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Modulation of Chicken Plaque-Forming Cells by Serotonin and Dopamine

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ABSTRACT Serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) are endogenous components of the central nervous and endocrine systems of the chicken. To determine the effects of these monoamines on antibody-mediated immunity, New Hampshire chickens of Line UNH 105 were injected intravenously with 5-HT (100 μg/kg of body weight) and DA (1 mg/kg of body weight). One milliliter of a 5% SRBC suspension was injected intravenously 30 min later. Both IgM and IgG splenic plaque-forming cells were assayed 5 days after antigen injection. For in vitro studies, spleen lymphocytes from SRBC-primed chicks were incubated with DA and 5-HT followed by quantitation of IgM and IgG plaque-forming cells. The in vitro incubation of splenic lymphocytes with specific antagonists was used to ascertain the presence of monoamine receptors on lymphocytes. The 5-HT significantly enhanced IgM plaque-forming cells compared with controls following in vivo [550 ± 85 (SE) cells/106 splenic lymphocytes versus 359 ± 44] but not in vitro exposure. The IgG plaque-forming cells were not affected by 5-HT. The DA significantly suppressed IgM plaque-forming cells responses following in vivo (284 ± 46 versus 499 ± 66) and in vitro (254 ± 57 versus 451 ± 51) exposure. Significant suppression of IgG plaque-forming cells was found in vivo (287 ± 40 versus 462 ± 75) and in vitro (153 ± 36 versus 371 ± 81) following treatment. Specific DA antagonists, apomorphine and metoclopramide, did not alleviate the in vitro suppressive effect of DA.

(Key words: serotonin, dopamine, plaque-forming cells, immunoglobulin, chickens)

INTRODUCTION

Experimental evidence supports the existence of communication between the immune and neuroendocrine systems. In rats, lesions in the anterior hypothalamus were correlated with alterations in lymphoid tissue cellularity and a decrease in the response to concanavalin A (Cross et al., 1980) but did not alter the release of corticosteroids. Preoptic lesions administered in rats impaired mitogen-induced lymphocyte blastogenesis (Roszman et al., 1982). These lesions in the rat brains were also associated with a decreased splenic natural killer activity (Cross et al., 1984). These data suggest a neuroendocrine pathway that is capable of modulating immune function.

Besedovsky and Sorkin (1977) described endocrine and neuroendocrine changes that followed antigenic stimulation. An increase in the circulating glucocorticoids was observed at the peak of the immune response. Two antigens (SRBC and trinitrophenylated hemocyanin) produced more than 100% increase in electrical activity of individual neurons in the ventromedial hypothalamus (Besedovsky et al., 1977). Furthermore, hypothalamic norepinephrine (NE) metabolism was altered by injection of an antigen or products of activated T-cells (Besedovsky et al., 1983b). Carlson et al. (1987) found that at the time the plaque-forming cell response peaked, NE decreased in the paraventricular nucleus (PVN) of the hypothalamus, whereas serotonin (5-hydroxytryptamine, 5-HT) was decreased in both the PVN and the supraoptic nucleus.

In vivo administration of 5-HT in mice suppressed the plaque-forming cell response as well as IgM and IgG antibody synthesis (DeVoino et al., 1975; Jackson et al., 1985). These authors suggested that 5-HT modulates

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the lymphocyte reactivity at peripheral sites rather than in the brain, as a limited penetration of 5-HT occurs across the blood-brain barrier. Interferon-γ-induced phagocytosis and phytohemagglutinin (PHA)-induced γ-interferon synthesis were altered by either 5-HT or dopamine (Andrade-Mena et al., 1987; Sternberg et al., 1987).

McCorkle et al. (1984) and Edens et al. (1987a,b) found that the levels of 5-HT, DA, and NE were depressed significantly in the brain, spleen, thymus, and bursa of Fabricius of turkey pouls infected with Bordetella avium, a mild upper respiratory pathogen that causes rhinotracheitis. This finding stimulated investigations of the effects of biogenic amines on avian immune responses. The PHA wattle response in chickens, a response mediated by T-cells (McCorkle et al., 1979), was significantly depressed by 5-HT, DA, or NE given 30 min prior to PHA administration (Lukacs et al., 1987). Using the same single-dose treatment, the leukocyte migration response was enhanced by 5-HT but suppressed by DA or NE given 30 min prior to PHA administration (McCorkle and Taylor, 1990a,b).

These earlier experiments investigated the monoamine effects on avian cellular immunity. So far, no studies have examined the possible effects of monoamines on antibody responses in chickens. Therefore an investigation was undertaken to determine whether in vivo and in vitro 5-HT and DA treatments would modulate chicken plaque-forming cells produced in response to SRBC.

MATERIALS AND METHODS

Animals

Six-week-old mixed sex New Hampshire chicks of Line UNH 105 were used in the present study. The line is maintained at the University of New Hampshire Poultry Research Farm. At hatching, chicks were vaccinated for Marek's disease and housed in brooder batteries with commercial feed and water available for ad libitum consumption. A light cycle of 12 h light: 12 h dark was used.

Antigen, Monoamines, and Antagonists

Sheep red blood cells from a single source were collected in Alsever’s solution. Cells were washed three times and were diluted to a 5% suspension in 9% saline. Serotonin, DA, apomorphine, a D1 receptor antagonist, and metoclopramide, a D2 receptor antagonist (Kilpatrick et al., 1986) were used for in vivo and in vitro treatments. Preliminary studies were used to determine the levels of monoamines and antagonists to be used for in vivo and in vitro experiments.

In Vivo Monoamine Treatment and Plaque Assay

The 5-HT and DA were injected intravenously at 100 μg/kg of body weight and 1 mg/kg of body weight, respectively, into two replicates of 10 chicks each. Twenty control chicks in two replicates were injected with sterile saline. Thirty minutes after monoamine administration, 1 mL of the 5% SRBC suspension was injected intravenously for antigenic stimulation. Chicks were killed by cervical dislocation and spleens removed 5 days after SRBC injection. Cells were collected in Minimum Essential Medium (MEM) by passing the spleens through a stainless steel screen. The cell suspensions were washed three times in MEM, assessed for viability by the trypan blue exclusion test (Tennant, 1964) and adjusted to a concentration of $10^6$ viable cells per milliliter of MEM. In a test tube, 150 μL of MEM, 100 μL of the lymphocyte suspension, 25 μL of 18% SRBC, and 25 μL of SRBC-absorbed chicken complement were mixed, transferred to modified Cunningham chambers, and sealed with melted paraffin. The chambers were incubated at 37 C for 60 min, after which the number of IgM plaque-forming cells were counted using a stereomicroscope (McCorkle and Leslie, 1983). The IgG plaque-forming cells were prepared according to the same procedure but with the addition of goat anti-chicken IgM antibodies to the reaction mixture. Each sample of IgG and IgM pfc cells had three chambers prepared and counted.
In Vitro Monoamine Treatment with Antagonists

Five days after intravenous immunization with SRBC, 15 birds were killed by cervical dislocation and spleens were removed. A cell suspension of $10^6$ cells per milliliter of MEM was prepared as described in the in vivo procedure. One milliliter of lymphocytes from each spleen cell suspension was placed in one of two different centrifuge tubes; control and monoamine treatment. The 5-HT (100 µg) or DA (1 mg) was then added to the monoamine tubes. All samples were incubated for 30 min on ice, washed, and resuspended in 1 mL of MEM. Both IgM and IgG plaque-forming cell assays were performed as described above. Each experiment was performed three times.

Six 1-mL splenic cell aliquots from each of 15 different chicks were prepared to test specific antagonists; control, DA, apomorphine (30 ng and 3 ng), and metoclopramide (100 ng and 10 ng). Antagonists were added to each designated tube and incubated for 30 min on ice. Cells were washed and resuspended in 1 mL of MEM. Control and DA treatments were handled in a similar manner without antagonists. The DA (1 mg) was then added to all the tubes except the controls. All samples were incubated for 30 min on ice, washed, and resuspended in 1 mL of MEM. The IgM and IgG plaque-forming cells were quantified as described.

Statistical Analysis

In vivo and in vitro treatment data were evaluated by Student's $t$ test. Analysis of variance was used to assess treatment effects in the antagonist study. Treatment means were compared with the control using the contrast procedure. In addition, antagonists treatments were compared with the DA mean.

RESULTS

Chicken IgM plaque-forming cell numbers were enhanced significantly by in vivo exposure to 100 µg 5-HT/kg of body weight (Figure 1). Plaques increased 53% above the controls after 5-HT injection. However, in vitro exposure to 5-HT had no significant effect on IgM PFC although PFC were increased 19%. The IgG plaque-forming cell numbers were not affected significantly by either in vivo or in vitro treatment with 5-HT (Figure 2).

Both in vivo treatment with 1 mg DA/kg of body weight and in vitro exposure to DA significantly suppressed IgM plaque-forming cell counts (Figure 3). Each of these treatments resulted in a 43% plaque-forming cell reduction. In vivo DA treatment reduced IgG counts by 38% and in vitro DA exposure produced 58% lower IgG counts (Figure 4). Both of these were significant suppressions.

To determine whether receptors on lymphocytes were mediating the monoamine effects, specific antagonists were used in vitro. Antagonists for 5-HT were not tested because in
**DISCUSSION**

Neuroendocrine alterations can affect the immune system. For example, hypothalamic lesions are associated with reduced lymphocyte blastogenesis and decreased natural killer cell activity in rats (Cross et al., 1980, 1984; Roszman et al., 1982). The reciprocal is true also because immune responses are correlated with neuroendocrine changes. Injection of antigen results in changes in hypothalamic electrical activity and lowered hypothalamic NE or 5-HT levels (Besedovsky et al., 1977; Carlson et al., 1983). Moreover, treatment with exogenous monoamines affects immunity as evidenced by reduced antibodies and plaque-forming cells (DeVoino et al., 1975; Jackson et al., 1985).
Evidence for immune-neuroendocrine communication exists in birds. Pathological conditions reduced 5-HT, DA, and NE in the brain of turkey pouls infected with *Bordetella avium* (McCorkle *et al.*, 1984; Edens *et al.*, 1987a,b). Cellular immune responses in chickens, such as the PHA wattle response or leukocyte migration, are also affected by experimental administration of exogenous monoamines. The PHA wattle response was suppressed by either a single dose or continuous administration of 5-HT or DA (Lukacs *et al.*, 1987; McCorkle and Taylor, 1990a,b). Serotonin enhanced leukocyte migration by either dosing method (McCorkle and Taylor, 1990a; McCorkle *et al.*, 1990). However, DA suppressed migration when given as a single dose but enhanced the response when given continuously (McCorkle *et al.*, 1990; McCorkle and Taylor, 1990b).

To the authors' knowledge, the present study is the first to demonstrate that monoamines can affect avian antibody-mediated immunity. Serotonin significantly enhances IgM plaque formation to SRBC in chickens when given as *in vivo* injection prior to immunization, but produces no effects after *in vitro* exposure when tested 5 days later. Neither *in vivo* nor *in vitro* exposure with 5-HT affects IgG plaque-forming cells. *In vitro* exposure of splenic cells was conducted 5 days after antigen injection when the plaque-forming cell responses would be at their peak. Therefore, it is unlikely that 5-HT acts through receptors on B-cells, because 5-HT did not affect the expression of plaque-forming cells when administered *in vitro*. The *in vivo* administration of 5-HT may release other hormones such as corticosterone, growth hormone, or prolactin, which could modulate the plaque-forming cell response. In the absence of receptors on plaque-forming cells, the enhanced IgM response to *in vivo* 5-HT could be due to the action of either 5-HT or a second messenger on antigen presentation, helper cell contribution, cell proliferation, suppressor activity, or subsequent differentiation of B-cells into plaque-forming cells.

Dopamine significantly suppresses both IgM and IgG plaque-forming cells after *in vivo* or *in vitro* exposure, which suggests the possibility of direct action on B-cells. This question is addressed by the *in vitro* exposure in the presence of DA antagonists. Incubation with apomorphine or metoclopramide, D<sub>1</sub> and D<sub>2</sub> receptor antagonists, respectively, does not block plaque suppression by DA at the concentrations of antagonists tested. The *in vitro* suppression of plaque-forming cells by DA, whether direct or through a second messenger, could be the result of enhanced capping of IgG on the surface of plaque-forming cells.

The cells involved in the immune response must operate in an environment containing numerous hormones and neurotransmitters. The importance of these substances may lie in concentration changes of the specific agents, or in their relationship to the concentration of other factors. An essential requirement for their participation in immunoregulation is the presence of receptors for these messengers. In mammals, receptors for corticosteroids, insulin, prolactin, growth hormone, estradiol, testosterone, NE, epinephrine, acetylcholine, and endorphins have been demonstrated on lymphoid or accessory cells (Besedovsky *et al.*, 1983a). On chicken leukocytes, the existence of receptors for 5-HT, DA, NE, and epinephrine has been suggested by leukocyte migration data (McCorkle *et al.*, 1990). The present study provides support for the existence of such receptors on immune cells with the exception of those of the B-cell lineage. The receptor concept is further strengthened by the limited ability of the monoamines to cross the blood-brain barrier and thereby bring about systemic effects.

The chicken's immune response is part of a homeostatic response that is subject to regulatory signals. The present study and the authors' previous work provide evidence that 5-HT and DA have a regulatory role in immunity. The results suggest that this action is mediated by receptors on cells of the immune system. However, it is possible that 5-HT and DA could indirectly influence the activation of other immunohormonal messengers that modify immunity.

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