Development of nucleoside phosphotransferase activity in the cerebral hemispheres of embryonal and adult chick

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Summary. In the cerebral hemispheres of the chick embryo, the level of nucleoside phosphotransferase activity is much higher than that of thymidine kinase and it increases progressively during development up to the adult stage. Therefore nucleoside phosphotransferase is not coupled with DNA synthesis.

Numerous enzymic activities, which exert their action in the area of metabolism linked with DNA synthesis, change during development. In proliferating and developing tissues, DNA polymerase shows characteristic variations which have been correlated with DNA accumulation[1-5]. Similar variations in activity have been shown also for carbamylphosphate synthetase and aspartate transcarbamylase[6]. Furthermore, in developing brains[7-12], the activities of both thymidylate synthetase and thymidine kinase, 2 enzymes which form d-TMP, by means of 'de novo synthesis' and of the 'salvage' of preformed thymidine respectively, have been correlated either with cellular proliferation or DNA synthesis.

Previously, we purified[13] (about 900-fold), and characterized[14] an aspecific nucleoside phosphotransferase of chick embryo - an allosteric enzyme regulated by the nucleotides - which catalyzes the transfer of phosphate ester from a nucleotide donor to a nucleoside acceptor.

In order to ascertain the metabolic role of this enzyme, we have studied the developmental pattern of the nucleoside phosphotransferase activity in the embryonal and adult brain of chick.

Materials and methods. Nucleotides and nucleosides were supplied by Boehringer, Biochemia, s.r.l. Milano. Radioactive thymidine was obtained from Sorin, Saluggia, Italy.

Fertile chicken eggs were placed in an incubator at 37 °C and 60% humidity, and turned at least twice daily. At the appropriate intervals, embryos or chickens (without regard to sex) were decapitated and the cerebral hemispheres quickly removed and weighed. The homogenates were made using a teflon pestle revolving at 3000 rev/min fitted to a Potter-Elvehjem homogenizer, in one of the media described below. The homogenates were then centrifuged at 105,000 × g for 30 min.

Thymidine kinase assay. The cerebral hemispheres were homogenized in a medium (2 µl/mg tissue) consisting of 250 mM sucrose, 100 mM potassium phosphate, pH 7.4, 15 mM/$MgCl_2$, 10 mM ATP, 1 mM dithiothreitol and 3 mM glutamine. The enzyme reaction mixture contained, in a final volume of 500 µl, 100 mM tris-HCl buffer pH 8.0, 5 mM $MgCl_2$, 5 mM ATP, 20 µM (0.5 µCi) (Me^3H)thymidine and 100 µl of the 105,000 × g supernatant. Samples were incubated at 37 °C for 10 min and the reaction stopped by adding 500 µl of trichloroacetic acid 10%.

Nucleoside phosphotransferase assay. The cerebral hemispheres were homogenized in a medium (2 µl/mg tissue) consisting of 5 mM tris-HCl buffer pH 8.8, 5 mM $MgCl_2$, 200 µM d-UMP. The enzyme reaction mixture contained, in a final volume of 500 µl, 40 mM tris-HCl buffer pH 8.8, 5 mM $MgCl_2$, 2 mM d-UMP, 20 µM (0.5 µCi) (Me^3H)thymidine and 50 µl of the 105,000 × g supernatant. Samples were incubated at 37 °C for 10 min and the reaction stopped by adding 500 µl of trichloroacetic acid 10%. TMP formed was measured as previously reported[13]. Proteins were determined by the Lowry colorimetric method[15].

Results and discussion. As previously reported[14], nucleoside phosphotransferase can employ efficaciously as phosphate donor a large number of nucleotides which include deoxyribonucleotides and pyrimidine ribonucleotides. Therefore we believe that the enzyme can assume a general role in controlling the pools of both purine and pyrimidine nucleotides and nucleosides.

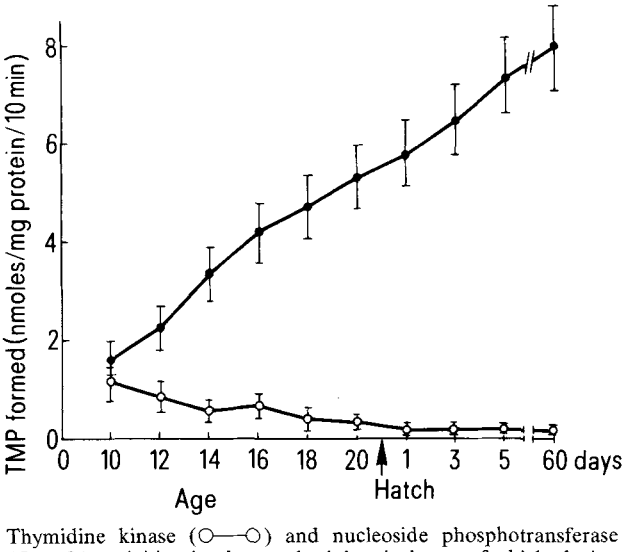
In addition it seems likely that the enzyme plays a part during development in the production of pyrimidine deoxyribonucleotides because it has a very high affinity for d-uridine and d-thymidine and it can produce d-UMP and d-TMP by means of a simple mechanism which is, unlike thymidine kinase[16], independent of ATP and not inhibited by d-TTP.

This paper demonstrates (see fig.) that in the cerebral hemispheres of chick embryo, nucleoside phosphotransferase increases progressively during development and this increase continues also after hatching until the adult age.

This is in contrast with the development pattern shown for thymidylate synthetase and thymidine kinase. In fact, as reported by Hyndman and Zamenhof[12], these activities show the highest level at day 10 of incubation. After this, the activities decrease until day 14, followed by a modest increment from day 14 to day 16 and, then by a definitive decrease from day 16 until day 22.

Our data (fig.) confirm these results for thymidine kinase (TK) and furthermore show that the level of nucleoside phosphotransferase activity (NP) is always, during development, much higher than the respective level of thymidine kinase activity. In addition, the NP/TK ratio increases progressively during development reaching, after hatching, a value of about 40.

In conclusion, although nucleoside phosphotransferase might assume a role in the production of deoxyribonucleotides, its activity is not correlated with the DNA production rate.



Thymidine kinase (O—O) and nucleoside phosphotransferase (●—●) activities in the cerebral hemispheres of chick during embryonal and postnatal development. Enzymatic activities, measured as described in the text, are expressed as nmoles of TMP formed/mg protein/10 min. Values are the mean ± SE of 7 independent determinations.

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Protein degradation in human T-lymphocytes

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Summary. Protein from resting or phytohemagglutinin-stimulated human peripheral blood T-lymphocytes, pulse-labeled 'in vitro' for 1 h with ^{3}H-leucine, had a half-life of 30 h.

Small, nondividing lymphocytes have been used to study the activation process induced by mitogens because metabolic, functional and morphological parameters undergo large-scale changes[1]. Therefore, these cells also provide a convenient model to clarify the regulation of protein metabolism, because protein synthesis can be regulated in a precise way during activation. Although there has been considerable work on protein synthesis in lymphocytes[1], little has been done in the area of protein degradation, a process which is of equal importance with synthesis in maintaining and regulating the protein content of the cells[2]. The present paper describes the general characteristics of protein degradation in human T-lymphocytes.

Material and methods. Human peripheral blood T-lymphocytes were obtained as previously described[3]. Cell viability, checked by trypan blue exclusion, was over 95% and differential counts showed 96.0% T-cells. Cells were incubated at 37 °C in an atmosphere of 5% CO_2 in RPMI 1640 medium (Flow Lag, Irvine, Scotland) (10^6 viable cells/ml) with 10% foetal bovine serum 100 U/ml penicillin and 100 µg/ml streptomycin. For phytohemagglutinin (PHA) stimulation, T-cells were incubated at 37 °C for 30 h in the same medium containing 25 µg/ml PHA-P (Difco Lag, Detroit, Michigan, USA). To study the time course of the stimulation, T-lymphocytes cultures were incubated as described above for 6, 24, 48, 72 and 96 h. 1 h before ending the incubation period, cultures were pulse-labeled with [methyl-^{3}H]-thymidine (2 µCi/ml, 2 Ci/mmole). Autoradiographs were made, as previously described[4], by the 'stripping film' technique. Labeled and unlabeled lymphocytes were evaluated in 3 smears per case (300 cells/case). Lymphocytes from untreated chronic lymphocytic leukaemia (CLL) diagnosed as T-cell leukaemia were isolated and incubated under the same conditions as the normal lymphocytes.

For radioactive labeling, cells were incubated in medium containing L- [4, 5-^{3}H] leucine (0.05 mM, 52 Ci/mmole) for 1 h in siliconized Erlenmeyer flasks at 37 °C with shaking. Then, the cells were washed 4 times with fresh medium containing 2 mM unlabeled leucine with 5 min incubations at 37 °C between resuspension of the cells in medium and centrifugations at 300 × g. After washing, the cells were chased at 37 °C in fresh medium containing 2 mM L-leucine for 24 h. Aliquots of medium were removed at different intervals, cooled in ice and centrifuged (300 × g, 5 min) at 4 °C. The cell pellet was resuspended in phosphate buffered saline. Supernatants and cells were precipitated with 5% trichloroacetic acid (TCA). Precipitates were dissolved in 0.2 N NaOH. Radioactivity was determined by liquid scintillation counting. All counts were corrected for quenching using an internal standard. The TCA-soluble radioactivity of supernatant and cells at each time-point was calculated as the difference between the value at each chase time and zero time and expressed as percent of total radioactivity (TCA-soluble plus TCA-insoluble radioactivity of both cells and supernatant). The half-lives were estimated from the slopes of the best fitted regression lines when the degradation curves were plotted semilogarithmically[5].

Results and discussion. Protein synthesis in resting T-lymphocytes is minimal, perhaps reflecting only the turnover of cell components. To study the mechanism/s of protein degradation in these cells, we have determined first their rate of protein degradation and then compared it with that found in CL (resting) and in PHA-transformed T-lymphocytes. A 30 h PHA incubation period was chosen so that most cells were in the G_1 phase (figure 1).

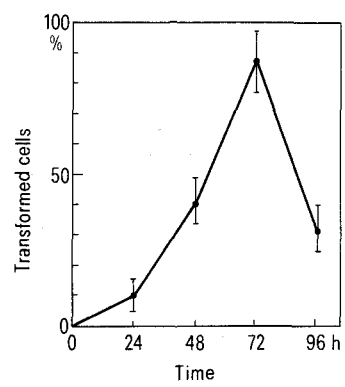

Fig. 1. Time course of T-lymphocyte stimulation by PHA.