The existence of different clones may reflect different stages of tumor progression. The possibility likewise cannot be ruled out that the system of intercellular interactions enable the tumor tissue to preserve a structure that resembles that of the original normal tissue. Further investigations of interclonal interactions in tumors will show which of these suggestions is right.

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INDUCTION OF Ca^{++} TRANSPORT IN HUMAN PLATELETS BY THYROID HORMONE RECEPTOR OF MALIGNANT CELLS

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Physiological effects of thyroid hormones are determined by their influence on transcription and translation [14, 15] and also their modifying action on the permeability of biological membranes [8, 13]. Several views are currently held on the mechanism of action of thyroid hormones on biological membranes [4, 10, 11]. It has been shown that these compounds influence biosynthetic processes by modulating the protein-lipid composition of the membranes and thereby modifying protein-lipid interactions in them [9]. Meanwhile the possibility of a direct membranotropic action of thyroid hormones has been investigated [8] and it has been shown that their physiological action is the result of a change in the physicochemical properties of biological membranes, leading to a change in their permeability [10].

There is no doubt that the effects of thyroid hormones are mediated through their interaction with specific receptor proteins. The isolation of thyroid hormone receptors of normal and malignant cells, and some of their properties, have been described previously [3, 5]. It has been shown that the hormone-receptor complex of cancer cells, irrespective of the location and etiology of the tumor, induces Ca^{++} conduction in experiments on artificial bilayer phospholipid membranes (BPM), whereas in the presence of other cations (H^+, K^+, Na^+, Ba^{2+}, Mn^{2+}, Mg^{2+}, Sr^{2+}) the resistance of BPM is unchanged [2, 6]. Under analogous experimental conditions the hormone-receptor complex of normal cells selectively induces only H^+-conductance of BPM [6].

It will be evident that the conditions of model experiments do not always adequately reflect physiological processes taking place in biological systems. Accordingly, it was necessary to study the effect of thyroid hormone receptors of normal and cancer cells on permeability of biological membranes.

This paper describes a study of the effect of thyroid hormone receptor on Ca^{++} transport in human platelets.

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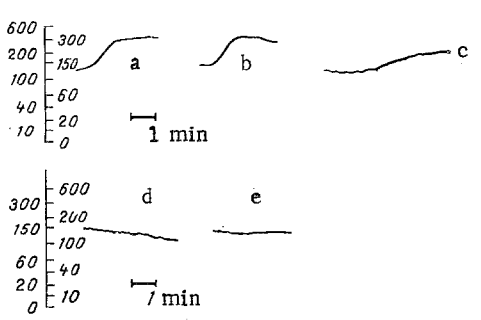

Fig. 1. Dependence of Ca^{++} concentration in platelets (in mM) on time after addition of complex of cancer cell receptor with T_4 (a), with T_3 (b), and with T_3 in the presence of 10^{-4} M verapamil (c). d, e) Effect of complex of normal cells with T_3 and T_4, respectively on intracellular Ca^{++} concentration. Receptors and hormones were preincubated before addition under conditions described in Experimental Method Section.

EXPERIMENTAL METHOD

In experiments to study functions of the normal thyroid hormone receptor, the receptor isolated from the cytoplasm of 6-10-week human embryos and thyroxine-binding blood serum prealbumin were used. The cancer cell receptor was isolated from the cytoplasm of HeLa and gastric adenocarcinoma cells. The receptors were isolated on CNBr-activated Sepharose 4B with immobilized thyroxine [5]. Final purification of the protein fractions after affinity chromatography was carried out by the method of preparative electrophoresis in 7% polyacrylamide gel, pH 8.3.

Human platelets were obtained from blood of healthy donors, who had taken no drugs for 10 days. The blood was mixed with an anticoagulant, containing 25 g of Na_3-citrate-$2H_2O$, 14 g of citrate-H_2O, and 20 g dextrose to 1 liter of water (six parts of blood to one part of anticoagulant) and centrifuged for 15 min at 190 g (37°C). Platelet-enriched plasma was centrifuged for 10 min at 1200 g. The residue was suspended in buffer containing 10 mM HEPES-Na (pH 6.55; 37°C), 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM glucose, 35% bovine serum albumin type V, 50 U/mg heparin, 150 mM NaCl, 2.7 mM KCl, 0.37 mM NaH_2PO_4, and 0.2 mg/ml of apyrase, with activity of 11 units per milligram protein. The volume of the platelet suspension was equal to the volume of platelet-enriched plasma used. To the platelet suspension 40 mM of a solution of the acetoxymethyl ester of quin-2 in dimethyl sulfoxide was addded up to a final concentration of 10 μM and the sample was incubated for 30 min at 37°C with periodic mixing. The platelets were then sedimented for 10 min at 1200 g. The residue was suspended in buffer containing 10 mM HEPES-Na (pH 7.4; 37°C), 150 mM NaCl, 2.7 mM KCl, 0.37 mM NaH_2PO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 5 mM glucose, 0.35% bovine serum albumin, and 0.12 mg/ml of apyrase. The number of platelets in the suspension was $(4-6)\cdot 10^8$ cells/ml.

To measure fluorescence the final suspension of platelets was diluted 10 times in buffer with pH 7.4, not containing albumin or apyrase. Fluorescence of the intracellular quin-2 was recorded on a Hitachi-850 spectrofluorometer at 30°C in a quartz cuvette (1 cm), with an exciting wavelength of 340 nm and an emission wavelength of 500 nm. The intracellular Ca^{++} concentration was calculated by the equation [9]:

$$Ca = 115\cdot(F - F_{min})/(F_{max} - F),$$

where F stands for the fluorescence measured in the experiment, F_{max} for fluorescence in the presence of 50 μM digitonin, and F_{min} fluorescence after addition of 0.5 mM $MnCl_2$.

The receptor of normal or cancer cells, in a concentration of 10^{-6} M, was incubated for 30-35 min in the presence of $5\cdot10^{-6}$ M thyroxine (T_4) or tri-iodothyronine (T_3) in buffer in which fluorescence was measured. The incubation mixture was then poured into the cuvette and the platelet suspension added to it.

EXPERIMENTAL RESULTS

The results of experiments to study the effect of thyroid hormone receptors on the calcium concentration in platelets saturated with quin-2, a fluorescent Ca^{++} ion chelating agent, are given in Fig. 1. The thyroid hormone receptor of cancer cells, added after preliminary preincubation for 30 min with T_3 or T_4 to the platelet suspension, led to a time-dependent increase in fluorescence of the dye, evidence of an increase in the Ca^{++} concentration in the cytoplasm (Fig. 1a, b). An increase in the intracellular Ca^{++} concentration was induced only by the hormone-receptor complex of the cancer cells. Addition of a complex of the normal cell receptor with T_4 or T_3 to the platelets did not cause the intracellular Ca^{++} level to rise

TABLE 1. Effect of Thyroid Hormone Receptor of Normal (RNC) and Cancer (RCC) Cells

Experimental conditions	Intracellular Ca^{++} conc. after addition of incubation mixture to platelets, nM
Control	110
Without preincubation	
RNC + T_3 (T_4)	110—112
RCC + T_3 (T_4)	105—110
Incubation for 30 min	
RNC + T_3 (T_4)	116—120
RCC + T_4 (T_4)	304—310
RCC + T_4 + 10^{-4} M verapamil	199
T_3	115
T_4	108
RNC	112
RCC	123

(Fig. 1d, e). Special experiments showed that thyroid hormones (T_3 or T_4 in a concentration of $5 \cdot 10^{-6}$ M), and also receptors of normal and cancer cells (10^{-6} M), added separately to the cell suspension, did not induce any change in the Ca^{++} concentration in the platelets (Table 1). The Ca^{++} concentration likewise was unchanged when the receptor was added together with the hormone after preliminary preincubation. This is evidence that the Ca^{++} concentration in the platelets was raised only by the action of the hormone-receptor complex. It is evident that the receptor, during interaction with the hormone, changes its conformation so that it becomes capable of affecting intracellular Ca^{++} accumulation.

On incubation of erythrocytes with the hormone-receptor complex of malignant cells a similar increase in the intracellular Ca^{++} concentration was observed [6]. It is not impossible that a similar effect will also be observed in other types of cells, i.e., that the ability of the hormone-receptor complex of cancer cells to accumulate Ca^{++} may be universal in character.

The increase in the Ca^{++} concentration in the cytoplasm may be due both to stimulation of the Ca channels of the plasma membrane and to release of Ca^{++} from intracellular sources. Several methods are now available for differentiating these processes. One generally accepted approach is inhibitor analysis. Several substances which cause selective blocking of the Ca channels of the plasma membrane and thereby prevent Ca^{++} from entering the cell are known. One such inhibitor is verapamil [12]. As will be clear from Fig. 1c verapamil, in a concentration of 10^{-4} M, delays the increase in the Ca^{++} concentration induced in platelets by the hormone-receptor complex of cancer vells, and reduces the absolute magnitude of this effect. Consequently, the increase in the intracellular Ca^{++} concentration in the platelets in this case is due to opening of the Ca channels of the plasma membrane.

We now know that metabolism of Ca^{++} ions in cancer cells has certain special features. In particular, it has been shown that the Ca^{++} concentration in cells which have undergone malignant transformation is higher than in normal cells [7]. Since thyroid hormone receptors of normal and cancer cells differ in their properties [1, 6], the possibility cannot be ruled out that the cause of the increase in the Ca^{++} concentration in the cells of malignant tumors is the ability of the thyroid hormone receptor of these cells to induce Ca^{++} transport through the plasma membrane.

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SELECTIVE GANGLIOSIDE SHEDDING FROM MOUSE ASCITES SARCOMA 37 CELLS

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Glycolipids, which are specific components of plasma membranes, undergo qualitative and quantitative changes during malignant transformation of cells. Under these circumstances, in particular, certain types of glycolipids such as G_{D3} of human melanoma [11], ceramide-trihexoside of Burkitt's lymphoma [10], fucose-containing monosialogangliosides in neoplasms of the gastrointestinal tract [9], ganglioside G_{M2} in neuroblastoma [12], and so on, which are specific markers of neoplasms, accumulate in tumors. We also know that gangliosides have the property of being continuously shed from the surface of tumor cells and certain normal cells into the extracellular space [1, 2, 13], where individual types of gangliosides may accumulate as the result both of shedding of products of "incomplete" synthesis and also of selective shedding of gangliosides by tumor cells.

This paper described the study of the shedding of gangliosides by cells of mouse ascites sarcoma 37.

EXPERIMENTAL METHOD

Experiments were carried out on male SHY/Kv mice weighing about 25 g with an intraperitoneally implanted ascites sarcoma 37. On the 8th day after transplantation of $5 \cdot 10^5$ tumor cells, the ascites fluid was withdrawn. The number of cells in the ascites fluid of carcoma 37 was $7.9 \cdot 10^7$/ml.

All subsequent procedures were carried out at a temperature of 0-4°C. The cell suspension was centrifuged at 800 g for 15 min. The supernatant (S_1) was removed and the cells resuspended in Hanks' solution and centrifuged as described above, to yield a supernatant (S_2) and cells (C_1). Supernatants S_1 and S_2 were centrifuged separately at 150,000g for 60 min. This yielded supernatants S_1' and S_2' and residues R_1 and R_2.

To determine galglioside shedding *in vitro*, carcoma 37 cells were sedimented by centrifugation at 800 g, washed twice as described above, and suspended in Hanks' solution. The number of cell suspensions was $7.7 \cdot 10^7$/ml. The number of viable cells, determined by the trypan blue test, was 85-90%. The cell suspension was incubated, with constant mixing, at 37°C for 60 min and centrifuged at 800g for 15 min, yielding cells (C_2 and supernatant S_3).

Gangliosides were separated from the tumor cells and lyophilized supernatants [6]. To determine the level of lipid-bound sialic acids (LSA) and to identify the gangliosides, micro-

KEY WORDS: gangliosides; ascites sarcoma 37; shedding form the cell surface.

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