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PHYSIOLOGY

Effects of Semax on Dopaminergic and Serotoninergic Systems of the Brain

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Short-term effects of the synthetic nootropic peptide Semax on the content and metabolism of monoamines in the brain of $C57/Bl_6$ mice and Sprague–Dawley rats were studied. Intraperitoneal injection of Semax at a dose of 0.15 mg/kg caused an increase in the tissue concentrations of 5-hydroxyindoleacetic acid (5-HIAA) in the hypothalamus and striatum of mice 0.5 and 2 h after the injection. Using brain microdialysis, we detected increased levels of extracellular 5-HIAA in the striatum of freely moving rats 1 h after administration of 0.15 or 0.6 mg/kg Semax. The effect lasted for an additional 3 h. These data suggest that Semax produces a modulatory effect on the serotoninergic systems of the animal brain, increasing the turnover rate of serotonin.

The search for drugs improving memory and enhancing cognitive functions in humans, and the study of the mechanisms of their effects are an important scientific problem. Semax, a recently developed nootropic drug, is a synthetic peptide Met–Glu–His–Phe–Pro–Gly–Pro, structurally analogous to region 4–10 of adrenocorticotropic hormone (ACTH) but devoid of its hormonal activity [1]. The Pro–Gly–Pro segment of Semax is responsible for its metabolic stability and the relatively long duration of its nootropic effect. As shown in a number of studies, Semax improves learning and memory in animals, increases the concentration and attention during information processing, and relieves mental fatigue in humans [1, 2]. However, the neurochemical mechanisms of Semax effects are still far from clear.

Recent studies demonstrated that various ACTH analogues, including Semax, possess the properties of antagonists of melanocortin (MC) receptors [3]. Convincing evidence has been provided for the existence of close functional and anatomical links between the melanocortin and monoaminergic systems of the brain [4, 5]. Intracerebroventricular administration of α-melanocyte-stimulating hormone induces grooming behavior in rats and simultaneously raises the intracellular level of dopamine in the striatum [6].

The goal of this study was to examine the effects of Semax on the neurochemical parameters of the dopaminergic and serotoninergic systems of the animal brain. Semax was administered at a dose of 0.15 mg per kilogram body weight to $C57/Bl_6$ mice, and their hypothalamus and striatum were analyzed for tissue levels of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as serotonin (or 5-hydroxytriptamine, 5-HT) and its metabolite (5-HIAA). The mice were sacrificed by decapitation 0.5 or 2.0 h after Semax injection. Brain structures were isolated on ice and stored in liquid nitrogen. The monoamines were identified by high-performance liquid chromatography (HPLC) using a Phenomenex 4-μm C18 column (150 × 4.6 mm) and an LC-4B electrochemical detector (BAS), as described previously [7]. In order to provide more detailed data, we used brain microdialysis to assess extracellular levels of DA, DOPAC, HVA, and 5-HIAA in the striatum of Spague–Dawley rats treated with 0.15 or 0.6 mg/kg Semax. A microdialysis probe was stereotactically implanted one day before the experiment. Dialysate samples were collected every 20 min. DA, DOPAC, HVA, and 5-HIAA were determined by HPLC (ESA 850) using a Phenomenex 4-μm C18 column (150 × 4.6 mm) and an electrochemical Coulochem II detector (ESA) [8].

Statistical analysis of the results was performed using the Statistica 5.0 software. In experiments where tissue levels of monoamines were measured, the significance of differences between the groups was assessed with the nonparametric Mann–Whithey *U* test; in microdialysis experiments, the significance of differences between groups at different time intervals was assessed using ANOVA for repeated measurements (Duncan's post hoc test). The data are shown as M ± SEM.

In the first series of experiments, we studied the effect of Semax injected intraperitoneally at a dose of 0.15 mg/kg on the tissue monoamine levels in the striatum and the hypothalamus of $C57/Bl_6$ mice. As follows from the data shown in Fig. 1, 30 min after the intraperitoneal injection, the tissue levels of DA and HVA in

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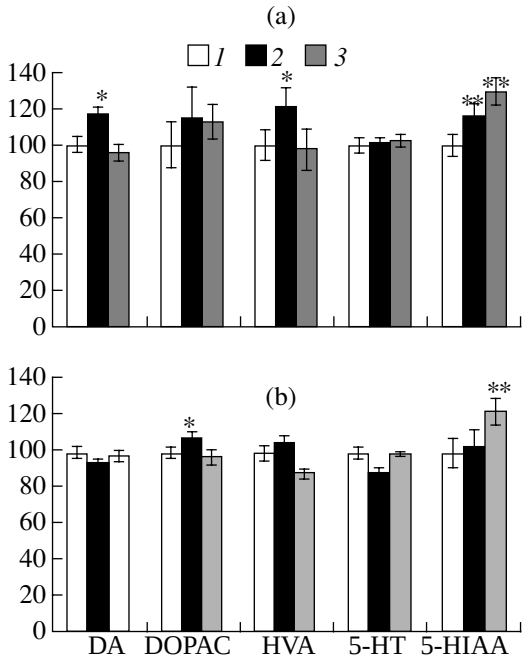

Fig. 1. Effects of intraperitoneal Semax administration (0.15 mg/kg) on tissue concentrations of monoamines and their metabolites in (a) the hypothalamus and (b) the striatum of C57/Bl_6 mice: (*1*) control animals; (*2*) animals sacrificed 30 min after Semax injection; and (*3*) animals sacrificed 120 min after Semax injection. Data are presented relative to the control (taken as 100%). One and two asterisks indicate differences from the control significant at $p < 0.1$ and $p < 0.05$, respectively.

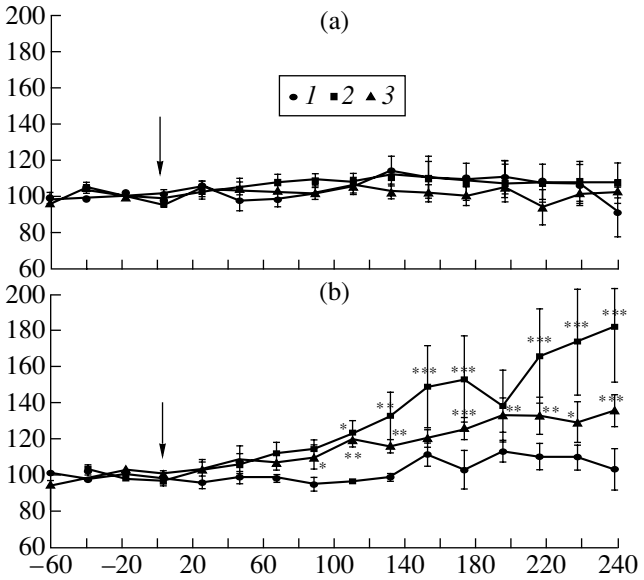

Fig. 2. Effects of Semax on extracellular concentrations of (a) DOPAC and (b) 5-HIAA in the striatum of freely moving Sprague–Dawley rats: (*1*) control; (*2*) Semax, 0.15 mg/kg; and (*3*) Semax, 0.6 mg/kg. Abscissa: time, min. Data are presented relative to the basal level (taken as 100%). One, two, and three asterisks indicate differences from the control significant at $p < 0.1$, $p < 0.05$, and $p < 0.01$, respectively.

the hypothalamus tended to rise ($p < 0.1$); the 5-HIAA level was significantly increased ($p < 0.05$). Its level in the hypothalamus became even higher 120 min after the injection. In the striatum, the 5-HIAA level tended to increase 30 min after the injection ($p < 0.1$) and, as in the hypothalamus, it was increased significantly 120 min after the injection ($p < 0.05$). In microdialysis experiments with rats, Semax injected at a dose of 0.15 or 0.60 mg/kg did not change significantly the extracellular concentrations of DA (data not shown) and its metabolites, DOPAC (Fig. 2a) and HVA (data not shown). The level of 5-HIAA, a metabolite of serotonin, increased gradually in the striatum between 1 and 4 h after the injection ($p < 0.05$; Fig. 2b). The effect was greater at the Semax dose of 0.15 mg/kg ($p < 0.05$, ANOVA).

It is generally accepted that the concentration of 5-HIAA, a metabolite of serotonin, rises with an increase in serotonin turnover, which may reflect activation of the brain serotoninergic system. Interestingly, the time course of 5-HIAA accumulation in the striatum of C57/Bl_6 mice (Fig. 1b) is closely correlated with the time course of the changes in the extracellular concentration of this metabolite observed in Spague–Dawley rats under the conditions of a microdialysis experiment (Fig. 2b). These data agree with our earlier results [9], according to which, the neurochemical parameters of the serotoninergic system of the brain remain altered even 24 h after Semax administration. The results of this study further confirm the assumption that the serotoninergic system of the brain is involved in the mechanism of action of Semax. Chaki *et al.* [10] suggested that MC_4 receptor antagonists activate the serotoninergic system of the animal brain. We also do not exclude that the effects of Semax on the serotoninergic system of the brain are related to its ability to act as an MC_4 receptor antagonist [3, 5].

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