RECOVERY OF CALCIUM CHANNELS FOLLOWING RAPID PHOTOINACTIVATION OF DIHYDROPYRIDINES IN MAMMALIAN VENTRICULAR MYOCYTES

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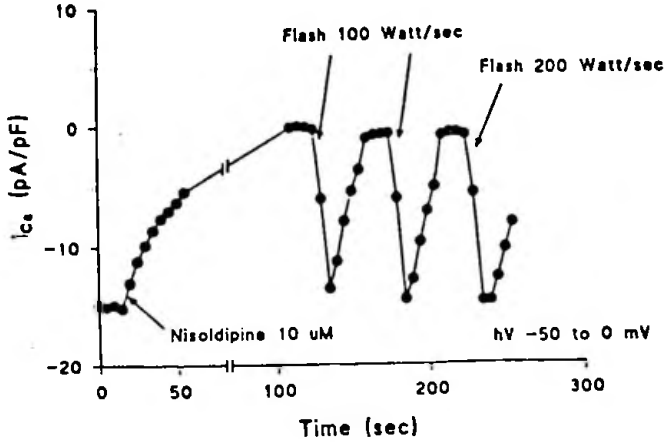
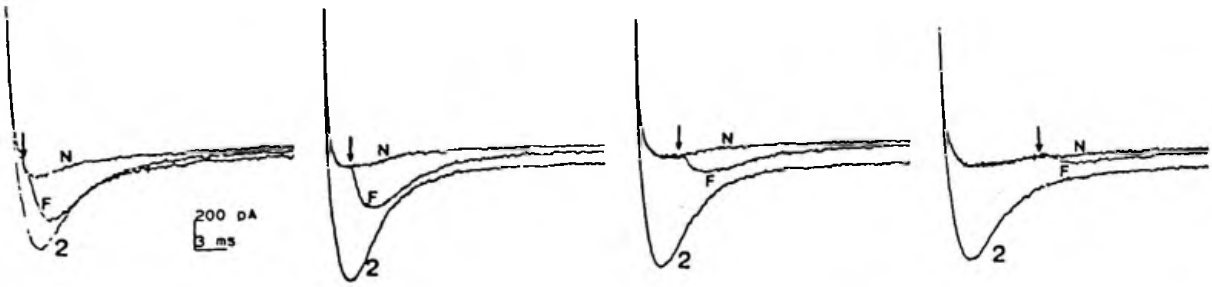
Dihydropyridine (DHP) calcium channels blockers are highly light sensitive. The aromatization of the dihydropyridine ring by UV light causes inactivation of the DHP and loss of their calcium channel blocking property. We have studied the kinetics of the recovery of calcium currents from nisoldipine block when a brief (180 μsec) light pulse was used to photoinactivate the drug. Inactivation of nisoldipine was independent on the intensity of the light pulse in the range of 50-200 J. In addition, the recovery of calcium current following photoinactivation of nisoldipine was dependent of the interval between the light pulse and the activation of the calcium current.

Single rat ventricular myocytes were enzymatically dissociated (Mitra and Morad, Am. J. Physiol. 249: H1056-H1060, 1985). Experiments were performed at room temperature (20-22 °C) using the whole cell patch-clamp technique (Hamill et al., Pflügers Arch. 391: 85-100, 1981). Patch pipettes had a resistance of 2-3 MΩ when filled with an intracellular solution containing (in mM): 10 NaCl, 100 CsAsp, 20 TEACl, 5 MgATP, 14 EGTA, 20 HEPES, 0.1 cAMP, pH 7.2. Extracellular solutions were exchanged rapidly (<50 msec) by using an electronically controlled concentration clamp system. Nisoldipine (1-10 μM) was added to the extracellular solution containing (in mM): 10NaCl, 127 CsCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, pH 7.4 and the solution protected from ambient light. Photolysis of DHP was achieved by the use of a Xenon arc lamp (240 J maximum output). Flashes (duration of about 0.180 msec) were focused onto the cell under investigation as described by Morad et al. (Mature 304, 635-638, 1983).

Figure 1 shows the effect of a light pulse of different intensity applied 10 msec prior to activation of Ca^{2+} channel by a 25 msec depolarizing pulse from -90 to 0 mV. Following addition of nisoldipine (10 μM) which blocked the Ca^{2+} current strongly, photoinactivation of the drug led to rapid recovery of I_{Ca}. The development of the block was significantly slower than unblocking the channel following the photoinactivation of nisoldipine. Maximum and often full recovery of Ca^{2+} current was consistently found only with the second depolarizing pulse following the light pulse. Figure 1 also shows significant difference in time course of nisoldipine-induced block of Ca^{2+} channel prior and following the photoinactivation of nisoldipine. This discrepancy in the time course of the block may be in part related to equilibration of the membrane with lipophilic drug following the 1st exposure to the drug.



The time course of recovery of Ca^{2+} current within the time course of depolarizing pulse was also tested by applying the photoinactivating pulse prior to, during and following the depolarizing pulse. Figure 2 shows original traces recorded in presence of nisoldipine (N) and after the application of a light pulse of 100 J (arrow) at different times during the depolarizing pulse (F). Maximum recovery was always reached at the second depolarizing pulse (2) after the light pulse application.



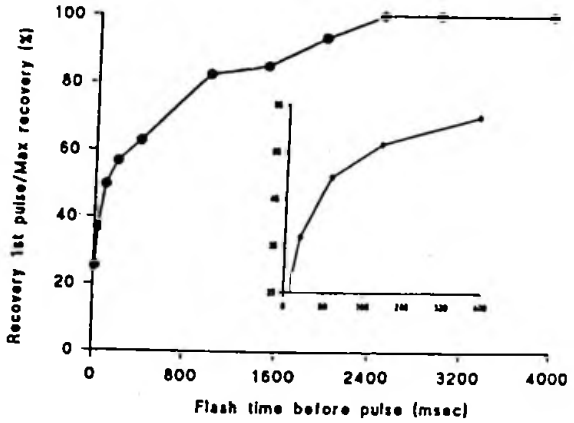


Figure 3 illustrates the time-dependency of the light-induced recovery of the calcium current when a 100 J light pulse was applied at different times before the application of a depolarizing pulse to 0 mV from a holding potential of -50 mV. Maximum recorevy was reached when the light pulse preceded 1.5-2 sec the depolarizing pulse. Similar results were obtained at a holding potential of -90 mV. Inset shows data for the first 400 msec using a different scale.

Our results showing that current recovery is bigger when photoinactivation of nisoldipine occurs earlier during the depolarizing pulse supports the idea that DHP calcium channel antagonists promote the movement of the Ca^{2+} channel to a drug-ocluded inactivated state (Morad and Rendt, Exp. Brain Res. (Suppl): 112-123, 1986). It has been suggested that DHPs block the Ca^{2} channel by enhancing the rate of inactivation of the channel. This may explain why flashes given early during depolarization are more effective in eliciting large recovery than those applied later during the depolarization pulse. With subsequent repolarization channels return to the closed state and are then available for full activation upon depolarization.

Our results are consistent with a scheme where DHP type calcium channel blockers appear to block the calcium channel by driving it into a drug-occluded inactivated state.

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