Effects of idarubicin and idarubicinol on rat coronary resistance and vasoconstrictor responsiveness of isolated aorta and mesentery

Michael Weiss[a], Christine Giessler[b] and Wonku Kang[a,*]

It has been hypothesized that coronary vasoconstriction is involved in the cardiotoxic action of anthracyclines. The purpose of this study was to determine whether an increase in coronary resistance induced by idarubicin (IDA) or its primary circulating metabolite idarubicinol (IDOL) is correlated with a decrease in vascular sensitivity to vasoconstrictor agonists. Coronary vasoconstriction was studied in single-pass perfused rat hearts after a 10-min infusion of 0.5 mg IDA or IDOL. In the endothelium-intact rat thoracic aorta and mesentery we measured the inhibition of phenylephrine (PE)- and KCl-induced contraction in the presence of IDA and IDOL, respectively. The increase in coronary vascular resistance evoked by IDOL (121%) exceeded that of IDA (75%). IDA (10–100 μmol/l) concentration-dependently diminished vascular sensitivity to PE and KCl due to a reduction in maximal contractile response (E_{max}), i.e. the antagonism by IDA of PE- or KCl-induced vasoconstriction was non-competitive, indicating a post-receptor cellular mechanism. These reductions of PE or KCl efficacy elicited by IDOL were significantly larger than those elicited by the corresponding doses of IDA. The decrease in efficacy of PE in the presence of IDA and IDOL was characterized by IC_{50} estimates of 44.3 and 30.7 μmol/l, respectively. With a 10-fold lower IC_{50}, IDA inhibited the reactivity of small mesenteric arteries to noradrenaline with 10-fold higher potency. The correlation between the increase in coronary resistance and the decrease in vasoconstrictor responsiveness may suggest that these anthracyclines act through a common cellular mechanism. ***Anti-Cancer Drugs*** **17:69–74 © 2006 Lippincott Williams & Wilkins.**

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[a]Section of Pharmacokinetics and [b]Department of Pharmacology, Martin Luther University Halle-Wittenberg, Halle, Germany.

Correspondence to M. Weiss, Section of Pharmacokinetics, Department of Pharmacology, Martin Luther University Halle-Wittenberg, 06097 Halle, Germany. Tel: +49 345 557 1657; fax: +49 345 557 1835; e-mail: michael.weiss@medizin.uni-halle.de

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Introduction

Anthracycline antibiotics are potent anti-tumor agents [1]. Idarubicin (IDA) is the first anthracycline that can be administered orally [2]. The clinical use of anthracyclines however is limited by a well-described, but incompletely understood, cardiac toxicity [3,4]. There is some evidence that vascular actions of anthracyclines contribute to both acute cardiotoxicity [5] and anti-tumor activity [6]. In the isolated rat heart, IDA increases the coronary vascular resistance [7]; however, nothing is known on the vascular actions of its primary circulating metabolite idarubicinol (IDOL). The purpose of this study was (i) to compare the effects of IDA and IDOL on rat coronary vascular resistance (*CVR*), and (ii) to determine whether they induce a decrease in vascular sensitivity to vasoconstrictor agonists in isolated rat aorta and mesentery. This latter action was previously reported for aclarubicin [8].

Methods

Materials

IDA was purchased from Pharmacia & Upjohn (Erlangen, Germany), and phenylephrine (PE) from Sigma (Deisenhofen, Germany). IDOL was kindly donated by Pharmacia & Upjohn (Erlangen, Germany). All other chemicals and solvents were of the highest grade available.

Isolated perfused rat heart

In this study, all animals received care in accordance with the European Community guidelines for the use of experimental animals. Prior approval was obtained by the Animal Protection Body of the State of Sachsen-Anhalt, Germany. As previously described [7,9], male Wistar rats (250–300 g) were heparinized and anesthetized with pentobarbital. After opening the chest, the ascending aorta was cannulated and perfused with Krebs–Henseleit buffer solution, pH 7.4, containing NaCl (118 mmol/l), KCl (4.7 mmol/l), $CaCl_2$ (2.52 mmol/l), $MgSO_4$ (1.66 mmol/l), $NaHCO_3$ (24.88 mmol/l), KH_2PO_4 (1.18 mmol/l), glucose (5.55mmol/l) and Na pyruvate (2.0 mmol/l). The solution was bubbled continuously with 95% O_2/5% CO_2 and maintained at 37°C. The pulmonary artery was incised to allow outflow of the perfusate. Coronary perfusion was initiated through a short cannula in the aortic root and maintained at a constant flow of 9.5 ± 0.4 ml/min by a peristaltic pump in a single pass method by the Langendorff technique. A

*Present address: Department of Pharmacy, Catholic University of Daegu South Korea

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latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve and connected to pressure transducer. The balloon was inflated with water to create a diastolic pressure of 5–6 mmHg. The hearts were beating spontaneously at an average rate of 280 beats/min. Coronary perfusion pressure, the left ventricular pressure and heart rate (*HR*) were measured continuously and a physiological recording system (Hugo Sachs Elektronik, March, Germany). *CVR* is calculated from perfusion pressure divided by coronary flow. After a 20-min equilibration period, IDA or IDOL (0.5 mg) was infused for 10 min with an infusion device into the perfusion tube close to the aortic cannula.

In-vitro responses of aorta thoracica and small mesenteric artery

Rat thoracic aortae and small mesenteric arteries were rapidly excised and cleaned of adherent tissue. The aorta was cut into helical strips 2 mm wide and 10 mm long (four strips per preparation). The strips were placed into 10-ml organ baths with modified Krebs–Henseleit buffer containing (in mmol/l): NaCl 119, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.25, D-glucose 10, EDTA 0.03 and ascorbic acid 0.1, equilibrated with carbogen at (95% O_2 and 5% CO_2) 37°C. The contractile force was measured isometrically using force transducers connected to amplifiers and recorders (Hellige, Freiburg, Germany). The resting tension of the vessels was adjusted to 9.81 mN. The strips were allowed to equilibrate for 60 min (bath fluid was replaced every 20 min during this period). Following equilibration, the aortic strips were contracted by exposure to 50 mmol/l KCl and 1 μmol/l PE to confirm the viability of the smooth muscle. When tension had reached a plateau, 10 μmol/l carbachol was added to verify the functional state of the endothelium (thoracic aortae 20.5 ± 2.4% relaxation with carbachol). Thereafter, the baths were washed several times with Krebs–Henseleit solution until the preparations reached their initial tension. After this equilibration period, the cumulative concentration–response curves to PE (10^{-9} to 10^{-5} M) or KCl (5×10^{-3} to 3×10^{-2} M) were assessed in the absence or presence of the test agents IDA and IDOL. IDA and IDIOL were added 30 min before constructing a concentration–response curve to PE or KCl. In order to exclude desensitization phenomena, only one concentration of test substance and one concentration–response curve for noradrenaline (NA) or KCl were examined on each strip [10]. From the mesenteric artery, a 2-mm ring of 125 ± 10 μm diameter was mounted in a myograph (J.P. Tading, Aarhus, Denmark) with 10 ml separated organ bath according to the standard procedure originally described by Mulvany and Halpern [11]. Vessel responses were measured continuously as changes in isometric force. After a 60-min stabilization period the internal diameter of each vessel was set to a tension corresponding to 0.9 times the estimated diameter at 100 mmHg effective transmural pressure [11]. The viability of the preparation was monitored by constriction with 10 μmol/l NA and 12.5 mmol/l KCl, and endothelium-dependent relaxation with 10 μmol/l carbachol. Following a 30-min washout period, a concentration–response curve to NA (10^{-10} to 10^{-5} M) was constructed. After a 30-min washout period, a further concentration–response curve was constructed in the presence of a single concentration of IDA or IDIOL (exposure started 30 min before the addition of contractile agonists). In control experiments, the concentration–response curve for NA was repeated without additional treatment and no significant difference between the first and the second cumulative concentration–response curves was observed.

Data analysis

Contraction is reported as a percentage of response to PE or KCl. The magnitude of the contractile responses was described by the general logistic equation:

$$E(C) = E_{max}/[1 + (EC_{50}/C)^n] \quad (1)$$

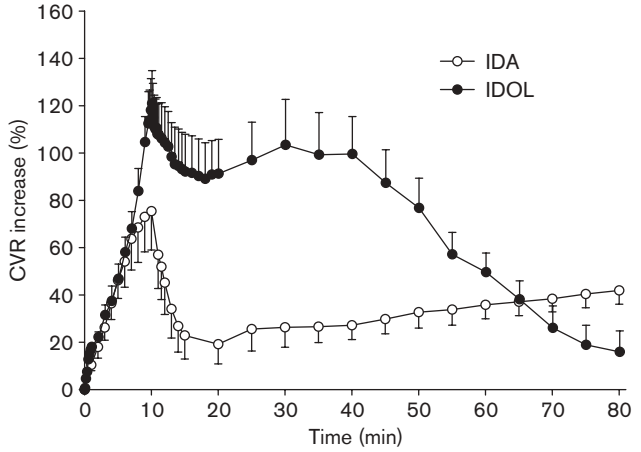
where $E(C)$ is the observed effect at concentration C, E_{max} is the maximal effect, EC_{50} is the concentration at half-maximal effect (pEC_{50}: negative logarithm of the EC_{50}) and n is the Hill factor (a constant expressing the sigmoidicity of the concentration–effect relationship). Parameter estimation and statistical analysis were performed using GraphPad InStat (GraphPad Software, San Diego, California, USA). Average data values of percent inhibition of E_{max} by IDA or IDOL were fitted to the two-parameter logistic function (Eq. 1), percent inhibition $= 100(1 - 1/(1 + IC_{50}/C_{IDA})^n$ using Scientist (MicroMath Scientific Software, Salt Lake City, Utah, USA). Data are presented as mean ± SD. $P < 0.05$ was considered statistically significant. When comparing two groups, an unpaired Student's *t*-test was used or, in concentration–response curves, analysis of variance with repeated measures. Analysis of variance followed by Student–Newman–Keuls *post-hoc* test was performed when comparing three or more groups. In all cases, $P \leq 0.05$ was considered statistically significant.

Results

Comparison of IDA- and IDOL-induced vasoconstriction in rat heart

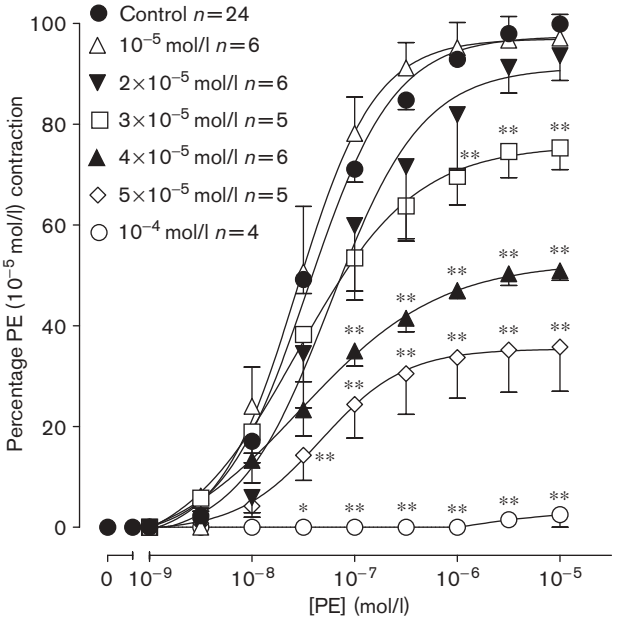
Figure 1 demonstrates the differences between IDA and IDOL regarding their vasoconstrictive potency. IDOL increased *CVR* to 221 ± 14% of the baseline level, exceeding that of IDA which changed *CVR* to 175 ± 16% ($P < 0.01$). Whereas in both cases this peak value was reached at the end of the 10-min infusion, a plateau value of about 100% increase was maintained for about 30 min after IDOL infusion, in contrast to the fast decrease of *CVR* at the end of IDA infusion which was followed by a slow secondary increase after 10 min.

Fig. 1



Effect of a 10-min infusion of 0.5 mg IDA and IDOL on *CVR* in perfused rat hearts (means ± SD, $n=5$ in each group).

Fig. 2



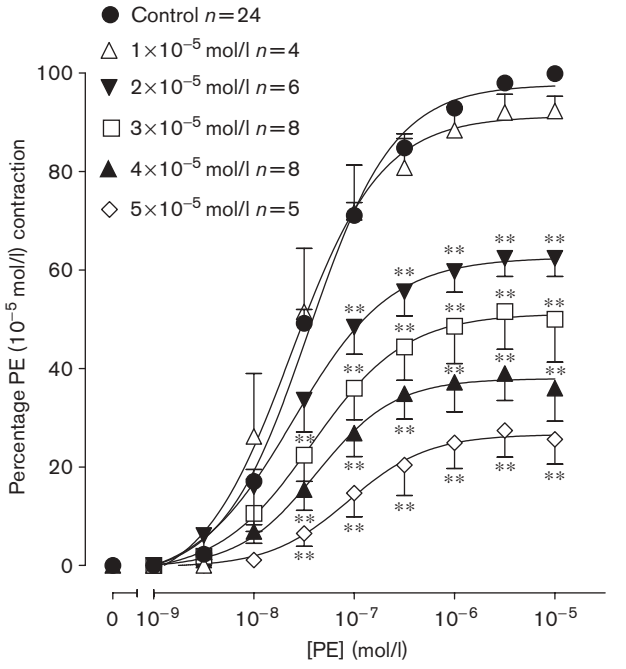
Concentration–response curve of contraction induced by PE on isolated rat aortic rings in the absence and presence of IDA. Points represent means ± SEM for the number of animals indicated. The asterisks indicate significant differences from control.

Although still visible, this biphasic pattern was much less pronounced for IDOL.

Effects of IDA and IDOL on contractile responses of rat aorta to PE and KCl

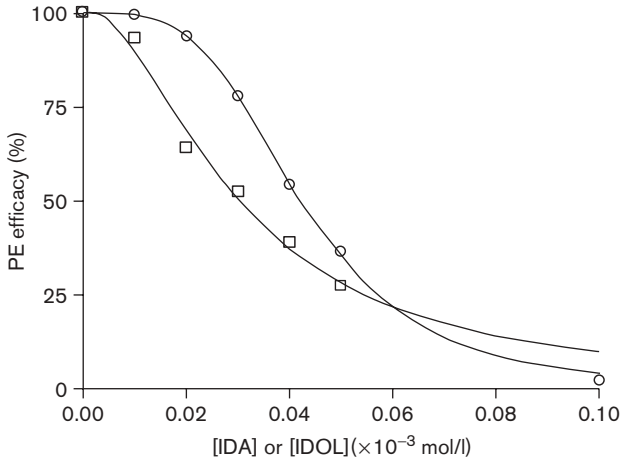
Exposure of rat aorta to IDA or IDOL produced only minimal increase in contraction and this response was characterized by high variability. For IDA this increase amounted to 3.1 ± 1.6 and 13.1 ± 5.4% at concentrations of 20 and 100 µmol/I, respectively. Similar results were observed for IDOL. The inhibition by IDA (IDOL) of contraction of rat aorta induced by PE and KCl, respectively, is demonstrated by concentration–response curves for PE and KCl measured in the absence and presence IDA (IDOL). As shown in Fig. 2, IDA produced concentration-dependent non-competitive inhibition of contractile responses of the aorta to PE and abolished it almost completely at a concentration of 100 µmol/l. None of the PE dose–response profiles displayed any significant shift in EC_{50} value ($pEC_{50} = 7.45 \pm 0.04$) or Hill factor ($n = 1.04$), which was not significantly different from 1. This holds also for the corresponding response curves to IDOL (Fig. 3). In both cases, there was a progressive decrease in maximal response (E_{max}) as the degree of concentration of IDA (IDOL) increased; this effect was significantly ($P < 0.01$) more pronounced for IDOL. This reflects a marked decrease in efficacy of the agonist, i.e. of PE in the presence of IDA, $e_{IDA} = E_{max,IDA}/E_{max,CONTR}$ with increasing IDA or IDOL concentrations from 10 to 50 µmol/l. This percent inhibition, i.e. e_{IDA} (%), could be well described by a logistic function (Fig. 4). A simultaneous computer fit of e_{IDA} and e_{IDOL} reduction data revealed IC_{50} values of 44.3 (5.6) and 30.7 µmol/l (5.2%) for IDA and IDOL, respectively (where the

Fig. 3



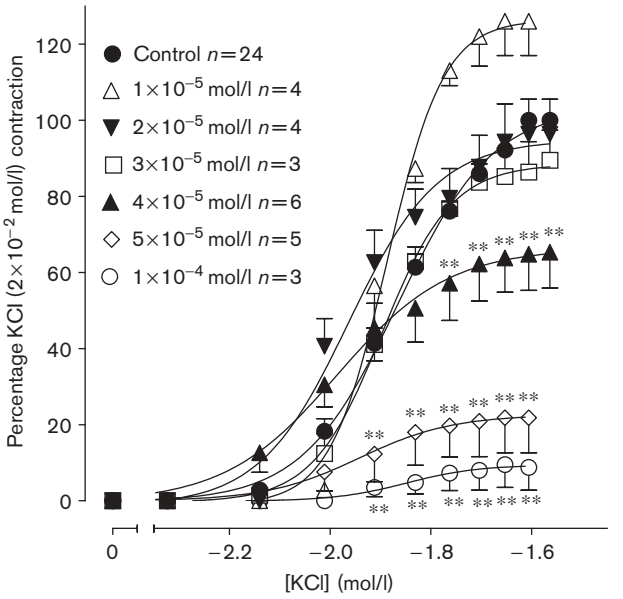
Concentration–response curve of contraction induced by PE on isolated rat aortic rings in the absence and presence of IDOL. Points represent means ± SEM for the number of animals indicated. The asterisks indicate significant differences from control.

Fig. 4



Decrease in efficacy of PE ($E_{max}/E_{max,cont}$) in the presence of IDA or IDOL as simultaneously fitted by a logistic function (solid lines) with respective 50% inhibition concentrations (IC_{50}) for IDA and IDOL of 44.3 and 30.7 μmol/l, respectively.

Fig. 5

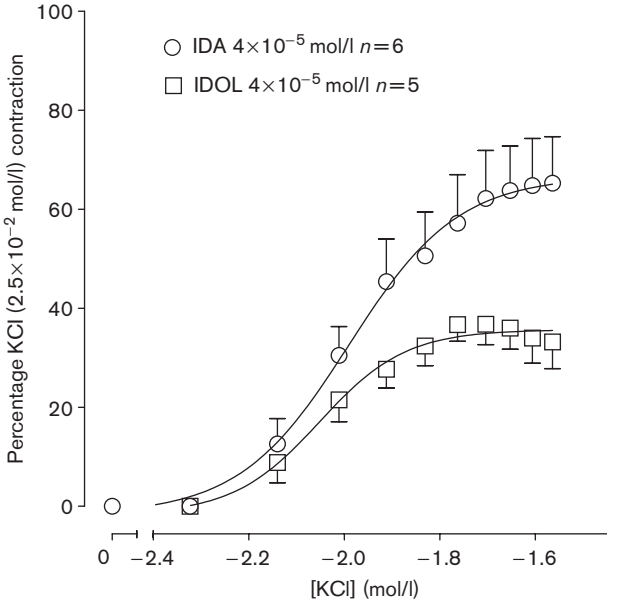


Concentration–response curve of contraction induced by KCl on isolated rat aortic rings in the absence and presence of IDA. Points represent means ± SEM for the number of animals indicated. The asterisks indicate significant differences from control.

number in brackets is the asymptotic coefficient of variation of the estimate). The estimate of the common Hill factor was 2.38 (11.4%).

The cumulative concentration–response curves constructed for KCl in the presence of IDA displayed an analogous behavior, except for the paradoxical effect in the presence of 10 μmol/l IDA where the response exceeded that under control conditions (Fig. 5). There was no detectable difference in the EC_{50} values for KCl (control: $pEC_{50} = 1.87 \pm 0.006$) and the slope index of the curves ($n = 4.33 \pm 0.25$). As in the case of PE, the reduction in maximal response (E_{max}) to KCl elicited by IDOL was significantly larger than that elicited by the corresponding concentration of IDA, as demonstrated in Fig. 6 for the concentration of 40 μmol/l.

Fig. 6



Comparison of KCl-induced contraction of rat aorta in the presence of 40 μmol/l IDA and IDOL showing the significantly higher reduction of E_{max} by IDOL.

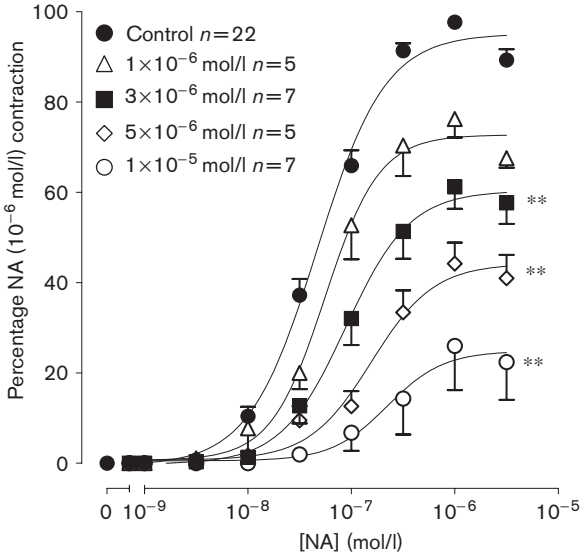
Effects of IDA on contractile responses of small mesenteric artery to NA

IDA had no effect on basal tone. The EC_{50} value of the mesentery response to NA ($pEC_{50} = 7.33 \pm 0.05$) and the Hill factor ($n = 1.3$) were not significantly different from the corresponding measures for PE found in aorta (Fig. 7). The reactivity of small mesenteric arteries to NA showed a similar pattern of E_{max} reduction by IDA with no effect of IDA on EC_{50} and the Hill factor (Fig. 6), but an IC_{50} value of 4.21 μmol/l (10.1%), i.e. a 10-fold lower IC_{50} than that estimated in rat aorta (Fig. 8).

Discussion

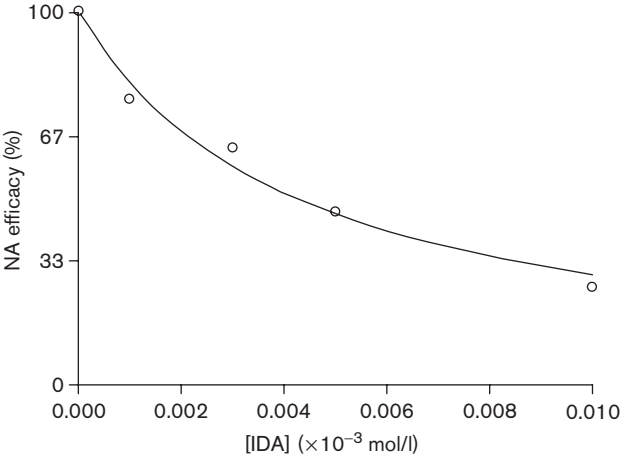
In the present study, a 0.5 mg dose of IDA infused within 10 min induced a higher increase in *CVR* than that observed after a 1-min infusion of the same dose (to 221 versus 138% of baseline level) [7]. The mean maximum outflow concentration of IDA at the end of the 10 min

Fig. 7



NA-induced contraction in mesenteric resistance arteries in the absence and presence of IDA. Points represent means ± SEM for the number of animals indicated. The asterisks indicate significant differences from control.

Fig. 8



Decrease in efficacy of NA-induced mesentery contraction (E_{max}/$E_{max,cont}$) in the presence of IDA as fitted by a logistic function (solid line) with respective 50% inhibition concentrations (IC_{50}) for IDA of 4.2 μmol/l.

infusion amounted 1.4 μg/ml and was accompanied by significant negative inotropic effect (about 50% decrease in left ventricular developed pressure) [9]. We report here the first evidence that IDOL, the primary circulating metabolite of IDA, evokes a higher coronary vasoconstriction than IDA (Fig. 1). This is important in view of the fact that in humans the area under the plasma concentration–time curve of the generated IDOL exceeds that of IDA about 4-fold [12]. Furthermore, even the relatively small amounts of IDOL generated in the heart [13] could contribute to the vasoconstrictive action of IDA, if IDOL would be formed in the vascular wall. It remains an open question as to whether this supports the alcohol metabolite hypothesis of cardiotoxicity [14]. Note that the same concentration of doxorubicin infused over 70 min rose *CVR* to 230% of baseline level [5,15]. Given the major role of nitric oxide (NO) in the physiological control of vascular tone, several lines of evidence suggest that inhibition of NO synthesis is responsible for the vasoconstriction produced by IDA [6]. Anthracyclines are potent nitric oxide synthase (NOS) inhibitors [16], whereby the endothelial NOS (NOS III) is particularly susceptible to this inhibition. Thus, in Langendorff-perfused hearts acute NOS inhibition increases *CVR* [17], valspodar (a derivative cyclosporine and an NOS inhibitor) potentiates the IDA-induced vasoconstriction [7], whereas rutin, probably due to its superoxide scavenging effect that protects NO from breakdown [16], attenuates any IDA-induced increase in *CVR* [13]. Analogously, melatonin almost completely prevented the vasoconstriction induced by doxorubicin in the perfused mouse heart [18]. In contrast to the acute vasoconstrictive action of IDA and IDOL in the perfused rat heart, only a minimal effect was observed in the isolated blood vessels. This is perhaps not surprising in light of recent evidence showing that short-term inhibition of NO synthesis produced a pronounced vasoconstrictive effect in rat (increase in blood pressure) but only a negligible response of rat aorta preparations [19]. This inhibition of NO synthesis, however, did not affect the vasoconstrictor response of rat aorta to PE. From this result we can infer that the reduced responsiveness to contractile agonists after the 60 min exposure of isolated blood vessels to IDA or IDOL is probably not related with NOS inhibition.

Because IDA and IDOL non-competitively reduced the contractions evoked by PE, NA and K^+, this is most likely due to a post-receptor inhibitory action. Thus, the attenuation of vasoconstrictor responsiveness by the anthracycline aclarubicin was explained by a several mechanism affecting signal transduction including reduction in Ca^{2+} influx and inhibition of phosphatidylinositol hydrolysis at a level of phospholipase C activation [6,8,20]. Interestingly, Lopez *et al.* [21] observed in rat aorta a reduced response to vasoconstrictor agonists KCl and PE after long-term pretreatment of rats with high doses of the NOS inhibitor L-NAME (NG-nitro-L-arginine methyl ester). These authors concluded that the reduction of smooth muscle contractility was endothelium independent and could be partly explained by a reduction in extracellular Ca^{2+} influx. Interestingly, the attenuation of vasoconstrictor responsiveness by aclarubicin was explained by a similar mechanism

including an inhibition of phosphatidylinositol hydrolysis at a level of phospholipase C activation [6,8,20]. The only difference between the response of rat aorta to PE and of mesentery to NA was the higher inhibitory potency of IDA in small mesenteric arteries as reflected by a 10-fold lower IC_{50}. Interestingly, the vasodilator effect produced by flavonoids in NA-pre-contracted vessels was also higher for the rat mesenteric resistance vascular bed than in conductance arteries [22].

Because of the close parallelism between the magnitudes of increase in coronary resistance and decrease in vasoconstrictor responsiveness observed here for IDA and IDOL [IDOL caused a 1.6-fold increase in *CVR* and a 1.4-fold decrease in IC_{50} for inhibition of PE-induced constriction (Figs 1 and 3)], it is likely that a common factor is involved in both processes which may be correlated, for example, with the ability of IDA and IDOL to inhibit NOS. This is particularly interesting with regard to possible structure–activity relations [6]. Despite the known differences between K^+ depolarization (KCl) and G-protein-coupled receptor activation (PE and NA), the complexity of the cellular signaling system [23] makes inferences on the basis of the interaction process difficult. Further studies are required to understand the mechanisms underlying the vascular actions of anthracyclines [6].

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