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Negative cooperativity may explain flat concentration-response curves of ATP-sensitive potassium channels

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Abstract. Blockage of ATP-sensitive K^+ channels by various drugs has been reported to exhibit a weak concentration dependence with Hill coefficients below unity. This phenomenon is interpreted by a negative cooperativity between K^+ channels whereby drug binding to one channel lowers the drug affinities of neighbouring channels. Results are presented for a dimeric and a tetrameric channel model and compared with published experimental data.

Key words: Potassium channel – Channel blockage – Hill coefficient – Negative cooperativity

Introduction

In patch clamp experiments currents through single membrane channels can be recorded, and it is usually assumed that the macroscopic current of a multi-channel preparation is obtained by multiplying the current through a single channel by the number of channels. This, however, is no longer valid if neighbouring channels interact with each other, thereby affecting the open channel conductance or the gating kinetics of the channels. Indeed, evidence for such cooperativity between channels has been described for various ion channels. Thus, Kolb and Bamberg (1977) suggested that electrostatic interactions between gramicidin A channels in lipid bilayers may explain different channel properties at low and high channel densities. Interactions between ion channels may also occur when large numbers of nicotinic acetylcholine receptors (Schindler et al. 1984) or voltage-sensitive Ca^{2+} channels (Hymel et al. 1988) are incorporated into lipid bilayers. Furthermore, indications for a cooperativity between ion channels or between subunits of ion channels in biological membrances have been found in several tissues, e.g. for Na^+ channels in the nodal membrane of frog nerve (Neumcke and Stämpfli 1983), for Cl^- channels in frog skeletal muscle (Woll and Neumcke 1987) and in human endothelial cells (Queyroy and Verdetti 1992), and for K^+ channels present in mouse skeletal muscle (Weik et al. 1989) or expressed in *Xenopus* oocytes (Tytgat and Hess 1992).

Interactions between ion channels may also account for a paradoxical dependence of the conductance of membrane channels on the concentration of channel blockers. Thus, the concentration-response curves of blockage of ATP-sensitive K^+ channels by some sulphonylureas, derivatives of nicotinic acid and bipyridines are very flat and have Hill coefficients below 1 (Zünkler et al. 1988; Hopkins et al. 1990; Bodewei et al. 1992). Such a weak concentration dependence cannot be described by the binding of an integral number of blocker molecules to a channel, because the Hill coefficient would then be equal to or larger than 1. It will be shown in this paper that concentration-response curves with Hill coefficients below 1 can arise from negative cooperativity between ion channels, and that the strength of the interactions may be regulated by intracellular factors.

Results

Figure 1 is reproduced from the paper by Zünkler et al. (1988) and shows the effects of the sulphonylurea tolbutamide on currents through ATP-sensitive K^+ channels (K_{ATP} channels) in pancreatic B-cells of the mouse. The figure illustrates that the hypoglycaemic drug reduces the currents, and that this effect is modulated by internal Mg · ADP complexes: Without this nucleotide in the internal solution the concentration-response curve is very flat and the Hill coefficient equals 0.29. With increasing Mg · ADP concentrations the response curves become steeper, and the values of the Hill coefficient increase up to 1.13.

Figure 2 shows another example of flat concentration-response curves of K_{ATP} channels which is taken from the paper by Bodewei et al. (1992). The curves describe the reduction of the open-probability of K_{ATP} channels in

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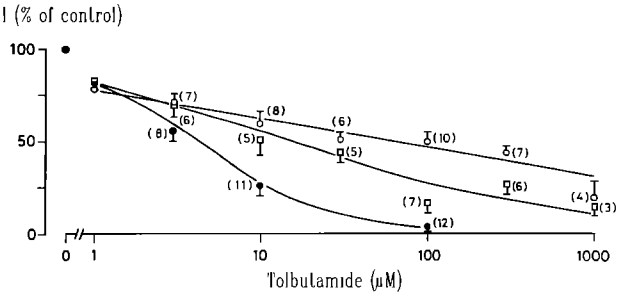

Fig. 1. Blockage of currents I through K_{ATP} channels in mouse pancreatic B-cells by tolbutamide. The internal Mg · ADP concentrations and the Hill coefficients of the concentration-response curves are 0 mM and 0.29 (○), 0.1 mM and 0.5 (□), 1 mM and 1.13 (●). [From Zünkler et al. 1988]

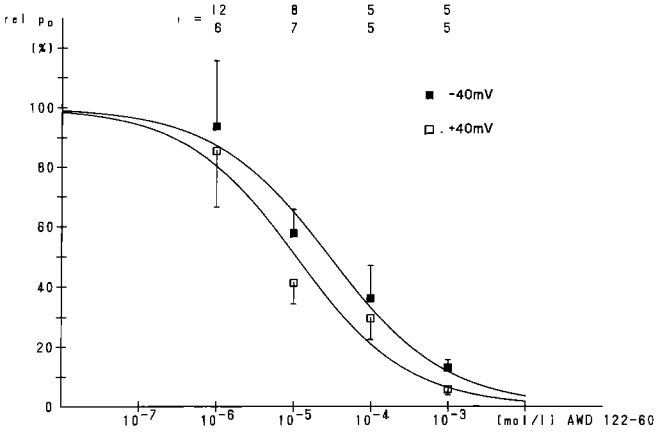

Fig. 2. Inhibition of K_{ATP} channels in mouse skeletal muscle by the cardiotonic bipyridine AWD 122-60 at +40 mV (□) and at −40 mV (■). rel p_0 denotes the relative open-probability of the channels in the presence of the drug with respect to drug-free conditions. Symbols and bars indicate the means and SEM values from i experiments at −40 mV (numbers in upper line) and +40 mV (numbers in lower line). The Hill coefficients of the concentration-response curves are 0.59 at +40 mV and 0.57 at −40 mV. [From Bodewei et al. 1992]

mouse skeletal muscle by a bipyridine at membrane potentials of +40 and −40 mV, and the Hill coefficients of the curves are clearly lower than 1 at both potentials.

To account for flat concentration-response curves of channel blockage, two models of ion channels with negative cooperativity will be analysed as illustrated in Fig. 3. The simplest case is a dimer of interacting ion channels (Fig. 3A). Here K denotes the equilibrium dissociation constant for the binding of one blocking particle to an empty dimer and αK the corresponding constant for the transitions between a state with one to the state with two particles. Without interactions between the channels the parameter α would be unity, and values $\alpha > 1$ would imply a weaker binding affinity of the second particle and consequently negative cooperativity between the channels. Details of the calculations for arbitrary values of α are outlined in Appendix A, and Fig. 4 shows the results for $\alpha = 1\,000$. A characteristic property of the calculated concentration-response curve is a "hump" at a blocker concentration at which the probability of one blocked channel in the dimer (curve $2p_1$ in Fig. 4) reaches a maximum.

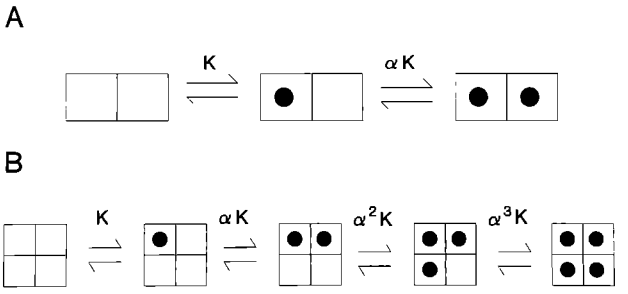

Fig. 3A, B. Models for negative cooperativity between ion channels with a dimeric **A** or tetrameric **B** arrangement of interacting channels. Open squares mark open channels and filled circles blocking particles bound to a channel. K denotes the equilibrium dissociation constant for the binding of the first blocking particle to the channel complex, and α is the interaction parameter

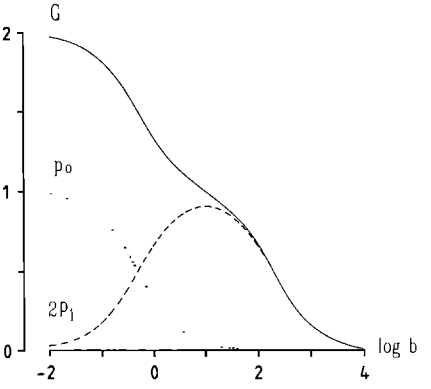

Fig. 4. Conductance G and probabilities p_0, $2 \cdot p_1$ of a dimeric channel complex for $\alpha = 1\,000$. The conductance of one open channel is taken as 1, and $b = B/K$ is the blocker concentration B divided by the equilibrium dissociation constant K. p_0 denotes the probability of an empty channel dimer and $2 \cdot p_1$ the probability of one and only one blocked channel in the dimer (left or right channel in Fig. 3A occupied by a blocking particle)

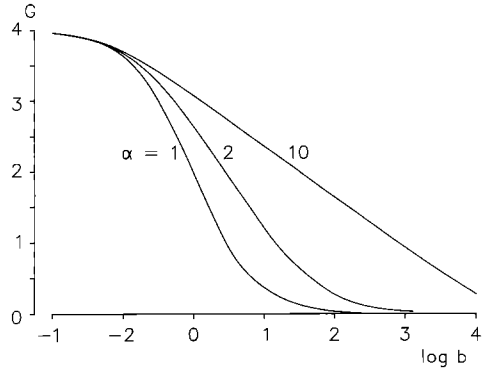

Fig. 5. Conductance G of a tetrameric channel complex for $\alpha = 1$, 2 and 10. The conductance of one open channel is taken as 1, and $b = B/K$ is the blocker concentration B divided by the equilibrium dissociation constant K

The extended tetrameric model illustrated in Fig. 3B closely resembles the Koshland-Némethy-Filmer model for a sequential binding of substrate molecules to a complex of four enzyme molecules (Koshland et al. 1966). For simplicity, the equilibrium dissociation constants for

the binding of 1, 2, 3 and 4 blocking particles to the channel complex were taken as K, αK, $\alpha^2 K$ and $\alpha^3 K$ to describe an increasing strength of negative cooperativity as the occupancy of the complex with blocking particles becomes higher. With this assumption the conductance of the tetrameric channel complex can be formulated by a simple analytical expression (see Appendix B). As expected, the slopes of the calculated concentration-response curves become less steep at increasing values of the interaction parameter α (Fig. 5). Owing to the presence of four subunits in the channel complex, "humps" no longer appear in the concentration-response curves, in contrast to the dimeric channel model. The Hill coefficients of the concentration-response curves plotted in Fig. 5 are 1 for $\alpha = 1$, 0.60 for $\alpha = 2$ and 0.32 $\alpha = 10$ in the range between $G = 0.2$ and 3.8.

Discussion

Flat concentration-response curves with Hill coefficients below 1 have been described for various enzyme reactions (e.g. see Fersht 1985). The weak concentration dependence does not necessarily imply a negative cooperativity between subunits of an enzyme complex. Instead, non-identical binding sites with different substrate affinities would also account for a broad concentration dependence of an enzyme reaction. Similarly, a weak dependence of the conductance of membrane channels on the concentration of channel blockers could arise from a population of heterogeneous channels with different binding constants for channel blockers. This possibility cannot be excluded by testing the effects of different concentrations of channel blockers on different membrane patches or by performing experiments on multi-channel preparations. Instead, it would be necessary to measure a complete concentration-response curve on a membrane patch containing only one ion channel. For K_{ATP} channels this type of experiment is rather unfeasible owing to the presence, in most cases, of several channels in a patch, to the uncertainty of whether the blocker effects are fully reversible, and to the limited duration of patch clamp experiments.

Therefore, other criteria are needed to distinguish between heterogeneous K_{ATP} channels and channel-channel interactions. The experiment shown in Fig. 1 might be useful in differentiating between these possibilities. Suppose K_{ATP} channels in pancreatic B-cells were heterogeneous with a broad range of tolbutamide affinities in the absence of Mg · ADP and an incomplete channel blockage even at a high tolbutamide concentration of 1 mM (open circles in Fig. 1). Then the nucleotide complex Mg · ADP would have to modify individual channels according to their tolbutamide affinities. Specifically, Mg · ADP should produce a strong increase of the tolbutamide sensitivity for a low-affinity channel and weaker increases for channels of higher affinities. Only then would Mg · ADP create a homogeneous channel population of uniform tolbutamide sensitivity and a concentration dependence with a Hill coefficient near 1 (filled circles in Fig. 1). Such a graded action of Mg · ADP on subpopulations of K_{ATP} channels seems to be rather unlikely. As an alternative, we suggest that negative cooperativity between K_{ATP} channels could account for the flat concentration-response curves of channel blockage by tolbutamide in the absence of Mg · ADP (Zünkler et al. 1988, see Fig. 1), by the hypoglycaemic drug U-56324 (Hopkins et al. 1990) and by the cardiotonic agent AWD 122-60 (Bodewei et al. 1992, see Fig. 2). The origin of the interactions between K_{ATP} channels could be channel clustering, possibly involving cytoskeletal elements as suggested for voltage-sensitive K^+ channels expressed in *Xenopus* oocytes (Honoré et al. 1992). The nucleotide complex Mg · ADP would then weaken the interactions between K_{ATP} channels as observed in the experiments on B-cells (Fig. 1) and obtained from the tetrameric channel model at lower values of the interaction parameter α (Fig. 5). Thus, Mg · ADP complexes would exert dual effects on K_{ATP} channels: The complexes stimulate the channels as originally found in insulin-secreting cells (Dunne and Petersen 1986; Kakei et al. 1986) by binding to an activatory channel site (Tung and Kurachi 1991; Schwanstecher et al. 1992). Furthermore, Mg · ADP would also act as an intracellular factor regulating channel-channel interactions. Such a modulation of the cooperativity between membrane channels may occur not only for K_{ATP} channels, but it could be a general phenomenon for all types of ion channels which are arranged at high densities or in clusters in biological membranes.

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Appendix A

Dimeric channel model

The probabilities of the states of the channel dimer illustrated in Fig. 3A are denoted by p_0 (both channels empty), p_1 (one blocking particle bound to the left channel) and p_2 (both channels occupied by two blocking particles). Since there are two possibilities of equal probabilities to have one and only one blocked channel in the dimer, one has:

$$p_0 + 2p_1 + p_2 = 1 . \tag{A1}$$

The laws of mass action read:

$$p_1/p_0 = b ; \qquad p_2/p_1 = b/\alpha \tag{A2}$$

where $b = B/K$ is the blocker concentration B divided by the equilibrium dissociation constant K.

If the conductance of an open channel is taken as unity, and if it is assumed that binding of a blocking particle to a channel produces channel closure, the conductance of the dimer is given by

$$G = 2(p_0 + p_1) . \tag{A3}$$

From Eqs. (A1), (A2) one can determine the probabilities as a function of b and α. Insertion into Eq. (A3) yields the

expression

$$G = \frac{2(1+b)}{1+2b+b^2/\alpha} \tag{A4}$$

which reduces to $G = 2/(1+b)$ in the absence of channel-channel interactions ($\alpha = 1$).

Appendix B

Tetrameric channel model

The probabilities of the states of the channel tetramer illustrated in Fig. 3B are denoted by p_0 (all channels empty), p_1 (one blocking particle bound to upper left channel), p_2 (two blocking particles bound to upper two channels), p_3 (three blocking particles bound to upper two channels and to lower left channel) and p_4 (all channels occupied by four blocking particles). Since there are $\binom{4}{n}$ possibilities of equal probabilities to have n and only n blocked channels in the tetramer, one has:

$$p_0 + 4p_1 + 6p_2 + 4p_3 + p_4 = 1\,. \tag{B1}$$

The laws of mass action read:

$$\begin{aligned} p_1/p_0 &= b; \quad & p_2/p_1 &= b/\alpha; \\ p_3/p_2 &= b/\alpha^2; \quad & p_4/p_3 &= b/\alpha^3 \end{aligned} \tag{B2}$$

with $b = B/K$ as in Appendix A.

The conductance of the tetramer is

$$G = 4p_0 + 3\cdot 4p_1 + 2\cdot 6p_2 + 4p_3 \tag{B3}$$

or after replacing the probabilities by the normalized blocker concentration b and the interaction parameter α

$$G = \frac{4(1+3b+3b^2/\alpha+b^3/\alpha^3)}{1+4b+6b^2/\alpha+4b^3/\alpha^3+b^4/\alpha^6}\,. \tag{B4}$$

In the absence of channel-channel interactions ($\alpha = 1$) one has $G = 4/(1+b)$.

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