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The vasodilator papaverine stimulates L-type Ca^{2+} current in rat tail artery myocytes via a PKA-dependent mechanism

Fabio Fusi [a,*], Fabrizio Manetti [b], Miriam Durante [a], Giampietro Sgaragli [a], Simona Saponara [a]

[a] Dipartimento di Scienze della Vita, Università degli Studi di Siena, via A. Moro 2, 53100 Siena, Italy
[b] Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, via A. Moro 2, 53100 Siena, Italy

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ABSTRACT

Papaverine is an opium alkaloid, primarily used as an antispasmodic drug and as a cerebral and coronary vasodilator. Its phosphodiesterase inhibitory activity promotes increase of cAMP levels mainly in the cytosol. As cAMP is known to modulate L-type Ca^{2+} channel activity, here we tested the proposition that papaverine could affect vascular channel function. An in-depth analysis of the effect of papaverine on Ba^{2+} or Ca^{2+} current through L-type Ca^{2+} channel [$I_{Ba(L)}$ or $I_{Ca(L)}$], performed in rat tail artery myocytes using either the whole-cell or the perforated patch-clamp method, was accompanied by a functional study on rat aorta rings. Papaverine increased current amplitude under both the perforated or whole-cell configuration. Stimulation of the current by papaverine was concentration-, V_h-, frequency-, and charge carrier-dependent, and fully reverted by drug washout. The PKA inhibitor H89, but not the PKG inhibitor Rp-8-Br-cGMPS, antagonised papaverine- as well as IBMX- (another phosphodiesterase inhibitor) induced $I_{Ba(L)}$ stimulation. In cells pre-treated with IBMX, application of papaverine failed to increase current amplitude. Papaverine sped up the inactivation kinetics of $I_{Ba(L)}$, though only at concentrations ≥30 μM, and shifted the voltage dependence of the inactivation curve to more negative potentials. In rings, the vasorelaxing activity of papaverine was enhanced by previous treatment with nifedipine. In conclusion, papaverine stimulates vascular L-type Ca^{2+} channel via a PKA-dependent mechanism, thus antagonising its main vasodilating activity.

1. Introduction

Papaverine (1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline) is an opium alkaloid that relaxes many types of smooth muscles [13]. Since it inhibits phosphodiesterase activity [19], its relaxing effect has been ascribed generally to the rise of intracellular cAMP levels. Due to its smooth muscle relaxant activity, papaverine has been used as a vasodilator [3] for relieving renal colics [14] and penile impotence [12]. Currently, it is approved for treating spasms of the gastrointestinal tract, bile ducts and ureter. During surgery for descending thoracic and thoraco-abdominal aortic aneurysm repairs, inclusion of intrathecal papaverine into the neuroprotective protocol may enhance spinal cord perfusion and provide effective spinal cord protection [20]. Furthermore, when angiographic and symptomatic vasospasm occurred after subarachnoid aneurysmatic haemorrhage, papaverine has been used as a vasodilator [17], alone or in combination with balloon angioplasty [21] and coronary artery bypass surgery [33].

Hundreds of scientific papers dealing with papaverine have been already published since its discovery in 1848. Since 1937, more than 1500 reports that contain the term papaverine as a title word are listed within PubMed. Though in the last decade the number of publications, having as the object papaverine, is drastically decreasing (about 10 to 20 per year), novel hypothetical, pharmacological activities are endeavoured beyond its classical myorelaxant activity. In fact, papaverine is a phosphodiesterase inhibitor selective for the PDE10A subtype, which is found mainly in brain striatum [22]. Although papaverine has only moderate potency (EC_{50} = 36 nM) and poor selectivity (9 fold) over the other PDE isoforms, it was the first compound used to explore the role of PDE10A in the CNS; this represents a new target for the treatment of some CNS diseases [25], such as schizophrenia and psychosis. Furthermore, papaverine may represent a suitable PET probe for imaging PDE10A in vivo.

Though papaverine is widely used in studies with smooth muscle preparations to produce maximal relaxation (akin to that achievable with a Ca^{2+}-free solution), only few studies have investigated its effect on Ca^{2+} currents. The first direct evidence of papaverine inhibition of voltage-dependent L-type Ca^{2+} channels was obtained from guinea

Abbreviations: AUC, area under the curve; $I_{Ba(L)}$, L-type Ba^{2+} current; $I_{ca(L)}$, L-type Ca^{2+} current.

* Corresponding author.
E-mail addresses: fabio.fusi@unisi.it (F. Fusi), fabrizio.manetti@unisi.it (F. Manetti), durante6@unisi.it (M. Durante), sgaragli@unisi.it (G. Sgaragli), simona.saponara@unisi.it (S. Saponara).

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pig trachea smooth muscle cells [13]; this effect was suggested to account for the drug-induced relaxation of tracheal smooth muscle. More recently, this activity was described also in rat basilar artery smooth muscle cells, though only at 100 μM concentration [11]. Nevertheless, the subcellular mechanisms underlying papaverine-induced block of $I_{Ca(L)}$ still remain unsettled. In the present study, we examined the effects of papaverine on the voltage-dependent Ca^{2+} channel current of single smooth muscle cells isolated from the rat tail main artery under the voltage clamp condition. Surprisingly, we found that papaverine effectively stimulated $I_{Ca(L)}$ in a PKA-dependent manner, thus partially counteracting its vasorelaxing activity in aorta ring preparations.

2. Materials and methods

2.1. Animals

All animal care and experimental protocols conformed to the European Union Guidelines for the Care and the Use of Laboratory Animals (European Union Directive 2010/63/EU) and were approved by the Italian Department of Health (666/2015-PR). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals [18,24]. A total of 32 animals were used in the experiments described here. Male Wistar rats (8–11 weeks old – 300-400 g, Charles River Italia, Calco, Italy) were anaesthetized (i.p.) with a mixture of Ketavet® (30 mg kg^{-1} ketamine; Intervet, Aprilia, Italy) and Xilor® (8 mg kg^{-1} xylazine; Bio 98, San Lazzaro, Italy), decapitated and exsanguinated. The tail was cut immediately, cleaned of skin and placed in physiological solution (namely external solution, containing in mM: 130 NaCl, 5.6 KCl, 10 Hepes, 20 glucose, 1.2 $MgCl_2 \cdot 6\ H_2O$, and 5 Na-pyruvate; pH 7.4) containing 20 mM taurine (prepared by replacing NaCl with equimolar taurine). The tail main artery was dissected free of its connective tissue.

2.2. Cell isolation procedure

Smooth muscle cells were freshly isolated from the tail main artery under the following conditions: a 5-mm long piece of artery was incubated at 37 °C for 40–45 min in 2 ml of 0.1 mM Ca^{2+} external solution containing 20 mM taurine, 1.35 mg ml^{-1} collagenase (type XI), 1 mg ml^{-1} soybean trypsin inhibitor, and 1 mg ml^{-1} bovine serum albumin, which was gently bubbled with a 95% O_2 - 5% CO_2 gas mixture to gently stir the enzyme solution, as previously described [6]. Cells, stored in 0.05 mM Ca^{2+} external solution containing 20 mM taurine and 0.5 mg ml^{-1} bovine serum albumin at 4 °C under normal atmosphere, were used for experiments within two days after isolation [26].

2.3. Whole-cell patch clamp recordings

Cells were continuously superfused with external solution containing 0.1 mM Ca^{2+} and 30 mM tetraethylammonium (TEA) using a peristaltic pump (LKB 2132, Bromma, Sweden), at a flow rate of 400 μl min^{-1}. Conventional [10] and amphotericin B-perforated whole-cell patch-clamp methods [28] were employed to voltage-clamp smooth muscle cells. 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. The internal solution for the conventional method (pCa 8.4) consisted of (in mM): 100 CsCl, 10 HEPES, 11 EGTA, 2 $MgCl_2$, 1 $CaCl_2$, 5 Na-pyruvate, 5 succinic acid, 5 oxaloacetic acid, 3 Na_2-ATP and 5 phosphocreatine; pH was adjusted to 7.4 with CsOH. For the perforated method, the internal solution (pCa 8.4) contained (in mM): 125 CsCl, 10 HEPES, 11 EGTA, 2 $MgCl_2$, 1 $CaCl_2$, amphotericin B (200 μg/ml); pH was adjusted to 7.4 with CsOH. Amphotericin B (100 mg/ml) was first dissolved in DMSO and then added to the internal solution.

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 and 80%), were low-pass filtered at 1 kHz and digitized at 3 kHz prior to being stored on the computer hard disk. Electrophysiological responses were tested at room temperature (20–22 °C).

The current through L-type Ca^{2+} channels was recorded in external solution containing 30 mM TEA and 5 mM Ca^{2+} or Ba^{2+}. Current was elicited with 250-ms clamp pulses (0.067 Hz) to 10 mV from a V_h of −50 mV or −80 mV. Cells wherein experiments at a V_h of −80 mV were performed, displayed L-type but not T-type Ca^{2+} currents (see [27]). To compare the effects of papaverine in the presence of either Ca^{2+} or Ba^{2+} as the charge carrier, preliminary experiments were performed to assess whether papaverine-induced stimulation of the current–voltage relationship was constant in the range 0–20 mV. The maximum of the relationship, in fact, falls within this range with both Ca^{2+} (see [26]) and Ba^{2+} (see [7]): therefore, 10 mV was chosen as the appropriate test pulse.

Data were collected once the current amplitude had been stabilised (usually 7–10 min after the whole-cell configuration had been obtained). At this point, the various experimental protocols were performed as detailed below. Under these conditions, the current did not run down during the following 40 min [8].

Steady-state activation curves were derived from the current–voltage relationships. 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 between −50 and 30 mV from V_h of −80 mV; E_m is the membrane potential; and E_{rev} is the reversal potential (estimated from the extrapolated current–voltage curves in Fig. 2B). G_{max} is the maximal Ca^{2+} conductance (calculated at potentials ≤30 mV). The G/G_{max} ratio was plotted against the membrane potential and fitted with the Boltzmann equation [16].

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 10 mV was delivered to evoke the current. The delay between the conditioning potential and the test pulse allowed the full or near-complete deactivation of the channels simultaneously avoiding partial recovery from inactivation.

The frequency-dependence of papaverine-induced effects on $I_{Ba(L)}$ was assessed applying ten or twenty depolarizing pulses of 50-ms duration to 10 mV from V_h of −80 mV at decreasing pulse intervals, ranging from 30, to 3 and 0.3 s (namely 0.033, 0.33, and 3.3 Hz) under control conditions. At the end of the protocols, papaverine was added to the bath solution and, after a 4-min interval without stimulation, the same protocols were repeated.

K^+ currents were blocked with 30 mM TEA in the external solution and Cs^+ in the internal solution. Current values were corrected for leakage and residual outward currents using 10 μM nifedipine, which completely blocked $I_{Ba(L)}$ and $I_{Ca(L)}$.

The osmolarity of the 30 mM TEA- and 5 mM Ca^{2+} or Ba^{2+}-containing external solution (320 mosmol) and that of the internal solution (290 mosmol; [32]) were measured with an osmometer (Osmostat OM 6020, Menarini Diagnostics, Florence, Italy).

2.4. Aorta rings preparation and functional experiments

Rings (2-mm wide) were prepared from the rat aorta as previously described [29]. The endothelium was removed by gently rubbing the lumen of the ring with the curved tips of a forceps. Contractile isometric tension was recorded as described elsewhere [4]. Control preparations were challenged with the drug vehicle only.

The vasodilating effect of papaverine was assessed on rings precontracted with 0.3 μM phenylephrine, either in the absence or presence of 30 nM nifedipine. After tone induction, nifedipine was added; this