Murrayafoline A modulation of rat vascular myocyte $Ca_v1.2$ channel: functional, electrophysiological and molecular docking analysis

S Saponara[1], M Durante[1], O Spiga[2], P Mugnai[1], G Sgaragli[1], T T Huong[3], P N Khanh[3], N T Son[3], N M Cuong[3] and F Fusi[1]

[1] *Dipartimento di Scienze della Vita, Università di Siena, via A. Moro 2, 53100 Siena, Italy;* [2] *Dipartimento di Biotecnologie, Chimica e Farmacia, Università di Siena, via A. Moro 2, 53100 Siena, Italy and* [3] *Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Cau Giay, Hanoi, Vietnam*

Methods

Whole-cell patch-clamp recordings

Recording electrodes were pulled from borosilicate glass capillaries (WPI, Berlin, Germany) and fire-polished to obtain a pipette resistance of 2-5 MΩ when filled with internal solution (see below). An Axopatch 200B patch-clamp amplifier (Molecular Devices Corporation, Sunnyvale, CA, USA) was used to generate and apply voltage pulses to the clamped cells and record the corresponding membrane currents. At the beginning of each experiment, the junction potential between the pipette and bath solution was electronically adjusted to zero. Current signals, after compensation for whole-cell capacitance and series resistance (between 70-75%), were low-pass filtered at 1 kHz and digitized at 3 kHz prior to being stored on the computer hard disk. Electrophysiological responses were assessed at room temperature (20-22°C).

$I_{Ba1.2}$ and $I_{Ca1.2}$ recordings

Steady-state inactivation curves were obtained using a double-pulse protocol. Once various levels of the conditioning potential had been applied for 5 s, followed by a short (5 ms) return to the V_h, a test pulse (250 ms) to 0 mV was delivered to evoke the current. The delay between the conditioning potential and the test pulse allowed full or near complete deactivation of the channels simultaneously avoiding partial recovery from inactivation.

Activation curves were derived from the current-voltage relationships (as shown in Figure 4B). Conductance (G) was calculated from the equation $G = I_{Ba(L)} / (E_m - E_{rev})$, where: $I_{Ba(L)}$ is the peak current elicited by depolarizing test pulses in the range -50 to 20 mV from V_h of -50 mV; E_m is the membrane potential; and E_{rev} is the reversal potential (181 mV, as estimated with the Nernst equation). G_{max} is the maximal Ba^{2+} conductance (calculated at potentials ≥5 mV). The ratio G/G_{max} was plotted against the membrane potential and fitted with the Boltzmann equation.

Results

Effect of murrayafoline A on aorta rings contracted by phenylephrine

The vascular effects of MuA were assessed on rat aorta rings contracted sub-maximally with 0.3 µM phenylephrine. When a steady tension was obtained, MuA was added cumulatively causing a concentration-dependent relaxation of vascular tone (Figure 1).

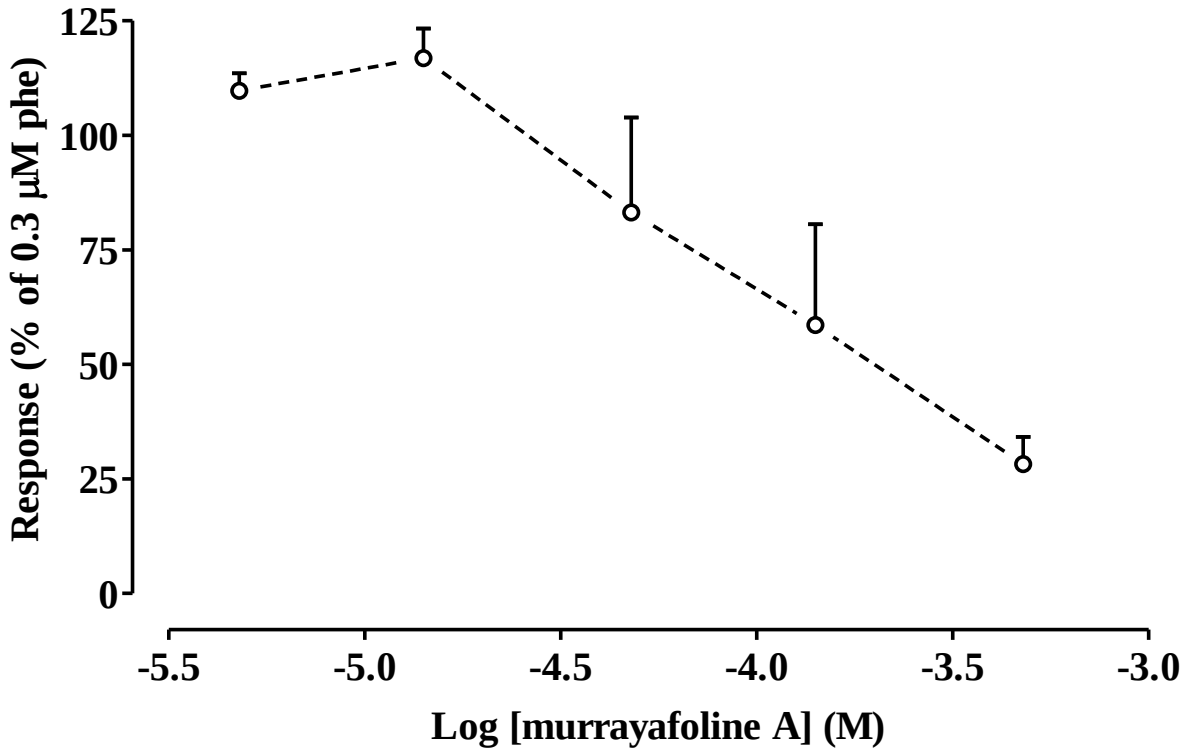

Figure 1 Effect of murrayafoline A on phenylephrine-induced contraction of rat aorta rings. Concentration-response curves of MuA in endothelium-denuded rings precontracted by 0.3 µM phenylephrine. In the ordinate scale, response is reported as percentage of the initial tension induced by phenylephrine (phe), taken as 100%. Data points are mean ± SEM (n = 6).

Effect of murrayafoline A on $I_{Ba1.2}$ in the absence or presence of PKC inhibitors

Since protein kinase C (PKC) plays a major role in the modulation of $Ca_v1.2$ channels, the effect of MuA on $I_{Ba1.2}$ was assessed in the absence or presence of the cell permeable, competitive PKC inhibitor GF109203X or the more selective, Ca^{2+}-dependent PKCα and PKCβ1 inhibitor Gö6976. The concentration-stimulation curve that characterized the effect of MuA (pEC_{50} 5.44 ± 0.03, *n* = 9) was only slightly, though not significantly affected when myocytes were pre-treated with either 5 µM GF109203X (pEC_{50} value of 5.25±0.11, *n* = 6; P>0.05 vs MuA alone) or 100 nM Gö6976 (pEC_{50} value of 5.28±0.12, *n* = 6; P>0.05) (Figure 2). The Ca^{2+} antagonistic effect exerted by 473.4 µM MuA was comparable under the above-mentioned, three different experimental conditions.

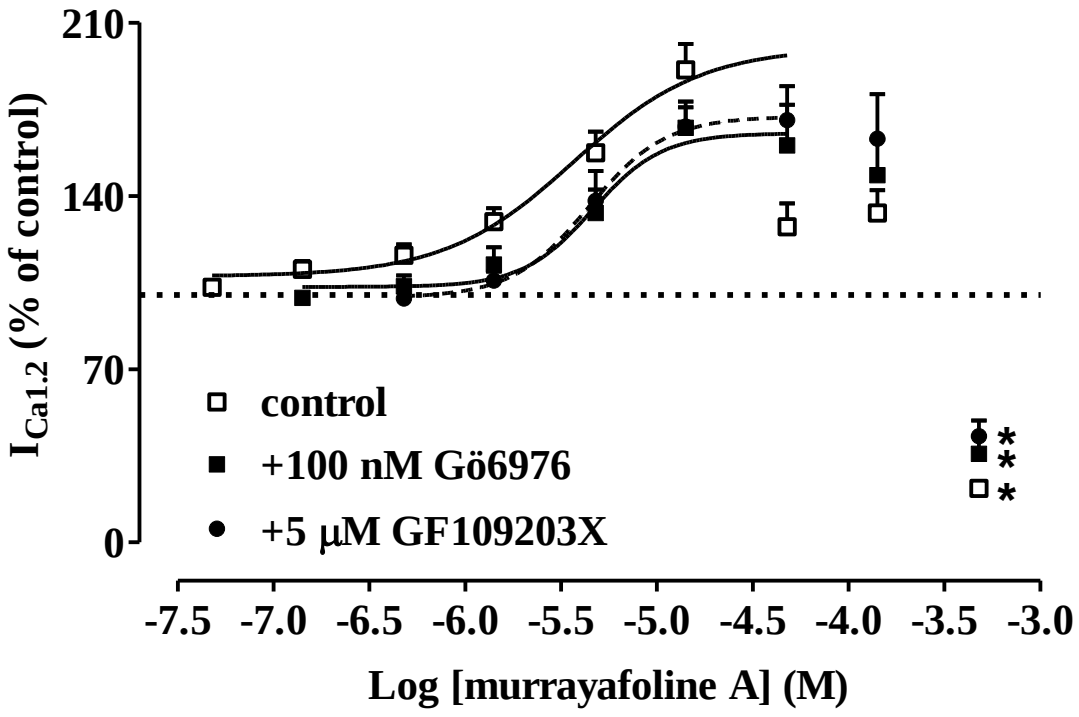

Figure 4 Murrayafoline A modulation of $I_{Ba1.2}$ of single rat tail artery myocytes. Effect of GF109203X and Gö6976 on MuA-induced modulation of $I_{Ba1.2}$. Concentration-dependent effect of MuA measured at V_h of -50 mV in the absence (control) or presence of either 5 µM GF109203X or

100 nM Gö6976. On the ordinate scale, response is given as a percentage of control. Data points are mean ± SEM. (*n* = 5-9). * P < 0.05 vs. control (100%), one sample *t* test.