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Involvement of nitric oxide in amiodarone- and dronedarone-induced coronary vasodilation in guinea pig heart

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Abstract

Amiodarone, a powerful antiarrhythmic compound, possesses coronary and peripheral vasodilator properties. The mechanisms responsible for these effects remain incompletely understood. In the present study, the coronary effects of amiodarone and dronedarone, a non-iodinated amiodarone-like compound, were investigated in isolated guinea pig hearts perfused at constant flow with high K^+ solution (40 mM). Amiodarone (0.01–10 μM), dronedarone (0.01–1 μM) and verapamil (0.01–10 μM) induced concentration-dependent decreases in coronary perfusion pressure. Amiodarone- and dronedarone-mediated reductions in coronary perfusion pressure were not modified by a cyclooxygenase inhibitor, indomethacin (3 μM). L-Nitro-L-arginine (L-NOARG; 3–100 μM) caused a rightward shift of concentration–response curves to amiodarone and dronedarone in a dose-dependent way; L-arginine, a nitric oxide (NO) precursor, reversed this effect. Furthermore, when guinea pigs were treated with N^G-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg), amiodarone could not reduce coronary perfusion pressure. NO synthase inhibition did not affect the coronary perfusion pressure response to verapamil. 1*H*-[1,2,4]Oxadiazole (4,3-*a*) quinoxalin-1-one (ODQ), a specific inhibitor of the guanylyl cyclase, inhibited the effects of amiodarone but not those of verapamil. In the presence of L-NOARG and ODQ, and in hearts from animals treated with L-NAME, a decrease in coronary perfusion pressure was still observed at the highest concentration of dronedarone. These results show that, in guinea pig hearts, the coronary vasodilation induced by amiodarone highly depends on nitric oxide. Dronedarone differs from amiodarone by a remaining relaxant effect, refractory to inhibition of NO synthase pathway, probably due to its Ca^+ antagonist properties.

Keywords: Amiodarone; Dronedarone; L-NOARG; Nitric oxide; (Guinea pig); Coronary pressure

1. Introduction

Amiodarone is the most effective antiarrhythmic compound currently available for clinical treatment of life threatening arrhythmias and suppression of atrial fibrillation. Initially marketed as an antianginal agent, amiodarone possesses coronary and peripheral vasodilator effects (Charlier et al., 1968) and exerts a negative chronotropic and dromotropic influence on the sinoatrial and atrioventricular nodes, respectively (Gloor et al., 1983). These pharmacological effects may be, in part, attributed to the blockade of calcium channels. Whereas calcium antagonistic properties of amiodarone have been demonstrated in myocardial cells (Nattel et al., 1987; Nishimura et al., 1989), there is no evidence that relaxant effects on smooth muscles are due solely to a blockade of calcium influx. Indeed, Radino et al. (1989) showed that amiodarone does not modify smooth muscle contraction in rabbit aorta strips precontracted with noradrenaline or potassium. On the contrary, Lubic et al. (1994) observed that amiodarone could completely antagonize depolarization-induced aortic ring contraction.

In our laboratory, previous in vitro studies performed in isolated Langendorff-perfused guinea pig, rat (Cosnier et al., 1998) and rabbit hearts have shown that amiodarone possesses powerful coronary vasodilation properties. But like Radino et al. (1989), we did not observe any relaxation induced by amiodarone in rabbit aorta rings previously contracted with KCl (40 or 100 mM). Moreover, it was shown that amiodarone (Grossmann et al., 1998) and its

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metabolite, *N*-desethylamiodarone (Grossmann et al., 2000), were able to induce endothelium-dependent vasodilation in human hand vein in vivo.

In the light of these observations, we have postulated that coronary vasodilation induced by amiodarone observed in isolated hearts could be endothelium-dependent. Therefore, the aim of the present study was to investigate whether the release of vasoactive substances such as prostaglandins and/or nitric oxide (NO) could be involved in the coronary vasodilation induced by amiodarone and dronedarone. The latter substance is a recently developed non-iodinated benzofurane derivative structurally related to amiodarone with similar antiarrhythmic (Finance et al., 1995), hemodynamic (Djandjighian et al., 2000) and electrophysiological characteristics (Sun et al., 1999; Aimond et al., 2000; Guillemare et al., 2000; Gautier et al., 2003).

To evaluate the pharmacological properties of amiodarone, dronedarone and verapamil on coronary resistance vessels, isolated guinea pig hearts perfused with a high concentration of potassium (40 mM) were used (Hoover, 1991).

2. Methods

2.1. Animals

Male Hartley guinea pigs weighing 450–550 g (Charles Rivers, France) were used in this study. The animals were housed in groups of two or three per cage for at least 5 days under controlled conditions of constant temperature/humidity and exposed to a 12-h light/dark cycle. They had free access to a standard diet (A04, UAR, Epinay-sur-Orge, France) and drinking water.

Our animal facilities and animal care and use programmes are in accordance with the principles laid down in the European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purpose and its appendix.

2.2. Isolated heart perfusion

Fifteen minutes after intraperitoneal injection of heparin sodium (1 μl/g body weight, 5000 UI/ml), the animals were exsanguinated after sedation with CO_2 90%, O_2 10%.

Then, hearts were excised and mounted in a Langendorff perfusion apparatus and were surrounded by a water-heated glass jacket maintained at 37 °C.

Coronary perfusion was performed at a constant flow rate of 8 ml/min using thermostatically controlled (37 °C) and oxygenated Tyrode's solution.

During the 30-min stabilisation period, the hearts were perfused with a normal Tyrode's solution (composition in mmol/l): NaCl 118, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5, glucose 11.5, pyruvic acid 2, pH 7.4 equilibrated with 95% O_2 and 5% CO_2.

After this period, hearts were perfused with high K^+ Tyrode's solution (composition mmol/l: KCl 40 and NaCl 83.4). Under these experimental conditions, the coronary perfusion pressure was concentration-dependently increased to a new baseline and the heart contractions were arrested, avoiding the direct and indirect influence of ventricular contraction on the coronary vasculature. All compounds were tested under these experimental conditions. They were administered into the perfusion stream through a side arm of the apparatus, just above the aortic cannula at a rate of 15 μl/min by means of syringe pumps. Since coronary flow rate and rate of the syringe pump were imposed, concentrations of the test compound were adjusted in the syringe.

The effects of amiodarone and dronedarone were also studied in five hearts taken from guinea pigs, which were chronically treated with N^G-nitro-L-arginine methyl (L-NAME) (20 mg/kg body weight per day in drinking water, for 20 days).

2.3. Measurement of coronary perfusion

Coronary perfusion pressure was measured using a pressure transducer (Baxter) attached to a side arm of the aortic cannula. The analogical electric signal of coronary perfusion pressure was converted into raw data (numerical) values and stored on the hard disc of the computer using IOX software (EMKA Technologies France). Every 5 s, processed data of coronary pressure values were collected in text files readable by spreadsheet Excel.

2.4. Experimental protocol

After a 30-min equilibration period with normal Tyrode's solution, perfusion was switched to hyper K^+ solution for the remainder of the experiment. Administration of compounds was started in a hyper K^+ medium when baseline coronary perfusion pressure was stabilised at a higher level. All preparations were challenged (2 min) with 1 μM acetylcholine to produce a transitory coronary perfusion pressure decrease in order to verify the responsiveness of the preparation. After testing with acetylcholine, amiodarone, dronedarone or verapamil was studied separately at increasing concentrations ranging from 0.01 to 10 μM. Perfusion with each concentration of compound was maintained, until a stable decrease in coronary perfusion pressure was observed.

To evaluate the role of endogenous prostacyclin as a modulator of amiodarone and dronedarone decrease in coronary perfusion pressure, the concentration–curve effects of amiodarone and dronedarone were obtained in the presence of the cyclooxygenase inhibitor indomethacin (3 μM, Hammartstrom et al., 1999).

To demonstrate the involvement of NO, we studied amiodarone-and dronedarone-induced decrease in coronary perfusion pressure in the presence of a NO synthase

inhibitor, L-nitro-L-arginine, (L-NOARG, from 1 μM to 100 μM) and in hearts obtained from guinea pigs chronically treated with L-NAME (20 mg/kg body weight per day in drinking water, for 20 days). In some experiments, L-arginine (100 and 300 μM) was added to restore the effects of amiodarone and dronedarone after NO synthase inhibition with L-NOARG. The concentrations of L-arginine were chosen according to preliminary experiments aimed to reverse the inhibitory effect of L-NOARG on the equipotent coronary vasodilations induced by amiodarone and dronedarone. In addition, the effects of amiodarone, dronedarone and verapamil were studied in the presence of a NO sensitive guanylyl cyclase inhibitor (1*H*-[1,2,4]oxadiazole (4,3-*a*) quinoxalin-1-one, ODQ, 1 and 3 μM). The ODQ concentrations inhibiting the decrease in CCP induced by amiodarone and dronedarone were chosen according to preliminary studies (not shown).

The concentration effect for amiodarone, dronedarone and verapamil started 15 min after the onset of antagonist perfusion. Only one concentration curve was performed by heart.

2.5. Treatments

Amiodarone, dronedarone, ODQ (RBI), were dissolved at the concentration of 5.4×10^{-3} M in dimetylsulfoxide (DMSO) 100%. Whereas verapamil (Sigma) and acetylcholine (Sigma) were dissolved in distilled water (5.4×10^{-3} M), indomethacin (Sigma) was dissolved at a concentration of 5.4×10^{-3} M in 70% ethanol. L-NOARG and L-arginine (RBI) were dissolved at a concentration of 5.4×10^{-2} M in HCl 0.1 N. For in vivo treatment, L-NAME (RBI) was dissolved in drinking water (220 mg/l).

The solvents used exerted no effects on coronary perfusion pressure at their perfused final concentrations.

This protocol has been approved by the Comité Expérimentation Animale (Animal Care and Use Committee) of Sanofi-Synthelabo Recherche.

2.6. Data analysis

Coronary pressure values in the presence of amiodarone and dronedarone were expressed as percentage decrease ± S.E.M., from the hyper K^+ coronary pressure state measured just before the administration of the test compound. Using computer software package Origin (Microcal Software, USA), concentration–response curves were analysed by fitting sigmoïdal curves using non-linear regression analysis. IC_{50} and maximum values were obtained for acetylcholine individual experiment and used to calculate the mean values of IC_{50} ± S.E.M.

To analyse the means coronary pressure and IC_{50} values, a Tukey–Kramer HSD test was used. $P < 0.05$ was considered statistically significant (Statistical Discovery Software, JMP, USA).

3. Results

3.1. Coronary perfusion pressure values according to experimental settings

At the end of the stabilization period, the mean coronary perfusion pressure of all the non treated hearts (29.4 ± 0.6 mm Hg; $n = 72$) was not statistically different from that of the L-NAME treated group (29.1 ± 2.2 mm Hg; $n = 10$).

Table 1 summarizes the coronary perfusion pressure values measured during the perfusion with high K solution (KCl 40 mM) in the absence or in the presence of treatment, just before the perfusion of amiodarone, dronedarone and verapamil. In KCl 40 mM, the mean coronary perfusion pressure of the non-treated group was statistically different only from the mean coronary pressure of the L-NAME group. On the other hand, the mean coronary perfusion pressure of the L-NAME treated group was the highest, and statistically different from all the other treated groups except for the L-NOARG 3 μM-treated group.

3.2. Effects of amiodarone, dronedarone and verapamil on coronary perfusion pressure

The digital recordings of four representative experiments are shown in Fig. 1A. In hearts perfused with a solution containing 40 mM KCl, acetylcholine (1 μM) induced a transitory decrease in coronary perfusion pressure. The top recording shows that neither vehicle, HCl 0.1 N solution nor DMSO modified the baseline of coronary perfusion pressure in high K medium. Amiodarone, verapamil (0.01–10 μM) and dronedarone (0.01–1 μM) produced concentration-dependent decreases in coronary perfusion pressure; at the concentration of

Table 1
Coronary perfusion pressure in isolated guinea pig hearts measured in high K solution (KCl 40 mM), in the absence or presence of indomethacin, L-NOARG or ODQ treatment and in hearts obtained from guinea pigs treated with L-NAME, just before the perfusion of amiodarone, dronedarone and verapamil

Treatment	*n*	KCl 40 mM
		Coronary pressure (mm Hg)
None	16	107.0 ± 2.0#
Indomethacin	8	107.0 ± 3.5#
L-NOARG 1 μM	5	109.4 ± 4.2#
L-NOARG 3 μM	5	117.5 ± 2.7
L-NOARG 10 μM	10	111.7 ± 4.3#
L-NOARG 30 μM	10	113.7 ± 1.5#
L-NOARG 100 μM	10	114.7 ± 1.6#
L-NAME	10	126.4 ± 2.5*
ODQ	8	108.9 ± 4.3#

Values are means ± S.E.M.
# $P < 0.05$ vs. L-NAME-treated group.
* $P < 0.05$ vs. no treatment.

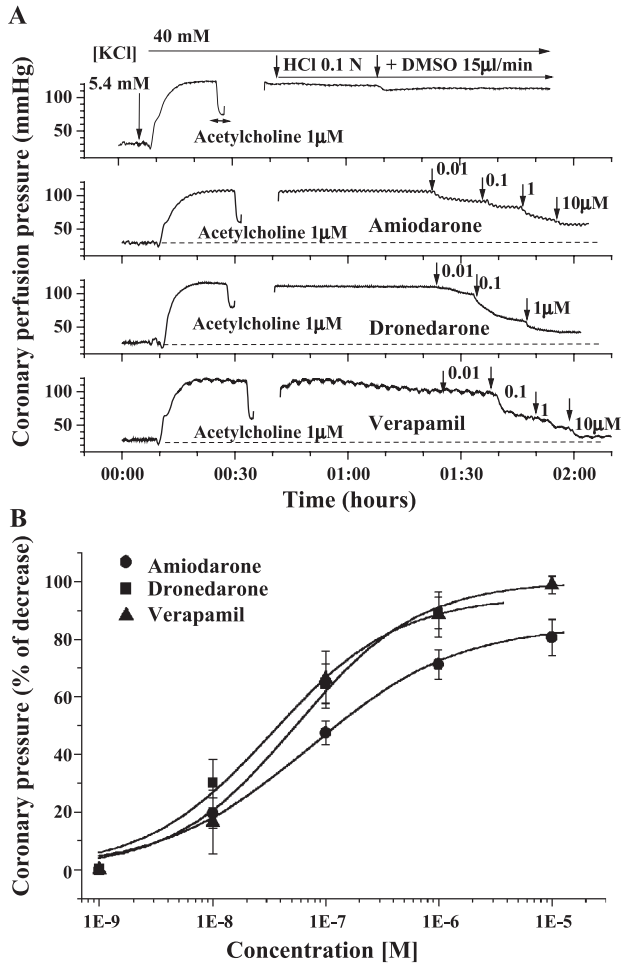


Fig. 1. Effects of amiodarone, dronedarone and verapamil on coronary perfusion pressure (coronary perfusion pressure) of guinea pig heart perfused with Tyrode's solution containing 40 mM KCl at constant flow (8 ml/min). (A) Representative experimental traces illustrating the brief perfusion of acetylcholine (1 μM) to test the endothelial dependent decrease in coronary pressure in each heart. Top recording shows the effects of vehicle, HCl 0.1 N + DMSO 100%, perfused at 15 μl/min. The three other recordings illustrate the perfusion of successive increasing concentrations (0.01–10 μM) of amiodarone ($n = 5$), dronedarone (0.01–1 μM, $n = 6$) and verapamil (0.01–10 μM, $n = 5$). (B) Summary of effects of amiodarone dronedarone and verapamil on coronary perfusion pressure. Concentration–response curves expressed as the percentage of decrease in coronary perfusion pressure from the level just before the administration of the test compound. Each data point represents the mean ± S.E.M. of n values from n hearts. Sigmoidal curves fitting were constructed by means of the Microcal Origin software.

1 μM, the decreases in coronary perfusion pressure were 71.3 ± 5.1% ($n = 5$), 89.1 ± 5.4% ($n = 6$) and 88.6 ± 1.4% ($n = 5$) for amiodarone, dronedarone and verapamil, respectively.

IC_{50} values calculated from the concentration curves (Fig. 1B) for dronedarone, verapamil and amiodarone were $4 \pm 1 \times 10^{-8}$ M ($n = 5$), $6 \pm 1 \times 10^{-8}$ M ($n = 6$), $9 \pm 2 \times 10^{-8}$ M ($n = 5$), respectively. No statistical difference both in maximal effects and IC_{50} were noted between the three compounds.

3.3. Effects of indomethacin on amiodarone- and dronedarone-induced decreases in coronary perfusion pressure

In hearts perfused with high K^+ medium, baseline coronary perfusion pressure was not affected by indomethacin at the concentration of 3 μM (Table 1). Indomethacin had no significant effect on amiodarone and dronedarone-mediated decreases in coronary perfusion pressure (Fig. 2A and B).

3.4. Effects of L-NOARG on amiodarone, dronedarone and verapamil-induced decreases in coronary perfusion pressure

L-NOARG (3–30 μM) caused a rightward shift of concentration–response curves obtained with amiodarone in a dose-dependent way and reduced the maximum response to amiodarone (Fig. 3A). In the presence of 30 μM of L-NOARG, amiodarone induced only about 20% decrease in coronary perfusion pressure vs. 80% in the absence of L-NOARG.

Concentration–response curves obtained with dronedarone were also shifted to the right by L-NOARG and maximal response to dronedarone was less inhibited when compared to amiodarone, even at the concentration of 100 μM of L-NOARG, 60% vs. 80% in the absence of L-

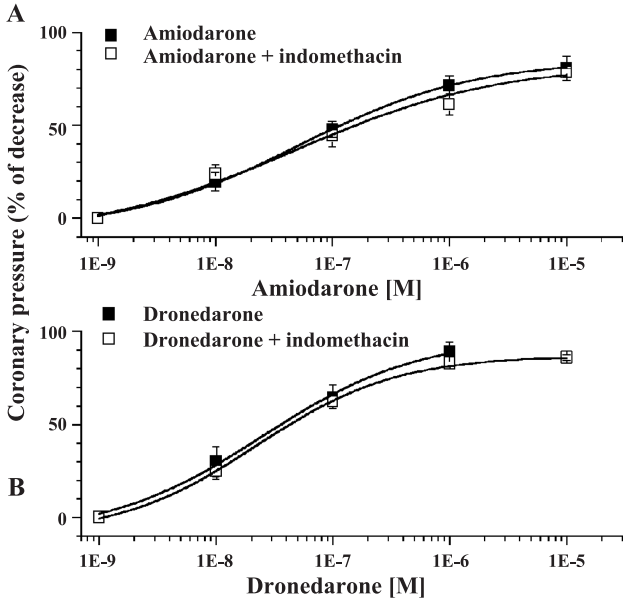
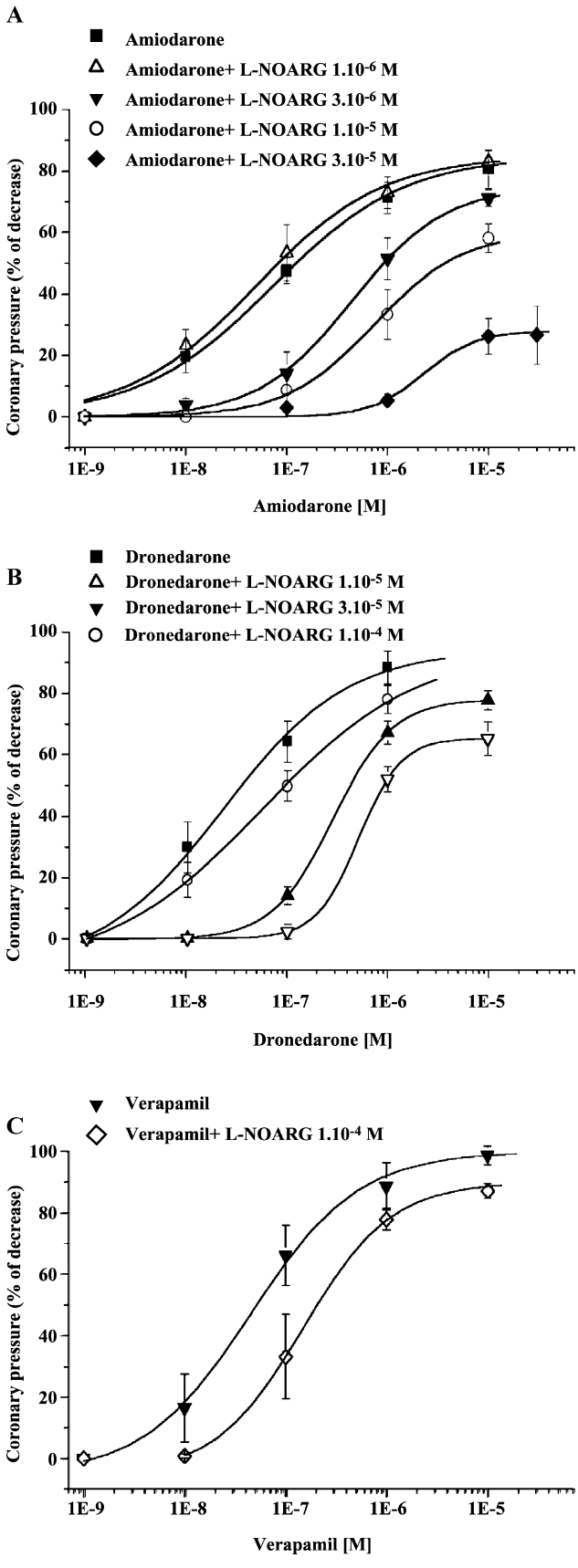


Fig. 2. Concentration–response curves decrease of coronary perfusion pressure to amiodarone (A) and dronedarone (B) in the absence and in the presence of indomethacin. Indomethacin (3 μM) does not modify significantly the decrease in coronary perfusion pressure induced by amiodarone and dronedarone in guinea pig heart perfused with Tyrode's solution containing 40 mM KCl at constant flow (8 ml/min). Each data point represents the mean ± S.E.M. of n values from n hearts. Amiodarone alone $n = 5$ plus indomethacin $n = 4$, dronedarone alone $n = 6$ plus indomethacin $n = 4$. Sigmoidal curves fitting were constructed by means of the Microcal Origin software.



NOARG (Fig. 3B). Concentration–response curve for verapamil was constructed in the presence of the highest concentration of L-NOARG (100 μM). As shown in Fig. 4C, only a weak shift to the right could be observed.

3.5. Concentration–response of amiodarone, dronedarone and verapamil in isolated hearts obtained from guinea pigs treated with L-NAME

Coronary pressure response to amiodarone, dronedarone and verapamil were tested in isolated hearts from guinea pigs treated with L-NAME (Fig. 4A and B). Inhibition of NO synthase was demonstrated by the absence of decrease in coronary perfusion pressure following perfusion of 1 μM Acetylcholine. Under NO synthase blockade, neither amiodarone nor dronedarone was able to reduce coronary perfusion pressure. Only a slight decrease was noted after dronedarone at the highest concentration (10 μM). On the contrary, coronary perfusion pressure response to verapamil (10 μM) was not abolished by L-NAME treatment (Fig. 4A and B).

3.6. Reverse effects of L-arginine on the L-NOARG-induced blockade of amiodarone and dronedarone responses

The recordings of four representative experiments are shown in Fig. 5. In the absence of L-NOARG, at equivalent effective doses (Fig. 3A and B), amiodarone (0.1 μM) and dronedarone (0.03 μM) induced a rapid and sustained decrease in coronary perfusion pressure (Fig. 5A and C), which was completely abolished when hearts were pre-treated with 10 or 30 μM L-NOARG (Fig. 5B and D). Addition of excess L-arginine (100 μM for amiodarone and 300 μM for dronedarone) restored the coronary effects of both amiodarone and dronedarone. It should be mentioned that L-arginine alone exerted no intrinsic effect on baseline coronary pressure in the presence of L-NOARG (data not shown).

Fig. 3. Effects of amiodarone ($4 \leq n \leq 5$) (A), dronedarone ($n = 5$) (B) and verapamil ($n = 5$) (C) in guinea pig hearts coronary perfusion pressure perfused with Tyrode's solution, at constant flow (8 ml/min), containing 40 mM KCl in absence and in the presence of L-NOARG. (A) Coronary perfusion pressure response obtained with amiodarone shifted to the right when the concentration of L-NOARG (1–30 μM) was increased. (B) Concentration of L-NOARG used to shift the coronary perfusion pressure with dronedarone was higher (10–100 μM) than those used with amiodarone. The maximum effects induced by dronedarone were less depressed by L-NOARG than those obtained with amiodarone. (C) Coronary perfusion pressure response to verapamil slightly shifted to the right with the highest concentration of L-NOARG (100 μM). Concentration–response curves are expressed as the percentage of decrease in coronary perfusion pressure from the level just before the administration of the test compound. Each data point represents the mean ± S.E.M. of n values from n hearts. Sigmoidal curves fitting were constructed by means of the Microcal Origin software.

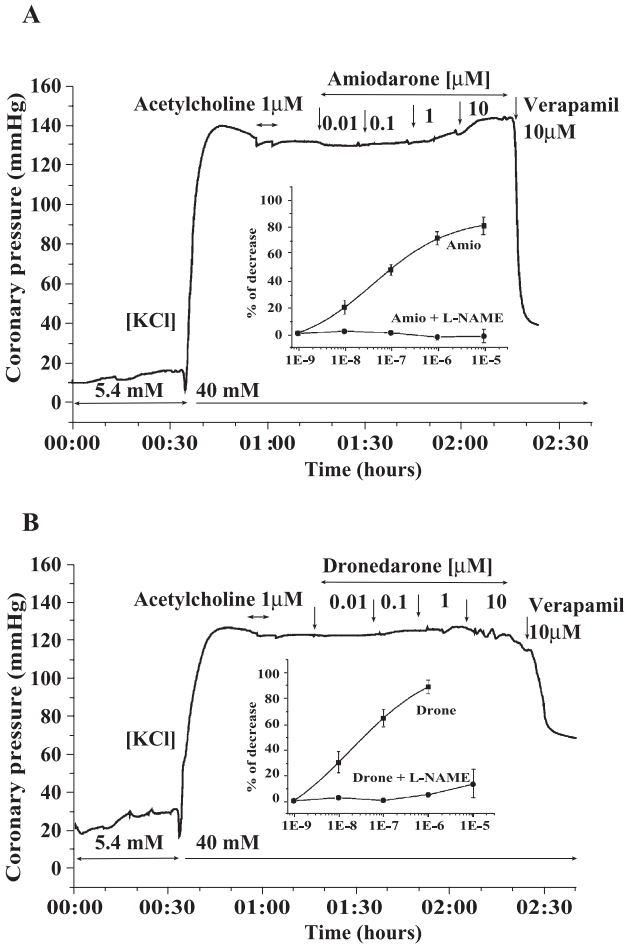


Fig. 4. Effects of acetylcholine, amiodarone, dronedarone and verapamil on coronary perfusion pressure of hearts taken from guinea pig chronically treated with L-NAME, which were perfused with Tyrode's solution containing 40 mM KCl at constant flow (8 ml/min). (A) Representative experimental traces illustrating the lack of effect on coronary perfusion pressure with acetylcholine (1 μM) and amiodarone (0.01–10 μM) on coronary perfusion pressure. (B) Representative experimental traces illustrating the lack of effect on coronary perfusion pressure with acetylcholine (1 μM) and dronedarone (0.01–1 μM). At the concentration of 10 μM, dronedarone induced a slight decrease in coronary perfusion pressure. Inset A: Concentration–response relationship curves of coronary perfusion pressure in the absence (Amio, $n=5$) and presence of L-NAME (Amio + L-NAME, $n=5$) in guinea pig hearts. Inset B: Concentration–response relationship curves of coronary perfusion pressure in the absence (Drone, $n=6$) and presence of L-NAME (Drone + L-NAME, $n=5$) in guinea pig hearts. Each data point represents the mean ± S.E.M. of n values from n hearts. L-NAME did not alter the coronary perfusion pressure response to verapamil in all cases.

3.7. Concentration–response of amiodarone and dronedarone in the presence of ODQ

The coronary perfusion pressure effects to increasing concentrations of amiodarone or dronedarone were studied in the presence of ODQ, a guanylyl cyclase inhibitor. ODQ, at the concentration of 1 μM, totally abolished the concentration effects for amiodarone, whereas the response to verapamil (10 μM) was not affected (Fig. 6A). At the concentration of 3 μM, ODQ abolished the coronary perfusion pressure decreases of 0.01 and 0.1 μM dronedarone, but partially inhibited the effects induced by 1 and 10 μM of dronedarone (Fig. 6B).

4. Discussion

The results of the present study show that both amiodarone and dronedarone effectively induce coronary vasodilation that involves nitric oxide but not prostaglandins or endothelium-derived hyperpolarising factor (EDHF) in isolated guinea pig hearts perfused with high K^+ solution. In the presence of the cyclo-oxygenase inhibitor, indomethacin, the decrease in coronary perfusion pressure induced by both amiodarone and dronedarone was not modified. This may suggest that, under our experimental conditions, prostacyclin was not implicated in the vasorelaxant effect of amiodarone and dronedarone in the guinea pig coronary bed. These findings are in contrast with those of Grossmann et al. (1998) who showed that, in the human hand vein, acetylsalicylic acid partially inhibited the amiodarone-induced vasodilation, suggesting that prostaglandins are likely to be involved in the effect of amiodarone. This difference may be due to the absence of prostacyclin release, or blockade of prostacyclin induced vasorelaxant effects in a hyper K^+ medium. Parkington et al. (1993) demonstrated that the guinea pig coronary artery bed was able to release prostacyclin, which hyperpolarizes the membrane of the smooth muscle. Moreover, in the bovine aortic endothelial cells, a high K^+ solution did not prevent the release of the prostacyclin; changes in KCl concentration from 0 to 80 mM in the incubation medium had no effect on the basal or ATP-stimulated release of prostacyclin (Boeynaems and Ramboer, 1989). Similarly, Hammarstrom et al. (1995) showed that the ability of NO or iloprost, a prostacyclin mimetic, to relax guinea pig coronary artery did not depend upon hyperpolarization of the smooth muscle. Furthermore, it was shown in human coronary artery that a high K^+ solution (45 mM) did not inhibit the vasorelaxant effects of iloprost (Merritt et al., 1991). Taken together, these findings demonstrate, on the one hand, that the guinea pig coronary artery bed is able to release prostacyclin and, on other hand, hyper K^+ medium does not inhibit the vasorelaxant effect of prostacyclin. According to these observations, the lack of effects of indomethacin on amiodarone and dronedarone decreases in coronary perfusion pressure, under our experimental conditions, suggests that these two compounds do not release prostacyclin in guinea pig coronary artery.

Subsequently, the decrease in coronary pressure induced by both amiodarone and dronedarone could not be due to vasodilation in response to the coronary smooth muscle hyperpolarization produced by endothelium-derived hyperpolarizing factor (EDHF). In fact, when the hearts were perfused with 40 mM K^+ solution, the driving force for K^+ was considerably reduced, thereby decreasing K^+ efflux

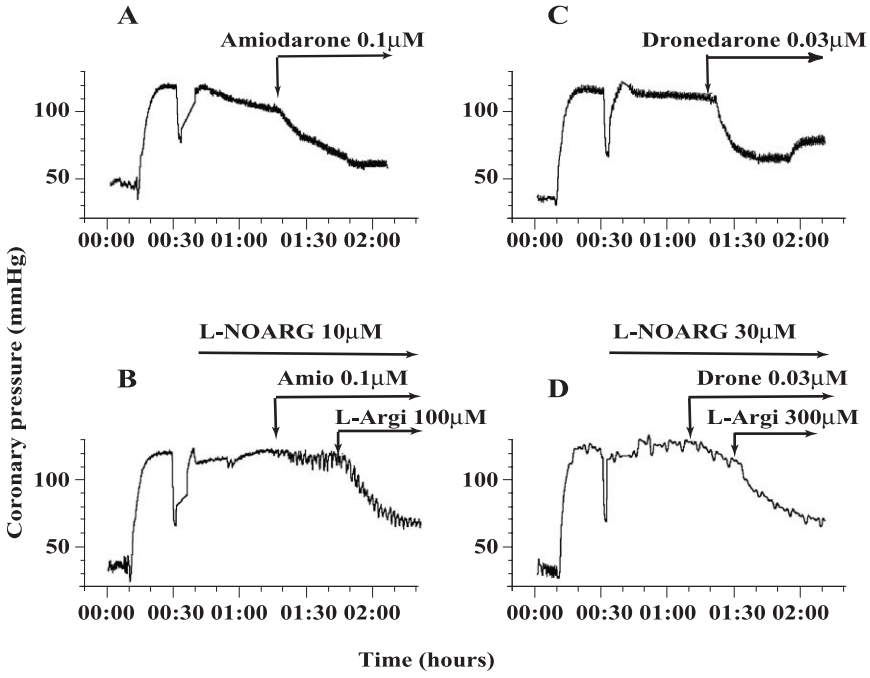


Fig. 5. Representative experimental traces illustrating the reverse effect of L-Arginine (NO precursor) on the blockage of NO synthase induced by L-NOARG in guinea pig hearts perfused with Tyrode's solution containing 40 mM KCl at constant flow (8 ml/min). (A and C) Control experiments showing the decrease in coronary perfusion pressure induced by amiodarone (0.1 μM) (A) and dronedarone (0.03 μM) (C). (B and D) Experiments showing that the effects on coronary perfusion pressure induced by amiodarone (0.1 μM) and dronedarone (0.03 μM) were inhibited by 10 and 30 μM of L-NOARG, respectively. The decrease in coronary perfusion pressure induced by amiodarone and dronedarone was restored by the administration of excess L-arginine. Similar results were obtained for both amiodarone and dronedarone in four different experiments.

and membrane hyperpolarization. Yajima et al. (1999) showed that the acetylcholine-induced hyperpolaryzation was abolished in high-K solution in smooth muscle of the guinea pig circumflex coronary artery. Similarly, Nagao and Vanhoutte (1992) showed that endothelium-dependent hyperpolarization of porcine coronary artery induced by bradykinin was abolished with high K^+ solution. Moreover, we verified that a K_{ATP} channel opener, SR47063 (Tourneur et al. 1994), had no effect on the coronary perfusion pressure in guinea pig heart perfused by Tyrode's solution containing KCl at the concentration of 40 mM (data not shown). Consequently, although it cannot be excluded that EDHF under normal conditions contributes to the endothelium dependent vasorelaxant effects of amiodarone and dronedarone, its involvement in the present experiments can be ruled out.

Finally, the contribution of NO in the coronary vasorelaxant effects of amiodarone and dronedarone were investigated. Inhibition of NO synthase with L-NOARG, antagonised the concentration-dependent decrease in the coronary pressure induced by both amiodarone and dronedarone, whereas the effects of verapamil were only slightly affected. This is the first evidence of involvement of NO in the decrease of coronary perfusion pressure induced by these two compounds. These results were strengthened by the fact that the L-Arginine, the substrate of NO synthase, reversed the inhibition induced by L-NOARG. Moreover, we showed that, in contrast to verapamil, both amiodarone and dronedarone, like acetylcholine, did not reduce coronary perfusion pressure in heart obtained from guinea pig treated with L-NAME. Corriu et al. (1998) showed a comparable result using bradykinin in aorta taken from guinea pigs treated L-NAME. It is noted that chronic in vivo administration of L-NAME was more effective than acute L-NOARG perfusion in inhibiting the effects of amiodarone and dronedarone. Our experimental conditions did not permit to attest that L-NAME was more efficient than L-NOARG. To be sure that L-NAME is more effective than L-NOARG, it would be appropriate to assess amiodarone and dronedarone vasodilation properties in the presence of acute L-NAME perfusion. Vial and Burnstock (1992), effectively observed that L-NAME was a more effective agent than L-NOARG in inhibiting the vasodilator actions of 5-HT and ATP in isolated guinea pig heart.

To reinforce previous results and add new evidence for the implication of nitric oxide pathway in the vasorelaxant effects of amiodarone and dronedarone, we showed in the present study that ODQ, a specific inhibitor of the guanylyl cyclase, the target of NO which leads to the formation of cGMP, was able to inhibit the effects of amiodarone and in part those of dronedarone but not verapamil. These findings may confirm the involvement of cGMP in the vasodilation of coronary bed induced by amiodarone and dronedarone.

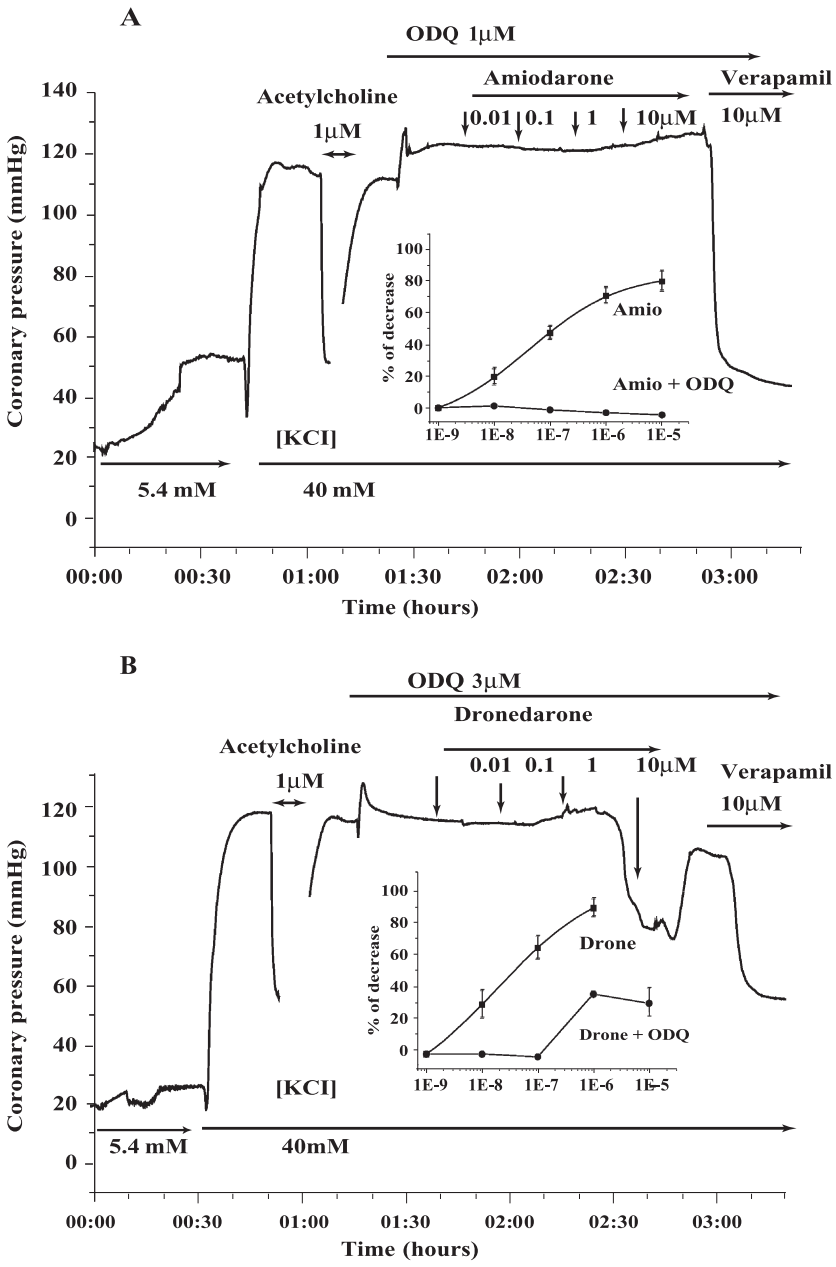


Fig. 6. Representative experimental traces illustrating the blockage of NO pathway by ODQ (guanylyl cyclase inhibitor) and corresponding effects on coronary perfusion pressure induced by both amiodarone and dronedarone in guinea pig hearts perfused with Tyrode's solution containing 40 mM KCl at constant flow (8 ml/min). (A) In the presence of ODQ (1 μM), perfused after acetylcholine test, the coronary perfusion pressure response to amiodarone (0.01–10 μM) was totally abolished. (B) The coronary perfusion pressure response to (0.01 and 0.1 μM) was abolished in the presence of ODQ (3 μM). However, at 10 μM dronedarone, the coronary perfusion pressure response was not completely abolished by ODQ. Inset A: Concentration–response relationship curves of coronary perfusion pressure in the absence (Amio, $n=5$) and presence of ODQ (Amio + ODQ, $n=4$). Inset B: Concentration–response relationship curves of coronary perfusion pressure in the absence (Drone, $n=6$) and presence of ODQ (Drone + ODQ, $n=4$). ODQ did not alter the coronary perfusion pressure response to verapamil in all cases.

We noted that neither L-NOARG nor ODQ significantly increased the coronary perfusion pressure in high-K medium, suggesting that, under these experimental conditions, the basal release of NO makes no contribution to coronary tone. Similar observation was made by Heijenbrok et al. (2000) in the rat carotid and mesenteric arteries.

Furthermore, it should be mentioned that a reduced coronary pressure induced by high concentration of dronedarone was not entirely suppressed by either L-NOARG or ODQ and in hearts taken from guinea pigs treated with L-NAME. The remaining coronary dilation with dronedarone after inhibition of the NO pathway is likely due to the inhibitory effect of L-type calcium current observed by Gautier et al. (2003) in guinea pig ventricular myocytes.

In conclusion, the present study showed that amiodarone and dronedarone elicit a concentration-dependent coronary

dilation. This effect appears to be mainly due to a release of NO. Dronedarone differs from amiodarone by a remaining relaxant effect refractory to the inhibition of NO synthase pathway probably due to its Ca^{+} antagonist property. Consequently, dronedarone may induce a coronary dilation involving a dual mechanism, stimulation of NO synthase pathway and putative Ca^{+} channels inhibition. Stimulation of the NO pathway appears to be the primary mechanism implicated in guinea pig coronary dilation induced by amiodarone. Nevertheless, further studies are required to elucidate the mechanism by which amiodarone and dronedarone stimulate the NO pathway.

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