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Nitric oxide donors induce calcium-mobilisation from internal stores but do not stimulate catecholamine secretion by bovine chromaffin cells in resting conditions

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Abstract

The potential role of nitric oxide (NO) donors and peroxynitrites on both basal catecholamine (CA) secretion and modulation of calcium levels has been investigated in primary cultures of bovine chromaffin cells. NO donors did not modulate catecholamine secretion, while peroxynitrites induced a time dose-dependent increase in basal CA secretion. Two facts may explain the lack of these compounds on basal CA secretion. NO donors induce, on the one hand, an increase in intracellular calcium levels by depletion of internal IP_3-stores from endoplasmic reticulum. On the other hand, a small calcium influx through N-type voltage-dependent calcium channels (VDCC), which seem not to be coupled to exocytosis of adrenaline and noradrenaline in chromaffin cells. Both effects, calcium-mobilisation from internal stores and calcium entry through N-type VDCC are mediated by cGMP synthesis. In contrast, peroxynitrites induce an increase in basal CA secretion by both a decrease of intracellular catecholamine content and a toxic effect on cellular membrane. All these results, taken together, could explain contradictory results in the literature on the role of NO on basal catecholamine secretion and on modulation of intracellular calcium in chromaffin cells.

Keywords: Nitric oxide; Chromaffin cells; Intracellular calcium-mobilisation; Peroxynitrite; Catecholamine secretion

1. Introduction

Nitric oxide (NO) is a highly reactive radical that plays an important role in the regulation of vascular tone, neuronal transmission and modulation of immunological and inflammatory reactions [1–3]. For the last 10 years, it has also been demonstrated that NO is involved in crucial physiological events such as the regulation of neurotransmitter release, and in pathological events underlying neurotoxicity and neurodegeneration [4–6].

NO is synthesised from L-arginine by nNOS in central and peripheral neural tissues. In adrenal chromaffin cells, Moro et al. [7] and Oset-Gasque et al. [8] demonstrated, by hystochemical and biochemical techniques, the presence of a constitutive NOS isoform, which has been identified as nNOS [9,10]. Moreover, Afework et al. [11] and Heym et al. [12] demonstrated the presence of NOS-containing fibres, closely associated with chromaffin cells.

NO has a well-established functional inhibitory role on CA secretion evoked by nicotine in chromaffin cells [8,13]. However, the nature of its functional role in the regulation of basal CA secretion is very controversial. Our previous studies showed that NO gas has an activatory effect on basal CA secretion in chromaffin cells [8], results which seem to be

Abbreviations: cADPR, cyclic adenosine diphosphate-ribose; ω-Aga, ω-agatoxin; BSA, bovine serum albumine; Caf, caffeine; $[Ca^{2+}]_i$, intracellular free calcium concentration; Ca^{2+}, calcium; CA, catecholamine; ω-Ctx, ω-conotoxine GVIA; $dONOO^-$, deactivated peroxynitrite; DMEM, Dulbecco's modified Eagle medium; Fura-2/AM, acetoxymethyl ester of fura-2; cGMP, 3′,5′-cyclic guanosine monophosphate; GSNO, *S*-nitrosoglutathione; Nife, nifedipine; NO, nitric oxide; $ONOO^-$, peroxynitrite; NOS, nitric oxide synthase; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; SNAP, *S*-nitroso-*N*-acetyl-penicillamine; SNP, sodium nitroprusside; thaps, thapsigargin; VDCC, voltage-dependent Ca^{2+} channels

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confirmed by the fact that NO donors mediate an increase in intracellular calcium levels [9], thus implying the possibility of an activatory effect on CA secretion. On the contrary, studies from Machado et al. [14] indicating that NO donors decrease basal CA secretion in single chromaffin cells and recent results from our group showing that L-arginine inhibits basal CA secretion while NOS inhibitors enhance it, seem to demonstrate that NO, via cGMP formation, is involved in the maintenance of low basal CA secretion at resting level [10].

The discrepancies above make us think of a different mechanism for NO donors and NO gas action on basal CA secretion. NO donor's mechanism is possibly mediated by cGMP and might be possibly carried out by a Ca^{2+} mobilization from internal stores. NO gas mechanism may involve the participation of oxidative species of NO, like peroxynitrites.

In order to test these hypothesis and clarify the above indicated dissenting results, we measured the effect of NO donors and peroxynitrites on basal CA secretion and studied the mechanisms by which NO donors produce an increase in $[Ca^{2+}]_i$ in chromaffin cell without modifying CA secretion.

2. Experimental procedures

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) and foetal calf serum (FCS) were from GIBCO (BRL, UK), collagenase from *Clostridium histolyticum* (EC 3.4.4.19) was from Boehringer Mannheim S.A. (Barcelona, Spain). The NO donors sodium nitroprusside (SNP), *S*-nitroso-*N*-acetyl-penicillamine (SNAP), *S*-nitroso-gluthatione (GSNO), antibiotics, Fura-2/AM, cytosine arabinoside, (+)5-fluorodeoxyuridine (FDU), nifedipine and ω-CTX GVIA were from SIGMA Chemical (Madrid, Spain) and ω-Aga IVA. ODQ was obtained from Tocris Cookson (Bristol, UK). Amphotericin B was from ICN Ibérica S.A. (Barcelona, Spain). Cyclic GMP kit was from Amersham International (Buckinghamshire, UK). All other chemicals were reactive grade products from Merck (Darmstadt, Germany).

2.2. Cell isolation and culture

Chromaffin cells were isolated from bovine adrenal glands and cultured as described by Bader et al. [15] with minor modifications. Briefly, glands supplied by a local slaughterhouse were trimmed off fat, cannulated through the adrenal vein and washed with a free-calcium Krebs-HEPES solution (Locke medium) containing (mM): NaCl 154, KCl 5.6, glucose 5.6 and HEPES 5.0, pH 7.5, at 37 °C. Digestion was performed with a 0.2% collagenase plus 0.5% bovine serum albumin solution in the above medium. After digestion, chromaffin cells were removed, dispersed and purified through a Percoll gradient. Cell viability was checked by trypan blue exclusion, and chromaffin cell purity was assessed by the specific incorporation of neutral red to the cells. Both parameters were routinely higher than 90%.

Cells were suspended in DMEM containing 10% FCS, antibiotics (100 units/ml penicillin, 100 mg/ml streptomycin, and 40 mg/ml gentamicin), and cytostatics (10 mmol/l FDU and 10 mmol/l cytosine arabinoside), and plated in 24 Costar cluster dishes at a density of 0.5×10^6 cells/well and used 3–5 days after plating.

2.3. Measurement of CA secretion

Cells were washed twice, at 10-min intervals, with 1 ml of a Krebs-HEPES solution (Locke medium) containing (mM): NaCl 140, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 11, ascorbic acid 0.5 and HEPES 15, pH 7.5, at 37 °C. This medium was removed from the wells and the cells were stimulated for 10 min at 37 °C with 0.5 ml of fresh Locke's medium (control cells) and Locke's medium containing different secretagogues, as indicated in each experiment. At the end of the test incubation period, the medium was taken and cells were lysed with 0.4 mol/l perchloric acid and scraped off the plates.

The incubation medium and the cell lysates were used for the determination of CA secretion and total CA content, respectively. Determination of CA secretion in both sample types, previously diluted and neutralised, was performed with an electrochemical detector (Metrohm 641 VA detector) adjusted to +580 mV and recorded (LKB recorder model 2210). A standard norepinephrine curve was used for calibration. Results were expressed as the percentages of CA release in the incubation medium over the total CA content (incubation medium plus pellet).

2.4. Measurement of nitrite production

Nitrites were determined as described Misko et al. [16], with minor modifications. This method is based on the measurement of the fluorescent product 1-(*H*)-naphtotriazole formed by the reaction of nitrites with 2,3-diaminonaphtalene (DAN) in acidic conditions.

Cells (0.5×10^6/condition) were stimulated for 10 min with 400 μl of the different NO donors mentioned above. After incubation, the medium was aspirated and 100 μl of the different samples were mixed with 50 μl of freshly prepared DAN (0.025 mg/ml in HCl 0.62 M) for 10 min in darkness. The reaction was stopped with 50 μl of NaOH 2.8N and the fluorescence was enhanced by incubating this solution in darkness for 5 min. The volume was completed to 1.5 ml with double-deionised water and the formation of 1-(*H*)-napthtotriazole was measured using a Perkin-Elmer LS50 fluorimeter with excitation and emission wavelengths set to 375 and 415 nm, respectively. The samples were calibrated with a standard curve of freshly prepared nitrites.