ping tool for new DNA markers in the *WAGR* region of 11p13, which will aid in the eventual identification of the Wilms' tumor and aniridia genes. Considering that aniridia and the translocated chromosome have been co-transmitted through at least three generations of this family without any evidence of Wilms' tumor or genitourinary abnormalities, we believe the deletion does not include or disrupt the *WT* gene. This constitutes the first description of a deletion that either disrupts or removes the aniridia gene and clearly spares the Wilms' tumor locus. Therefore markers included in this deletion become likely candidates for the aniridia gene. The DNA segments *D11S93* and *D11S95*, mapping between *CAT* and *FSHB* in a position either very near or within the aniridia gene, should prove to be valuable tools in the eventual identification of the genes involved in the *WAGR* complex, beginning with the aniridia gene.

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Inactivation and Block of Calcium Channels by Photo-released Ca^{2+} in Dorsal Root Ganglion Neurons

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Calcium channels are inactivated by voltage and intracellular calcium. To study the kinetics and the mechanism of calcium-induced inactivation of calcium channels, a "caged" calcium compound, dimethoxy-nitrophen was used to photo-release about 50 μM calcium ion within 0.2 millisecond in dorsal root ganglion neurons. When divalent cations were the charge carriers, intracellular photo-release of calcium inactivated the calcium channel with an invariant rate [time constant (τ) $\approx$ 7 milliseconds]. When the monovalent cation sodium was the charge carrier, photorelease of calcium inside or outside of the cell blocked the channel rapidly ($\tau \approx$ 0.4 millisecond), but the block was greater from the external side. Thus the kinetics of calcium-induced calcium channel inactivation depends on the valency of the permeant cation. The data imply that calcium channels exist in either of two conformational states, the calcium- and sodium-permeant forms, or, alternatively, calcium-induced inactivation occurs at a site closely associated with the internal permeating site.

CALCIUM CHANNELS ARE HIGHLY Ca^{2+} selective. Currents carried by monovalent ions through the channel are blocked by low external Ca^{2+} concentrations ($2 \times 10^{-6} M$) (*1, 2*). Internal free Ca^{2+} also modulates the inactivation of the Ca^{2+} channel (*3–6*). However, little is known about the kinetics or the molecular mechanisms regulating these processes. We have examined the kinetics of interaction of Ca^{2+} with a neuronal Ca^{2+} channel by rapid "photo-release" of Ca^{2+} in the intra- or extracellular space from a novel caged Ca^{2+} compound, dimethoxy (DM)-nitrophen, which on photolysis changes its Ca^{2+}-binding affinity.

Cultured chick (1- to 2-day-old) dorsal root ganglion (DRG) neurons were voltage clamped with the whole-cell patch clamp technique (*7*). Cells were dialyzed with internal solutions buffered either with EGTA to give an internal Ca^{2+} concentration

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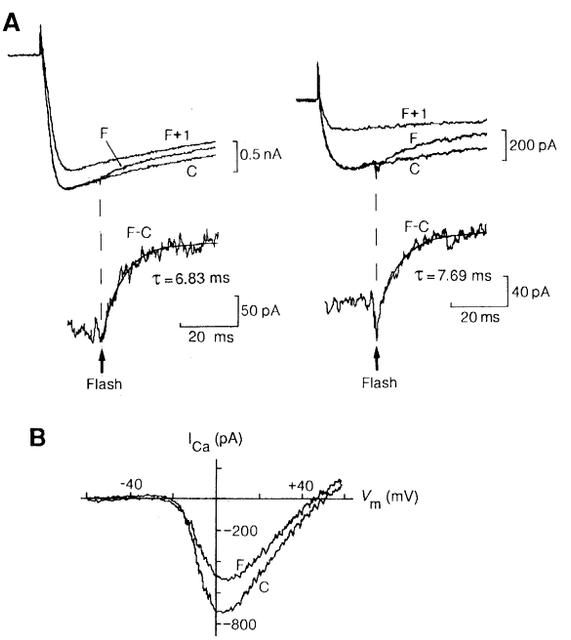

Fig. 1. Photo-release of intracellular Ca^{2+}. (**A**) Ca^{2+} current through high-threshold Ca^{2+} channels in two different cells was suppressed following light-induced increase in $[Ca^{2+}]_i$. The external solution contained 120 m*M* NaCl, 5 m*M* $CaCl_2$, 20 m*M* Hepes, and 3 μM (tetrodotoxin) TTX, at *p*H 7.3, and the pipette solution contained, in addition to high Cs^+ and Hepes concentrations, 2.25 m*M* DM-nitrophen and 2 m*M* $CaCl_2$. The effect of a rapid increase in intracellular Ca^{2+} on the I_{Ca} is shown by the F traces. The C traces represent I_{Ca} before, and traces F + 1 are the I_{Ca} 3 s after, the photo-releasing light pulse. The time course of the effect caused by elevation of Ca^{2+} (traces F − C) was fitted by a single exponential with τ values of 6.83 and 7.69 ms, respectively. (**B**) A voltage-clamp ramp from +60 to −85 mV over 90 ms was applied immediately after a depolarizing command pulse and used to generate the *I-V* relation of the Ca^{2+} current before (trace C) and after (trace F) the photo-release of intracellular Ca^{2+}. The largest difference between curves C and F occurred at the peak of the I_{Ca}, indicating that the release of intracellular Ca^{2+} did not activate an outward or a nonspecific current. There was a larger flash artifact (A, right tracing) because a different voltage source for the light was used. In both cells the holding potential was −80 mV and the command potential was to 0 mV.

($[Ca^{2+}]_i$) of about $10^{-8}M$ or with DM-nitrophen (*8, 9*). DM-nitrophen is an EDTA-based molecule with an inserted σ-nitrobenzyl group that renders it light sensitive. The affinity of DM-nitrophen for Ca^{2+} decreases sharply on photolysis from about $5 \times 10^{-9}M$ to $2 \times 10^{-3}M$ at *p*H 7.0. The affinity of DM-nitrophen for Mg^{2+} was much lower and changes from $2.5 \times 10^{-6}M$ to $2 \times 10^{-3}M$ on photolysis (*9*). We omitted Mg^{2+} from external or internal solutions, reducing possible complications secondary to the presence of internal Mg^{2+}. We used absorption of a Ca^{2+}-sensitive dye, antipyrylazo III, to measure the speed of the photolytic reaction and found that the half time ($t_{1/2}$) of Ca^{2+} release was about 200 μs (*10*), which is at the limiting rate of antipyrylazo-Ca^{2+} reaction (*11*).

Internal solutions contained the Ca^{2+} buffer plus 50 m*M* Hepes, 80 m*M* CsCl, 20 m*M* tetraethylammonium chloride, 1 m*M* adenosine 3′,5′-monophosphate (cAMP), and 5 m*M* sodium adenosine triphosphate (ATP). Tetrodotoxin ($3 \times 10^{-6}M$) was added routinely to the external solution to block the Na^+ channel, and in some experiments 5 m*M* 4-amino-pyridine was used to suppress possible Ca^{2+}-induced K^+ currents (legend to Fig. 1). Internal and external solutions were designed to optimize the measurement of the Ca^{2+} current (I_{Ca}) and to minimize any contaminating K^+ and Na^+ currents.

Ultraviolet light (330 to 370 nm) was focused through a ×40 oil-immersion objective (Nikon, capable of 60 to 80% transmission at the above wavelengths) of an inverted microscope (Zeiss IM). The light beam for photo-release was about 2 mm in diameter with an intensity of 3 to 4 mJ mm^{-2}. Released Ca^{2+} concentration was measured with double-barreled Ca^{2+}-selective microelectrodes inserted into a droplet, 20 to 50 μm in diameter, suspended from the opening of a micropipette filled with the intracellular solution containing DM-nitrophen. The free Ca^{2+} concentration in this solution, as measured with a Ca^{2+}-sensitive microelectrode, was $3 \times 10^{-7}M$. The magnitude of release of Ca^{2+} under such conditions ranged between 50 and 100 μ*M*, with a solution containing 2.25 m*M* DM-nitrophen and 2 m*M* Ca^{2+}.

The $[Ca^{2+}]_i$ was rapidly increased in two different DRG neurons by photolysis with a 150-μs light pulse (Fig. 1, arrows). The I_{Ca} inactivated rapidly after the light pulse with a time constant of about 6 to 8 ms (Fig. 1, A and B). To examine whether the Ca^{2+}-induced attenuation of the inward current after photo-release was contaminated by Ca^{2+}-activated outward currents, we measured the current-voltage (*I-V*) relation of the cell with a ramp clamp pulse from +60 to −85 mV applied immediately after a depolarizing pulse. Figure 1B shows that the *I-V* relations, measured before and after the photo-release of Ca^{2+}, were significantly different only near the peak of the Ca^{2+} current. Thus the increase in $[Ca^{2+}]_i$ did not appear to activate an outward current, suggesting that under the ionic conditions used here the suppression of the Ca^{2+} current after photo-release of Ca^{2+} is due primarily to the effect of $[Ca^{2+}]_i$ on the Ca^{2+} channel.

There was some variability in the degree to which the I_{Ca} that was recorded 5 to 10 s after the light pulse was suppressed (compare trace F + 1 in Fig. 1A, left and right). This variability may result from Ca^{2+}-induced "run down" of the Ca^{2+} current. The initial inactivation time course was well fit with a single exponential with a τ of 6 to 8 ms, suggesting a first-order process. Since $t_{1/2}$ of photo-release of Ca^{2+} from DM-nitrophen was about 200 μs (*10*), the time course of Ca^{2+}-induced inactivation of I_{Ca} probably represents primarily the kinetics of the inactivation process. Similar results to those shown in Fig. 1 were obtained when Ba^{2+} was the charge carrier through the Ca^{2+} channel.

We also examined the effect of photo-release of Ca^{2+} in neurons where the external Ca^{2+} was buffered to submicromolar levels so that Na^+ was the only charge carrier through the Ca^{2+} channel (*1, 2*). Under these conditions, photo-release of intracellular Ca^{2+} did not induce a time-dependent inactivation; rather, the current appeared to be step blocked. The Na^+ current through the Ca^{2+} channel was suppressed with a time constant of 0.48-ms (Fig. 2B, trace F − C). Considering that the light pulse lasts about 150 μs and the photo-

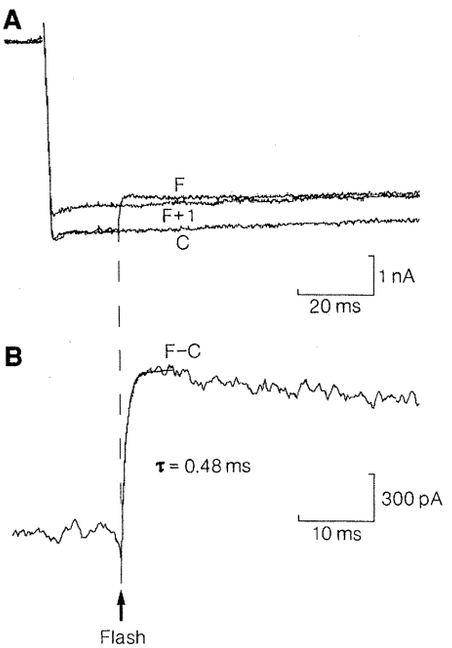

Fig. 2. Na^+ current through high-threshold Ca^{2+} channel was rapidly reduced following photo-release of internal Ca^{2+}. The extracellular solution of Fig. 1 was modified to contain in addition 5 m*M* EGTA and 0.25 m*M* $CaCl_2$, and at *p*H 7.3 had a calculated free Ca^{2+} concentration of $5 \times 10^{-9}M$. The pipette solution also contained 2.25 m*M* DM-nitrophen and 2 m*M* $CaCl_2$, *p*H 7.0. (**A**) Na^+ current decreased by 20% after the photo-releasing pulse (trace F). Trace C is the current before and trace F + 1 is the current 5 s after the photo-release of Ca^{2+}. (**B**) The difference trace F − C shows the time course of the effect of the photo-released internal Ca^{2+} on the Na^+ current. The rapid reduction of the current was fitted by a single exponential with a τ of 0.48 ms, which was similar to that obtained when Ca^{2+} was released on the external side (Fig. 3). Currents through the Ca^{2+} channel were activated by depolarizing pulses to −40 mV from holding potentials of −90 mV. We estimated that 50 to 100 μ*M* Ca^{2+} was released in this experiment.

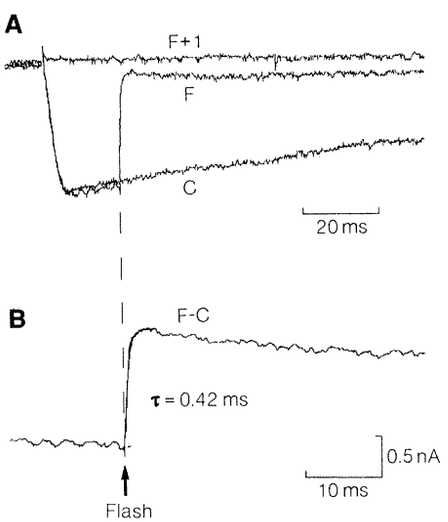

Fig. 3. Na^+ current through high-threshold Ca^{2+} channel was suppressed after photo-release of extracellular Ca^{2+}. The extracellular solution was similar to that of Fig. 1 but had 1.125 m*M* DM-nitrophen and 1 m*M* Ca^{2+}, which gave a free Ca^{2+} concentration of $4 \times 10^{-7}M$ at *p*H 7.3. The pipette solution was somewhat different and contained 100 m*M* CsCl, 20 m*M* tetraethyl ammonium chloride, 10 m*M* glucose, 10 m*M* EGTA, 0.25 m*M* Ca^{2+}, and 10 m*M* Hepes, *p*H 7.3. (**A**) Trace C shows the Na^+ current through the Ca^{2+} channel before, and trace F + 1 is 5 s after the photo-release pulse. Trace F shows that rapid photolysis of DM-nitrophen leads to marked reduction of Na^+ current. Currents were activated by depolarizing to −40 mV from holding potentials of −90 mV. (**B**) Subtraction of C from F represents the time course of reduction of Na^+ current induced by photo-released Ca^{2+}. The segment of the trace for 5 ms after the flash was fitted by a single exponential with a τ of 0.42 ms. Photo-release of Ca^{2+} measured with double-barreled Ca^{2+} microelectrodes gave values in the range of 100 μ*M* Ca^{2+}. The external solution containing DM-nitrophen was not continuously perfused between traces C and F + 1.

lytic reaction has a τ of 280 μs (*10*), the 0.48-ms τ (Fig. 2) approaches the limit of the release technique and suggests that the suppression of Na^+ current by Ca^{2+} occurs well within this time. Thus the response of the Ca^{2+} channel to a step elevation of intracellular Ca^{2+} was markedly different when Na^+ was the charge-carrying ion than when Ca^{2+} was the permeating species. We never found evidence for a slower component of Ca^{2+}-induced inactivation of Ca^{2+} channel when Na^+ was the charge carrier, such as that seen when divalents were the permeating cations. The step decrease in the Na^+ current induced on photo-release of intracellular Ca^{2+} (Fig. 2A) also supports the assertion that the slower time course of inactivation when Ca^{2+} or Ba^{2+} were the charge carriers (Fig. 1) was not limited by the kinetics of photo-release and cytoplasmic redistribution of the released Ca^{2+}.

We also measured the kinetics of suppression of Na^+ current through the Ca^{2+} channel when Ca^{2+} was increased stepwise in the external bathing solution surrounding the cell (Fig. 3). Photo-release of Ca^{2+} reduced the Na^+ current stepwise through the Ca^{2+} channel with a τ of 0.42 ms (Fig. 3B, trace F − C). Note also that the current remains completely blocked 5 s after the photo-releasing light pulse (Fig. 3A, trace F + 1) when the cell is not continuously perfused with unphotolyzed DM-nitrophen. The similarity in the kinetics of reduction of Na^+ current through the Ca^{2+} channel (Figs. 2 and 3) was consistently seen in all experiments and indicates that, when the charge carrier is Na^+, the Ca^{2+}-induced reduction of the current resembles a rapid block rather than the slower process generally associated with inactivation (*3*). Comparison of Figs. 2 and 3 also shows that, although the kinetics of the block are similar, Ca^{2+} applied from the extracellular side is more effective in blocking the channel than the intracellular Ca^{2+} (if we assume that 50 to 100 μM Ca^{2+} was photo-released in both cases).

We attempted to compare the increase in Ca^{2+} concentration induced by photolysis in the internal dialyzing solution to that in extracted cytoplasm from freshly prepared DRG neurons. The DRG cytoplasm was obtained by solubilizing the neurons (10 to 20 cells) with 0.4% saponin in 120 mM KCl, 10 mM glucose, 20 mM Hepes, and 0.02 mM EGTA (pH 7.5), centrifuging the solution for 15 min at 45,000g at 4°C, then dissolving the pellet in 4 μl of saponin and 2 μl of internal dialyzing solution. The Ca^{2+} released in such a solution, in response to the light pulse, was 50 to 80 μM, similar to that released in a corresponding internal solution without the cytoplasm. These results suggested that the neuronal cytoplasm does not have sufficient buffering capacity to significantly sequester the Ca^{2+} released by photolysis of DM-nitrophen. Our results therefore suggest that the dissociation constants (K_d) of the block of the Ca^{2+} channel by Ca^{2+} from the extra- and intracellular sites are markedly different. This observation is consistent with the measurements of unitary currents of the low-threshold Ca^{2+} channel in outside-out patches of DRG neurons, where $[Ca^{2+}]_i$ in the range of 1 mM were ineffective in blocking the Na^+ current through the Ca^{2+} channel (*12*).

We were surprised to find that the kinetics of Ca^{2+}-induced inactivation or block of Ca^{2+} channels in part depends on the ionic charge carrier through the Ca^{2+} channel. When Na^+ was the charge carrier, the intracellular Ca^{2+}-induced block was immediate and showed little time dependence, characteristics previously assumed to be inherent to the Ca^{2+}-induced inactivation process (*3*) (Fig. 1). This suggests that Ca^{2+}-dependent inactivation is either absent (for example, Ca^{2+} channels are in different conformational states) or that those channels that are blocked by Ca^{2+} would have been the channels that inactivated. The latter possibility implies that inactivation takes place at a site closely associated with the entry sites of the blocking Ca^{2+} ions, that is, the internal channel mouth. On the other hand, the differential block of the high-threshold Ca^{2+} channel by ω-conotoxin [depending on whether they are permeant to Na^+ or Ca^{2+} in DRG neurons (*13*)] supports the possibility that Ca^{2+} channels may be in different conformational states when they transport monovalent ions. Irrespective of the exact mechanism of Ca^{2+}-induced inactivation, our data show that Ca^{2+}-induced inactivation exhibits a τ of 7 to 8 ms and that the kinetics of this process depend on the valency of the ionic species transported.

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