Effect of Continuous Melatonin Administration on the Diurnal Rhythms of the Peripheral Blood Leukocyte Counts in Chicks

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Abstract Effect of melatonin implanted in the femoral region on the diurnal rhythmic patterns of the peripheral leukocyte counts was studied in chicks. The diurnal rhythmic patterns of lymphocyte count and monocyte count, which shows two peaks of counts in a day, respectively, disappeared by the continuous melatonin administration. In the granulocyte count, rhythmic pattern was altered by the melatonin treatment. The results suggest that melatonin is involved in rhythm formation of the peripheral blood leukocyte numbers in chicks by acting upon some target sites which have been assumed to be concerned with the rhythm formation of peripheral blood leukocyte count.

Key words: Diurnal rhythm, Melatonin, Leukocyte count, Chick

Diurnal variations have been reported in the field of hematology7). Melatonin has been thought to be a humoral factor controlling the locomotor rhythm in birds from the facts that circadian locomotor rhythms of several species of birds become to be indistinct by melatonin treatment6,10). In the present report, we examined the effect of continuous melatonin administration on the rhythmic patterns of leukocyte (lymphocyte, monocyte and granulocyte) counts in the peripheral blood of chicks.

Materials and Methods

Chicks and laboratory conditions

Male chicks of the White Leghorn Julia Strain, 11 to 14 weeks old, were used. Chicks were implanted subcutaneously with a small piece (about 2.5 cm length) of silastic tube (Silastic medical grade tubing; Dow Corning, CAT. No. 602-235, 0.077 inches outer diameter) filled with approximately 10 mg of melatonin (Sigma) at the right femoral region. After the treatment, chicks were transferred to a closed room (1.8 × 2.2 × 2 m), and maintained on, and adapted to, an artificial light condition (70 to 100 lux) of 14 hour light (lights on from 06.00 to 20.00 hour) and 10 hour darkness and room temperature at 23±1°C. Chicks were subjected to the experiments described later.
Leukocyte Count Rhythms in Melatonin-treated Chicks

Counting of leukocytes in the peripheral blood

After 10 days to 14 days of the melatonin implantation, 0.45 ml of the peripheral blood was drawn from a wing vein of melatonin-implanted White Leghorn chicks with a syringe containing 0.05 ml heparin at the 4 hour-interval for 24 hours (07.00, 11.00, 15.00, 19.00, 23.00 and 03.00 hour). Each chick was subjected to continuous blood sampling during 24 hours. Each blood sample was mixed with 0.45 ml of Mg, Ca(-)-Hank's balanced salt solution added with 1.3 mM EDTA. The samples collected from eighteen chicks at each determination point were stained with Natt & Herrick staining solution, then the numbers of lymphocytes, monocytes and granulocytes per 1 ml of blood were counted with hemocytometer. Analysis of variance was used to determine significant differences between means from 07.00 hour to next day at 07.00 hour. All significant differences were determined at the 5% level. All results are given as means and standard errors.

Results and Discussion

Fig. 1 shows the diurnal changes of lymphocyte count, monocyte count and granulocyte count in the peripheral blood of melatonin-implanted chicks. Lymphocyte count showed constant values (11.27 to 13.08 $\times 10^6$/ml) in a day (Fig. 1-A). Monocyte count also showed constant values (0.86 to 1.17 $\times 10^6$/ml) in a day (Fig. 1-B). In the both peripheral blood leukocyte counts, there were no significant differences among the values at 6 measured times. Lymphocyte count$^1$ and monocyte count$^2$ in the peripheral blood of non-treated White Leghorn chicks under the same light condition as the present experiment have been reported to have similar diurnal rhythmic pattern which show a peak at light period and dark period, respectively. Granulocyte count in the peripheral blood of the melatonin-implanted chicks showed a peak level at light period (Fig. 1-C). The peak value at 15.00 hour was

Fig. 1. Diurnal changes of the peripheral blood leukocyte counts in the continuously melatonin-administered chicks. A: lymphocyte counts, B: monocyte counts, C: granulocyte counts. *=Mean significantly differs from the means at other measured times (p<0.05).
significantly higher than the values at 7.00, 19.00, 23.00 and 03.00 hour. Granulocyte count in the peripheral blood of non-treated chicks also showed a rhythmic pattern\(^2\). However, the rhythmic pattern of granulocyte count in the non-treated chicks differed from that of the melatonin-implanted chicks with respect to the peak value of the count observed during dark period. The present results indicate extrinsic melatonin influences on the peripheral leukocyte numbers in chicks. In the chicken, plasma melatonin concentration showed a rhythmic change synchronized with 12 hr light and 12 hr dark photoperiod\(^4\). The number of melatonin granules in the pineal gland of chicken also showed a daily fluctuation which was higher at midnight than at midday\(^5\). These results suggest that, in the present experiment, continuous melatonin administration abolished rhythmic fluctuation in the plasma melatonin concentration of chicken, and the diurnal rhythmic changes in the peripheral blood leukocyte counts were influenced by the flattening of plasma melatonin concentration in a day.

Although factors involved in diurnal fluctuation of the peripheral leukocyte counts are not exactly clarified, it has been assumed that a possible factor of the fluctuation is periodic circulation of leukocytes between the peripheral blood and the bone marrow, which is estimated by the fact that circulating lymphocytes are sequestered in the bone marrow of mice under a given condition\(^3\). Another possible factor of the fluctuation is diurnal variation of proliferation activities of leukocytes in the bone marrow\(^9\). Melatonin may produce daily variation in the peripheral leukocyte number by influencing on re-homing of leukocytes into the bone marrow, and/or on proliferation activities of leukocytes in the bone marrow. Difference in the influences of continuous melatonin administration among the counts of three kinds of circulating leukocytes, namely abolishment of rhythmic fluctuations in lymphocyte and monocyte counts, and shift of the peak from dark period to light period in granulocyte count, may suggest that relative significance of the above two factors in the formation of diurnal fluctuation differs between lymphocyte-monocyte group and granulocyte group. Further experiments are necessary for clarification of these postulations.

References