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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.
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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 noninvasively 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

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

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

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

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

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

where $\vec{Y}$ denotes the vector of CBV-values for each measurement, $\Delta CBV_{rel}(D_i)$ 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. $CBV_{rel,0}$ is the normalized baseline blood volume, which is used as a dummy parameter in the model. The dimensions of the vectors $\vec{Y}$, $\Delta CBV_{rel}(D_i)$, and $CBV_{rel,0}$ corresponds to the number of voxels, that of $\vec{X}$ to the number of conditions.
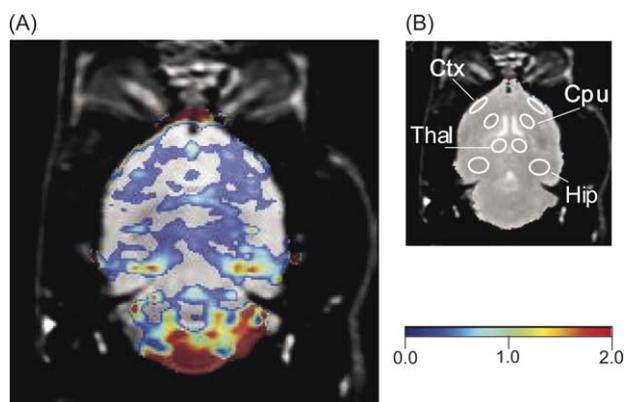
Fig. 1. Functional MRI of the rat brain. (A) Coronal section through rat brain illustrating the changes in CBV_{rel} elicited by D-serine injection in percent of pre-drug administration. (B) Regions-of-interest (ROIs) in the cortex (Ctx), caudate-putamen (CPu), hippocampus (Hip) and thalamus (Thal) used for quantitative analysis of the CBV changes. The ROIs correspond to images taken from the rat brain atlas [26].

Fig. 2. CBV_{rel} changes after D-serine injection. (A) Averaged changes of CBV_{rel} in percent of pre-D-serine administration values ($\Delta CBV_{rel}\%$) for the four ROIs in cortex (Ctx), caudate-putamen (CPu), hippocampus (Hip) and thalamus (Thal). D-Serine (50 mg/kg, i.p.) was administered 2 h before the MRI measurement. A statistically significant ($^{*}p < 0.03$) increase in the CBV_{rel} was observed in the hippocampal ROI. Data are given as the mean ± S.E.M., $n = 10$. (B) Another set of animals ($n = 10$) received the same treatment plus an administration of the L-701,324 (20 mg/kg, i.p.) 30 min before the MRI measurement. These animals did not show a statistically significant change in the CBV_{rel} in the ROIs studied.

Finally, regions-of-interest (ROIs) were manually defined on the anatomical reference image for the following regions: cortex (Ctx), hippocampus (Hip), caudate putamen (Cpu) and thalamus (Thal). They were transferred onto the ΔCBV-estimate-map for statistical analysis. All data analyses were performed using imaging analysis software developed in-house (Biomap, M. Rausch).

Systemic infusion of D-serine (50 mg/kg) led to region-specific changes in local CBV_{rel} values. As illustrated in a coronal brain section (Fig. 1A), distinct CBV_{rel} increases have been observed bilaterally in hippocampus. Caudate putamen, on the other hand, displayed only a weak and diffuse increase. A strong change was observed in the cerebellum; however, this is likely due to the contribution of large vascular structures [18] and artifacts generated by the geometrical processing. It is noteworthy that such strong changes in the cerebellum were not observed when the animals were individually assessed for signal changes before geometrical processing.

Regions showing D-serine distribution closely resembling that of NMDA receptors with low or undetectable glycine levels [31,32] were selected for the quantitative analysis of the fMRI response. ROIs were defined in cortex, caudate-putamen, hippocampus, and thalamus (Fig. 1B). The averages of changes of CBV_{rel} in percent of pre-drug administration values ($\Delta CBV_{rel}\%$) are shown in Fig. 2. In the hippocampus, D-serine elicited a statistically significant increase in local CBV values of $\Delta CBV_{rel}\% = 2.38 \pm 1.22\%$ ($p < 0.03$; ANOVA; $n = 10$) (Fig. 2A). Signal changes in the other cerebral ROIs did not display significant alteration with regard to the baseline $CBV_{rel,0}$ values (Fig. 2A).

Provided the CBV_{rel} increases in the hippocampus were induced by the interaction of D-serine with the glycine-binding site of NMDA receptors, this effect should be prevented by the addition of a site-specific antagonist. In fact, treatment of the animals with the specific full antagonist
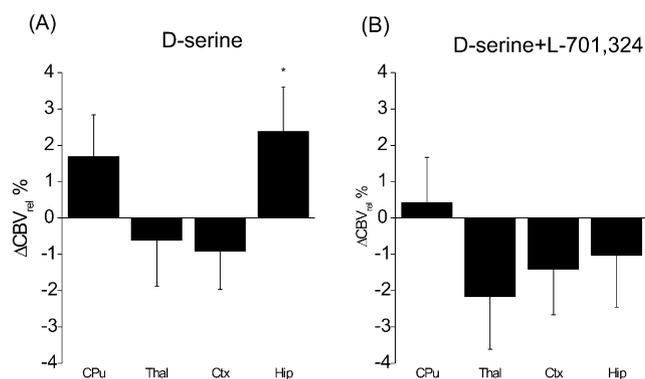
L-701,324 [29] of the co-agonist glycine site 30 min prior to MRI measurements, completely inhibited the hippocampal CBV_{rel} changes induced by D-serine (Fig. 2B). In spite of the presence of a general trend toward lower levels of activation, no statistically significant signal change was observed with antagonist treatment. On the other hand, measurement in another group of animals ($n = 8$) that received only L-701,324 did not elicit significant changes in the fMRI signal (data not shown).

Placebo-treated animals ($n = 8$) were measured using the identical protocol, with vehicle (saline and/or PEG) administration on the second day. These animals did not show any significant difference in the CBV_{rel} values as obtained from the first and second measurement (data not shown).

D-Serine injection did not cause any global hemodynamic changes in the rats during the experiments. Similarly, physiological parameters, such as body temperature, blood pressure and blood gases were not affected by the amino acid administration (data not shown). Also the L-701,324 administrations were not followed by any statistical significant changes in the physiological parameters studied (data not shown).

The N-methyl-D-aspartate (NMDA) receptor is a subtype of the ionotropic glutamate receptor that plays an important role in several cerebral functions such as neuronal plasticity and regulation of neurotransmitter release. Opening of the channel requires both the binding of glutamate and the activation of a co-agonist site by either D-serine or glycine [19]. Administration of D-serine stimulated NMDA receptor-related effects both in vitro and in behavioral experiments in animals (reviewed in [7]). This co-agonist was also shown to have clinical effects [39]. D-Serine has even been proposed as a new neuro-modulator of the NMDA receptors since it is synthesized and released in the vicinity of the synapse and it has a recognition site which affects receptor function [42]. While

glycine can also activate inhibitory strychnine-sensitive receptors, in particular in the spinal cord and the brain stem, the NMDA receptors are considered to be the exclusive target for D-serine [12].

Animal studies have demonstrated that low doses of peripherally administered D-serine induced central effects [7,13]. Intraperitoneal (i.p.) injection of D-serine at the dose utilized in our study (50 mg/kg) caused a sustained increase in the amino acid level in the infant rat cerebral cortex for at least 24 h [36]. In a recent paper Hashimoto and Chiba [11] observed a prolonged (3 days) elevation of D-serine in the hippocampus and others cerebral structures after a single i.p. injection in adult rats. Based on these observations, we decided to study the effect of D-serine in the brain activity at 2 h after i.p. administration.

Our results show hippocampal activation following peripheral administration of D-serine. This effect could be blocked by L-701,324, a specific antagonist of the glycine-binding site. These results indicate that under the present controlled experimental conditions in the hippocampus, the co-agonist glycine-binding sites of NMDA receptors are not endogenously saturated. fMRI signal changes in other brain regions evaluated did not reach statistical significance. It seems probable that in such regions the co-agonist sites were already fully activated or the increase in the NMDA receptor activity was too low to be detected.

The observed activation of the hippocampus is congruent with prior reports of effects on agonists of the glycine-binding site of NMDA receptors. Hippocampus-related effects include improvement in learning processes [33,40], enhancement of long-term potentiation in the CA1 region [37], and increase in field potential [27]. Stimulation of the glycine-binding site is assumed to be of special importance for the formation of memory and long-term potentiation. Both glycine [23] and D-serine [1] were shown to have positive effects on learning and memory in rats, while glycine-binding site antagonists have the opposite effect inducing memory deficits in animals [6,8]. On the other hand, humans showed improved performance in a word recall test following administration of agonists of the glycine-binding site [33,40]. In fact, the awareness of the importance of the glutamatergic system to learning and memory processes prompted the efforts to develop a novel therapy approach for Alzheimer's disease [4].

Hippocampus activation by NMDA receptor co-agonists is furthermore supported by electrophysiological data. For instance, intra-hippocampal administration of D-serine induced a slight increase in the magnitude of long-term potentiation recorded extracellularly in anesthetized rats [37].

Since L-701,324 is a full antagonist of the co-agonist site of NMDA receptors, one could expect that it would reduce the brain activity by itself. However, in the present report, we did not observe a significant statistical decrease in the measured signal following L-701,324 administration. Other fMRI studies also failed to measure negative signals in response to antagonist drugs [28,34,35]. These findings can be correlated to fMRI technique limitation to detect declines in brain functional activity. It is noteworthy that in a previous report in rats, MK-801, an NMDA receptor antagonist, elicited significant fMRI signal decreases restricted to few neocortical regions [17].

In summary, specific activation of the rat hippocampus following systemic administration of D-serine has been demonstrated non-invasively using fMRI techniques. The effect was shown to be caused by D-serine interaction with the glycine-binding site of NMDA receptors as it could be completely inhibited by administration of the selective antagonist L-701,324. Activity in this brain region can be related to behavioral and biochemical effects of D-serine.

Acknowledgments

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