increased cerebral oxygen consumption rates, which result in better local blood oxygenation in the activated brain area. As deoxygenized hemoglobin is paramagnetic (thus constituting an

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endogenous contrast agent), alterations of blood oxygenation levels lead changes in MRI signal intensity, the fMRI signal [2,25]. Alternatively, the local perfusion and cerebral blood volume (CBV) changes caused by neuronal activity could be measured directly using intravascular contrast agents such as superparamagnetic iron-oxide nanoparticles [21,30].

Neuro-pharmacological stimulation has been demonstrated to be an effective stimulation paradigm in anesthetized rats. This approach can be used to study the effect of drugs at the level of its specific receptor [5,30].

In this article we describe the effects of D-serine administration on neuronal activation in rats as monitored by assessing changes on relative local CBV (CBV_{rel}) under controlled physiological conditions. We could identify areas displaying CBV increases induced by the amino acid, which is indicative that the co-agonist glycine sites were not fully activated under our experimental conditions. These activated regions could be related to circuits involved with the clinical effects of the site agonists.

All animal experiments were carried out in strict adherence to the Swiss law for animal protection. Male Fisher rats (Iffa-Credo, L'Arbresle, France) weighing between 200 and 250 g were used in this study. Animals were anesthetized with 1.2–1.5% isoflurane (Abbott, Cham, Switzerland) in air: oxygen mixture (2:1) administered via face mask and positioned in a cradle made from Plexiglas. The tail vein was cannulated with a 20-gauge needle for intravenous (i.v.) infusions. Body temperature was maintained around 36.5 °C using warm air, regulated by a rectal temperature probe (DM 852, Ellab, Copenhagen). Blood CO_2 was monitored non-invasively throughout the experiment using a transcutaneous monitoring device (TCM3, Radiometer Copenhagen) adopting an experimental protocol described previously [22].

T_2-weighted MR images were acquired on a 7 T Pharmascan system (Bruker Medical Systems, Ettlingen, Germany) using a three-dimensional (3D) Rapid Acquisition with Relaxation Enhancement (RARE) sequence [14] with the following parameters: repetition time (TR) = 900 ms, effective echo time (TE) = 60 ms, RARE factor = 16, field-of-view (FOV) = 3.28 cm × 3.00 cm × 2.5 cm, matrix size (MS) = 96 × 80 × 42, single slice thickness 2 mm, and number of averages (NA) = 2. The readout direction was caudal-rostral. The radiofrequency probe was a birdcage resonator of 35 mm inner diameter. Total acquisition time for one 3D dataset was 6 min 28 s. Images were reconstructed to 128 × 128 × 64 voxels. After acquisition of a pre-contrast dataset, 0.7 ml of Endorem (Guerbet, Aulnays-sur_Bois, France) were administered via the tail vein. A post-contrast dataset was acquired after 10 min of contrast agent injection. The CBV map is computed from the pre- and post-contrast scans as described below.

At day 0 a first set of CBV maps was measured as baseline. The following day a second set of CBV maps was obtained 2 h after intraperitoneal administration of D-serine (Aldrich, Steinheim, Germany) at a dose of 50 mg/kg. D-Serine was diluted in saline. For competition experiments, the glycine site antagonist L-701,324 (7-chloro-4-hydroxy-3-[3-phenoxy]-phenylquinolin-2[1*H*]-one) (Sigma, St. Louis, USA) was diluted in polyethylenglycol (PEG) 25% with NaOH (pH ∼ 8) in distilled water and administered by intraperitoneal injection (20 mg/kg) 30 min before the MRI measurements. The placebo-treated group received saline (1 ml/kg) and PEG (3 ml/kg) solutions applied with the same infusion protocol.

The intravascular contrast agents lead to a change in the transverse relaxation time T_2 in the voxel (x, y, z) according to

$$\frac{1}{T_2(x, y, z)} = \frac{1}{T_{20}(x, y, z)} + r_2 c_p \, \mathrm{CBV}(x, y, z)$$

where $T_2(x, y, z)$ and $T_{20}(x, y, z)$ are the transverse relaxation times prior and after contrast agent administration, r_2 is the molar relaxivity of the contrast agent and c_p is the plasma concentration of the contrast agent. Assuming equilibrated plasma levels of the contrast agents, we find that T_2 becomes proportional to CBV. Hence, relative CBV-maps were calculated according to

$$\mathrm{CBV}_{rel}(x, y, z) \propto -\ln\left(\frac{S_{post}(x, y, z)}{S_{pre}(x, y, z)}\right)$$

where $S_{pre}(x, y, z)$ and $S_{post}(x, y, z)$ are the signal amplitudes prior to and after administration of contrast agent.

Geometrical processing of the data comprised the following steps: first, the voxels that did not belong to the brain were removed by an automatic segmentation based on intensity thresholding. Second, the segmented pre-contrast datasets were automatically coregistered to a target dataset using the method described by Woods et al. [43]. The registration parameters were transferred to the CBV maps, which finally were resliced to the target space using 3D-bilinear interpolation. This process provided accurate co-registration of all datasets recorded.

The general linear model was used for statistical analysis of the fMRI data. Pre-processing comprised first a spatial filtering with a sinc-shaped digital filter with a cutoff at 0.4 of the Nyquist-frequency, followed by a normalization of the voxel intensities by the global mean of the CBV-map. CBV-change induced by the drug were modeled on a pixel-by-pixel level according to

$$\vec{Y} = \Delta\mathrm{CBV}_{rel}(D_i)\vec{X}(D_i) + \mathrm{CBV}_{rel,0}$$

where $\vec{Y}$ denotes the vector of CBV-values for each measurement, $\Delta\mathrm{CBV}_{rel}(D_i)$ is the estimate for the compound related blood volume change at dose D_i, $\vec{X}$ is the vector denoting the condition to which a measurement belongs: if measurement k was acquired after treatment with dose D_i, the corresponding value in the vector $X(D_i)$ is 1; otherwise, it is 0. $\mathrm{CBV}_{rel,0}$ is the normalized baseline blood volume, which is used as a dummy parameter in the model. The dimensions of the vectors $\vec{Y}$, $\Delta\mathrm{CBV}_{rel}(D_i)$, and $\mathrm{CBV}_{rel,0}$ corresponds to the number of voxels, that of $\vec{X}$ to the number of conditions.