# Dopaminergic Mechanisms in the Immunostimulating Effect of μ-Opioid Receptors

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Immunogenesis was appreciably stimulated in CBA mice injected the μ-opioid receptor agonist DAGO. This stimulation was prevented by transecting the pituitary peduncle. Immunoactivation induced by opioid peptide was due to interaction with the dopaminergic system, because the effect is canceled by preliminary haloperidol blocking of $D_2$-postsynaptic receptors.

**Key Words:** *μ-opioid receptors; immunostimulation; dopaminergic system*

Our previous studies demonstrated the contribution of the central δ- and κ-opioid receptors to immunosuppression [3,4]. The neurotransmitter serotoninergic system inhibiting immunogenesis has been shown to be involved in this effect [1]. On the other hand, recent studies have revealed interactions between the μ-opioid peptidergic and dopaminergic (DA) systems in various structures of the brain [5]. The functional role of the DA system in neuroimmunomodulation has been shown to be activating [1]. In order to elucidate the role of the μ-opioid system in immunogenesis and its clinical significance [15], we investigated the central mechanisms of immune system regulation during interactions of μ-opioid and DA receptors in the course of immunogenesis.

## MATERIALS AND METHODS

Experiments were carried out with 120 male CBA mice weighing 22-24 g. The specific agonist of μ-opioid receptors DAGO [D-Ala$^2$, N-ME-Phe$^4$,Gly$^5$-ol]-Enkephalin (Vektor, Novosibirsk) was injected intraperitoneally 30 min before immunization of sheep red cells ($5 \times 10^8$) in a dose of 0.1 mg/kg. Haloperidol (Gedeon Richter), a selective blocker of the postsynaptic type $D_2$ DA receptors, was administered in a dose of 2 mg/kg 2 h before the antigen. If the agents were injected together, the interval was 10 min. Control mice were injected normal saline in the same doses (0.2 ml) as the agents according to the same protocol. The immune response was tested at the peak of its development by the number of plaque-forming cells (day 4 postimmunization) and of rosette-forming cells (day 5) in the spleen of each mouse. The contribution of the pituitary to the effect of DAGO on immunogenesis was studied in mice in which the pituitary peduncle was cut 10-14 days before the experiment. The operation was performed transauricularly under Nembutal narcosis (50 mg/kg). The accuracy of destruction of the pituitary peduncle was assessed visually after instantaneous decapitation of animals. The results were statistically processed using Student's *t* test. Differences were considered reliable at $p \leq 0.05$.

## RESULTS

The immunomodulating role of μ-opioid receptors was assessed by using the synthetic opioid peptide DAGO. This agent is highly selective for these receptors [14] and is most frequently used for *in vivo* studies. A single injection of DAGO appreciably (almost 3 times) boosted the immune reaction (Table 1) under conditions of activation of μ-opioid receptors. Table 1 shows that this process is associated with a sharp increase in the number of IgM-plaque-forming cells and total rosette-forming cells. Published data on the effects of μ-receptor agonists on the immune response are contradictory and depend, among other things, on their dose and on the conditions of the experiment [6].

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**TABLE 1.** Prevention of Immunostimulating Effect of DAGO in CBA Mice Immunized with Sheep Red Cells by Transection of the Pituitary Peduncle and Haloperidol (HP) Administration (*M±m*)

| Agent | Plaque-forming cells | | Rosette-forming cells per $10^3$ cells |
|---|---|---|---|
| | per $10^6$ cells | per spleen | |
| Normal saline (control) | 95.3±10.0 | 7969.0±1093.7 | 18.8±1.0 |
| DAGO* | 257.0±30.0 | 28478.7±4472.8 | 52.8±2.2+ |
| HP** | | | 14.4±0.3++ |
| HP+DAGO | | | 19.5±1.3 |
| Transection of pituitary peduncle | 103.3±11.7 | 8080.2±965.0 | 21.8±1.8 |
| Operation+DAGO | 99.6±13.2 | 8583.1±1180.6 | 22.3±0.8 |

**Note.** Each experimental series included at least 10 animals. *DAGO was injected in a single dose of 0.1 mg/kg 30 min before immunization. **HP was injected in a dose of 2 mg/kg 2 h before immunization. +$p<0.001$, ++$p<0.002$ *vs.* the control.

There are many μ-opioid receptors in the brain. Autoradiographic examination of mouse brain using $^3$H-dihydromorphine revealed the highest density of high-affinity μ-receptors in the striatum, nucleus accumbens, thalamus, and, less so, in the hypothalamus [12]. The majority of these receptors contribute to the processes of immunomodulation [1]. On the other hand, some reports indicate that immunocompetent cells of heterogeneous populations possess sites for binding opioid receptor ligands, including those for the μ-type receptors [13]. Hence, it is not clear whether the immunostimulating effect of DAGO is a result of its direct effect on the receptors situated on the cells or whether it is realized through the central mechanisms, specifically, involving the hypothalamo-pituitary complex, as was shown for neurotransmitter systems [1]. That study proved the involvement of μ-opioid receptors of the brain in neuroimmunomodulation. Severing of the connection between the hypothalamus and pituitary prevented stimulation of the immune response under the effect of DAGO in comparison with that in sham-operated animals (Table 1). The control level of rosette-forming cells remained the same under these conditions. The operation proper did not influence the activity of the immune response.

According to published data, the effects of μ-opioid agonists on DA neurons may be the opposite. The presynaptic effect of morphine is believed to inhibit the release of DA [9], whereas DAGO boosts DA turnover in some structures of the brain [11]. μ-Opioid receptors are known to play the principal role in animal behavior mediated by the DA system. As for immunomodulation, our experiments with DAGO injection to mice with DA receptors blocked by haloperidol showed no activation of immunogenesis (Table 1). The immune response of such animals was at the level of the control, this permitting us to conclude that DA mechanisms are involved in the manifestation of the immunostimulating effect of DAGO. According to the literature, there are at least 2 types of DA receptors: $D_1$ and $D_2$. Since the effect of haloperidol is related to postsynaptic $D_2$-receptors [16], a relationship between the μ-opioid and DA systems at the level of these receptors may be hypothesized. The detected interactions might be determined by the existing morphofunctional relationships of the enkephalinergic and DA systems in such brain structures as the striatum or nucleus accumbens [8,10], whose contribution to neuroimmunomodulation has already been reported.

Hence, experiments with agents affecting the activity of the μ-opioid peptidergic and DA systems showed that modulation of the immune reactions is mediated by interactions of these systems.

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