Differential c-Fos induction by different NMDA receptor antagonists with antidepressant efficacy: potential clinical implications

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Abstract

Preclinical and clinical studies have shown that N-methyl-D-aspartate (NMDA) receptor antagonists can exert antidepressant effects. Thus, a single intravenous injection of ketamine, a non-competitive NMDA receptor antagonist, has been recently demonstrated to produce a rapid and relatively sustained antidepressant effect in patients. Therefore, the role of NMDA receptors and their signalling pathways for pathophysiology and therapy of depression are under intense research.

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Introduction

The NMDA receptor (NMDAR) is a ligand-gated ion channel. It is thought to be a tetramer composed of two NR1 subunits combined with two NR2 subunits (NR2A–NR2D) (Dingledine et al. 1999). Within the ion channel, two sites have been identified, the ‘s’ site and the phencyclidine (PCP) site, the latter is also the binding site for ketamine and MK-801. The NR2 subunits have been identified as glutamate-binding sites. Whereas the NR1 subunit is ubiquitously expressed throughout the CNS due to its obligatory presence in all NMDARs, NR2 subunits show brain region-specific expression patterns, probably implicating functional specificity. Recent experiments in mice have demonstrated that the antidepressant effects of the channel blockers ketamine and MK-801 can also be achieved by the NR2B subunit specific antagonist Ro 25-6981 (Maeng et al. 2008). This may be clinically important, since non-selective inhibition of NMDARs by ketamine and MK-801 can evoke psychotomimetic effects (delusions, hallucinations, cognitive deficits) in psychiatric patients and healthy probands, which has so far not been reported for Ro 25-6981. Thus, treatment with an NR2B antagonist may have less side-effects while offering the same antidepressant potential (Preskorn et al. 2008). The neurobiological mechanisms underlying this hypothesis have so far not been addressed.

Methods and Results

An established method for identifying brain systems responding to a pharmacological treatment, in particular with respect to the glutamatergic system, are expression studies of the transcription factor c-Fos, an immediate early gene and neuronal activation marker induced by a plethora of stimuli (Herdegen & Leah, 1998). Indeed it has been shown that PCP, MK-801 and ketamine induce c-Fos in brain areas that have been implicated in schizophrenia, such as the retrosplenial, entorhinal and prefrontal cortices and the thalamus (Vaisanen et al. 2004). We have investigated here, whether this is also true for Ro 25-6981. For this purpose we injected male C57BL/6 mice (Charles River, Germany) with the same doses of MK-801 [(+)-MK-801 hydrogen maleate, 0.2 mg/kg i.p.], ketamine [(±)-ketamine hydrochloride, 10 mg/kg i.p.] and Ro25-6981 ([R-(R*,S*)]-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinopropanol hydrochloride, 10 mg/kg i.p.) that have previously been shown to have an antidepressant effect in mice (Maeng et al. 2008).
Subsequently, we studied the expression of c-Fos by immunocytochemistry in the brain as described earlier, using a polyclonal antiserum (Gass et al., 1993). As previously reported, the PCP-like drugs ketamine and MK-801 induced 90 min after injection a prominent c-Fos expression in distinct neuronal cell populations such as retrosplenial and posterior cingulate cortices, entorhinal and piriform cortices, scattered neurons of the neocortex (in particular layers IV–VI), midline thalamic nuclei and basolateral nucleus of the amygdala (Fig. 1). Ketamine and MK-801 evoked a very similar c-Fos expression pattern. Therefore c-Fos induction by ketamine probably results from its NMDAR channel-blocking activity, although ketamine has also a number of other pharmacological actions, e.g. modulation of GABA_A receptors (Hevers et al. 2008; Machado-Vieira et al. 2009). In contrast to ketamine and MK-801, c-Fos induction was missing in these brain areas in animals treated with Ro 25-6981. These animals demonstrated only a few scattered c-Fos-positive cells also found in sham-treated control animals (Fig. 1). Although one has to consider that Ro 25-6981 might have a differential brain penetration compared to ketamine or MK-801, and that the differential effects on c-Fos expression observed could be attributable to this kinetic feature rather than to subtype specificity, this interpretation seems unlikely, since all three substances were used in the same dosages, respectively, in which they had antidepressant effects in murine depression models (Maeng et al., 2008).

**Discussion**

These results indicate that the NR2B antagonist Ro 25-6981 does not act on the same neuronal substrates as the non-specific NMDAR channel blocker ketamine, at least not to the same extent, as indicated by the activation marker c-Fos. Since the expression of the NR2B subunit is more restricted than the more common NR2A subunit, NR2B antagonists probably only block a subset of NMDARs in specific neurons, affecting less brain regions when compared to PCP-like drugs such as ketamine (Loftis & Janowsky, 2003). Moreover, while the latter substance is a non-competitive channel blocker, Ro 25-6981 inhibits NR2B-containing NMDARs by an allosteric mechanism (Loftis & Janowsky, 2003). The subunit specificity
of NR2B antagonists may account for less adverse behavioural effects in experimental animals and a better tolerability in clinical studies. This may also hold true for other NR2B antagonists already used in clinical trials, although there is evidence that two distinct classes of NR2B-directed NMDAR antagonists may exist, one which binds to NMDA NR2 receptors regardless of the NR2 subunit composition (e.g. Ro 25-6981), and one in which binding to NR2B-containing NMDARs is altered by the presence of other NR2 subunits than NR2B (e.g. CP-101,606) (Chazot et al. 2002; Nikam & Meltzer, 2002). In this respect, our conclusions in regard to NR2B mechanisms are compound specific (to Ro 25-6981).

Two clinical studies using a single injection of ketamine as a monotherapy in severe or treatment-resistant depression, respectively, reported psychotogenic effects in some patients early in the treatment (Berman et al. 2000; Zarate et al. 2006). In contrast, a first placebo-controlled trial with a single injection of the NR2B antagonist CP-101,606 as add-on to paroxetine in 30 non-responding severely depressed patients achieved a rapid and robust antidepressant response without producing severe dissociative reactions that had been observed in the trials using ketamine (Preskorn et al. 2008). This indicates that it may be possible to achieve a pharmacological dissociation between the antidepressant and psychotomimetic effects of NMDAR antagonists. In this respect it is highly speculative that the brain regions showing c-Fos induction after treatment with ketamine (and also the similarly acting MK-801) might be those that are involved with the psychotomimetic effects, i.e. retrosplenial and posterior cingulate cortices, entorhinal and piriform cortices, deeper layers of the neocortex and midline thalamic nuclei (Vaisanen et al. 2004). In contrast one might speculate that the antidepressive effects of non-selective as well as NR2B-specific NMDAR blockers are mediated via the hippocampus, since this important region of the limbic system is spared from c-Fos induction by ketamine and MK-801.

Conclusion

NMDAR channel blockers can inhibit glutamate-induced immediate early gene expression, such as c-Fos induction, under a variety of physiological and pathophysiological conditions (Herdegen & Leah, 1998). However, NMDAR antagonists have themselves the potential to induce immediate early genes in restricted but specific neurons, indicating that they do not only inhibit glutamate-induced synaptic activation, but also activate specific synapses, potentially by an indirect mechanism (Loftis & Janowsky, 2003). We have demonstrated here that the specific induction of c-Fos observed after treatment with non-specific NMDAR channel blockers is greatly reduced using the subunit-specific NR2B antagonist Ro 25-6981. In experimental animals, and as far as one can speculate from first clinical trials, these two classes of NMDAR antagonists may be similar with respect to antidepressant effects, but quite different with regard to their side-effect profile. c-Fos labelling may turn out under such experimental conditions as a molecular correlate for the latter.

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Statement of Interest

None.

References


