SHORT COMMUNICATION

Circadian rhythmicity of delayed hypersensitivity to oxazolone in the rat

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Summary

1. A marked circadian rhythm was detected in the ear swelling of rats immunized and then challenged with oxazolone.
2. The peak response observed at 10.00 hours was over eight times the minimum at 16.00 hours.
3. As related tests are used frequently in man greater attention to clock time is necessary in clinical immunology.

Key words: cellular immunity, circadian rhythm, oxazolone, skin tests.

Introduction

Some components of cellular immunity exhibit rhythmicity when isolated and tested in the laboratory. Tavadia, Fleming, Hume & Simpson (1975), using a purified lymphocyte preparation, found a circadian rhythm in the transformation by phytohaemagglutinin with a nadir at midnight and a peak at 08.00 hours. Eskola, Frey, Molnar & Soppi (1976), however, obtained conflicting results with a method based on whole blood which they considered to simulate the conditions in vivo more closely, the mitogen-stimulated response being lowest at 08.00 hours and highest at 02.00 hours. These rhythms were not due to long-established variation in leucocyte count during the course of the day but to changes in the activity of the sampled lymphocytes. These two reports only show that there is cyclic activity in a mixed lymphocyte population; they do not establish that there is circadian variation in lymphocytes sensitized to one particular antigen, nor that the observed rhythms could cause variation in the expression of immunity. Does a cell-mediated immune response exhibit circadian variation when assessed in the whole organism? We have studied the degree of delayed hypersensitivity shown by sensitized rats to the contact-sensitizing agent oxazolone. The ear swelling, which is due to the arrival at the challenged ear of antigen-sensitive T-cells and macrophages (Allwood, 1975), was measured at six different times in the day.

Methods

Adult male Wistar rats, approximately 250 g, were used in groups of six for each time of day, and an additional six control rats at 10.00 hours. They were housed three per cage with free access to a standard laboratory diet and water. The bedding of sawdust was changed twice weekly in the late morning or early afternoon. A regime of light between 10.00 and 22.00 hours and total darkness from 22.00 to 10.00 hours was used. Investigations during the dark period were carried out elsewhere after the quiet removal of the two appropriate cages without illumination in the main room. The rats were then returned immediately to the standard conditions. Procedures were carried out under light ether anaesthesia, taking approximately 5 min per rat.

Rats were actively sensitized between 10.00 and 12.00 hours with 0.5 ml of a 10% (w/v) solution (approx. 0.42 mol/l) of oxazolone (2-phenyl-4-ethoxymethylene-oxazolone; BDH Chemicals Ltd, Poole, Dorset, U.K.) in ethanol. The hapten was
applied in drops over a closely shaved area of abdominal skin approximately 25 mm x 75 mm. The six control rats were not sensitized. Nine days later all rats were challenged at the appropriate time of day. The challenge dose was 10% (w/v) oxazolone in an acetone/olive oil (4:1, v/v) mixture and was administered as three drops (approx. 0.07 ml) from a Pasteur pipette, which was also used to spread the chemical across the dorsal surface of the ear. Left ears only were treated. Oxazolone was applied similarly at 10.00 hours to the six control rats not previously sensitized. Immediately before challenge three measurements were taken on a constant area of both ears with an engineer’s micrometer (model 964 MB, Moore and Wright, Sheffield, U.K.). The immune response to oxazolone has been shown to be maximal 24 h after challenge (unpublished observations), in common with similar delayed hypersensitivity reactions (Turk, 1967; Phanuphak, Moorhead & Claman, 1974). Therefore the thickness of each ear was measured again exactly 24 h after challenge and any change calculated. Values are given as mean ±1 SEM. Comparisons are made by Bessel corrected t-test (Moroney, 1951).

Results

In immunized rats the increase in ear thickness measured after 24 h varies markedly with the timing of the oxazolone challenge (Fig. 1). The maximum value for day 9 was seen at 10.00 hours. This value was repeatable in other rats on day 10 at 10.00 hours. There was a rapid decline to a minimum at 16.00 hours, which is an eightfold reduction (P < 0.001). The untreated ears in these rats showed practically no change. In nonsensitized control rats the change in thickness of oxazolone-treated ears at 24 h was only 16 ± 9 μm. The increases in thickness in the responding ears of the sensitized rats are therefore significant (P < 0.005, or better) at all times of challenge except 16.00 and 22.00 hours.

The rats studied at 22.00 hours on day 9 were challenged again at 10.00 hours on day 21. Their ear thickness then increased by 142 ± 21 μm, which is comparable with the earlier response in other rats challenged at 10.00 hours (Fig. 1). This indicates that their failure to respond significantly on day 9 was due to the timing of the challenge at 22.00 hours rather than their intrinsic failure to recognize oxazolone as an immune stimulus.

Measurements at the six circadian stages on day 9 were analysed to determine the best-fitting cosine curve and its rhythm characteristics. A copy of the resultant cosinor (Halberg & Katinas, 1973) has been lodged as Clinical Science and Molecular Medicine Table no. 77/25 with the Librarian, the Royal Society of Medicine (1 Wimpole Street, London W1M 8AE), who will issue copies on request.

The calculated acrophase (timing of the peak) for the immune response was 08.03 hours and the amplitude (half the peak-trough difference) of 48.7 μm about the mesor (24 h mean) of 65 μm was significant at the 0.1% level.

Discussion

Circadian variations of lymphocyte numbers and plasma corticosteroid concentrations are known in the rat (Guillemin, Dear & Liebelt, 1959; Scheving & Pauly, 1966). It is apparent that the response to oxazolone was weakest in rats challenged during that period of the day when circulating lymphocyte numbers were relatively low and corticosteroid
concentrations high, and conversely the response was maximal when the blood lymphocyte count was close to maximal and corticosteroid concentrations were low. One possible explanation for our findings is that oxazolone administered at a time of low circulating lymphocyte population may produce a weak response, as smaller numbers of the relevant lymphocytes were available. Their ability to proliferate and recruit other cells involved in the response may also be lower at certain times of day. Other factors, such as the corticosteroid concentration, may modify the response by an influence at either a central or a peripheral level. Rhythmicity in the activity of skin epidermal cells may also contribute. The antigenicity of low-molecular-weight haptens, such as oxazolone, probably depends on their combination with skin protein (Turk, 1967) and variations in the way the antigen is processed before detection might contribute to the rhythmicity of the observed response, as might other circadian variations in local responses, e.g. in capillary permeability.

Our results suggest that the time to onset, magnitude and duration of delayed hypersensitivity may well be influenced by the timing of a challenge. At 10.00 hours the response to oxazolone in the sensitized rat resembles the classical tuberculin response. At 16.00 and 22.00 hours the responses are so weak that they are not statistically different (P > 0.05) from that seen in non-immunized rats. If similar circadian rhythms occur in other delayed hypersensitivity reactions then other immunological tests may need to be standardized with regard to clock time. Different circadian patterns in immune response might be expected in man since, unlike nocturnally active rats, in the human corticosteroid concentrations fall during daylight hours (Knapp, Keane & Wright, 1967) and absolute lymphocyte numbers rise (Tavadia et al., 1975; Eskola et al., 1976). Clinical decisions are made on the basis of similar skin tests, as for example the quantitative response to the contact sensitizer dinitrochlorobenzene as a method of detecting patients most likely to reject renal allografts (Diamandopoulos, Briggs & Hamilton, 1977). The rhythmic change in the immune response may also have important therapeutic implications. If cell-mediated immune responses are more vigorous at certain times of the day it seems logical that immunosuppressive treatment should be planned to take account of these times.

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References


