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IMPACT OF CHANGES IN INTRACELLULAR Ca^{2+} AND K^+ CONCENTRATION ON THE DEVELOPMENT OF HORMONAL IMPRINTING IN A TETRAHYMENA MODEL SYSTEM

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Abstract. The influence of Ca^{2+} antagonists (nifedipine, verapamil) and Ca^{2+} and K^+ ionophores (A 23187, valinomycin) on hormonal (insulin) imprinting of Tetrahymena pyriformis Zeuthen and Tetrahymena pyriformis W cells was examined by determination of labeled hormone binding. Reduced intracellular Ca^{2+} and elevated intracellular K^+ equally inhibited hormonal imprinting, probably as a result of various effects in themselves, or in combination, more precisely as the issue of the influences of the agents on membrane structure and polarity, membrane reassembly associated with ion-dependent intracellular events, and certain nuclear level events. Nifedipine, A 23187 and valinomycin diminished the hormone binding capacity of the membrane also in themselves, by a durable effect lasting over several subsequent generations.

Key words: Ca^{2+}, K^+, Tetrahymena, ionophore, hormonal imprinting

EFFETS DES VARIATIONS EN CONCENTRATION Ca^{2+} ET K^+ INTRACELLULAIRE, SUR LE DÉVELOPPEMENT DE L'EMPREINTE HORMONALE EN TANT QUE SYSTEME MODELE CHEZ TÉTRAHYMENA

Résumé. L'influence des antagonistes de Ca^{2+} (nifédipine, vérapamil) et des ionophores de Ca^{2+} de Ca^{2+} et K^+ (A 23187, valinomycine) sur l'empreinte hormonale (insuline) de Tetrahymena pyriformis Zeuten et Tetrahymena pyriformis W, a été examinée par la détermination de la liaison de l'hormone en question. L'empreinte hormonale a été bloquée par diminution du Ca^{2+} intracellulaire et par augmentation du K^+ intracellulaire, probablement par suite d'effets variés ou en combinaison, tels que les influences de corps agissant sur la structure de la membrane et sur sa polarité, sur la formation de la membrane en relation avec des phénomènes ionodépendant, du type intracellulaire et par suite de certaines actions ayant eu lieu au niveau du noyau. La nifédipine, le A 23187 et la valinomycine ont diminué la capacité de la liaison hormonale de la membrane par un effet durable se prolongeant sur plusieurs générations successives.

Mots - clefs: Ca^{2+}, K^+, Tetrahymena, ionophore, l'empreinte hormonale

INTRODUCTION

Certain cations and anions play an important role in the life processes of the cell, above all in maintaining a dynamic equilibrium between the extra-and intracellular space. Ion transport across the cell membrane may influence the latter's electrochemical parameters, and the cations and anions entering the cell may serve as activators, or, conversely, inhibitors, of enzymes. Ion transport, particularly Ca^{2+} and K^{+} transport, has been extensively studied, with special regard to its interrelationship(s) with the membrane potential, and with certain pathways of intracellular reactions. One experimental approach to the problem has been based on the artificial induction of changes in the intracellular concentration of a given ion, either by inhibiting its entry into the cell with an antagonist, to elicit a concentration decrease, or conversely, by enhancing transmembraneous ion transport with a membrane active ionophore, to elicit a concentration increase. Both treatments alter the intracellular ion milieu, and modify thereby specifically certain intracellular reaction series.

Hormonal imprinting occurs at the primary interaction of a cell with a given hormone, and induces a cellular "memory", which alters (usually increases) cellular response to the hormone on reexposure (Csaba, 1980; Csaba, 1984; Csaba, 1985) either in respect of hormone binding capacity (Csaba et al.,1982), or in respect of some other function (Csaba,1985).

Hormonal imprinting being an extraordinarily complex process, some of its details are still obscure. It has been suggested that it involves a hormone-induced change in membrane structure and composition (Holmreich, 1976; Nozawa et al.,1985) and/or certain gene level effects of the hormone (Csaba et al.,1980, 1984). The transfer of information from the cell membrane to the nucleus involves a series of biochemical events (Kudo et al.,1982; Berridge and Irvine, 1984) among others the collaboration of those intracellular enzyme systems, whose functional state depends on the ion saturation of the controlling mechanisms (e.g. the operation of the guanylate cyclase - calmodulin - Ca^{2+} system). These considerations prompted us to approach the problem of the development of, and possible modifications in, hormonal imprinting, form the angle of the ion sensitivity of intracellular information transfer, by inducing intracellular ion concentration changes parallel to the induction of imprinting.

We used in the experiment cultures of the unicellular Tetrahymena, which proved to be a reliable model cell for investigations into hormonal imprinting. Changes in insulin-imprinting were studied in presence of two Ca^{2+} -antagonists (nifedipine, verapamil), and of two ionophores (A23187, valinomycin) known to enhance the penetration of Ca^{2+} and K^{+}, respectively, across the cell membrane. The ion-dependence of imprinting was assessed parallely from the hormon binding values of two slightly dissimilar taxa (Tetrahymena pyriformis Zeuthen; Tetrahymena pyriformis W).

MATERIALS AND METHODS

Tetrahymena pyriformis Zeuthen and Tetrahymena pyriformis W cultures, grown in 0.2 per cent yeast extract containing 2 per cent Bacto Tryptone medium (Difco, Michigan, USA), at 28°C were used in the logarithmic phase of growth. The following ion transport influencing agents were tested: nifedipine (BAYER, FRG) 100 ug/ml; verapamil, (Eisai Co., Japan) 100 ug/ml; A23187 (Calbiochem-Behring Co., USA) 10^{-6}M; valinomycin (Sigma, USA) 10^{-6}M.

Apart from one untreated and one insulin-treated (Semilente, Novo, Copenhagen, Denmark) control group, four other groups were set up for treatment with the ion transport-influencing agents, either alone of in combination with insulin. Insulin was applied at 10^{-6}M concentration, and each exposure lasted 1 h.

The cells were sheltered from light during exposure to nifedipine and verapamil, for these compounds are photosensitive.

After treatment, the cells were returned to plain medium for 24 h, were subsequently fixed in 4 per cent neutral formaline, washed in three change of phosphate buffer (PBS=0.05 M phosphate buffer, ph 7.2; 0.9 per cent NaCl), incubated in presence of fluorescein-isothiocyanate (FITC-BDH Chemical Ltd London, England) -labeled insulin, washed again in several changes of PBS and spread on slides.

The binding of FITC-labeled hormone was determined by cytofluorimetry using an Olympus MMPS cytofluorimeter connected with a Canon Canola SX 320 apparatus for data processing and evaluation.

Thirty cells were assayed for the intensity of fluorescence in each group, and each test was performed in three replicates, thus the values shown in the Figures represent means of 90 measurements.

RESULTS

Certain intracellular processes depending on the ion permeability of the membranes are also involved in the complex mechanism of hormonal imprinting. In the present study we investigated the impact of the changed membrane penetrance of Ca^{2+} and K^{+} on the course of insulin-induced imprinting.

Since both Ca^{2+} -antagonists accounting for the storage (retention) of Ca^{2+} on specific membrane structures are photosensitive, the cells were sheltered from light during treatment: The examined two taxa responded differently to the short-term effect of the antagonists which were applied in face of the environmental changes elicited by imprinting.

While the Tetrahymena pyriformis Zeuten cells bound somewhat less insulin than the control, the Tetrahymena pyriformis W cells bound considerably more, owing to the influence of imprinting. Since, according to previous studies (Kőhidai et al., 1987), the hormone binding capacity changed similarly in the two taxa, the difference found can be attributed to a masking effect, which does not interfere with the comparability of the results.

The two Ca^{2+} -antagonists tested had a dissimilar impact on imprinting (Fig.1).

Nifedipine reduced the binding of labeled hormone both alone and in combination with insulin, and the extent of reduction did not appreciably differ between the two schemes of treatment.

Verapamil reduced insulin binding considerably when applied in combination with the hormone, but enhanced it rather slightly in both cells types when applied alone.

The two ionophores also acted differently on imprinting (Fig.2). A 23187 reduced insulin binding in combination with the hormone, and to a still greater degree alone, especially in the cultures of Tetrahymena pyriformis W cells.

The other ionophore, valinomycin, known to promote the intracellular entry of K^{+} ions, also had a negative influence on insulin binding, both alone and in combination with insulin.

DISCUSSION

The cytoplasmic membrane separates the cell and its functions from the environment, but plays simultaneously a primary role in the mediation of cell-environment interactions. The permeability and the potential of the membrane have a decisive influence on the intracellular events, and thereby also on most cellular functions.

The intracellular de-codeing of the information carried by extracellular signal molecules (hormones) is of vital importance for the cell. Hormonal effect on the target cell depends greatly on the qualitative and quantitative aspects of hormone-receptor relationship (Csaba,

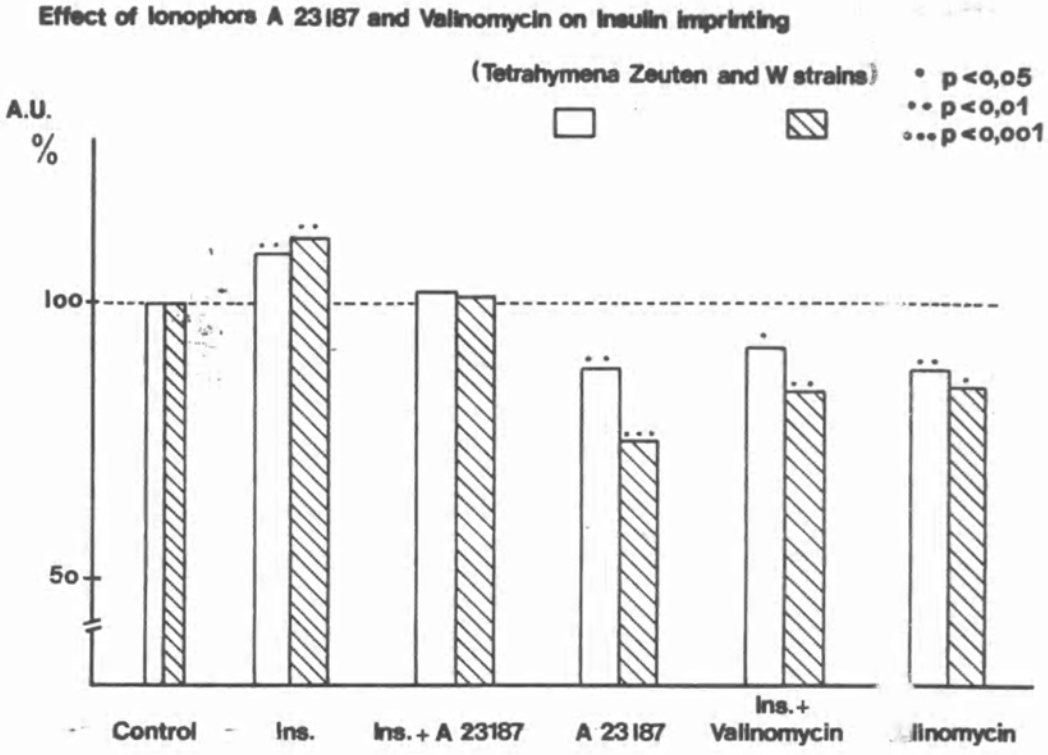

fig. 1.

1985) in the case of both membrane-associated and cytosolic receptors. The hormone-receptor relationship depends again on the fluidity (Nozawa et al.,1985) potential (Kőhidai et al., 1986) and other qualitative parametes of the membrane, but the activities of intracellular enzymes or enzyme groups may also have an influence on the development of hormone action.

The presence or absence, bound or unbound state, and membrane-associated or cytoplasmic localization of certain ions, among others of Ca^{2+} and K^{+}, plays an important role in material and energy transport between the cell entity and its environment.

Among the multiple functions of Ca^{2+}, the following are most important from the biological point of view:(i) as a structural element, it links with phospholipids or other membrane componensts, or determines the structural characteristics of certain proteins and nucleic acids (Harrison and Harrison, 1974; Li et al., 1977) (ii) its electrochemical role seems to be clear, since it is able to poverfully influence membrane potential (Eckert and Brehm 1979; Meech,1976) (iii) it acts on certain extracellularly localized surface regulating proteins (Fearon and Austen, 1976) (iiii) it influences, as intracellular regulator, the enzymic functions,membrane permeability and division of the cell (Meech,1976).

The main role of K^{+} is its collaboration in the transmembraneous Na^{+}/K^{+} pump mechanism, which furnishes the membrane potential, but it is also involved in several other cellular functions. It is a component of certain enzyme systems, and the concentration changes of intracellular K^{+} may even affect the gene level through the influence of K^{+} on the condensation of intracellular chromatin, and thereby on the conditions of information transfer.

FITC-Insulin binding of Insulin, Nifedipine and Verapamil pretreated cells.

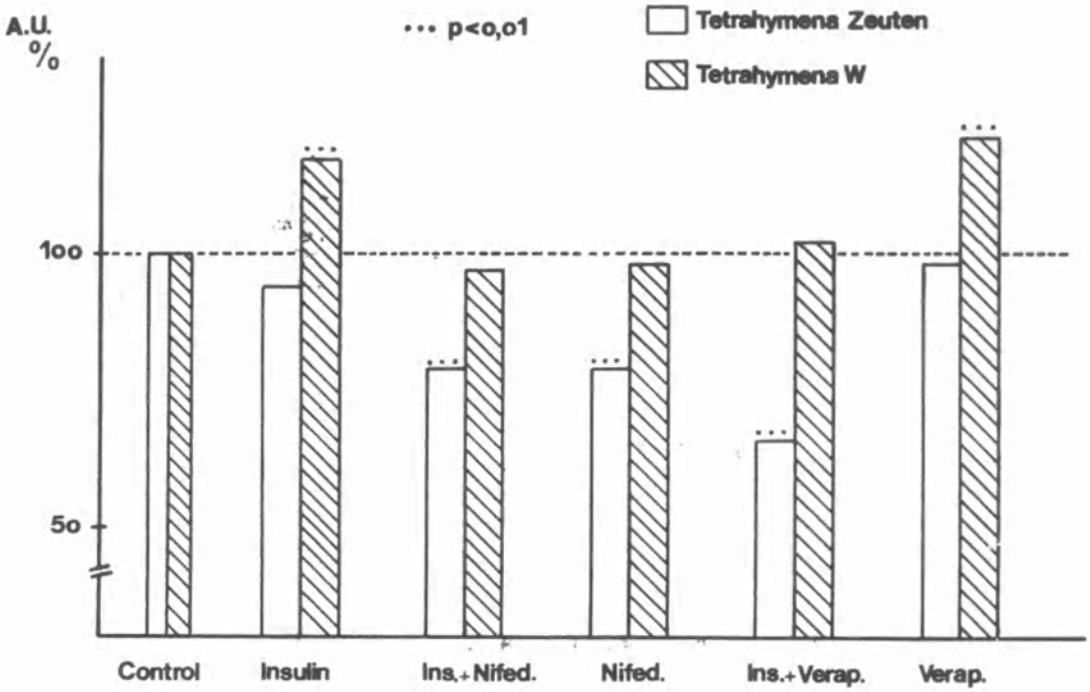

fig. 2.

It follows that Ca^{2+} and K^{+}, and the conditions of their transmembraneous transport, may have an impact on practically all stages of information transfer between cell membrane and nucleus. In this light it does not seem surprising that the applied antagonists and ionophores had a demonstrable influence on the course of hormonal imprinting,which is itself a multifactorial process presupposing the well coordinated function of the entire cell. We demonstrated earlier that interference with the plasticity of the membrane, or with certain intracellular processes,either hampered, or inhibited altogether, the development of hormonal imprinting (Kovács et al.,1984).

Nifedipine and verapamil, used in the present study as Ca^{2+}-antagonists, and previously been tested for action on various target organs (cells), and had been found to inhibit Ca^{2+} - influx into myocardial cells (Kohlhardt et al.,1972), smooth muscle(Fleckenstein and Nakayama) 1975) vascular endothelium (Lonchampt et al.,1982) thrombocytes Addonizio et al.,1980) and glandular cells (Sommers et al., 1976) as well.

Cellular insulin binding capacity was found to differ between the two taxa studied.While the Tetrahymena pyriformis W cells showed, exactly as in earlier studies, an increase in binding capacity after primary exposure to insulin, the Tetrahymena pyriformis Zeuthen cells showed no binding change relative to the control. The cause of that irresponsiveness may have been an environmental change consequent upon treatment in the dark and, ultimately, on the dissimilar photosensitivity of the two taxa. A decreased intensity of insulin imprinting in

cultures sheltered from light was already observed earlier (Kőhidai et al.,1987),and was found to be due to the synthesis of haem, or its precursor protoporphyrine IX, in the Tetrahymena cells. The synthesis of protopohyrine IX is light-dependent,and its incorporation into the membrane may modify the structural and fluidity conditions (Ruben et al.,1982)altering thereby also membrane availability for imprinting. The examined two taxa obviously differed in that respect, inasmuch as while haem synthesis was of a considerable degree in the T.Zeuthen cells, accounting probably even for a photosensitive transformation of the membrane, it appeared to be negligible in the W cells, to judge from their relative insensitivity to changes in illumination, i.e. equal imprinting behaviour in light and dark environment. Nevertheless,changes in the absolute value of insulin binding were of similar tendency, and therefore comparable, between Zeuthen and W cells.

Of the Ca^{2+} -antagonists tested, nifedipine reduced hormone binding in the Zeuten cells,but had no influence on it in the W cells. Conversely, verapamyl stimulated hormone binding significantly in the W cells, but did not alter it in the Zeuthen cells. Since the binding values were assessed 24h after treatment with the Ca^{2+} -antagonists, the action of these can be regarded as durable (lasting over 5-6 generations), conform to pertinent earlier observations (Csaba et al.,1982).

The binding values assessed after imprinting in presence of nifedipine did not differ between the groups treated with the Ca^{2+} -antagonist alone and with Ca^{2+} -antagonist-nifedipine. It follows that no imprinting took place in presence of nifedipine. The same was observed with verapamil, except that combination of the antagonist with the hormone accounted later for a decrease in binding capacity, which indicated a negative tendency of imprinting,if it had taken place at all.

Consideration should also be given to the membrane-level events which take place during the period of imprinting. The hormone has different effects on the cell membrane during primary interaction; further to activation of endocytosis, it induces structural transformation by direct influence. Insulin, for example, causes lasting changes in the saccharide and protein components of the membrane (Kovács and Csaba,1986). The hormonal influence may alter not only the structural elements of the membrane, but also the quantity, distribution or functional state of the enzyme molecules bound to its outer surface (Gammeltoft,1984). However, imprinting itself is not merely a membrane-level event,for it also involves certain intracellular mechanisms, which collaborate in the transfer of information from membrane to nucleus and can,in turn, influence membrane reassembly though the membrane flow.

The Ca^{2+} -antagonists studied may impair the above outlined mechanism at various stages. They may, already at membrane level, account for abnormal relations of membrane-bound Ca^{2+}, and interfere thereby with the hormonal course of membrane polarisation and depolarisation, and/or with the function of certain enzymes or other proteins (Campbell, 1983a). These changes can in themselves bias hormonal imprinting, which presupposes an intact functional state of the cell. On the other hand, the Ca^{2+} antagonists may, through depression of the intracellular Ca^{2+} level by inhibiting Ca^{2+} - influx, prevent the transfer of information from the membrane to intracellular structures, and block the effector mechanisms (Campbell,1983c).

The similar responses to the two antagonists indicate the extraordinary sensitivity of hormonal imprinting, i.e. of membrane-hormone interaction, to even minor changes in the ion milieu, which may alter the binding affinity by modifying the molecular structures. Effects deve-

loped through mechanisms other than Ca^{2+} -antagonization also deserve mention, for they may characteristically modify certain cellular functions. For example, modification of the hormone binding capacity of the membrane is the issue of various membrane-level effects.

The dissimilar activity of the two antagonists studied has also been substantiated by the clinical observation that nifedipine was superior to verapamil in therapeutic effect (Taira, 1978).

It is known from other experiments that hormone effects, including hormonal imprinting, give rise to a lasting depolarisation of the membrane, which persists over several generations in unicellular model systems (Kōhidai et al., 1986; Schlatz and Marinetti, 1972). Accepting depolarisation as one prerequisite of hormone action facilitates the understanding of the impairment of imprinting by simultaneous exposure to Ca^{2+} -antagonists. These molecules not only hamper the influx of Ca^{2+}, but they also alter the membrane potential by promoting the storage of Ca^{2+} in certain membrane sites. The changed potential necessarily modifies the imprinting behaviour. It should be noted that the two antagonists tested also differ in binding affinity to the membrane, which can in itself explain part of the dissimilarities in their action.

The examined Ca^{2+} and K^{+} ionophores, too, interfered with the normal course of imprinting, but in that case intracellular ion excess rather than ion deficiency was responsible for the changes.

The agent A 23187 promotes the influx of Ca^{2+} ions into the cell. It reduced the hormone binding capacity of both Zeuthen and W cells considerably when applied in itself, and negligibly when applied in combination with the hormone.

Evaluating the impact of A23187 on imprinting, effects other than ionophoric should also be taken into consideration. A 23187 is known to influence the transmembraneous transport of Ca^{2+} and, to a lesser degree (Pressman, 1976), of Mg^{2+}, to enhance membrane conductancy (Eckert and Brehm, 1979) for K^{+}, and to facilitate the transport of certain amines and amino acids across the hydrophobic bilayer (Pfeiffer et al., 1974). Moreover, it promotes migration across gap junctions, influencing thereby the localization of membrane proteins (Rose and Loewenstein 1976). It interferes with the second messenger mechanisms in two stages, by increasing the cGMP level and depressing phosphatidyl-inositol turnover, which ultimately inhibits inositol trisphosphate synthesis (Goldberg and Haddox, 1977; Michel et al., 1977).

Among the nuclear effects of A 23187, the marked (5-10 fold) DNA synthesis stimulating action deserves special mention (Campbell, 1983b).

Nevertheless, apart from, or in association with, the above effects, the main effect of A 23187 remains the promotion of Ca^{2+} influx by ion release from the intracellular pools, elicited by micromolar concentrations of the ionophore. Both intracellular Ca-elevating mechanisms should be taken into consideration for the evaluation of the present experiments.

As pointed out earlier, no positive imprinting occurred in presence of A 23187, although there was a slight increase in hormone binding over cells exposed to the ionophore alone. In this light, the difference between the response of ionophore-and ionophore+hormone -treated cells could probably be ascribed to imprinting. On the other hand, a protective effect of the hormone (protein) on the membrane may also have reduced the latter's vulnerability to the ionophore. The consequences of intracellular Ca^{2+} -elevation may have had a lesser impact on structural modification, than in presence of the ionophore alone.

The non-ionophoric effects of the molecule deserve consideration also in this context. Altered hormone binding may have been consequent not only upon the ion concentration change, but also upon a structural change elicited in the membrane by A 23187, to judge from the greater binding capacity of the cells treated with inophore+hormone compared to those treated with the inophore alone.

Valinomycin, which influences the influx of K^+ ions, acts similar to A 23187 as a mobile carrier. The valinomycin-induced elevation of intracellular K^+ may alter the membrane potential by modifying the K^+ -Na^+ pump mechanism. Moreover, several intracellular enzymes require presence of K^+ for development of action (Lehninger, 1975).

Unlike A 23187, valinomycin inhibited the formation of insulin binding structures to a practically similar degree in itself and in combination with the hormone, in both taxa studied. In other words, it inhibited the development of imprinting altogether. The dissimilar behaviour of A 23187 and valinomycin can probably be ascribed to different targets of action. While A 23187 biassed imprinting presumably by altering a membrane-level event or a membrane-level manifestation of an intra cellular event, valinomycin may have inhibited it by a less direct effect.

Although depression of hormone binding by the membrane potential modifying effect cannot be disregarded in this context either, there is reason to postulate that the ionophore-induced elevation of intracellular K^+ gave rise to far-reaching nuclear changes, which ultimately resulted in heterochromatinization of the nuclear chromatin, more precisely in a gene-level blocking of the nuclear stage of hormonal imprinting, and/or of the nuclear control over the dynamic disassembly and reassembly of the membrane. The dual target of action, more precisely the modification of the membrane potential and of the nuclear chromatin activity, can explain the marked decrease in binding capacity shown by both taxa in presence of valinomycin.

It follows from the above experimental observations that cellular function and its membrane-level manifestations depend greatly on changes in the intracellular ion - Ca^{2+}, K^+ - concentration. Shift of that equilibrium in any direction modifies and/or biases certain life processes and responses of the cell, among others the primary interaction with a hormone, i.e. the phenomenon of hormonal imprinting. The normal course of hormonal imprinting is influenced by several iondependent intracellular events, or by the modifications of these, by ion-dependent changes in the membrane potential, and by ion concentration-dependent intracellular reactions, which ultimately lead to alterations in the membrane structure. Gene-level effects also play a role. The antagonists and ionophores used in this study (nifedipine, A 23187, valinomycin) can in themselves modify the membrane structure for a reduced hormone binding. The Ca^{2+} -antagonists nifedipine and verapamyl, and the K^+ elevating ionophore valinomycin, prevented the development of imprinting, whereas A 23187 promoted an increase relative to its in itself depressive effect on binding capacity. The foregoing considerations support the implication that presence of Ca^{2+} and an adequate level of K^+ are essential prerequisites of the normal course of hormonal imprinting.

REFERENCES

Addonizio, C.A., Fischer, C.A. and Edmunds, L.H., Effects of verapamil and nifedipine on platelet activation. Circulation, 1980, 62: supp. III., Abst. 326.

Berridge,M.J.and Irvine,R.F.,Inositol trisphosphate, a novel second messenger in cellular signal transduction.Nature,1984, 312: 315-321.

Campbell,R.A.,Intracellular Calcium.Its Universal Role as Regulator.Chapter 4.John Wiley and Sons Ltd.,Chichester, 1983a, pp.150-177.

Campbell, R.A., Intracellular Calcium. Its Universal Role as Regulator.Chapter 8.John Wiley and Sons Ltd.,Chichester, 1983b, p. 378.

Campbell, R.A.,Intracellular Calcium. Its Universal Role as Regulator.Chapter 9.John Wiley and Sons Ltd.,Chichester,1983c, pp. 393-454.

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

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

Csaba,G., The unicellular Tetrahymena as a model cell for receptor research.Internat.Rev.Cytol. 1985, 95: 327-377.

Csaba,G.,Németh,G.and Vargha,P.,Development and persistence of receptor "memory" in a unicellular model system.Exp.Cell Biol.1982, 52: 291-294.

Csaba,G.,Sudár,F. and Pados,R.,Binding and internalization of ^{3}H-epinephrine in Tetrahymena. Endocrinologie, 1980, 76: 340-344.

Csaba,G.,Török,O. and Kovács,P., Hormonal imprinting in cell culture II. Evidence of hormonal imprinting and thyrotropin(TSH) - gonadotropin (FSH) overlap in a Chinese hamster ovary (CHO) cell line. Acta physiol.hung.1984, 64: 135-138.

Eckert,R. and Brehm,P.,Ionic mechanisms of excitation in Paramecium.Ann.Rev.Biophys.Bioeng. 1979, 8:353-383.

Fearon,D.T.and Austen,K.F.,The human complement system: biochemistry, biology and pathology. Assays med.Biochem.1976, 2: 1-35.

Fleckenstein,A.and Nakayama,K.,Interaction of vasoactive ions and drugs with Ca dependent excitation - contraction coupling of vascular smooth muscle.In:Calcium Transport in Contraction and Secretion. Carafoli E.(ed.) North Holland Publisher Co.,New York,1975,pp.555-556.

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

Goldberg,N.D.and Haddox,M.K.,Cyclic GMP metabolism and involvement in biological regulation. Ann.Rev.Biochem.1977,48:823-896.

Harrison,H.E.and Harrison,H.C.,Calcium.Biomembranes,1974,4B:743-846.

Holmreich,E.J.M.,Hormone-receptor interactions.FEBS Lett.1976, 61:1-5.

Kőhidai,L.,Darvas,Zs. and Csaba,G., Effect of light and darkness on the imprintability of Tetrahymena. Acta microbiol.hung.1987, (accepted at 20.9.1986).

Kőhidai,L.,Muto,Y.,Nozawa,Y.and Csaba., Studies into the interrelationship between the hormonal imprinting and membrane potential in model experiment in Tetrahymena.Cell.mol.Biol. 1986,32:641-646.

Kohlhardt;M.,Bauer,B.,Krause,H.and Fleckenstein,A.,New selective inhibitors of the transmembrane Ca conductivity in mammalian myocardial fibres. Experientia,1972, 28: 288-298.

Kovács,P.and Csaba,G.,Is the saccharide component of the insulin receptor involved in hormonal imprinting? Cell biochem.Funct.1986, 4: 111-114.

Kovács,P.,Csaba,G. and Nozawa,Y., Influence of membrane fluidity changes upon the imprinting of polypeptide hormones in Tetrahymena. Comp.Biochem.Physiol.1984, 78A: 736-766.

Kudo, Sh.,Muto,Y.,Nagao,S.,Naka,M.,Hidaka,H.,Sano.M.and Nozawa,Y.,Specificity of Tetrahymena calmodulin in activation of calmodulin regulated enzymes. FEBS Lett. 1982, 149:271-276.

Lehninger,A.L.,Biochemistry,Worth Publisher Inc.,New York,1975, p. 185.

Li,H.J.,Hu,A.W.,Maciewicz,R.A.,Cohen,P.,Santella,R.M.and Chang,P.,Structural transition in chromatin induced by ions in solution.Nucleic Acid.Res.1977, 4:3834-3854.

Lonchampt,M.O.,Romquin,N.,Bonne,C. and Regnault,R.,Inhibition of prostacyclin release from endothelial cells by verapamil.Vth Intern.Conf.Prostaglndins,Florence,1982,Abst.447.

Meech,R.W.,Intracellular calcium and the control of membrane permeability.Symp.Soc.exp.Biol. 1976, 30: 161-191.

Michel,R.H.,Jones,L.M. and Jafferji,S.S., A possible role for phosphatidyl inositol breakdown in muscarinic cholinergic stimulus-response coupling.Biochem.Soc.Trans.1977,5:77-81.

Nozawa,Y.,Kovács,P.,Okhi,K. and Csaba,G., Effect of internalisation of insulin encapsulated and empty liposomes on hormone binding and its imprinting in Tetrahymena pyriformis.Cell.mol. Biol. 1985, 31: 7-11.

Pfeiffer,D.R.,Reed,P.W.and Lardy,H.A.,Ultraviolet and fluorescent spectral properties of devalent cation ionophore A 23187 and its ion complexes. Biochemistry,1974, 13:4007-4013.

Pressman,B.C.,Biological applications of ionophores. Ann.Rev.Biochem.1976,501-530.

Rose,B. and Loewenstein,W.R.,Permeability of a cell junction and the local cytoplasmic free ionised calcium concentration:a study with aequorin.J.Membr.Biol.1976, 28: 87-119.

Ruben,L.,Lageson,J.Hyzy,B. and Hooper,A.B.,Growth cycle dependent overproduction and accumulation of protoporphyrin IX. in Tetrahymena: Effect of heavy metal.J. Protozool.1982,29: 233-238.

Sommers,G.,Davis,G.,Van Obberghen,E.and Malaisse,W.J.,Calcium antagonists and islet function. Endocrinology, 1976, 99: 114-124.

Schlatz,L. and Marinetti,G.V.,Hormone-calcium interaction with the plasma membrane of rat liver cells.Science,1972, 176: 175-177.

Taira,N.,Effects of diltiazem and other calcium-antagonists on cardiac functions and coronary blood flow as assessed in blood - perfused dog - hearth preparation. New Drug Therapy with a Calcium Antagonist,Diltiazem,Hakone Symposium '78.Bing.R.J.(ed.) Excerpta Medica - Amsterdam,1978, pp. 91-105.