Effects of 6-Hydroxydopamine on the Antibody Response to the Hapten Dinitrophenyl in the Chicken

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ABSTRACT. Antibody responses to the hapten dinitrophenyl (DNP) and the concentration of catecholamine in chickens received a single injection of 6-Hydroxydopamine (6-OHDA) into the chorioallantoic cavity at the embryonal stage were evaluated. A significantly enhanced anti-DNP IgA response and a markedly decreased level of noradrenaline in the peripheral blood were observed in chickens treated with 400 μg of 6-OHDA at 14 days of incubation. These results suggested the immunomodulatory influence of the sympathetic nervous system. — KEY WORDS: anti-DNP antibody, chicken, 6-Hydroxydopamine.


6-Hydroxydopamine (6-OHDA) is a neurotoxin that selectively destroys noradrenergic nerve fibers with the catecholamine high affinity carrier found on noradrenergic nerve terminals in the periphery [1]. Chemical sympathectomy in the rodent reduced both cell-mediated immunity and antibody production to T-dependent antigens in vivo and in vitro, and increased antibody responses to T-independent antigens [8, 11, 12, 16]. Neonatal sympathectomy that results in a long lasting depletion of peripheral noradrenergic nerve fibers, is associated with the enhancement of antibody responses in the adult rodent [3, 14]. There is, however, little information concerning the effects of catecholamine on ontogeny of the immune system. In the present study, therefore, we attempted to investigate the effects of 6-OHDA on antibody responses and the level of catecholamine in the peripheral blood to elucidate the interactions between the nervous system and the immune system in the chicken.

6-Hydroxydopamine hydrobromide (Sigma, St. Louis, MO, U.S.A.) was dissolved in sterile saline containing 0.01% ascorbate as an anti-oxidant. Fertilized eggs of line V chickens (MHC: B1B1B1), originally derived from the University of Turku, Finland and kept at our institute, were used. On day 7 or 14 of incubation, embryos received a single injection of 50 or 400 μg of 6-OHDA into the chorioallantoic cavity (treatment group). Embryos injected with saline at the same procedure were used as a control group.

Dinitrophenyl-keyhole limpet hemocyanin (DNPKLH) and dinitrophenyl-bovine serum albumin (DNPBSA) were prepared by coupling 2,4-dinitrobenzen sulfonic acid sodium salt (Eastman Kodak Co., Rochester, NY, U.S.A.) with KLH (Calbiochem Behring Co., San Diego, CA, U.S.A.) and BSA (Sigma, St. Louis, MO, U.S.A.), respectively, as described previously [5]. The protein contents of these antigens were determined by optical density values measured at 280 nm. Hapten contents were also determined at 360 nm. The final conjugation ratios were DNP_BKHL and DNPBSA. One hundred μg/0.5 ml of DNPKSLH was mixed with 0.5 ml of 20 mg Al (OH)_3, and used for immunization. Five-week-old chickens were immunized intraperitoneally with DNPKSLH, and bled at a 7 day-interval after immunization. Anti-DNP antibodies were determined by the enzyme-linked immunosorbent assay as described previously [17]. In brief, each well of a 96-well-microplate (Immunoplate, Nunc, Roskilde, Denmark) was coated with 60 μl of DNPBSA (50 μg/ml). Subsequently, serially 10-fold diluted antisera in phosphate buffer saline containing 1% BSA were added into the antigen-coated wells, and incubated for 1 hr at 37°C. The wells were then washed, and rabbit anti-chicken IgM, IgG or IgA (1/2,000) was added. After incubation at 37°C for 1 hr, the wells were washed, supplemented with peroxidase-labeled goat anti-rabbit Ig (1/4,000; Zymed Lab. INC., San Francisco, CA, U.S.A.), and then incubated for 1 hr at 37°C. The wells were washed, supplemented with 60 μl of substrate buffer (100 μl/ml) of ABTS [2,2-azino-bis (3-ethylbenzthiazolone-6-sulfonic acid); Sigma, St. Louis, MO, U.S.A.] containing 5 μl of H_2O_2 (SantoKu Chemical Industries, Co., Ltd., Tokyo, Japan), and then incubated for 30 min at room temperature. Subsequently, the plates were read at 405 nm by a microplate reader (Bio-Rad Model 450: Bio-Rad Lab., Richmond, CA, U.S.A.). The concentration expressing 50% binding activity was calculated, and the unit of normal chicken serum was taken as 1 unit. The concentrations of noradrenaline (NA) and dopamine (DA) in plasma samples from 9-week-old chickens treated with 6-OHDA were also determined by a high performance liquid chromatography (Coulochem II 5100A; Shimadzu, Kyoto, Japan), as described previously [7]. The separations of NA and DA were performed by using a STR ODS-II column (particle size, 5 μm; 150 × 4.6 mm inner diameter; Shimadzu, Kyoto, Japan). The results were expressed as mean ± standard error (SE), and differences among the various parameters were analyzed by the repeated measure ANOVA and post-hoc analysis. P<0.05 was considered...
significant.

As shown in Fig. 1, the effects of 6-OHDA on anti-DNP responses in chickens were dependent on the embryonal age and doses of this drug to be injected. Neither IgM- nor IgG-class specific anti-DNP antibody responses were significantly different between 6-OHDA treated chickens and control chickens. IgM class of anti-DNP antibody was inclined to enhance at 1 week after immunization in chickens treated with 400 µg of 6-OHDA at 14 days of incubation, as compared with control (Fig. 1A). These treated chickens also showed a tendency of enhanced anti-DNP IgG responses at 3 and 4 weeks after immunization, as compared with control (Fig. 1B). Interestingly, anti-DNP IgA responses were significantly enhanced at 1 week after immunization in these treated chickens (Fig. 1C).

The results of NA and DA levels in plasma samples of 9-week-old chickens treated with 400 µg of 6-OHDA at 14 days of incubation are shown in Fig. 2. NA levels in 9-week-old chickens treated with 6-OHDA were significantly lower than those of control chickens of the same age (P<0.05). On the other hand, DA levels were not significantly different when compared between the two groups.

Regarding the avian lymphogenesis, the inflow of the lymphoid stem cells into the thymus rudiment starts on the second half of the 7th day of embryonic development [9]. In a similar way, the colonization of the bursal primordium by stem cells occurs mainly 8 to 14 days in the chicken embryo [13]. In the present study, therefore, treatment with 6-OHDA was carried out at 7 or 14 days of chicken embryos on the basis of the histogenesis of these central lymphoid organs during the embryonic development.

Jankovic et al. [10] showed that chemical sympathectomy of chicken embryos with 6-OHDA at 12 days of incubation resulted in a decrease in the number of direct plaque-forming cells (PFC) to sheep red blood cells (SRBC), an increase in the level of NA and a decrease in DA level in the spleen at 18 days of embryonal age. These results suggested that the decreased number of PFC was associated with a depletion of lymphocytes and an increase in NA level in the spleen. However, the further investigations of PFC responses and the level of catecholamine in developing chickens treated with 6-OHDA at the embryonal stage have not been followed. Their results were different from ours in present study in which developing chickens treated with 400 µg of 6-OHDA at 14 days of embryonal ages showed significantly
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REFERENCES


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