ACTA PROTOZOOLOGICA
Vol. 25, No. 3, pp. 331–338 (1986)

Modelling the Insulin Receptor in the *Tetrahymena*. Time-dependence of Receptor Formation, Down-regulation and Imprinting[1]

György CSABA and László KÖHIDAI

Department of Biology, Semmelweis University of Medicine, H-1445, Budapest, Hungary

Received on 26 November 1985

Synopsis. The insulin receptor formed (amplified) in *Tetrahymena* during primary exposure to the hormone has shown the phenomenon of down-regulation, most distinctly after preexposure for 3–6 h. The establishment of hormonal imprinting induced by primary interaction takes a certain time: the effect of 1-h preexposure (imprinting) reached a statistically significant degree after 24 and 48 h. It depends on the duration of down-regulation whether or not the effect of imprinting is measurable after 24 h.

The unicellular *Tetrahymena*, which represents a very low level of phylogenesis, is able to respond to hormones of higher organisms (Csaba 1981). At its primary interaction with the hormone, hormonal imprinting takes place, which increases the binding capacity of the hormone receptor, and cellular response to hormone reexposure(s) as well (Csaba 1980, 1984, 1985). Thus imprinting gives rise to amplification of the binding structures, which persist over as many as 500 subsequent cell generations (Csaba et al. 1982 a). In earlier experiments with amino acid type hormones, even a very low (10^{-18}M), in itself functionally inactive hormone concentration gave rise to imprinting, indicating that, between certain limits, the amount of interacting hormone plays little role, while the time factor is decisive, for at least 1-h exposure was required to induce imprinting (Csaba et al. 1982 b).

In the present study a polypeptide hormone, insulin was examined for interdependence between imprinting and the time factor, in other words, for the length of exposure required to induce imprinting in the *Tetrahymena*. Much information has been accumulated on the insulin binding capacity and binding properties of hepatocytes and adipocytes (Gammeltoft et al. 1978). The receptor binds the insulin molecule specifically, and binding elicits cellular response to insulin through poorly understood mediator mechanism. Subsequently, the bound insulin becomes

[1] Supported by the Scientific Research Council, Ministry of Health, Hungary 16/1-04/432/Cs

internalized in coated vesicles, and becomes degraded intracellularly, while the receptor structure re-enters the cytoplasmic membrane via recycling (Gammeltoft et al. 1978, Gorden et al. 1980). Binding of a larger dose of insulin (and of other polypeptide hormones in general) (Gorden et al. 1980, Djiane et al. 1979) accounts for down-regulation, i.e., for a temporary decrease in, or failure of, insulin binding (Marshall and Olefsky 1980, Pezzino et al. 1980). Down-regulation is presumably consequent upon increased internalization and decreased recyclization of the receptor, and on certain postreception events (Marshall and Olefsky 1980) as well, although inhibition of receptor neogenesis could also play a role (Reed and Lane 1980).

We demonstrated earlier that binding of FITC-labelled insulin to *Tetrahymena* can be either displaced or inhibited by non-labelled insulin (Csaba et al. 1985). Since, like mammalian cells, *Tetrahymena*, too, possesess internalizable coated pits and coated vesicles (Csaba et al. 1984 a), we expected that those changes in binding capacity, which characterize the mammalian insulin receptor, could also appear in *Tetrahymena*. Since imprinting is a proof of receptor formation, we used it as index to study insulin receptor behaviour in *Tetrahymena* and simultaneously, the influence of possible down-regulation on hormonal imprinting at the unicellular level.

Material and Methods

Tetrahymena pyriformis GL cells, cultured in 0.1% yeast extract containing 1% Bacto Tryptone medium (Difco, Michigan, USA) at 28°C, were used in the logarithmic phase of growth. After 24 h, the cells were treated on three different schemes, as follows:

First series. The cells were exposed to 10^{-6}M insulin (Semilente, Novo, Copenhagen, Denmark), for 1 h, after which they were returned to plain medium and examined for insulin binding immediately, and 1, 3, 6, 24 and 48 h after the exchange of medium.

Second series. The cells were exposed to insulin for 1, 3, 6, 24 or 48 h, and were examined for insulin binding immediately after treatment, without return to plain medium.

Third series. The cells were treated with insulin for the same lengths of time as in the second series, but were returned to plain medium for 24 h, and sampled for insulin binding tests only thereafter.

All samples were fixed in 4% formaline, washed in two changes of PBS (0.05 M phosphate buffer in 0.9% saline), and exposed to fluoresceine-isothiocyanate-labelled insulin (FITC, BDH Chemicals Ltd., Poole, England) for 1 h. After incubation, the samples were washed in several changes of PBS, spread on slides, and dried for determination of the intensity of fluorescence in a Zeiss Fluoval cytofluorimeter, which was connected with a HP-41C calculator for statistical evaluation of the results. The values given by the cytofluorimeter are arbitrary units. This renders possible the comparison of treated cells with control ones, but the calculation of absolute values of insulin bound is impossible. Twenty cells were assayed for fluorescence at each level of insulin treatment, and each experiment was performed in five replicates, thus the enclosed Figures show the mean of 100 measurements in each series. The mean values are given (in the Figures) in percentage, related to the control as 100.

Results

Six hours after 1 h insulin exposure the binding of labelled insulin did not differ from the control. At 24 and 48 h the difference in binding is biologically and mathematically significant alike (Fig. 1).

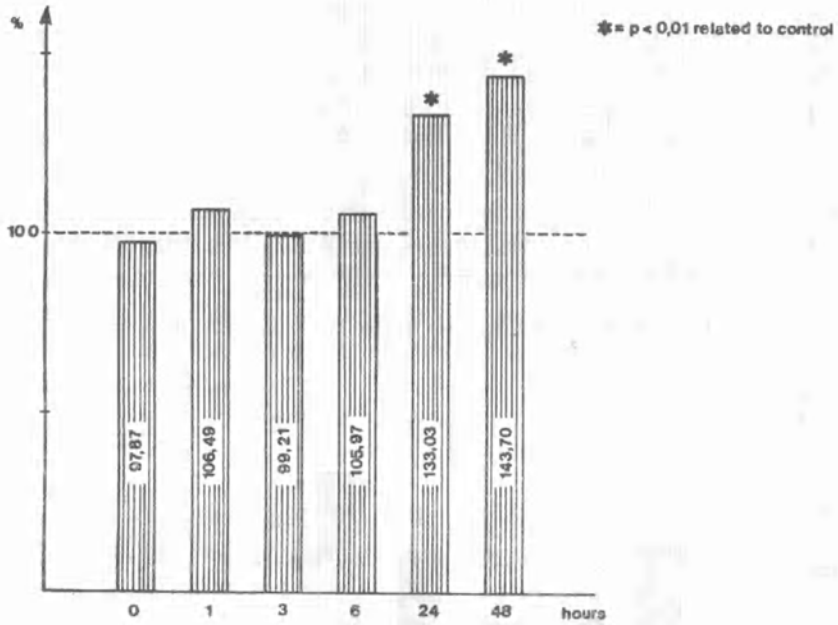

Fig. 1. Effect of 1 h insulin pretreatment (imprinting) on the binding of FITC-labelled insulin to *Tetrahymena* after staying for different periods in normal medium

In the permanent presence of hormone (and measuring labelled insulin binding without delay) the 1 h treatment did not cause down regulation. Although a biologically significant decrease had been observed between 3 and 24 h due to the high deviation only the 48 h difference (from the control) was significant mathematically (Fig. 2).

Twenty four hours after 1 to 48 h insulin treatment, the cells treated for 3and 6 h decreased, treated for 48 h increased their insulin binding, significantly (Fig. 3).

Discussion

The establishment of imprinting took a relatively long time. Insulin binding still did not differ from the control at 6 h after the 1-h exposure, either because the lasting presence of insulin on the surface of the cells prevented further (labelled) insulin binding, or because the negative influence of down-regulation counteracted

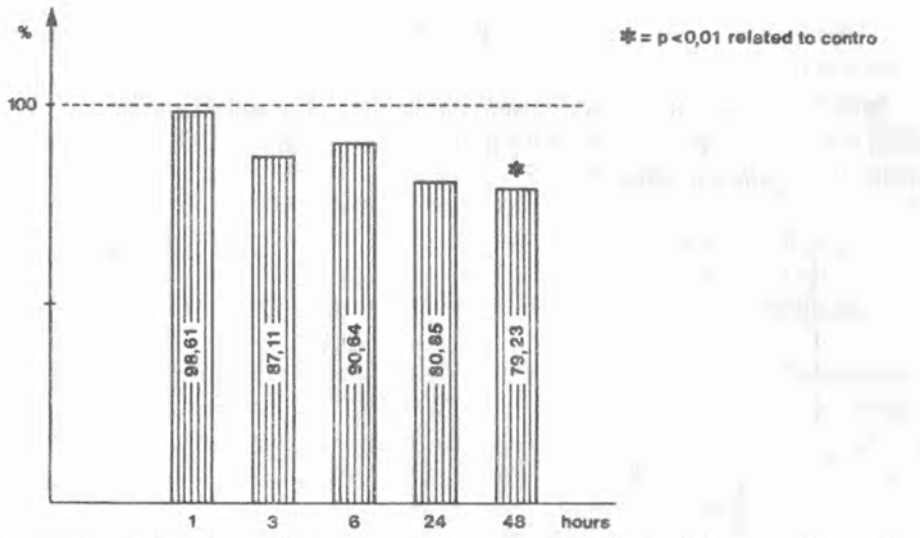

Fig. 2. Binding of FITC-insulin to *Tetrahymena* treated with insulin for different periods of time

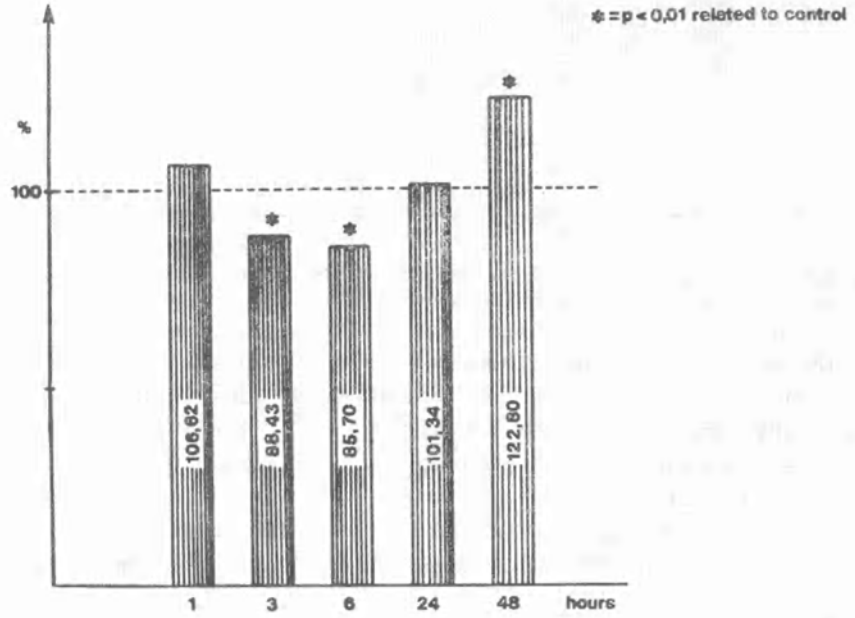

Fig. 3. Effect of insulin treatment of different periods of time to insulin binding after 24 h stay in normal medium

the positive influence of insulin, or thirdly, simply because the establishment of imprinting required a longer time than 6 h. The effect of the imprinting was in fact measurable at 24 h.

The *Tetrahymena* cells divide every 3–4 h in culture. This fact, and internali-

zation of the bound ligand, which is characteristic of polypeptide hormones (Pezzino et al. 1980), seem to disprove that the insulin used for the induction of imprinting was still present on the surface of the daughter cells in a fully inhibitory concentration at 6 h. At the same time, the fact that imprinting took full effect by 48 h, during which more than 10 generation changes had taken place, strongly suggests that imprinting, once it had occurred, becomes, an endogenous property of the cell.

The direct effect of down-regulation was not demonstrable immediately after exposure, but increased measurably with the progression of time. One might, naturally, postulate that fewer receptors had become saturated in 1 than in 48 h, but the possibility of this saturation difference is low since, in principle, 1 h is sufficient for full receptor saturation. If, however, the actual number of active receptors depended on the quantitative relation (equilibrium) between internalized and recycled receptor structures, increase in internalization and a parallel decrease in recyclization could well occur with the progression of time. Such shift of equilibrium between the actual numbers of active and inactive (internalized) receptor structures could account for a time dependent decrease in FITC-labelled insulin binding at reexposure. The applied insulin concentration, 10^{-6}M, was relatively high, but it was required to produce a well measurable effect in the given conditions of experiment. Extracellular insulin decomposition is low (1–6 %/h), but there is evidence that the liver cells degrade 40 % of bound insulin within 1 h (Gammeltoft et al. 1978, Gammeltoft 1984). No similar data are available on the *Tetrahymena*, but the present findings unequivocally suggest that down-regulation can also occur in unicellular organisms.

The third experimental series has thrown a light on the interrelationships between different lengths of primary exposure and establishment of imprinting within 24 h. While preexposure for 1 h did induce imprinting within 24 h, preexposure for 3 or 6 h had a negative rather than positive influence on insulin binding within the same time period. The binding capacity of the cells preexposed for 24 h, did not differ from the control after 24 h, but increased considerably over it by 48 h. An interaction between imprinting and down-regulation can well be held responsible for this phenomenon. One-hour preexposure to insulin did not elicit down-regulation in the *Tetrahymena*, whereas 3-h and 6-h preexposure did. It appears that the 24-h hormone withdrawal period following upon preexposure was too short for expiration of the effects of downregulation (by these short treatments), and for manifestation of the effects of prolonged imprinting as well, whence the latter were not strong enough to supersede the negative influence of the former.

It was interesting to note that the effects of down-regulation were still demonstrable in the hepatocellular membrane of rats 24 h after *in vivo* insulin treatment (Vigneri et al. 1978). Thus the period of 24 h might generally be too short for the expiration of down-regulation phenomena.

Remarkably, while preexposure (imprinting) for 24 h had counter-balanced

down-regulation to the extent of maintaining the control level, preexposure for 48 h had, despite a much stronger down-regulating influence, induced an appreciable degree of imprinting, which was already demonstrable 24 h later. However, with the continuous internalization and degradation of the hormone taken into consideration, the explanation lies close at hand that all insulin added to the medium for preexposure had been degraded between 6 and 24 h, and the *Tetrahymena* cells "consuming" it had increased in number (hence the return of the binding value to the control level), thus insulin was no longer present in the next 24 h, which were sufficient for the establishment of imprinting.

The foregoing considerations support the earlier implication (Csaba et al. 1982 b) that the time factor is of primary importance for the establishment of hormonal imprinting. The dynamic membrane structures of the unicellular are, in all probability, incessantly scanning the environment for information, and require a certain time to assemble to configurations capable of an adequate response. The present findings strongly suggest that the mechanism of hormonal imprinting involves a stage of down-regulation not only in higher organisms, but also in the *Tetrahymena*, from which we imply that the "inherent" insulin receptor of mammals, and the induced insulin receptor of the *Tetrahymena*, are similar structures, as already suggested by earlier studies along this line (Csaba et al. 1984 b, Kovács et al. 1985).

REFERENCES

Csaba G. 1980: Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. Biol. Rev., 55, 47–63.

Csaba G. 1981: Ontogeny and Phylogeny of Hormone Receptors. Karger, Basel.

Csaba G. 1984: The present state in the phylogeny and ontogeny of hormone receptors. Horm. Metab. Res., 16, 329–335.

Csaba G. 1985: The unicellular *Tetrahymena* as model cell for receptor research. Int. Rev. Cytol., (in press).

Csaba G., Gruszczynska M., Madarász B. and Sudár F. 1984 a: Coated pits and coated vesicles in unicellulars (*Tetrahymena*, *Critidia*) and in Hydra. Acta Morphol. Acad. Sci. Hung., 32, 181–186.

Csaba G., Kovács P. and Inczefi-Gonda Á. 1984 b: Insulin binding sites induced in the *Tetrahymena* by rat liver receptor antibody. Z. Naturforsch., 39 e, 183–185.

Csaba G., Németh G. and Vargha P. 1982 a: Development and persistence of receptor "memory" in a unicellular model system. Exp. Cell Biol., 50, 291–294.

Csaba G., Németh G. and Vargha P. 1982 b: Influence of hormone concentration and time factor on development of receptor memory in a unicellular (*Tetrahymena*) model system. Comp. Biochem. Physiol., 73 B, 357–360.

Csaba G., Németh G., Kovács P., Vargha P. and Vas Á. 1985: Receptor level action of polypeptide hormones (insulin, glucagon, FSH, TSH, ACTH) and nonhormone polypeptides on the *Tetrahymena*. BioSystems, 17, 227–231.

Djiane J., Clauser H. and Kelly P. A. 1979: Rapid down regulation of prolactin receptors in mammary gland and liver. Biochem. Biophys. Res. Commun., 90, 1371–1378.

Gammeltoff S. 1984: Insulin receptors: binding kinetics and structure-function relationship of insulin. Physiol. Rev., 64, 1321–1378.

Gammeltoff S., Kristensen L. O. and Sestoft L. 1978: Insulin receptors in isolated rat hepatocytes. J. Biol. Chem., 253, 8406–8413.

Gorden P., Carpentier J.-L., Freychet P. and Orci L. 1980: Morphologic probes of polypeptide hormone receptor interactions. J. Histochem. Cytochem., 28, 811–817.
Kovács P., Csaba G. and Bohdaneczky E. 1985: Immunological evidence of the induced insulin receptor in *Tetrahymena*. Comp. Biochem. Physiol., 80 A, 41–42.
Marshall S. and Olefsky J. M. 1980: Effects of insulin incubation on insulin binding, glucose transport and insulin degradation by isolated rat adipocytes. J. Clin. Invest., 66, 763–772.
Pezzino V., Vigneri R., Pliom N. B. and Goldfine I. D. 1980: Rapid regulation of plasma membrane insulin receptors. Diabetologie, 19, 211–215.
Reed B. C. and Lane M. D. 1980: Insulin receptor synthesis and turnover is differentiating 353-L1 preadipocytes. Proc. Natl. Acad. Sci. USA, 77, 285–289.
Vigneri R., Pliam N. B., Cohen D. C., Pezzino V., Wong K. Y. and Goldfine I. D. 1978: *In vivo* regulation of cell surface and intracellular binding sites by insulin. J. Biol. Chem., 253, 8192–8197.