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Full Length Research Paper

The effect of the bioflavonoid quercetin on voltage-gated calcium channels in *Periplaneta americana* Df motoneuron

Mehrdad Roghani[1]*, Mohammad Reza Vaez Mahdavi[1] and Tourandokht Baluchnejadmojarad[2]

[1]Department of Physiology, School of Medicine, Shahed University, Tehran, Iran.
[2]Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

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The effect of the natural bioflavonoid quercetin on voltage operated calcium channels was investigated in American cockroach (*Periplaneta americana*) fast depressor motoneuron (Df) using electrophysiological and pharmacological methods. For blockade of outward potassium currents, tetraethylammonium and cesium leakage from microelectrode tip was used. Under this condition and application of command potentials, current-potential relationship was obtained in the absence and presence of quercetin (10 µM). Meanwhile, frequency and amplitude of action potentials (calcium spikes) under current clamp were also determined. In the presence of quercetin, inward calcium current showed a significant increase ($p<0.05$-0.01) in the potential range of -40 to 0 mV as compared to vehicle. Meanwhile, the frequency of calcium spikes in the presence of quercetin significantly increased relative to vehicle ($p<0.01$) and quercetin did not significantly change their amplitude. On the other hand, it caused a significant reduction of afterhyperpolarization (AHP) ($p<0.05$). Quercetin also shifted the voltage dependence of the inactivation and activation curves to more negative potentials and caused a signicant increase in the slope of activation, which reflects a yield of more current for a given potential. Quercetin through augmentation of inward calcium currents (Ica, L), increasing frequency of calcium spikes, and reduction of AHP amplitude could increase excitability and firing of Df motoneurons and this may be of benefit in those brain diseases in which neuronal excitability is depressed.

Key words: Quercetin, calcium channel, electrophysiology, *Periplaneta americana.*

INTRODUCTION

Voltage-gated calcium channels are responsible for calcium influx and regulate intracellular processes such as neuronal excitability and neurotransmitter release. Structurally, they are members of a gene superfamily of transmembrane ion channel proteins that also include voltage-gated potassium and sodium channels (Borjesson and Elinder, 2008; Catterall, 1995). In humans, mutations in calcium channel genes have been linked to a number of serious neurological disorders. These channels are also considered as a potential target for the treatment of various neurological disorders, such as epilepsy, insomnia, and neuropathic pain and for this reason, drugs capable to affect these channels may be of therapeutic value (Cain and Snutch, 2011; Liao et al., 2009). Quercetin, a natural polyphenolic compound (bioflavonoid), is a strong antioxidant and radical scavenger which is abundant in edible fruits and vegetables and also in beverages (that is, tea and red wine) (Boots et al., 2008). Interest in dietary phenolics has increased greatly, owing to their possible beneficial implications in human health, such as in the treatment and prevention of cancer, cardiovascular diseases and other pathologies (Crozier et al., 2009).

Quercetin has been shown to possess biological properties responsible for its beneficial effects on the cardiovascular system. In this regard, its administration to spontaneously hypertensive rats has been shown to produce antihypertensive effect (Perez-Vizcaino et al., 2009). Until now, the effect of this flavonoid on the

*Corresponding author. E-mail: mehjour@yahoo.com. Fax: 00982188966310.

electrophysiological properties of Ca^{2+} channels in the cardiovascular system has been much investigated and a decreased transmembrane Ca^{2+} influx (Formica and Regelson, 1995; Morales and Lozoya, 1994) was indicated as a possible mechanism for its vasodilator effect. Although quercetin could increase Ca^{2+} currents (I_{Ca}) in clonal rat pituitary GH4C1 cells (Summanen et al., 2001) and in rat pituitary tumor GH(3) cells (Wu et al., 2003), but in neuronal NG108-15 cells, it attenuates such currents (Wu et al., 2003). Considering these differntial and contrasting effects in mind and since its exact effect on the neurons has not been well-delineated yet, therefore, the aim of this study was to investigate the effect of quercetin on voltage-dependent Ca^{2+} channels in *Periplaneta americana* Df motoneuron to elucidate more how quercetin modulates neuronal I_{ca}.

MATERIALS AND METHODS

All experiments were performed on the fast metathoracic coxal depressor Df motor neuron of adult male cockroaches (*P. americana*) that were kept in colonies within cylindrical containers and all experiments were carried out at room temperature (22 to 24°C). Animals (n = 37) were immobilized in a cold box, pinned, decapitated, dissected dorsally, the mesothoracic and metathoracic ganglia and the first three abdominal ganglia were dissected out and then the metathoracic ganglion was desheathed for electrophysiological recording in a recording chamber with a volume of about 2 ml. Experiments were performed in circulating oxygenated saline containing (in mM) (Mills and Pitman, 1997): 214 NaCl, 3.1 KCl, 9 CaCl2, and 10 N-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer, pH adjusted to 7.2, with a osmolality of about 281 m*Osm/L*. A few drops of saturated neutral red were applied to the desheathed ganglion for about 10 min to make the cell bodies stain pink. This has been proved not to affect obviously the activity of the cell (Washio, 2002). This aided easy observation and penetration of the neuron somata with microelectrodes. For experiments using a barium saline, CaCl2 was replaced with equimolar BaCl2. Oxygenation system was designed in such a way that it mixed and diluted used agents before they reached the preparation. Concentrations were expressed as final values attained after agents had mixed in the experimental chamber. Final concentration of quercetin in the chamber (optimum concentration) was 10 micromolar, as determined by previous studies on its effect in isolated cells (Saponara et al., 2002). Quercetin was dissolved in Dimethyl sulfoxide (DMSO) as vehicle.

Df somata were penetrated by two thin-walled, fiber-filled borosilicate glass microelectrodes (WPI, USA). Microelectrodes for current recording experiments contained 2 M potassium acetate and had a resistance of 12 to 20 MΩ. For voltage-clamp recordings, the microelectrodes were filled with 2 M cesium chloride; the voltage electrode (for monitoring membrane potential had a resistance of 8 to 15 MΩ, whereas the current electrode (used to apply current) had a resistance of 6 to 8 MΩ. Outward potassium currents were blocked by applying 50 mM tetraethylammonium chloride (TEA, Sigma) to the saline and by the leakage of cesium ions into the cell from the microelectrodes. This leakage of cesium ions was facilitated by applying a train of positive pulses. Current and potential were monitored using TEC-05 amplifier (NPI Instruments, Germany). Data from all experiments were digitized using AD/DA PCI-6024e board (National Instruments, USA), captured and analyzed using Cell works software (NPI Instruments, Germany). This system provided a sampling frequency of 8 to 10 kHz, which was optimized automatically by the software. All statistical data were presented as means ± SEM. For statistical analysis, paired and unpaired t-tests were used. A statistical p value less than 0.05 was considered significant.

Chemicals

Neutral red, TES, barium chloride, quercetin, potassium acetate, cesium chloride and TEA were purchased from Sigma Chemicals (USA). All other chemicals were procured from Merck, Germany.

RESULTS AND DISCUSSION

Inward currents were measured using two-electrode voltage-clamp technique and with minimized outward potassium currents. The resting membrane potential recorded in these experiments was -68.5 ± 0.9 mv (n = 51). When Df soma was depolarized from a holding potential of -70 mV, which was near its normal resting membrane potential, to progressively more positive membrane potentials, inward currents were elicited in saline containing either 9 mM calcium chloride or in saline in which equimolar barium chloride had replaced the calcium chloride. The related current-voltage (I-V) relationship for saline containing calcium and for saline containing barium was shown in Figure 1. In our study, I-V relationship before quercetin addition showed that maximum I_{ca} (-125.6 ± 7.1 nA) occurred at a potential of about +10 mV and it reached to zero at a potential of -56.8 mV. After addition of quercetin at a concentration of 10 µM, it shifted markedly downward and leftward at negative potentials (that is, to more negative potentials) and shifted less markedly downward and rightward at positive potentials (ithat is, to more positive potentials) and the existing difference before and after addition of quercetin was significant at a potential range of -40-0 mV ($p<0.05$ to 0.01) (Figure 2). Quercetin also shifted the voltage dependence of the inactivation and activation curves to more negative potentials and caused a signicant increase in the slope of activation, which reflects a yield of more current for a given potential (data not shown).

Following depolarizing of Df motoneuron soma under current clamp, about 1 to 2 h after dissection and isolation, action potentials were observed. These spikes had a threshold between about -55 and -45 mV (with an average of -49.1 mV) with their final potentials at an average level of -36.5 mV. Addition of nifedepine (50 µM) and cadmium (100 µM) nearly abolished these spikes, indicating their dependency on inward calcium currents. Addition of quercetin at its optimum concentration (10 µM) significantly increased calcium spikes frequency from 17.4 ± 1.5 to 28.9 ± 2.1 Hz ($p<0.01$), with no significant change in their amplitude. In addition, quercetin application significantly decreased AHP amplitude from an average of 5.31 mV to 3.89 mV under current-clamp condition ($p<0.05$). In our study, quercetin increased

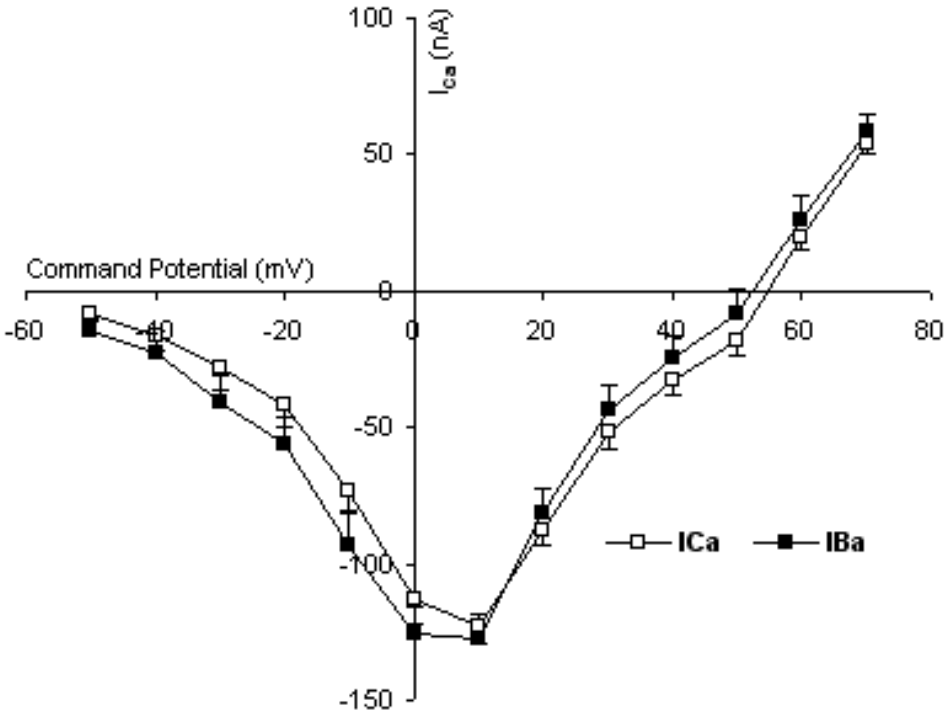

Figure 1. Calcium and barium currents recorded from Df motoneuron (n = 11). These currents were elicited by stepping from a holding potential of -70 mv to the indicated command potential.

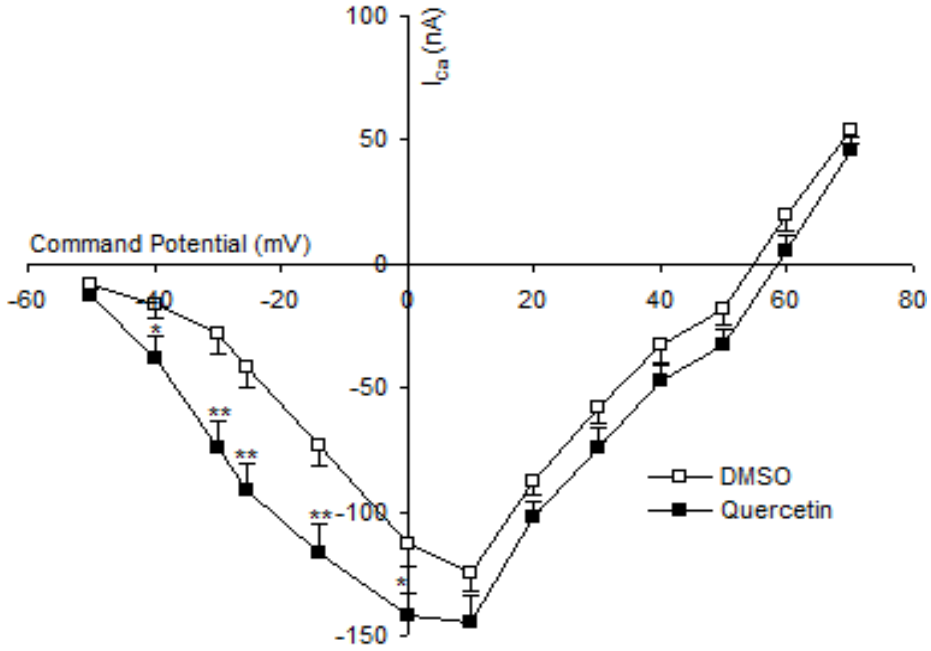

Figure 2. Current voltage relationship for calcium currents recorded from Df motoneuron in the absence (DMSO) (n=10) and presence of quercetin (n=12). These currents were elicited by stepping from a holding potential of -70 mv to the indicated command potential. * $p<0.05$, ** $p<0.01$ (versus DMSO).

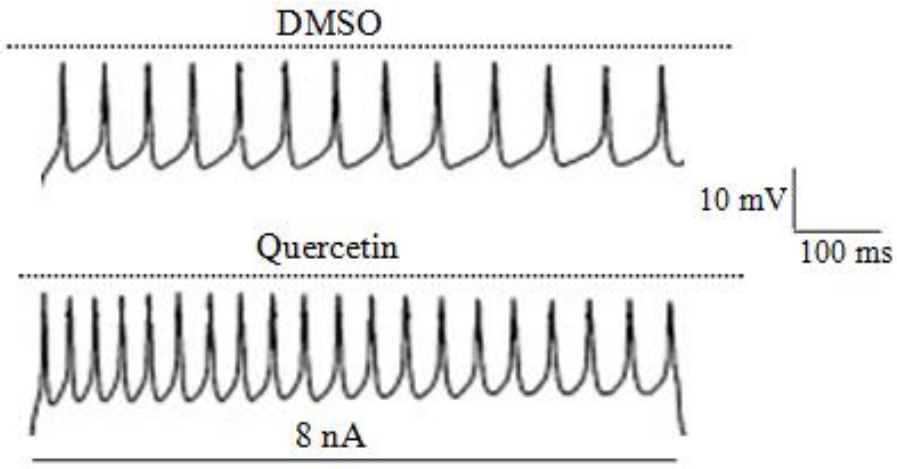

Figure 3. Effect of quercetin on calcium spikes of Df motoneurons under current clamp. Final concentration of quercetin was 10 µM.

(Figure 3) inward calcium current (I_{ca},L) and frequency of calcium spikes, did not significantly change their amplitude, and reduced AHP. This indicates that it may have acted as an activator of L-type Ca^{2+}-channels in Df motoneuron. The calcium current in the soma of Df has been known to consist of at least two different components: one that activates positive to -60 mV, peaks between -10 and 0 mV, and is preferentially blocked by nifedipine, and another component that activates positive to -50 mV, peaks between -10 and -20 mV, and is inhibited by cadmium (Mills and Pitman, 1997). It is possible that more than one conductance may underlie each component, especially the cadmium-sensitive component, because cadmium has been shown to block a number of high-threshold calcium channels in preparations from other organisms. The calcium current in Df motor neuron is less sensitive to nifedipine, and displays steady-state inactivation at more negative membrane potentials than a typical vertebrate L-type current (Mills and Pitman, 1997).

The soma of Df can display both calcium-dependent action potentials and calcium-dependent plateau potentials. Plateau potentials can be recorded immediately after dissection of the central nervous system (CNS), whereas action potentials can normally only be observed at periods 2 to 4 h after isolation (Mills and Pitman, 1997), which was also observed in our study. The plateau potential can be blocked by nifedipine but not by micromolar cadmium, whereas the action potentials are only blocked by a combination of both drugs, suggesting that the nifedipine-sensitive current underlies the plateau potential and that both the nifedipine sensitive and cadmium-sensitive currents contribute to the spikes (Mills and Pitman, 1997). Although quercetin increased calcium current in Df motoneuron in our study, but this property contradicts with its well-known vasodilatory effect in the vascular system (Formica and Regelson, 1995; Roghani et al., 2004), which is partly attributed to its inhibition of calcium channels. In agreement with our results, Summanen et al. (2001) demonstrated that quercetin could increase calcium current in clonal rat pituitary GH4C1 cells, possibly via a cyclic amplifier (AMP)-induced activation of protein kinase A (Summanen et al., 2001). There is also some evidence indicating that stimulation of L-type calcium current in the presence of quercetin in some preparations is not mediated by diffusible intracellular factors but is rather the consequence of its direct interaction with the channel proteins (Wu et al., 2003). In this study, quercetin induced a peak current enhancement which was greatest at weak depolarization and became progressively smaller with increasing depolarization, and shifted the maximum of the current-voltage relationship towards more negative potentials.

The effect of quercetin on the current-voltage relationship might be the consequence of the hyperpolarizing shift in the activation curve. Quercetin also shifted the voltage dependence of the inactivation and activation curves to more negative potentials and caused a signicant increase in the slope of activation, which reflects a yield of more current for a given potential. These results indicate that quercetin may alter the voltage sensitivity of the channel (data not presented). The effect of the flavonoid quercetin on voltage-gated I_{Ca},L in rat pituitary GH3 cells has also been investigated using whole-cell voltage-camp technique (Wu et al., 2003). In this regard, quercetin stimulated I_{Ca},L in a concentration-dependent manner and under current-clamp, quercetin could increase actions potentials frequency (Wu et al., 2003) which is also consistent with our results. Conversely, in NG108-15 neuronal cells, quercetin suppresses the amplitude of I_{Ca},L (Wu et al., 2003). It has also been reported that quercetin-mediated stimulation of I_{Ca},L in GH3 cells is

presumably not associated with intracellular level of cyclic AMP, or with the activity of tyrosine or phosphoinositide 3-kinases (Wu et al., 2003). Therefore, the effect of quercetin on ion currents in neurons may, at least, be contributed to the underlying mechanisms through which it affects neuronal function and excitability (Wu et al., 2003). Taken together, quercetin through augmentation of inward calcium currents and increasing frequency of calcium spikes and reduction of AHP amplitude could increase excitability and firing of Df motoneurons and this may be of benefit in those brain diseases in which neuronal excitability is depressed.

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