Electrical bistability of skeletal muscle membrane
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Cells have at their disposal many complex mechanisms to exercise their functions. Skeletal muscles carry out important functions for the whole body. A well-described function is muscle contraction, which ensures movement. A different function is potassium storage. This is achieved, because muscles have a large volume in comparison to the whole body and because they have direct access to the circulation. An important factor for both functions is the membrane potential. The membrane potential can adopt two stable values, which is called bistability. The interesting back-ground of bistability is the subject of this thesis.

Inwardly Rectifying Potassium Channel
The inwardly rectifying potassium channel (IRK) has an important role in this type of bistability in heart tissue. This was the starting assumption, which was backed-up by data of indirect nature and by literature, for this study in skeletal muscle.

IRK is a potassium selective channel. It differs from other channels, because it transports \( K^+ \) more readily into the cell than out of the cell. Briefly, this phenomenon is known as rectification. Research over the past fifty years has shown that rectification is determined by the molecular interaction between IRK, as a membrane protein, and the combination intracellular magnesium and polyamine concentrations.

Research Set-up
In order to execute a study into the properties, which are responsible for bistability, an approach should be undertaken, that maximises these properties to full extent. Three factors should be considered. Firstly, the factors, which are directly responsible for bistability. Secondly, factors, which can have an indirect effect on bistability. Finally, conditions, which are experimentally achievable for maximising the properties influencing bistability. Therefore, a combined approach was undertaken of intracellular microelectrodes, as voltmeters, of the cell-attached patch clamp technique to record currents through single IRK channels and of computer simulations. In this day and age it is difficult to conceive avoiding computer simulations to analyze the great amount of data, which results from the interaction and interdependence of membrane proteins, molecules and possibly genes.

Results from a General Perspective
A considerable influence by the \( Na^+K^+/2Cl^- \) cotransporter on the membrane potential is described in chapter two. This cotransporter transports two positive charges (\( Na^+ \) and \( K^+ \)) and two negative (2 \( Cl^- \)) charges in the same direction. How does a mem-
brane protein, which by itself does not transport net charges still influence the membrane potential? Secondly, it seems obvious that the transport of ions does not occur independently. These two observations are studied in chapter three by means of computer simulations. This resulted in the design of specific experimental protocols, which made it possible to study the relation between cotransport and bistability in more detail in chapter four. An adrenaline-like substance (isoprenaline) appeared able to obscure bistability. This was explained by the fact that due to this adrenergic signal the cell replaces IRK by a different K⁺ channel, which does not show rectifying properties, that can result in bistability. Considering that the cell-attached patch clamp technique does not have access to the inside of the cell, it can be determined that this K⁺ channel resembles properties displayed by the calcium activated K⁺ channel. In the last chapter the model for bistability is refined with the use of a kinetic scheme, which can mimic single IRK channel behaviour over a large voltage range.

Checking Assumptions
After it has been determined that bistability occurs in many tissues (Table 1.1), but before a function or mechanism can be discussed, it is important to check the starting assumptions. Several steps confirm the starting assumptions:
1 bistability is shown in one cell (Fig. 4.1)
2 IRK is identified on the skeletal muscle membrane by a direct technique (Fig. 5.1A-B)
3 IRK activity is appropriately high in a relevant voltage range (Fig. 5.3A)
4 a direct relation between IRK and bistability is crucial (Fig. 4.7)

Short Synthesis
Bistability is shown in skeletal muscle with excursions of 20 to 50 mV (Fig. 4.3A). Additionally, bistability is variable by experimental conditions, which are within physiological limits. It is variable indirectly by alterations in chloride transport (Fig. 4.5 & 4.6) or by calcium activated K⁺ channel activity (chapter 5), and directly by the application of barium (Fig. 4.7) or hormone activity (chapter 5). Components are identified and characterized, and subsequently integrated in the refined model.

Physiological Function
A clear function for bistability is not entirely at hand. Evident functions are the influence on driving forces of ions over the membrane, and the fact a change in membrane potential is by far the simplest way of (de)-activating any channel. However,
both the variability and the broad tissue distribution of bistability indicate that a general physiological function is observed rather than a tissue specific function. In analogy to studies executed by famous physiologists, bistability seems related to K\(^+\) homeostasis. In this regard the K\(^+\) storage facility can be very useful.