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Involvement of K^+ channels in the quercetin-induced inhibition of neuroblastoma cell growth

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Abstract. The effects of the flavonoid quercetin on cell proliferation and voltage-dependent K^+ current were studied on mouse neuroblastoma×glioma hybrid cells. In the presence of 1% fetal calf serum, quercetin inhibited both cell proliferation and K^+ current with effective doses inducing half-maximum effects of 10 μM and 70 μM respectively. Valinomycin (1 nM) antagonized 80% of the growth-inhibitory effects of 10 μM quercetin. The results suggest that at least a part of the antiproliferative effect of quercetin is mediated by a K^+ channel blockade. They further confirm a role for K^+ channels in mitogenesis and cell proliferation.

Key words: Potassium channels – Neuroblastoma cells – Quercetin – Cell proliferation

Introduction

Quercetin, a flavonoid commonly found in fruits and vegetables consumed in the human diet, inhibits growth and proliferation of malignant cells in vitro [20] and chemically induced murine carcinogenesis [7, 22]. While quercetin is known to interact with a broad spectrum of enzymes and receptors, including protein kinase C [10], Na^+/K^+-ATPase [20] and type-II oestrogen-binding sites [14], which are thought to be involved in cell division, its antitumour mode of action is not clear. Recently it has been shown that the infusion of *Ruta graveolens* (which contains quercetin-3-rutinoxide) blocked K^+ channels in myelinated nerve fibres [2]. Since K^+ channels are assumed to be involved in mitogenesis (for a review see [8]), we have tested the hypothesis that the antitumoral action of quercetin could be mediated by a K^+ channel blockade.

We report here that quercetin blocks both proliferation and voltage-dependent K^+ channels of mouse neuroblastoma cells. Moreover, its growth-inhibitory action can be suppressed by the K^+ ionophore, valinomycin.

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Materials and methods

The electrophysiological experiments were performed at room temperature on differentiated or undifferentiated neuroblastoma×glioma NG 108-15 cells. All methods of cell culture and electrophysiological recording were as previously described [16, 17], except that the cells were grown in Dulbecco's modified Eagle culture medium containing 1% fetal calf serum (FCS). Voltage-dependent K^+ current and membrane potential were recorded with the whole-cell patch-clamp technique. External and internal solutions had the following compositions: external, 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES); internal, 140 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES. External and internal pH were adjusted to 7.3 using NaOH. Electrophysiological experiments were carried out either in the external solution or in culture medium. Immediately before experiments, quercetin (Sigma, Saint Quentin Fallavier, France) was dissolved in 1 M NaOH at a concentration of 100 mM and then diluted either in the external solution or in the culture medium to give a final concentration ranging between 1 μM and 100 μM. The cell under investigation was continuously superfused by control or test solutions. The rate of cell growth was calculated between day 0 and day 2 of culture, by counting trypan-blue-excluding cells with a Mallassez grid. Each count was carried out eight times. In some experiments, valinomycin (Sigma) was added to the dishes at a final concentration of 1 nM. Experimental values are given as mean ± standard error of n experiments.

Results and discussion

The delayed current we recorded in undifferentiated or differentiated NG 108-15 cells essentially consists of a voltage-activated and voltage-inactivated K^+ current [17, 18]. Indeed, Na^+ current is too fast and Ca^{2+} current is too small to interfere noticeably with the K^+ current described here. We also exclude the involvement of K^+ current types other than voltage-dependent K^+ current, i. e. M current and Ca^{2+}-activated K^+ current (see [3]).

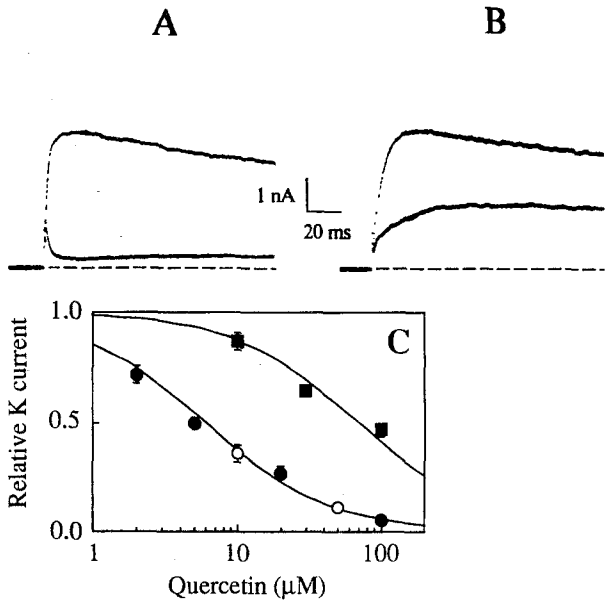

Fig. 1A–C. Quercetin blocks the voltage-dependent K^+ current. **A, B** Traces of K^+ current recorded on differentiated cells during depolarizations to + 50 mV from a holding potential of −80 mV under control conditions (*upper traces*) and 6 min after addition of 100 μM quercetin to the external medium (*lower traces*). The external medium was the FCS-free external solution (**A**) or the DMEM culture medium containing 1 % FCS (**B**). **C** Dose/response curves of the effect of quercetin (QU) on the peak current recorded at + 50 mV on differentiated (●, ■) or undifferentiated (○) cells in FCS-free external solution (●, ○) or in DMEM culture medium (■). Each point is the mean of three to seven experiments. Curves were drawn from the equation: relative K^+ current = $1/(1 + [QU]/K)$ with K = 6 μM (FCS-free medium) or 70 μM (DMEM culture medium)

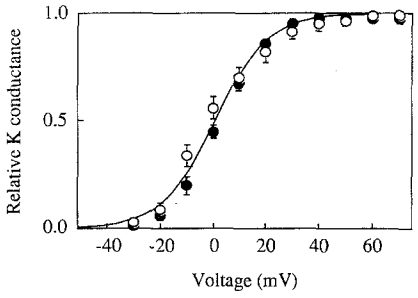

Fig. 2. Peak K^+ conductance/voltage curve in control (●) and in the presence of 20 μM quercetin (○). The K^+ current was recorded on differentiated cells superfused with the FCS-free solution during voltage pulses of various amplitudes from a holding potential of −80 mV. Mean of five experiments. The curve was drawn from the Boltzmann equation: relative K^+ conductance = $1/\{1 + \exp[(V_{0.5} - V)/k]\}$ with $V_{0.5}$ = 1 mV and k = 10 mV. The K^+ conductance was calculated from the equation: $g_K = I_K/(V - V_K)$ where I_K is the peak K^+ current, V the voltage and V_K = −85 mV

Whereas an M current can be recorded in prostaglandin-differentiated NG 108-15 cells [3, 15, 19], it is absent in undifferentiated [15] or dimethylsulphoxide-differentiated (personal observation) NG 108-15 cells. Along the same lines, there is no indication of the existence of a Ca^{2+}-activated K^+ current in our cells.

In the micromolar concentration range, quercetin reduced the amplitude of the voltage-dependent K^+ current

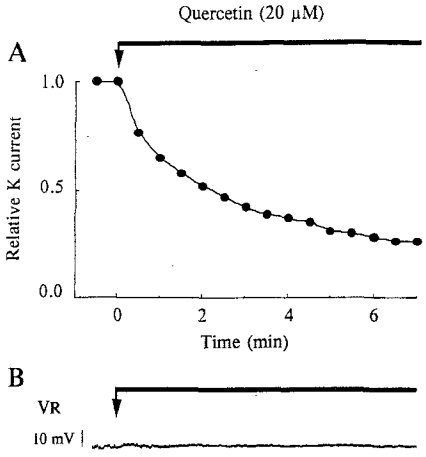

Fig. 3A, B. Quercetin-induced K^+ current inhibition is not associated with change in resting potential. Peak K^+ current at + 50 mV (**A**) and resting potential (**B**) were recorded on two different differentiated cells before and during the application of 20 μM quercetin. The resting potential was −60 mV. The time scale is the same in **A** and **B**

and slightly slowed its activation and inactivation kinetics (Fig. 1) without significant modification of its voltage sensitivity (Fig. 2). Figure 1A, B shows the effects of 100 μM quercetin on the peak K^+ current recorded on differentiated cells respectively in serum(FCS)-free medium and in culture medium containing 1 % FCS. In agreement with [20], FCS decreased the effective quercetin concentration, probably by interaction of quercetin with albumin. The K^+ current was blocked with an apparent dissociation constant of 6 μM in FCS-free medium and 70 μM in the presence of 1 % FCS (circles and squares respectively in Fig. 1C). The effect of quercetin on the K^+ current was similar in both differentiated and undifferentiated cells (open and filled symbols in Fig. 1C). It developed within few minutes after the addition of quercetin to the external medium (Fig. 3A) and was reversible upon washing with a quercetin-free solution (not shown). In contrast with most eukaryotic cells, the blockade of K^+ channels in neuroblastoma cells with classical K^+ channel blockers does not induce membrane depolarization [12, 18]. This is also the case for quercetin, which did not modify the resting potential (Fig. 3B).

In order to minimize the protective effect of FCS on the quercetin action described above [20], FCS was added to the culture medium at a minimal concentration compatible with satisfactory cell growth, i. e. 1 % instead of the 5 % used in our previous work [16, 18]. In these conditions, quercetin, added to the culture medium of proliferating cells, decreased the rate of cell growth (Fig. 4). A 50 % effect was achieved with a quercetin concentration of about 10 μM. At a concentration of 20 μM, quercetin completely inhibited cell growth. This steep relationship between quercetin concentration and growth inhibition is similar to those reported by [20] in various cell lines. It may reflect a synergism between several molecular effects of the flavonoid. From this point of view, it should be noted that quercetin had both cytostatic and cytotoxic effects, since cell viability at

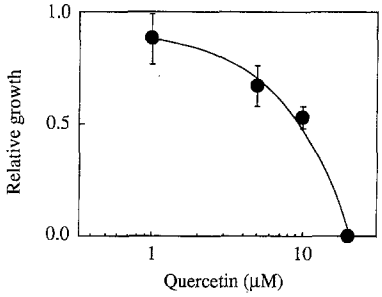

Fig. 4. Quercetin blocks the cell proliferation. Relative rate of cell growth as a function of quercetin concentration. Each point is the mean of five to eight experiments. The curve was drawn by eye

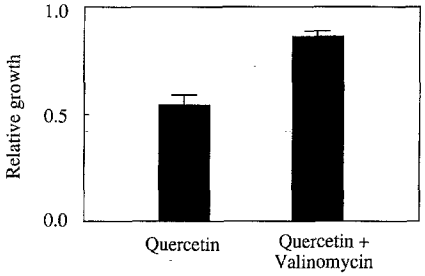

Fig. 5. Valinomycin (VAL) antagonizes the growth-inhibitory effect of quercetin (QU). Rate of cell growth calculated in four different conditions: drug-free, 10 μM QU, 1 nM VAL, and 10 μM QU + 1 nM VAL, and expressed as ratios QU/drug free (*left column*) and (QU + VAL)/VAL (*right column*). Mean of six experiments

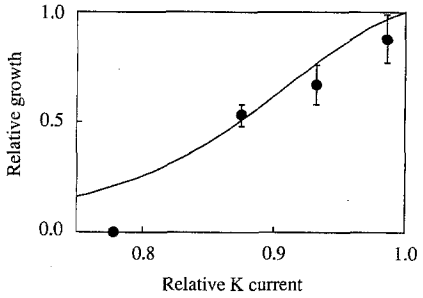

Fig. 6. Relative rate of cell growth as a function of the relative K^+ current. Data from Figs. 1 and 4. The curve was drawn according to the model we recently proposed [8, 18] from the equation: relative growth = $(1 + X)/\{1 + X\ [1 + Z\ (1-\text{relative } K^+ \text{ current})]^n\}$ with $n = 4$, $Z = 8$ and $X = 0.07$. Compared with our previous results obtained in the presence of 5 % FCS and various K^+ channel blockers [18], the theoretical growth/K^+ current relationship was made steeper by increasing Z and X values by 1.6 and 7 respectively

day 2 of culture had decreased from 82 % under control conditions to 73 % and 33 % in the presence of 10 μM and 20 μM quercetin respectively.

The fact that quercetin blocks both K^+ channels and cell growth does not necessarily imply a causal relationship between these two effects since, on the one hand, quercetin is known to interact with several enzymes and receptors involved in mitogenesis [10, 14, 20] and, on the other hand, in the presence of FCS quercetin is more efficient in inhibiting the cell proliferation than the K^+ current. If K^+ channel blockade is involved in the growth-inhibitory effect of quercetin, an increased passive membrane permeability to K^+ should restore cell growth. In order to test this hypothesis, we used the K^+ ionophore valinomycin. Since valinomycin is cytotoxic and inhibits cell proliferation at concentrations larger than 1 nM [5, 6], we chose this concentration to study its action on the growth-inhibitory effect of quercetin. When applied alone, 1 nM valinomycin did not modify the resting potential (not shown) and decreased the rate of cell growth to 89 ± 3 % ($n = 6$) of its control value. When valinomycin was added to the medium containing 10 μM quercetin, the rate of cell growth increased from 51 ± 4 % to 75 ± 3 % (relative to cell growth in drug-free conditions) and to 84 ± 3 % (relative to cell growth with valinomycin alone) (Fig. 5). It thus appears that 1 nM valinomycin has two opposite effects on cell growth. It decreases cell proliferation by about 10 % and suppresses almost 80 % of the growth-inhibitory effect of 10 μM quercetin. The former effect is probably due to its action on cellular ATP, since valinomycin is known to produce an ATP depletion [13]. The latter effect can be attributed to its action on the membrane permeability to K^+. On the basis of this interpretation, the above results demonstrate that the major part of the growth-inhibitory effect of 10 μM quercetin is mediated by a K^+ channel blockade. Obviously this does not mean that the antitumour action of quercetin, especially at large concentrations (e. g. 20 μM in neuroblastoma cells), is not in part due to the effect of the flavonoid on molecular targets other than K^+ channels. While quercetin is known to decrease the growth of both normal and transformed cells, it seems to act more efficiently on the latter [20]. In the light of the present results, it would be interesting to know if differences in K^+ channel properties and/or K^+ channel types of normal and tumoral cells are involved in this differential action of quercetin.

If, in various preparations, K^+ channel blockers always inhibit DNA synthesis, the role of K^+ channels in mitogenesis is not clear. K^+ channels, with an open probability and/or density increased by mitogens, are assumed to play a role in the progression through the cell cycle at a critical period in the G_1 phase, before the entry into the S phase [1]. Their possible role may result either from a maintenance of a hyperpolarized resting potential and a control of nutrient or Ca^{2+} uptake or a regulation of cell volume and the intracellular concentration of a solute directly involved in DNA synthesis (see [8, 9, 11]). Our present results, showing that, on the one hand, quercetin and valinomycin do not modify the resting potential and, on the other hand, that valinomycin antagonizes the growth-inhibitory effect of quercetin, suggest that, of the two hypotheses outlined above, the former is not valid. On the basis of the latter hypothesis, we recently proposed a model to take account of the role of K^+ channels in mitogenesis [8, 18]. According to this model, the rate of cell growth is a function of the intracellular concentration of a solute involved in the activation of DNA synthesis and is controlled by K^+ flux through K^+ channels. Qualitatively, this model can take account of the effects of quercetin on cell proliferation. However, while, according to our previous results [18], growth inhibition of neuroblastoma cells is almost pro-

portional to K^+ channel blockade, a quercetin-induced reduction of the current by about 10 % is associated here with a 50 % inhibition of cell growth (Fig. 6). This may be due to the fact that the flavonoid exerts its growth-inhibitory action through several mechanisms including K^+ channel blockade. On the basis of the antagonist action of valinomycin, we showed above that this seems unlikely at least for quercetin concentrations lower than 20 μM. A more tenable interpretation of the steepness of the relationship between growth and K^+ current inhibition is that the cell proliferation was assayed here in media containing a FCS concentration (1 %) that does not maximally stimulated mitogenesis in the absence of quercetin. In such conditions, our model, which predicts that a small reduction in K^+ flux is associated with an important growth inhibition [8], can quantitatively take account of the effects of quercetin (Fig. 6). A direct confirmation of this prediction would be given by studying the effects of changing the rate of control cell growth (for instance with various FCS concentrations) on the relationship between cell proliferation and K^+ current inhibition at a constant K^+ channel blocker concentration. However, since quercetin interacts with FCS, this type of experiment should be done with another K^+ channel blocker.

In conclusion, our results strongly suggest that at least a part of the antiproliferative effect of quercetin on neuroblastoma cells is due to a blockade of K^+ channels. They further confirm that K^+ channels do play a role in mitogenesis and support the conclusion that characterization of K^+ channel properties may help our understanding of the mechanisms underlying proliferation of normal and tumour cells [4, 8, 21].

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