Dual Actions of the Voltage-Dependent Potassium Channel Modulators on Cell Proliferation in SH-SY5Y Neuroblastoma Cells

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Voltage-dependent (Kv) and Ca^{2+}-activated (BKCa) K^+ channel modulators are proposed in CNS and peripheral neuromuscular disorders. As recently shown K^+ channel blockers enhance cell viability (Curci et al., 2014). We investigated the actions of 4-aminopyridine (4AP), riluzole (RIL), acetazolamide (ACTZ), resveratrol (RESV), dichlorphenamide (DCP), bendroflumethiazide (BFT) combined with dimethyl sulfoxide (DMSO) on the viability of a human neuroblastoma cell line(SH-SY5Y). We performed cell viability assays by incubating these cells with different concentrations of drugs over a period of 3, 6 and 24h. We also investigated the effects of the application of increasing drug concentrations on Kv/BKCa currents by using patch-clamp technique in whole-cell configuration. To identify the intracellular pathways involved in the regulation of neuronal proliferation by the Kv/BK channel modulators, the activity of two protein kinases, PKA and PKC, was investigated after 6h of incubation in cell lysates treated with the drugs under investigation by ELISA assays.
We observed that the incubation of cells with ACTZ(10^{-7}-$2x10^{-4}$M), BFT(10^{-9}-10^{-5}M), DCP(10^{-12}-$2x10^{-4}$M) and TEA(10^{-6}-10^{-3}M) over a period of 3h induced cell proliferation with almost all tested concentrations and the percentage changes of cell viability was inversely related to drug concentration for ACT, DCP and TEA. RESV(10^{-7}-$2x10^{-4}$M) and 4AP(10^{-10}-10^{-4}M) failed to induce cell proliferation at 3h.
A peak of cell proliferation was observed after 6h with all drugs.
As opposite, the incubation of cells with these drugs over a period of 24h reduced cell viability, with the exception of the lowest tested concentration of 4AP that enhanced it. Therefore all drugs under investigation showed bell-shaped concentration- and time-dependent cell viability relationships.
In contrast, RIL(10^{-6}-10^{-4}M) caused a concentration- and time-dependent decrease of cell viability in all experimental conditions.
DMSO($7x10^{-10}$-$1.5x10^{-1}$%) tested at the percentages corresponding to the amount of DMSO used as a co-solvent, induced a time- and concentration-dependent cell proliferation with a peak of proliferation after 6 h. After 24h we observed cell proliferation at the highest concentrations tested of DMSO($7x10^{-3}$ and $1.5x10^{-1}$%).
The application of ACTZ, BFT, RESV, DCP and 4AP to neurons inhibited the whole-cell K^+-currents at +30mV(Vm) in a concentration-dependent manner, with the exception of DCP for which the percentage of inhibition was reduced by increasing concentration. Otherwise RIL induced a concentration-dependent activation of K^+-currents at +30mV. We analyzed also the effects of the application of DMSO to SH-SY5Y cells at the same concentrations used in cell viability assays; it decreased K^+-currents recorded at +30 mV. In some cells DMSO activated outward chloride currents.
At 3h and 24 h incubation time we found a linear correlation between cell viability and K^+-currents inhibition for ACTZ(r^2=0.85/0.6) and RESV(r^2=0.98/0.9); DCP instead, showed an inverse correlation (r^2=0.86/0.9). A low correlation was found for BFT. At 24h, we found a linear correlation between cell viability and K^+-current activation for RIL(r^2=0.98).
The PKA/PKC assays showed that all of tested drugs including TEA and DMSO enhanced the activity of these kinases, with the exception of RIL that reduced the activity of PKC. We can conclude that PKA and PKC may participate to the intracellular cascades affected by these drugs that regulate cell viability and K^+-currents in this cell line. ACTZ, BFT and RESV were the most powerful proliferating drugs within 6h of incubation, while RIL was a potent antiproliferative drug. These effects could be related with their inhibitory actions, mostly for ACTZ and RESV, and activatory action for RIL, respectively. Our findings may have relevance in neuromuscular disorders where the Kv/BKCa-PKA/PKC pathways may play a role in cell repair processes.

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