Regulation of Transmitter Function: Basic and Clinical Aspects

Edited by
E. S. Vizi and K. Magyar

REGULATION OF TRANSMITTER FUNCTION: BASIC AND CLINICAL ASPECTS

Proceedings of the Fifth Meeting of European Society for Neurochemistry, held in Budapest, Hungary, on 21–26 August 1984

Edited by

E. S. Vizi

and

K. Magyar

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PREFACE

This volume contains the papers presented at the 5th Meeting of European Society for Neurochemistry (Budapest, 21-26 August 1984). It has become a tradition to make the printed material available to the participants at the time the congress is held, in order to contribute to the top level discussions that are characteristic of our meetings. The excellent cooperation between the Programme Committee and the Publishing House has ensured the publication of this book in good time.

The scientific programme of the Meeting comprises plenary lectures, symposia, different workshops, round table discussions and posters.

The main goal of the Programme Committee was to afford the opportunity to summarize new findings and to promote fruitful exchanges between clinicians and researchers.

When arranging the programme the Committee tried to avoid parallel sections. This was achieved in the case of the plenary talks and the symposia but it was unavoidable to schedule the round table conferences and workshops at the same time. We do hope, however, that we succeeded in minimizing the overlap between these two programmes.

The Organizing Committee and the Board of the Society thank the participants and the Publishing House for their cooperation, as well as the institutes who have provided moral and financial support, for their help with publishing this volume and with organizing this congress in Budapest.

Budapest, June 1984

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IONIC CHANNELS - NEW PROBLEMS

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Introduction

Extensive investigations performed during last decades on many excitable cells firmly established the main principles concerning the mechanisms of active cellular responses to external stimuli. These principles can be stated as follows: 1) The ionic content of the cytoplasm largely differs from that of the extracellular medium. In order to maintain this uneven distribution of ions produced by enzymatic transporting systems ("ionic pumps"), a considerable amount of metabolic energy must be expended by the cell. 2) In conditions of relatively low passive membrane permeability, the uneven distribution of ions on both sides of the surface membrane creates for the cell a source of potential energy in the form of transmembrane electrochemical gradients. In order to transform it into an active response, it is only necessary for the external stimulus to open a way for the down-hill movement of ions through the membrane. 3) The pathways for ionic currents driven by electrochemical gradients are produced by the presence of special molecular structures ("ionic channels") in the membrane. These structures have a component which determines their ability to select one or another type of penetrating ions ("selectivity filter") and a component which opens or blocks the way through the channel in response to external influence ("gating mechanism").

Resulting from these principles, a general scheme of functioning of excitable membranes has been developed, according to which, ionic channel and cell metabolism are operationally independent in the mechanism of maintaining cell excitability. The genetic apparatus of the cell synthetizes the corresponding protein macromolecules

which are incorporated in the surface membrane and exist there for a while being replaced by new ones with time. Metabolism creates the necessary prerequisites for their function - the electrochemical gradients. As long as these gradients are maintained, the membrane keeps the ability of reacting to external stimuli by active responses due to conformational changes in channel structure which open the way for ionic currents. This scheme looked universal for a long time; however, recently more experimental data became available indicating that it considerably simplifies the reality and that the functioning of ionic channels itself may be under direct control of intracellular metabolic processes.

The present lecture is a brief review of such data obtained for different types of ionic channels and discusses some general problems concerning the mechanisms of cellular activity.

Opening of ionic channel from the cytoplasmic side of the membrane

The main types of ionic channels have been widely discussed; their characteristics are well known nowadays and do not need special description. Their gating mechanisms are put into action by either a decrease in the transmembrane potential difference (sodium, potassium and calcium electrically-operated channels) or by interaction of chemical substances coming from the external medium with a special receptor group (different types of chemically-operated channels).

However, as far back as 1970 Meech et al. have shown on Aplysia neurons the presence of a membrane ion-conducting mechanism put into action not by an external stimulus but by a cytoplasmic change, namely by the increase in the intracellular level of ionized calcium (15). The corresponding channels differ essentially in their functional properties from "usual" potential-dependent potassium channels; it was supposed that they are, as a matter of fact, chemically-operated channels governed by Ca^{2+} ions from inside the cell. Now similar channels are known to be widely distributed also in other types of cells. A more thorough examination of the activity of these channels in mollusc nerve cells under intracellular perfusion has shown, however, a more complex nature of their gating mechanism. Besides the response to intracellular calcium, they also react to the changes in transmembrane electric field (10).

A detailed study of single ionic channels of this type has been recently performed using the patch-clamp technique. It has been shown that the mean open time of the channel is a linear function of the concentration of ionized calcium, and the mean closed time is an inverse function of this concentration (16).

One more experimental finding concerning these channels is of great importance. De Peyer et al. have found on perfused snail neurons that intracellular introduction of the catalytic subunit of the cAMP-activated protein kinase (the enzyme phosphorylating certain membrane proteins) increased the described ionic current (17). Inactivation of the catalytic subunit abolished the effect as well as a reduction in the extracellular calcium followed by a decrease in inward flux of these ions through calcium channels. In fact, introduction of the catalytic subunit reproduced the effect of increase in the intracellular calcium level. The authors suggested that phosphorylation of some proteins at the inner mouth of the channels is important for their activation; may be it increases the sensitivity of the gating mechanism towards intracellular calcium ions.

In 1975 Liberman et al. have shown (also on snail neurons) that intracellular injection of cAMP (or extracellular application of membrane-permeable dibutyryl cAMP) produced a reversible membrane depolarization (13). The depolarization was caused by the induction of a stationary inward current. cGMP produced no effect. A detailed investigation of this phenomenon performed in our laboratory by Kononenko et al. (8) has shown that the cAMP-induced current is due to the opening of channels passing predominantly sodium (but to some extent also potassium and calcium) ions. Wide range changes in holding potential do not disturb the generation of the current. Tolbutamide (a protein kinase inhibitor) and lowering of temperature decrease considerably the rate of rise and a maximal amplitude of the current; theophylline (a phosphodiesterase inhibitor), on the contrary, increases its amplitude. Obviously, in this case we again deal with the chemically-operated channels opened from the cytoplasmic side of the membrane. Data about the participation of protein kinases in the generation of the described effects indicate that phosphorylation of some proteins on the inner membrane surface is important for the conversion of the channels into a conducting state.