Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 496 (2004) 151–158

www.elsevier.com/locate/ejphar

Cocaine-induced relaxation of isolated rat aortic rings and mechanisms of action: possible relation to cocaine-induced aortic dissection and hypotension

Wenyan Li[a], Jialin Su[a], Swati Sehgal[a], Bella T. Altura[a,b], Burton M. Altura[a,b,c,*]

[a] *Department of Physiology and Pharmacology, State University of New York, Downstate Medical Center, Box 31, 450 Clarkson Avenue, Brooklyn, NY 11203-2056, USA*

[b] *The Center for Cardiovascular and Muscle Research, State University of New York, Downstate Medical Center, Brooklyn, NY 11203-2056, USA*

[c] *Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, NY 11203-2056, USA*

Received 19 January 2004; received in revised form 27 May 2004; accepted 8 June 2004

Abstract

Cocaine HCl is well known for its toxic effects on the cardiovascular system, but little is known about its effects on different regional blood vessels. We designed experiments to determine if cocaine HCl could influence the tension of isolated aortic rings, i.e., induce contraction or relaxation. Surprisingly, cocaine HCl (1×10^{-5} to 6×10^{-3} M) relaxed isolated aortic rings precontracted by phenylephrine in a concentration-dependent manner. No significant differences were found between intact or denuded isolated aortic rings ($P>0.05$). The maximal % relaxations of intact vs. denuded isolated aortic rings were $108.9 \pm 24.3\%$ vs. $99.5 \pm 8.3\%$ ($P>0.05$). Cocaine HCl, 2×10^{-3} M, was found to inhibit contractions by phenylephrine; EC_{50}s were increased ($P<0.01$) and E_{max}'s were decreased ($51.3 \pm 16.4\%$ vs. $89.8 \pm 10.6\%$, $P<0.01$). A variety of amine antagonists could not inhibit the relaxant effects of cocaine HCl ($P>0.05$). The cyclooxygenase-1 inhibitor, indomethacin, also failed to inhibit relaxations induced by cocaine HCl ($P>0.05$). Neither L-arginine, N(G)-monomethyl-L-arginine (L-NMMA), nor methylene blue could inhibit the relaxations induced by cocaine HCl ($P>0.05$), suggesting cocaine HCl does not relax isolated aortic rings by inducing the synthesis or release of nitric oxide (NO) or prostanoids from either endothelial or vascular muscle cells. Inhibitors of cAMP, cGMP and protein kinase G (PKG) also failed to inhibit cocaine-induced relaxations. Cocaine HCl (1×10^{-5} to 6×10^{-3} M) could also relax isolated aortic rings precontracted by phenylephrine in high K^+ depolarizing buffer. Surprisingly, calyculin A, an inhibitor of myosin light chain (MLC) phosphatase, inhibited cocaine-induced relaxations in a concentration-dependent manner, suggesting the probable importance of cocaine-induced MLC phosphatase activation in rat aortic smooth muscle cells. It was also found that cocaine HCl could dose-dependently inhibit Ca^{2+}-induced contractions of isolated aortic rings in high K^+–Ca^{2+}-free buffer, suggesting that cocaine HCl may inhibit Ca^{2+} influx and/or intracellular release.

Keywords: Aortic ring; Cocaine; Relaxation; Phosphatase; Ca^{2+} entry; Aortic dissection

1. Introduction

Cocaine HCl abuse has increased dramatically during the last decade, with an abuse population of more than 6,000,000 in the USA alone and it has many adverse effects (Gold, 1993). Among the many complications exhibited by cocaine HCl (in almost every human system and behavior), cardiovascular toxicities are very prominent, especially compared with other controlled drugs. Short-term or long-term abuse of cocaine HCl can cause or exacerbate hypertension, atherosclerosis (Norris et al., 2001; Fogo et al., 1992), coronary artery spasm, myocardial ischemia, cardiac infarction, myocarditis, arrhythmias including ventricular fibrillation (Kliner et al., 1992), cerebral infarction (Daras et al., 1994), nontraumatic intracranial haemorrhage (Mangiardi et al., 1988; Nolte and Gelman, 1989), cerebrovasospasm (Altura et al., 1985; Madden and Powers, 1990; He et al., 1993), stroke and sudden death (Polis et al., 1987; Altura and Gupta, 1992). Cocaine HCl also exerts effects on

* Corresponding author. Tel.: +1-718-270-2194; fax: +1-718-270-3103.

E-mail address: baltura@downstate.edu (B.M. Altura).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.06.015

functions of platelets and coagulation systems, which are also thought to contribute to its cardiovascular toxicities (Konzen et al., 1996; Zurbano et al., 1997). Although high plasma concentrations of cocaine HCl are often associated with dissecting aneurysm (Cohle and Lie, 1992; Alspaugh, 1995), vasculitis and rupture of various vessels (Frederichs and McQuiuen, 1991; Park, 1992) and hypotension (Eisenberg et al., 1996), followed by death, the mechanisms for these adverse phenomena have not been elucidated. Studies of the effects of cocaine HCl on the cardiovascular system, its related mechanisms and possible treatments are thus very important scientifically, clinically and socially.

Our laboratory and several others have focused on cocaine-induced cardiovascular complications and their mechanisms of action. Almost 20 years ago, we first reported that cocaine HCl can induce cerebral vessels to contract and to undergo spasm, brain ischemia and stroke (Altura et al., 1985; He et al., 1993). Similar cerebral vasoconstrictive actions have been observed in a number of mammals (Madden and Powers, 1990; Wagerle et al., 1990; Wang et al., 1990; Kurth et al., 1993; Schreiber et al., 1994; Salom et al., 1996). Evidence has been presented to suggest that several possible mechanisms of contraction include increased transmembrane influx and intracellular release of Ca^{2+} (Zhang et al., 1996) coupled to intracellular decreases of magnesium (Huang et al., 1990; Altura and Gupta, 1992; Altura et al., 1993). Cocaine HCl, however, exerts no effects on the tensions of canine or sheep mesenteric arteries (He et al., 1993). Other researchers have found that cocaine HCl can also contract coronary arteries, which may be related to the mechanism of cocaine-induced cardiac ischemia and infarctions (Vongpatanasin et al., 1997).

Cocaine abusers tend to suffer from hypertension. Intravenous (i.v.) or intranasal administration of cocaine HCl (4 to 8 mg) to volunteer subjects can cause increases of blood pressure (Fischman et al., 1976; Javaid et al., 1978). Several acute animal experiments into the haemodynamic effects of cocaine HCl also showed that cocaine HCl can increase blood pressure of conscious or sedated dogs as well as rats and other mammals (Catravas et al., 1978; Bedotto et al., 1988; Wilkerson, 1988; Auer et al., 2001). The mechanism of cocaine-induced hypertension is thought to include a blockade of reuptake of epinephrine, norepinephrine and dopamine at the presynaptic level, whose concentrations can then increase in plasma (Billman, 1990; Trouve et al., 1990). It is not known if cocaine HCl can contract all or only some peripheral arterial vessels or arterioles directly. However, hypotension is one of the clinical manifestations of toxicated cocaine abusers when the dosage approaches high levels (Campbell, 1988; Merlin et al., 1988; Weicht and Bernards, 1996), and i.v. injection of high doses of cocaine HCl (9 to 10 mg/kg) to dogs can cause rapid decreases of blood pressure (Koerker and Moran, 1971). In view of the later considerations, we wondered whether different concentrations of cocaine HCl can induce relaxation or dilation of the aorta. This study was, therefore, designed to test this hypothesis by using isolated rat aortic segments. This study was also designed to investigate the possible mechanisms of such action by using different receptor antagonists and inhibitors of signal transduction pathways related to vascular smooth muscle relaxation. Surprisingly, calyculin A, a MLC phosphatase inhibitor, was found to reduce the relaxant effects of cocaine HCl.

2. Materials and methods

2.1. Animals, vessel preparations and solutions

Male Wistar rats, 250–350 g, were sacrificed by decapitation. After the thoracolaparotomy, the descending thoracic aortae were excised from heart to diaphragm, similar to previous studies and placed in Petri dishes containing normal Krebs–Ringer bicarbonate (NKRB) solution at room temperature and carefully cleaned of loose connective tissue (Yang et al., 1998). Segments of about 3–4 mm were then cut. Each thoracic descending aorta was cut into four segments, with two of them leaving the endothelium intact, and the other two being denuded of endothelium. For intact aortic ring preparations, extreme care was taken to avoid damage of endothelial cells. For denuded aortic rings, the endothelial intima of the rings were gently rubbed off with a wire (Zhang et al., 1992a). The composition of the NKRB was (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 10 and $NaHCO_3$ 25. When Ca^{2+}-free or Mg^{2+}-free NKRB was utilized, $CaCl_2$ or $MgSO_4$ was replaced by isosmolar amounts of NaCl, respectively. High K^+ solution was also utilized in some experiments; the composition was (in mM): KCl 4.7, K_2SO_4 143, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 10. In additional experiments, $CaCl_2$ contractile concentration–effect curves were obtained on tissues subjected to Ca^{2+}-free NKR followed by high K^+, Ca^{2+}-free K^+ NKB as described previously (Altura and Altura, 1974b).

2.2. Assessment of vascular reactivity to cocaine HCl

Prepared rat aortic rings were mounted in muscle chambers containing 20 ml of NKRB, tied to force-displacement transducers (Grass, Model FT03C), which were connected to polygraphs (Grass, Model 7C) to record the tensions of the aortic rings (Zhang et al., 1992a). The tissues were incubated at 37 °C, gassed with 95% O_2 and 5% CO_2 and suspended isometrically under resting tensions of 2 g. All tissues were initially equilibrated for 2 h. During the incubation period, the loading tensions were adjusted periodically and maintained throughout the equilibration period. The incubation media were routinely changed every 15 min as a precaution against interfering metabolites (Altura and Altura, 1970). The stable level of tension developed in response to the addition of 80 mM KCl was always

measured before testing the reactivity to cocaine HCl. This usually occurred over a 90-min period. To examine the functional viability of an intact endothelium, vascular rings were precontracted by 0.1 μg/ml phenylephrine HCl, and the presence and absence of endothelium was confirmed by testing for relaxation to 1 μg/ml acetylcholine, which generally resulted in 90% relaxation in rat aortic rings with intact endothelium. As for endothelium-denuded aortic rings, the relaxation to acetylcholine was always less than 10% (Zhang et al., 1992a).

After equilibration and contraction to 80 mM KCl, to get stable levels of contraction and testing for intact or denuded endothelium, the rat aortic rings were exposed to NKRB containing cocaine HCl (10^{-9} to 6×10^{-3} M) to determine the effects of cocaine HCl on rat aortic basal tension. When testing to determine whether cocaine HCl can induce relaxation, the aortic rings were precontracted by 0.1 μg/ml phenylephrine, and cocaine HCl was added after the phenylephrine-induced contractions have reached their plateaus.

2.3. Effects of antagonists and inhibitors

To investigate whether cocaine-induced relaxation could be attributed to endogenous release of specific types of vasoactive amines from the blood vessels [e.g. norepinephrine, dopamine, acetylcholine, histamine, opiates and serotomine (5-HT)] and prostaglandins, specific pharmacologic antagonists of these vasoactive substances as well as a cyclooxygenase inhibitor (indomethacin) were examined by incubating (10 min) with these drugs before adding cocaine HCl. These antagonists were added in concentrations that produce specific antagonisms to their respective agonists and cyclooxygenase (10^{-7} to 10^{-5} M) (Altura and Altura, 1974a,b; Zhang et al., 1993). Haloperidol and indomethacin were dissolved in DMSO, and all other drugs were dissolved in NKRB.

To determine if extracellular magnesium ($[Mg^{2+}]_o$) is important in cocaine-induced relaxation, paired segments of rat aortic rings were incubated with or without $[Mg^{2+}]_o$, followed by exposure to cocaine HCl.

2.4. Drugs

The following drugs were used: cocaine HCl (National Institute of Drug Abuse); propranolol HCl (Aldrich Chemical, Milwaukee, WI); atropine sulfate (Mann Res. Labs, New York); diphenhydramine HCl (Benadryl, Parke-Davis, Ann Arbor, MI); cimetidine HCl (Smith Kline Beckman, Philadelphia); methysergide maleate (Sandoz, Basel, Switzerland); indomethacin (Merck, Rahway, NJ); haloperidol (Sigma); D-tetraethylammonium chloride (T-2265), thapsigargin (Sigma), methylene blue (MB) (Sigma), calyculin A (Sigma), L-NMMA (Sigma), L-arginine (Sigma), PTIO (Sigma), protein kinase K inhibitor (KT-5823) (Calbiochem), 9*(Tetrahydro-2-furanyl)-9H-purin-6-amine (SQ 22538) (Sigma), Vitamin E (Sigma), pyrrolidine dithiocarbarmate (PDTC, Sigma), methylene blue (Sigma), Rp-cGMPs triethylamine (Sigma), Sp-cGMPs triethylamine (Sigma), and glybenclamide (Sigma). All other organic and inorganic chemicals were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and were of the highest purity.

2.5. Calculations and statistics

Where appropriate, means ± S.E.M. were calculated and compared for statistical significance by means of Student's *t*-test, paired *t*-tests or ANOVA using Sheffe's contrast-test. A probability value of 0.05 or less was considered significant.

3. Results

3.1. Cocaine and basal tension

Cocaine HCl exerted no effects on baseline tension of isolated rat aortic rings. The resting tensions of isolated rat aortic rings were 2.0 g. When exposed to cocaine HCl (10^{-9} to 10^{-3} M), neither intact nor denuded tissues showed any changes in their tensions ($n = 10-14$ each).

3.2. Cocaine induces relaxation of phenylephrine-contracted tissues

After induction of peak contraction by 0.1 μg/ml phenylephrine, concentrations of cocaine HCl (10^{-5} to 6×10^{-3} M) were found to dose-dependently relax both the intact and the denuded rat aortic rings precontracted by phenylephrine (Fig. 1). There was, however, no significant difference between the magnitudes of the relaxation of intact vs. denuded tissues or their EC_{50}s. The maximal % relaxations of intact vs. denuded rat aortic rings were $108.9 \pm 24.3\%$ vs. $99.5 \pm 8.3\%$ ($P > 0.05$), respectively. Their EC_{50}s were $7.8 \pm 2.6 \times 10^{-4}$ M and $7.3 \pm 1.9 \times 10^{-4}$ M, respectively ($P > 0.05$). The EC_{min}s were $1.9 \pm 1.0 \times 10^{-4}$ M and $1.7 \pm 0.7 \times 10^{-4}$ M, and the ED_{max}s were $2.4 \pm 1.6 \times 10^{-3}$ M and $3.2 \pm 1.3 \times 10^{-3}$ M, respectively ($P > 0.05$). The relaxation effects of cocaine HCl could be completely washed out, i.e., isolated rat aortic rings could still be contracted by 0.5 μM phenylephrine or 80 mM KCl to almost the same extent after treatment with cocaine HCl (10^{-5} to 6×10^{-3} M) as before. So, the effects of cocaine HCl on aortic rings are not a consequence of vascular muscle cell or vascular wall damage.

3.3. Cocaine inhibits contractions induced by phenylephrine

Cocaine HCl clearly affected the contractile concentration–response curves of phenylephrine -induced contrac-

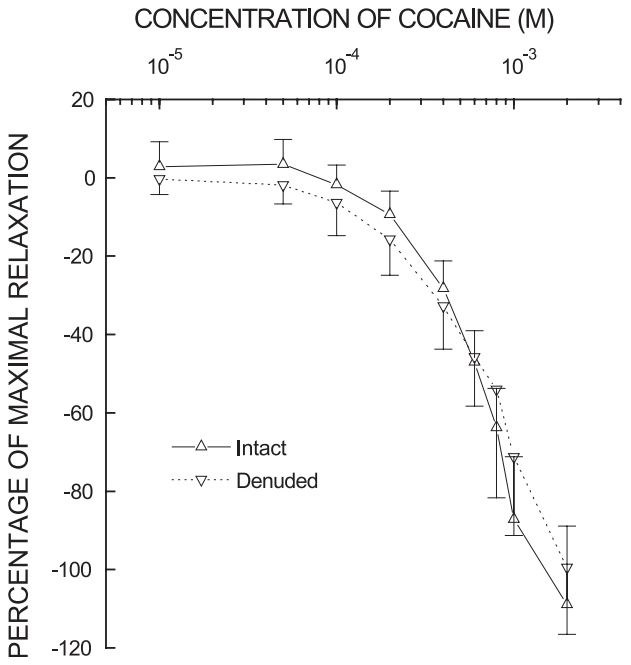

Fig. 1. Effects of cocaine HCl on isolated rat aortic rings. Cocaine HCl can relax both intact and denuded rings contracted by phenylephrine ($n=5$). There is no significant difference between intact and denuded tissues ($P>0.05$).

tions. For intact aortic rings, although 4×10^{-4} M cocaine HCl did not affect the concentration–response curves of phenylephrine-induced contractions, higher concentrations of cocaine HCl shifted the concentration–response curves of phenylephrine to the right (Fig. 2). We also tested higher concentrations of cocaine HCl (4×10^{-4} M) and found these concentrations inhibited contractions induced by phenylephrine (1×10^{-5} M) to greater extents: EC_{50}s were increased ($3.0\pm4.0\times10^{-1}$ μg/ml vs. $1.9\pm1.1\times10^{-2}$ μg/ml, $p<0.01$) and the E_{max}'s were decreased (51.3 ± 16.4% vs. 89.8 ± 10.6%, $p<0.01$). cocaine HCl, 1×10^{-3} M, also increased the EC_{50}s ($3.0\pm4.0\times10^{-2}$ μg/ml vs. $1.9\pm1.1\times10^{-2}$ μg/ml, $p<0.01$), but did not decrease the E_{max} (88.6 ± 7.0% vs. 89.8 ± 10.6%, $P>0.05$). A quantitatively, and qualitatively, similar effect of cocaine HCl on phenylephrine-induced contractions was also seen on denuded rat aortic rings ($n=8$, data not shown).

3.4. A variety of pharmacologic antagonists, antioxidants and nitric oxide (NO) inhibitors failed to affect cocaine-induced relaxations

A variety of amine antagonists, such as propranolol (beta-adrenoceptor antagonist)—10^{-7} M, diphenhydramine (histamine H_1-receptor antagonist)—10^{-7} M, cimetidine (histamine H_2-receptor antagonist)—10^{-6} M, haloperidol (dopamine D_1-receptor antagonist)—10^{-7} M, and methysergide (5-HT_1-receptor antagonist)—10^{-7} M all failed to interfere with the cocaine -induced relaxation ($P>0.05$, $n=6$, data not shown). Indomethacin—10^{-5} M, L-arginine—1 mM and L-NMMA—150 μM also all failed to attenuate the effects of cocaine HCl ($n=5$, $P>0.05$). Use of a stable radical scavenger for NO, i.e., PTIO—50 μM and antioxidants, such as PDTC—1 μM, and vitamin E—100 μM, also did not affect the relaxations induced by cocaine HCl ($n=5$, $P>0.05$). In addition, none of the antagonists used altered the phenylephrine contraction plateaus (data not shown).

3.5. Effects of cocaine HCl on Ca^{2+}-induced contractions in high K^+-/Ca^{2+}-free buffer

$CaCl_2$ (10^{-5} to 10^{-2} M) was found to dose-dependently contract rat aortic rings in high K^+-/Ca^{2+}-free buffer, as has been reported previously (Godfraind and Kaba, 1969; Altura and Altura, 1974b). Cocaine HCl (1×10^{-3} to 4×10^{-3} M) was found to dose-dependently inhibit Ca^{2+} induced contractions (Fig. 3). In addition, cocaine HCl (4×10^{-3} M) increased the EC_{50}s of Ca^{2+}-induced contractions ($1.5\pm0.9\times10^{-3}$ M vs. $8.5\pm5.1\times10^{-4}$ M, $p<0.05$).

3.6. Effects of Mg^{2+}-free NKRB and a sarcoendoplasmic reticulum Ca^{2+}-ATPase inhibitor

Vascular relaxation may be the result of increased Mg^{2+} influx into the vascular cells or Ca^{2+} influx into the sarcoendoplasmic reticulum. So, we also tested whether cocaine HCl can still relax rat aortic rings in Mg^{2+}-free NKRB or in the presence of thapsigargin, which inhibits

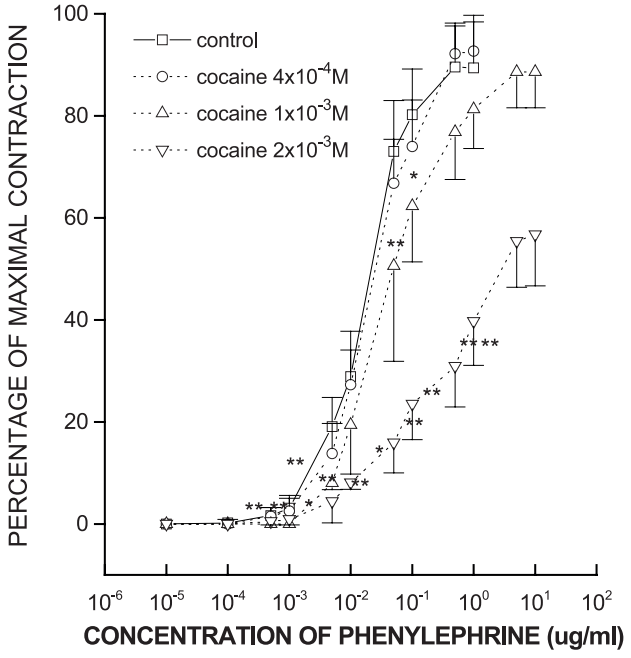

Fig. 2. Effects of cocaine HCl on contractile concentration–response curves to phenylephrine on intact rat aortic rings ($n=8$). Cocaine HCl can inhibit contractions induced by phenylephrine. *$p<0.05$, **$p<0.01$ vs. Control.

sarcoendoplasmic reticulum Ca^{2+}-ATPase and, thus, inhibits uptake of Ca^{2+} from the cytosol to the sarcoendoplasmic reticulum (Treiman et al., 1998). We found that without $[Mg^{2+}]_o$ in the buffer, cocaine HCl could still relax the rings ($n=6$, $P>0.05$, data not shown). With or without thapsigargin (10^{-7} M), the % relaxation of cocaine HCl was not significantly different ($n=5$, $P>0.05$, data not shown).

3.7. Effects of a MLC phosphatase inhibitor

A very low concentration of calyculin A (5 nM) did not influence cocaine-induced relaxations ($P>0.05$), but use of 50 and 100 nM calyculin A, respectively, dose-dependently inhibited the relaxation effects of cocaine HCl, causing the concentration–response curves of cocaine HCl to shift to the right (Fig. 4). Although not shown, calyculin A (50–100 nM) failed to either attenuate or inhibit acetylcholine- or bradykinin-induced relaxations of phenylephrine-induced rat aortic contractions ($n=4$–6).

3.8. Effects of cAMP, cGMP and PKG inhibitors

Use of the cGMP inhibitor methylene blue (5.0 μM), PKG inhibitors (KT 5823-50 nM, Rp-cGMPS triethylamine—20 μM and Sp-cGMPS triethylamine—20 μM) or the adenylate cyclase inhibitor (SQ 22538—50 μM) all failed to inhibit or attenuate the effects of cocaine HCl ($n=5$, $P>0.05$, data not shown).

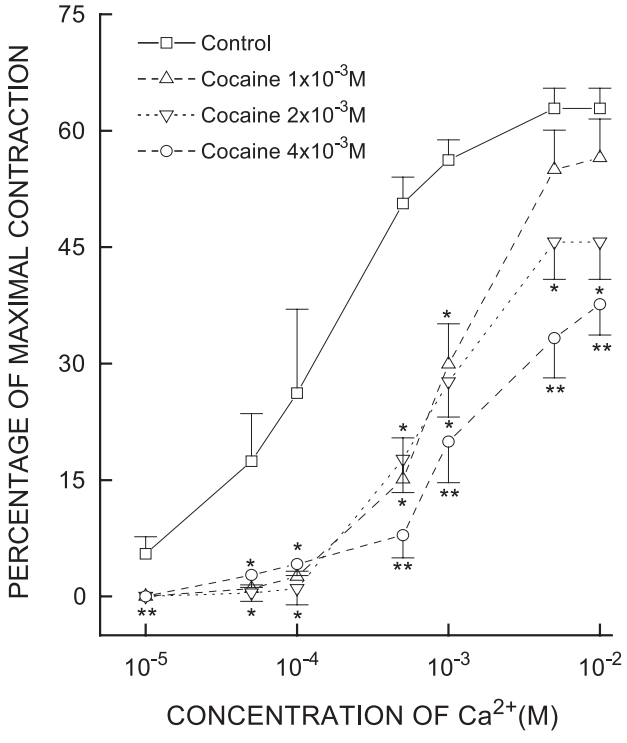

Fig. 3. Effects of cocaine HCl on $CaCl_2$ induced contractions of rat aortic rings in high K^+-/Ca^{2+}-free buffer ($n=5$). $*p<0.05$, $**p<0.01$ vs. Control.

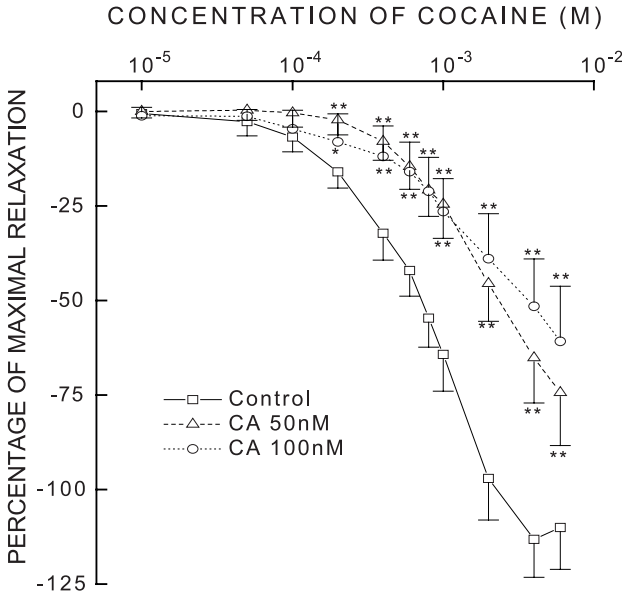

Fig. 4. Cocaine -induced relaxation can be inhibited by calyculin A (CA) in a concentration-dependent manner ($n=6$). $*p<0.05$, $**p<0.01$ vs. Control.

3.9. Effects of high-K^+ NKRB and K^+ channel blockers

When rat aortic rings were incubated in high-K^+ NKRB, cocaine HCl could still relax the precontracted tissues, without significant changes in maximal magnitude or EC_{50}, as in normal NKRB ($n=5$, $P>0.05$, data not shown). Both a nonselective K^+-channel blocker (teraethylammonim chloride), and a selective ATP-sensitive K^+ channel blocker (glybenclamide) failed to influence the relaxation effects of cocaine HCl ($n=4$, $P>0.05$, data not shown).

4. Discussion

In this study, we demonstrate that cocaine HCl (10^{-9} to 10^{-3} M) has no effect on the baseline tension of rat aortic rings. We believe that this result is very interesting, and seems, at first glance, to be paradoxical with most of the clinical cardiovascular findings. But the present results remind us that cocaine HCl can probably have different effects on vascular tension in different regional blood vessels. Although cocaine HCl cannot contract rat aorta directly, it can directly contract canine, ovine, bovine, rat, porcine, monkey and human cerebral arteries (Huang et al., 1990; Madden and Powers, 1990; Wagerle et al., 1990; Wang et al., 1990; Salom et al., 1996; Auer et al., 2001; He et al., 1993). Others have recently measured the diameter of aortae of cocaine HCl abusers with transesophageal echocardiography, and found that the diameter is not affected by cocaine HCl (Eisenberg et al., 1996), which is in accordance with our results that cocaine HCl has no direct contraction effect on rat aorta. Egashira et al. studied the effects of cocaine HCl on excitation–contraction cou-

pling of aortic smooth muscle from ferrets by measuring the tension of aortic strips stimulated by 4 Hz which resulted, primarily, in activation of adrenergic nerve endings rather than direct stimulation of smooth muscles (Egashira et al., 1991). Cocaine HCl (10^{-6} to 10^{-4} M) could increase the tension of the aortic strips significantly more from ferrets not pretreated with reserpine than from ferrets pretreated with reserpine, indicating that cocaine could increase aortic tension by its presynaptic action. This helps to explain why cocaine HCl can increase blood pressure when injected without any direct contractile effect. Moreover, the $t_{1/2}$ of cocaine HCl is quite short, about 1 h, and thereafter it will be metabolized into benzoylecgonine and ecgonine methyl ester (Madden and Powers, 1990). Whether the metabolites of cocaine HCl may have different vascular effects and thus influence blood pressure still needs to be elucidated.

We also demonstrate here, for the first time, that high doses of cocaine HCl can dose-dependently relax isolated rat aortic rings. The minimal concentration for the relaxant effects of cocaine HCl on isolated aortic rings is about 10^{-5} to 10^{-4} M, and the maximal relaxation can be reached at a concentration of $\sim 10^{-3}$ M to 6×10^{-3} M. This concentration range is relatively higher than the concentration range needed to contract isolated or intact cerebral blood vessels (10^{-7} to 10^{-4} M) (Huang et al., 1990; Madden and Powers, 1990; Wang et al., 1990; He et al., 1993; Salom et al., 1996). However, from the study of He et al. (1993), it could also be noticed that when the concentrations continued to increase to 10^{-3} M, the tensions decreased. The blood levels of cocaine HCl required to produce euphoric effects are approximately 10^{-7} M to 10^{-5} M (Van Dyke et al., 1976), which is also, for the most part, lower than the concentrations needed to relax rat aortic rings in our study. Schwartz et al. studying the effects of i.v. cocaine HCl, in awake and anesthetized dogs, found that only anesthetized dogs could tolerate the highest cocaine HCl doses. The blood pressure increased in the lowest dose range, remained unchanged at intermediate doses, and fell at the highest doses (Schwartz et al., 1989). The similar hypotensive effects of high doses of cocaine HCl (9 to 10 mg/kg) have also been studied by other researchers (Koerker and Moran, 1971). Since only high doses of cocaine HCl relax rat aortic rings, it may explain why many drug abusers have hypertension resulting from increased sympathetic activity, rather than have hypotension.

How do high concentrations of cocaine HCl cause relaxation of isolated rat aortic rings? The tension of blood vessels or the contraction of vascular smooth muscle cells are regulated by many factors and signaling pathways (Horowitz et al., 1996). It is well established that MLC phosphorylation and dephosphorylation is one of the most fundamental mechanisms of smooth muscle contraction and relaxation. We attempted to examine many of these in order to determine the possible mechanisms of the relaxant action of cocaine HCl. Surprisingly, only one drug that we tested turned out to exert an inhibiting effect on the cocaine-induced aortic relaxation. Calyculin A, which is known to inhibit myosin light chain phosphatase in vascular smooth muscle cells (Mills et al., 1994; Bolz et al., 2003), dose-dependently inhibited the relaxation effects of cocaine HCl. This result suggests to us that cocaine HCl may increase the activity of light chain myosin phosphatase. In order for myosin to be phosphorylated, a MLC kinase specifically phosphorylates Ser 19 in myosin, and in turn increases muscle tension. Conversely, myosin light chain phosphatase can catalyze the dephosphorylation of the phosphorylated myosin, and thus relax the vessel. When myosin light chain phosphatase activity is increased, the vessel tension may thus be reduced (Somlyo and Somlyo, 1994). Several investigators, using concentrations of calyculin A similar to those herein, have recently been using calyculin A as a tool to study the activation of MLC phosphatase in smooth muscle cells, including vascular smooth muscle cells (VSMCs) (Ozaki et al., 1991; Iizuka et al., 1999; Adragna et al., 2000; Bolz et al., 2003). So, cocaine HCl may relax rat aorta by increasing the activity of MLC-PP, decrease phosphorylated myosin, and therefore relax the vessel. The fact that neither acetylcholine- nor bradykinin-induced relaxations were attenuated or inhibited by 50–100 nM calyculin A supports our tenet.

Intracellular-free Ca^{2+} and Mg^{2+} are key divalent ions regulating the tensions of blood vessels. When intracellular Ca^{2+} is decreased or intracellular Mg^{2+} is increased, blood vessels will relax (Altura and Altura, 1974a; Zhang et al., 1992b; Horowitz et al., 1996; Yang et al., 2000). Cytoplasmic Ca^{2+} increases occur by either influx into the cells or by release from the sarcoplasmic reticulum, and decreases occur by either an extrusion of Ca^{2+} out of the cell or uptake into the sarcoplasmic reticulum. Cocaine HCl was found in the present study to inhibit contractions induced by Ca^{2+} in high K^+, Ca^{2+}-free solution, suggesting that it may inhibit Ca^{2+} influx and thus inhibit the contraction of the aortic rings. Thapsigargin, a good inhibitor of sarcoendoplasmic reticulum Ca^{2+}-ATPases, which is pivotal in Ca^{2+} uptake (Treiman et al., 1998), failed to influence the relaxant effects of cocaine HCl. So, the cocaine -induced relaxation may not be via an increase of sarcoplasmic reticulum Ca^{2+} uptake. Mg^{2+} increases in the vascular cells can either be the result of increasing influx or the result of a release inside the cell (Altura and Altura, 1974a; Altura, 1991; Yang et al., 2000). Cocaine HCl, however, was found in our study to still relax aorta in Mg^{2+}-free NKRB, supporting the idea that the relaxation is not due to increased Mg^{2+} influx.

A number of drugs and agonists acting on VSMCs are thought to induce relaxation by increasing intracellular cAMP (Horowitz et al., 1996). Some agonists relax vascular smooth muscle by increasing intracellular cGMP, thus activating PKG (for example, NO). But, neither a potent cAMP inhibitor, nor several potent cGMP inhibitors, nor a PKG inhibitor were able to inhibit the relaxations induced

by cocaine HCl. Thus, we could not find any evidence to support either cAMP or cGMP formation or release as mediators or second messengers for cocaine-induced relaxation of isolated aortic rings.

NO is thought to be a very important intrinsic relaxing factor, produced mostly by endothelial cells (for recent review, see Russo et al. (2002)). Cocaine HCl was found, however, in our study to relax intact and denuded aortic rings almost equally, indicating that it has no effect on the production of NO by rat aortic endothelial cells. VSMCs may also produce some amounts of NO (Buchwalow et al., 2002), but neither L-arginine, L-NMMA, nor PTIO exerted any effects on the relaxations induced by cocaine HCl. So, cocaine HCl does not appear to be relax isolated aortic rings by increasing or releasing intrinsic NO production. Indomethacin also failed to inhibit the effect of cocaine HCl, suggesting that cocaine-induced relaxation is not due to activation of cyclooxygenase and production of prostanoids. A variety of amine antagonists also failed to antagonize cocaine HCl, and there is still no evidence to show that cocaine HCl has specific receptors on blood vessels.

In conclusion, high concentrations of cocaine HCl can relax isolated rat aortic rings in a concentration-related manner, which appears to be, in part, due to activation of myosin light chain phosphatase and dephosphorylation of myosin and inhibition of Ca^{2+} influx and its intracellular release. Our results might prove useful in models of cocaine-induced aortic dissection and hypotension, and as such could prove useful in the design of therapeutic agents for these pathological conditions.

Acknowledgements

This study was supported in part by an NIH Research Grant (AA-08674) to B. M. Altura.

References

Adragna, N.C., White, R.E., Orlov, S.N., Lauf, P.K., 2000. K-Cl cotransport in vascular smooth muscle and erythrocytes: possible implication in vasodilation. Am. J. Physiol. Cell Physiol. 278, C381–C390.

Alspaugh, J.A., 1995. Cocaine-associated chest pain: a case of aortic dissection. J. Tenn. Med. Assoc. 88, 271.

Altura, B.M., 1991. Basic biochemistry and physiology of magnesium: a brief review. Magnes. Trace Elem. 10, 167–171.

Altura, B.M., Altura, B.T., 1970. Differential effects of substrate depletion on drug-induced contractions of rabbit aorta. Am. J. Physiol. 219, 1698–1705.

Altura, B.M., Altura, B.T., 1974a. Magnesium and contraction of arterial smooth muscle. Microvasc. Res. 7, 145–155.

Altura, B.M., Altura, B.T., 1974b. Peripheral vascular actions of glucocorticoids and their relationship to protection in circulatory shock. J. Pharmacol. Exp. Ther. 190, 300–315.

Altura, B.M., Gupta, R.K., 1992. Cocaine induces intracellular free Mg deficits, ischemia and stroke as observed by in-vivo 31P-NMR of the brain. Biochim. Biophys. Acta 1111, 271–274.

Altura, B.M., Altura, B.T., Gebrewold, A., 1985. Cocaine-induced spasms of cerebral blood vessels: relation to cerebral vascular accidents, strokes and hypertension. Fed. Proc. 44, 1637.

Altura, B.M., Zhang, A., Cheng, T.P.O., Altura, B.T., 1993. Cocaine induces rapid loss of intracellular free Mg2+ in cerebral vascular smooth muscle cells. Eur. J. Pharm. 246, 299–301.

Auer, J., Berent, R., Eber, B., 2001. Cardiovascular complications of cocaine use. New Engl. J. Med. 345, 351–358.

Bedotto, J.B., Lee, R.W., Lancaster, L.D., Olajos, M., Goldman, S., 1988. Cocaine and cardiovascular function in dogs: effects on heart and peripheral circulation. J. Am. Coll. Cardiol. 11, 1337–1342.

Billman, G.E., 1990. Mechanisms responsible for the cardiotoxic effects of cocaine. FASEB J. 4, 2469–2475.

Bolz, S.-S., Vogel, L., Sollinger, D., Derwand, R., de Wit, C., Loirand, G., Pohl, U., 2003. Nitric oxide-induced decrease in calcium sensitivity of resistance arteries is attributable to activation of the myosin light chain phosphate and antagonized by the RhoA/Rho kinase pathway. Circulation 107, 3080–3087.

Buchwalow, I.B., Podzuweit, T., Bocker, W., Samoilova, V.E., Thomas, S., Wellner, M., Baba, H.A., Robenek, H., Schnekenburger, J., Lerch, M.M., 2002. Vascular smooth muscle and nitric oxide synthase. FASEB J. 16, 500–508.

Campbell, B.G., 1988. Cocaine abuse with hyperthemia, seizures and fatal complications. Med. J. Aust. 149, 387–389.

Catravas, J.D., Waters, I.W., Walz, M.A., Davis, W.M., 1978. Acute cocaine intoxication in the conscious dog: pathophysiologic profile of acute lethality. Arch. Int. Pharmacodyn. Ther. 235, 328–340.

Cohle, S.D., Lie, J.T., 1992. Dissection of the aorta and coronary arteries associated with acute cocaine intoxication. Arch. Pathol. Lab. Med. 116, 1239.

Daras, M., Tuchman, A.J., Koppel, B.S., Samkoff, L.M., Weitzher, I., Marc, J., 1994. Neurovascular complications of cocaine. Acta Neurol. Scand. 90, 124–129.

Egashira, K., Morgan, K.G., Morgan, J.P., 1991. Effects of cocaine on excitation-contraction coupling of aortic smooth muscle from the ferret. J. Clin. Invest. 87, 1322–1328.

Eisenberg, M.J., Yakel, D.L., Mendelson, J., Redberg, R.F., Jones, R.T., Foster, E., 1996. Immediate effects of intravenous cocaine on the thoracic aorta and coronary arteries. A transesophageal echocardiographic study. Chest 110, 147–154.

Fischman, M.W., Schuster, C.R., Resnekov, L., Shick, J.F., Krasnegor, N.A., Fennell, W., Freedman, D.X., 1976. Cardiovascular and subjective effects of intravenous cocaine administration in humans. Arch. Gen. Psychiatry 33, 983–989.

Fogo, A., Superdock, K.R., Atkinson, J.B., 1992. Severe atherosclerosis in the kidney of a cocaine addict. Am. J. Kidney Dis. 20, 513–515.

Frederichs, R.K., McQuiuen, J.B., 1991. Cerebral vasculitis associated with cocaine abuse. Stroke 22, 1437–1439.

Godfraind, T., Kaba, A., 1969. Blockade or reversal of the contraction induced by calcium and adrenaline in depolarized arterial smooth muscle. Br. J. Pharmacol. 36, 549–560.

Gold, M.S., 1993. Drugs of Abuse: A Comprehensive Series for Clinicians. Cocaine, vol. 3. Plenum Medical Bood, New York, pp. 1–10.

He, G.Q., Zhang, A., Altura, B.T., Altura, B.M., 1993. Cocaine-induced cerebrovasospasm and its possible mechanism of action. J. Pharm. Exp. Ther. 268, 1532–1539.

Horowitz, A., Menice, C.B., Laporte, R., Morgan, K.G., 1996. Mechanisms of smooth muscle contraction. Physiol. Rev. 76, 967–1003.

Huang, Q.F., Gebrewold, A., Altura, B.T., Altura, B.M., 1990. Cocaine-induced cerebral vascular damage can be ameliorated by Mg^{2+} in rat brain. Neurosci. Lett. 109, 113–116.

Iizuka, K., Yoshii, A., Samizo, K., Tsukagoshi, H., Ishizuka, T., Dobashi, K., Nakazawa, T., Mori, M., 1999. A major role for the Rho-associated coiled coil forming protein kinase in G-protein-mediated Ca^{2+} sensitization through inhibition of myosin phosphatase in rabbit trachea. Br. J. Pharmacol. 128, 925–933.

Javaid, J.I., Fischman, M.W., Schuster, C.R., Dekirmenjian, H., Davis, J.M., 1978. Cocaine plasma concentration: relation to physiological and subjective effects in humans. Science 202, 227–228.

Kliner, R.A., Hale, S., Alker, K., Rezkalla, S., 1992. The effects of acute and chronic cocaine use on the heart. Circulation 85, 407–419.

Koerker, R.L., Moran, N.C., 1971. An evaluation of the inability of cocaine to potentiate the responses to cardiac sympathetic nerve stimulation in the dog. J. Pharmacol. Exp. Ther. 178, 482–496.

Konzen, J.P., Levine, S.R., Garcia, J.H., 1996. Vasospasm and thrombus formation as possible mechanisms of stroke related to alkaloidal cocaine. Stroke 26, 147–148.

Kurth, C.D., Monitto, C., Albuquerque, M.L., Feuer, P., Anday, E., Shaw, J., 1993. Cocaine and its metabolites constrict cerebral arterioles in newborn pigs. J. Pharmacol. Exp. Ther. 265, 587–591.

Madden, J.A., Powers, R.H., 1990. Effect of cocaine and cocaine metabolites on cerebral arteries in vitro. Life Sci. 47, 1109–1114.

Mangiardi, J.R., Daras, M., Geller, M.E., Weitzner, I., Tuchman, A.J., 1988. Cocaine-related intracranial hemorrhage. Report of nine cases and review. Acta Neurol. Scand. 77, 177–180.

Merlin, S.I., Brissie, R.M., Kapila, A., Hauser, M.T., 1988. Hyperpyrexia, seizures, and hypotension in a 31-year-old man. Ala. J. Med. Sci. 25, 274–279.

Mills, I., Murata, K., Pacher, C.S., Sumpio, B.E., 1994. Cyclic strain stimulates dephosphorylation of the 20 kDa regulatory myosin light chain in vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 205, 79–84.

Nolte, K.B., Gelman, B.B., 1989. Intracranial hemorrhage associated with cocaine abuse. Arch. Pathol. Lab. Med. 113, 812–813.

Norris, K.C., Thornhill-Joynes, M., Robinson, C., Strickland, T., Alperson, B.L., Witana, S.C., Ward, H.J., 2001. Cocaine use, hypertension, and end-stage renal disease. Am. J. Kidney Dis. 38, 523–528.

Ozaki, H., Gerthoffer, W.T., Publicover, N.G., Fusetani, N., Sanders, K.M., 1991. Time-dependent changes in Ca^{2+} sensitivity during phasic contraction of canine antral smooth muscle. J. Physiol. 440, 207–224.

Park, H., 1992. Rupture of splenic artery aneurysm. Am. J. Forensic Med. Pathol. 1.13, 230–232.

Polis, A., Maginn, D., Barr, J.L., 1987. Tissue deposition of cocaine in man: a report of five fatal poisonings. Forensic Sci. Int. 33, 83–88.

Russo, G., Leopold, J.A., Loscalzo, J., 2002. Vasoactive substances: nitric oxide and endothelial dysfunction in atherosclerosis. Vascul. Pharmacol. 38, 259–269.

Salom, J.B., Torregrosa, G., Barbera, M.D., Jover, T., Orti, M., Alborch, E., 1996. Effects of cocaine on human and goat isolated cerebral arteries. Drug Alcohol Depend. 42, 65–71.

Schreiber, M.D., Madden, J.A., Covert, R.F., Torgerson, L.J., 1994. Effects of cocaine, benzoylecgonine, and cocaine metabolites in cannulated pressurized fetal sheep cerebral arteries. J. Appl. Physiol. 77, 834–839.

Schwartz, A.B., Janzen, D., Jones, R.T., Boyle, W., 1989. Electrocardiographic and hemodynamic effects of intravenous cocaine in awake and anesthetized dogs. J. Electrocardiol. 22, 159–166.

Somlyo, A.P., Somlyo, A.V., 1994. Signal transduction and regulation in smooth muscle. Nature. 372, 231–236.

Treiman, M., Caspersen, C., Christensen, S.B., 1998. A tool coming of age: thapsigargin as an inhibitor of sarcoendoplasmic reticulu Ca2+-ATPases. Trends Pharmacol. Sci. 19, 131–135.

Trouve, R., Nahas, G.G., Manger, W.M., Vinyard, C., Goldberg, S., 1990. Interactions of nimodipine and cocaine on endogenous catecholamines in the squirrel monkey. Proc. Soc. Exp. Biol. Med. 193, 171–175.

Van Dyke, C., Barash, P.G., Jatlow, P., Byck, R., 1976. Cocaine: plasma concentrations after intranasal application in man. Science 191, 859–861.

Vongpatanasin, W., Lange, R.A., Hillis, L.D., 1997. Comparison of cocaine-induced vasoconstriction of left and right coronary arterial systems. Am. J. Cardiol. 79, 492–493.

Wagerle, L.C., Kurth, C.D., Roth, R.A., 1990. Sympathetic reactivity of cerebral arteries in developing fetal lamb and adult sheep. Am. J. Physiol. 258, H1432–H1438.

Wang, A.M., Suojanen, J.N., Colucci, V.M., Rumbaugh, C.L., Hollenberg, N.K., 1990. Cocaine- and methamphetamine-induced acute cerebral vasospasm: an angiographic study in rabbits. Am. J. Neuroradiol. 11, 1141–1146.

Wilkerson, R.D., 1988. Cardiovascular effects of cocaine in conscious dogs: importance of fully functional autonomic and central nervous systems. J. Pharmacol. Exp. Ther. 246, 466–471.

Yang, Z.W., Zheng, T., Zhang, A., Altura, B.T., Altura, B.M., 1998. Mechanisms of hydrogen peroxide-induced contraction of rat aorta. Eru. J. Pharmacol. 344, 169–181.

Yang, Z.W., Gebrewold, A., Nowakowski, M., Altura, B.T., Altura, B.M., 2000. Mg^{2+}-induced endothelial-dependent relaxation of blood vessels and blood pressure lowering: role of NO. Am. J. Physiol., Regul. Integr. Comp. Physiol. 278, R628–R639.

Zhang, A., Altura, B.T., Altura, B.M., 1992a. Endothelial-dependent sexual dimorphism in vascular smooth muscle: role of Mg^{2+} and Na^{+}. Br. J. Pharmacol. 105, 305–310.

Zhang, A., Cheng, T.P.O, Altura, B.T., Altura, B.M., 1992b. Magnesium regulates intracellular free ionized calcium concentration and cell geometry in vascular smooth muscle cells. Biochim. Biophys. Acta 1134, 25–29.

Zhang, A., Altura, B.T., Altura, B.M., 1993. Ethanol-induced contraction of cerebral arteries in diverse mammals and its mechanism of action. Eur. J. Pharmacol. 248, 229–236.

Zhang, A., Cheng, T.P.O, Altura, B.T., Altura, B.M., 1996. Acute cocaine results in rapid rises in intracellular free calcium concentration in canine cerebral vascular smooth muscle cells: possible relation to etiology of stroke. Neurosci. Lett. 215, 57–59.

Zurbano, M.J., Heras, M., Rogol, M., Epelde, F., Miranda, F., Sanz, G., Escolar, G., Ordinas, A., 1997. Cocaine administration enhances platelet reactivity to subendothelial components: studies in a pig mode. Eur. J. Clin. Investig. 27, 116–120.