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MINIREVIEW ARTICLE

Cell proliferation, potassium channels, polyamines and their interactions: a mini review

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Abstract Polyamines, which are obligatory molecules involved in cell cycling and proliferation, are subject to a change in their free intracellular concentrations during the cell cycle. Potassium (K^+) channels are also considered, but less well recognized, to be necessary for cell proliferation by either hyperpolarizing or depolarizing cells during the cell cycle. A block of polyamine synthesis as well as block or knockout of K^+ channels can halt cell proliferation. K^+ channels like BK (maxi calcium (Ca^{2+})-activated K^+), Kir (inward rectifier), M-type K^+-and TASK (two-pore domain K^+) channels or the delayed rectifier K^+ channels are modulated in their electrical properties by polyamines. Polyamines are most effective in blocking these channels when applied to the intracellular face of these channels except for TASK channels where they act only from the extracellular side. Quinidine, a general K^+ channel blocker, was found to reduce putrescine concentrations, to block the ornithine decarboxylase and halt cell proliferation. From these results, the question arises if there is an interaction between polyamines, K^+ channels and proliferation. It might be speculated that a decrease of intracellular polyamines allows more K^+ channels to be active, thus inducing hyperpolarization, while an increase of the polyamine concentration may block K^+ channel activity leading to depolarization of the membrane potential. On the other hand, a block or a deletion of K^+ channels may cause a decrease of the polyamine concentration in cells. More research is needed to test these hypotheses.

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Introduction

Regulation of cell proliferation is of high biological significance in the animal as well as in the plant kingdom. Cell proliferation is a necessity during development or wound healing and repair under normal physiological conditions, but its control has also great relevance for neoplastic diseases like cancer. In contrast to a tremendous knowledge about the biochemical and molecular mechanisms controlling the cell cycle, far less is known about bioelectrical events modulating cell cycle checkpoints. Polyamines as well as K^+ channels have been found to be important factors for a controlled proliferation process. They are not the only players in the web of proteins, enzymes and other molecules needed for a cell to cycle. However, without polyamines proliferation comes to a halt. In this mini review, we will summarize functions and modulatory properties of polyamines and K^+ channels involved in cell proliferation and focus on their interactions.

Polyamines and cell proliferation

The polyamines putrescine, spermidine and spermine are small, polycationic, organic molecules carrying two, three or four positive charges at physiological pH. They are found in prokaryotic as well as eukaryotic cells in plants and animals and have a vast number of physiological functions. For instance, they are mandatory for cell proliferation, modulate ion channels and interact with DNA, RNA, ATP, phospholipids or proteins. Although most

polyamines in the cells are present in a bound form, only 5.1 % of spermine and 15 % of spermidine occur as free molecules as found in bovine lymphocytes (Igarashi and Kashiwagi 2010). Ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase (SAMDC) and spermidine/spermine N^1-acetyltransferase (SSAT) are the key enzymes for polyamine synthesis and interconversion, respectively. Intracellular polyamine concentrations are adjusted by an inhibitory protein called antizyme which blocks ODC and stimulates its degradation, favors polyamine excretion and inhibits polyamine uptake via a specific polyamine transporter system. Increased levels of polyamines are found in cancers. For recent reviews on polyamine physiology, see Alm and Oredsson (2009), Bachrach (2005), Igarashi and Kashiwagi (2010), Mangold (2005) and Morgan (1999).

The cell cycle is divided into defined phases, namely G1 (first gap), S (synthesis), G2 (second gap) and M (mitosis), while a post-mitotic cell in G0 is considered to be in a non-dividing status (quiescent). It is well established that polyamine depletion interrupts cell cycle progression with most cells arrested at the G1/S checkpoint. One of the reasons for the retarded cycle progression is an increase of inhibitors ($p27^{Kip1}$ and $p21^{Cip1/WAF1}$) of cyclin-dependent protein kinases CDK2 and CDK4 (Ray et al. 1999). Polyamine concentrations of non-cycling cells are low during the G0 phase. In cycling cells, polyamine biosynthesis increases at the G1/S transition at the end of the S phase and during the G2-phase. The contribution of polyamines was found to be more important at the G1/S boundary than during the G2/M phase, but it should be kept in mind that polyamines play an important role during all phases of the cell cycle (Yamashita et al. 2013). During the cell cycle, antizyme activity was reported to oscillate opposite to the ODC activity. This explains the biphasic activity of ODC during the course of the cell cycle (Linden et al. 1985). Polyamine levels increase during the cell cycle to peak during the G2 phase and decline thereafter as summarized in Alm and Oredsson (2009).

K^+ channels and proliferation

In all living cells, K^+ channels are involved in the maintenance of the membrane resting potential. There are two basic types of K^+ channels which contribute to the membrane resting potential: 1) the so-called leak or background channels which are mostly time and voltage independent and belong to the superfamily of the two-pore domain K^+ channels (K_{2P}) and 2) several other K^+ channels like voltage-activated K^+ channels (e.g., the delayed rectifier or Kv channels), Ca^{2+}-activated K^+ channels, or ATP-sensitive (K_{ATP}) K^+ channels, to name a few (Hille 2001). While the job of K^+ channels as modulators and housekeepers of the membrane potential is broadly established, their mitogenic role as important players in controlling cell proliferation is far less recognized.

The first paper indicating that K^+ channels may play some role in cell proliferation was published in 1984 by DeCoursey et al. They observed that the mitogen phytohaemagglutinin (PHA) alters K^+ channel gating in T-lymphocytes by shifting the voltage activation curve to more negative potentials and that 3H-thymidine incorporation following a PHA stimulation is inhibited by K^+ channel blockers such as tetraethylammonium (TEA), 4-aminopyridine (4-AP) or quinine. Ever since, the body of evidence indicating a number of K^+ channels to have an important regulatory function in cell proliferation is growing; for reviews see Blackiston et al. (2009), Dubois and Rouzaire-Dubois (1993), Girault et al. (2012), Jehle et al. (2011), Rodríguez-Rasgado et al. (2012), Sundelacruz et al. (2009), Wang et al. (1998) and Wonderlin and Strobl (1996). K^+ channel activity seems to be necessary for cells to progress through the G0/G1 checkpoint of the cell cycle, which can be prevented by specific K^+ channel blockers such as glibenclamide for K_{ATP} channels (Nunez et al. 2013), $BaCl_2$ for Kir4.1 channels (Higashimori and Sontheimer 2007), triarylmethane (TRAM-34) for intermediate Ca^{2+}-activated K^+ channels (IK) (Zhang et al. 2012) or paxilline for maxi Ca^{2+}-activated K^+ channels (BK) (He et al. 2011) (for reviews see also Dubois and Rouzaire-Dubois 1993; Wallace et al. 2011; Wonderlin and Strobl 1996). Further K^+ channels found to modulate cell proliferation are TASK1 channels (Bittner et al. 2012). More recent studies using knockout design or short-interfering RNAs (siRNAs) further support the contribution of K^+ channels such as Kv channels, ether-à-go–go (Eag) K^+ channels implicated in the pathogenesis of various cancers via association of cell cycle alterations or IK channels to cell cycling and proliferation (Asher et al. 2011; Grgic et al. 2009; Koeberle et al. 2010; Weber et al. 2006).

While most papers report a reduction of cell proliferation by a block of K^+ channels, the contrary effect has been found in MCF-7 breast cancer cells. Low concentrations of tamoxifen in the nanomolar concentration range activated BK channels and stimulated cell proliferation (Coiret et al. 2007). Another more unspecific way to stimulate cell proliferation is to depolarize the cells slightly by increasing the amount of extracellular K^+ (10–30 mM), which results according to the Nernst equation in a more positive membrane potential (Basrai et al. 2002). In this study with human 1321N1 astrocytoma cells, a block of BK channels using the specific blocker iberiotoxin (IBTX) or 1 mM tetraethylammonium prohibited cell proliferation—but interestingly, only if cells were first activated by more depolarized membrane potentials. In undisturbed cells at

control resting potential, BK channel blockers did not interfere with proliferation rates. Depolarization with high extracellular K^+ appears to model a situation as in rapidly growing tumors, indicating a specific modulatory role of BK channels for cell proliferation in this experimental setting. However, it was recently also observed that a block of BK channels can increase cell proliferation and an activation of the same channel may cause an inhibition of cell proliferation (Chang et al. 2011). K^+ channels have also been found to be important for a cell to proceed from G1 to the S phase in the cell cycle (Kv1.1 channels (Chittajallu et al. 2002), IK1-like channels (Ouadid-Ahidouch et al. 2004)).

Decreased Kir channel expression coupled with membrane depolarization was found in models of astrocyte injury during active gliosis, while in resting non-proliferating astrocytes Kir channels led to membrane hyperpolarization (Bordey et al. 2001; MacFarlane and Sontheimer 1997). In spinal cord astrocytes down-regulation of Kir accompanied with a depolarization was observed to promote cell cycle progression through the G1/S checkpoint, indicating depolarization to be necessary for entering the S phase (MacFarlane and Sontheimer 2000). A block of Kir channels in actively proliferating cells caused an accumulation in G2/M. However, a block of the delayed rectifier K^+ channel by TEA or 4-AP caused proliferating astrocytes to arrest in G0/G1.

In summary, there is strong evidence that several different types of K^+ channels—ligand—as well as voltage gated or combinations of these channels are necessary for cells to progress through the cell cycle. The reason for this effect was attributed to K^+ diffusion through K^+ channels out of the cells as shown in theoretical models (Dubois and Rouzaire-Dubois 1993; Wonderlin and Strobl 1996) resulting in hyperpolarization of the membrane potential. Hyperpolarization in addition increases the driving force for Ca^{2+} into the cells according to the Nernst equation, which makes sense since Ca^{2+} is another major factor in cell-promoting proliferation. Increased Ca^{2+} entry into the cells via T-type Ca^{2+} channels at low membrane resting potential has been called "T-type window current" (for review see Capiod 2011). On the other hand, Ca^{2+}-sensitive K^+ channels like BK or IK channels may serve as regulatory sensors by hyperpolarizing cells and in this way limit the action of voltage-operated Ca^{2+} channels.

Early doubts that more general types of K^+ channel blockers such as quinidine, TEA or 4-AP cause the antiproliferative effect due to an unknown unspecific side effect are still arguments which have to be discussed, but in the light of modern channel knockout experiments (as described above) they do not refute the basic ideas of this concept. The idea of K^+ channels regulating cell proliferation might be interesting for treating diseases of uncontrolled neoplastic cell proliferation such as cancer. For instance in the breast cancer cell lines MCF-7 and T47D, estrogen is required for proliferation. In these cells. estrogen up-regulates the expression of the pH-sensitive two-pore domain TASK2 channels. Blocking the expression of these channels with small interfering RNA not only reduced the amount of current, but also prevented cell proliferation (Alvarez-Baron et al. 2011). In human breast cancer cells, the active form of vitamin D, calcitriol inhibits ether-à-go–go (Eag1) channel expression via a vitamin D receptor-dependent mechanism resulting in a reduction of cell proliferation. Although in some cancers, cell proliferation may be controlled by blocking K^+ channels, others may not benefit from this approach as recently described for gliomas (Abdullaev et al. 2010). To make matters more complicated, in some cells K^+ channel activation might be the most useful strategy for reducing proliferation or inducing apoptosis as found in human ovary cancer cells where a specific BK channel opener (NS1619) resulted in a reduction of cell proliferation (Han et al. 2008). Taken together, this perspective, in combination with a precise targeting, might open new vistas for treatment of cancer which is resistant to other chemotherapies.

K^+ channels and polyamines

The modulation of K^+ channels by polyamines was recently reviewed in detail (Weiger and Hermann 2009). In brief: polyamines modulate channels like N-methyl-D-aspartate (NMDA) receptors, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate receptors or Ca^{2+} channels (for review see Johnson 1996; Williams 1997) and a number of diverse K^+ channels such as Kir channels, BK channels, the delayed rectifier, KCNQ channels or TASK channels.

Delayed rectifier K^+ channels

In neurons, this channel is mainly responsible for repolarizing the action potential. The first report demonstrating the modulatory action of polyamines on K^+ channels dates back to 1990 when Drouin and Hermann reported that in neurons from *Aplysia californica* intracellular but not extracellular spermine blocked the delayed rectifier K^+ current in a dose- and voltage-dependent manner. The minimal concentration of spermine required to show an effect on the delayed rectifier was 200 μM. They suggested that this block was caused by spermine slipping rapidly in and out of the channel pore, a phenomenon called a "fast block" (Drouin and Hermann 1990, 1994).

M-type K^+ (KCNQ) channels

M-type K^+ channels (KCNQ, Kv7) are low-threshold voltage-activated channels. They control the resting membrane potential as well as excitability of cells. Neurotransmitters like acetylcholine or hormones activating the receptor switch off these channels via a phospholipase C (PLC), phosphatidylinositol bisphosphate (PIP2)-dependent pathway (Delmas and Brown 2005). Putrescine and spermine were found to inhibit these channels in a dose-dependent manner. The half maximal inhibitory concentrations (IC50 values) were 2.4 and 0.86 mM, respectively (Suh and Hille 2007). Their data further indicate that polyamines do not decrease KCNQ currents by a direct block of the channel pore from inside the cell like the delayed rectifier, but instead act by binding electrostatically to negative phosphates of PIP2.

BK channels

BK channels (B stands for big because of their large unitary conductance up to 200–300 pS and K for potassium) are also found in the literature under the synonyms Slo, KCa1.1, Slowpoke (in *Drosophila*) or KCNMA1 in the human genome. These channels are voltage and Ca^{2+} sensitive and serve as Ca^{2+} sensors. Once activated by an increase in voltage and Ca^{2+}, they quickly hyperpolarize cells, shut down voltage-sensitive Ca^{2+} channels and hence limit the entry of Ca^{2+} into the cell. They are found in almost all excitable and non-excitable cells and are involved in a vast number of physiological and pathophysiological processes such as action potential repolarization, hormone secretion, blood pressure control, cell proliferation, epilepsy or erectile dysfunction to name a few (Hermann et al. 2012; Ghatta et al. 2006; Weiger et al. 2002). The modulation of a Ca^{2+}-activated K^+ current in *Aplysia* neurons and BK channels in GH3 pituitary tumor cells by polyamines was reported in parallel by Drouin and Hermann (1994) and by Weiger and Hermann (1994). The order of blocking efficacy was spermine > spermidine > putrescine. Polyamines blocked the channels in a voltage- and dose-dependent manner in GH3 cells with a K_d of 0.7 mM for the reduction of the open probability by spermine. Polyamines did not exhibit any blocking action when applied from the extracellular side of the membrane. This was attributed to the relatively close spatial distribution of positive charges within the polyamines when compared with 1,12-diaminododecane. The latter blocked the channels effectively from outside, because in contrast to polyamines it probably can strip off its hydration shell when entering the channel pore, whereas spermine cannot and is therefore unable to enter the channel (Weiger et al. 1998). In rabbit pulmonary arteries Snetkov et al. (1996) found polyamines to be responsible for rectification of BK currents. The rectification was released in cells treated with the ornithine decarboxylase (ODC) blocker difluoromethylornithine (DFMO) which inhibits internal polyamine synthesis (Snetkov et al. 1996). Similar results indicating a rectification of BK currents by polyamines were reported in myocytes (Catacuzzeno et al. 2000).

BK channels possess a ring of negative charges which attract K^+ to the inner vestibule of the channel. These charges located at the channel entrance facilitate the spermine block through a preferential electrostatic attraction of polyamines over K^+. Increasing voltage drives the positively charged polyamines into the channel pore, similar to the case for Kir channels (see below) causing blockade and rectification of the channel increasing at more positive voltages (Zhang et al. 2006). A recent study in humans revealed that a block of BK channels by polyamines may be a reason and/or target for the treatment of the overactive bladder syndrome (Li et al. 2009).

In the majority of cases, polyamines block BK channels when applied from the cytoplasmic side. However, they were found to be ineffective in Müller glia cells of the retina (Biedermann et al. 1998). The reason for this result is unclear, but may be due to the relatively low polyamine concentration used in that particular study or to a less sensitive BK channel splice variant. In another report using myocytes from the guinea pig stomach, extracellular application of 100 μM spermine activated the BK channels at voltage above +60 mV by 14 % (Li et al. 2000). No further concentrations or other polyamines were tested and spermine was ineffective at low voltages. It is also not clear if this result is specific for BK channels, since it was measured in the whole cell patch clamp mode recording total outward current, which could be contaminated by some other unknown outward current other than BK.

TASK channels

Leak K^+ channels determine and control the resting membrane potential which is important to be kept within a limited range in excitable cells such as neurons or heart. One key property of these leak channels is their voltage independence. A polyamine-modulated member of these types of channels which belong to the KCNK K^+ channel family (potassium channel subfamily K) are TASK channels (TWIK-related acid-sensitive K^+, where TWIK stands for tandem pore domains in a weak inwardly rectifying K^+ channel). TASK channels are pH-sensitive K^+ channels with very fast activation kinetics and weak inward rectification. They are inhibited by protons applied to the extracellular face of the channel (O'Connell et al. 2002). In

contrast to an earlier nomenclature which included five different TASK channels more recently, only TASK-1, TASK-3 and TASK-5 are considered as TASK channels sensu strictu (Enyedi and Czirják 2010). While TASK-1 channels are not affected by polyamines, TASK-3 channels are blocked by extracellular, micromolar application of spermine. The insensitivity of TASK-1 channels against polyamines appears to be due to a positive charge at the outer vestibule. As described above for BK channels, a ring of 13 negative charges at the, in this case, outer opening of TASK-3 channels seems to be responsible for the blocking properties of polyamines. These charges attract under normal conditions K^+ in high concentrations to the channel. Shielding by spermine or divalent cations prevents the local increase of K^+ which in turn contributes to a lower conductance of the channel. Addition of spermine to the bath solution changes the firing pattern of thalamocortical relay neurons from a burst-like mode to tonic discharge behavior. Hence, extracellular spermine at postsynaptic TASK-3 channels may increase neuronal excitability (Musset et al. 2006). It should be emphasized that in contrast to almost all other K^+ channels, TASK channels are modulated by extracellular application of polyamines.

Kir channels

The inward rectifier K^+ channel family (Kir) is divided into seven different sub-classes (Kir1–7). These channels are involved in maintaining the membrane resting potential. Two mechanisms regulate their K^+ conductance: (a) it is increased at more hyperpolarized membrane potentials and (b) at a given membrane potential their conductance is augmented with increasing extracellular K^+ concentration.

The term inward rectification refers to the fact that these channels favor K^+ ions to enter the cell at more negative potentials but block their exit at more positive potentials. These channels keep the membrane potential at more negative values, but close once more positive potentials are reached as for instance during an action potential. Kir channels play a role in controlling neuronal excitability, hormone and transmitter release as well as in regulating cardiac pacemaker activity (Hille 2001; Stanfield and Sutcliffe 2003).

Lopatin et al. (1994), Ficker et al. (1994) and Fakler et al. (1994, 1995) concurrently discovered that the inward rectification of Kir channels is caused by polyamines at the cytoplasmic side of the channels in a voltage- and dose-dependent fashion. Spermine is the most potent of the three polyamines acting at submicromolar concentrations. Furthermore, spermidine and spermine decrease mean channel open times and prolong channel closed times. In these early experiments, it was found that Kir2.1 (IRK1) channels exhibit a very strong rectification by polyamines, while Kir1.1 (ROMK) channels were less sensitive. From all Kir channels tested so far, Kir2.1 displays the strongest rectification caused by polyamines. These ground-breaking experiments shifted the understanding of the mechanism of rectification from an intrinsic channel feature or a magnesium (Mg^{2+}) block to a new function assigned to polyamines.

Ever since, modulation of Kir channels by polyamines has received extensive attention which is reflected in a number of reviews (i.e., Hibino et al. 2010; Lu 2004; Wang et al. 2011). The mode of action of polyamines on Kir channels is assumed to result from the slow blocking and unblocking of the channels. Depolarization corresponds to polyamines causing a time-dependent decrease of outward current. On hyperpolarization, the inward Kir increases due to slow polyamine unblocking preceded by fast Mg^{2+} unblocking. Kir channel blockade by polyamines is probably one of the best understood mechanisms of this kind. In a most recent publication, the blocking mechanism is described as follows: polyamines enter and traverse the long (>70 Angström) channel pore from the intracellular side up into the channel displacing multiple permeant ions en route to a high-affinity binding site deep within the channel (Kurata et al. 2013). Kir channels which show a strong rectifying behavior have an acidic residue (Asp-172 in Kir2.1 channels) at the inner cavity of the channel, which is also known as the rectification controller and is an important determinant for the voltage-dependent polyamine block. The deep binding site of spermine is now believed to be located between the rectification controller and the selectivity filter. Binding of spermine at this site involves most likely interactions of a hydrogen bond with the rectification controller's carboxylate functional group (Kurata et al. 2013). In a very recent study, it was shown that Kir2.1 channels are also blocked by extracellular spermine. In contrast to the block from the intracellular side, spermine applied to the extracellular side does not inhibit outward currents by entering the cytoplasmic pore. Mutagenesis studies support the notion that spermine may directly interact with the extracellular domain of the channel (Chang and Shieh 2013).

Is the cellular free polyamine concentration sufficient to modulate K^+ channels?

Most experiments described above use arbitrary polyamine concentrations irrespective of the polyamine concentration of the cell investigated. Electrophysiological experiments, where cell free systems such as inside out or outside out patch configurations are used, allow for tight control of the polyamine concentrations. Polyamine concentrations reported

from cells or tissues mostly do not differentiate between bound and free polyamines, but only the latter are important and available for interaction with ion channels. This raises the question whether K^+ channels are modulated in vivo by free polyamines present in a cell. Watanabe et al. (1991) measured free polyamine concentrations in rat liver and bovine lymphocytes. They found depending on their experimental conditions, with varying amounts of K^+ and Mg^{2+} and the cell type investigated, free spermine concentrations ranging from 8.2 to 75.5 μM and free spermidine concentrations of 38.2–199 μM. However, it has to be kept in mind that this is a snapshot showing polyamine concentrations at a single time point, while cellular polyamine concentrations vary during the cell cycle as described above. For polyamine concentrations applied in the course of electrophysiological experiments as reviewed above, the following values were reported: For Kir2.1 (IRK1) channels, a K_d for spermine of 7.5 nM, for spermidine of 17.9 nM and for putrescine of 9,800 nM was found (Ficker et al. 1994). This indicates that Kir2.1 channels are fully modulated by naturally occurring free intracellular polyamines. BK channels with a K_d of 0.7 mM for spermine (Weiger and Hermann 1994) are probably not very strongly affected by polyamines in resting cells, but their polyamine sensitivity starts in a range which is near the free polyamine concentrations reported by (Watanabe et al. 1991). Zhang et al. (2006) found 10–100 μM spermine sufficient to produce a partial BK channel block with a higher efficiency at more positive voltages. A value of at least 200 μM spermine needed to block delayed rectifier as found by Drouin and Hermann (1990, 1994) in *Aplysia californica* may not be comparable to the other data presented here, which were obtained mainly in mammalian systems for two reasons: First, the BK channel in *Aplysia* might be a splice variant differing from mammalian BK channels and, second, polyamine concentrations in marine snails are unknown. M-type channels exhibit an IC50 of 2.4 and 0.86 mM for a block by putrescine or spermine, respectively (Suh and Hille 2007). For TASK channels, intracellular polyamines are not relevant since they are modulated only by extracellularly applied polyamines. Concluding, the question if the amount of free intracellular polyamines is sufficient to modulate potassium channels can be answered with yes, but differences in the strength of modulation of individual channel types exist, with some being affected only when intracellular polyamines reach higher concentration as in the case during the cell cycle, in tumor cells or during development.

Conclusions and future perspectives

Bioelectrical signaling provides a rich and interesting communication system for inter- and intracellular communication next to and in combination with chemically supported transduction pathways. Most exciting are the interfaces between these two message systems. Particularly, the regulation of the cell cycle needs concurrence of both types of signaling to work properly. Polyamines as representatives of the chemical system are well known to modulate, besides other channels, a number of K^+ channels as representatives of the bioelectrical side. To hyperpolarize cells K^+ channels must be opened for conduction, i.e., unblocked to set the electrochemical driving force into motion. On the other hand, depolarization can be caused by obstruction of the passage of K^+, which can be achieved by a block of K^+ channels, for instance via intracellular polyamines.

In this way, both polyamines and K^+ channels act together to adjust the desired membrane potential during cell cycling. However, there is basically no information available if K^+ channel activity may modulate polyamine concentrations in cells via a feedback mechanism. To our knowledge, there exists only the publication by Weiger et al. (2007) suggesting that polyamine—K^+ channel signaling may work in both directions. In this study, we used a classical approach blocking K^+ channel activity pharmacologically with quinidine to stop proliferation of C6 glioma cells. Quinidine depolarized the cells. In addition, quickly, within the first 6 h, cellular putrescine content decreased beyond detection levels and ODC activity was reduced significantly, whereas spermidine and spermine concentrations remained unaffected. The experiments suggest that the antiproliferative effect of quinidine is not just due to a simple membrane depolarization by blocking K^+ channels, but the drug also inhibits ODC activity. It remains questionable, however, if polyamine synthesis was affected by quinidine directly or if the block of K^+ channel activity initiated an unknown signaling pathway leading to decreased ODC activity and reduction of the putrescine content. The data so far give rise to the speculation that K^+ channel activity may modulate polyamine concentrations via a feedback loop. We hypothesize that a reduced or blocked K^+ channel conductance leads to a decline of the cellular polyamine concentration. Low polyamine concentrations cause unblocking of polyamine-sensitive K^+ channels leading to hyperpolarization of the membrane potential. On the other hand, an increased intracellular polyamine concentration blocks polyamine-sensitive K^+ channels in a dose-dependent manner and results in membrane depolarization. Looking at cell cycling, it is evident that besides polyamines and other factors, bioelectrical signaling for proper cycling is needed. If polyamines are low, hyperpolarization mediated by K^+ channels could be this signal in many tissues. An increased polyamine concentration would go hand in hand with a depolarizing signal after a dose-dependent block of polyamine-sensitive K^+ channels by polyamines. Cancer cells exhibit high polyamine concentrations together with a depolarized membrane potential (Bachrach 2004). Our model of a

feedback loop between polyamines and K^+ channels predicts that a blocked K^+ conductance by polyamines may cause this depolarization, but in contrast to normal cells where a depolarization would give the signal to reduce polyamines, in cancer cells this loop is disrupted and hence may be the trigger for the cells to become carcinogenic. An experiment to test this hypothesis of a polyamine—K^+ channel feedback may be to knock out K^+ channels and compare the polyamine concentrations of these cells to wild-type cells. Clearly, more research is needed to solve some of these questions.

A more detailed understanding of the interaction between polyamines, polyamine-sensitive K^+ channels and cell proliferation is apt to contribute to a better understanding of cellular events during cell cycling and may be a rewarding task for the development of new medication in the treatment of neoplastic diseases like cancer or to improve wound healing.

Conflict of interest The authors declare that they have no conflict of interest.

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