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Flow Stimulates Nitric Oxide Release in Guinea Pig Heart: Role of Stretch-Activated Ion Channels

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Blood flow regulates vessel tone triggering the release of nitric oxide; however, the mechanism involved in this phenomenon is unknown. We investigated whether coronary flow induces nitric oxide release in the isolated perfused guinea pig heart and the role of the stretch-activated ion channels in the effect of flow. We used gadolinium (3 μM) in order to block these channels, and estimated nitric oxide release by an oxyhemoglobin method. The results have shown a flow-dependent stimulation of nitric oxide release (fivefold increase at perfusion flow of 25 ml/min). Gadolinium inhibited this effect in a dose-dependent fashion. Acetylcholine was able to stimulate nitric oxide release in presence of gadolinium. We concluded that coronary flow stimulates nitric oxide release in the guinea pig heart. Stretch-activated ion channels mediate this effect. Acetylcholine and flow stimulate nitric oxide release by different mechanisms of action.

Key Words: **nitric oxide release; stretch-activated ion channels; coronary flow.**

Blood flow regulates vessel tone mainly by the action of shear stress on the endothelium (for review see 1). The dependence of flow-mediated vasodilation on an intact endothelium has been confirmed in large conduit arteries as well as in resistance-sized vessels (2–10). The mechanism of flow-induced vasodilation involves the enhanced release of endothelial-derived relaxing factors (EDRF), the principal component of which is nitric oxide (NO) and closely related nitroderivatives (11–13). NO is the principal EDRF in the vasculature as determined by an overwhelming number of *in vivo*, *ex vivo* and *in vitro* experiments (reviewed in Refs. 11 and 14). NO is formed from L-arginine (12) by the activity of NO synthase which is inhibited by the analog N^{ω}-nitro-L-arginine methyl ester (L-NAME) (15). Endothelial NO synthase is constitutively expressed at basal level, and its activity is calcium/calmodulin dependent (16, 17). Consideration of the mechanism(s) by which flow induces NO-mediated vasodilation is focused on the regulation of endothelial NO synthase activity, however, mechanism for the transduction of the hemodynamic force into NO release remains unknown. Stretch-activated nonselective cation channels have been demonstrated in a wide variety of cells including vascular endothelium (18) and can be blocked by Gd^{3+} (19). Opening of these channels causes Ca^{2+} and Na^{+} influx and membrane depolarization which may contribute to the activation of NO synthase with the concomitant increase in NO release. We thus hypothesize that blockade of stretch-activated ion channels with Gd^{3+} can modify the flow-induced NO release. In the present study, to test this possibility, we study the effect of Gd^{3+} in the flow-induced NO release curve in the isolated perfused guinea pig heart. The results obtained provide the first evidence that flow-induced NO release is mediated by activation of stretch-activated ion channels.

METHODS

Isolated guinea pig hearts. Guinea pigs weighing 300-350g were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg body weight), xylazine (20 mg/kg body weight), and heparin (500 U). The heart was removed and retrogradely perfused via a nonrecirculating perfusion system at constant flow. Coronary flow was adjusted to different values by varying the output of a variable-speed peristaltic pump (model 1215, Harvard Apparatus). An initial perfusion rate of 25 ml/min for 5 min was followed by a 25-min equilibration period at a perfusion rate of 10 ml/min. All experimental measurements were initiated after this period of equilibration. The perfusion medium was Krebs-Ringer-bicarbonate solution with the following composition (mM): NaCl 117.8, KCl 6, $CaCl_2$ 1.6, $NaHCO_3$ 24.2, $MgSO_4$ 1.2, NaH_2PO_4 1.2, Na_2EDTA 0.027, and glucose 5.0. This solution was equilibrated with 95% O_2, 5% CO_2 (37°C at pH 7.4). This method has been described before (20). The coronary perfusion pressure was continuously recorded via a sidearm of the perfusion cannula. Coronary vascular resistance was estimated as the ratio between perfusion pressure (mmHg)/perfusion flow (ml/min).

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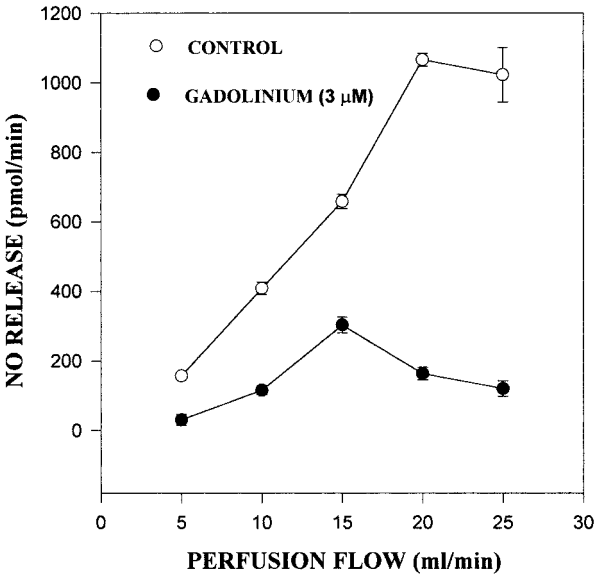

FIG. 1. Influence of flow on nitric oxide release in the absence or presence of gadolinium. Hearts were perfused as indicated in Methods. Changes in perfusion flow were induced by changing pump perfusion rate to obtain those rates indicated in the figure. Nitric oxide release was measured in the absence (open circles) and in the presence (solid circles) of 3 μM $GdCl_3$. Each point represents the mean ± SE, n = 6.

Measurement of NO. NO release was quantified in the effluent perfusate by a specific difference spectrophotometric assay, which is based on the rapid oxidation of oxyhemoglobin (HbO_2) to methemoglobin by NO (21, 22). Since HbO_2 traps the entire amount of NO released in less than 100 ms (22), measurement of the extinction difference (λ_1 401 nm, λ_2 411 nm) in a double beam spectrophotometer (SLM-Aminco, Urbana, IL, U.S.A.) in samples obtained from the venous effluent of perfused hearts, permits an estimation of the NO released. The NO concentrations were calculated from the extinction coefficient, which was determined under the conditions used in this study to be 38 mM^{-1}.

RESULTS

Effect of flow on nitric oxide release. Isolated perfused hearts at a coronary flow of 10 ml/min, constantly released NO at a basal rate of 409 ± 18 pmol/min (n = 6). This value was in the same range to that reported by Kelm and Schrader using similar experimental conditions (21). Figure 1 shows the influence of flow on nitric oxide release before and after the infusion of $GdCl_3$ (3 μM). Increases in coronary flow stimulated NO release in a flow-dependent fashion ranging from 157 ± 4 pmol/min at coronary flow of 5 ml/min to 1023 ± 78 pmol/min at coronary flow of 25 ml/min (Fig. 1 open circles). Infusion of $GdCl_3$ decreased NO release at a basal flow of 5 ml/min and inhibited the flow-induced NO release (Fig. 1 solid circles).

Effect of gadolinium on acetylcholine-induced NO release. Figure 2 shows the effect of a change on perfusion flow from 10 to 20 ml/min on NO release in presence of acetylcholine (4 μM), $GdCl_3$ (3 μM) or both substances. In this group of experiments, an increase of perfusion flow from 10 to 20 ml/min produced an increase in NO release of 160% (flow 10: 409 ± 17.8 pmol/min, flow 20: 1066 ± 18.7 pmol/min). Acetylcholine stimulated the flow-induced NO release in both tested flow rates in 60 and 100% respectively (flow 10: 657 ± 32, flow 20: 2156 ± 158). Infusion of $GdCl_3$ depressed NO release in both flow rates, however, addition of acetylcholine in presence of gadolinium reestablished NO release to control values.

Effect of gadolinium on coronary resistance. Perfusion flow increases coronary perfusion pressure in control conditions. Infusion of $GdCl_3$ (3 μM) had a pressor effect on coronary perfusion pressure (Fig. 3, panel A). Coronary resistance (perfusion pressure/coronary flow) did not change with flow in control. Gadolinium produced an increase in coronary resistance at the lower perfusion flow rate, however, coronary resistance values returned close to control values as flow rates were increased. (Fig. 3, panel B).

DISCUSSION

In the present study, we demonstrated that an increase in steady flow rate augments the release of nitric oxide by the isolated perfused guinea pig heart

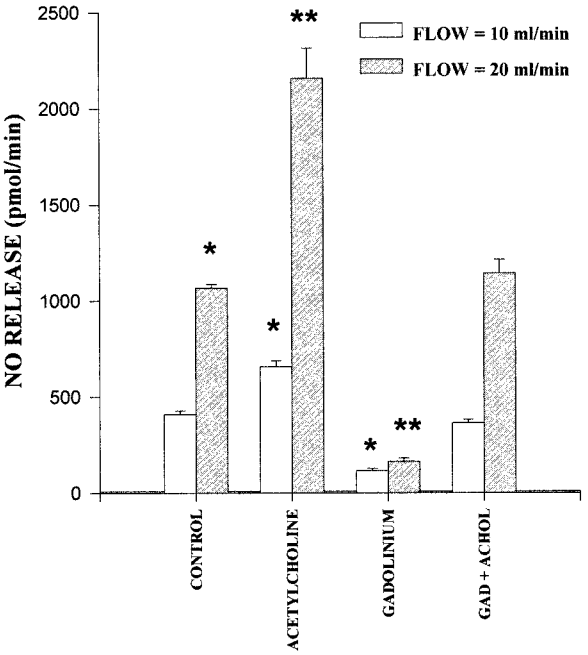

FIG. 2. Effect of gadolinium on acetylcholine-induced nitric oxide release. In separate series of experiments, coronary flow was changed from 10 to 20 ml/min in the absence and in the presence of 4 μM acetylcholine, 3 μM gadolinium, or acetylcholine plus gadolinium as indicated in the figure. Bars represent means ± SE, n = 6. (*) $p \leq 0.05$ vs. control at flow, 10 ml/min; (**) $p \leq 0.05$ vs. control at flow, 20 ml/min.

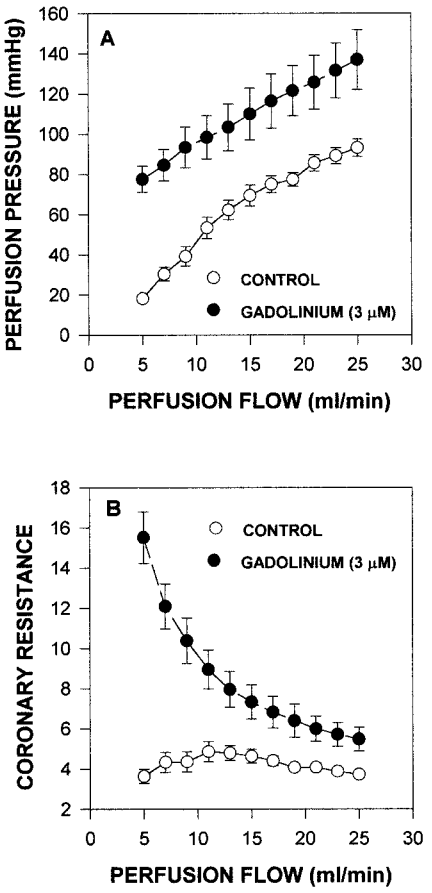

FIG. 3. Influence of blockade of stretch-activated ion channels on the flow-pressure (A) and flow-coronary resistance (B) curves. Hearts were perfused at different flow rates in the absence (open circles) and in the presence (solid circles) of 3 μM $GdCl_3$. Each point represents means ± SE, n = 6.

and, this flow-induced NO release is inhibited by gadolinium, a specific stretch-activated ion channel blocker. Kelm and Schrader described the method utilized here to measure NO release in the guinea pig heart (21). We obtained similar values of basal NO release (coronary flow rate of 10 ml/min, 409 ± 17 pmol/min, n = 15) to that reported before (216 ± 36 pmol/min, ref. 21). An increase in coronary perfusion flow stimulated the basal NO release in a dose-response fashion (Fig. 1). This indicates that coronary circulation also responses to flow releasing NO. Increase in NO release involves activation of NO synthase by an increase in calcium/calmodulin. We hypothesized that, opening of stretch-activated ion channels causes Ca^{2+} and Na^{+} influx and membrane depolarization which may contribute to the activation of NO synthase with the concomitant increase in NO release. Blockade of these channels by gadolinium (3 μM) inhibited the flow-induced NO release supporting this hypothesis. Gadolinium concentration used here is in the range reported to block stretch-activated ion channels (19) and utilized by others to test participation of stretch-activated ion channels in responses to mechanical stress (24–26). Acetylcholine was able to induce NO release in presence of gadoliniumCoronary perfusion pressure was increased by gadolinium and it was reflected in coronary resistance (Fig. 3A and 3B). We expected this effect since gadolinium inhibits NO release, however, it is interesting that coronary resistance in the gadolinium treated heart, started 3 fold higher than the control heart and it was decreasing when perfusion flow was increased. This decrease in coronary resistance is not due to NO release since it is inhibited by gadolinium (Fig. 3), therefore, participation of another relaxing factor, in order to maintain coronary resistance, is suggested.

In conclusion, we first demonstrate that coronary flow stimulates NO release and stretch-activated ion channels could mediate this phenomenon.

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