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Continuous administration of dopamine alters cellular immunity in chickens

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Dopamine administered continuously through osmotic pumps altered the PHA wattle response and in vitro leukocyte capillary tube migration in UNH 105 chickens. The PHA wattle response was suppressed significantly by 48 hr exposure to dopamine at a dose of 1 µg/hr. Administration of 10 µg/hr dopamine for 48 hr enhanced significantly in vitro leukocyte migration.

Key words: Dopamine; Chicken; Leukocyte migration; PHA wattle response; Cellular immunity; Biogenic amines; Osmotic pumps; Neurotransmitter.


Introduction

Neurotransmitters of the sympathetic nervous system, such as 5-hydroxytryptamine (serotonin, 5-HT), 3-hydroxytyramine (dopamine, DA) and arterenol (norepinephrine, NE), affect immune responses. 5-HT suppresses mouse IgM and IgG plaque-forming cell (PFC) responses to sheep red blood cells when administered 30–60 min prior to immunization (Bliznakov, 1980; Devoino et al., 1975; Jackson et al., 1985). Two related compounds, NE and DA, also perturb the anti-SRBC response. DA enhances PFC following immunization whereas NE significantly suppresses the in vitro anti-SRBC response of murine spleen cells (Besedovsky et al., 1979). Furthermore, 5-HT modulates phagocytosis and suppresses interferon-induced Ia expression in mice (Steinberg et al., 1987).

Biogenic amine concentrations were diminished by bacterial infections which stimulated turkey immune responses. Levels of 5-HT, DA and NE were significantly depressed in the brain, spleen, thymus and bursa of Fabricius of Bordetella avium infected turkey poults (Eden et al., 1987a,b). This mild respiratory pathogen causes rhinotracheitis. The B. avium infection which altered 5-HT, DA and NE levels also enhanced leukocyte migration (McCorkle and Simmons, 1984). These results prompted investigation of avian immune response alterations by biogenic amines.

Lukacs et al. (1987) found that the PHA wattle response in chickens, a T-cell-mediated response (McCorkle et al., 1979), was significantly depressed by 5-HT, DA or NE given 30 min prior to PHA administration. After in vitro treatment of peripheral blood leukocytes, the migration response was enhanced by 5-HT but suppressed by DA or NE (McCorkle et al., 1990). In vivo
exposure to these compounds did not affect leukocyte migration (McCorkle et al., 1990). Gray et al. (1991) discovered that 5-HT significantly enhanced IgM but not IgG plaque-forming cells (PFCs) in vivo whereas DA significantly suppressed both IgM and IgG PFCs in vivo.

The effect of continuous exposure to biogenic amines was examined because previous research demonstrated that elevations in biogenic amines levels modulated avian immune responses in birds. The PHA wattle response was significantly suppressed by continuous 5-HT administered through osmotic pumps (McCorkle and Taylor, 1989). On the other hand, in vitro leukocyte migration was significantly enhanced by the same treatment (McCorkle and Taylor, 1989). The PHA wattle response and leukocyte migration were evaluated following continuous dopamine (DA) administration in the current study.

Materials and Methods

Animals

Line UNH 105 chicks, a line of New Hampshires (Gallus domesticus) maintained at the University of New Hampshire Poultry Research Farm, were used throughout this study. Chicks of mixed sexes were housed in heated brooder batteries with commercial feed and water available ad libitum. All experiments were performed when the chicks were 6 weeks old.

Catecholamine administration

Dopamine (DA) (Sigma Chemical Company, St Louis, MO) was dissolved in acidified 0.9% saline (pH 4.0). Alzet mini-osmotic pumps Model 2001 (Alza Corporation, Palo Alto, CA), having a mean pumping rate of 1.07 μl/hr calculated according to manufacturers' directions, were used.

Dose-time experiment

DA was loaded into osmotic pumps to deliver controlled doses of 100 ng, 1 μg or 10 μg/hr. Pumps were implanted subcutaneously at the base of the neck of individual chicks after injection of a local anesthetic. Control birds received pumps containing the acidified physiological saline vehicle. Two cell-mediated responses (PHA wattle response and leukocyte migration) were tested after the pumps had been in place for 24, 48 or 72 hr. Five birds were used for each treatment for each time period.

PHA wattle response

Phytohemagglutinin (PHA-P, Difco Laboratories, Detroit, MI) was prepared as a 1 mg/ml stock solution in sterile phosphate-buffered saline (PBS, pH 7.2). This stock was stored in aliquots at -20°C until used. The PHA wattle response, as described by McCorkle and co-workers (1979), was performed at the designated interval after pumps were implanted. Using a micrometer, the initial thickness of each wattle was measured to the nearest 0.1 mm. Immediately after measurement, the right wattle was injected subcutaneously with 0.1 ml of the stock solution of PHA (100 μg). The left wattle was injected with 0.1 ml PBS. At 24 hr post-injection, wattle thickness was measured again.

The change in thickness for each wattle was calculated using the formula (wattle thickness at 24 hr post-injection - initial wattle thickness). A swelling greater than 0.3 mm was considered a positive response in the PHA-injected wattles, since the saline-injected wattle never exceeded this measurement at 24 hr (Goto et al., 1978; McCorkle et al., 1979). A stimulation index for PHA-injected wattles was calculated with the formula (change in wattle thickness/initial wattle thickness).

Leukocyte migration

Blood was collected into a heparinized syringe by cardiac puncture 24, 48 or 72 hr after the pumps were placed in birds. Leukocytes were separated by slow-speed centrifugation (15 min, 100 g), removed as the buffy coat and washed three times in RPMI 1640 cell-culture medium (pH 7.4). Cell numbers were adjusted to approximately 2 x 10⁶ cells/ml RPMI (McCorkle and Simmons, 1984) after assessment of cell viability by Trypan Blue exclusion (Tennant, 1964).

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

### Optimum dose experiments

Based on the results of the dose-time experiments, separate trials were conducted using the most effective dose and exposure time of DA. Each trial consisted of 10 DA-treated and 10 saline-treated chicks. Three trials were conducted for both the PHA wattle test and leukocyte migration, which were performed as described above.

### Statistical analysis

Experimental data were evaluated by analysis of variance with dose and exposure time as main effects. Dunnett’s test, at the 0.05 level of significance, was used to assess differences between DA treatments and controls in the dose-time study.

### Results

DA was administered to UNH 105 chickens via osmotic pumps in doses of 100 ng, 1 µg or 10 µg/hr for 24, 48 or 72 hr. The lowest dose administered for the shortest time which demonstrated an effect in the dose-time studies was chosen for subsequent trials. Table 1 shows the effects of different DA doses and exposure times on the PHA wattle response. Significant suppression of the response was found when 1 µg/hr DA was given for either 48 or 72 hr. No other dose or exposure time had a significant effect. Three subsequent trials of the PHA wattle response used 1 µg/hr DA given for 48 hr. Figure 1 shows significant suppression of the PHA wattle response.

The effect of continuous exposure to DA on in vitro leukocyte migration is shown in Table 2. The 100 ng/hr DA dose did not significantly effect migration at any exposure time. On the other hand, 1 µg/hr DA significantly enhanced migration after 48 or 72 hr. DA at 10 µg/hr significantly enhanced migration in all three exposure times. In three additional trials, 48 hr exposure to 10 µg/hr DA significantly

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

<table>
<thead>
<tr>
<th>Dose</th>
<th>Length of exposure†</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>1.8 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>DA 100 ng/hr</td>
<td></td>
<td>1.5 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>DA 1 µg/hr</td>
<td></td>
<td>1.8 ± 0.4</td>
<td>1.0 ± 0.1*</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td>DA 10 µg/hr</td>
<td></td>
<td>1.8 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
</tbody>
</table>

†Each value represents the mean ± standard error of five birds.

*Value significantly different from the control within a column at \( P < 0.05 \).

### Table 2. Mean leukocyte migration area from line UNH 105 chicks following continuous exposure to DA at different doses and exposure times

<table>
<thead>
<tr>
<th>Dose</th>
<th>Length of exposure†</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>4.4 ± 0.8</td>
<td>5.6 ± 0.6</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>DA 100 ng/hr</td>
<td></td>
<td>3.3 ± 0.5</td>
<td>6.3 ± 1.0</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>DA 1 µg/hr</td>
<td></td>
<td>4.8 ± 0.5</td>
<td>7.3 ± 0.7*</td>
<td>8.0 ± 0.3*</td>
</tr>
<tr>
<td>DA 10 µg/hr</td>
<td></td>
<td>7.2 ± 0.5*</td>
<td>11.1 ± 0.3*</td>
<td>10.2 ± 0.4*</td>
</tr>
</tbody>
</table>

†Each value represents the mean ± standard error of five birds. Peripheral blood leukocytes migrated for 1 hr at 37°C.

*Value significantly different from the control within a column at \( P < 0.05 \).
increased \textit{in vitro} leukocyte capillary tube migration (Fig. 2).

\section*{Discussion}

Using the Alzet osmotic pump, DA significantly depresses the PHA wattle response (Table 1 and Fig. 1). This test is a type of delayed hypersensitivity response that requires T-lymphocytes (McCorkle \textit{et al.}, 1979). The participating T-cells are affected by continuous administration of DA; a result which could occur in several ways. First, DA 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, hormones or second messengers such as cyclic AMP, which could affect the wattle reaction, may be stimulated by DA.

\textit{In vitro} leukocyte capillary tube migration is significantly enhanced by continuous DA administration (Table 2 and Fig. 2). Enhancement of migration suggest either that the leukocytes possess receptor(s) for DA or that second messengers are activated which stimulate leukocyte migration. The leukocyte migration assay utilizes a mixed population of cells, but heterophils are the primary cell type migrating in this \textit{in vitro} assay system. Heterophil migration to an infection site is important because these phagocytic cells represent the major initial defense against a pathologic process. During a disease state in chickens, high levels of DA triggered by pathologic processes could increase leukocyte (heterophil) migration to areas of infection.

This is the first report of continuous administration of DA at physiological levels in chickens. Edens \textit{et al.} (1987b) reported that DA levels in turkey poult lymphoid tissues were 60 ng/g of spleen, 20 ng/g of thymus and 55 ng/g of bursa of Fabricius at 28 days of age following \textit{B. avium} infection. In the present study, DA was released at 1 ng/hr, a lower concentration than in the previous report. At this lower level, the PHA wattle response was suppressed whereas leukocyte migration was enhanced. These results are similar to the effects of 5-HT on these two immune responses (McCorkle and Taylor, 1989). NE and E, administered continuously, also demonstrated comparable outcomes: suppression of the PHA wattle response and enhancement of leukocyte migration (McCorkle and Taylor, 1993). Therefore, three compounds from the same biosynthetic pathway DA, NE and E, administered continuously, also affected two tests of cellular immune function.

The apparent difference in the manner in which these tests were modulated has two possible explanations. First, two different cell types respond in these tests: T-cells in the PHA wattle response and heterophils in leukocyte migration. Second, the PHA wattle response occurs \textit{in vivo} and the DA effects may be direct or indirect. The response is measured 24 hr after PHA injection so effects which occur more slowly may be evident. Leukocyte migration, \textit{an in vitro} test, occurs quickly (1 hr) so any effect delayed beyond that time would not be seen. The results indicate that DA appears to have a role in regulating the chicken's immune response as measured in these \textit{in vivo} and \textit{in vitro} tests.

\section*{Acknowledgements}

The authors thank Carol Langschwager and Jackie Packard for clerical assistance. This project was funded in part by FRCE grant No. 4-22892 from Central Michigan University. This is Scientific Contribution No. 1867 from the New Hampshire Agricultural Experimental Station.
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