Tohoku J. Exp. Med., 2005, **206**, 141-150

Suppressive Effects of Red Wine Polyphenols on Voltage-Gated Ion Channels in Dorsal Root Ganglionic Neuronal Cells

YAN L. WU,[1,2] ATSUSHI OHSAGA,[1] TAKAKO OSHIRO,[1] KAZUIE IINUMA,[2] YOSHIAKI KONDO,[2] SATORU EBIHARA,[3] HIDETADA SASAKI[3] and YOSHIO MARUYAMA[1]

[1]Department of Physiology, [2]Department of Pediatrics, and [3]Department of Geriatric and Respiratory Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

WU, Y.L., OHSAGA, A., OSHIRO, T., IINUMA, K., KONDO, Y., EBIHARA, S., SASAKI, H. and MARUYAMA, Y. *Suppressive Effects of Red Wine Polyphenols on Voltage-Gated Ion Channels in Dorsal Root Ganglionic Neuronal Cells*. Tohoku J. Exp. Med., 2005, **206** (2), 141-150 —— Polyphenols are ubiquitous plant metabolites with multiple pharmacological properties. Using whole-cell patch-clamp current recording techniques, we studied the effects of polypnenols extracted from red wine (purity > 90% from Cabernet Sauvignon grape wine) on the activities of voltage-operated Na^+-, K^+-, and Ca^{2+}-channel currents in mouse dorsal root ganglionic neuronal cells. The polyphenols suppressed all of the channel activities with half-effective concentrations of about 2.5, 4.0, and 0.8-1.5 μg/ml, respectively. In contrast, they showed no noticeable effects on the ion channels in other types of cells, including large conductance K^+-channels in mouse lacrimal acinar cells. Thus, the polyphenols suppress firings of the action potential in the neuronal cells and could show a sedative effect on the excitation. We expect that red wine can be used as a remedy for excessive sensory stimuli ——— red wine; polyphenols; dorsal root ganglion; voltage-dependent ion channels; neuronal excitation

Polyphenols or phenolics are a class of multi-potent compounds with a wide spectrum of pharmacological and biological properties. They are ubiquitously present in all plants and are abundant in tea and red wine (Bravo 1998). They act as antioxidants (Frankel et al. 1993; Corder et al. 2001; Etus et al. 2003) and anticancer reagents (Soleas et al. 1997; Bradlow et al. 1999; Yang et al. 2001), inhibit cell kinase activity (Soleas et al. 2002), evoke vasodilatation, and reduce endothelin synthesis. The latter two properties are thought to account for the so-called "French paradox" (Renaud et al. 1992), the widely known benefit of red wine. The major constituents of polyphenols are quercetin, resveratol, D,L-catechin, and D,L-epicatechin, and additional anthocyanins particularly in red wine. Among the multiple actions of polyphenols, an individual constituent can act on a variety of cell functions including the ion channel activities. That is, quercetin activates Ca^{2+}-releasable channels located in skeletal muscle sarcoplasmic reticulum (Ferriola et al. 1989)

Received March 1, 2005; revision accepted for publication March 23, 2005.
Correspondence: Yoshio Maruyama, Department of Physiology, Tohoku University Graduate School of Medicine, Seiryomachi 2-1, Aoba-ku, Sendai 980-8575, Japan.
e-mail: ymaruyama@cellphysiol.med.tohoku.ac.jp.

and L-type Ca^{2+}-channel current in smooth muscle cells (Saponara et al. 2002), resveratol stimulates the large conductance Ca^{2+}-activated K^+-channels in endothelial cells (Granados-Soto et al. 2002) and inhibits Ca^{2+}-fluxes in blood platelets (Dobrydneva et al. 1999), and catechins enhance voltage-gated Ca^{2+}-channel currents in chromaffin cells (Pan et al. 2002). These seemingly divergent and multi-directed effects, in which some promote and others demote the channel activities, are so far inexplicable by a unified mechanism but are presumably due to the respective mechanisms ascribed to the individual constituents. The effect of polyphenols in this context may emerge through the combined or integrated effects of these constituents or through an entirely different mechanism unique to polyphenolic compounds. We scarcely know how they affect the ion channel activities.

Using polyphenols extracted from red wine in the present study, we examined their effects on three distinct voltage-operated ion channels, Na^+-, K^+- and Ca^{2+}-channels, in freshly isolated single dorsal root ganglion cells. The red wine polyphenols suppressed or inhibited all the channel activities more or less in a concentration-dependent manner, suggesting their potential for a control of excessive sensory stimuli in clinical use.

Materials and Methods

Cell dissociation

From male albino mice (ddy strain 5 - 7 weeks old), stunned and sacrificed by cervical dislocation, we collected 20 - 30 cervical, thoracic and lumbar dorsal root ganglia by manual dissection under a stereo-microscope. The protocol for animal use was approved by the Animal Care and Use Committee of Tohoku University Graduate School of Medicine. After a minor cut on the individual ganglia, we transferred them into a small container filled with L-15 solution (1.5 ml) containing 3 mg/ml collagenase (Wako, Tokyo). We kept the container at 37°C for 25 min in a temperature-controlled heating bath. After incubation, we dispersed the preparation into single cells by passing them through a thin plastic tube several times. We subsequently washed the cells with L-15 solution three times to prepare them for the current measurement. By this procedure we obtained a number of single cells of various sizes. The smallest single dorsal root ganglion (DRG) neuronal cells, with a diameter of about 30 - 40 μm without axon attachments were used for patch-clamp whole-cell recordings.

Solutions

The external solution used for sodium channel current measurements contained (in mM) 120 NaCl, 25 TEA-Cl (tetraethylammonium-Cl), 4 KCl, 2 $MgCl_2$, and 5 Tris (Tris [hydroxylmethyl] aminomethane), and the pipette solution contained (in mM) 125 Cs-glutamate, 25 TEA-Cl, 2 $MgCl_2$, 5 Tris, 0.5 ATP, and 1 BAPTA (1,2-Bis [2-aminophenoxy] ethane-N,N,N',N'-tetraacetic acid tetrapotassium salt; a Ca^{2+}-chelator). The external solution used for potassium channel current measurements contained (in mM): 140 NMDG-Cl, 4 KCl, 2 MgCl2, 5 Tris, and the pipette solution contained (in mM): 145 K-glutamate, 2 $MgCl_2$, 5 Tris, 0.5 ATP, and 1 BAPTA. The external solution used for calcium channel current measurements contained (in mM) 80 NMDG-Cl (N-methyl D [-]-glucamine-Cl), 25 TEA-Cl, 20 $BaCl_2$, 4 KCl, 2 MgCl2, and 5 Tris; and the pipette solution contained (in mM) 125 Cs-glutamate, 25 TEA-Cl, 2 $MgCl_2$, 5 Tris, 0.5 ATP, and 1 BAPTA. The pH of all these solutions was adjusted to 7.3 by HCl. These solutions were designed to distinguish among individual different channel currents.

Electrical measurements

We used standard patch-clamp whole-cell current recording techniques (Hamill et al. 1981; Maruyama et al. 1983a,b) for the measurements of ion channel currents from a single DRG neuronal cell. In brief, patch pipettes, fabricated using a two-stage vertical puller (PP-83, Narishige, Tokyo), had a resistance of 2 - 5 MΩ when filled with the pipette solution. We discarded cells with axons and selected small single cells to keep the space clamp condition optimal. We did not compensate the leakage currents, which were negligibly small, for the estimation of the effect of polyphenols. The whole-cell recordings of DRG cells were remarkably stable and the access conductance was over 80 nS throughout the experiments that lasted more than 40 min.

Using the internal (pipette) and external solutions described in the previous section, we could separate each of the voltage-operated Na^+-, K^+-, or Ca^{2+}-channel currents from others. All of the above currents lasted undiminished more than 50 min without noticeable changes in their size and time course, and no possible "channel rundown" was prominent in the present experiments.

We clamped the DRG cells at -80 mV and activated the channels by applying positive voltage-steps. The activation was repeated every 1 min which was enough time for the recovery of the channels from their inactivation. Currents were measured with a patch-clamp amplifier (EPC-7, HEKA, Gettingen, Germany), low-pass filtered at 500 Hz, digitized at 2 kHz, and fed into a built-in computer. All the experiments were carried out at room temperature (22°C).

Red wine polyphenols

Polyphenols or phenolics used in the present study were extracted from red wine made of Cabernet Sauvignon grapes (red wine polyphenols, RWP, supplied by Mercian, Tokyo), and its purity as quantified by the Folin-Ciocateu method was over 90% (83.3% Gallic acid equivalent).

Application of RWP

The concentrated RWP (20 times the final bathing concentration) dissolved in the external solution was added sequentially to the edge of the recording chamber at a volume of 1.5 ml (sequential bath application). Based on the added volume of the RWP solution and the final bathing volume, we calculated the chamber RWP concentration. The added RWP spread over the entire chamber within 3 min. Waiting 3 - 5 min, we started the current measurements. Repeating the procedure, we completed a set of experiments within roughly 40 min. We affirmed beforehand in several experiments that the above application gave the same results as those obtained from a bath perfusion with pre-determined RWP concentrations. From this type of experiments, we found that 50% recovery from the RWP effects took 10 - 20 min with the control perfusion after cell exposure to high RWP concentrations. Thus, we adopted the sequential bath application in most cases to estimate the RWP effects in a wide range of concentrations using the same cell with patch-clamp whole-cell recordings.

Results

RWP suppressed all voltage-operated Na^+-, K^+-, and Ca^{2+}-channel currents in the DRG neuronal cells in a concentration dependent manner, the details of which are described together with the relevant experiments. The suppression developed steadily with increasingly higher RWP concentrations. We evaluated the mode of suppression in terms of the current amplitude relative to the control as I_{RWP}/I_C, where I_{RWP} and I_C represent the current amplitude with or without RWP, respectively.

Inhibition of voltage-gated Na^+-channel current

We studied the effects of RWP on the voltage-operated Na^+-channel currents with a solution designed to isolate them from the K^+- and Ca^{2+}-channel currents (see, Method, solutions). Fig. 1A shows the current traces evoked by voltage-steps to -40, -20, and -10 mV from the holding potential of -80 mV with RWP concentrations of 0, 1.0, 2.0, and 3.0 μg/ml, respectively. Fig. 1B depicts the peak amplitudes of the individual Na^+-current plotted against the membrane potential (the current/voltage diagram, I/V-curve). The maximum point in the I/V-curves was attained at -10 mV and the curves seemed to reduce in size progressively with increasingly higher concentrations of RWP. When we examined the individual current trace carefully, we found that RWP decreased the current without changing its time course. That is, currents at the same voltage with or without RWP were super-imposable when an appropriate scaling factor for the amplitude axis was applied. Fig. 2A shows that the current trace with RWP (2 μg/ml) coincided with the control trace with an enlargement factor of 2. Such similarity in shape emerged in all the other combinations of traces with different RWP concentrations. The result suggests that RWP has no effect on the gating of the Na^+-channel current but decreases its size presumably by a reduction of either the effective number of channels in the membrane or the single channel current amplitude.

The Na^+ current reduction evaluated at -10 mV provided an estimate of the half-maximal concentration for RWP-induced inhibition (IC50), and of about 2.5 μg/ml (Fig. 2B).

Inhibition of voltage-gated Ca^{2+}-channel current

We studied the effects of RWP on the voltage-gated Ca^{2+}-channel currents with a solution designed to isolate them from the Na^+- and K^+-channel currents (see, Method, solutions). We used Ba^{2+} as permeant ions. DGR neuronal cells

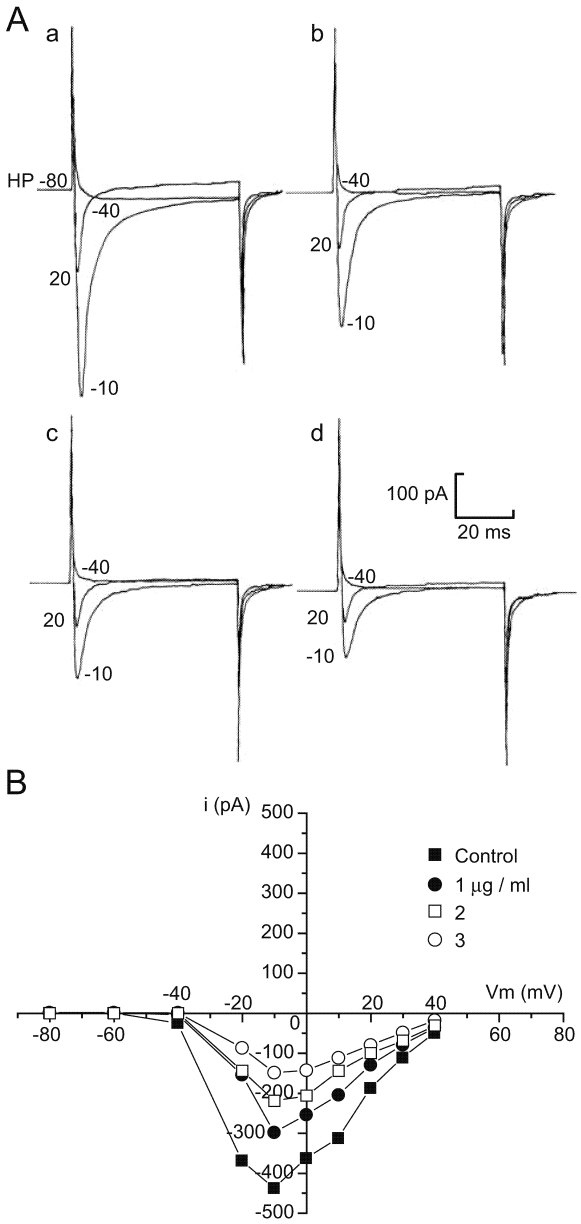


Fig. 1. Effect of red wine polyphenols (RWP) on whole-cell Na^+-channel current in single DRG neuronal cells. A: superimposed current traces at different voltages with or without RWP. The currents were elicited by voltage-steps to -40, -10, and 20 mV from the holding potential of -80 mV. The pulses were applied at 60 s intervals. The final bathing concentrations of RWP were, 0, 1, 2, and 3 μg/ml, for *a, b, c,* and *d,* respectively. B: Current/voltage diagram of the peak Na^+ currents with or without RWP. The data points were obtained from the records represented in A.

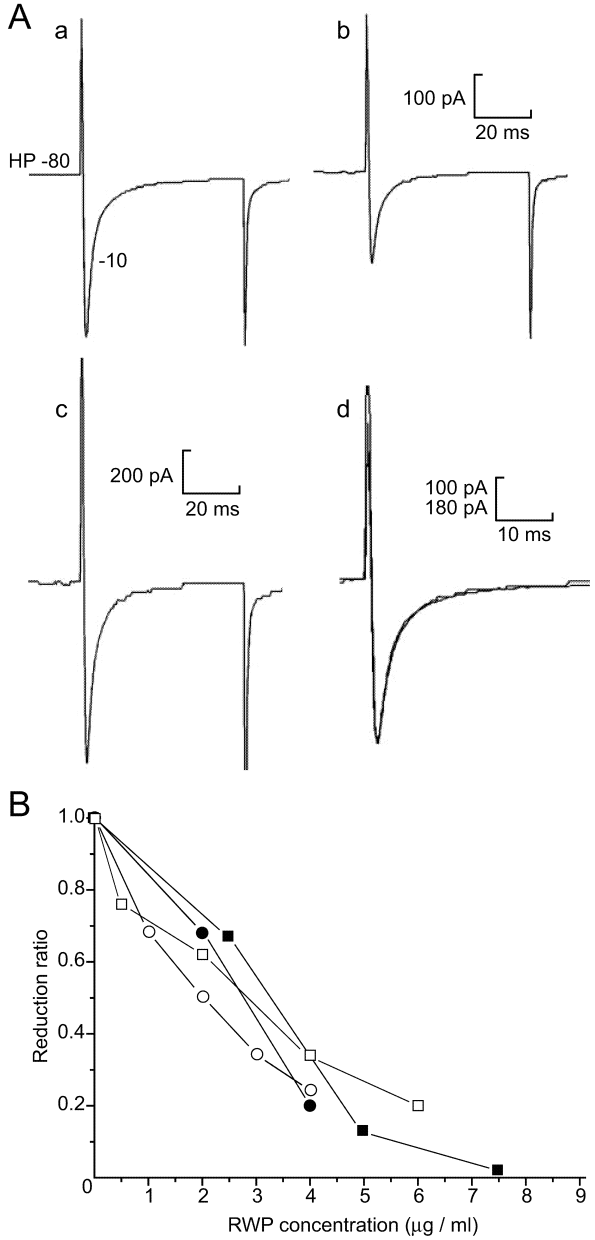


Fig. 2. Mode of RWP action on voltage-operated DRG Na^+-channel current. A: reduction in size of the Na^+-current with RWP. The Na^+-current at -10 mV with 2 μg/ml-RWP (b) was enlarged by a scaling factor of 2 in the amplitude axis (c) and superimposed on the trace without RWP (a, control; d, superposition). B: Current reduction rate in terms of the RWP concentration. The peak Na^+ current at -10 mV was evaluated. The IC50 was about 2.5 μg/ml.

have been reported to possess several Ca^{2+}-channels such as L, N, P/Q, R and T types (Hille 2001) according to Tsien type classification. However, the present study was only concerned with a coarse classification including high- and low-threshold voltage-activated Ca^{2+}-channel currents (HVA and LVA). Our DRG neuronal cells contained these two channels to various degrees, some exhibiting predominantly HVA and others a mixture of the two. We separated each current by voltage-pulses at either high or low voltage levels.

We determined beforehand that the Ba^{2+}-currents lasted undiminished for more than 50 min without noticeable changes in the whole-cell recordings. Fig. 3A represents either LVA, characterized by low activation voltages and rapid decay, and HVA with by high activation voltages and slow decay. The currents evoked by either -40 or 20 mV were unchanged for 50 min and they responded to the subsequent application of RWP (Fig. 3B). Thus, no possible "rundown" of Ca^{2+}-channel currents (Hille 2001) was prominent in our experiments.

Fig. 4A shows the differential effect of RWP on the two types of Ba^{2+} currents simultaneously appearing in single DRG cells, in which 1 μg/ml-RWP completely abolished LVA evoked at -40 mV but not HVI evoked at 0 mV. The abolishment of HVI required a higher RWP concentration (3 μg/ml-RWP), indicating that HVL is less sensitive to RWP.

In some cells which apparently manifested only HVA (Fig. 4B), 1 μg/ml-RWP diminished an initial current notch that could presumably be ascribed to the N-type HVA activation. This effect was prominent at 0 mV where the notch was completely lost (Fig. 4B*b*). Further increases in the RWP concentration progressively eliminated the notches with a concomitant reduction of the steady-state currents supposedly composed of L-type HVA (Fig. 4B*c* and *d*). This indicated that N-type HVA (the current responsible for the notches) is more sensitive to RWP than L-type HAV. The presence of two HVA currents and their differential sensitivity to RWP are reflected by the distorted HVA I/V-curves shown in Fig. 4C, in which the potential level providing the maximal amplitude shifted to a positive direction from 0 to 20 mV as the RWP concentration increased.

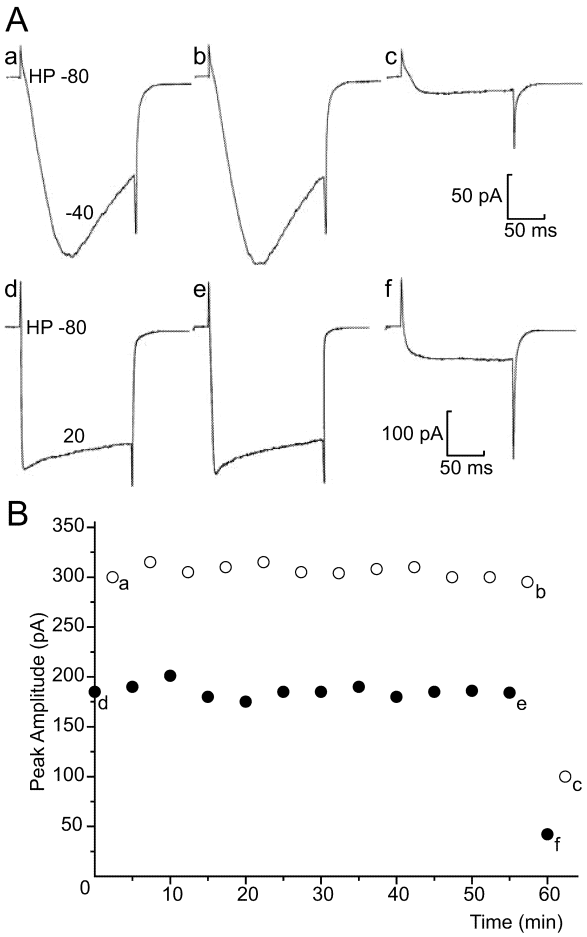
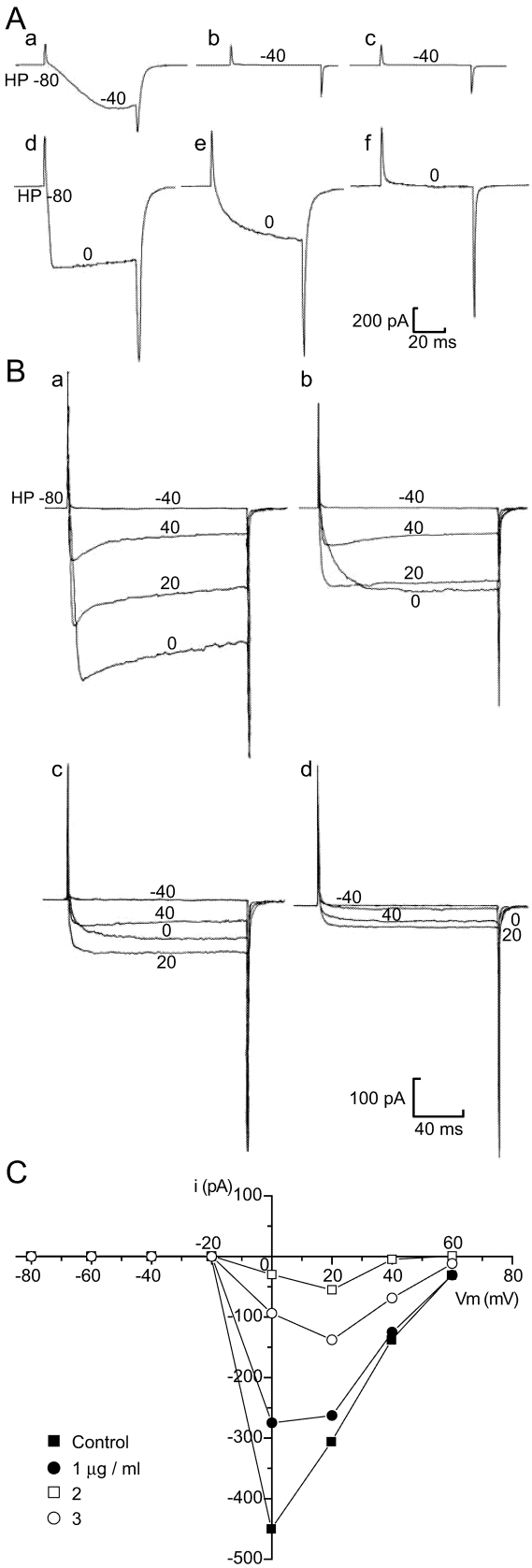


Fig. 3. Absence of "rundown" in Ca^{2+}-channel currents in DRG neuronal cells. A: Low and high voltage activated Ba^{2+} currents monitored in the same cell at intervals of 2.5 min for 50 min before the application of 2 μg/ml-RWP. LVA was elicited at -40 mV (upper traces), and HVA at 20 mV (lower traces). B: Peak Ba^{2+}-current plotted against time in whole-cell recording configuration. The currents corresponding to *a* to *f* in A are indicated in the diagram. Filled circles represent the peak LVA currents, and open circles those of HVA currents. No clear rundown was detected and the current responded to the application of RWP 60 min after the establishment of the whole-cell recording configuration.

The Ba^{2+}-current reduction evaluated at -40 mV and at 0 mV provided an IC50 of roughly 0.8



for LVA and 1.5 μg/ml for HVA (Fig. 5).

Inhibition of voltage-gated K^+-channel current

We studied the effects of RWP on the voltage-gated K^+-channel currents with a solution designed to isolate them from the Na^+- and Ca^{2+}-channel currents (see, Materials and Methods, *solutions*). RWP decreased the voltage-gated K^+-channel currents in a concentration dependent manner. At low concentrations, RWP abolished an initial upheaval notch of the current (Fig. 6A*b*), and at high concentrations it induced the reduction of the stationary current amplitude in a concentration-dependent manner (Fig. 6A*c* and *d*). The latter feature was reflected in the I/V-curves (Fig. 6B), evaluated at the end of the voltage-pulse. Several types of K^+-channel currents, including transient and sustained types, have been demonstrated in DRG neuronal cells (Gold et al. 1996). The differential sensitivity of these channels to RWP emerged in the changes in the time course of the currents, in which the transient type was more sensitive.

The K^+-current reduction evaluated at 60 mV provided an IC50 of about 4 μg/ml (Fig. 6C).

Overall current responses to RWP in DRG neuronal cells

We carried out a series of experiments to survey the overall current response, the simultaneously appearing Na^+-, Ca^{2+}-, and K^+-currents in

Fig. 4. Effects of RWP on low and high voltage activated Ca^{2+}-channel currents (LVA and HVA) in DRG neuronal cells. A: RWP effect on LVA evoked at -40 mV (*a,b,c*) and HAV at 0 mV (*d,e,f*) in the same DRG cell, where *a* and *d* represent control traces without RWP, *b* and *e* with 1 μg/ml-RWP, and *c* and *f* with 3 μg/ml-RWP. B: RWP effect on HAV elicited at several voltage levels. Currents elicited at 2 min intervals by each voltage-step are represented in the superposition. RWP concentrations were 0, 1, 2, and 3 μg/ml for *a, b, c,* and *d,* respectively. C: Current/voltage diagram (I/V-curve) of HVA. The data were collected from the traces shown in B. The maximum currents, either at the peak if detected or at the pulse-end, were plotted against the membrane potential.

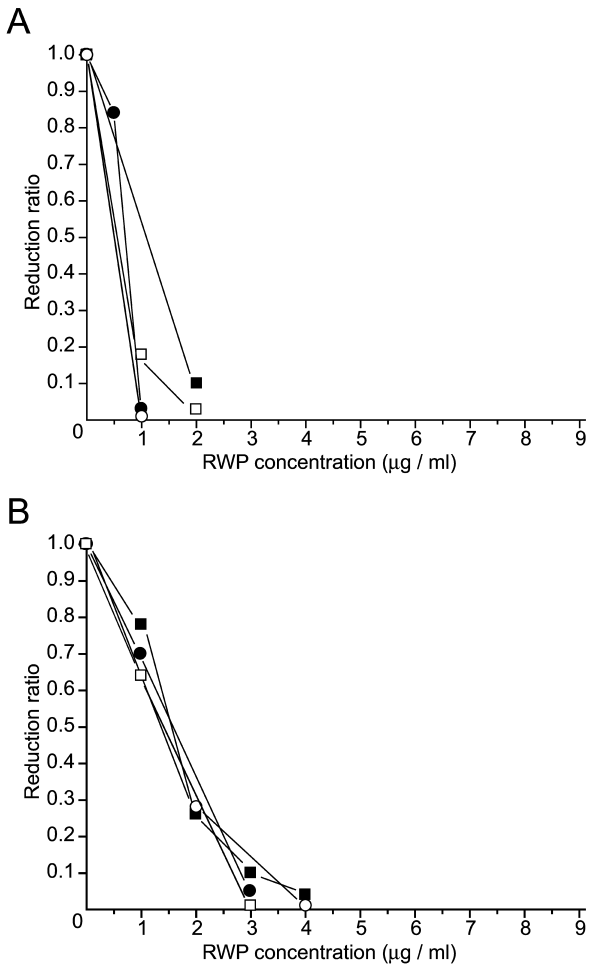


Fig. 5. The RWP-induced current reduction of the voltage-activated Ca^{2+}-channel currents in DRG neuronal cells. A: RWP effect on LVA elicited at -40 mV. The IC50 of LVA was about 0.8 μg/ml. B: RWP effect on HVA elicited at 0 mV. The IC50 of HVA was about 1.5 μg/ml.

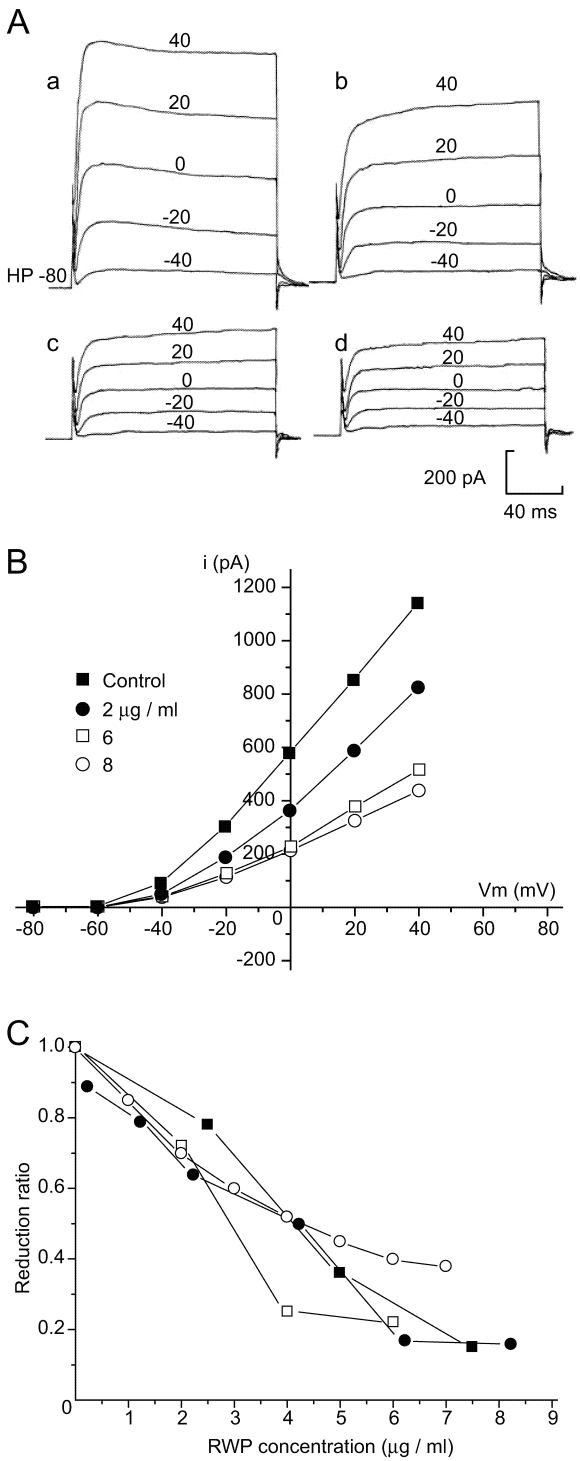


Fig. 6. Effects of RWP on voltage-operated K^+-channel currents in single DRG neuronal cells. A: The K^+ current traces in superposition with or without RWP. The currents were elicited by 20 mV voltage-step to -40, -20, 0, 20, and 40 mV at 60 s intervals from the holding potential of -80 mV. The final bathing concentrations of RWP were, 0, 2, 6, and 8 μg/ml for a, b, c, and d, respectively. B: Current/voltage diagram of the K^+ currents with or without RWP. The maximum current amplitude in A, either at the peak if detected or at the pulse-end, was plotted against the membrane potential. C: The current reduction at 40 mV by RWP plotted against the concentration. The IC50 was about 4 μg/ml.

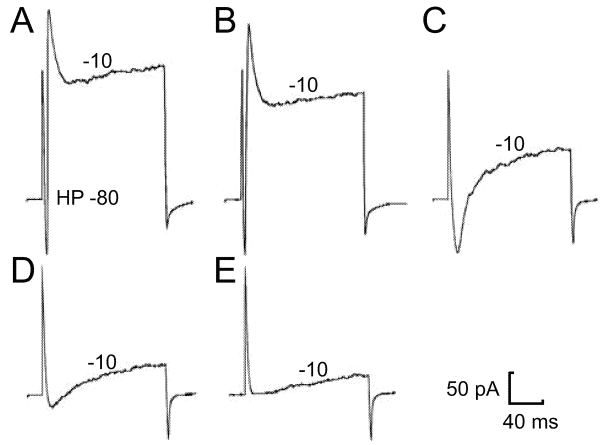


Fig. 7. Overall current response to RWP in single DRG neuronal cells. The external solution contained (in mM): 130 NaCl, 5 $CaCl_2$, 4 KCl, 2 $MgCl_2$, 5 Tris (Tris(hydroxylmethyl)aminomethane), and the pipette solution contained (in mM): 140 K-glutamate, 2 $MgCl_2$, 5 Tris, 0.5 ATP, 5 BAPTA. The currents elicited at -10 mV were monitored. The RWP concentrations in each trace were 0, 2, 3, 5, and 7 μg/ml for A, B, C, D, and E, respectively.

single DRG cells. This determined the contribution of each current and predicted the effect of RWP on the action potential of DRG cells. The solutions used were a normal NaCl solution outside and K-glutamate solution inside (detailed composition in Fig. 7 legend). The cells contained all three channel currents as depicted in Fig. 7A. The initial sharp inward deflection indicated the activation of the voltage-operated Na^+ current, a substantial part of which was counterbalanced by an outward current composed of the K^+ current, and the downward deflection retained in the outward current was the HVA Ca^{2+} current. Increases in the RWP concentration eliminated each current component one after another according to their differential sensitivity (Fig. 7C, 7D, and 7E). The shape of the current at each epoch seemed so complicated that it was difficult to point out which component had been diminished. However, we found that RWP eventually abolished all current components and that a major shape transformation took place during the concentration change from 2 to 3 μg/ml-RWP. In this transition, we can predict that the upstroke of the action potential is diminished concomitant with the reduction of the speed of the repolarization so that the action potential becomes progressively blunt and depressed. On the other hand, the cell maintains the resting membrane potential relatively intact since the K^+-current remained even after the abolishment of the other components (Fig. 7E), which contributed to the cell depolarization. Thus, RWP at 3 μg/ml was enough to evoke a reduction in the excitability of DRG cells.

Effects of RWP on ion channels in other cell types

Based on the results from the neuronal cells, we examined the effects of RWP on ionic currents from other three cell types freshly isolated from mice, the two belonging to epithelial secretory cells and the one to a blood cell group. The examined currents were acetylcholine-induced Ca^{2+}-sensitive currents, composed of non-selective monovalent-cation- and Cl^--channel currents from pancreatic acinar cells (details of the channels and experiments, see, Maruyama and Petersen 1982; Oshiro et al. 2005), large-conductance K^+-channel currents (Maruyama et al. 1983a, b; Trautman and Marty 1984) from lacrimal acinar cells, and delayed-rectifier K^+ currents in megakaryocytes (Ikeda et al. 1992). Under the whole-cell recording configuration, all of these currents were not noticeably affected by the application of RWP (1 - 50 μg/ml) (data not shown). These results suggest that the neuronal cells are particularly sensitive to RWP, but not other cell types.

Discussion

The present study showed that RWP suppressed the voltage-gated Na^+-, K^+-, and Ca^{2+}-channels in DRG neuronal cells with an IC50 of 2.5, 4, and 0.8-1.5 μg/ml, respectively. The above channels can be classified into two subtypes of Na^+-channel currents (Rush et al. 1998) including TTX-sensitive and –resistant currents, six subtypes of K^+-selective channel currents including transient and sustained currents (Gold et al. 1996), and five subtypes of Ca^{2+}-channel currents, T-, L-, N-, P/Q-, and R-type (Fox et al. 1987; Hille 2001). Although we did not separate the currents

to each subtype, RWP consequently suppressed all the currents encountered, presumably containing some of the subtype currents not detected in the present experiments.

Each constituent of RWP has been reported to act on specific ion channels of different cell types; quercetin activated Ca^{2+} releasable channels in sarcoplasmic reticulum (Ferriola et al. 1989), resveratol stimulated the large conductance Ca^{2+}-activated K^+-channels in endothelial cells (Saponara et al. 2002), and catechin enhanced voltage-gated Ca^{2+}-channels in chromaffin cells (Pan et al. 2002). None of these fits with our results. Thus, we can expect that the effect of RWP involves a totally different mechanism since it equally suppressed all the current types in DRG cells. This suggests that an unknown mechanism, not related to the specificity of the each channel type, would be involved in the RWP-induced current suppression. A possible mode, exemplified by its action on the Na^+-channel current (Fig. 2A), would be a reduction in the functional channel number in the plasma membrane, presumably through a modification of the lipid-bilayer or related structure which serves as a common base for all the membrane proteins including ion channels. That is, the functional channels may be sequestrated from the ordinary membrane surface to a specialized region, perhaps in a form of minute vesicles, not affected by the change in the membrane potential in the whole-cell recording assembly.

DRG neuronal cells mediate sensory information from the skin, muscle, and visceral organs. An immediate effect of RWP would be a diminution of the nerve cell excitability. This is reminiscent of the action of anaesthetics or sedative reagents on the nervous system. A report has so far shown that intake of 100 ml of red wine increased the plasma phenolic concentration to about 2.5 μg/ml within 50 min (Duthie et al. 1998). This level is within the range of RWP concentrations, which show the suppressive effects on the neuronal ion channels. Collectively, red wine may have potential as a tranquilizing reagent for sensory stimuli including pain.

Acknowledgments

The study was supported in part by the Grant-in-Aid for Scientific Research (B) (No. 14370241) and Specific Research on Priority Area (B) (No. 12144207) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

Bradlow, H.L., Telang, N.T., Sepkovic, D.W. & Osborne, M.P. (1999) Phytochemicals as modulators of cancer risk. *Adv. Exp. Med. Biol.,* **472**, 207-221.

Bravo, L. (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.*, **56**, 317-333.

Corder, R., Douthwaite, J.A., Lees, D.M., Khan, N.Q., Viseu dos Santos, A.C., Wood, E.G. & Carrier, M.J. (2001) Endothelin-1 synthesis reduced by red wine. *Nature,* **414**, 863-864.

Dobrydneva, Y., Williams, R.L. & Blackmore, P.F. (1999) trans-Resverareol inhibits calcium influx in thrombin-stimulated human platelets. *Br. J. Pharmacol.*, **128**, 149-157.

Duthie, G.G., Pedersen, M.W., Gardner P.T., Morrice, P.C., Jenkinson, A.M., McPhail, D.B. & Steele, G.M. (1998) The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. *Eur. J. Clin. Nut.*, **52**, 733-736.

Etus, V., Altug, T., Belce, A. & Ceylan, S. (2003) Green tea polyphenol (-)-epigallocatechin gallate prevents oxidative damage on periventricular white matter of infantile rats with hydrocephalus. *Tohoku J. Exp. Med.*, **200**, 203-209.

Ferriola, P.C., Cody, V. & Middleton, E. (1989) Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem. Pharmacol.*, **38**, 1617-1624.

Fox, A.P., Nowycky, M.C. & Tsien, R.W. (1987) Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurons. *J. Physiol.*, **394**, 149-172.

Frankel, E.N., Kanner, J., German, J.B., Parks, E. & Kinsella, J.E. (1993) Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*, **341**, 454-457.

Gold, M.S., Schuster, M.J. & Levine, J.D. (1996) Characterization of six voltage-gated K^+ currents in adult rat sensory neurons. *J. Neurophysiol.*, **75**, 2629-2646.

Granados-Soto, V., Arguelles, C.F. & Ortiz, M.I. (2002) The peripheral antinociceptive effect of resverateol is associated with activation of potassium channels. *Neuropharmacology,* **43**, 917-923.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F.J. (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch.*, **391**, 85-100.

Hille, B. (2001) Voltage-gated calcium channels. In: *Ion Channels of Excitable Membrane*. Sinauer Associates Inc. New York, 95-129.

Ikeda, M., Kurokawa, K. & Maruyama, Y. (1992) Cyclic AMP dependent regulation of agonist-induced calcium increases in mouse megakaryocytes. *J. Physiol. (Lond.)*, **447**, 711-728.

Maruyama, Y. & Petersen, O.H. (1982) Cholecystokinin activation of single-channel currents is mediated by internal mes-

senger in pancreatic acinar cells. *Nature*, **300**, 61-63.

Maruyama, Y., Gallacher, D.V. & Petersen, O.H. (1983a) Voltage and Ca^{2+}-activated K^+ channel in basolateral acinar cell membranes in mammalian salivary glands. *Nature,* **302**, 827-829.

Maruyama, Y., Petersen, O.H., Flanagan, P. & Pearson, G.T. (1983b) Quantification of Ca^{2+}-activated K^+ channels under hormonal control in pig pancreatic acinar cells. *Nature,* **305**, 228-232.

Oshiro, T., Takahashi, H., Ohsaga, A., Ebihara, S., Sasaki, H. & Maruyama, Y. (2005) Delayed expression of large conductance K^+ channels reshaping agonist-induced currents in mouse pancreatic acinar cells. *J. Physiology (Lond.)*, **563**, 379-391.

Pan, C.Y., Kao Y.H. & Fox, A.P. (2002) Enhancement of inward Ca^{2+} currents in bovine chromaffin cells by green tea polyohenol extracts. *Neurochem. Int.*, **40**, 131-137.

Renaud, S. & De Lorgeril, M. (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet,* **339**, 1523-1526.

Rush, A.M., Bräu, M.E., Elliott, A.A. & Elliot, J.R. (1998) Electrophysiological properties of sodium channel subtypes in small cells from adult rat dorsal root ganglia. *J. Physiol.,* **511**, 771-789.

Saponara, S., Sgaragli, G. & Fusi, F. (2002) Quercetin as a novel activator of L-type Ca^{2+} channels in rat tail artery smooth muscle cells. *Br. J. Pharmacol.*, **135**, 1819-1827.

Soleas, G.J., Diamandis, E.P. & Goldberg, D.M. (1997) Wine as a biological fluid: history, production, and role in disease prevention. *J. Clin. Lab. Anal.*, **11**, 287-313.

Soleas, G.J., Grass, l., Josephy, P.D., Goldberg, D.M. & Diamandis, E.P. (2002) A comparison of the anticarcinogenic properties of four red wine polyphrnols. *Clin. Biochem.*, **35**, 119-124.

Trautman, A. & Marty, A. (1984) Activation of Ca-dependent K channels by carbamylcholine in rat lacrimal gland. *Proc. Natl. Acad. Sci. USA*, **81**, 280-288.

Yang, C.S., Landau, J.M., Huang, M.T. & Newmark, H.L. (2001) Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu. Rev. Nutr.,* **21**, 381-406.