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THE ENDOTHELIUM-DEPENDENT AND THE ENDOTHELIUM-INDEPENDENT VASODILATORS IN THE ISOLATED, PERFUSED GUINEA PIG HEART.

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The endothelium-dependent (acetylcholine, bradykinin, substance P) and the endothelium-independent (gliceryl trinirate, 3-morpholinsydnominine, sodium nitroprusside) vasodilators were studied in the Langendorff-perfused heart of the guinea pig. The involvement of prostanoids and EDRF in the endothelium-dependent responses were assessed by using indomethacin, an inhibitor of cyclooxygenase, and N^G-nitro-L-Arginine, an inhibitor of NO synthase. The endothelium-independent agents were used as reference compounds. Both indomethacin and N^G-nitro-L-Arginine elevated significantly baseline coronary perfusion pressure, indicating that prostanoids (most likely PGI_2 and PGE_2) and EDRF modulate the resting tone of the guinea pig coronary circulation. N^G-nitro-L-Arginine, but not indomethacin, considerably reduced receptor-stimulated responses. It is concluded that acetylcholine, bradykinin or substance P-induced vasodilation is mediated by EDRF. In contrast, prostanoids do not contribute to endothelium-dependent responses. In addition, short-term tachyphylaxis to bolus injection of gliceryl trinitrate but not of sodium nitroprusside was demonstrated, suggesting that this preparation may be of value for studying nitrate tolerance.

Key words: *coronary microcirculation, endothelium-dependent relaxation, EDRF, prostacyclin, gliceryl trinitrate, tachyphylaxis.*

INTRODUCTION

Endothelium-derived-relaxing-factor, EDRF, (1) is a potent vasorelaxant owing to the stimulation of soluble guanylate cyclase and an increase in intracellular level of cyclic GMP (2). Nitric oxide (NO) or NO containing moiety (3—5) accounts for the biological activity of EDRF. Nitric oxide is formed by a dioxygenase (NO-synthase) through oxidation of L-Arg to NO

and citrulline (6). It is well established that in conductive arteries and veins EDRF mediates the vasodilatory action of many endogenous vasodilators such as acetylcholine (Ach), bradykinin (Bk) or substance P (SP) (7, 8). However, the role of EDRF in the microcirculation is less clear. In the coronary circulation Ach (9,10) or Bk (11, 12) were claimed to release EDRF or its mediation was not established (13). Apart from EDRF endothelial cells produce prostacyclin (14—16), which also relaxes vascular smooth muscle, and moreover the release of PGI2 and EDRF is coupled (17). However, there is no consensus on the role of prostanoids as the mediators of vasodilator responses to Ach, Bk, SP in the coronary circulation (18—23). Therefore, this study was designed to reevaluate the participation of EDRF and prostanoids in the coronary vasodilation induced by Ach, SP, Bk in the isolated, perfused heart of the guinea pig. The endothelium-independent vasodilators (e. g. gliceryl trinitrate — GTN, 3-morpholinosydnonimine — SIN-1 and sodium nitroprusside — NaNP) were used as reference compounds, while L-G^G-nitro-arginine (NO_2Arg) an inhibitor of NO synthase (24—26) and indomethacin (IND), an inhibitor of cyclooxygenase (27), were used as pharmacological tools for studying the participation of EDRF or prostanoids in the endothelium-dependent responses.

MATERIALS AND METHODS

Guinea pigs of both sexes (body weight 200—300g) were killed by a blow to the neck. After the thorax was opened, the heart was rapidly excised, placed in ice-cold saline, and retrogradely perfused through the ascending aorta according to the Langendorff technique. Sixty seconds after the heart was removed it started to be perfused with Krebs-Hanseleit solution of the following composition (in mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.52, Mg SO_4 1.64, $NaHCO_3$ 24.88, KH_2PO_4 1.18, Glucose 5.55, Sodium pyruvate 2.0. The perfusate was equilibrated with 5% CO_2: 95% O_2 at 37°C using a disc oxygenator (Hugo Sachs Elektronik-HSE). Subsequently the pericardium and adherent lung tissue were removed and the pulmonary artery was cannulated to allow coronary venous drainage. The fluid-filled balloon connected to a pressure transducer (Isotec HSE) was inserted into the left ventricle for the measurement of left ventricular pressure (LVP). The volume of the balloon was adjusted to maintain the end-diastolic pressure below 10 mmHg. The heart was paced at 273/min via a coaxial stimulation electrode which was connected to a HSE stimulator P and placed on the surface of the right atrium. The stimulation parameters were: stimulus amplitude less then 10V (minimum voltage needed to override the intrinsic beat of the heart), duration 5 msec. Coronary perfusion pressure (CPP) was monitored by a second pressure transducer (Isotec HSE) connected to the side arm of the perfusion line. Changes in CPP were used to monitor changes in coronary tone and were continuously displayed on a line recorder (TZ 4620). The heart was equilibrated for 15 min at a constant pressure perfusion (55 mmHg) and afterwards perfused at a constant flow by means of a roller pump. The pump was set initially to the flow observed under constant pressure perfusion and gradually increased to obtain a starting

CPP between 60—70 mmHg. The heart was used for the experiment only when the following conditions were fulfilled: (1) CPP was higher than 55 mmHg, (2) bolus injection of sodium nitroprusside (3 nmoles) decreased CPP by more than 20 mmHg, (3) occlusion of the coronary flow for 3 sec evoked reactive vasodilation. At the end of the experiment the capacity of the coronary vessel to dilate was checked by the same standard test for reactivity of the coronary smooth muscle and coronary endothelium. Within 3 hours of starting the perfusion reactivity of the heart did not decline.

Bolus injections of the investigated vasodilators were administered into the perfusate, proximally to the aortic cannula. The transit time from the site of injection to the coronary circulation was less than 30 sec. The inhibitors used in the study were added to the perfusate reservoir (IND) or infused through a side arm of the perfusion line at a rate of 0.1 ml/min (NO_2Arg) and perfused through the heart for a minimum period of 10 min before obtaining responses to the drugs.

The inhibition of vasodilatory effect was determined on the basis of area of dilation defined as area under the curve of CPP line representing the fall of coronary perfusion pressure. This area was calculated by weighing the tracing paper in the shape of the area of dilation and expressed in square cm. In order to check this method it was compared with Simpson's numerical integration method of calculating the area under the curve. The results obtained by both methods were almost identical. All experiments were completed in less than 3h.

The following drugs were purchased from Sigma Chemical Co.: acetylcholine (Ach), bradykinin (Bk), substance P (SP), gliceryl trinitrate (GTN), 3-morpholinosydnonimine (SIN-1), sodium nitroprusside (NaNP), indomethacin (IND), and L-N^G-nitro-arginine (NO_2Arg). All vasodilators were dissolved in saline. Indomethacin was dissolved in 5% solution of $NaHCO_3$. NO_2Arg was dissolved in saline at 40°C with shaking. Fresh solutions of vasodilators were prepared every day. The solution of NaNP was kept on ice and in the dark throughout the experiments. The solution of SIN-1 was prepared immediately before its administration.

All results are presented as means and their standard errors of n determinations. The differences between means were evaluated by unpaired Student t-test and those of P values of 0.05 or less were considered statistically significant.

RESULTS

Baseline coronary perfusion pressure (CPP) in the isolated guinea pig heart was 65.2 ± 0.46 mmHg ($n = 42$). Bolus injections of GTN, SIN-1, NaNP (0.1—30 nmoles), Ach (10—300 pmoles), Bk (0.1—10 pmoles) and SP (1—100 pmoles) induced a dose-dependent vasodilation expressed as the fall in CPP *(Fig. 1)*. In general, the endothelium-dependent vasodilators were one to three orders of magnitude stronger than the endothelium-independent agents. Among the endothelium-dependent vasodilators Bk was more potent than SP and Ach, while potencies of the endothelium independent vasodilators were similar. The fall in CPP (28.4 ± 2 mmHg) elicited by maximally effective dose of Ach (300 pmoles) was smaller than those caused by the maximal effective doses of other drugs which ranged from 35 to 40 mmHg (*Tab. 1*). Repetitive

administration of bolus injection of GTN showed tachyphylactic response which was not observed with other drugs *(Fig. 2)*. All studied vasodilators (with the exception of Ach) had no significant effect on heart rate or left ventricular function (LVP). Ach in doses higher than 600 pmoles had negative chronotropic and inotropic action.

Table 1. Maximum vasodilator responses which were achieved by the endothelium-dependent (Ach, SP, Bk) and endothelium-independent (GTN, SIN-1, NaNP) vasorelaxants in the perfused guinea pig heart. Data presented as mean and s. e. mean of n = 3—5 experiments.

Vasodilator	dose	maximum decrease in perfusion pressure (mmHg)
Ach	300 pmoles	28.48 ± 2.08
SP	100 pmoles	35.2 ± 0.68
BK	10 pmoles	36 ± 1.8
GTN	30 nmoles	34.8 ± 0.8
SIN-1	30 nmoles	34.5 ± 0.28
NaNP	30 nmoles	39.68 ± 0.32

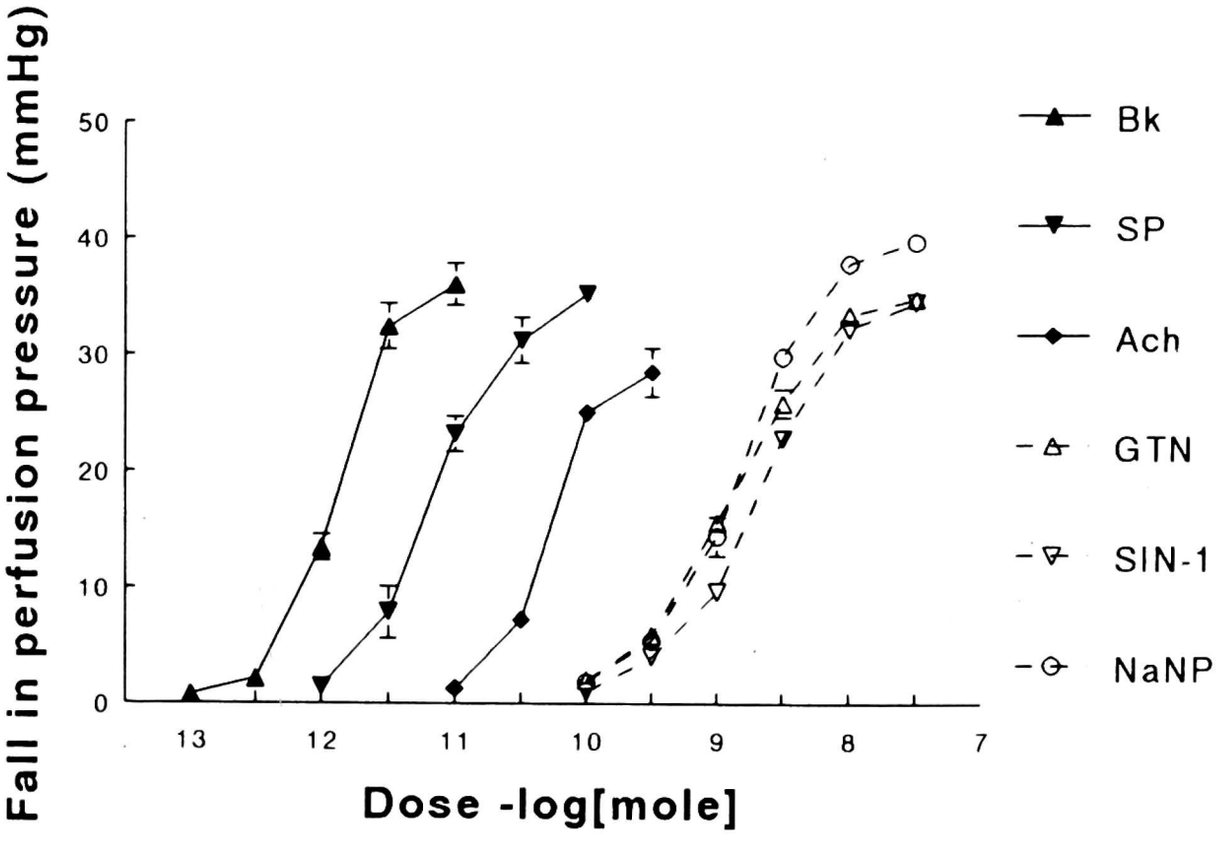

Fig. 1. The dose response curves for a decrease in perfusion pressure induced by the endothelium-dependent vasodilators (Ach, SP, Bk) and endothelium-independent vasodilators (GTN, SIN-1, NaNP) in the coronary bed of the isolated guinea pig heart. Each point represents the mean and vertical bars standard errors of the mean of n = 3—5 experiments.

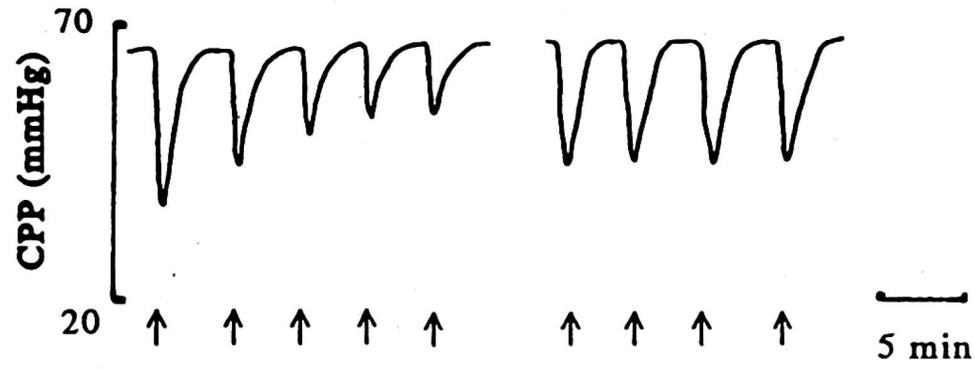

Fig. 2. Repetitive administration of bolus injection of GTN (10 nmoles) but not of NaNP (3 nmoles) produced tachyphylaxis of vasodilator responses in the isolated guinea pig heart.

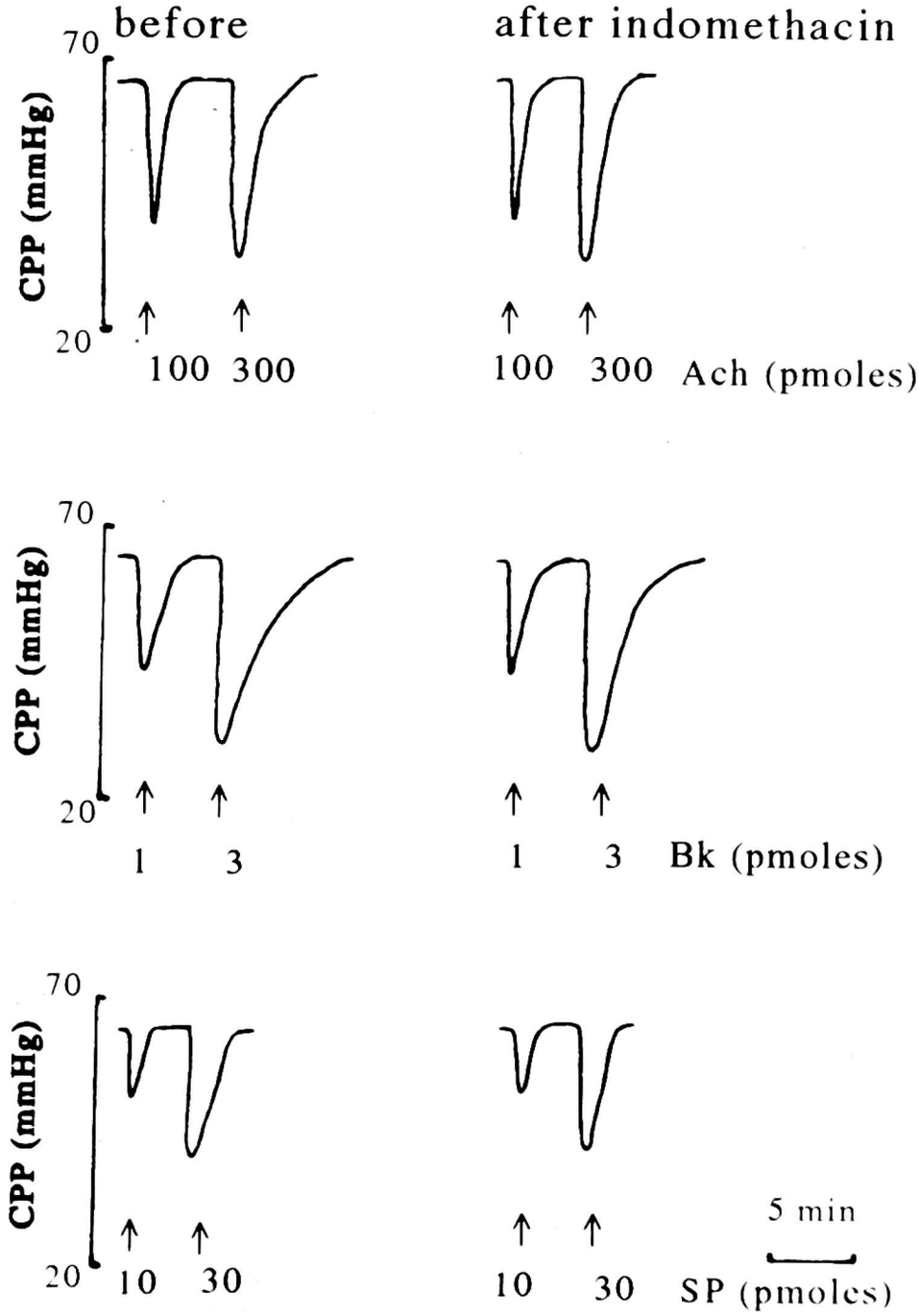

Fig. 3. Lack of the effect of indomethacin (5 μM) on the decrease in coronary perfusion pressure induced by the endothelium-dependent vasorelaxant (Ach, Bk and SP) in the isolated guinea pig heart. Similar results were obtained in other 5 experiments.

Infusion of indomethacin (5 μM) slightly although significantly increased CPP (by 3.7 ± 0.36 mmHg $n=7$) but did not alter the relaxant responses to Ach, SP, Bk (*Fig. 3*).

Infusions of NO_2Arg (10—100 μM) caused a concentration-dependent elevation of CPP (*Fig. 4*) without affecting either LVP or heart rate. At the maximal concentration used (100 μM), NO_2Arg increased CPP by 7.92 ± 1 mmHg ($n=8$). This elevation was long-lasting usually persisting to the end of

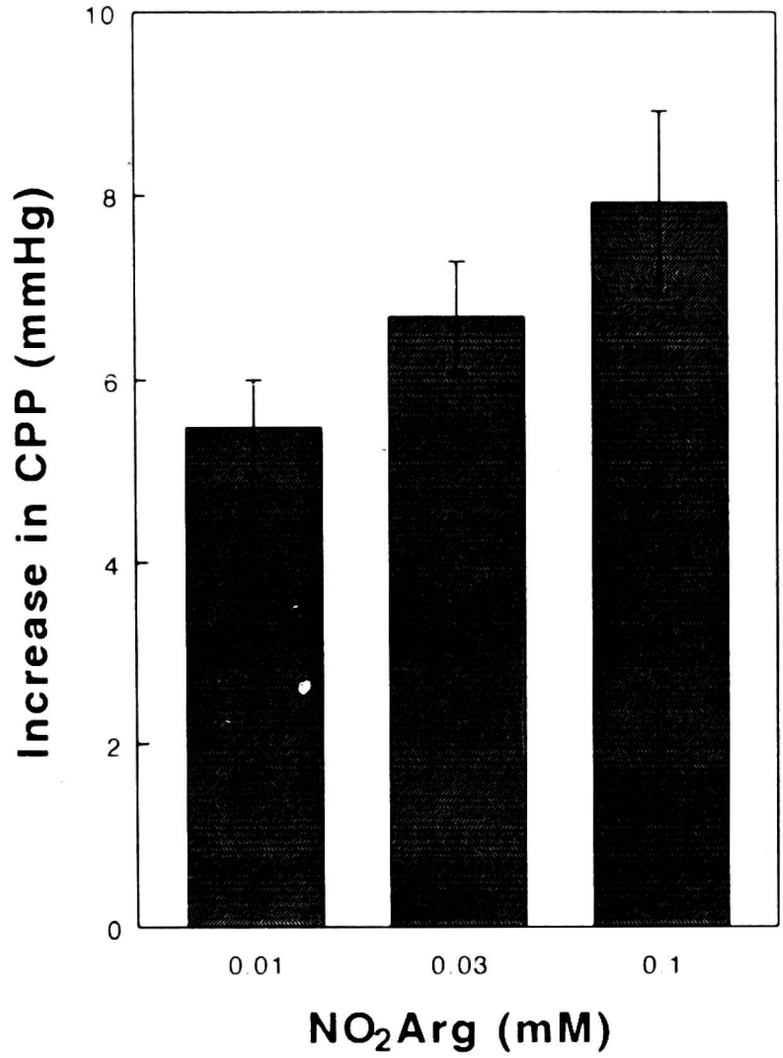

Fig. 4. Concentration-dependent increase in basal coronary perfusion pressure (CPP) in the isolated guinea pig heart following 15 min long infusion of NO_2Arg (0.01-0.1mM). Each column represents the mean and vertical bars s.e. mean of n = 5-8 experiments.

the experiment. The responses to Ach, Bk and SP were abolished or considerably reduced (*Fig. 5*) after the 15 min long infusion of NO_2Arg (100 μM). In some experiments NO_2Arg revealed a vasoconstrictor effect of Ach. This was not observed neither with SP nor with Bk. The coronary dilation in response to the endothelium-independent agents were not significantly reduced by NO_2Arg.

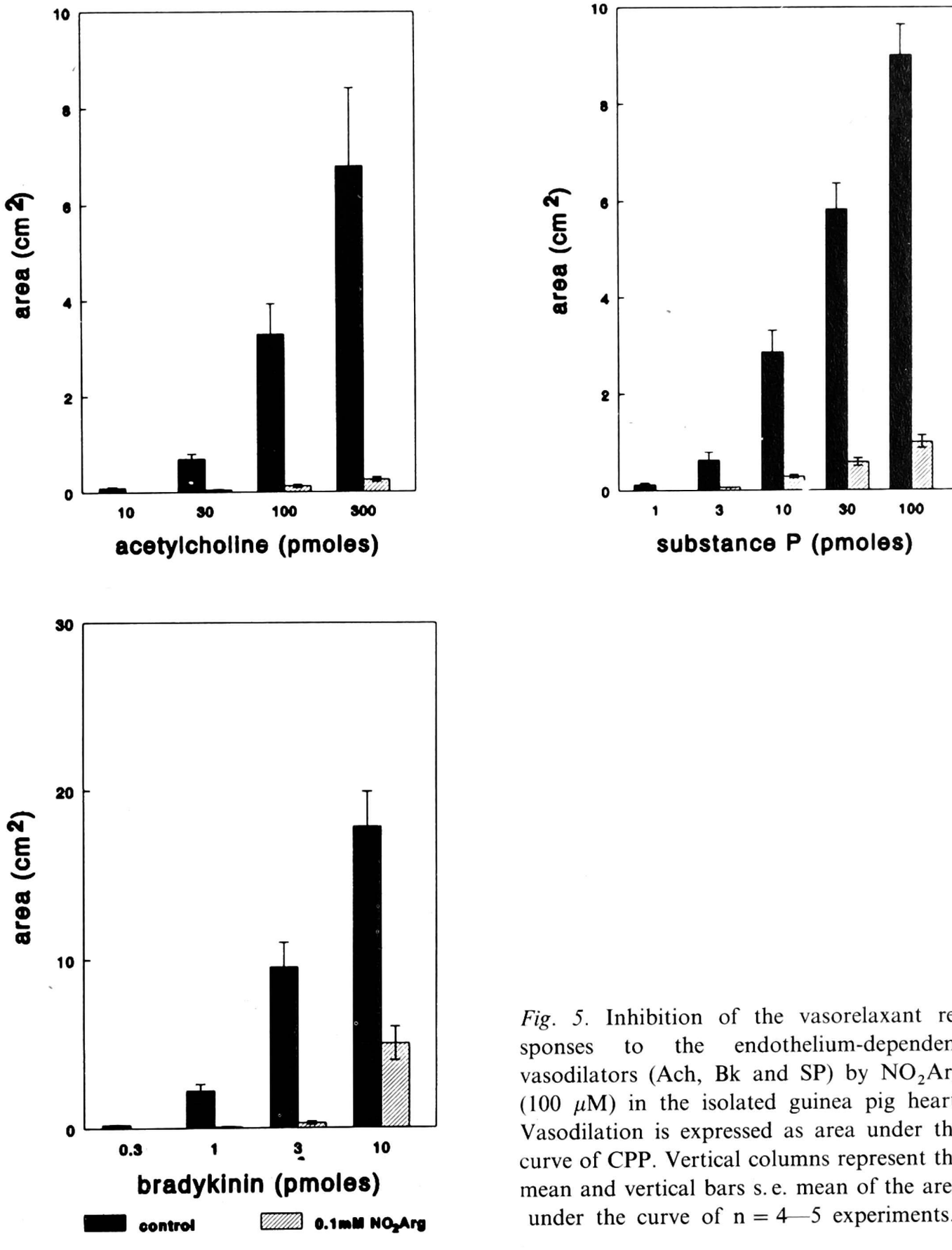

Fig. 5. Inhibition of the vasorelaxant responses to the endothelium-dependent vasodilators (Ach, Bk and SP) by NO_2Arg (100 μM) in the isolated guinea pig heart. Vasodilation is expressed as area under the curve of CPP. Vertical columns represent the mean and vertical bars s.e. mean of the area under the curve of n = 4—5 experiments.

DISCUSSION

The results reported above show that in the isolated, perfused heart of the guinea pig (1) the coronary vasodilation induced by Ach, Bk or SP is mediated by EDRF, but not by prostanoids, (2) continuous generation of EDRF and to

a lesser extent that of prostanoids influence basal coronary tone, (3) the endothelium-dependent vasodilators (Ach, Bk, SP) are remarkably more potent coronary vasorelaxant than the endothelium-independent vasodilators (GTN, SIN-1, NaNP), (4) even a short term exposure to GTN (bolus injections) is sufficient to induce tachyphylaxis.

NO_2Arg substantially reduced vasodilatory responses to Ach, Bk and SP while indomethacin did not affect them. These findings clearly indicate that in the microcirculation of the guinea pig heart, as in the case of large conductive arteries (7, 8) the relaxation induced by Ach, SP and Bk is mediated by EDRF. These results are consistent with previous studies which proved EDRF involvement in the Ach induced vasodilation in the isolated rabbit heart (9, 10) and in the Bk-induced vasodilation in the isolated guinea pig heart (11, 12). In contrast to the above findings, it has been recently claimed (13) that NO_2Arg reduces the duration but not the magnitude of Bk responses in the isolated rat heart and both haemoglobin and SOD have no effect on these responses. The authors conclude that Bk does not produce vasodilation in the isolated rat heart by releasing NO. In our experiments with the guinea pig heart the relaxant responses to the maximal effective dose of Bk (10 pmoles) was strikingly less inhibited by NO_2Arg than the responses to smaller doses of Bk or other endothelium — depended vasodilators. Thereby, we speculate that in contrast to the Ach and SP-induced vasodilation presumably mediated exclusively by EDRF, Bk may have an endothelium-independent component of its relaxation. A number of studies have recognized that Bk releases by interacting with B2 receptors which are present on the endothelial cells, while the activation of B1 receptors may contribute to the vasodilator action of Bk through EDRF-independent mechanism (28—31). In contrast to the coronary vascular bed of the rat, in the guinea pig heart the vasodilation induced by Bk is mediated by EDRF. However, Bk at higher doses may elicit vasodilation partly through a NO-independent pathway, possibly by acting on B1 receptors.

NO_2Arg at a concentration as low as 10 μM produced a significant increase in CPP. This suggests that in the guinea pig isolated heart there exists a significant basal production of EDRF, which contributes to the regulation of coronary vascular tone. This has been recently shown not only in the coronary circulation (10,11), but also in other vascular beds (32) including peripheral circulation in man (33). IND produced a small, albeit significant increase in CPP. Thus vasodilatory prostanoids, most likely PGI_2 and PGE_2 (34), also participate in maintaining the coronary resting tone. However, as mentioned above, prostanoids hardly play a role in receptor-stimulated responses. This is in accordance with the data of Stewart & Piper (22) but not with those of Lee et al. (23). There is no ready explanation for these contradictory findings aside from pointing out that Lee et al. (23) performed their experiments on the

potassium arrested, isolated heart of the guinea pig. This non-physiological high potassium concentration might have influenced the endothelium-dependent relaxations (35).

Interestingly, a substantial difference in vasodilator potencies between the endothelium-dependent and the endothelium-independent vasodilators was observed, although both evoke relaxation by increasing intracellular level of cGMP. Even such a powerful nitrovasodilator as GTN was weaker by three orders of magnitude than Bk. This finding indicates that constitutive NO synthase (36) is present in the endothelial cells of the coronary vasculature and its activation through the receptor mechanisms yields a substantial amount of NO at a right site for the induction of powerful vasodilation. As shown above, in the isolated guinea pig heart Bk is the more potent releaser of NO than SP or Ach. Interestingly, the endothelium-dependent vasodilators differ in their rank order of potency between species and between various vascular beds. For instance in the rabbit aortic endothelium SP is the most potent releaser of EDRF (37) while in the cultured bovine aortic endothelial cells (17) there is no muscarinic receptor for Ach and Bk is also the strongest releaser of NO. In contrast to the guinea pig and rabbit (38) coronary circulations, SP is more potent than Bk in the porcine coronary vessels (39). Similarly, there is a growing body of evidence for SP being the most potent releaser of NO from human coronary vascular bed (40). As shown above, Ach is not only the weakest vasodilator in guinea pig heart coronary circulation, but also the maximal response to Ach in this preparation is lower than those elicited by either Bk or SP. Again this pattern of endothelium-dependent responses differs from that in the rabbit hindlimb microcirculation (41), where Ach is more potent than SP. This heterogeneity might be due to different endothelial receptor populations or a difference in their capacity to transduct a signal for the activation of NO synthase or different relaxing factors released.

In our experiments the endothelium-independent vasodilators, i.e. GTN, SIN-1 and NaNP, were equipotent, although GTN has to be reduced to NO in the vascular smooth muscle cells (42—44), SIN-1 releases NO spontaneously (45) and NaNP seems to interact with soluble guanylate cyclase directly (46). The different activation of the smooth muscle guanylate cyclase by all three nitrovasodilators in conjunction with their vasodilator equipotency might suggest that their penetration through the endothelial layer to target cells would blunt the developement of their pharmacological action.

In addition, a marked decrease of the relaxant responses to GTN upon the repetitive administrations of bolus injections of GTN was observed. GTN tolerance has been studied extensively for the past 20 years. It seems to be associated with the depletion or unavailability of thiol groups involved in the denitration of GTN and in the formation of S-nitrosothiols, which are

essential for the stimulation of guanylate cyclase (42, 47, 48). The lack of tachyphylaxis to direct NO-donor (NaNP) leaves unlikely the possibility that the desentization of the soluble guanylate cyclase is responsible for GTN tachyphylaxis (49). Rapid development of tachyphylaxis to GTN, demonstrated in this paper, might indicate that enzymatic pathway of GTN metabolism in the guinea pig heart is prone to fast desentization. It may well be that in coronary smooth muscle cells of the guinea pig the reducing equivalents for GTN are scarce and therefore a long time exposure to high concentration of GTN (50, 51, 53) is not required to induce tolerance. Thus the model of the perfused guinea pig heart might be useful for studying tachyphylactic response to organic nitrates, particularly since the view that NAC or other thiols might be able to prevent the development of nitrate tolerance is still controversial (51—55).

In conclusion, it has been demonstrated that in the isolated perfused guinea pig heart continuous generation of EDRF and to a lesser extent of vasodilatory prostanoids serve to maintain the coronary bed in the dilated state, but the receptor mediated endothelium-dependent relaxation induced by Ach, SP or Bk is mediated by the release of EDRF only. EDRF which is released from endothelial cells in the closest vicinity of vacular smooth muscle is by far a more powerful vasodilator than NO derived from nitrovadilators. The model of the quinea pig heart might be useful for studying nitrate tolerance.

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