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The Role of Dopaminergic System in the Immunostimulatory Effects of Substance P and Its Analog

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Systemic administration of synthetic substance P or its analog EC-1 to CBA mice results in considerable stimulation of immune reactions. No stimulation is observed after disconnection of the hypothalamus from the pituitary. It is concluded that the immunostimulating effect of these peptides is mediated by the dopaminergic system, since it is abolished by the D_2 receptor blocker haloperidol.

Key Words: *neuropeptides; immunostimulation; dopaminergic system; pituitary*

It is known that substance P stimulates both T and B cells [6,13]. On the other hand, this substance may have a role in the etiology and pathogenesis of various mental disorders and in stress reactions [3,11]. Several analogs of substance P exhibit biological activity in the central nervous system [10]. However, the neurochemical mechanisms by which tachykinins (for example, substance P) modulate immunogenesis remain obscure. Functional interactions between P-peptidergic terminals and dopamine (DA) cell bodies have been demonstrated in various brain structures [5,15]. The receptors for substance P have been identified in the substantia nigra and neostriatum [15]. In the present study we attempted to evaluate the contribution of the interactions between tachykinins and dopaminergic system to the immune response.

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MATERIALS AND METHODS

CBA mice (n=161, body weight 20-22 g) were used. Substance P (Vektor) or its analog EC-1 (a generous gift of Dr. O. S. Papsuevich from the Institute of Organic Chemistry, Riga) were injected in doses of 1, 10, and 100 μg/kg 30 min before immunization. The mice were treated with 2 mg/kg haloperidol (Gedeon Richter) 2 h before the antigen administration. All substances were injected intraperitoneally in 0.2 ml of physiological saline. In experiments with more than one test substance, the interval between injections was 10 min. The mice were immunized with sheep erythrocytes (5×10^8 cells, intravenously). The immune response was evaluated by counting plaque-forming cells [7] and rosette-forming cells (RFC) [2] on days 4 and 5 postimmunization, respectively.

The pituitary stalk was destroyed transauricularly under Nembutal anesthesia (50 mg/kg) 10 days

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TABLE 1. Stimulatory Effects of Substance P and Its Analog EC-1 on IgM Antibody-Producing Cells (APC) in the Spleen of CBA Mice on Day 4 After Immunization with Sheep Erythrocytes (5×10^8, $M\pm m$)

Treatment	Dose, μg/kg	IgM APC/10^6 cells	IgM APC/spleen
Physiological saline (control)		73.4±6.0	8916.0±830.2
Substance P	1	255.6±24.3**	35528.4±2055**
	10	243.4±32.7**	32205.6±3857**
	100	203.4±50.8*	31642.0±2378**
EC-1	1	235.8±13.8**	29845.0±1943**
	10	279.7±14.0**	36270.0±4450**
	100	250.3±16.5**	32500.0±1697**

Note. There were no less than 10 mice in each series; *p=0.02, **p=0.001 compared with control.

before experiments. The precision of destruction was verified after instantaneous decapitation of the mouse.

The results were analyzed by Student's t test.

RESULTS

Both neuropeptides stimulated immune reactions. In all studied doses substance P and EC-1 increased the number of splenic cells secreting antigen-specific IgM antibodies (Table 1) as well as the number of RFC (Table 2). The intensity of immune response did not depend on the dose of substance P, but was higher at higher doses of its analog. These results agree with the observation that substance P stimulates the production of IgM and IgG [13].

TABLE 2. Prevention of the Immunostimulatory Effects of Substance P and Its Analog EC-1 by Pituitary Stalk Transection and by Haloperidol Injection on Day 5 After Immunization with Sheep Erythrocytes (5×10^8, $M\pm m$)

Treatment	Dose, μg/kg	RFC/1000 cells
Physiological saline (control 1)		12.2±1.2
Substance P	1	24.2±0.6*
	10	23.4±1.4*
	100	23.2±1.1*
EC-1	1	22.1±1.0*
	10	26.7±1.0*
	100	26.5±1.1*
Operation (transection of pituitary stalk)		10.8±0.4
Operation+substance P	1	9.1±1.3
Operation+EC-1	10	10.7±1.0
Physiological saline (control 2)		24.7±0.5
Substance P	1	40.0±1.8**
Haloperidol	2 mg/kg	14.2±0.4**
Haloperidol+substance P		24.4±0.5

Note. There were no less than 10 mice in each series; p<0.001: *compared with the control 1, **compared with the control 2. RFC = rosette-forming cells.

From the available physiological, pharmacological, and anatomical data it can be concluded that substance P meets most criteria characterizing neurotransmitters presumably involved in immunomodulation. Substance P is widely distributed in the central nervous system, and binding sites for it have been identified in a number of brain structures, including the hypothalamus. It is likely that substance P plays a role in the regulation of the synthesis of pituitary hormones [9]. On the other hand, murine T and B cells have receptors for substance P [14] and can be stimulated by this neuropeptide [13]. Therefore, it was interesting to examine the immunomodulating effects of substance P and its analog. A series of experiment was performed in which the connection between the hypothalamus and pituitary was interrupted before administration of substance P or EC-1. The immune response of operated mice remained at the control level, suggesting that substance P and its analog produce a central effect on the immune response development (Table 2). Similarly to our previous findings [1], destruction of the pituitary stalk had no effect on the immune response.

Recent studies have shown that substance P modulates the release and activity of DA from nigrostriatal DA neurons [8,11]. Specifically, local injection of substance P into the substantia nigra increases DA release in the ipsilateral caudate nucleus [12]. It has been suggested that the interaction of nigral dopaminergic neurons and terminals of the descending P-peptidergic pathway in the substantia nigra [11] is mediated by D_2 receptors [4]. Previously, we showed that a group of A9 neurons located in the compact zone of this structure is involved in dopaminergic immunomodulation; bilateral electrolytic destruction of A9 led to a substantial reduction

of the immune response [1]. It seems likely that the stimulation of immunogenesis observed in mice injected with substance P is associated with the dopaminergic system that contributes considerably to neuroimmunostimulation [1]. In fact, blockade of postsynaptic D_2 receptors with haloperidol prevented the increase in the number of RFC observed in substance P-treated mice (Table 2). As in a our previous study [1], this blocker markedly suppressed the immune response. Single and prolonged haloperidol administration produces different effects on the substance P levels in various brain structures, including the substantia nigra [11]. There is clinical evidence that neuroleptics (particularly, haloperidol) alters the substance P concentrations in the specific brain structures. From our results and the published data it can be concluded that the effects of substance P are DA-dependent and are mediated via D_2 receptors.

Thus, stimulation of immune reactions involves the interaction between the central P-peptidergic and dopaminergic systems.

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