advances in the treatment of cardiovascular disease and bronchial asthma.

Conclusion

K channels represent the most diverse group of ion channels so far investigated. Biochemical, electrophysiological and pharmacological approaches are now being applied to clarify the mechanisms responsible for gating K channels. The very diversity of these channels suggests that synthetic agents specific for individual types of K channel will be developed. Such a prospect offers fascinating opportunities both for basic research and for clinical exploitation.

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Functions of gap junction channels in the open and closed states

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Introduction

Gap junctions allow a direct cell-to-cell communication, and are sometimes thought to confer a syncytial behaviour to a coupled ensemble of cells. This view is of course far too simple: like the other ionic channels, junctional channels can adopt at least two states, open and closed, and one cannot understand the function of such channels without considering the transitions between these different states. The existence of coupling between cells of a tissue in resting conditions means that a large number of gap junction channels in the tissue are normally open. These open channels could therefore be involved in the maintenance of the steady-state conditions. However, the functional significance of the closed state of these channels should also be examined. In addition, there are examples where in resting conditions, gap junction channels are closed, and open only in stimulated states (Giaume & Korn, 1983; Margiotta & Walcott, 1983). In this paper, I shall review the functions of the open and closed states of gap junction channels, with special attention to the possible involvement of protein phosphorylation in the modulation of gap junction function.

Possible functions of gap junction channels

Gap junction channels are large-sized pores which discriminate very poorly between cations and anions (Neyton & Trautmann, 1985), and which allow the intercellular circulation of molecules of up to 1200 Da (for globular molecules) (see for review, Loewenstein, 1981). The large size of these channels makes them suitable for different potential functions. As will be seen, the tissue specificity of intercellular communication and its regulation is much more marked than in species specificity (at least among vertebrates). This functional tissue specificity may be related to the differences between the gap junction proteins found in various organs, differences that have progressively emerged during evolution, under different selective pressures: gap junctions are present in large numbers in liver, heart and lens for instance, and their channel-forming proteins share several antigenic determinants (Willecke et al., 1985), but they are also clearly

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Abbreviations used: PKC, protein kinase C; DAG, diacylglycerol; AC, acetylcholine.
different in these three organs, according to their molecular mass and to Northern blot analysis (Paul, 1986; Kumar & Gilula, 1986).

Electrical coupling

In some tissues, the most obvious function of gap junctions is the maintenance of an electrical coupling between two or more cells. Such an electrical coupling is involved in the fast propagation of action potentials between coupled neurons (Furshpan & Potter, 1959; Watanabe & Grundfest, 1961), in synchronization of the contraction of heart (Barr et al., 1965) or smooth muscles (Garfield et al., 1977). In the retina, electrically coupled horizontal cells form a functional network (Byzov, 1975) directly related to the size of the receptive field (Piccolino et al., 1982). In a tissue where cells have a very high input impedance, the opening of a few gap junction channels would tend to stabilize the resting potential of groups of coupled cells. This could be the case in exocrine glands (Petersen, 1985).

Metabolic co-operation

Metabolic co-operation is another possible gap junction function: in vitro, normal cells are able to complement the enzymic deficiency of mutant cells in conditions where only gap junctions can allow a functional complementation (Subak-Sharpe et al., 1969; Sheridan et al., 1979). Such a co-operation has not been demonstrated in vivo, but it might well play a role in organs which are not vascularized, such as lens (Goodenough et al., 1980).

Transfer of second messengers

Gap junctions might play a third type of role in the cell-to-cell transfer of second messengers. Such a function implies some heterogeneity between the coupled cells. For instance, in the coelenterate Obelia, some cells are endowed with calcium channels, and other cells contain a Ca-dependent photoliuminescent protein (obelin), but have no Ca channels. In this coelenterate, light emission seems to involve Ca entry into one cell, followed by diffusion of Ca from this cell to its neighbour, where it can react with obelin (Dunlap et al., 1987). Cyclic nucleotides are also second messengers which are small enough molecules to pass through gap junction channels (Tsiens & Weingart, 1976). Does this second messenger transfer through gap junctions play a role in embryonic development? Much more has been written about the theoretical aspects of this hypothesis than about the experimental facts to support it. In amphibian embryos, well-defined groups of cells coupled together, but not to their neighbours, are also committed to a similar developmental fate (see for review, Lo, 1985). These results are certainly intriguing and deserve further study. However, the coupling-commitment correlation does not seem to be an absolute rule, and correlation does not mean causal relationship.

It has recently been shown that antibodies to the major rat liver gap junction protein are effective in blocking junctional communication between the coelenterate Hydra cells. It has been observed that these antibodies induce a perturbation in the head inhibition gradient in grafting operations, suggesting that gap junctions play a role in this tissue patterning process (Fraser et al., 1987).

How and why should gap junction channels close?

Gap junction closure has often been studied independently from its physiological relevance. For instance, long chain alcohols, like octanol, cause channel closure in many gap junctions through a still unknown mechanism (see for review, Spray & Bennett, 1985). On the other hand, voltage, calcium, protons and protein phosphorylation are among four factors which have been shown to affect gap junction conductance, and which, in principle, could have some physiological importance.

A role for voltage

Three groups of gap junctions may be distinguished according to their transjunctional voltage dependence. Most gap junctions are voltage insensitive, like the junctions present in exocrine glands and in heart (see for review, Spray & Bennett, 1985). Other gap junctions are voltage sensitive, but the importance of this sensitivity is not known. Junctions between embryonic cells are one example of this class. Gap junctions between hepatocytes are probably another example (Young et al., 1987), although their voltage sensitivity was not observed in a former study (Spray et al., 1986). Finally, only few gap junctions present a voltage dependence which undoubtedly has physiological importance: some electrical synapses rectify in such a way that they prevent an antidromic propagation of action potentials (Furshpan & Potter, 1959). However, it is not the case in all electrical synapses (see Watanabe & Grundfest, 1961).

A role for protons?

Gap junction channels are also sensitive to protons. It is sometimes forgotten that this property is not specific to gap junction channels, but is shared by several other ionic channels, like K channels (Wanke et al., 1979; Blatz, 1984). In some tissues in which the pH sensitivity of the gap junction is weak around pH 7, a role for pH in gap junction modulation seems excluded (Schuetze & Goodenough, 1982; Spray et al., 1986). When gap junction channels are very sensitive to internal pH, like those between amphianathan blastomeres (Turin & Warner, 1980; Spray et al., 1981), pH changes may play a physiological role in gap junction modulation, although no such role has ever been demonstrated.

A role for calcium?

The Ca sensitivity of gap junctions is also variable from tissue to tissue. There are junctions where millimolar Ca is required to close the channels (Spray et al., 1982), and it is difficult to think that this phenomenon has any physiological relevance. In other junctions, channel closure can be triggered by an intracellular Ca concentration between 1 and 10 μmol (Dahl & Isenberg, 1980; Neyton & Trautmann, 1986). However, it is still unclear whether a direct Ca-induced closure of gap junction channels takes place in vivo (except at the time of cell death, if one may call this an in vivo phenomenon). Similarly, it has been suggested that an intracellular Ca increase might lead to gap junction closure by activating a Ca-dependent protein like calmodulin (Peracchia & Girsch, 1985).

Functions of protein phosphorylations

In the past few years, a number of reports have dealt with the possible role of various protein kinases in the regulation of gap junction permeability. Cyclic AMP affects junctional communication in many tissues. There is now increasing evidence that, in most (though not in all) cases, cyclic AMP exerts its action by activating protein kinase A (see for review, Beebe & Corbin, 1986); this probably applies to gap junction modulation, too. The effects of cyclic AMP on junctional communication include slow increases of coupling, over periods of hours or days (reviewed in Loewenstein, 1985), fast increases in coupling (De Mello, 1984; Saez et al., 1986), or fast decreases in coupling (Teranishi et al., 1982;
Piccolino et al., 1984). This fast decrease in coupling observed in fish and turtle retinas is of particular interest, because it is one of the few examples where the significance of gating of gap junction channels has been understood: the size of the receptive field in the retina is determined by the extension of the network of coupled horizontal cells. Opening or closing of gap junction channels between these cells modifies the receptive field size. A shrinkage of this field can be achieved physiologically by cyclic AMP and dopamine (presumably via an increase in cyclic AMP concentration) which both uncouple horizontal cells (Piccolino et al., 1984).

Another kinase, which is a tyrosine protein kinase, has been shown to cause a reduction in the junctional permeability in different cell lines. This phenomenon could play a role in the action of some transforming viruses (see Loewenstein, 1985 for a review).

Finally, phorbol esters also reduce gap junction permeability in various cell lines, presumably by activating protein kinase C (PKC) (see, for example Enomoto et al., 1981; Yada et al., 1985). But the direct demonstration of the role of PKC on gap junction function was still lacking. Our recent findings suggest that PKC might mediate the muscarinic-induced closure of gap junction channels in rat lacrimal glands (C. Randriamampita, J. Neyton, C. Giaume & A. Trautmann, unpublished work). The evidence is as follows: activators of PKC (various diacylglycerols (DAG) or phorbol dibutyrate) induce a closure of gap junction channels. The effect of DAG is specific: it is neither mimicked by those DAGs which lack the stereospecificity required to activate PKC, nor by the degradation products of DAG itself. After a prolonged incubation of the cells in DAG, gap junction channels reopen: this is probably due to the down-regulation of PKC (see for review, Nishizuka, 1986). In these conditions, the efficiency of activating G proteins in closing gap junction channels is markedly reduced, as one would expect if PKC mediates the ACh effect on gap junction channels. Finally, the requirement for divalent cations of the ACh-induced uncoupling is precisely the one predicted for a PKC-mediated effect. On one hand, intracellular Ca is not absolutely necessary, although it slightly potentiates both the ACh and the DAG effects. On the other hand, Mg is absolutely required for the ACh-induced uncoupling, which, if it involves a phosphorylation step, should indeed be blocked in the absence of MgATP.

What would be the physiological relevance of this ACh-induced closure of gap junction channels? It is quite possible that during a sustained secretion, all the cells are not simultaneously active, and that they alternate between the resting and secreting states, while their neighbours could be in a different state. More precisely, secretion might require large transients in the concentration of a second messenger like Ca. Such transients could be achieved in an isolated cell, but would be dampened in normally coupled acini.

The literature concerning the gating of gap junction channels has long been dominated by the ‘Ca hypothesis’ and the ‘pH hypothesis’ (see e.g. Loewenstein, 1981; Spray & Bennett, 1985). We have seen that the number of instances in which Ca or protons have been shown to play a physiological role in gap junction modulation is extremely reduced. The potential role of various protein kinases in regulating the number or the gating of gap junction channels has become more and more likely in the past few years. But a large effort is still needed to demonstrate the physiological implications of these putative regulation mechanisms.

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