Internalization of triiodothyronine-bovine serum albumin-colloidal gold complexes in human peripheral leukocytes

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Summary. Complexes of triiodothyronine-bovine serum albumin-colloidal gold were specifically internalized in human peripheral leukocytes after 5 min of incubation. The specifity was partially lost after a longer incubation.
Key words. Triiodothyronine; internalization; affinity cytochemistry.

Early publications postulated that thyroid hormones (TH) enter the target cell by passive diffusion[1-3]. Further biochemical research proved that TH binds on plasma membranes[4,5], and it was suggested that this could be related to specific internalization of TH. A study describing the entry of triiodothyronine (T_3) into the cell postulated an uptake of T_3 with two transport constants, saturability and energy dependency of the transport mechanism[6]. Moreover, T_3 transport was shown to be separated from that of thyroxine (T_4), and to be energy and Na^+ gradient sensitive[7]. It was proved, using drug inhibition, that transport of T_3 is active[8] and stereospecific with respect to D and L enantiomers[9]. By biochemical means, the transport of T_3 was shown to occur in plasma membrane vesicles[10]. It was suggested that internalization of T_3 is due to receptor-mediated endocytosis[11,12].

We studied the internalization of T_3 by electron microscopy affinity cytochemistry using complexes of colloidal gold stabilized with conjugate of T_3 and bovine serum albumin (GT_3A).

Materials and methods. Human leukocytes from heparinized peripheral blood of euthyroid donors were obtained after 60 min of sedimentation at room temperature and 3 washings in Eagle's minimal essential medium (MEM) for 10 min at 200 × g. Colloidal gold (diameter 15 nm) was prepared as described by Frens[13]. 24 ml of colloidal gold were stabilized with conjugate of T_3 and bovine serum albumin[14] similarly as immunogold complexes[15] at pH 9. Each molecule of bovine serum albumin was associated with 17 molecules of T_3, as determined analytically. Each gold particle was calculated to be associated with 600–1000 molecules of T_3, which was immunoreactive in RIA. The purified complexes were resuspended in phosphate buffer, pH 7.6. 10×10^6 leukocytes were incubated for 5 and 60 min with GT_3A complexes in MEM containing 4% of polyvinylpyrrolidone. Competitive controls were incubated in the presence of 50 nM of free T_3. The cells were then washed 3 times in phosphate-buffered saline (PBS) and processed for electron microscopy. Parallel sets of samples were incubated under similar conditions with complexes of colloidal gold stabilized with bovine serum albumin only (complexes GA). The concentration of gold particles in GT_3A and GA were determined spectrophotometrically. Thin sections from each sample were systematically photographed and the presence of complexes on plasma membrane and in particular cell structures was determined.

Results. GT_3A complexes were bound on the plasma membrane of lymphocytes and granulocytes after 5 min of incubation. Some complexes were found in the tubulovesicular structures and less frequently in the Golgi region (figs 1, 2, 3). After 60 min of incubation, the complexes were found in the same structures as after 5 min. In addition, a large number of complexes was observed in the lysosomes (fig. 4).

Morphometric analysis of the binding and internalization of the complexes in the experimental and control preparations revealed specific binding to the plasma membrane in both incubation periods and specific internalization after 5 min of incubation (fig. 5). The total number of complexes inter-

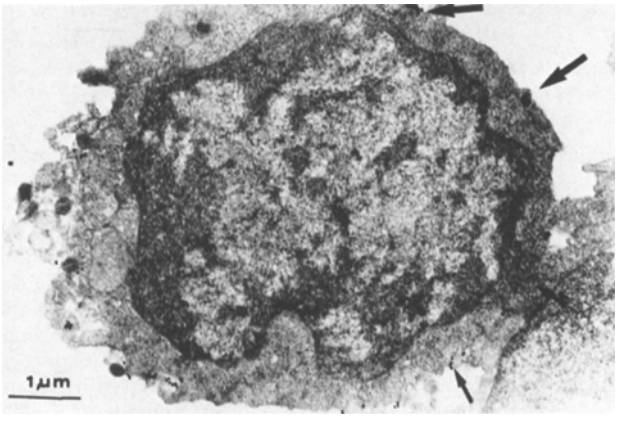


Figure 1. Lymphocyte incubated with GT_3A for 5 min. Binding of individual complexes on plasma membrane (arrow) and clustering of complexes (large arrows).

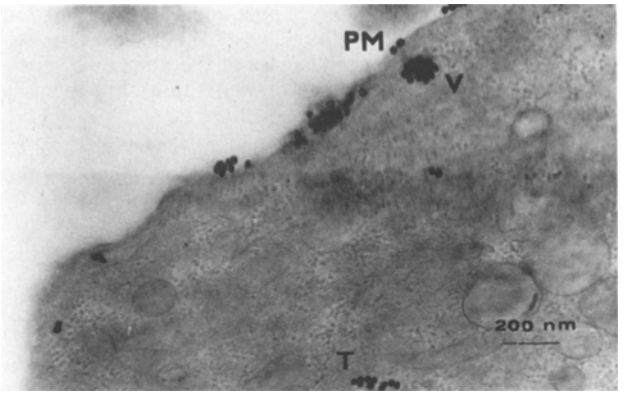


Figure 2. Detail of a granulocyte incubated for 5 min with GT_3A complexes. Frequent binding of complexes on plasma membrane (PM), clustering of complexes in a vesicular structure (V) and internalization in a tubular structure (T).

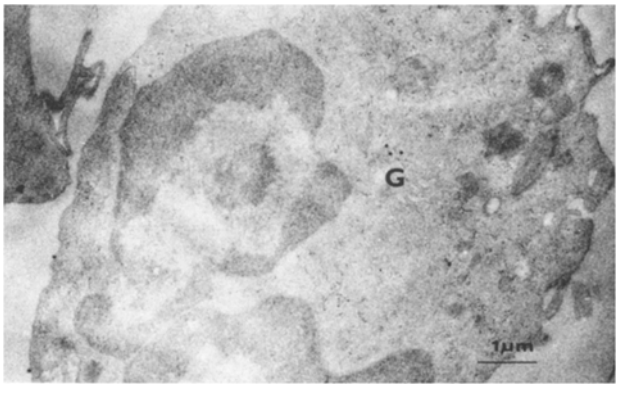


Figure 3. Detail of a granulocyte incubated for 5 min with GT_3A. Complexes in Golgi region (G).

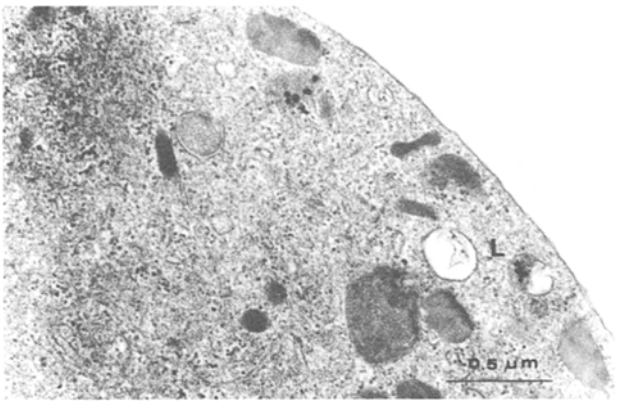
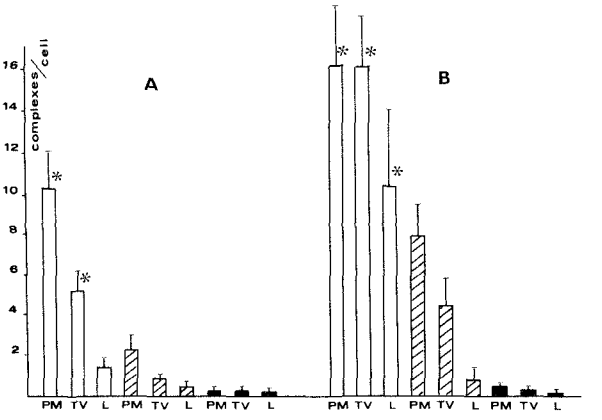


Figure 4. Detail of a granulocyte incubated for 60 min with GT_3A complexes. Complexes in lysosomes (L).

nalized was, however, higher in competitive controls after 60 min of incubation.

Discussion. Our results show that complexes of triiodothyronine-bovine serum albumin-colloidal gold are suitable for the study of specific T_3 internalization. During a short-time (5 min) incubation free T_3 competed with the binding of the complexes on the plasma membrane. The decrease of the presence of GT_3A complexes in vesicular and tubulovesicular structures indicates a partial specifity of T_3 transport; it indicates also that T_3 enters the cell at least in part by receptor-mediated endocytosis, as previously suggested[11, 12]. The higher number of internalized particles found with free T_3 after 60 min of incubation than were found without it may be caused by a stimulatory effect of free T_3 on the internalization of GT_3A complexes, a phenomenon similar to that observed by Rao[6]. Our results suggest in some cases a possibility of GT_3A exocytosis (decrease of the number of internalized complexes after 60 min of incubation), but the data are not sufficient to allow clear conclusions.



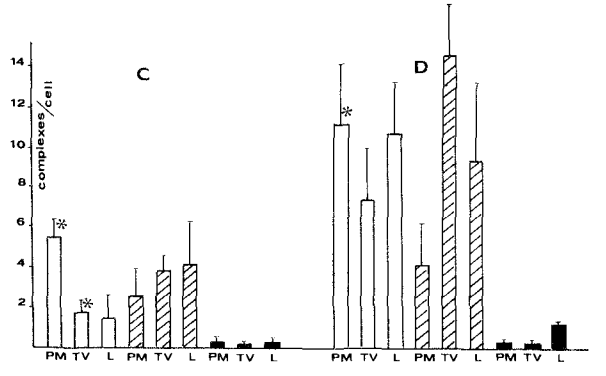


Figure 5. Mean number+SE of complexes bound on plasma membrane (PM), internalized in tubulovesicular structures (TV) and in lysosomes (L) (per mid-plane cell section). *A* Lymphocytes incubated with GT_3A for 5 min. *B* Granulocytes incubated with GT_3A for 5 min. *C* Lymphocytes incubated with GT_3A for 60 min. *D* Granulocytes incubated with GT_3A for 60 min. White columns: incubation with GT_3A only; hatched columns: incubation with GT_3A in the presence of free T_3; black columns: incubation with complexes GA only. *indicate significant differences on 0.5% level between values for incubations with GT_3A and GT_3A with free T_3. (Student's t-test and f-test used).

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