

RECEPTORS AND ION CHANNELS

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Receptors and ion channels are membrane-bound proteins involved in fundamental regulatory mechanisms in cells. They govern both a cell's homeostatic activity and its programmed responses to intra- and extracellular stimuli. Specific receptor molecules in a cell's plasma membrane can be activated by signal molecules present either in the medium surrounding the cell or even generated within the cell itself. The activated receptors sometimes directly bring about changes in the conductances of ion channels. On other occasions the receptors generate changes in a cascade of intracellular second messengers that subsequently activate the ion channels or effect appropriate biochemical changes. In many cases the receptors themselves are actually part of the ion channel complex and in other cases the receptor properties reside in distinct molecular entities. Ion channels may also be regulated by changes in the potential across the membrane in which they are found and also by changes in the concentrations of specific ionic modulators within the cell. Thus the actions of receptors and ion channels in the regulation of cellular function are inextricably intertwined.

Our concepts of the molecular configurations and functioning of ion channels and receptors have altered dramatically over the last few years due to a number of important breakthroughs. For instance, the application of molecular biological techniques to the nervous system has revealed the complete amino acid sequence of the sodium channel and of all the subunits of the acetylcholine receptor–ion channel complex. Such findings are increasing our understanding of how these important molecules function in their membrane environment. Equally important insights have come from recent studies on the actions of novel second messenger systems turned on by cellular receptors and from studies on the kinetic properties of single ion channels. The present symposium is devoted to new advances in the two latter fields. It concentrates on the enormous diversity of ion channel and receptor molecules that are now known to be involved in the control of cellular processes, and emphasizes the modifiability of their responses by changes in both the extra- and intracellular environments. A unifying theme throughout the symposium will be a consideration of the significance of changes at the molecular level in the functioning of ion channels and receptors in terms of the properties of whole cells and in terms of their behavioural consequences to the intact animal.

Over the past 10 years, since the introduction of the extracellular patch-clamp technique by Neher & Sakmann, our understanding of the reactions involved in the control of channel gating and its modulation has increased dramatically. The introduction of the 'giga-seal' modification of the technique in 1980 by Sigworth & Neher opened up the field even further and allowed the development of novel modifications of the original technique including the use of 'excised patches' and of 'whole-cell recording'. Using the cell attached patch-clamp technique it can be shown that hormones or neurotransmitters evoke K^+ loss due to the *opening* of K^+ channels in the plasma membranes of exocrine acinar cells (see chapters by Petersen, Findlay, Suzuki & Dunne and by Marty, Evans, Tan & Trautmann), whereas in the insulin-secreting pancreatic β -cells stimulation by glucose or glyceraldehyde evokes an ATP-mediated *closure* of K^+ channels (see chapter by Petersen *et al.*). Interesting information on the properties of Ca^{2+} -activated K^+ channels has been obtained by the complementary technique of introducing the channels into planar phospholipid bilayers (see chapter by Golowasch, Kirkwood & Miller). Further evidence for the versatility of the patch-clamp technique comes from its application in the study of cation channels and in the role of protein kinase C in the process of secretion from peptidergic nerve terminals (see chapter by Lemos & Nordmann) and from its application in the study of the modifiability of gap junction permeability (see chapter by Neyton & Trautmann).

Patch-clamp techniques have also been used extensively in the study of the diversity of Ca^{2+} channel types in various tissues (see chapter by McCleskey, Fox, Feldman & Tsien). Thus in cardiac cells voltage-dependent Ca^{2+} channels can be shown to be modulated by β -adrenergic receptors in a cyclic AMP-dependent fashion and by 1,4-dihydropyridine in a cyclic AMP-independent fashion (see chapter by Reuter, Kokubun & Prod'hom). By contrast, in various identified neurones in the nervous system of the snail, *Helix aspersa*, transmitter-induced modulation of Ca^{2+} channels does not involve cyclic AMP but appears to be mediated by cyclic GMP and by calcium ions themselves (see chapter by Gerschenfeld, Hammond & Paupardin-Tritsch). In addition the finding that a toxin (ω -CgTX) from the marine snail *Conus geographus* selectively blocks some types of Ca^{2+} channel provides a very useful tool to dissect their different physiological functions and to serve as a biochemical probe for their isolation and purification (see chapter by McCleskey, Fox, Feldman & Tsien).

Another fundamental change in our thinking about the way in which nervous systems function has also occurred in the last 10 years. This is the realization of the extent to which synaptic interactions between cells can be modified by factors in their immediate hormonal environment. The exact definition of a modulatory compound has been the subject of much debate as various workers struggled to distinguish between the terms neurotransmitter, neuromodulator and neurohormone. The most useful concept would appear to be to consider neurotransmitters and neurohormones as the extremes of a continuum of chemical interactions between cells. Neuromodulators would lie somewhere in the middle of this continuum, but it is impossible to define the point where a neurotransmitter becomes a neuromodulator and likewise

a neuromodulator becomes a neurohormone. Modulatory compounds such as biogenic amines and peptides interact in a complex way to produce their integrated effects on the behaviour of an animal. Multiple modulators can be released from identified neurones, or arrive as neurohormones, at target sites where they interact with a multiplicity of specific receptors which may each have a different mode of action (see chapters by Beltz & Kravitz and by Evans & Myers). In addition, a single neurotransmitter may interact in the nervous system with a number of pharmacologically distinct receptor subtypes each with its own specific mode of action (see chapters by Schwartz, Arrang, Garbarg & Korner and by Evans & Myers). Furthermore, neurotransmitters may also produce both rapid and slow synaptic interactions at the same synaptic junction by activating different receptors whose modes of action bring about postsynaptic effects with different time courses. Sensory information from primary afferents in the spinal cord, for instance, is conveyed by the release of sensory transmitters that elicit both fast and slow responses in spinal neurones. The rapid interactions in this system may be mediated by amino acids and ATP (see chapter by Jessel, Yoshioka & Jahr). This same system has recently been suggested to exhibit a novel form of 'agonist-receptor' interaction during development whereby cell surface glycoconjugates on the dorsal root ganglion cells may interact with specific carbohydrate-binding proteins (lectins) during the formation of their connections with spinal neurones (see chapter by Dodd & Jessell). Slow synaptic interactions between neurones are now well documented and in the sympathetic ganglion of the bullfrog the various ionic conductances underlying these slow potentials have been worked out to such an extent that a complete reconstruction of the effects of slow synaptic transmission on electrical behaviour can be made (see chapter by Adams, Jones, Pennefather, Brown, Koch & Lancaster).

Throughout this symposium a unifying idea is the interactions of receptors and ion channels and the modifiability of this connection. In recent years our knowledge of the different types of second messengers turned on by receptor activation and of their effects on the conductances of ion channels has much increased (see chapters by Siegelbaum, Belardetti, Camardo & Shuster and by Lotshaw, Levitan & Levitan). Thus we have examples of single neurotransmitter receptors acting *via* a single intracellular messenger to modulate several classes of ion channels in a single nerve cell and also of a single class of ion channel being modulated by multiple intracellular messengers. These observations highlight the complexity of neuronal regulatory pathways which underlie the 'fine tuning' of neuronal electrical activity.

It is perhaps in the field of novel receptor-activated second-messenger systems that some of the most exciting developments have emerged in recent years. The finding of a new signalling pathway, independent of the ones using cyclic AMP or calmodulin, has revolutionized this field and provided a unifying concept for the disparate actions of a considerable number of biologically-active molecules. This new pathway involves a combination of second messengers including Ca^{2+} and two substances, inositol trisphosphate (IP_3) and diacylglycerol (DG), generated by the breakdown of a specific membrane phospholipid. The IP_3 is released into the cytosol where it acts as a second messenger to release calcium from endoplasmic reticulum-derived stores

(see chapters by Berridge and by Drummond). The DG remains in the membrane where it activates the enzyme protein kinase C which can regulate various ionic mechanisms such as Ca^{2+} -dependent K^+ channels or the Na^+/H^+ exchanger (see chapters by Berridge, by Drummond and by Kaczmarek). It is interesting to note that certain growth factors also appear to act by stimulating the production of IP_3 whereas others appear to activate specific tyrosine kinase molecules in the cell membrane (see chapter by Moolenaar, Defize & de Laat).

The articles in this symposium serve to illustrate some of the new advances that are occurring in the field of ion channel and receptor research. They emphasize how intimately the functioning of ion channels and of receptors is tied together *via* second messenger systems. Thus we are now beginning to understand how changes at the molecular level in cells can be translated into complex cellular activity patterns such as growth and secretion. In the future our understanding of the functioning of these key membrane-bound protein molecules will undoubtedly advance with respect to further details of their interactions. In addition, it seems very likely that molecular biological techniques will allow us to ask questions about how the primary structures of these proteins are arranged in the membrane, and which parts of the molecule are responsible for their various specific properties. Further revelations about the significance of the molecular mechanisms underlying the physiological functioning of cells are eagerly awaited.