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REVIEW

MODE OF ENTRY OF STEROID AND THYROID HORMONES INTO CELLS

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Characteristics of the processes by which steroid and thyroid hormones enter tissues, cells and membrane vesicles are reviewed. Several authors suggest that entry is by passive diffusion: the accumulation within cells is attributed to cytoplasmic binding proteins. Other authors, however, propose a membrane-mediated process of entry. The involvement of saturability, high specificity, sensitivity to temperature, sulfhydryl and cell-surface-perturbing reagents and hydrolytic enzymes support the latter view. Purified plasma-membrane vesicle preparations retain several characteristics of entry shown by intact cells. Intracellular hormone-binding proteins would not contribute to processes observed with these preparations.

Keywords: steroid, thyroid-hormone entry; tissue, intact cells, membrane vesicles; membrane mediation; diffusion.

The study of the mechanism of action of steroid and thyroid hormones at the molecular level has attracted many investigators. These hormones appear to be effective only when they are present inside cells. By contrast, peptide and polypeptide hormones appear to initiate their physiological effect by remaining on the exterior of the cell bound to the plasma membrane. It is generally accepted that the steroid first reacts with a receptor protein in the cytoplasm, forming a steroid–receptor complex. Activation of the complex occurs in the cytoplasm. The active form then enters the nucleus and binds to "acceptor" sites. (A critique of the different models of steroid hormone action has been discussed by Gorski and Gannon, 1976.) Proteins in the cytoplasm also bind thyroid hormones, but a hormone–protein complex is not essential for the binding to nuclear sites (Woeber and Braverman, 1977). The net result of the interaction between the hormone–receptor complex and the nucleus is a stimulation of the production of mRNA or its enhanced utilization. However, these different intracellular events are preceded by the translocation of the hormone across the plasma membrane into the cell. The process by which this translocation is achieved has been studied recently. Arguments in favor of mediated transport are confronted with those against this view. (Transport or entry is defined as translocation or migration of the hormone from one phase across the plasma membrane to another; if components or carrier system(s) in the plasma membrane are involved in the translocation or migration process it is referred to as

mediated transport. For a detailed explanation refer to Lehninger, 1975; Christensen, 1975.) In this review an attempt is made to present some contemporary thoughts on the entry mechanisms of steroid and thyroid hormones into cells. Hopefully these will provide a better understanding of the very first step in the chain of events in the mechanism of hormone action, namely, the interaction between the free hormone and the plasma membrane.

More than 90% of steroid hormones and 99% of thyroid hormones that circulate in blood are bound to plasma proteins. The biological activity, however, is attributed to the fraction of the hormone that is not bound. This free hormone must penetrate the plasma membrane in order to act within the cell. The lipophilic nature of the hormone facilitates easy permeation of the plasma membrane which is rich in lipids. The popular view attributes entry to simple diffusion, and accumulation to binding to proteins or receptors inside the cell. The present consensus maintains that the plasma membrane is not a barrier for the movement of lipophilic hormones in and out of cells. Methods to study enzyme kinetics have served for the analysis of the rates of uptake or transport by intact cells or plasma-membrane vesicles. (Uptake is defined as the association or binding of the hormone with a biological system or with some component of the plasma membrane irrespective of the nature of association; uptake may be coupled with transport.) The following are properties characteristic of mediated transport and uptake processes: saturation kinetics, temperature sensitivity, high affinity, specificity for the ligand, transport against a gradient, requirement or involvement of metabolic energy, inhibition by thiol-group-blocking agents and transport into the lumen of the membrane vesicle. The simple diffusion process differs considerably in its properties from the above-mentioned characteristics. (For details the reader is requested to consult Stein, 1967; Christensen, 1975; Neame and Richards, 1972; Lehninger, 1975.)

Uptake of steroid hormones has been studied in lymphocytes (Munck and Brinck-Johnsen, 1968), anterior pituitary cell suspensions (Leavitt et al., 1973), rat kidney (Pasqualini and Sumida, 1977), toad bladder (Crabbé, 1977), neuronal and glial cells from the brain of chicken in culture (Vernadakis and Culver, 1976), uterine cell suspensions (Williams and Gorski, 1973; Müller and Wotiz, 1979) and whole uteri (Williams and Gorski, 1974), rat uterus and diaphragm (Peck et al., 1973), rat uterine horns (Milgrom et al., 1973), normal mammary parenchymal and cancer cells of the rat (Grubbs and Moon, 1974), cultured bovine mammary cells (Tucker et al., 1971), a mouse pituitary cell line (AtT-20/D-1) (Harrison et al., 1975), human and canine erythrocytes (Brinkmann et al., 1971; Jacobsohn et al., 1975; Philip and Marotta, 1974), canine prostate (Giorgi, 1976), cultured hepatoma cells (Baxter et al., 1975; Plagemann and Erbe, 1976; Graff et al., 1977), liver cells (Rao et al., 1976a, c, 1977a, b; Pietras and Szego, 1979a), isolated endometrial cells (Pietras and Szego, 1977) and the bacterium *Pseudomonas testosteroni* (Watanabe et al., 1973). In addition, a variety of other tissues and cells have been used (King and Mainwaring, 1974). Uptake by plasma membranes has also received a considerable amount of attention. Membranes were obtained from rat liver (Suye-

mitsu and Terayama, 1975; Alléra et al., 1979), from ascites hepatomas (Terayama et al., 1976), from rat anterior pituitary gland (Koch et al., 1978), from *Pseudomonas testosteroni* (Lefebvre et al., 1976), from rat uterus (Pietras and Szego, 1979b; Müller and Wotiz, 1979) and from human placenta (Fant et al., 1979).

STUDIES WITH STEROID HORMONES

Steroid hormones are rapidly taken up by tissues and cells at 37°C; hence, accurate measurement of initial rates of uptake poses a technical problem. Even at low temperatures the linear phase of uptake lasts only for seconds; for example, the uptake of estrone, estradiol-17β and testosterone by isolated rat-liver cells at 15°C is linear up to 10 sec (Rao et al., 1977a). When whole rat uteri are used, the uptake of estradiol-17β is linear up to 15 min at 37°C (Milgrom et al., 1973); however, isolated cells from rat uterus take up estradiol-17β linearly up to 20 sec at 35°C (Müller and Wotiz, 1979). Isolated pituitary cells of the rat take up 30% of incubated estradiol-17β within 5 min at 37°C (Leavitt et al., 1973). Intact AtT-20/D-1 cells take up triamcinolone acetonide maximally within 2 min at 4°C. At 25°C 3 times more is taken up after 30 min than at 4°C (Harrison et al., 1975). Novikoff rat hepatoma cells in suspension culture take up prednisolone maximally within 15 min at 18°C (Plagemann and Erbe, 1976); at 37°C maximal uptake occurs after about 15 min (Graff et al., 1977). The uptake of cortisol by isolated rat-liver cells is linear up to 45 sec at 37°C (Rao et al., 1976a); the uptake of corticosterone is linear up to 17 sec at 14, 27 and 37°C (Rao et al., 1977b). The conclusion that may be drawn from these time-course experiments is that the phase of the initial rate of uptake of steroids at physiological temperature is extremely short-lived.

Transport of ions and nutrients, membrane receptor function and activity of membrane enzymes are affected by the fluidity of membrane lipids which react sensitively to changes in temperature (Cooper, 1977). Mediated-transport processes may be compared with biological reactions that have a Q_{10} of 2 or 3. Such reactions have activation energies between 42 and 84 kJ/mole (Bray and White, 1966). This type of transport often exhibits discontinuity (phase transition) in the Arrhenius plot. Simple diffusion processes have a Q_{10} of 1.4 (Lehninger, 1975; Christensen, 1975) and are not sensitive to lipid-phase transition (Graff et al., 1977). The process of uptake of cortisol and corticosterone by intact rat-liver cells (Rao et al., 1976b, 1977b, 1979) and of triamcinolone acetonide by mouse pituitary tumor cells (Harrison et al., 1975) shows discontinuities in the Arrhenius plots. The Q_{10} values for cortisol and corticosterone are in the range observed for biological reactions. Although the Q_{10} value for the uptake of prednisolone by cultured Novikoff hepatoma cells is in keeping with that of a biological reaction, it is thought that the effect of temperature is on the rate of diffusion through the membrane, decreasing at lower temperatures owing to a decrease in the fluidity of the membrane lipids; in this case the Arrhenius plot shows no discontinuities and is linear (Graff et al.,

1977). The effects of temperature on the uptake of estradiol-17β by uterine cells (Q_{10} of 2.0) are thought to reflect the binding properties of the cytoplasmic estrogen receptor (Müller and Wotiz, 1979). In contrast with these findings, the uptake of estradiol-17β by the rat uterus is found to be dependent on temperature but is thought not to be due to the limitation of the estrogen–receptor interaction (Milgrom et al., 1973). Although sensitivity to temperature serves to differentiate to some extent between mediated uptake and simple diffusion, the significance of this intrinsic membrane property is held in doubt.

An important criterion of a mediated-uptake process is saturability. Estrone, estradiol-17β, testosterone, cortisol and corticosterone interact with component(s) of the plasma membrane of the isolated rat-liver cell, and the interaction exhibits saturation kinetics (Rao et al., 1976a, c, 1977b). The uptake of estradiol-17β by the rat uterus (Milgrom et al., 1973), by rat pituitary cells (Leavitt et al., 1973) and by isolated rat endometrial and liver cells also exhibits saturation kinetics (Pietras and Szego, 1977, 1979a). Bovine mammary cells in culture have 2 high-affinity components for the uptake of cortisol (Tucker et al., 1971). One feature that is noteworthy in these studies is that the uptake system has more than one saturable component, a high-affinity and a low-affinity uptake component; the presence of a diffusion component is not unusual. Uptake due to the latter probably takes place even under physiological conditions. For corticosterone this is estimated to be 10% of the total uptake (Rao et al., 1977b). Diffusion, non-specific binding and adsorption do not exhibit saturability. For example, uptake of prednisolone by cultured Novikoff hepatoma cells (Plagemann and Erbe, 1976; Graff et al., 1977), uptake of cortisol by rat liver, kidney, spleen and brain (Bellamy et al., 1962), uptake of estradiol-17β by rat uterus and diaphragm (Peck et al., 1973), the uptake of estradiol-17β by isolated uterine cells (Müller and Wotiz, 1979), and the uptake of estradiol-17β by rat uterine cell suspension and whole uteri (Williams and Gorski, 1974) are all non-saturable processes. The obvious conclusions from these studies are (a) that steroid hormones may interact with a component(s) of the plasma membrane and exhibit saturability which is characteristic of mediated-transport systems; (b) that steroid hormones do not interact with components of the plasma membrane and that entry is by simple diffusion. The problem is undoubtedly complex.

An integral part of the saturability experiments is the differentiation between specific and non-specific uptake processes; to achieve this, advantage is taken of the fact that a large excess of the non-labeled hormone competes with the radioactive hormone for specific processes which can be saturated, leaving uptake by non-specific and diffusion processes, which are not saturated, unaffected. Specific uptake is then the difference between total and non-specific uptake. The method of distinguishing between the 2 types of uptake is applicable to uptake or binding systems which do not exhibit positive co-operativity. The apparent K_t values (concentrations of the steroid at which uptake is half-maximal) for the uptake of estrone, estradiol-17β and testosterone by liver cells are in the micromolar range (Rao et al., 1977a, 1979), and for cortisol and corticosterone in the nanomolar range (Rao et

al., 1976a, 1977b). When cells are allowed to react with the steroid hormone till equilibrium is reached, the apparent dissociation constant, K_d, for the binding of estradiol-17β to hepatocytes at 22°C is 2 pM, and the total number of binding sites per cell is 3400 (Pietras and Szego, 1979a). The specific binding component in pituitary cells for estradiol-17β has a K_d of 0.43 nM (Leavitt et al., 1973). The cells have about 19000 binding sites. The K_d for the saturable process of uptake of estradiol-17β by uterine horns is 3.21 nM (Milgrom et al., 1973). 2 glucocorticoid-binding components with K_d values of 4.64 nM and 15.7 μM for triamcinolone acetonide have been reported with mouse pituitary tumor cells (Harrison et al., 1976). The bovine mammary cell has about 7500 specific binding sites (Tucker et al., 1971). These data fulfill 2 criteria for mediated transport, namely (a) sensitivity to temperature, and (b) saturation. That rat endometrial and liver cells bind estradiol-17β immobilized by covalent linkage to albumin-derivatized nylon fibers is good evidence supporting the hypothesis of an initial interaction between the estrogen and component(s) of the plasma membrane before the steroid is internalized (Pietras and Szego, 1977). Uterine cells do not appear to contain such specific binding proteins in/on the membrane; this conclusion is arrived at from the result of the inability of estradiol-17β-carboxymethyloxime–bovine serum albumin to reduce the uptake of estradiol-17β (Müller and Wotiz, 1979). An interesting observation is that the concentration of estrone, estradiol-17β, testosterone (Rao et al., 1977a) and prednisolone (Plagemann and Erbe, 1976) at the initial phase of uptake is larger in cells than in the external medium. The ratios of the concentrations of testosterone, androstenedione and dehydroepiandrosterone in human benign hypertrophic prostate tissue to the concentration in plasma are 5.91, 5.04 and 6.89, respectively, suggesting that the tissue concentrates androgens (Farnsworth, 1971). Canine prostate tissue slices also concentrate androgens when subjected to superfusion with medium containing the hormone (Giorgi, 1976). Several other steroids are also accumulated by their target organs. The uptake of sex hormones is sensitive to metabolic inhibitors (Rao et al., 1977a; Milgrom et al., 1973), but that of corticosterone (Rao et al., 1977b) and prednisolone (Plagemann and Erbe, 1976) is not affected. The concentrative process may involve cellular energy, but it is also likely that the translocated steroid is bound in the cytoplasm and the decrease in uptake by metabolic inhibitors may well be an effect on uptake and binding.

Cells treated with thiol-group-blocking agents take up less hormone. The conclusion drawn from this result is that thiol groups in the plasma membrane may be involved in the uptake process. For example, the uptake of estrone and estradiol-17β is reduced by several thiol-group-blocking agents; *N*-ethylmaleimide appears to be most effective. Uptake of testosterone is not affected by any of the reagents (Rao et al., 1977a). α-Iodoacetamide and iodoacetate inhibit the uptake of estradiol-17β by the rat uterus by more than 90% (Milgrom et al., 1973). Uptake of cortisol is reduced by 1-fluoro-2,4-dinitrophenol and *p*-chloromercuribenzoate but not by *N*-ethylmaleimide (Rao et al., 1976a); uptake of corticosterone is reduced 50% by *N*-ethylmaleimide (Rao et al., 1977b). These effects may be considered as addi-

tional evidence for the protein nature of the uptake system, because it is inconceivable that a simple diffusion process is affected by thiol-group-blocking agents. However, it must be remembered that these reagents, depending on their concentration, may affect the viability of the cell, resulting in a decrease in uptake and thus lead to erroneous conclusions.

Cell-surface components, such as glycoproteins, mucopolysaccharides and glycolipids, exercise vital functions in an organism (Nicolson and Poste, 1976; Popper et al., 1977). These components also appear to take part in the uptake of steroid hormones, because enzymes that perturb them lead to a reduction of uptake (Rao et al., 1977b; Harrison et al., 1974, 1976). Uptake of prednisolone, however, by glucocorticoid-unresponsive Novikoff hepatoma and Reuber hepatoma cells is not affected by treatment with neuraminidase or phospholipase C (Plagemann and Erbe, 1976).

A paramount factor that needs careful consideration in investigations on the uptake of steroids by intact viable cells is the binding of steroids by intracellular proteins and organelles; the steroid is thought to diffuse rapidly through the cell membrane and to bind immediately in the cytoplasm. Experiments to demonstrate that the mediated-transport process actually occurs and is followed by binding to cytoplasmic proteins are technically difficult to carry out because methods are required for accurate measurement of uptake within fractions of a second or seconds, that is at a time when the hormone, after crossing the plasma membrane, is still not bound in the cell. However, one approach did throw some light on this aspect. The kinetics of uptake of cortisol by viable cells and the binding to cytosol were studied under identical conditions (Rao et al., 1976b), with findings as follows. (1) Uptake by cells exhibits a much greater sensitivity than binding by cytosol toward changes in temperature. The activation energy for uptake between 5 and 20°C is 75.1 kJ; for binding, the value is 5.0 kJ between 5 and 32°C; moreover, the Arrhenius plot of the uptake by cells displays a transition in temperature at 20°C, whereas binding by cytosol does not. (2) Uptake of cortisol by cells shows two saturable components and a diffusion component, whereas binding is proportional to the concentration of cortisol. (3) Treating cells with 2,4-dinitrophenol or KCN reduces uptake, but binding is not affected. Therefore, if uptake by cells were due to binding by cytoplasmic components there would be marked similarities in the two processes with the plasma membrane functioning as a permeable membrane for hormones. The differences strongly suggest that uptake is different from binding; the former is a membrane-mediated process and the latter an intracellular reaction.

Another approach to permit differentiation between mediated transport and binding to cytoplasmic components is to study uptake by plasma-membrane fractions (Suyemitsu and Terayama, 1975; Watanabe and Po, 1974; Lefebvre et al., 1976; Koch et al., 1978; Müller et al., 1979; Alléra et al., 1979; Fant et al., 1979). The advantage is that such a membrane preparation contains, if any at all, extremely low amounts of contamination by subcellular organelles and cytoplasmic steroid-binding proteins. A highly purified plasma-membrane vesicle preparation

from rat liver (Eckel et al., 1979) took up corticosterone extremely rapidly at 15 or 23°C (Alléra et al., 1979). In the concentration range of corticosterone in the medium, between 30 and 8100 nM, uptake occurs both by a mediated process and by simple diffusion. At low concentrations of the hormone, between 2 and 227 nM, and in the presence of a 200-fold molar excess of non-labeled corticosterone, uptake exhibits several saturable components. The apparent K_d values of the uptake systems are 7.2 ± 2.0 and 234 ± 67 nM (Alléra et al., 1979). The K_m for the uptake by placental membrane vesicles is 7 nM (Fant et al., 1979). The K_d of 7.2 nM agrees well with the concentration of free corticosterone in the rat (3–14 nM; Keller et al., 1966). Uptake of a steroid is often accompanied by adsorption onto the external surfaces of cells and membrane vesicles. It is possible to distinguish between adsorption and mediated transport by a procedure applicable to membrane vesicles, which are normally susceptible to changes in the osmolarity of the external medium. Increasing the osmolarity will lead to a reduction in vesicle volume and to a decrease in steroid uptake, but adsorption will not be affected (Alléra et al., 1979). With this approach the total uptake of corticosterone by rat-liver plasma-membrane vesicles consists of 45–60% of transport into the vesicle lumen and the rest is adsorption onto the vesicle exterior. The transport portion may be due to simple diffusion or to a membrane-mediated process. A distinction between the two processes cannot be made from this type of experiment. Because uptake is saturable, transport of corticosterone into the vesicle is a membrane-mediated process. The uptake of corticosterone by human placental membrane vesicles is also dependent on the intravesicular volume; 84% of the total corticosterone taken up is in the free form in the vesicles (Fant et al., 1979).

At 8 nM of corticosterone in the external medium, the concentration inside the vesicle is 10–19-fold higher; it is highly improbable that this accumulation against an apparent concentration gradient occurs by simple diffusion. Estimation of the contamination of membrane vesicles by cytoplasmic binding proteins varies between 0 and 3% as judged by the activity of glucose-6-phosphate dehydrogenase, this enzyme being used as marker for the soluble portion of the cytoplasm (Lehninger, 1975; Müller and Wotiz, 1979). Thus the contribution of binding by cytoplasmic components to uptake is estimated to be less than 0.7% (Alléra and Rao, unpublished observations). Specific and saturable binding of estradiol-17β to purified uterine membranes obtained from whole uteri has been attributed to cytoplasmic estrogen receptor contamination (about 5%; Müller et al., 1979). In a series of controlled experiments, the binding of estradiol-17β to a partially purified plasma-membrane fraction obtained from isolated rat uterine cells is saturable and specific. In contrast with the previous finding (Müller et al., 1979), only a small fraction of binding is due to retention or adsorption of cytosol protein by the plasma-membrane fraction (Pietras and Szego, 1979b). These results suggest that (1) transport of steroid into the vesicle occurs even in the absence of cytoplasmic binding proteins by a mediated process; (2) component(s) of the transport system have high affinity for the steroid.

Several structural analogs of corticosterone, other corticosteroids and sex hormones reduce the specific uptake of corticosterone by liver-cell plasma-membrane vesicles. Steroids with 5α configuration inhibit the uptake to a larger extent than do their 5β counterparts (Alléra et al., 1979). Specific binding sites for corticosterone, cortisol and cortisone have been found in rat-liver membranes (Suyemitsu and Terayama, 1976) and in a crude fraction of rat-pituitary plasma membranes (Koch et al., 1978). Testosterone is transported into membrane vesicles from *Pseudomonas testosteroni* by a saturable process; the transport process requires NAD^+ and is inducible by the androgen; the vesicles accumulate testosterone against a gradient (Watanabe and Po, 1974). Together, the studies document that steroid hormones are transported into the cell by a membrane-mediated process and by passive diffusion. A single mode of entry cannot be postulated.

STUDIES WITH THYROID HORMONES

The normal thyroid gland secretes mainly L-thyroxine (T_4) and a lesser amount of L-triiodothyronine (T_3; ref. Werner and Ingbar, 1978). Both hormones circulate, in blood, bound to plasma proteins to an extent of more than 99%. The major amount of T_3 is derived from the monodeiodination of T_4 in peripheral organs (Visser, 1978). Thyroid hormones are also known to stimulate transcription of DNA (Oppenheimer and Dillmann, 1978; DeGroot and Stanbury, 1975); that is, they must cross the plasma membrane to reach the site of action. Like steroid hormones, they are bound by proteins in the cytoplasm with high affinity. However, a cytoplasmic protein–hormone complex appears not to be essential for the hormone to bind to nuclear sites; the free hormone reacts with receptors in the nucleus. In addition to its role as the major organ of metabolism of thyroid hormone, the liver is also a target organ (Shambough III, 1978). The process of entry of T_3 has, in recent times, received some attention. Uptake of T_3 by isolated rat-liver cells is a membrane-mediated process (Rao et al., 1976c). Further studies showed the following. (1) Uptake is temperature sensitive; a transition in temperature is found at 16°C. The activation energies of the high-affinity system between 3 and 16°C and between 16 and 37°C are 72.8 and 47.4 kJ/mole. (2) Uptake displays several saturable components with increasing concentrations of T_3. The apparent K_t and V_{max} values are 86 pM and 33 fmole/(min × mg of protein), respectively. At low concentrations of T_3 (4–80 pM), the apparent K_t is 8.6 pM, a concentration that is close to the physiological concentration of free T_3 (Rao et al., manuscript in preparation). (3) Thiol-group-blocking agents, hydrolytic enzymes and cell-surface-perturbing agents reduce the uptake of T_3. (4) The uptake system of T_3 exhibits a fair degree of specificity; T_4 does not compete with T_3 (Eckel et al., 1979). Two saturable uptake systems for T_3 in cultured rat-liver parenchymal cells have been described. The K_m values are 21 nM and 1.8 μM at 21°C; T_4 affects the uptake of T_3 (Krenning et al., 1978). T_3 and T_4 are taken up by isolated rabbit adipocytes by

saturable processes; the apparent K_a for T_3 and for T_4 are $4.2 \pm 0.8 \times 10^{11}$ M^{-1} and $5.5 \pm 1.8 \times 10^{11}$ M^{-1}, respectively (Parl et al., 1977). Human adipose tissue cells also take up T_3 and T_4 apparently through a common system (Hušek and Felt, 1974). Accumulation of T_3 also takes place; at 5 pM of T_3 in the incubation medium a 35-fold accumulation is observed after 7 sec of incubation (Rao et al., manuscript in preparation). Metabolic inhibitors reduce the uptake of T_3 by the high-affinity system (Eckel et al., 1979; Krenning et al., 1978). Taken together, the results suggest that uptake of T_3 by rat-liver cells is consistent with mediated transport. The physiological significance of the accumulation of T_3 against a gradient is not known. Can it be to extract T_3 from the blood and maintain a pool in a bound form? Or are high intracellular concentrations of T_3 needed to saturate the low-affinity, high-capacity sites? Several questions remain unanswered.

Cytoplasmic proteins of the liver cell bind T_3 with lower affinity than the nuclear receptor; binding of T_3 is not due to serum proteins (Dillmann et al., 1974) or to thyroxine-binding globulin (Defer et al., 1975). An important question is, to what extent do the cytoplasmic binding proteins contribute to uptake by cells? A comparison of the uptake by intact cells with binding to the cytosol fraction shows that, at 23°C, uptake is 3 fmole/(min × mg of cell protein), whereas binding by the cytosol fraction is only 0.8 fmole/(min × mg of cytosol protein) (Rao et al., manuscript in preparation. Binding is not influenced by metabolic inhibitors. Uptake of T_3 by cultured rat-liver parenchymal cells due to binding by cytoplasmic proteins is excluded (Krenning et al., 1978).

Transport of T_3 can also be demonstrated by plasma-membrane vesicles of liver cells. Uptake follows a sigmoidal curve as a function of increasing concentration of T_3. The apparent K_t is 96 pM, which is similar to the value obtained with intact cells. At low concentrations (4–80 pM), uptake of T_3 shows an apparent K_t value of 4.2 pM; the concentration of free T_3 in the plasma of female rats is about 5 pM; therefore this uptake system may be of physiological significance. High-affinity, low-capacity binding sites (K_d 3.2 nM) for T_3 have been reported to be present on plasma membranes of rat liver (Pliam and Goldfine, 1977). Human red blood cell ghosts take up T_3 involving two saturable components with K_m values of 0.16 and 3.3 μM (Holm and Jacquemin, 1979). At a concentration of 8.6 pM of T_3 in the incubation medium, plasma-membrane vesicles accumulate the hormone against a gradient (450-fold; Rao et al., manuscript in preparation). Red blood cell ghosts accumulate T_3 6-fold (Holm and Jacquemin, 1979). How, in the absence of an external source of energy, the vesicles build up a gradient is not known. That T_3, which is taken up by plasma-membrane vesicles of the liver cell or the red blood cell ghosts, is indeed transported into the lumen of the vesicles is seen by the decrease in uptake with decrease in vesicle volume (Eckel et al., 1979; Holm and Jacquemin, 1979). In the liver cell, T_4 is converted into T_3 (Silva and Larsen, 1978); interestingly two sets of high-affinity binding sites for T_4 (K_d 0.39 ± 0.06 nM and 23 ± 5 nM) are found in purified rat-liver plasma membranes. The binding is stereospecific and is inhibited by thiol-group-blocking agents, proteases and phospholipase A (Gharbi and Torresani, 1979).

What is the physiological relevance of mediated transport for "gene-activating" hormones? There is now considerable evidence that strongly supports an interaction between gene-activating hormones and component(s) in the plasma membrane, resulting in the transport of the hormone into the cell. Transport and binding to intracellular components are two different but closely linked processes. Transport functions in the absence of cellular binding proteins in a mediated fashion, but binding is dependent on the delivery of the hormone into the cell; binding alone fails to explain the membrane-mediated process. Because the hormone can enter the cell by mediated and non-mediated processes, the subsequent steps will proceed; that is, the entry mechanism has no apparent regulatory function. However, the K_t values at low concentration of the hormone are not very different from the concentration of free hormones in rat plasma. The K_d of the uptake systems of the membrane vesicles also lie in the physiological range of the free hormones (corticosterone as well as T_3). These results certainly bestow considerable physiological importance on the mechanism of entry. In addition, if entry is hindered or blocked at the membrane level, the subsequent steps should remain at a standstill; on the other hand if entry is enhanced, the level of the hormone would be diminished in the blood and increased in a responsive cell. The evidence that a protein(s) in the plasma membrane is involved in the entry of hormones gives ample scope for experiments to provide a better understanding of its function. Suffice it to say that exploration of the physiology of hormone transport deserves much more earnest attention.

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REFERENCES

Alléra, A., Rao, G.S., and Breuer, H. (1979) J. Steroid Biochem. 12, 259–266.

Baxter, J.D., Higgins, S.J., and Rousseau, G.G. (1975) Methods Enzymol. 36, 240–248.

Bellamy, D., Phillips, J.G., Chester Jones, I., and Leonard, R.A. (1962) Biochem. J. 85, 537–545.

Bray, H.G., and White, K. (1966) in: Kinetics and Thermodynamics in Biochemistry, Eds.: H.G. Bray and K. White (Churchill, London).

Brinkmann, A.O., Mulder, E., and van der Molen, H.J. (1971) Res. Steroids 4, 91–99.

Christensen, H.N. (1975) in: Biological Transport, Ed.: H.N. Christensen (Benjamin, London).

Cooper, R.A. (1977) N. Engl. J. Med. 297, 371–377.

Crabbé, J. (1977) in: Receptors and Mechanism of Action of Steroid Hormones, Ed.: J.R. Pasqualini (Marcel Dekker, New York) pp. 530–532.

Defer, N., Dastugue, B., Sabatier, M.M., Thomopoulos, P., and Kruh, J. (1975) Biochem. Biophys. Res. Commun. 67, 995–1004.

DeGroot, L.J., and Stanbury, J.B. (1975) in: The Thyroid and its Diseases, Eds.: L.J. DeGroot and J.B. Stanbury (Wiley, New York).
Dillmann, W.H., Surks, M.I., and Oppenheimer, J.H. (1974) Endocrinology 96, 492–498.
Eckel, J., Rao, G.S., Rao, M.L., and Breuer, H. (1979) Biochem. J. 182, 473–491.
Fant, M.E., Harbison, R.D., and Harrison, R.W. (1979) J. Biol. Chem. 254, 6218–6221.
Farnsworth, W.E. (1971) Invest. Urol. 8, 367–372.
Gharbi, J., and Torresani, J. (1979) Biochem. Biophys. Res. Commun. 88, 170–177.
Giorgi, E.P. (1976) J. Endocrinol. 68, 109–119.
Gorski, J., and Gannon, F. (1976) Annu. Rev. Physiol. 38, 425–450.
Graff, J.C., Wohlhueter, R.M., and Plagemann, P.G.W. (1977) J. Biol. Chem. 252, 4185–4190.
Grubbs, C.J., and Moon, R.C. (1974) Eur. J. Cancer 10, 707–712.
Harrison, R.W., Fairfield, S., and Orth, D.N. (1974) Biochem. Biophys. Res. Commun. 61, 1252–1257.
Harrison III, R.W., Fairfield, S., and Orth, D.N. (1975) Biochemistry 14, 1304–1307.
Harrison, R.W., Fairfield, S., and Orth, D.N. (1976) Biochim. Biophys. Acta 444, 487–496.
Holm, A.-N., and Jacquemin, C. (1979) Biochem. Biophys. Res. Commun. 89, 1006–1017.
Hušek, P., and Felt, V. (1974) Klin. Wochschr. 52, 505–506.
Jacobsohn, G.M., Siegel, E.T., and Jacobsohn, M.K. (1975) J. Clin. Endocrinol. Metabolism 40, 177–185.
Keller, N., Sendelbeck, K.R., Richardson, U.I., Moore, O., and Yates, F.E. (1966) Endocrinology 79, 884–906.
King, R.J.B., and Mainwaring, W.I.P. (1974) in: Steroid–Cell Interactions, Eds.: R.J.B. King and W.I.P. Mainwaring (Butterworth, London).
Koch, B., Lutz-Bucher, B., Briaud, B., and Mialhe, C. (1978) J. Endocrinol. 79, 215–222.
Krenning, E.P., Docter, R., Bernard, H.F., Visser, T.J., and Hennemann, G. (1978) FEBS Lett. 91, 113–116.
Leavitt, W.W., Kimmel, G.L., and Friend, J.P. (1973) Endocrinology, 92, 94–103.
Lefebvre, Y., Po, L., and Watanabe, M. (1976) J. Steroid Biochem. 7, 867–868.
Lehninger, A.L. (1975) in: Biochemistry – The Molecular Basis of Cell Structure and Function, Ed.: A.L. Lehninger (Worth, New York) pp. 779–805.
Milgrom, E., Atger, M., and Baulieu, E.-E. (1973) Biochim. Biophys. Acta 320, 267–283.
Müller, R.E., and Wotiz, H.H. (1979) Endocrinology 105, 1107–1114.
Munck, A., and Brinck-Johnsen, T. (1968) J. Biol. Chem. 243, 5556–5565.
Neame, K.D., and Richards, T.G. (1972) in: Elementary Kinetics of Membrane Carrier Transport, Eds.: K.D. Neame and T.G. Richards (Blackwell, Oxford).
Nicolson, G.L., and Poste, G. (1976) N. Engl. J. Med. 295, 330–333.
Oppenheimer, J.H., and Dillmann, W.H. (1978) in: Clinics in Endocrinology and Metabolism, Ed.: R. Volpe (Saunders, London) Vol. 7, pp. 145–165.
Parl, F., Korcek, L., Siegel, J.S., and Tabachnick, M. (1977) FEBS Lett. 83, 145–147.
Pasqualini, J.R., and Sumida, C. (1977) in: Receptors and Mechanism of Steroid Hormones, Ed.: J.R. Pasqualini (Marcel Dekker, New York) pp. 413–414.
Peck Jr., E.J., Burgner, J., and Clark, J.H. (1973) Biochemistry 12, 4596–4603.
Philip, E.L.I., and Marotta, S.F. (1974) Steroids 23, 35–47.
Pietras, R.J., and Szego, C.M. (1977) Nature (London), 265, 69–72.
Pietras, R.J., and Szego, C.M. (1979a) J. Cell Physiol. 98, 145–160.
Pietras, R.J., and Szego, C.M. (1979b) J. Steroid Biochem. 11, 1471–1483.
Plagemann, P.G.W., and Erbe, J. (1976) Biochem. Pharmacol. 25, 1489–1494.
Pliam, N.B., and Goldfine, I.D. (1977) Biochem. Biophys. Res. Commun. 79, 166–172.
Popper, H., Bianchi, L., and Reutter, W. (1977) in: Membrane Alterations as Basis of Liver Injury, Eds.: H. Popper, L. Bianchi and W. Reutter (MTP Press, Lancaster).
Rao, M.L., Rao, G.S., Höller, M., Breuer, H., Schattenberg, P.J., and Stein, W.D. (1976a) Hoppe-Seyler's Z. Physiol. Chem. 357, 573–584.

Rao, G.S., Schulze-Hagen, K., Rao, M.L., and Breuer, H. (1976b) J. Steroid Biochem. 7, 1123–1129.

Rao, G.S., Eckel, J., Rao, M.L., and Breuer, H. (1976c) Biochem. Biophys. Res. Commun. 73, 98–104.

Rao, M.L., Rao, G.S., and Breuer, H. (1977a) Biochem. Biophys. Res. Commun. 77, 566–573.

Rao, M.L., Rao, G.S., Eckel, J., and Breuer, H. (1977b) Biochim. Biophys. Acta 500, 322–332.

Rao, M.L., Rao, G.S., Höller, M., and Breuer, H. (1979) Acta Endocrinol. 90, 648–657.

Shambough III, G.E. (1978) in: The Thyroid, Eds.: S.C. Werner and S.H. Ingbar (Harper and Row, New York) pp. 115–124.

Silva, J.E., and Larsen, R.R. (1978) J. Clin. Invest. 61, 1247-1259.

Stein, W.D. (1967) in: The Movement of Molecules across Cell Membranes, Ed.: W.D. Stein (Academic Press, New York).

Suyemitsu, T., and Terayama, H. (1975) Endocrinology 96, 1499–1508.

Terayama, H., Okamura, N., and Suyemitsu, T. (1976) in: Control Mechanisms in Cancer, Eds.: W.E. Criss, T. Ono and J.R. Sabine (Raven, New York) pp. 83–97.

Tucker, H.A., Larsen, B.L., and Gorski, J. (1971) Endocrinology 89, 152–160.

Vernadakis, A., and Culver, B. (1976) 6th Symposium of the International Society of Psychoneuroendocrinology, 1975. Cellular and Molecular Basis of Neuroendocrinological Processes, Ed.: E. Endroczi (Akad. Kiádo, Budapest) pp. 1–3.

Visser, T.J. (1978) Mol. Cell. Endocrinol. 10, 241–247.

Watanabe, M., and Po, L. (1974) Biochimica et Biophysica Acta 345, 419–429.

Watanabe, M., Philips, K., and Chen, T. (1973) J. Steroid Biochem. 4, 613–621.

Werner, S.C., and Ingbar, S.H. (1978) in: The Thyroid. Eds.: S.C. Werner and S.H. Ingbar (Harper and Row, New York).

Williams, D., and Gorski, J. (1973) Biochemistry 12, 297–306.

Williams, D., and Gorski, J. (1974) Biochemistry 13, 5537–5542.

Woeber, K.A., and Braverman, L.E. (1977) in: The Year in Endocrinology, Ed.: S.H. Ingbar (Plenum, New York) pp. 73–108.