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Pharmacological Modulation of Beta-Endorphin in Rat Peritoneal Macrophages

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The neuropeptide beta-endorphin is present in cells of the immune system, i.e., lymphocytes and monocytes, and its expression can be induced by immunological stimuli. In the present study, we showed that the increase of the serotoninergic availability induces an increase of beta-endorphin concentrations in rat peritoneal macrophages that is blunted by the administration of serotonin receptor antagonists. A significant increase of beta-endorphin concentrations is also evident after blocking the dopaminergic receptors, whereas a dopaminergic agonist decreases the concentrations of the peptide. Our data are consistent with a similar modulation of beta-endorphin concentrations in central nervous system and in immune cells, e.g., rat peritoneal macrophages.

Key words: Beta-endorphin, serotonin, dopamine, macrophages

INTRODUCTION

Proopiomelanocortin (POMC)-derived peptides, e.g., beta-endorphin (BE) and adrenocorticotropic hormone (ACTH) are present in cultured cells of the immune system, i.e., lymphocytes and monocytes (1–3). Receptors on immune cells for these peptides have also been described (4,5) and their transduction partially elucidated (6). The expression of POMC-derived peptides in the immune system was originally shown to be inducible in vitro by immunological stimuli, e.g., Newcastle virus or concanavalin-A (1–3). Several studies showed that the POMC peptides present in immune cells are synthetized by the cells, and not absorbed from plasma (7). In the present study, we investigated whether BE was expressed in resting rat peritoneal macrophages in vivo. Moreover, because the pharmacological modulation of BE in the central nervous system is well elucidated (8,9), we wondered if the peptide could be similarly modulated in the immune system.

MATERIALS AND METHODS

Collection of Macrophages

Sprague-Dawley male CD rats, 200 g body weight (Charles River, Calco, Italy), ten in each experiment, were used. After decapitation, peritoneal cells were recovered by injection of 50 ml phosphosaline buffer (PBS), 10 μ/ml heparine, into the rat peritoneal cavity and withdrawal of 30 ml. Cells obtained by this peritoneal lavage were washed, resuspended in a small volume of PBS, and differentially counted (10,11).

Measurement of BE in Macrophages

Cell aliquots (20×10^6) were suspended in 1 ml 0.1 N acetic acid, homogenized in a blade homogenizer, and sonicated. The supernatant was separated after 10 minute centrifugation at 10,000 × g and kept frozen at −20° until the moment of assay. BE was measured by radioimmunoassay with a C-terminal-specific antibody raised in our laboratory. The entire radioimmunoassay procedure has been previously described and validated (12). BE identity was assured by previous HPLC separation of samples. The high-pressure liquid chromatography (HPLC) procedure is the same used in our laboratory for plasma or tissue samples as has been previously described and validated (13).

Pharmacological Treatments

Rats were treated intraperitoneally for 15 days with either saline or the serotonin precursor 5-hydroxytryptophan at the dose of 30 mg/kg twice daily, the blocker of serotonin reuptake chlorimipramine at the dose of 20 mg/kg twice daily, or the serotonin receptor antagonist metergoline at the dose of 7.5 mg/kg twice daily. The serotonin receptor antagonist was also administered together with 5-hydroxytryptophan or chlorimipramine. To study the role of the dopaminergic system, rats were treated subcutaneously with either saline or the dopaminergic receptor agonist bromocriptine at the dose of 5 mg/kg, the antagonist haloperidol at the dose of 2 mg/kg, or the combination of the two drugs. Animals were killed 12 hours after the last treatment.

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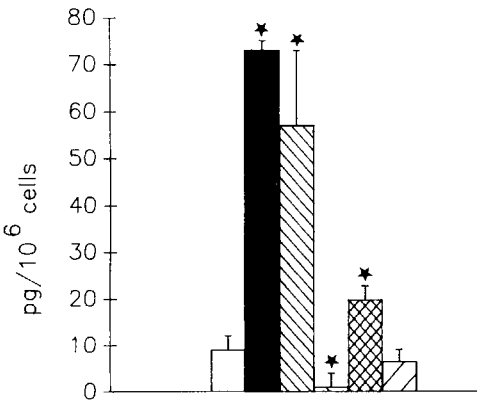

Fig. 1. Beta-endorphin concentrations in rat peritoneal macrophages after chronic treatment with serotoninergic and antiserotoninergic drugs: □, saline; ■, 5HTP; ▨, chlorimipramine; ▨, metergoline; ▨, 5HTP + metergoline; ▨, chlorimipramine + metergoline. * = $P <$ 0.01 vs. saline. Dunnet's test for multiple comparisons.

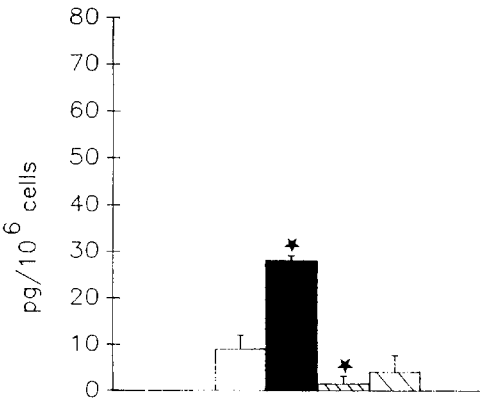

Fig. 2. Beta-endorphin concentrations in rat peritoneal macrophages after chronic treatment with dopaminergic and antidopaminergic drugs: □, saline; ▨, bromocriptine; ■, haloperidol; ▨, haloperidol + bromocriptine. * = $P < 0.01$ vs. saline. Dunnet's test for multiple comparisons.

RESULTS

Figure 1 shows that both 5-hydroxytryptophan and chlorimipramine induce an increase of BE concentrations in macrophages that is partially blunted by the concomitant treatment with the serotoninergic antagonist metergoline. This figure also shows that metergoline, when administered alone, decreases the concentration of BE in macrophages. As shown in Figure 2, BE concentrations increased after administration of the dopaminergic antagonist haloperidol, whereas they decreased after bromocriptine. When administered together, bromocriptine and haloperidol do not modify BE concentrations. HPLC analysis of the peritoneal macrophage extracts showed a peak with the same retention characteristics of synthetic BE (data not shown).

DISCUSSION

The data presented indicate that BE is present in resident peritoneal macrophage in vivo, and its expression can be modulated both by immunological and non-immunological stimuli. The measurement of BE in resting immune cells is an interesting finding. In previous experiments, the presence of BE was observed mainly when immune cells had been stimulated, e.g., lymphocytes in vitro exposed to Newcastle virus (2,14). In contrast, we were able to measure BE also in resting cells obtained from normal animals that were free of any experimental immunological stimuli. The present data also offer some new insight into the possibility of modulating BE concentrations in immune cells. Both serotonin and dopamine seem to have important roles in the expression of the opioid peptide. Similar to that observed in the central nervous system, serotonin has a stimulatory effect on BE, whereas dopamine is inhibitory (8,9). We observed, in fact, that both the serotonin precursor 5-hydroxytryptophan and the blocker of serotonin reuptake chlorimipramine induce an increase of BE concentrations in macrophages that is blunted by the serotonin antagonist metergoline. The inhibitory role of dopamine is suggested by the observation that bromocriptine, a dopamine receptor agonist, induces a decrease in BE concentrations that is blunted by the receptor antagonist haloperidol.

Interestingly, both metergoline and haloperidol exert their effects also in basal conditions, suggesting that expression of BE in resting peritoneal macrophages is under tonic dopaminergic and serotoninergic control. This observation is consistent with the presence in the immune system of receptors for the two neurotransmitters and of receptors for tricyclic antidepressants on lymphocytes (15–17).

Because all of the drugs we used freely cross the blood-brain barrier, the problem arises as to whether the effects we observed are exerted directly on macrophages or if they are mediated through the central nervous system. The presence of dopamine and serotonin receptors on macrophages is consistent with a direct effect; however, there are multiple evidences for modifications of the immune system following pharmacological or surgical interventions on neurotransmitters in the central nervous system (18). Further studies with drugs that do not cross the blood-brain barrier are necessary in order to elucidate this point.

In conclusion, our data offer a new perspective in the modulation of neuropeptides contained in immune cells. Because peptides have been shown to exert multiple modulatory effects on the immune system, the possibility of a new pharmacological approach to the modulation of immune responses is predictable.

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