Continuous administration of 5-hydroxytryptamine alters cellular immunity in chickens

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CONTINUOUS ADMINISTRATION OF 
5-HYDROXYTRYPTAMINE ALTERS CELLULAR IMMUNITY IN CHICKENS

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Abstract—1. 5-Hydroxytryptamine administered continuously through osmotic pumps altered the PHA wattle response and in vitro leukocyte capillary tube migration in UNH 105 chickens.
2. Administration of 100 ng/hr 5-hydroxytryptamine for 48 hr significantly suppressed the PHA wattle response.
3. In vitro leukocyte migration was significantly enhanced by 72 hr exposure to 5-hydroxytryptamine at a dose of 100 ng/hr.

INTRODUCTION

Physiologic functions including those of the immune system are subject to hormonal regulation. Acute involution of lymphoid organs caused by adrenocortical, androgenic, and estrogenic steroid hormones alters both antibody and cell-mediated immune responses in birds and mammals (Gisler, 1974; Glick et al., 1977; Glick and Sadler, 1961; Solomon et al., 1974; Wilson and Glick, 1966). Excitatory neurotransmitters such as 5-hydroxytryptamine (serotonin, 5-HT), 3-hydroxytryptamine (dopamine, DA) and artemol (norepinephrine, NE) also affect immune responses. In mice, 5-HT modulates phagocytosis and suppresses interferon-induced 1a expression (Steinberg et al., 1987). Systemic administration of 5-HT 30 to 60 min. before immunization suppresses mouse IgM and IgG plaque-forming cell (PFC) responses to sheep red blood cells (Bliznakov, 1980; Devoine et al., 1975; Jackson et al., 1985). Two products of the same biosynthetic pathway, NE and DA also disturb the anti-SRBC response. Norepinephrine (NE) strongly suppresses the in vitro anti-SRBC response of murine spleen cells but dopamine (DA) enhances PFC following immunization (Besedovsky et al., 1979). 5-HT also enhances human natural killer (NK) cell cytotoxicity (Hellstrand and Hermodsson, 1987).

Similar results have been found in experiments using avian species. Single doses of 5-HT, DA or NE given 30 min prior to phytohemagglutinin (PHA) each depressed subsequent wattle responses (Lukacs et al., 1987). Using the same single dose treatment, IgM and IgG PFC against SRBC were significantly lower in 5-HT and DA treated chicks compared to controls (Gray et al., 1986; McCorkle et al., 1986). Leukocyte migration from capillary tubes was not altered by 30 min in vitro exposure to monoamines but in vitro exposure of peripheral blood leukocytes to 5-HT, DA or NE produced significant effects. Relative migration areas were significantly higher after exposure to 100 ng 5-HT (p < 0.05) but were significantly lowered by 1 µg 5-HT (p < 0.05). Migration was also suppressed by DA but enhanced by NE (McCorkle et al., 1988).

Additional studies revealed significantly lower 5-HT concentrations in the brain, spleen, thymus and bursa of Fabricius of turkey poults infected with Bordetella avium, a mild upper respiratory pathogen that causes rhinotracheitis (Ekus et al., 1987a and b; McCorkle et al., 1984). In that study, immune stimulation by a pathogen reduced 5-HT concentrations. Since changes in monoamine levels modulated immune responses and immune stimulation affected monoamine levels, the effect of continuous exposure to monoamines became of interest. The objective of this study was to examine the effect of continuous 5-HT administration on two chicken cellular immune responses: the PHA wattle response and leukocyte migration.

MATERIALS AND METHODS

Animals. Chicks of mixed sexes from line UNH 105, a non-inbred line of New Hampshires (Gallus domesticus) were used throughout this study. The line, having commercial origin, is maintained at the University of New Hampshire Poultry Research Farm. Chicks were housed in brooder batteries with free access to feed and water. All experiments were performed when the chicks were six weeks old.

Monoamine administration. 5-Hydroxytryptamine (serotonin, 5-HT), purchased from Sigma Chemical Company, St. Louis, Missouri, was dissolved in acidified 0.9% saline (pH 4.0). Alzet mini-osmotic pumps. Model 2001, were obtained from the Alza Corporation, Palo Alto, California. The mean pumping rate of 1.06 µl/hr was calculated according to manufacturers' directions.

Dose-time experiment. Osmotic pumps were loaded with 5-HT to deliver controlled doses of 10 ng, 100 ng or 1 µg/hr. Using a local anesthetic, pumps were implanted subcutaneously at the base of the neck of individual chicks. Control
birds received pumps containing the acidified physiological saline vehicle. The cell-mediated responses (PHA wattle response and leukocyte migration) were tested after the pumps were in place 24, 48 or 72 hr. Five birds were used for each treatment for each time period.

**PHA wattle response.** A stock solution (1 mg/ml) of phytohemagglutinin (PHA-P, Difco Laboratories, Detroit, Michigan) was prepared in sterile phosphate-buffered saline (PBS, pH 7.2) and stored in aliquots at −20°C until used. At the designated interval after pumps were implanted, the PHA wattle response was performed as described by McCorkle and co-workers (1979). Initial thickness of each wattle was measured to ±0.1 mm with a micrometer. Immediately after measurement, 0.1 ml of the stock solution of PHA (100 μg) was injected subcutaneously into the right wattle while the left wattle received 0.1 ml PBS as a negative control. Wattle thickness was measured again 24 hr post-injection.

Change in thickness for each wattle was calculated by subtracting the initial thickness from the thickness at 24 hr. In PHA-injected wattles, a swelling greater than 0.3 mm was considered a positive response since in these trials the saline-injected wattle never exceeded this measurement at 24 hr. A stimulation index for PHA-injected wattles was calculated by dividing the change in wattle thickness by the initial thickness.

**Leukocyte migration.** Twenty-four, 48 or 72 hr after the wattle response was tested, but before saline was removed as the buffy coat and washed three times in RPMI 1640 media (pH 7.4). Cell viability was assessed by the trypan-blue-exclusion (Tennant, 1964) after which cell numbers were adjusted to approximately 2 x 10⁸ cells/ml and exposure time for 5-HT from the dose-time experiment, capillary tubes were used for each bird (McCorkle and Simmons, 1984).

Non-heparinized capillary tubes (1.25 x 75 mm) were filled with the cell suspension, sealed with a clay-type tubing sealer and centrifuged at 500 x g for 3 min. The cell packs were cut evenly at the cell-fluid interface and placed into 24-well plates supported by a small amount of vacuum grease (Dow Corning Corp., Midland, Michigan). After the wells were filled with RPMI 1640, plates were incubated at 37°C for 1 hr. Three replicate capillary tubes were used for each bird (McCorkle and Simmons, 1984). The length and width of each migration zone was measured using an ocular micrometer on a dissecting microscope. The relative area of cell migration was calculated by the formula [(length x width)/10,000]. The results were expressed as the mean migration area ± standard error of mean.

**Optimum dose experiments.** Using the most effective dose and exposure time for 5-HT from the dose-time experiment, separate trials were conducted. Each trial consisted of ten 5-HT-treated and ten saline-treated chicks. Four trials were performed for the wattle test while two trials were conducted for leukocyte migration. Both the wattle test and leukocyte migration were performed as described above.

**Statistical analysis.** Experimental data were evaluated by analysis of variance with dose and exposure time as main effects. Differences between 5-HT treatments and controls in the dose-time study were assessed by Dunnett's test at the 0.05 level of significance.

### RESULTS

Osmotic pumps were used to administer 5-HT to UNH 105 chickens at doses of 10 ng, 100 ng, and 1 μg/hr for 24, 48 or 72 hr. The effect of continuous 5-HT on the PHA wattle response is presented in Table 1. Twenty-four hour exposure to 5-HT did not affect the reaction but all three doses significantly suppressed the PHA wattle response after 48 hr. After a 72 hr exposure time, only the highest dose (1 μg/hr) significantly suppressed the response (Table 1). The 100 ng/hr 5-HT administered for 48 hr was selected for subsequent trials since this dose gave the greatest suppression. Combined data for four trials, shown in Fig. 1, revealed significant suppression of the PHA wattle response compared to saline controls.

Table 1. Mean PHA wattle stimulation indices from line UNH 105 chicks following continuous exposure to 5-HT at different doses and exposure times

<table>
<thead>
<tr>
<th>Dose</th>
<th>Length of exposure*</th>
<th>1 Day</th>
<th>2 Days</th>
<th>3 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
<td></td>
<td>1.8 ± 0.3</td>
<td>3.2 ± 0.2</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>10 ng</td>
<td></td>
<td>2.2 ± 0.5</td>
<td>2.2 ± 0.2</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>100 ng</td>
<td></td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>1 μg</td>
<td></td>
<td>2.0 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

* – Each value represents the mean ± standard error of 5 birds.

### DISCUSSION

When 5-HT is given with the Alzet osmotic pump over two days, the PHA wattle response is significantly depressed (Table 1 and Fig. 1). This data...
suggests that the T-lymphocytes involved in the PHA wattle response are affected by the continuous administration of 5-HT. This result could be manifested in several ways. First, 5-HT may affect receptors found on the responding T-cells and/or basophils. Second, the response of infiltrating T-cells to chemotactic factors released after PHA injection may be diminished or the amount of chemotactic factors generated may be reduced. Third, other hormones or second messengers such as cyclic AMP which could depress the wattle reaction may be stimulated by 5-HT.

In vitro leukocyte capillary tube migration is significantly enhanced by continuous 5-HT administration (Table 2 and Fig. 2). This suggests that the leukocytes have a receptor for 5-HT or that a second messenger hormone is activated that stimulates the ability of leukocytes to migration. During a disease state in chickens, high levels of 5-HT triggered by pathologic processes could increase leukocyte migration to areas of infection.

This is the first report of continuous administration of 5-HT at physiological levels. Ringer and Meyer (1976) found circulating levels of 5-HT in chickens to be 3.2–6.9 μg/ml of blood. Edens et al. (1987b) reported that 5-HT levels in turkey poult lymphoid tissues were 800 μg/g of spleen, 70 μg/g of thymus, and 200 μg/g of bursa Fabricius at 28 days of age. In the present study, 5-HT was released at 100 ng/hr, a concentration lower than either previous report. At this lower level, the PHA wattle response, a T-cell dependent response (McCorkle et al., 1979; Edelman et al., 1986), was suppressed. The reduction in a T-cell reaction by elevated levels of 5-HT could compromise the response to disease organisms. On the other hand, leukocyte migration was enhanced by 5-HT which could increase the movement of phagocytic cells toward invading foreign organisms. The population or subpopulation of cells responding in these two assays are probably divergent since one cellular response was suppressed while another cellular reaction was enhanced by continuous 5-HT infusion. Despite these differences between an in vitro and in vivo response, 5-HT appears to have a role in regulation of the chicken’s immune response.

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### REFERENCES


