Stimulating Effect of Mercuric Chloride and Nickel Sulfate on DNA Synthesis of Thymocytes and Peripheral Lymphoid Cells from Newborn Guinea Pigs

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Abstract

The metal allergens mercuric chloride and nickel sulfate were found to stimulate DNA synthesis of different in vitro cultured lymphoid cells from newborn guinea pigs. In contrast to earlier findings in adult animals (where spleen cells were most consistently stimulated), in newborn animals thymocytes were the most clearly stimulated lymphoid cells. When separating thymocytes by peanut agglutinin agglutination, both agglutinated and nonagglutinated cells were stimulated, indicating that both functionally immature and mature thymocytes are the target cells for this effect of metal allergens.

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In a previous investigation [5], the effects of different metal allergens were tested on DNA synthesis of unsensitized adult guinea pig lymphoid cells cultured in vitro. At certain concentrations in the range of 10^{-8} – 10^{-6} M, cobalt chloride, mercuric chloride, nickel sulfate and potassium dichromate stimulated the DNA synthesis of both thymocytes and peripheral lymphocytes. Spleen cells were the most consistently stimulated cell type.

Guinea pigs are believed to be physiologically and immunologically mature at birth [6, 10] and at this time lymphocytes from thymus, spleen and lymph nodes respond well to the mitogens PHA and Con A [2].

In the present study, mercuric chloride and nickel sulfate, in concentrations which in the previous investigation [5] were stimulatory on lymphocyte DNA synthesis, were tested on thymocytes and peripheral lymphocytes from newborn guinea pigs. In order to further characterize the responding lymphocyte cell type, thymocytes in some experiments were separated according to their ability to become agglutinated by peanut agglutinin (PNA) before testing. PNA can be used for separation of subpopulations of thymocytes in different species [7–9, 12], the agglutinated cells being immunologically immature and the nonagglutinated cells mature, mitogen responsive [8–9, 12].

The final concentrations of mercuric chloride (HgCl_2) in the cultures were 1.4×10^{-6} and 3.3×10^{-8} M

and of nickel sulfate ($\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$) were 7.6×10^{-6} and 3.8×10^{-6} M. The dilutions from stock solutions, all in 0.9% saline, were made with saline as the diluent.

Thymocytes, spleen cells and lymph node cells from newborn male guinea pigs (weight 90–100g) were prepared as described previously [1], washed with buffer, resuspended in RPMI 1640 with addition of L-glutamine ($2 \mu\text{mol/ml}$), streptomycin ($100 \mu\text{g/ml}$), penicillin (100 IU/ml) and L-alanine ($0.5 \mu\text{mol/ml}$) [4, 11] to a concentration of 5×10^6 cells/ml.

The agglutination with PNA was performed according to Reisner et al. [7] with some minor modifications. Thymocytes (1×10^8) were suspended in 0.25 ml H-D medium consisting of equal parts of Hanks' balanced salt solution and Dulbecco's phosphate buffered saline (PBS), and incubated with 0.25 ml PNA solution (PNA; Boehringer, Mannheim, FRG; 2mg/ml in 0.9% saline) at room temperature for 15 min. The suspension was then diluted to 0.5 ml with H-D medium and gently layered over 4 ml of 25% heat-inactivated homologous serum in PBS. The sedimented aggregates were collected after 30–45 min at room temperature. Nonagglutinated cells were aspirated from the top of the serum cushion. The enriched cells were freed from PNA by two washings in a solution of 0.3 M D-galactose and PBS (2:1 v/v). Thymocytes incubated in parallel without PNA and mixed with 25% inactivated homologous serum underwent

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Table I. Stimulating effect of mercuric chloride and nickel sulfate on DNA synthesis of lymphoid cells from newborn guinea pigs

Metal compound Thymocytes

Spleen cells

Lymph node cells

Control

100 100 100

(281 ± 13) (304 ± 26) (411 ± 78)

100 100 100

(443 ± 85) (490 ± 47) (499 ± 1

100 100 100

(333 ± 50) (343 ± 70) (509 ± 25)

Different concentrations of mercuric chloride and nickel sulfate were tested on the incorporation of tritiated thymidine in DNA of different lymphoid cells cultured for various times. The mean activity of five experiments is given in percent of the control (mean ± SEM), and for the control also in cpm (mean ± SEM) (in parenthesis).

Table II. Stimulating effect of mercuric chloride and nickel sulfate on DNA synthesis of thymocytes from newborn guinea pigs separated by PNA agglutination

Different concentrations of mercuric chloride and nickel sulfate were tested on the incorporation of tritiated thymidine in DNA of unseparated, PNA agglutinated and nonagglutinated thymocytes, cultured for 48 and 72 h. The mean activity of three experiments is given in percent of the control, and for the control also in cpm (in parenthesis).

the same procedure and served as control cells. Unseparated, agglutinated and nonagglutinated cells were finally washed with buffer and resuspended in medium to a concentration of 5×10^6 cells/ml. The agglutinated cells comprised 59–63% of the recovered cells.

The various lymphoid cells were incubated for 48, 72 and in some experiments up to 96 h in a Linbro microtitration plate at 37 °C in an atmosphere of 5% CO₂ in air; for details see Söder and Sandberg [1]. After a preincubation of 30 min, 20 µl of the metal-salt solution to be tested was added, and the same volume of 0.9% saline to control cultures. 2 h before interrupting the cultures, 0.5 µCi of 3H-thymidine (5 Ci/mmol; Radiochemical Centre, Amersham) in 10 µl saline was added to each well. The incubation was interrupted on a Skatron multiple cell collector using glass fiber filters. Incorporated radioactivity was determined by liquid

scintillation in a Packard liquid scintillation spectrometer. The cultures were performed in triplicate.

The spontaneous incorporation of tritiated thymidine into the control cultures of the various lymphocyte types are given in table I and II. After addition of mercuric chloride and nickel sulfate, thymocytes were the most consistently stimulated cell type at all investigated times (table I). Spleen cells were stimulated at 48 h while lymph node cells were found to be slightly stimulated at 72 h. After agglutination of thymocytes, agglutinated cells were stimulated by the metal compounds at both investigated times while nonagglutinated cells were stimulated at 72 h (table II).

Compared to an earlier report in adult guinea pigs [5] where spleen cells were the most consistently stimulated cell type after addition of metal compounds, in this investigation on newborn animals, thymocytes

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were the most clearly stimulated cell type. This might be due to a postnatal peripheralization of thymocytes responsive to metal allergens. In an earlier study [3], guinea pig thymocytes showed a maximum response to PH A and Con A in the first week after birth, the response thereafter decreased up to the age of 1 month. The findings after addition of metal compounds to PNA separated thymocytes indicate that in newborn guinea pigs both functionally immature and mature thymocytes are stimulated by metal compounds.

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