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Cell Membrane Permeability Change: An Important Step in Hormone Action*

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Many hormones, i. e. the large group of polypeptide hormones, interact with specific receptor sites on the outside of the cell membrane of their respective target cells[1–4]. The intracellular effects of hormone action must therefore be mediated by some second messenger. The most well known and well studied second messenger is cyclic AMP[5] (cyclic adenosine 3′,5′-monophosphate), but other substances such as cyclic GMP[6] (cyclic guanosine 3′,5′-monophosphate) and Ca^{2+} [7] have recently been mentioned as candidates. It has also been mentioned that hormone-receptor interaction could cause cell membrane depolarization[7], but until very recently this has hardly been studied, and the importance of evoked membrane permeability changes have scarcely received any notice. Since real progress recently has occurred in this field, it seems important to extract the general implications for the understanding of hormone action from these studies.

In our laboratory the membrane effects of 3 different hormones have been studied. Adrenaline on salivary gland cells and liver cells, glucagon on liver cells and cholecystokinin-pancreozymin (CCK-Pz) on pancreatic acinar cells. These actions have been compared with those of the neurotransmitter acetylcholine on salivary gland cells and pancreatic acinar cells, and with the nucleotide cyclic AMP on liver cells. The effects of these substances on cell membrane potential and resistance together with other relevant information taken from recent publications is compiled in the Table.

To this list one could have added insulin and ACTH (adrenocorticotrophic hormone), although in these cases there is no information available about evoked membrane resistance changes. It has been reported, however, that insulin hyperpolarizes both the fat cell membrane[34] and the striated muscle cell membrane[35]. ACTH depolarizes the cell membranes of the adrenal cortex and evokes action potentials during exposure

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Important parameters relevant to the understanding of the mechanism of action of some hormones

Hormone	Target cell	Result of hormone action	Result of hormone action mimicked by cAMP	Result of hormone action mimicked by Ca^{2+} ionophore	Hormone effect on adenyl cyclase activity	Hormone effect on cell membrane resistance	Hormone effect on cell membrane potential	Hormone effect on ^{45}Ca efflux from prelabelled tissue
Adrenaline (α)	Salivary acinar cell	Fluid secretion	No[8]	Yes[9]	No effect[8]	Decrease[10]	Hyperpolarization[11]	Increase[12]
Adrenaline (α)	Liver cell	Glucose release	Yes[a 5]	?	Decrease[5]	Decrease[13,14]	Hyperpolarization[13,15]	?
ACh	Salivary acinar cell	Fluid secretion	No[8]	Yes[9]	No effect[8]	Decrease[16,17]	Hyperpolarization[b 16,17]	Increase[12],
ACh	Pancreatic acinar cell	Enzyme secretion	No[18,19]	Yes[20]	No effect[22]	Decrease[23,24]	Depolarization[23,25]	Increase[26,27]
CCK-Pz	Pancreatic acinar cell	Enzyme secretion	No[18,19]	Yes[20]	Increase[22]	Decrease[23,28]	Depolarization[23,25,29]	Increase[26,27]
Adrenaline (β)	Salivary acinar cell	Enzyme secretion	Yes[30]	No[9]	Increase[31]	Decrease[10]	Depolarization[11]	?
Adrenaline (β)	Liver cell	Glucose release	Yes[5]	?	Increase[5]	Decrease[14]	Hyperpolarization[15]	Increase[32]
Glucagon	Liver cell	Glucose release	Yes[5]	?	Increase[5,33]	Decrease[14]	Hyperpolarization[15]	Increase[32]
cAMP	Liver cell	Glucose release	—	?	—	Decrease[14]	Hyperpolarization[15]	Increase[32]

[a] This is only because the result of both α and β actions on liver cells is the same. [b] In submaxillary acinar cells this hyperpolarization is preceded by a short-lasting depolarization.

of the tissue to potassium-free solution[36,37]. In the adenohypophysis, it has been shown that hypothamlaic extracts depolarizes the cell membranes[38]. To the examples of adrenaline effects investigated in our laboratory many others, notably those on smooth muscle and heart muscle, could have been added. It is well established that adrenaline in these cells causes well defined changes in potential and resistance with the possible exception of the β-adrenergic effect on certain smooth muscle cells[39]. In nearly all cases, hormone action thus involves a change in membrane potential, reflecting a change in membrane permeability for one or more ions. The methods involved in such studies have recently been reviewed[40].

The case of the polypeptide hormone CCK-Pz is of considerable theoretical interest, since all necessary data on the action of this hormone in the pancreas are available. It has been known for a long time that both cholinergic agents and pancreozymin cause enzyme secretion from the exocrine pancreas[41]. The effects of ACh and pancreozymin on the pancreatic acinar cell membrane potential are indistinguishable[29] and the dose-response relationships for the evoked depolarization are parallel[29,25]. The membrane resistance changes evoked by ACh and CCK-Pz are of the same magnitude[23]. Both agonists evoke an increase in ^{45}Ca efflux from the prelabelled tissue[26,27]. The dose response curves for agonist evoked cell membrane depolarization, increase in ^{45}Ca efflux and increase in amylase release are similar[26]. There is no increase in ^{45}Ca influx after stimulation[27], so the increase in ^{45}Ca efflux must be explained by intracellular release of bound Ca. The marked cell membrane resistance change evoked by both ACh or CCK-Pz is not a consequence of the granule membrane insertion into the plasma membrane which occurs during the exocytosis process, but is due to a specific agonist-induced increase in Na and K conductance[24]. It has been suggested that the Na influx which occurs during the excitation of the receptors triggers the release of bound intracellular Ca[27], but other possibilities exist[42]. Anyway the agonist-evoked amylase release from the pancreas is strictly dependent on the extracellular Na concentration[43]. However, one should also consider the possible involvement of cyclic AMP or cyclic GMP in the action of CCK-Pz. There is no doubt that there is a pancreatic

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adenyl cyclase which is stimulated by CCK-Pz, probably localized to the acinar cells[22,44]. Cyclic AMP and cyclic GMP phosphodiesterases are also present[45]. The intracellular concentration of cyclic AMP is markedly increased after both ACh and CCK-Pz stimulation[21]. However, while the effect of ACh, but not CCK-Pz, on membrane resistance, potential[25,29], Ca flux[26] and amylase secretion[41] are all abolished in the presence of atropine, the ACh-evoked increase in cellular cyclic AMP level, found in one study, was uninfluenced by atropine[21]. A stimulatory effect of cyclic AMP on amylase secretion has been described[46], but could not be reproduced in many other experiments even using the same type of preparation[19,47]. Cyclic AMP had no effect on cell membrane potential and Ca flux in pancreatic acinar cells[25,19]. There is only one report mentioning cyclic GMP but this had no effect on amylase secretion[19]. Thus we have here an example of a polypeptide hormone having an immediate effect on protein extrusion[26], and this is an effect which is not mediated by cyclic AMP. This does not exclude, however, that other effects of CCK-Pz, e.g. increased protein synthesis, could be mediated by cyclic AMP.

The other polypeptide hormone that we have investigated, i. e. glucagon, is of course an example of a hormone that acts through the second messenger cyclic AMP[5]. However, even in this case, there are marked membrane permeability and potential changes involved, as indicated in the Table. One could postulate that these membrane permeability changes were unimportant; but, to prove this, one must demonstrate uncoupling of membrane permeability change and effect on cell metabolism in such a way that the metabolic response in the intact cell is observed under circumstances where no permeability change is induced. It is difficult to see how this could be achieved.

β-Adrenergic actions are generally mediated by cyclic AMP[5], and, as seen from the Table, also here effects on membrane permeability and potential are found. It is apparent from the Table that we can distinguish 2 subclasses within the important class of hormones that act on receptors localized to the outside of the plasma membrane: 1. the hormones that work through the second messenger cyclic AMP (here cyclic AMP mimicks the hormone effect) and the hormones which work through the second messenger Ca^{2+} (here Ca^{2+} ionophores simulate hormone action). In both groups of hormone actions, however, marked membrane permeability changes causing distinct potential changes are involved. In one particular case, the action of CCK-Pz, it is likely, as already mentioned, that the ionic fluxes caused by the membrane permeability change are directly responsible for the altered intracellular Ca distribution. In other cases, e.g. the action of adrenaline on salivary α-receptors, it is possible that it works the other way around, i.e. that Ca released from the inside of the plasma membrane and/or from other organelles induces the permeability change for K and possibly Na, which is required for the transcellular salt and water flux[9,11,16,48]. Anyway, it is clear now that in the action of those hormones acting through the second messenger Ca^{2+}, the membrane permeability change is an essential step, whereas the role of the membrane permeability change in the action of those hormones working through cyclic AMP remains unexplained. The general recognition of the fact that all hormone actions so far investigated involve marked changes in surface cell membrane permeability will, one hopes, stimulate intensified research into the biophysics of hormone actions which have been almost completely neglected in comparison with the huge interest into the biochemistry of hormone action.

The astonishing similarity between the mechanism of action of the neurotransmitter ACh and the polypeptide hormone CCK-Pz on the same target cell, the pancreatic acinar cell, emphasizes that there is no clear distinction between hormones and transmitter substances with respect to their mechanism of action. The very concept of chemical or humoral transmission as a means of communication between cells was established in endocrinology (secretin) by STARLING and BAYLISS[49] many years earlier than in the field of synaptic transmission (vagus on heart)[50]. However, the biophysics of *synaptic* transmission were described in great details in the 1950's, whereas, with respect to the action of *hormones* on cell membranes, such electrophysiological studies have only recently been started. This development is understandable in view of the tremendous difficulties encountered when applying microelectrode techniques to cells from other tissues than muscle or nerve, mainly because of their small size. Recently, however, many of these difficulties have been overcome[40] and the field now seems wide open for detailed electrophysiological studies.

Zusammenfassung. Nachweis, dass die Hormon-Membran-Rezeptor-Interaktionen meistens von einer bedeutenden elektrischen Widerstandsänderung in den Zellmembranen begleitet sind. Es wird der Zusammenhang zwischen Membranpermeabilitätsänderung, Ca^{2+}-Transport und Adenylcyclaseaktivierung diskutiert.

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