The Neuroprotective Effects of Semax in Conditions of MPTP-Induced Lesions of the Brain Dopaminergic System


This report describes studies of the effects of the ACTH(4–10) analog Semax (MEHFPGP) on the behavior of white rats with lesions to the brain dopaminergic system induced by the neurotoxin MPTP. Neurotoxin was given as single i.p. doses of 25 mg/kg. Neurotoxin injections were shown to decrease movement activity and increase anxiety in the animals. Daily intranasal administration of Semax at a dose of 0.2 mg/kg decreased the severity of MPTP-induced behavioral disturbances. The protective activity of Semax in MPTP-induced lesions of the brain dopaminergic system may be associated with both its modulating effect on the dopaminergic system and the neurotrophic action of the peptide.

KEY WORDS: ACTH fragments, Semax, dopaminergic system, MPTP, rats.

ACTH/MSH-like peptides, collectively known as “melanocortins,” represent a class of endogenous regulatory peptides currently subject to active study. This class of peptides includes adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), and their fragments and synthetic analogs. These compounds are interesting because peptides of this class have marked actions on central nervous system (CNS) function. The first data on the neurotrophic effects of melanocortins were obtained from studies of their effects on animal behavior. These studies showed that these peptides act on learning, motivational processes, and the concentration and attention abilities of animals. The behavioral effects of melanocortins are not associated with their hormonal activity, but result from their direct actions on the CNS [9]. Structural-functional studies identified the amino acid sequence for a minimal ACTH fragment retaining its behavioral activity. The most marked effects on behavior were seen with the fragment ACTH(4–10) – Met-Glu-His-Phe-Arg-Trp-Gly, which retains the behavioral effects of the whole molecule. Tetrapeptide ACTH(4–10) has almost the same activity [4, 11].

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The literature now contains many reports on the effects of melanocortins on nervous tissue development and regeneration processes [12]. Peptides of this class promote neuron survival and the growth of neurites in tissue cultures [19]. It has also been demonstrated that ACTH, MSH, and their fragments and analogs have neurotrophic and neuroprotective influences on the central and peripheral nervous systems during early ontogenesis and in neuronal damage in adult animals. Peripheral administration of melanocortins during the neonatal period accelerates maturation of the neuromuscular system, affects CNS development, and leads to long-term changes in animal behavior [18]. In peripheral nerve lesions in adult animals, administration of melanocortins accelerates nerve regeneration and muscle reinnervation, as demonstrated by electrophysiological, morphological, biochemical, and functional tests [21]. Positive influences of melanocortins have also been noted in experiments with central nervous system lesions induced both by transection of various parts of the brain and by administration of neurotoxins. Peripheral administration of ACTH fragments and their analogs accelerates functional recovery after damage to the hippocampus, labyrinthectomy, and bilateral lesions and section of the fornix. In addition, it has been demonstrated that administration of the ACTH(4–9) analog ORG 2766 has marked protective actions in lesions to the substantia nigra due to administra-
tion of the neurotoxin 6-OHDA. Animals given the peptide showed accelerated recovery of behavioral, morphological, and biochemical parameters as compared with controls [6].

Thus, extensive experimental data have now been accumulated providing evidence of the neurotrophic influences of melanocortins on the developing and regenerating nervous system. Use of short ACTH fragments and their analogs lacking hormone activity allows the endocrine properties of the hormones to be separated from their neurotrophic effects. Structural-functional studies have demonstrated that as in the case of the behavioral effects, the neurotrophic activity is due to the N-terminal part of the ACTH molecule [12]. Immunochemical methods have demonstrated the existence of an endogenous ACTH(4–10) fragment in the rat brain during the early neonatal period. In adult animals, immunoreactivity to ACTH(4–10) has been noted after lesioning of the nervous system. It has been suggested that the endogenous ACTH(4–10) fragment is formed in the brain during the period of nervous system development and during the regeneration of nerve tissue [7].

A significant disadvantage of natural melanocortins is their short duration of action. Many investigators have tried to create highly effective analogs of ACTH fragments by introducing various modifications of the primary structure of the molecule. These experiments resulted in the development of analogs of natural peptides lacking hormonal effects but having marked neurotrophic activity and high protease stability. Examples of such compounds include the ACTH(4–9) analogs ORG 2766 and HOE-427, as well as the ACTH(4–10) analog BIM 22015, which have been shown to have high levels of behavioral, neurotrophic, and neuroprotective activity in in vivo and in vitro experiments. These peptides improve learning and memory, accelerate regeneration of peripheral nerves after transection, and have neuroprotective actions in various models of pathology [16, 17, 20]. Preliminary clinical studies have supported the data obtained in animal experiments. However, none of the synthetic analogs listed above has yet been introduced into clinical practice. The ACTH(4–10) heptapeptide analog Semax (Met-Glu-His-Phe-Pro-Gly-Pro) has been developed and studied at the Institute of Molecular Genetics, Russian Academy of Sciences and the Faculty of Biology, Moscow State University. Studies of the physiological activity of this peptide have demonstrated that it improves memory and attention, has antihypoxic and antihemorrhagic effects, and promotes decreases in the severity of the clinical and neurophysiological manifestations of experimental ischemic insult. Thus, Semax retains the spectrum of activity of natural ACTH fragments and the effects are manifest for prolonged periods [8]. Semax is currently used in medicine as a nootropic agent. Clinical studies have shown its high efficacy in the treatment of cognitive/memory disorders of different origins and in the prophylaxis and treatment of post-anesthesia memory impairments [1]. Administration of Semax has marked positive actions in the treatment of stroke [3]. Recent studies have established that the peptide can increase the lifetime of nerve cells in primary cultures from rat embryo brain [2].

Studies of the neurotrophic effects of Semax continue, using a variety of experimental models, the aim being to widen the spectrum of its clinical application. The present study addresses the effects of Semax on an MPTP-induced model of parkinsonism. Parkinson’s syndrome arises as a result of damage to dopaminergic neurons in the substantia nigra. Parkinson’s syndrome arises as a result of damage to dopaminergic neurons in the substantia nigra, leading to decreases in dopamine levels in the striatum [13]. These lesions can be modeled experimentally by administration of the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which specifically damages dopaminergic neurons in the substantia nigra, inducing the development of parkinsonian symptoms. Biochemical and structural changes induced by systemic administration of MPTP are accompanied by disorders of behavioral reactions and the development of depressive behavior [14]. The aim of the present work was to study the efficacy of Semax in conditions of MPTP-induced lesions to the brain dopaminergic system.

**METHODS**

Studies were performed on male white mongrel rats weighing 180–230 g. Animals were kept in standard animal-house conditions and all experiments were performed between 11:00 and 18:00. Lesions of the dopaminergic system were induced by single injections of MPTP (25 mg/kg, i.p., in physiological saline). Animals received intranasal Semax at doses of 0.05 and 0.2 mg/kg 30 min after neurotoxin administration. Semax was subsequently given daily for four days one hour after testing. Each series of experiments involved four groups of animals (18–20 rats per group); controls received i.p. physiological saline and intranasal water; the Semax group received i.p. physiological saline and intranasal Semax; the MPTP group received i.p. MPTP and intranasal water; the combined treatment group received i.p. MPTP and intranasal Semax.

One day after MPTP administration, the animals’ behavior was measured in an open field test (in stress-free conditions) followed by testing in the burrow box 1 test. Rats were subsequently tested once daily using the following methods: an elevated maze test at two days, burrow box 2 at three days, and in the open field test (stressful version) at four days.

**Open Field Test (OFT).** The experimental chamber was a round arena 80 cm in diameter with a wooden floor divided by two concentric circles and eight diameters. A 500-W electric lamp was placed 80 cm above the arena, along with a domestic electric bell and a red 15-W lamp. For testing, animals were placed in the center of the arena and horizontal movement activity was monitored visually.