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Unitary Recordings of TRP and TRPL Channels From Isolated *Drosophila* Retinal Photoreceptor Rhabdomeres: Activation by Light and Lipids

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Delgado R, Bacigalupo J. Unitary recordings of TRP and TRPL channels from isolated *Drosophila* retinal photoreceptor rhabdomeres: activation by light and lipids. *J Neurophysiol* 101: 2372–2379, 2009. First published March 4, 2009; doi:10.1152/jn.90578.2008. Transient receptor potential (TRP) channels play key roles in sensory transduction. The TRP family founding members, the *Drosophila* light-dependent channels, were previously studied under voltage clamp, but had not been characterized in intact rhabdomeres at single-channel level. We report patch-clamp recordings from intact isolated photoreceptors of *wt* and mutant flies lacking TRP (trp^{343}), TRPL ($trpl^{302}$), or both channels (trp^{313}; $trpl^{302}$). Unitary currents were activated by light in rhabdomere-attached patches. In excised rhabdomeral patches, the channels were directly activated by molecules implicated in phototransduction, such as diacylglycerol and polyunsaturated fatty acids. Currents recorded from *trpl* photoreceptors are blocked by external Ca^{2+}, Mg^{2+} (1 mM), and La^{3+} (20 μM), whereas those from *trp* photoreceptors are not. Rhabdomeric patches lacked voltage-dependent activity. Patches from *trp;trpl* mutants were devoid of channels. These characteristics match the macroscopic conductances, suggesting that the unitary currents from *Drosophila trpl* and *trp* photoreceptors correspond to TRP and TRPL.

INTRODUCTION

Drosophila compound eyes contain ~800 ommatidia, each having eight elongated photoreceptor cells arranged in a stereotypical array (Ready 1989). Each cell contains a thin, longitudinal column of tightly packed, light-sensitive microvilli, termed the rhabdomere, which faces the intraommatidial cavity. Phototransduction, the process in which light absorption leads to channel opening initiating the visual electrical response, takes place in the rhabdomere (Hardie and Postma 2008). It involves a second-messenger-mediated biochemical cascade the constituents of which, organized in molecular complexes, are confined to the microvilli (Hardie and Raghu 2001; Tsunoda et al. 1997). On absorbing a photon, the photopigment rhodopsin activates a G_q-protein that in turn activates phospholipase C (PLC), generating inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP_2) (Hardie and Postma 2008). The central role of PLC is evidenced by the fact that photoreceptors from mutants lacking functional PLC (*norpA*) do not respond to light (Bloomquist et al. 1988; Pearn et al. 1996). Commonly IP_3 releases Ca^{2+} from intracellular stores, whereas DAG activates protein kinase C. However, in *Drosophila* photoreceptors, IP_3 increases generated by flash photolysis have given negative results (Hardie 1995; Hardie and Raghu 1998), and the light response of a null mutant for the IP_3-receptor gene seems normal (Acharya et al. 1997; Raghu et al. 2000), suggesting that either increases in concentration of DAG or its hydrolysis products, the polyunsaturated fatty acids (PUFAs) (Chyb et al. 1999), or depletion of PIP_2 (Estacion et al. 2001) may gate the transduction channels, transient receptor potential (TRP) and TRP-like (TRPL) (Reuss et al. 1997). These final steps of the light transduction cascade remain as a major enigma. The PUFAs linolenic acid (LNA) and arachidonic acid (AAc) activate the light-dependent current when puffed onto intact isolated ommatidia. Intriguingly, similar applications of the DAG had no effect, suggesting PUFAs as the most likely channel agonist candidates (Chyb et al. 1999).

TRP was the first member of the extensive TRP channel family to be discovered (Hardie and Minke 1992; Montell and Rubin 1989). It is the principal light-activated channel in *Drosophila* phototransduction, being 10-fold more abundant than TRPL and carrying most of the transduction current (Reuss et al. 1997). Single-channel recordings from the cell-attached light-sensitive membrane have been unsuccessful because the microvilli within the ommatidia were inaccessible to patch-clamp electrodes. Unitary current recordings have been attempted in heterologous systems, but only single TRPL-dependent currents have been measured (Kunze et al. 1997). Noise analysis of TRP- and TRPL-dependent whole cell currents recorded from *trpl* and *trp* photoreceptors, yielded estimated unit conductances of 8 and 35 pS, respectively, in the presence of divalent cations (Reuss et al. 1997), but these values are higher in the absence of divalents (Hardie and Mojet 1995). More recently, Haab et al. (2000) recorded spontaneous multiple-conductance events (4–144 pS) in patches excised from isolated rhabdomeres of *trpl* mutants with Ba^{2+} as the only permeant cation, likely corresponding to TRP channels.

To characterize the transduction channels further and re-examine the role of lipid compounds as agonists, we performed patch-clamp recording directly from rhabdomeres of isolated ommatidia that were mechanically dissociated to allow access to this light-sensitive membrane. Our procedure let us test the effect of light on the channels present in the rhabdomere-attached membrane patches as well as of putative agonists on excised inside-out rhabdomeric patches; we show light-induced channel activity in the former condition and lipid-induced channel activity in the excised patches. We also found that both TRP and TRPL can be activated by DAG and PUFAs. Our work provides long awaited answers to some fundamental questions in *Drosophila* phototransduction.

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METHODS

Dissection

Eyes were removed from adult *Drosophila melanogaster* flies under dim red illumination, and their retinae were disrupted by gently hitting them with a sharp tungsten wire under divalent cation-free solution (DVF, see following text). The large majority of the isolated ommatidia looked healthy, but intact (Fig. 1*Aa*); a few of them exhibited individual photoreceptor cells partly dissociated; although their cell bodies looked evidently traumatized, some rhabdomeres seemed virtually undamaged and retained their responsiveness to light (Fig. 1*A, b* and *c*). Rhabdomeres of these cells were used in our experiments immediately after dissociation.

The following fly strains were utilized: wild-type white Oregon (WOR), $trpl^{302}$, trp^{343}, and trp^{313};$trpl^{302}$ (kindly donated by R. Hardie, Cambridge University and C. Montell, Johns Hopkins University).

Electrophysiology

Single-channel currents were recorded with an Axoptach-200B patch-clamp amplifier (Axon Instruments). Cells were viewed with an Olympus IX70 inverted microscope, equipped with DIC optics (×60 objective) and phase contrast (×100 objective). Recording pipettes

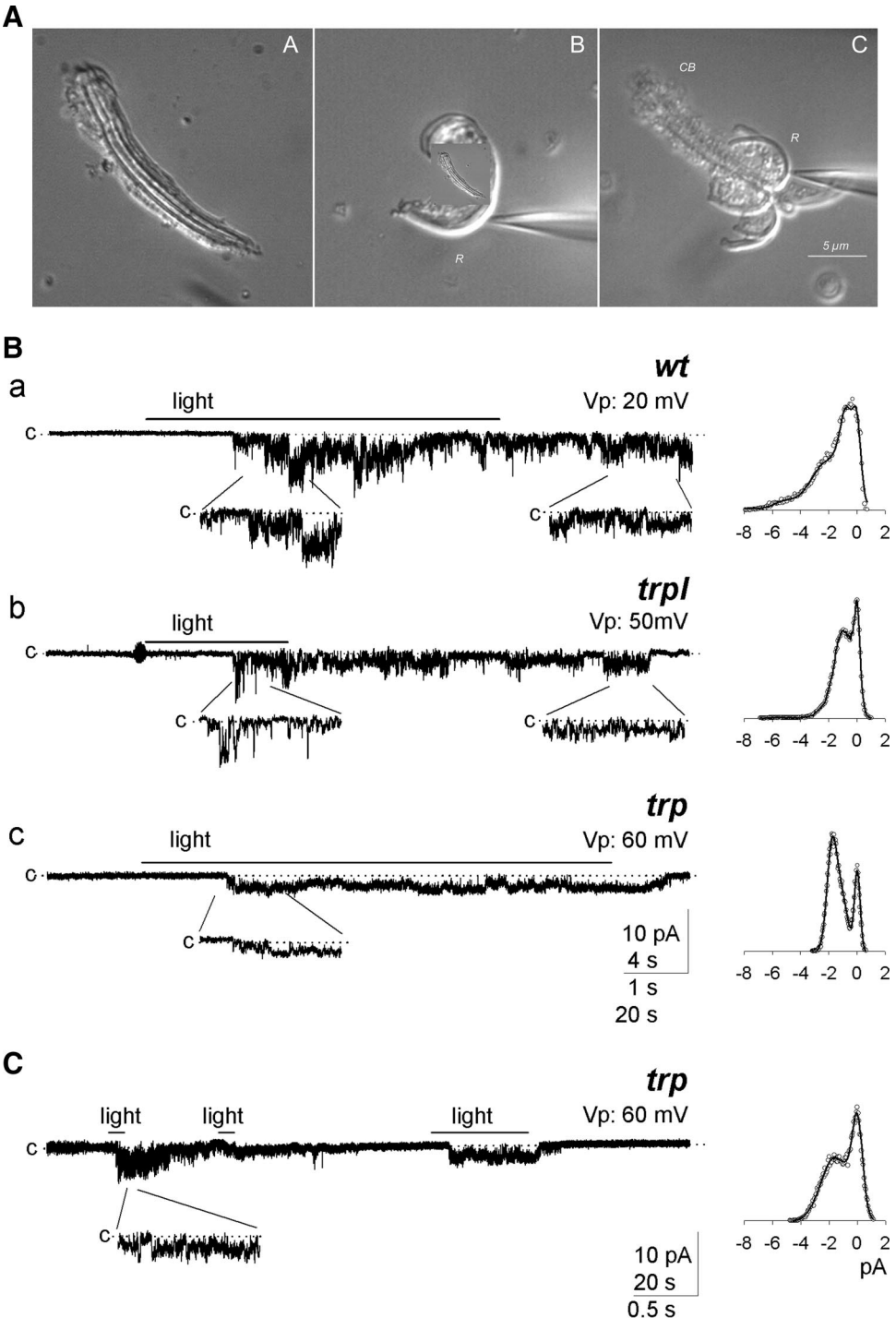

FIG. 1. Exposed *Drosophila* rhabdomeres. *A*: dissociated ommatidium under DIC optics (*a*). Partially dissociated ommatidia, with a patch pipette positioned on one of the rhabdomeres (*b* and *c*). R, rhabdomere; CB, cell body. *B*: light-activated channels in rhabdomere-attached membrane patches. Representative examples of light responses from *wt* (a; $n = 4$), *trpl* (b; $n = 8$), and *trp* (c; $n = 5$) photoreceptor rhabdomeres are shown. *C*: responses to successive light stimuli ($n = 3$). ··· labeled "c" indicates the current intensity for the closed state of the channels. Expanded segments of the current traces are shown under each record. Bath: normal solution; pipette: divalent-free solution. Pipette potential is indicated.

were made of borosilicate glass capillaries with internal filament, and pulled with a horizontal glass puller (Sutter Instruments) to tip resistances of ≥50 MΩ. Seal resistances were ≥5 GΩ. Pipettes were filled with divalent cation-free solution (see following text) unless indicated.

Cells were maintained in darkness for ≥5 min before starting the rhabdomere-attached experiments. Every membrane patch studied was polarized to +50 or +60 mV (pipette potential, V_p) and was tested for the presence of voltage-gated channels with a depolarizing voltage pulse (to $V_p = -60$ mV) at the beginning of the experiment. A fraction of the membrane patches presented spontaneous channel activity (~80%); of these, in our experiments we only utilized those in which activity was absent or low. Patches that remained silent after lipid application were briefly exposed to air, inducing spontaneous activity in approximately half of them, suggesting the presence of a vesicle, while the seal of other half broke. All voltages are expressed as pipette voltages (V_p). Data were digitized with a LabMaster interface (Scientific Solutions) and stored in a PC. pClamp 6.0 software (Axon Instruments) was utilized for acquisition, stimulation protocols and analysis. The current was sampled at 20,000 Hz and low-pass filtered at 2,000 Hz for analysis (8-pole Bessel filter). Light stimuli were applied with a superbright white light-emitting diode (LED, N21BY Agilight) positioned 3 cm from the preparation; luminance was 1.6 cd/cm^2 (dimmer lights were not tested).

Solutions

Normal external solution contained (in mM) 120 NaCl, 5 KCl, 8 $MgSO_4$, 1.5 $CaCl_2$, 25 L-proline, 2.5 sucrose, and 10 HEPES, pH 7.15; divalent cation-free solution (DVF) contained (in mM) 120 NaCl, 5 KCl, 25 L-proline, 2.5 sucrose, 10 HEPES, and 2 mM Mg-ATP, pH 7.15. In the blockage studies, the pipette was filled with DVF solution plus the desired divalent (1 mM) or La^{3+} (20 μM), while the bath contained DFV.

Lipid stock solutions of 1-oleoyl-2-acetyl-sn-glycerol (OAG, 125 mM) and arachidonic acid (AAc, 80 mM) were prepared in DMSO. LNA (20 mM), oleic acid (140 mM), and elaidic acid (50 mM) were prepared in ethanol. Appropriate amount of the desired stock solution was dissolved in the DVF to the desired final concentration.

Application of solutions

In most experiments, a single- or double-barreled puffer pipette positioned at ~2 μm from the ommatidium was used to apply the appropriate solutions by pressure, using a custom made computer-driven picospritzer. In some experiments, instead of being added to the puffer pipette the lipids were included in the recording pipette solution, for the sake of simplicity.

Chemicals

The membrane-permeant DAG analogue OAG was used instead of DAG. All chemicals were from Sigma Chemicals.

RESULTS

The use of partly dissociated photoreceptors from isolated *Drosophila* ommatidia was crucial for assessing the study of the rhabdomeric channels at the unitary level. Because it is highly likely that the only channels residing in the light-sensitive region are the light-dependent channels, like in other photoreceptors (Johnson and Bacigalupo 1992; Nasi and Gomez 1992; Stern et al. 1982), we expected that single-channel currents recorded from this region of the photoreceptor corresponded to TRP and TRPL. To test this, we compared the characteristics of the rhabdomeric channels with those previously reported of their respective macroscopic currents in whole cell voltage-clamp studies.

Light-activated single-channel currents from rhabdomeres

We tested whether single-channel currents could be evoked by light in the exposed rhabdomeres of the dissociated photoreceptors. Figure 1*B* shows representative examples of light responses from *wt* (Fig. 1*Ba; n* = 4), *trpl* (*Bb; n* = 8), and *trp* photoreceptors (*Bc; n* = 5). Recordings were obtained with patch electrodes attached to the rhabdomere, under positive pipette potentials (20–60 mV; the rhabdomere membrane potential was assumed to be 0 mV, as the cell bodies were severely damaged and the magnitudes of elementary events were not different after than before excision). Light stimuli were applied after several minutes of darkness. Channel activity was typically preceded by a latency of a few seconds after the onset of the stimulus, persisted for some time, slowly declining to the dark level. The time course of the light-induced currents was orders of magnitude longer than that of a light response recorded in an intact photoreceptor. Light activation was transient in all cases, and a few patches (n = 3) were capable of responding to more than one stimulus (Fig. 1*C*), indicating that channel activation was not a result of some irreversible process caused by illumination.

Effect of lipids on excised rhabdomeric patches

With the knowledge that rhabdomeral single-channel currents were light-activated, we examined whether any of the lipids that have been proposed as possible agonists of the light-dependent channels were capable of activating the rhabdomeric channels. Figure 2 shows that all of them, namely OAG, AAc, and LNA, were capable of triggering channel activity when puffed onto inside-out membrane patches excised from *wt Drosophila* rhabdomeres. After delays of several seconds (5–60 s), 4 of 6 patches responded to AAc (10 μM; Fig. 2*Aa*), 49 of 60 to the membrane-permeable DAG analog, OAG (2–10 μM; *Ab*), and 26 of 35 to LNA (2–10 μM; *Ac*). Channel activity typically declined to prestimulus level with a variable time course (20 to hundreds of seconds). We also tested in the same manner the monounsaturated fatty acids oleic acid (10 μM; n = 5) and elaidic acid (10 μM; n = 4), both of which failed to activate channels (Fig. 2*A, d* and *e*). Nevertheless, all patches tested with oleic or elaidic acids responded to LNA or OAG (not shown). Control experiments applying lipid-free carrier solution (same DMSO or ethanol concentrations, using 2-barreled theta glass pipettes) had no effect (n = 6 of each). Similar effects of OAG and LNA were observed in membrane patches from *trp* and *trpl* mutants rhabdomeres (n = 4); in these cases, the lipids were added to the pipette solution rather than puffed onto the patches, for simplicity. The results indicated that not only the PUFAs, but also DAG, can activate rhabdomeric channels.

The effect of the lipids was clearly dose-dependent, as illustrated by the example of Fig. 2*Ca,* in which 2 and 5 μM OAG induced moderate channel activation, whereas 10 μM substantially increased it. The lipid dose-response relation was difficult to quantify because the number of channels in the patches was unknown. Nevertheless, we plotted the total charge mobilized across the membrane per second as a func-

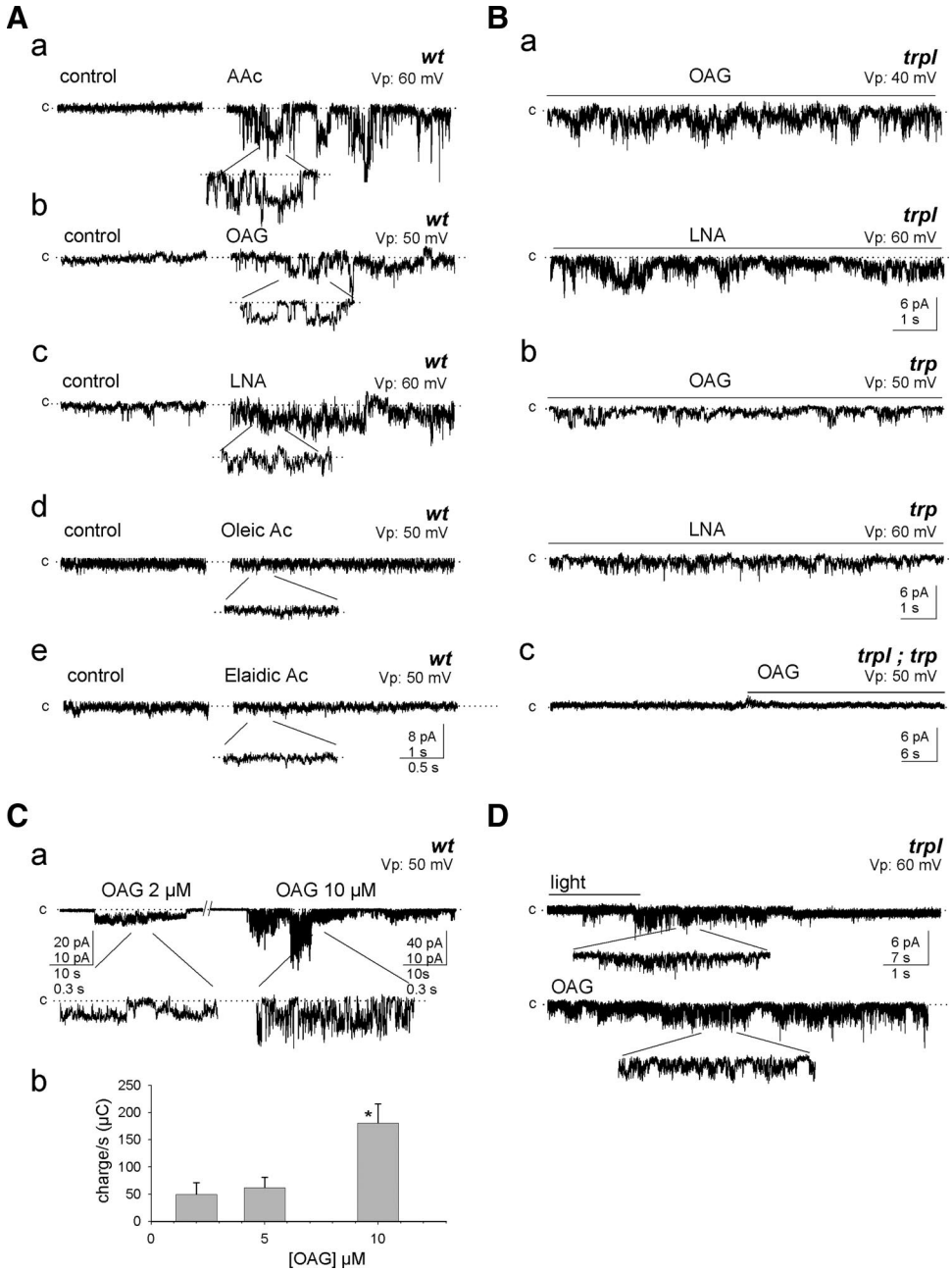

FIG. 2. Diacylglycerol (DAG) and polyunsaturated fatty acid (PUFA) activate ion channels in inside-out excised rhabdomeric membrane patches. *A*: representative recordings from different *wt* excised patches, previous and during channel activity, illustrating channel activation by arachidonic acid (Aac, *a;* $n = 4$), membrane-permeant DAG analogue 1-oleoyl-2-acetyl-sn-glycerol (OAG, *b;* $n = 49$), linolenic acid (LNA, *c;* $n = 26$) and the monounsaturated fatty acids oleic acid (d; $n = 5$) and elaidic acid (e; $n = 4$; 10 μM of each). *Insets*: segments of them shown at faster timescale. In each case, a control trace (before application of the lipid) followed by a trace during lipid exposure are shown. Lipids were applied for 20 s, and channel activation started in a range of 5–60 s after the end of the application. Control experiments applying lipid-free carrier solution for 20 s were done (not shown). *B*: channel activity from *trpl* (*a;* $n = 4$) and *trp* (*b;* $n = 4$) membrane patches triggered by OAG and LNA. *c*: a current traces from a *trpl;trp* patch ($n = 6$). *C*: OAG-induced activity in the same *wt* patch at 2 and 10 μM (a; $n = 4$). Same protocol as in *A* was used. Quantification of dose dependence of OAG on channel activity (expressed as charge per second) from different membrane patches is shown in *b*. ANOVA test was used for statistics analysis ($P < 0.05$). Error bars: SE. *D*: comparison of light- and OAG-induced channel activity in the same *trp* membrane patch ($n = 3$). ··· labeled "c" denote channel closed state. Bath: DVF; pipette: DVF solution without (*A, C,* and *D*) and with the indicated lipid (*B*).

tion of lipid concentration for several experiments ($n = 4$ for each condition). A dose dependency can be appreciated in the plot (Fig. 2*Cb*). In addition, we observed that further current levels seemed to appear at higher lipid concentrations (Fig. 2*Ca*), an observation that requires more investigation.

To determine whether the channels activated by light and by lipids corresponded to the same molecular entities, we compared recordings from separate membrane patches exposed to either light or OAG, under rhabdomere-attached and excised conditions, respectively. A representative example is shown in Fig. 2*D*. The single-channel events look closely similar in both cases (Fig. 2*D*). Two more patches for each condition in which individual events could be distinguished were analyzed, giving consistent results. It appears that the single-channel events under both conditions were due to activation of the same channel species, in this case, TRP. Equivalent results were observed in an analogous experiment on a *trp* mutant, indicating that light and lipids activate the TRPL channel (not shown).

We recorded from rhabdomeric patches excised from the double mutant lacking the TRP and TRPL channels, in the presence of 5 μM OAG (Fig. 2*Bc;* $n = 6$). None of these patches exhibited spontaneous activity, and no channel events were induced on puffing the lipid onto them, in agreement with the notion that the single-channel currents from the *trpl* and *trp* fly strains corresponded to the two light-dependent channels species, TRP and TRPL.

The tested lipids that activated the channels are products of the PIP_2 hydrolysis by PLC, which in the fruit fly photoreceptors is triggered by light. Light and direct application of such lipids activated channels in rhabdomeral membranes but not in the double mutant *trp;trpl*. On the other hand, genetic analysis

indicates that TRP and TRPL are the light-dependent channels in *Drosophila* (Niemeyer et al. 1996; Reuss et al. 1997). Together this evidence and the present results strongly support the view that the rhabdomere's light-activated channels are opened by lipids.

TRP and the TRPL channels expressed in the trpl and the trp mutants

Recordings obtained from an excised *trpl* rhabdomeric patch exposed to 5 μM OAG under symmetrical DVF solution and different voltages, are displayed in Fig. 3*A*. Channel activity seems complex, showing different current levels. The *I-V* relationship built from the most visited current amplitude value at each potential was linear, with a slope conductance of 56.8 $\pm$ 3.7 pS ($n = 4$; Fig. 3*B*).

Similarly, An *I-V* plot (Fig. 3*D*) was built with data from a *trp* patch (Fig. 3*C*), determining a conductance of 49.0 $\pm$ 5 pS ($n = 4$).

Blockage of the rhabdomeric channels by divalent cations and La^{3+}

Macroscopic studies have shown that *Drosophila* TRP-dependent currents are blocked by millimolar Mg^{2+} and low micromolar La^{3+}, while TRPL is unaffected (Hardie and Minke 1994; Hardie and Mojet 1995). Extracellular Ca^{2+} blocks TRP and inhibits TRPL-dependent currents (Hardie and Postma 2008; Reuss et al. 1997; Scott et al. 1997). We examined the effects of these three ions separately on excised *trpl* and *trp* rhabdomeric channels exposed to 5 μM OAG (Fig. 4*B*). The desired cation was added to the pipette DVF solution while the bath contained DVF, and the currents were examined at two opposite voltages. Calcium (1 mM) blocked the single-channel currents from a *trpl* rhabdomeral patch at +60 mV (voltage that impelled Ca^{2+} out of the pipette), but not at −60 mV (Fig. 4*A, top*). The same happened when 1 mM Mg^{2+} or 20 μM La^{3+} was added to the pipette solution instead of Ca^{2+} (Fig. 4, *medium* and *bottom*). In contrast, when the same experiment was performed on *trp* membranes, the single-channels currents were unaffected in any of the tested conditions (Fig. 4*B*). These results are quantified in the plots of Fig. 4, *C* and *D,* which show the average nPo values measured for each experimental condition ($n = 4$ of each). These single channel results are in agreement with the previous whole cell recording measurements with regard to the effect of Mg^{2+} and La^{3+}, supporting the correspondence between both channels and the respective macroscopic conductances, but they are in apparent disagreement with the inhibitory effect of extracellular Ca^{2+} on the TRPL-dependent current.

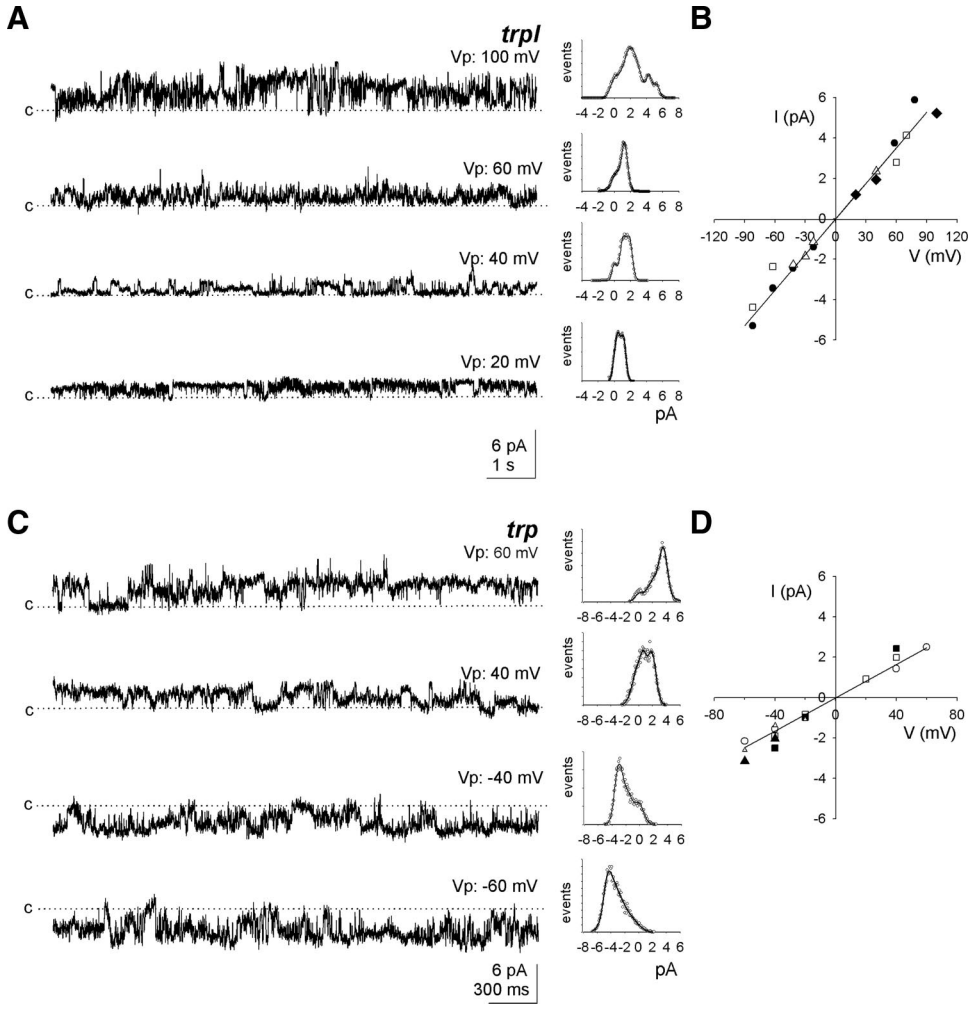

FIG. 3. *I-V* relations of OAG-activated channels from *trpl* and *trp* membrane patches. *A*: recording obtained under 5 μM OAG at different pipette potentials. Amplitude histograms are shown by each trace. *B*: *I-V* curve built from 4 individual *trpl* patches, using the peak values of the histograms at each potential. The straight line corresponds to an average of 4 experiments; slope conductance: 56.8 $\pm$ 3.7 (mean $\pm$ SD) pS. *C*: single-channel currents recorded from a *trp* patch at different pipette potentials. *D*: *I-V* from data of four *trp* patches. Slope conductance: 49.0 $\pm$ 5.0 pS. Bath: DVF solution, pipette: DVF supplemented with 5 μM OAG.

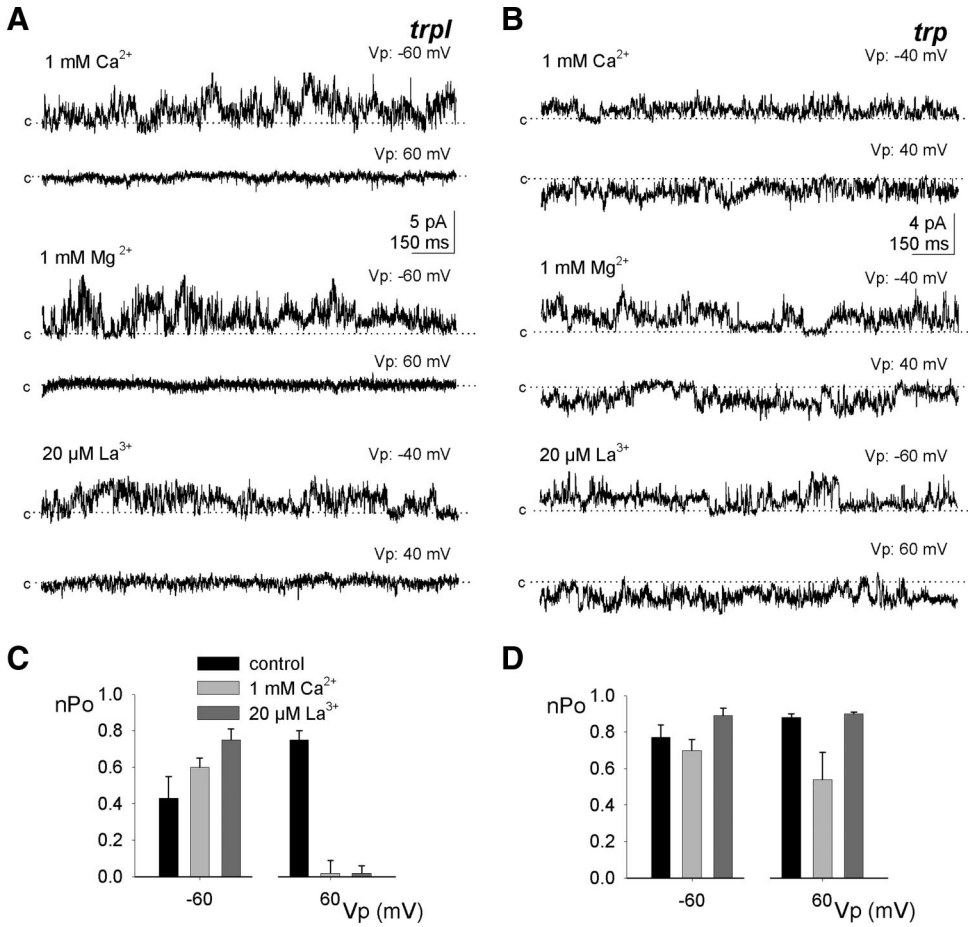

FIG. 4. Ca^{2+}, Mg^{2+}, and La^{3+} blockade of OAG-activated channels in *trpl* and *trp* membrane patches. *A*: effect of the cations on a *trpl* patch at 2 equal but opposite potentials. *B*: effect of the cations on a *trpl* patch at 2 equal but opposite potentials. *C*: quantification of 4 experiments as in *A*. Because the number of channels (*n*) in the membrane patches was unknown, the ordinate values are expressed as nPo, which correspond ratio of the total time the current departed the closed level and the total time period of the recording. *D*: quantification of 4 experiments as in *B*. The tested cations were added to the pipette solution at the indicated concentrations. Statistical analysis: ANOVA test, $P < 0.05$.

We also examined the effects of the same three cations (same concentrations) from the internal (bath) side of the channels. All three of them strongly abolished the channels in both *trpl* and *trp* mutants (not shown), suggesting that TRP and TRPL have one or more blocking binding sites on their inner face.

DISCUSSION

Considerable progress has been made on the characterization of the light-dependent channels based on whole cell recordings from intact isolated ommatidia of *wt*, *trpl,* and *trp* mutant flies (Reuss et al. 1997). However, a detailed characterization of these channels could only be attained by single-channel studies. In this work we are reporting single-channel currents recorded directly from the native light-sensitive membrane of partly dissociated *Drosophila* photoreceptors using high-resistance patch electrodes. In effect, we show that light triggered transient channel activity in rhabdomere-attached patches in *wt*, *trpl,* and *trp* photoreceptors. Most membrane patches exhibited spontaneous channel activity that varied from patch to patch, except those derived from the double mutant *trp;trpl*, which lacked both spontaneous and light-induced channel activity. The spontaneous activity was made of similar events than those that were activated by light or lipids (not shown). These observations are consistent with the concept that the only channels residing in the rhabdomere are the light-dependent channels and that what we recorded corresponded to TRP and TRPL.

Kinetics of the light-induced channel activation

The latency and duration of the light-induced channel activity were considerably longer than in a normal light response from an intact photoreceptor. The reason for these major discrepancies between the micro- and the macroscopic responses is unknown. Nevertheless, it is conceivable that the process of cell dissociation and sealing of the patch pipette against the rhabdomeric membrane may seriously distort the extremely fine molecular arrangement of the transduction machinery within the microvilli, which is thought to critically determine the time course of the light response (Hardie and Postma 2008). Similar observations were previously made in the activation of light-dependent rhabdomeric channels in *Limulus* and scallop photoreceptors under comparable experimental conditions (Bacigalupo and Lisman 1983; Nasi and Gomez 1992). However, in spite of the alterations in the response kinetics, our results offer valuable information about the characteristics of the light-dependent channels and their possible agonists.

Blockage by multivalent cations

It has been documented that *Drosophila* TRP is blocked by extracellular Ca^{2+}, Mg^{2+} (1 mM), and La^{3+} (20 μM), whereas TRPL is relatively insensitive to Mg^{2+} and La^{3+} ions (Hardie and Minke 1992; Hardie and Postma 2008; Reuss et al. 1997). Likewise, we found that single-channel currents from *trpl* photoreceptors were blocked extracellularly by these cations,

whereas those from *trp* rhabdomeres were insensitive to them. However, macroscopic studies have shown that external Ca^{2+} inhibits the TRPL-dependent current, whereas we found that Ca^{2+} had no effect on the TRPL channel in excised rhabdomeral patches. The reason for the discrepancy may be that lipids (LNA, among others) remove divalent cations block of TRPL, abolishing the outward rectification of its *I-V* curve (Parnas et al. 2009). It is also conceivable that the action of Ca^{2+} on TRPL in vivo, which is mediated by calmodulin (Reuss et al. 1997; Scott et al. 1997), may not be operating in excised patches because calmodulin and TRPL are thought not be tightly attached to the transducisome protein complex (Hardie and Postma 2008; Li and Montell 2000). In addition, the two channels were blocked by the three tested cations when added to the internal side (bath), suggesting that they possess intracellular blocking sites for them. Lanthanum had not been previously tested from the intracellular side in *Drosophila* TRP or TRPL, but it was shown to block heterologously expressed TRPC3 at submillimolar concentrations, while millimolar levels were required to block that channel extracellularly (Halaszovich et al. 2000). This is probably due to the existence of La^{3+} binding sites with different affinities at either side of the channel.

Characteristics of TRP and TRPL unitary currents

The conductance of the channel recorded in *trpl* rhabdomeres in the absence of divalent cations (56.8 ± 3.7 pS) is considerably larger than that derived by noise analysis of the TRP-dependent macroscopic current under physiological ionic conditions (8 pS) (Reuss et al. 1997) but similar to that estimated for divalent-free conditions (Hardie and Postma 2008). This is expected because under divalent cations, the TRP channel is largely blocked, whereas our measurements were performed in the absence of divalents. On the other hand, the most frequently observed unitary conductance of the channel recorded from *trp* patches under low lipid concentrations was found to be 49.0 ± 5 pS, somewhat lower than estimates obtained by noise analysis (Hardie and Postma 2008; Reuss et al. 1997) as well as with measurements done in single-channel patch-clamp recordings of TRPL expressed in Sf9 cells (Kunze et al. 1997). In our recordings we also observed, although less frequently, events of other sizes, which may account for the larger conductance values reported by the previous studies. For the moment, attempts to obtain unitary TRP channel recordings expressed heterologously have been unsuccessful (Minke and Parnas 2006).

DAG and PUFA as possible light-dependent channels agonists

The PUFAs AAc and LNA are considered as possible agonists of *Drosophila* light-dependent channels because they activate the light-dependent conductance when puffed onto isolated ommatidia in darkness. Surprisingly, OAG (DAG) was without effect when tested in identical experiments (Chyb et al. 1999). We found that these three lipids were capable of activating rhabdomeric channels when applied to the intracellular side of excised rhabdomeric patches. The dose-response relation of the lipid effect was comparable to that reported by Chyb et al. (1999). The discrepancy may rely on the fact that we applied the lipids directly onto the luminal face of the excised patches rather than externally. A possible explanation is that the microvilli might be equipped with a strong pool of DAG-kinase that prevents luminal DAG to reach the threshold level for channel activation when added externally, whereas DAG concentration may rise above threshold when added from the inner side of excised patches. In the latter case, any kinase activity would become overwhelmed during DAG application. On the other hand, oleic and elaidic acids were unable to induce channel activity in excised rhabdomeric patches. However, oleic acid evoked currents under whole cell conditions (20 μM) (Chyb et al. 1999). It is possible that the lower concentration used in the present work (10 μM) was insufficient to activate rhabdomeric channels. Our results strongly reinforce DAG as a possible agonist of the light-dependent channels without ruling out a similar role for PUFAs. A function of DAG as agonist of other channels of the TRPC subfamily has also been proposed (Venkatachalam and Montell 2007). Estacion et al. (2001) suggested that DAG may indirectly activate TRPL by enhancing PLC activity because its effect was reduced by pharmacological inhibition of PLC in Sf9 cells heterologously expressing TRPL. Our findings cannot exclude an additional role of DAG such as this in phototransduction. The same authors also reported no channel activation by OAG in excised patches from Sf9 cells expressing TRPL, contrary to our observations. This discrepancy may be due to the different preparations used in both works.

It is possible that the mechanism for TRP gating involves direct binding by lipids such as DAG and PUFA at specific sites on the channels proteins. However, it is alternatively possible that these lipids might transiently alter the structure of the microvillar membrane as their concentration rises during a light response (or on an experimental application of exogenous lipids), modifying as a consequence the transduction protein complex structure, somehow leading to the opening of the light-dependent channels. Interestingly, DAG causes a negative curvature in lipid monolayers, generating inverted micelles (Carrasco and Merida 2007). Ion channel gating by changes in membrane structure occur in some mechanosensitive TRP channels (Christensen and Corey 2007; Venkatachalam and Montell 2007). It is therefore possible that the light-dependent channels in *Drosophila* photoreceptors are mechanically gated, an idea that has to be investigated.

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