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Basal tonic release of nitric oxide coupled to cGMP production regulates the vascular reactivity of the mesenteric bed

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Abstract

To reveal a basal production of nitric oxide (NO) and guanosine 3′,5′ cyclic monophosphate (cGMP) in the rat arterial mesenteric bed, mesenteries were perfused in the absence and in the presence of selective blockers of the L-arginine cascade. Endothelium removal or inhibition of NO synthase significantly reduced the release of NO and tissue cGMP. A significant correlation between these messengers was shown. Blockade of soluble guanylyl cyclase with 0.3–10 μM 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) only reduced basal cGMP production; 1–100 nM sildenafil (Sild), an inhibitor of phosphodiesterase V, increased basal tissue cGMP without modifying the release of NO. Acetylcholine (0.01–10 μM) caused a concentration-dependent rise in NO and cGMP evoking a proportional vasodilatation, demonstrating the interdependence between these messengers and vascular reactivity. Endothelium removal or NO synthase blockade reduced the acetylcholine-induced increase of messengers and the vasodilatation. ODQ attenuated only the increase in cGMP and the vasodilatation, while sildenafil increased cGMP without significantly altering luminal NO release. The present results highlight a tonic release of NO and its involvement in endothelial-smooth muscle signaling; NO and cGMP are determinants of vascular reactivity.

Keywords: Nitric oxide (NO); cGMP; Vasodilatation; Nitric oxide (NO) synthase; Guanylyl cyclase; Phosphodiesterase V; Endothelial signaling

1. Introduction

A variety of chemicals, including hormones, neurotransmitters, drugs and toxins, produce cellular responses through guanosine 3′,5′ cyclic monophosphate (cGMP) signaling. The biochemical mechanisms underlying these responses include the synthesis of the nucleotide following the activation of either the soluble or the particulate guanylyl cyclase (Lucas et al., 2000) and its degradation by numerous phosphodiesterases (Beltman et al., 1995). The recent availability of selective and potent phosphodiesterase inhibitors highlights the role of cGMP in cellular biology opening broad clinical applications. Sildenafil is a high affinity and specific inhibitor of phosphodiesterase V (Chuang et al., 1998; Ballard et al., 1998), the isoenzyme that metabolizes most selectively cGMP (Turko et al., 1999). While this enzyme was originally described in the corpus cavernosum, it is now known to be widely distributed along the vascular system (Wallis et al., 1999).

Since the discovery that endothelium-derived relaxing factor (EDRF) is nitric oxide (NO), an avalanche of publications has recognized its key role in cell to cell communication, including endothelium and neuronal signaling and the immune response following pathogen infection (Rees et al., 1989; Lancaster, 1992; Moncada and Higgs, 1995). Due to its paramagnetic properties (Ignarro, 1990), NO is an endogenous activator of soluble guanylyl cyclase, raising the intracellular levels of cGMP which relax smooth muscle (Moncada et al., 1991). This nucleotide activates protein kinases related to the intracellular transport of calcium; the specificity of the cellular response to cGMP is dictated by cGMP-binding motifs in target proteins (Lincoln et al., 1994; Carvajal et al., 2000). The unraveling of the L-arginine/NO/cGMP pathway and its involvement in endothelial-smooth muscle signaling provides new clinical opportunities in the therapy of cardiovascular, urogenital and central nervous system disorders (Schmidt et al., 1993).

Acetylcholine and bradykinin were first characterized as endogenous activators of the L-arginine cascade (Furchgott and Zawadzki, 1980; Cherry et al., 1982). Shear stress acting on the endothelium is another critical determinant of

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endothelium-smooth muscle signaling. Endothelial cells sense the changes in blood flow, facilitating the activation of the L-arginine cascade, imposing a tonic regulation on blood flow (Kuo et al., 1991; Davies and Tripathi, 1993). In the search of a tonic production of basal NO and tissue cGMP, Buvinic and Huidobro-Toro (2000) consistently observed that the sole perfusion of the isolated rat mesenteric bed with drug-free buffer evidenced a basal release of NO and cGMP to the lumen. In further support of a tonic activation of the L-arginine cascade, we now document that pharmacological blockade of the L-arginine pathway alters the basal release of luminally accessible NO and the corresponding tissue cGMP content. Moreover, we expand this concept to include acetylcholine, a prototype of a receptor-mediated process known to activate the L-arginine cascade, in a further attempt to establish an inter-relationship between the luminal release of NO, tissue cGMP production and vascular reactivity. The present results allow the proposal that blood vessels are under a basal NO/cGMP-tone, which is determinant in the control of vascular reactivity.

2. Materials and methods

2.1. Perfusion of the rat arterial mesenteric bed

Adult male Sprague–Dawley rats (250–300 g) bred in our Animal Reproduction Laboratories were anesthetized with 40 mg/kg sodium pentobarbital. The abdominal cavity was opened at the midline; the superior mesenteric artery was cannulated with polyethylene tubing and perfused with Tyrode buffer bubbled with 95% O_2/5% CO_2 at 37 °C. Perfusion of the arterial bed was performed using a peristaltic pump at a flow of 2 ml/min. The mesenteric bed was next excised from the intestines and placed in a dish specially designed for perfusate collection (Donoso et al., 1996). A pressure transducer was placed close to the entrance of the artery; the transducer was connected to a Grass polygraph. Fluctuations in the perfusion pressure were interpreted as changes in the resistance of the arterial bed. Mesenteries were perfused for 20 min prior to drug challenges. Drugs were dissolved in Tyrode buffer and applied by perfusion; concentrations are expressed as moles/liter.

Basal messenger levels refer to the amount of NO accessible to the perfusate or the tissue content of cGMP following a 20-min perfusion of the mesenteries with drug-free buffer at a flow of 2 ml/min. We consider that luminally accessible NO is a reflection of its endothelial production, since NO is unique in its ability to diffuse quickly in both aqueous and lipid phases, allowing a rapid three-dimensional spread (Wood and Garthwaite, 1994). Likewise, the tissue production of cGMP reflects NO-stimulated guanylyl cyclase activation. Results compare the mean changes in luminal NO and tissue cGMP production in separate protocols performed in the presence and in the absence of the several L-arginine cascade blockers.

2.2. Quantification of luminally accessible NO, tissue cGMP and vasodilatation

2.2.1. Measurements of NO by chemiluminescence

Samples of the mesenteric perfusate were collected in 5-ml test tubes every minute and immediately sealed with parafilm. The sample NO content was quantified using a Sievers 280 analyzer within 60 min. To reduce the nitrites in each sample, the instrument reaction chamber was filled with 8 ml of glacial acetic acid containing 100 mg of potassium iodide at room temperature. Fifty microliters of a sample was injected to the chamber; a stream of N_2 carried the resulting NO to a cell in which the specific chemiluminescence generated by the NO–ozone reaction was quantified (Boric et al., 1999). Calibration of the equipment was performed with 10–1000 nM sodium nitrite. The equipment allows detecting 0.5–1 pmol NO (10–20 pmol/ml). Background buffer readings were subtracted to determine the net NO release. Results are expressed either as the basal value of the luminally accessible NO (pmol/ml), or as the integrated area of the NO peak produced by acetylcholine over basal values (ΔNO, pmol).

2.2.2. Radioimmunoassay of cGMP

The nucleotide was quantified using a 10-fmol threshold RIA for acetylated cGMP. As a tracer 2′-*O*-succinylguanosine 3′,5′-cyclic monophosphate tyrosyl methyl ester was used; the nucleotide was labeled locally with ^{125}I (Boric et al., 1999).

Following the determination of luminally accessible NO, all mesenteries were processed to determine tissue cGMP content. The mesenteries were homogenized in 3-ml 10% trichloroacetic acid and centrifuged 30 min at 3000 rpm (4 °C). The aqueous phase was extracted four times with 4 vol. of ethyl ether each run. The samples were dried on a speed-vac and stored at −20 °C for less than a week until the radioimmunoassay was performed. Results are expressed as picomoles per gram of wet tissue.

2.2.3. Acetylcholine-induced vasodilatation

To assess the acetylcholine-induced vasodilatation, mesenteries were precontracted with 10 μM noradrenaline (NA); once the vasoconstriction reached a plateau, 0.01–10 μM acetylcholine was added for 6 min, maintaining noradrenaline in the buffer. The preparations were next perfused with drug-free buffer for additional 15 min, the time required to reach basal perfusion pressure. An acetylcholine concentration–response curve was performed applying three to four concentrations in each preparation. Vasodilatation was expressed as a percentage of the noradrenaline-induced contraction.

To assess the viability of the preparations, mesenteries were routinely contracted with 10 μM noradrenaline, a concentration that caused a sharp 40 mm Hg rise in perfusion pressure. The few noradrenaline-resistant preparations were discarded.

2.3. Pharmacological blockade of the L-arginine / NO / cGMP cascade and removal of endothelium cell layer

2.3.1. General protocol

Three groups of rats were used to perform the following protocols: (1) determination of the basal luminal release of NO and cGMP tissue content, (2) determination of the luminal release of NO and tissue cGMP induced by a 6-min perfusion with 0.1–1 μM acetylcholine in non-precontracted tissues, (3) determination of the vasorelaxation evoked by 1 μM acetylcholine in noradrenaline pre-contracted tissues. Each group of animals was composed of four subgroups in which we assessed: (A) the effect of endothelium removal, (B) inhibition of NO synthase with *N*ω-nitro-L-arginine, (C) blockade of soluble guanylyl cyclase with ODQ, and (D) blockade of phosphodiesterase V with sildenafil. Results compare the mean changes in luminal NO, tissue cGMP production and acetylcholine-induced vasodilatation in the absence and in the presence of L-arginine cascade blockers and endothelium denudation.

2.3.2. Endothelium removal

Endothelium denudation was achieved applying 0.1% saponin for 55 s followed by drug-free buffer perfusion for the next 30 min (Peredo and Enero, 1993; Donoso et al., 1996).

2.3.3. Nω-nitro-L-arginine (L-NNA)

Endothelial NO synthase was blocked with 100 μM L-NNA perfused for 45 min (Boric et al., 1999).

2.3.4. 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ)

The concentration of ODQ required to block the synthesis of cGMP in the mesenteric bed was assessed perfusing the mesenteries with 0.3–10 μM ODQ for 20 min prior to cGMP measurements (Garthwaite et al., 1995). Most of the protocols were performed using 3 μM ODQ.

2.3.5. Sildenafil studies

To assess the concentration of this drug required to block cGMP degradation in this preparation, cGMP production was quantified following 1–100 nM sildenafil perfusion for 25 min, according to the studies of Ballard et al. (1998). Most of the protocols were performed with 10 nM sildenafil.

2.4. Drug sources

The composition of the Tyrode buffer is (mM): NaCl 118, KCl 5.4, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 23.8 and glucose 11.1. The buffer's reagents were obtained from Merck, Chile. Acetylcholine and noradrenaline hydrochlorides, saponin, *N*ω-nitro-L-arginine (L-NNA) and 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) were purchased from Sigma (St. Louis, MO, USA). Pfizer Central Research (Sandwich, UK) generously donated a sample of sildenafil citrate.

2.5. Statistical analysis

One and two-way ANOVA's, linear correlation analysis and Student's "*t*-test" were used throughout. Dunnett's tables for multiple comparisons with a single control were used when appropriate. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Luminally accessible NO and tissue cGMP basal values before and after blockade of the L-arginine cascade

3.1.1. NO release

Basal luminally accessible NO ranged from 41 to 228 pmol/ml; the mean value was 104 ± 5.6 pmol/ml ($n = 54$). Following endothelium denudation, the mean luminally accessible NO was reduced to 28 ± 3 pmol/ml ($P < 0.01$, $n = 12$). Likewise, L-NNA caused a 55% reduction in the mean basal NO production (47 ± 3 pmol/ml, $P < 0.01$, $n = 12$). Neither 0.3–10 μM ODQ nor 1–100 nM sildenafil caused significant changes in basal NO levels (Figs. 1A and 2).

3.1.2. cGMP tissue content

Basal content of cGMP was 13.7 ± 0.5 pmol/g ($n = 54$); the nucleotide also showed individual variability, ranging from 3.9 to 32.5 pmol/g (Fig. 1C). After endothelium removal or blockade of NO synthase, mean tissue cGMP content was reduced to 3.7 ± 0.6 ($P < 0.01$, $n = 12$) and 1.0 ± 0.2 pmol/g ($P < 0.01$, $n = 12$), respectively (Fig. 1B). While ODQ decreased cGMP in a concentration-dependent manner (Figs. 1B and 2A), sildenafil increased concentration-dependently the tissue cGMP content (Figs. 1B and 2B).

3.1.3. Interdependence between basal endothelial NO release and tissue cGMP production

Emphasizing the inter-relationship between NO release and cGMP production, a significant correlation ($r = 0.79$, $P < 0.01$) was found between these messengers (Fig. 1C). Likewise, following endothelium removal, or NO synthase blockade, a significant correlation between luminal NO and tissue cGMP was evidenced ($r = 0.57$ and $r = 0.58$, respectively; $P < 0.05$, $n = 12$ in each case). No correlation between basal NO release and tissue cGMP was

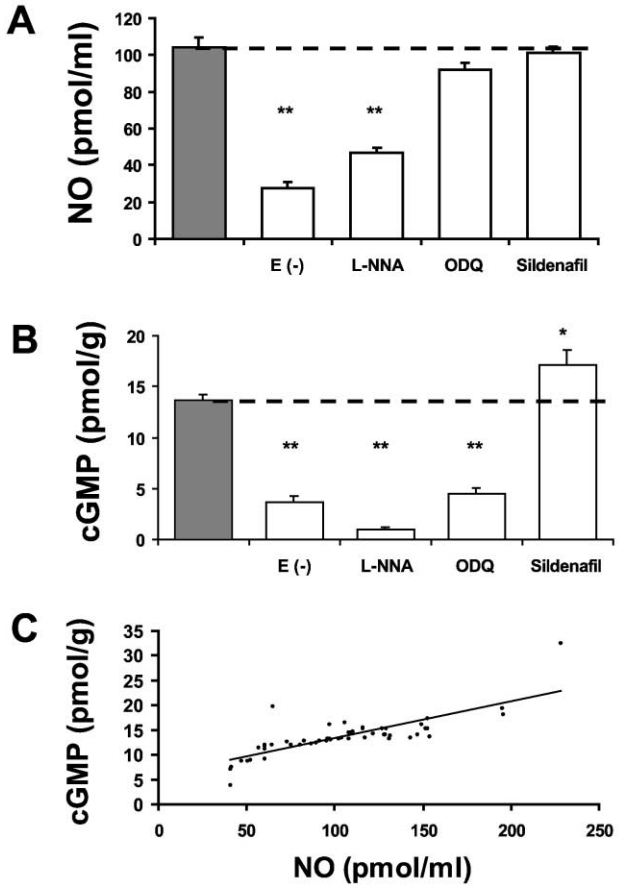

Fig. 1. Basal release of luminal NO and tissue cGMP production following perfusion of the rat arterial mesenteric bed with buffer at a flow of 2 ml/min; effect of L-arginine cascade inhibitors. Fifty-four separate mesenteries were perfused with drug-free buffer (basal conditions, dark columns). Separate protocols studied the effect of: (i) endothelium removal ($n=12$, E(−)), (ii) NO synthase inhibition with 100 μM L-NNA ($n=12$), (iii) guanylyl cyclase blockade with 3 μM ODQ ($n=15$), or (iv) phosphodiesterase V blockade with 10 nM sildenafil ($n=15$). Perfusate samples were collected to measure luminally accessible NO (A) and tissue cGMP (B); (C) correlation between basal luminal NO and basal tissue cGMP production. Columns denote mean values; bars the S.E.M.; $^{*}P<0.05$, $^{**}P<0.01$ as compared to the controls (dark columns); Dunnett's tables.

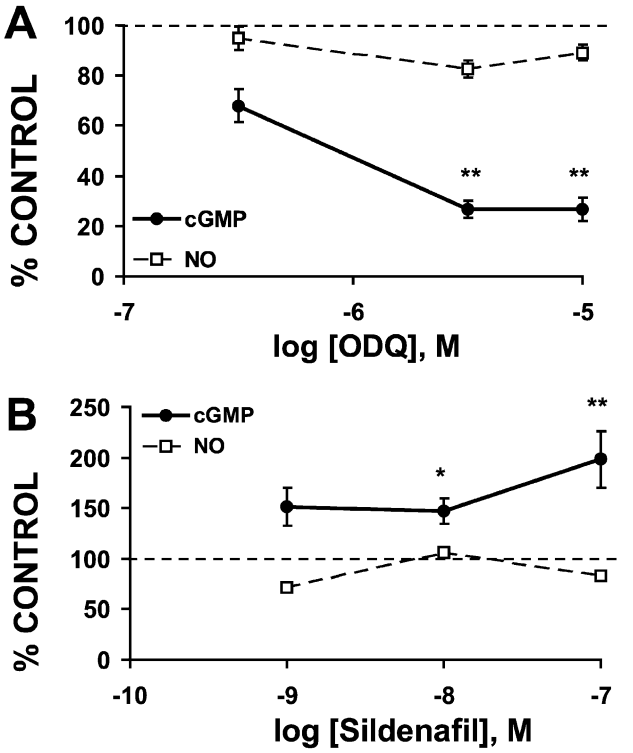

Fig. 2. ODQ and sildenafil concentration–response curves. ODQ (A) and sildenafil (B) concentration–response protocols were performed in separate mesenteries to quantify basal levels of luminally accessible NO and tissue cGMP. Results are expressed on a percentage basis as compared to controls perfused with drug-free buffer. Symbols represent mean values; bars S.E.M. $^{*}P<0.05$; $^{**}P<0.01$; Dunnett's tables.

attained after ODQ or sildenafil, suggesting blockade at a distal step of the cascade.

3.2. Effect of the L-arginine pathway blockade on the noradrenaline-induced vasoconstriction

Endothelium removal, or perfusion with L-NNA or ODQ augmented significantly the noradrenaline-evoked vasoconstriction (Table 1); to cause an equivalent rise in perfusion pressure, its concentration was reduced 10-fold allowing the proper assessment of the acetylcholine-induced vasodilatation (Table 1). After 10 nM sildenafil, even 100 μM noradrenaline failed to contract the mesenteric bed (Fig. 3, Table 1).

3.3. Acetylcholine-induced activation of the L-arginine cascade

Acetylcholine caused a sharp and concentration-dependent vasodilatation associated to a rise in the luminal accessible NO and tissue cGMP (Figs. 3 and 4A); a significant correlation was found between these messengers ($r=0.90$, $P<0.01$, $n=22$). While 1 μM acetylcholine induced full vasodilatation, the rise in NO and

Table 1
Noradrenaline-induced rise in the perfusion pressure of the rat arterial mesenteric bed; effect of L-arginine cascade inhibitors
Values show the mean ± S.E.M.

	Increase in perfusion pressure (mm Hg)	
	1 μM noradrenaline	10 μM noradrenaline
Control	14 ± 2 (7)	40 ± 1 (110)
Endothelium removal	54 ± 2[a] (21)	155 ± 7[a] (23)
100 μM L-NNA	31 ± 2[a] (34)	143 ± 4[a] (20)
3 μM ODQ	31 ± 6[a] (15)	113 ± 26[a] (5)
10 nM sildenafil		0[a] (6)

Numbers in parenthesis indicate the times the experiment was repeated in separate preparations.
[a] $P<0.01$ significantly different from its control (Dunnett's tables).

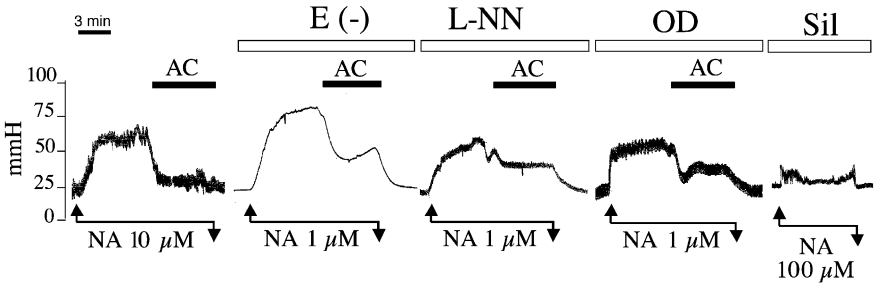

Fig. 3. Representative recordings show the vasodilator action of acetylcholine in mesenteries pretreated with L-arginine cascade blockers. Separate mesenteries were perfused with noradrenaline (NA) to raise the perfusion pressure about 40 mm Hg in order to examine the vasodilatation evoked by 1 μM acetylcholine (AC). Representative tracings show that endothelium removal (E(−)), 100 μM L-NNA, or 3 μM ODQ attenuated the AC-induced vasodilatation. Following these manipulations, the 40-mm Hg rise in vasomotor tone was now elicited with 10 times less NA. 10 nM sildenafil (Sild), annulled the vasomotor response evoked by 100 μM noradrenaline, impeding testing the AC-evoked vasodilatation.

cGMP did not attain the maximal production; 10 μM acetylcholine elicited a 2.5-fold larger increase in NO release and a 4-fold larger increase in cGMP production.

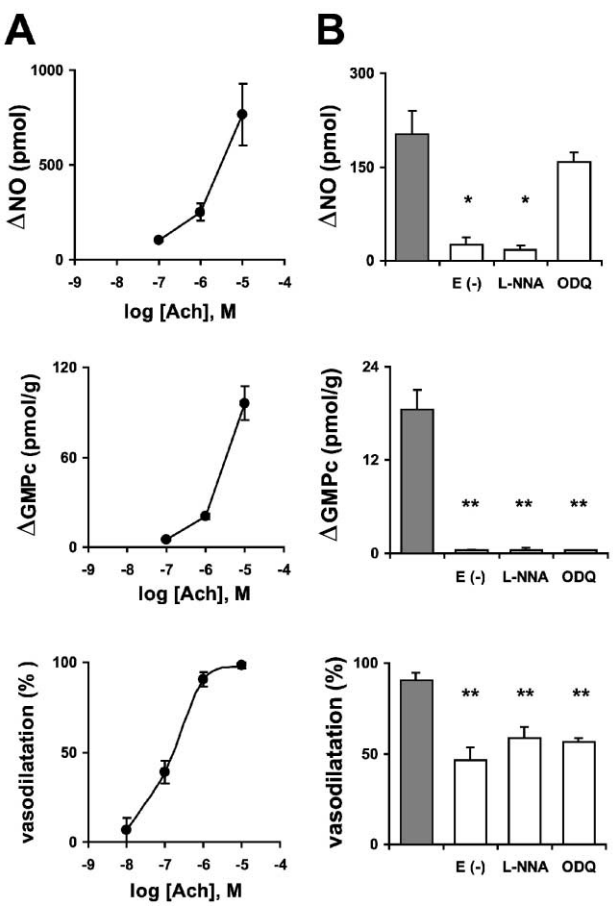

Fig. 4. Concentration-dependent increases in luminal accessible NO, tissue cGMP and vasodilatation induced by acetylcholine and its blockade by L-arginine cascade inhibitors. (A) Concentration-dependent rise in luminally accessible NO (upper panel), tissue cGMP content (middle panel), and vasodilatation (lower panel) elicited by acetylcholine. Symbols represent mean values; bars the S.E.M. (B) The 1 μM acetylcholine-evoked luminally accessible NO release, tissue cGMP and vasodilatation was significantly reduced by endothelium denudation (E(−), $n=4$), 100 μM L-NNA ($n=4$), or 3 μM ODQ ($n=4$). Dark columns represent mean values in the absence of inhibitors; bars the S.E.M. $^{*}P<0.05$, $^{**}P<0.01$; Dunnett's tables.

The acetylcholine-evoked production of NO and tissue cGMP was reduced by about 90% after endothelium removal or blockade of NO synthase (Fig. 4). The vasodilatation was reduced only between 35% and 50%, nonetheless, the reduction was significant (Figs. 3 and 4B, $P<0.01$). ODQ did not modify statistically the peak of NO released while abolishing the drug-induced rise of tissue cGMP ($P<0.01$, $n=4$, Fig. 4B) and reduced 38% the vasodilatation ($P<0.01$, $n=4$). Sildenafil more than doubled the rise in tissue cGMP elicited by 0.1 μM acetylcholine (4.98 ± 0.95 vs. 11.68 ± 2.11 pmol/g, $n=4$, $P<0.05$), without altering the NO released luminally.

4. Discussion

The present results reveal the tonic release of endothelial NO linked to basal guanylyl cyclase activity, which is related to the vascular reactivity of the rat arterial mesenteric bed. This notion relies on the direct measurement of basal NO release and cGMP production and on the recording of perfusion pressure, allowing us to analyze the inter-relationships of these messengers following blockade of key enzymes of the L-arginine cascade. Three experimental results support the interdependence between these messengers: (i) endothelium damage or NO synthase blockade reduces NO and tissue cGMP production, while augmenting significantly the vasomotor action of noradrenaline; (ii) blockade of soluble guanylyl cyclase with ODQ, a procedure which did not interfere with NO production, decreased tissue cGMP and increased the sensitivity of the mesentery to the vasomotor action of noradrenaline; (iii) Sildenafil increased tissue cGMP without modifying NO production. Interestingly, sildenafil antagonised the noradrenaline-induced vasoconstriction.

In view of the three-dimensional spread of NO (Wood and Garthwaite, 1994), the luminal accessible NO is a reflection of the fraction of endothelial NO that reaches the vascular lumen, while the tissue cGMP production denotes the fraction that diffuses to the smooth muscle and activates guanylyl cyclase. The significant inter-relationship

between NO and cGMP observed in the 54 mesenteries used to study the basal production of these messengers, highlights their physiologic interdependence. The extrapolation of the plot shown in Fig. 1C towards the *Y*-axis probably indicates other sources of cGMP generation, which might include the participation of particulate guanylyl cyclase. In support of the direction of the cascade, significant correlation between the production of these molecules was found after endothelium damage or L-NNA, but not after ODQ or sildenafil blockage, consonant with the action of these drugs at a step distal in the cascade. Regarding the clinical application of this concept to human vascular homeostasis, no combined measurements of basal NO and tissue cGMP are available at present in vivo, except for the multiple indirect evidence derived from enzyme inhibition protocols (Moncada and Higgs, 1995).

To further assess the interdependence of NO and cGMP release and their inter-relationship with vascular reactivity, we compared the basal release of NO and cGMP to that elicited through a receptor-mediated process. For this purpose, we chose the endothelial muscarinic receptor, since the acetylcholine-induced vasodilatation is a classical response linked to the L-arginine cascade. The present results indicate that acetylcholine evokes a significant interdependence between luminally accessible NO and tissue cGMP production. As with the basal release protocols, studies with L-NNA, ODQ and sildenafil fully support this interrelationship.

The present results consistently show that the maximal acetylcholine-induced vasodilatation is paralleled with only a fraction of NO and cGMP tissue production. Several factors can account for this discrepancy. Analytical and methodological limitations plus rapid tissue metabolism of NO or cGMP may hinder our interpretations. Likely, an excessive synthesis of either NO or cGMP may play other roles in endothelium-smooth muscle signaling. NO might prolong the duration of the dilatation as a mechanism to counteract physiologically the contraction, in view that NO spreads rapidly in a three-dimensional manner (Wood and Garthwaite, 1994). Finally, we cannot ignore that acetylcholine may release other endothelium mediators, which can contribute substantially to the dilatation.

The present data demonstrate that about 50% of the maximal acetylcholine-induced vasodilatation is related to the activation of the L-arginine pathway. The rest of the acetylcholine-induced dilatation must be accounted by the release of other mediators, including the endothelium-derived hyperpolarizing factor (Chen et al., 1988; Adeagbo and Triggle, 1993; Woodman et al., 2000). In our hands, only 50% of the acetylcholine-induced vasodilatation can be attenuated by endothelial denudation with saponin, a procedure that destroys endothelium integrity. Since the literature details that the acetylcholine-induced vasorelaxation is fully endothelium dependent (Vanhoutte, 1983), it is necessary to assume that the denudation of the endothelial cell layer was incomplete, although histological analysis of the larger vessels show a substantial detachment of the endothelial cells following saponin-treatment (M.V. Donoso, personal communication). Since saponin causes a sharp and maintained increase in perfusion pressure, it is possible that the vasoconstriction partially prevents the exposure of part the mesentery microcirculation to the detergent. Furthermore, the pre-contraction with noradrenaline, a requirement to elicit the acetylcholine-induced vasorelaxation, might change the resistance distribution favoring the perfusion of areas that could have been less accessible to the detergent. We cannot discard that acetylcholine, acting presynaptically on sensory nerve terminals, favors the release of endothelium-independent endogenous vasodilators such as calcitonin gene-related peptide or other compounds.

The physiological mechanisms underlying the maintenance of the tonic release of NO and cGMP remain to be elucidated, but likely, the pulsatile nature of flow plays a pivotal role in determining the activity of the L-arginine/NO/cGMP cascade both in vivo and in vitro. Rees et al. (1989) demonstrated for the first time that endothelium NO production plays a role in the regulation of blood pressure, suggesting a tonic release of NO in blood vessel physiology. Considering that flow is a tonic stimulus to the vessel wall, its participation in the permanent activation of the L-arginine cascade was proposed. In this regard, assays in isolated vessels (Kuo et al., 1991) and in cultured endothelial cells (Kuchan and Frangos, 1994), corroborated the relevance of flow in the maintenance of a tonic NO production involved in blood vessel tone. Moreover, Figueroa et al. (2000) demonstrated that in the resistance vessels of the rat mesenteric bed endothelial NO synthase activity is increased in response to sustained shear stress. In vivo evidences linking the role of flow to endothelial-smooth muscle signaling is lacking at present.

We consistently observed that 10 nM sildenafil results in an insurmountable inhibition of the 10–100 μM noradrenaline-evoked vasoconstriction. In our laboratory, P. de la Cerda (unpublished observations), has observed that 100 nM sildenafil reduces in about 50% the 0.1 μM noradrenaline and 20 mM KCl-evoked contractions in isolated rat aortic rings. Furthermore, the i.v. administration of 0.1 mg/kg sildenafil to anesthetized cats attenuates the baroreflex sympathetic response (D. Capurro, personal communication), suggesting that sildenafil might interfere non-specifically with the contractile machinery. The cellular bases of these findings are unknown; it is plausible to propose that excessive intracellular cGMP interferes with the contractile machinery, as a case of physiological antagonism. Secondary drug effects cannot be discarded.

In conclusion, the tonic basal release of NO and its interdependence with basal tissue cGMP and vascular reactivity was unveiled in the isolated rat arterial mesenteric bed. The rat mesentery, a preparation used 35 years ago to study the pharmacology of sympathetic transmission (McGregor, 1965), is now used as a model to study the

inter-relationship between intracellular messengers and endothelium-mediated vasodilatation. This preparation offers attractive possibilities to study endothelial-smooth muscle signaling, and its pharmacological manipulation. The use of this simple preparation coupled to the analytical determination of intracellular messengers is a valid model for the study of the physiology and pharmacology of novel vascular regulatory mechanisms.

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