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BRAIN RESEARCH

Research Report

Coadministration of bicuculline and NMDA induces paraplegia in the rat

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ABSTRACT

Motor neurons (MNs) of an adult rat are normally insensitive to the neurotoxic action of NMDA. Meanwhile, the experiments in non-motor neurons showed that sensitivity to NMDA can be increased by bicuculline, an antagonist at $GABA_A$ receptors. The aim of the present work was to examine whether bicuculline would produce such an effect in the adult MNs. In adult Wistar rats, intrathecal injection of bicuculline and NMDA individually failed to affect motor activity of the extremities. In contrast, bicuculline–NMDA combination dose-dependently impaired hindlimb functions. At the 9th day after injections of the combination, a paraplegia with persistent bilateral spastic extension developed in all animals. Light microscopic assessment showed that the development of the motor deficit is associated with pathological changes in spinal motor neurons (swelling, accumulation of the Nissl substance near nucleus, hyperchromatosis, shrinkage, and chromatolysis), mainly in the lumbar ventral horns. Additionally, distinct abnormalities were observed in the white matter of the lumbar cords. The bicuculline–NMDA combination induced a loss of spinal cord MNs while sparing the dorsal horn neurons. The effects of the combination were reversed by muscimol, a $GABA_A$ agonist. Thus, an inhibition of $GABA_A$ergic processes can induce NMDA sensitivity in adult MNs. The present data may provide new insights into the mechanism of motor disorders in amyotrophic lateral sclerosis and other states wherein the combination of glutamatergic overstimulation and $GABA_A$ergic understimulation takes place.

1. Introduction

Glutamate is an essential neurotransmitter in the mammalian nervous system (Fonnum, 1984). An increased level of this factor, however, can damage neurons. As was shown, overstimulation of glutamate receptors can initiate a toxic metabolic cascade resulting in neuronal death (Choi, 1987; Choi, 1992; Estevez et al., 1995; Lu et al., 1996; Randall and Thayer, 1992; Regan and Choi, 1991; Van Den Bosch and Robberecht, 2000; Van Den Bosch et al., 2000). The glutamate neurotoxicity is thought to be implicated in a variety of pathological processes including brain trauma, cerebral ischemia, status epilepticus, and neurodegenerative diseases (Meldrum, 2000). The neurotoxic activity of glutamate is mediated largely by glutamate

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receptors of NMDA- and AMPA-subtype named from their selective unnatural agonists, N-methyl-D-aspartate and δ-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, respectively.

A significant body of evidence supports glutamate representing a high toxicant for motor neurons (MNs). A notable result of these studies was a possible dependence of the toxic mechanism on cell maturity. In embryonic MNs, the neurotoxicity was found to be mediated by receptors of both NMDA- and AMPA-subtype (Carriedo et al., 1996; Regan and Choi, 1991; Urushitani et al., 2001; Van Den Bosch et al., 2000). In adult animals, MNs were damaged with agonist of AMPA receptors while a stimulation of NMDA receptors induced no (Corona and Tapia, 2004; Kalb, 1994) or only weak (Martin et al., 1977) effect. These results could be explained by the small number of NMDA receptors in the adult MNs since the binding of labeled NMDA receptor agonists to the rat MNs was shown to be substantially decreased by aging (Kalb et al., 1992).

Meanwhile, there are the indications that the sensitivity of neuronal NMDA receptors is elevated when the activity of gamma-aminobutyric acid (GABA) falls. Bicuculline, an antagonist of $GABA_A$ receptors (Bowery et al., 1984), reportedly stimulated the expression of NMDA receptors in the rat hippocampal CA1 pyramidal cells (Swearengen and Chavkin, 1989). Similar bicuculline effects were obtained in the cells of the rat primary somatosensory cortex (Luhmann and Prince, 1990), layer V neurons of the rat anterior cingulate cortex (Wang et al., 2005), in the noradrenergic neurons of the rat locus coeruleus (Shiekhattar and Aston-Jones, 1992), nociceptive neurons of the mouse spinal cord (Cao et al., 2011). In light of this, it can be of interest to evaluate whether a GABAergic understimulation influences the effects of NMDA on the MNs. The present work addressed this issue.

2. Results

Intrathecal administrations of vehicle failed to induce any perceptible changes in motor behavior. Injection of bicuculline at a dose of 2 μg/rat (n=10) in all animals produced body tremor and heavy clonic convulsions of limbs, 6 rats further manifested tonic seizures of limbs. This dose of bicuculline was excluded from further tests.

No behavioral effects were induced by bicuculline (50 and 250 ng/rat), NMDA (60 and 300 ng/rat), and muscimol (250 ng/rat) individually; the combination of bicuculline and NMDA at the doses of 50 and 60 ng/rat, respectively was ineffective as well. In contrast, the combination of the large doses of bicuculline and NMDA (250 and 300 ng/rat, respectively) induced periodic spasms of the tail and lower extremities. These spasms appeared once the animals recovered from anesthesia and lasted for about 1.5 h.

On the 4th day after the large-dose combination treatment, certain impairments in the hindlimb functions were noted in some rats. During the next days, the signs of hindlimb motor deficit progressed, and by the 9th day a paraplegia with persistent bilateral spastic extension developed in all animals. In result, the rats used only their forelimbs for locomotion. No visible deteriorations of the forelimb functions were observed in paraplegic animals. Motor impairment induced by the large-dose combination was significantly reversed by muscimol. During 12 days of observation, the ambulation of the animals treated with vehicle, bicuculline, NMDA, muscimol, or the low-dose bicuculline–NMDA combination was almost normal (no significant difference among these groups, $p>0.05$; Table 1).

24 h, 3, 8 and 12 days after the intrathecal injections of vehicle, bicuculline, NMDA, muscimol, and the bicuculline–NMDA combinations, the spinal cords of animals were examined.

Macroscopic observation of the spinal cords did not reveal any signs of hemorrhage. Histological examination found distinct pathological alterations in spinal cord of the animals treated with the large-dose bicuculline–NMDA combination (Figs. 1 and 2). Bicuculline, NMDA, and muscimol alone, as well as the low-dose combination of bicuculline and NMDA, did not induce any histological abnormalities.

Changes in the gray matter. Damage to the MNs was found in the lumbar and, to a lesser extent, thoracic spinal cord while the cervical cord was very weakly affected. The motoneurons were changed mainly in the ventral horns. The injured MNs were scarce in the lateral horns and absent in dorsal ones.

24 h after injection, two kinds of alterations were found in some MNs. A distinct swelling of perikaryon with an accumulation of the Nissl substance near nucleus can be seen, these cells became rounded (Fig. 2b). Another form of abnormality was a hyperchromatosis and cell shrinkage (Fig. 2c).

Table 1 – The ability of rats to walk with lower extremities.

Drug (dose)	Motor function score at different days after i.t. injection		
	4th	9th	13th
Vehicle	5	5	5
Bicuculline (250 ng)	5	4.82±0.69	4.69±0.71
NMDA (300 ng)	4.87±0.62	5	4.73±0.67
Muscimol (250 ng)	4.76±0.64	5	5
Bicuculline (50 ng), NMDA (60 ng)	4.84±0.70	4.97±0.73	4.74±0.68
Bicuculline (250 ng), NMDA (300 ng)	2.17±0.35†	0†	0†
Bicuculline (250 ng), NMDA (300 ng), muscimol (250 ng)	4.61±0.74	3.97±0.64	4.18±0.66

Measurements are expressed as means±SEM (n=10–16).
The groups were compared at the same time point.
†Significantly different from all other groups, $p<0.01$.