Mechanisms of coronary vasoconstriction induced by Na arachidonate in experimentally diabetic dogs[1]

L. Sterin-Borda, E. Borda, E.J. del Castillo, M.F. Gimeno and A.L. Gimeno[2]

Centro de Estudios Farmacológicos y de Principios Naturales (CEFAPRIN), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Serrano 665/69, 1414 Buenos Aires (Argentina), 9 November 1981

Summary. Na arachidonate (NaA) enhanced the resting basal tone of isolated coronary arteries from diabetic dogs and depressed it in coronary arteries from normal controls. Inhibitors of thromboxane A_2 biosynthesis and of lipoxygenases abolished the vasoconstrictor effect of NaA on diabetic arteries, whereas inhibitors of cyclooxygenase activity and PGI_2 biosynthesis blocked the vasodilating action of NaA on normal arteries.

Arachidonic acid (AA) is the biological precursor of diverse biologically active products, such as stable prostaglandins, prostacyclin (PGI_2), thromboxanes, hydroperoxy acids and leukotrienes[3,4]. The transformation of AA into cyclic endoperoxides by the cyclooxygenase system is a pivotal step in the biosynthesis of the 1st 3 groups of compounds, whereas the latter 2 are lipoxygenase metabolites.

Thromboxane A_2 (TXA_2) a metabolite of AA, is synthetized by platelets, spleen, lung[5], white cells[6], brain[7] and umbilical arteries[8] and has been described as a potent vasoconstrictor agent in all the vascular beds studied[9,10].

On the other hand, PGI_2 synthetized by vascular endothelial cells[11] is a potent dilator of isolated blood vessels, including coronary arteries[12-14]. Alterations in vascular function and increased platelet aggregability have been suggested as factors involved in the vasculopathy that accompanies diabetes[15,16]. TXA_2 has been implicated as a possible mediator of both complications[16]. In addition, abnormal reactivity to vasoactive compounds in association with diabetes has also been reported[17].

In view of these findings, it was decided to study the mechanical response of isolated coronary arteries from experimentally diabetic dogs to Na arachidonate (NaA) alone or in the presence of inhibitors of PGI_2 and TXA_2 synthesis as well as after blockade of the cyclooxygenase and lipoxygenase pathways.

Methods. Coronary arteries were obtained from mongrel dogs weighing between 9 and 20 kg. 2 groups of animals were used: a) normal controls (n = 9) and b) totally pancreatectomized dogs (n = 9).

The pancreatectomized animals were fed with 40 g/kg of raw beef heart and 70 g of raw pancreas daily[18]. The experiments with totally pancreatectomized dogs were performed 5-7 days after the operation and 16 h after the last meal. Blood sugar was determined by Somogyi's method. As previously reported[18] glycemia was normal in controls (113 ± 10) and elevated in pancreatectomized animals (328 ± 26) (figures are mg 100 ml^{-1}; means $\pm$ SEM). Dogs were anesthetized with pentobarbital (25-30 mg·kg^{-1}) and the beating heart was rapidly removed from the chest[19,20].

After excision of the heart, the aortic root and its attached left anterior descending coronary were dissected in bloc and immersed in Krebs-Ringer-Bicarbonate (KRB) solution composed as reported elsewhere[21]. One end of a helically-cut strip was attached to a stationary glass holder and the other was connected by means of a thread to a force transducer coupled to an oscillographic recorder via a carrier preamplifier. Strips were immersed in a muscle bath containing 20 ml of KRB solution gassed with 5% CO_2 in oxygen and kept at 37 °C and pH 7.4.

The contractile activity was recorded as previously reported[19-21]. After an initial preload of 1 g, the preparations underwent a strees relaxation, and tension stabilized after 90 min at about 600 mg. The tonic changes of the vessels elicited by experimental additions were expressed as mg of tension above or under the basal resting tension or initial preload (0 mg in the figure).

Cumulative dose-response curves for Na arachidonate (NaA) were constructed for coronaries derived from control dogs and diabetic dogs, each treated or untreated with drugs known to inhibit PGI_2 and TXA_2 synthesis or cyclooxygenase and lipoxygenase activity. Indomethacin at 10^{-6} M (Merck Sharp and Dohme); acetylsalicylic acid (ASA) at 1.8×10^{-4} M, imidazole at 10^{-3} M, nordihydroguaiaretic acid (NDGA) at 10^{-5} M (Sigma Chemical Co.); L-8027 at 10^{-6} M (Labaz Lab.); 5-8, 11-14-eicosatetraynoic acid (ETYA) at 10^{-7} M (Hoffmann-La Roche)and tranylcypromine at 2.5×10^{-4} M (Smith-Kline-French), were delivered to the tissue bath 30 min before NaA (Sigma Chemical Co.).

Results were compared employing Student's t-test. Mean values were expressed in mg and experimental values were considered significantly different when $p = 0.05$ or less.

Results. Figure 1 and 2 shows in a dose-related manner the contractile influence of Na arachidonate on coronary arteries from diabetic (upper panel) and normal dogs (lower panel). The PG precursor enhanced the resting basal tone

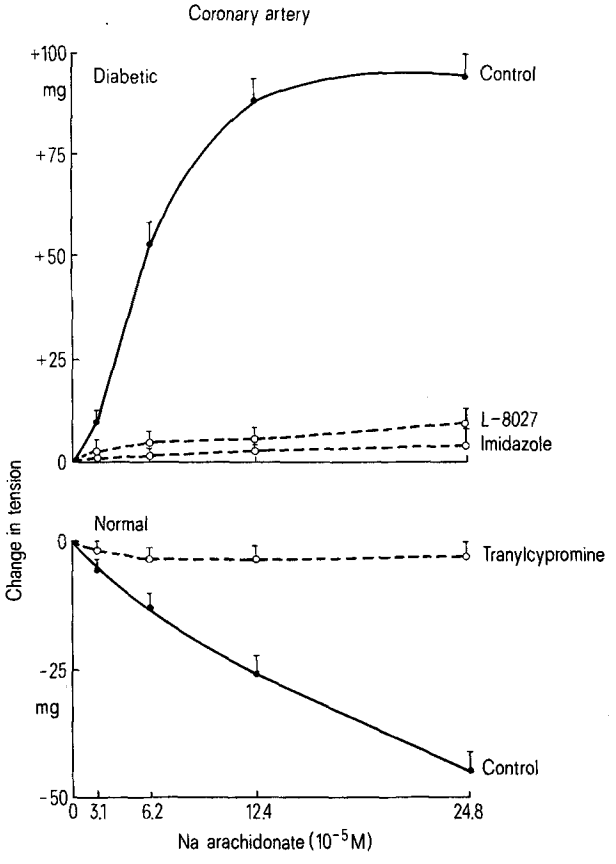

Figure 1. Cumulative dose-response curves of Na arachidonate on diabetic (upper panel) and normal (lower panel) coronary arteries. Influences of L-8027, imidazole and tranylcypromine. Points and bars represent the mean and the SEM, respectively, n = 7 in each group.

of diabetic preparations and depressed that of the normal controls. Inhibitors of TXA_2 synthesis, namely L-8027 and imidazole (fig. 1 upper panel) completely blocked the vasoconstrictor effect of NaA on coronaries from diabetic dogs. On the other hand the vasodilator effect of NaA on normal vessels was prevented by tranylcypromine, an inhibitor of PGI_2 synthesis (fig. 1 lower panel).

As can been seen in figure 2, the tonic increment of tension induced by NaA during diabetes was blocked both by ETYA and NDGA, inhibitors of the lipoxygenase pathway (fig. 2 upper panel). On the other hand, inhibitors of cyclooxygenase activity, such as indomethacin and ASA, failed to modify the vasoconstrictor effect of NaA on diabetic vessels (upper panel), but prevented the vasodilating action of the precursor on normal arteries (lower panel).

Discussion. The present report documents the fact that coronary arteries isolated from diabetic dogs exhibit a different contractile response to NaA in comparison to that observed in normal controls. Indeed, NaA produced relaxation of normal coronary arteries and contracted diabetic vessels.

It has been suggested that arachidonic acid dilates the coronary vascular bed of most species[22] due to the generation of PGI_2[12].

Our results are in keeping with previous reports, because inhibitors of cyclooxygenase activity i.e. indemethacin and acetylsalicylic acid[23] abolished the vasodilatation induced by NaA suggesting that the cyclooxygenase system is the initial reaction in the biosynthesis of vasoactive prostaglandins in normal coronary arteries. In addition, the incubation of normal vessels with tranylcypromine, at a concentration that is known to block prostacyclinsynthetase[24], reduced the relaxation evoked by NaA suggesting that PGI_2 may be responsible for this effect.

On the contrary, the fact that imidazole or L-8027, inhibitors of the biosynthesis of TXA_2[25,26], abolished completely the vasoconstrictor influence induced by NaA on isolated coronary arteries from diabetic dogs, permits us to advance the notion that TXA_2 is the metabolite involved in this action. Although it is recognized that all the blocking drugs used have nonspecific actions it is possible to suggest as a working hypothesis that coronary vessels from diabetic dogs might be able to generate TXA_2 or a vasoconstrictor eicosanoid. The observation that the reactivity of diabetic artery to NaA is abolished by inhibitors of lipoxygenase, such as NDGA[27] or ETYA[28], appears to indicate that the active substance producing vasoconstriction on diabetic coronary arteries could be derived from arachidonic acid via a lipoxygenase catalyzed pathway.

Previous reports have documented that some groups of compounds originated from AA by the action of lipoxygenases have a vasoconstrictor effect in guinea-pig skin[29], and produce a strong and long-lasting contraction of the lung parenchymal strip which proved to be mediated by the release of thromboxanes[30]. Our results support the notion that, in isolated coronary arteries from diabetic dogs, NaA probably induces vasoconstrictions via TXA_2 generated by cyclooxygenase or lipoxygenase catalyzed reactions. However, the fact that indomethacin and ASA failed to modify the stimulatory effect of NaA on the diabetic condition suggests the participation of compounds presumably derived from a lipoxygenase and able to produce a direct vasoconstrictor effect.

The present results suggest that NaA dilates normal coronary arteries and that PGI_2 could be the metabolite responsible for this action; on the contrary, NaA contracts coronary arteries from diabetic dogs, presumably due to the generation of TXA_2 and/or compounds formed via the operation of lipoxygenases pathways.

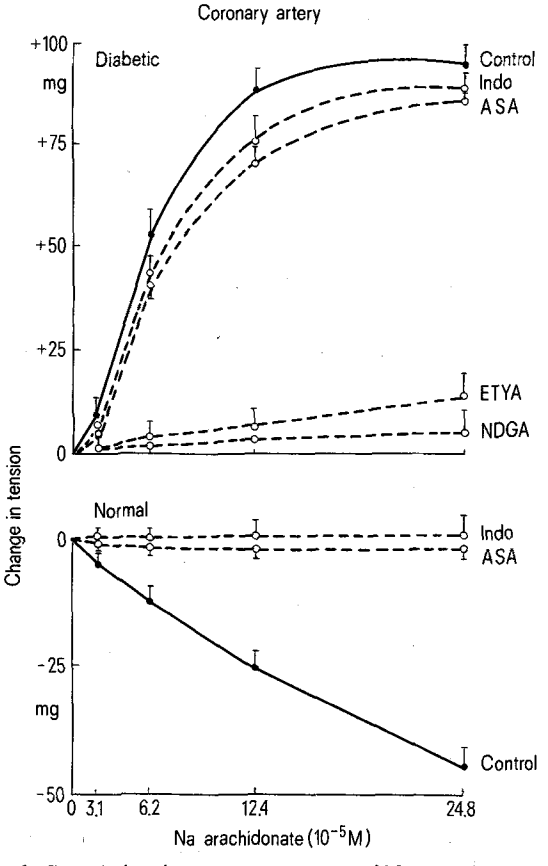

Figure 2. Cumulative dose-response curves of Na arachidonate on diabetic (upper panel) and normal (lower panel) coronary arteries. Influence of indomethacin (INDO); acetylsalicylic acid (ASA); nordihydroguaiaretic acid (NDGA) and 5-8,11-14-eicosatetraynoic acid (ETYA). For further explanations see legend of figure 1.

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2 To whom reprint requests should be addressed.
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Evidence for enzymatic reduction of 1-nitropyrene by rat liver fractions

J.P. Nachtman[1] and E.T. Wei

School of Public Health, University of California, Berkeley (California 94720, USA), 4 November 1981

Summary. 1-Nitropyrene, a mutagenic compound found in diesel exhaust and photocopy toners, is reduced anaerobically by rat liver fractions to 1-aminopyrene. This reaction is stimulated in the microsomal fraction by NADPH and in the cytosol by FMN. Addition of both cofactors produced more reduction in S-9, microsomes, or cytosol than either cofactor alone.

The nitroaromatic compounds 4,4′-dinitrobiphenyl[2], 2-nitrofluorene[3] and 4-nitrobiphenyl[4] produce cancer when fed to experimental animals. The mechanisms of carcinogenicity are thought to be mediated by reduction of the nitro group, followed by conversion of the chemical to reactive intermediates similar to those formed from oxidation of the corresponding carcinogenic amines: (4,4′-diaminobiphenyl), 2-aminofluorene[4] and 4,4′-diaminobiphenyl[2]. Nitroreductases have been studied in relationship to the mutagenic and carcinogenic potentials of nitrofurantoins[5], nitroimidazoles[6], and 4-nitroquinoline-N-oxide, but less is known about the reduction of nitroarenes. Earlier work showed minimal conversion of 2-nitrofluorene to 2-aminofluorene by liver preparations[4]. Poirier and Weisburger[7], however, demonstrated the enzymatic reduction of 2-nitronaphthalene to 2-aminonaphthalene by rat and mouse liver fractions.

Recently, potent mutagenic nitroaromatics have been detected in materials such as photocopy toners[8], engine exhausts[9,10] and urban aerosols. The mutagen bioassay used was the Ames *Salmonella typhimurium* test, a system which contains bacterial nitroreductases. 1 compound, 1-nitropyrene, is of particular interest because its presence in diesel engine exhaust particulates may account for up to 30% of the total engine mutagenic activity in the sample. In order to study the toxic potential of 1-nitropyrene, we tested the ability of mammalian enzymes to reduce 1-nitropyrene (1-NP) to 1-aminopyrene (1-AP).

Liver homogenates were prepared from male Sprague-Dawley rats weighing 150–250 g. Animals were decapitated and the livers rapidly excised and weighed. Tissue samples were homogenized with a Polytron ultrasonic homogenizer using 20 mM Tris-HCl containing 1.15% KCl (pH 7.4) buffer, 4 ml/g of liver wet wt. These homogenates were centrifuged at 9000 × g for 10 min to obtain the S-9 fraction. NADPH, $MgCl_2$ and a glucose-6-phosphate dehydrogenase preparation were added according to the method of Poirier and Weisburger[6]. Protein concentrations were measured by the method of Bradford[11]. Cytosol and microsomal fractions were prepared by centrifugation at 105,000 × g for 1 h at 4 °C. To exclude O_2 from the incubation mixture, tissue and co-factor preparations were kept on ice and gassed with N_2 for at least 20 min. Incubation tubes were also gassed with N_2 and sealed with screw-cap tops. The reaction, with 300 nmoles 1-NP as substrate, was conducted by mechanically shaking the mixture at 37 °C for 40 min (final volume = 3 ml). The reaction was terminated by the addition of 0.1 ml of 10 N HCl.

The sample was extracted with 5 ml cyclohexane for 15 min to remove 1-NP, washed with another 5 ml cyclohexane, and then made basic with 0.6 ml of 2 N NaOH. This was re-extracted with 5 ml cyclohexane to obtain the reduced product 1-AP. The cyclohexane extracts were placed in an American Instruments Co. reflecting fluorimeter (Model SPF-125) with excitation wavelength set at 283 nm, and the percent relative fluorescence was measured. Cyclohexane extracts of incubation mixtures that did not contain 1-NP served as blanks. 1-AP spiked buffer samples were extracted to provide a linear standard curve. The reduction rate was linear with time to approximately 40 min.

1-AP, dissolved in cyclohexane, has an emission maximum at 403 nm. The fluorescence spectra of alkaline extracts of

Subcellular distribution of nitroreductase in rat liver

nmole 1-aminopyrene formed mean (SD)/mg protein			
Cofactors Added	S-9	Cytosol	Microsomes
None	0.03 (0.009)	0.06 (0.012)	0.02
NADPH	0.07 (0.01)	0.07 (0.02)	0.30 (0.05)
FMN	0.36 (0.11)	0.16 (0.05)	0.02
NADPH + FMN	0.54 (0.05)	0.73 (0.09)	7.34 (0.8)
mg protein/fraction	1280	1020	32

The reaction mixture consisted of 300 nmoles of 1-nitropyrene in 50 µl ethanol incubated anaerobically (40 min) in 20 mM Tris-HCl, 1.15% KCl (pH = 7.4) buffer. The final volume was 3.0 ml which contained 1.0 µmoles NADPH and/or FMN, co-factors. Microsomes from 120 mg rat liver; cytosol or S-9 from 40 mg rat liver. 2 determinations per point were performed.