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REVERSAL OF THE POSTISCHAEMIC SUPPRESSION OF CORONARY FUNCTION IN PERFUSED GUINEA PIG HEART BY ISCHAEMIC PRECONDITIONING

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In the isolated guinea pig hearts suppression of endothelium-dependent (Acetylcholine, Substance P, postocclusive hyperaemia) and endothelium-independent (Sodium nitroprusside, PGE_1) responses after 30 min subglobal ischaemia (reduction of coronary flow to 5%) were analysed in hearts which were not preconditioned or preconditioned by various protocols. Preconditioning consisted of single 5 min ischaemia (IP5) or single 10 min ischaemia (IP10) or double 5 min ischaemia (IP5+5). Thirty minutes of ischaemia followed by reperfusion reduced both endothelium-dependent and endothelium-independent responses approximately by 30—50 % and slightly suppressed basal coronary flow by 10%. IP5 and IP5+5 protected against postischaemic suppression of responses to NaNP but not against postischaemic impairment of SP, ACh, and POH responses. The endothelium-dependent responses and postischaemic suppression of basal coronary flow were protected by IP10 only. In summary, in the isolated guinea pig heart the 30-min ischaemia impairs vasodilator responses to both endothelium-dependent and endothelium-independent agents. Ischaemic preconditioning protects both endothelial and smooth muscle cells function against this impairment, though endothelial cells require a more extensive preconditioning to put in motion protective mechanisms than smooth muscle cells do. Independent mechanisms of IP in endothelial cells and in smooth muscle cells are suggested.

K e y w o r d s: *ischaemic preconditioning, guinea pig heart, coronary circulation, endothelium, vascular smooth muscle.*

INTRODUCTION

Over a decade ago Murry et al. (1) reported that exposing of myocardium to a brief transient ischaemia prior to a subsequent longer ischaemia would considerably diminish the area of myocardial necrosis. This cardioprotective phenomenon was named ischaemic preconditioning (IP). Later it was demonstrated that IP not only diminished area of postischaemic necrosis but

also that of apoptosis (2), prevented ischaemia-induced or reperfusion-induced arrhythmias (3), and enhanced postischaemic recovery of cardiac function (4, 18).

However, the effect of ischaemic preconditioning on coronary vasculature has not been fully explained so far. There is no clear-cut evidence for the IP-induced protection of vascular smooth muscle cells against ischaemic injury, although protection of skeletal muscle by IP was demonstrated (4). As for the effect of IP on postischaemic endothelial cell injury, there are studies demonstrating protection (5—9) or lack of protection of coronary endothelium against the ischaemic insult (10—12). Discrepancies between results of these studies do not find their explanation in variation in animal species or experimental models used (5, 8, 10, 11).

Single ischaemia/reperfusion shot of various durations (2—10 min) or ischaemia/reperfusion cycles repeated up to twelve times were applied to induce IP. In rabbits (13) and dogs (14), but not in pigs (15) reduction of infarct size by IP seems to obey rule of "all or nothing" response up to several ischaemia/reperfusion cycles. On the contrary, postischaemic arrhythmias seem to be reduced by IP in step-wise mode depending on the intensity of IP (3).

Presently, we are looking for a possible protective effect of IP against functional injury of endothelial and smooth muscle cells in coronary vascular bed in the postischaemic isolated guinea pig heart. We have also undertaken an attempt to spot differences between „single shot IP" or „repeated cycles IP" procedures in protection of coronary vascular bed against ischaemia-induced trauma.

MATERIALS AND METHODS

Perfusion of isolated guinea pig heart

The method was described more in detail elsewhere (16). Briefly, guinea pig hearts were removed and perfused at the constant pressure of 60 mm Hg retrogradely through the ascending aorta in the Langendorff apparatus (Hugo Sachs Elektronic — HSE) with the Krebs-Henseleit buffer of the following composition (mM) :NaCl 118.00, $CaCl_2$ 2.52, $MgSO_4$ 1.64, $NaHCO_3$ 24.88, KH_2PO_4 1.18, glucose 10.00, sodium pyruvate 2.0, equilibrated with 95% O_2 + 5% CO_2 at 37°C. The heart was paced at 273 impulses per minute through platinum electrodes inserted into the right atrium. Left ventricular pressure (LVP) was measured using the fluid-filled balloon inserted into the left ventricle and connected to a pressure transducer (Isotec, HSE). End diastolic pressure was set to 5—10 mm Hg. The values of dp/dt_{max} (systolic contractility) and dp/dt_{min} (diastolic contractility) were obtained from LVP signal by an analogue differentiation amplifier (DIF module, HSE). Coronary flow (CF) was monitored by Narcomatic Electronic Flowmeter (HSE). Coronary flow, left ventricular pressure, dp/dt_{max} and dp/dt_{min} were continuously measured and displayed throughout the experiment. For data analysis especially designed software (PSCF-IGEL, Poland) was used. All experiments were completed in less than three hours.

Chemicals

Acetylcholine (ACh), substance P (SP) and sodium nitroprusside (NaNP) were obtained from Sigma Chemical International and PGE_1 (Prostavasin) from Schwarz Pharma, Germany,.

Experimental protocol

Isolated hearts were equilibrated at the perfusion pressure of 50 mm Hg for about 10 min and then pressure was adjusted to 60 mm Hg and that heart was equilibrated again for 10—15 min before the beginning of experiment. Inclusion rules were as follows:

(1) basal CF higher than 4 ml/min, (2) increase of CF to bolus injection of 300 pmoles of ACh $\geq$ 2ml/min, (3) no arrhythmias present before subglobal ischaemia.

The preischaemic basal coronary flow in all hearts was 8.3 ± 0.3 ml/min (total n = 71). The increase in coronary flow evoked by all vasodilator agents and POH was of similar magnitude (4—6 ml/min). However, the magnitude of vasodilator responses was expressed as an area under the curve of an increase in coronary flow (from start of vasodilation until flow returned to normal), rather than an increase in flow as assayed in ml/min. This first method better represent the magnitude of vasodilator response than simple measurement of an increase in coronary flow only (17). Area under the curve was calculated by a specially designed program (PSCF-Igel, Poland) in arbitrary units. Data on the effect of IP on CF responses to vasodilators in postischaemic period were presented as percent of preischaemic responses.

Standard endothelium-dependent vasorelaxant responses were evoked by intracoronary one minute infusion at a rate of 0.1 ml/min of ACh, at final concentration of 100 nM, or SP (10 nM or 30 nM) or by post-occlusive (10 sec) hyperaemia (POH). On the other hand, endothelium-independent vasorelaxant responses were evoked by one minute infusion of NaNP (3 μM) or by a bolus injection of PGE_1 (100 pmoles).

Hearts were subjected to three different preconditioning protocols: single subglobal ischaemia, which lasted 5 min (IP5), 10 min (IP10) or double 5-min ischaemic periods which were separated by a 10-min reperfusion period (IP5+5). Control group was not exposed to preconditioning (IP-0). Hearts of the above groups were subjected to 30 minutes of subglobal ischaemia, followed by 90 minutes of reperfusion. During this last period endothelium-dependent and endothelium-independent responses were evoked again and compared with the initial preischaemic responses. A scheme of experimental protocols is shown in *Fig. 1.* In all hearts subglobal ischaemia, lasting 5 10 or 30 min, were evoked by reduction in coronary inflow by 90—95% from basal coronary flow.

To exclude the influence of short lasting ischaemia itself on vasodilator responses additional control groups of hearts were included (IP5C, IP10C and IP5+5C), which hearts were not subjected to subglobal 30-min ischaemia, but were exposed to vasodilator agents at the same time course as IP5, IP10, IP5+5 group, respectively.

Pilot experiments showed that vasodilator responses to ACh, NaNP, PGE_1 or POH were of approximately equal magnitude during the whole reperfusion period, as exemplified by responses to ACh and NaNP in *Fig. 2.* This is why we decided to test each vasorelaxant response only twice in further experiments: firstly during preischaemic period and then between 40—50 minutes of reperfusion (see *Fig. 1*). In the absence of ischaemia both endothelial-dependent and endothelial-independent responses as well as basal coronary flow did not decline in our experimental set-up up to three hours of experiment as shown earlier (17).

In addition to the vasodilator responses, basal CF and heart systolic contractility (dp/dt_{max}) were recorded throughout the experiment. Values of CF and contractility at various time intervals of reperfusion were taken and calculated as percent of preischaemic value.

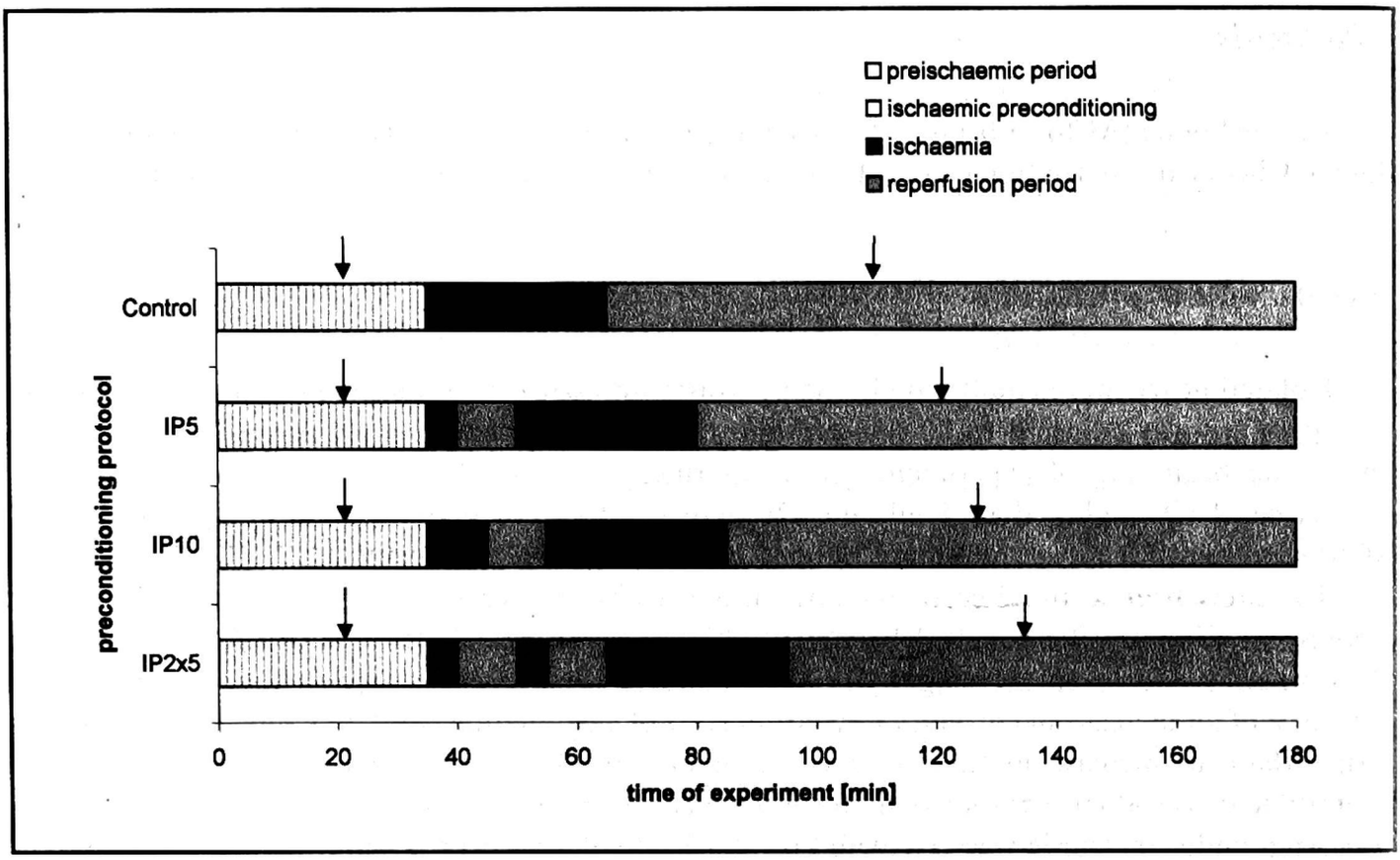

Fig. 1. Scheme of experimental protocol. Endothelium-dependent and endothelium-independent responses were evoked during preischaemic period and reperfusion that followed 30-min ischaemia.

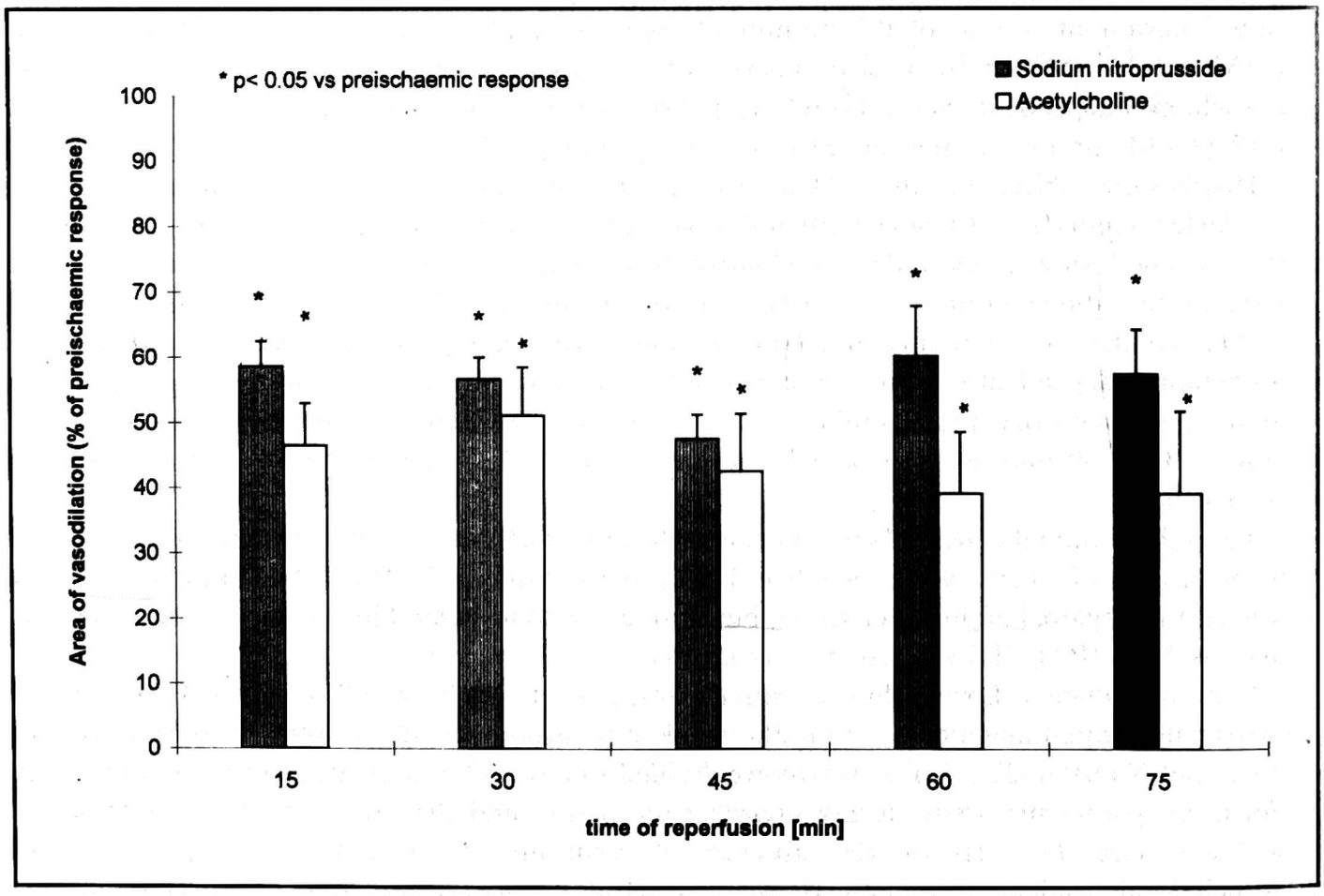

*Fig. 2.*Time course of suppression of endothelium-dependent (ACh) and endothelium-independent (NaNP) responses after subglobal ischaemia lasting 30 minutes. ACh-induced responses (100 nM) and NaNP-induced responses (3 μM) were suppressed approximately by half during whole reperfusion period lasting 90 minutes. Data represent mean ± SEM for at least 6 experiments.

Statistics

All figures represent mean ± S.E. of response during reperfusion (% of preischaemic value) for at least 5—6 experiments, unless stated otherwise. For evaluation of statistical significance within the group paired Student's t test was used. Significance of differences between groups were established by single factor analysis of variance (ANOVA) followed by t test for multiple comparison.

RESULTS

Effects of 30 minutes ischaemia on vascular function and contractility in the isolated guinea pig heart.

Subglobal ischaemia lasting 30-min suppressed significantly ($p < 0.05$) endothelium-dependent and endothelium-independent vasorelaxant responses to the drugs approximately by half and that induced by POH only by one quarter (*Fig. 3*). The depression of basal CF was modest (by 10%) but significant ($n = 27$, $p < 0.001$) and it was observed at any time of reperfusion (*Fig. 4*).

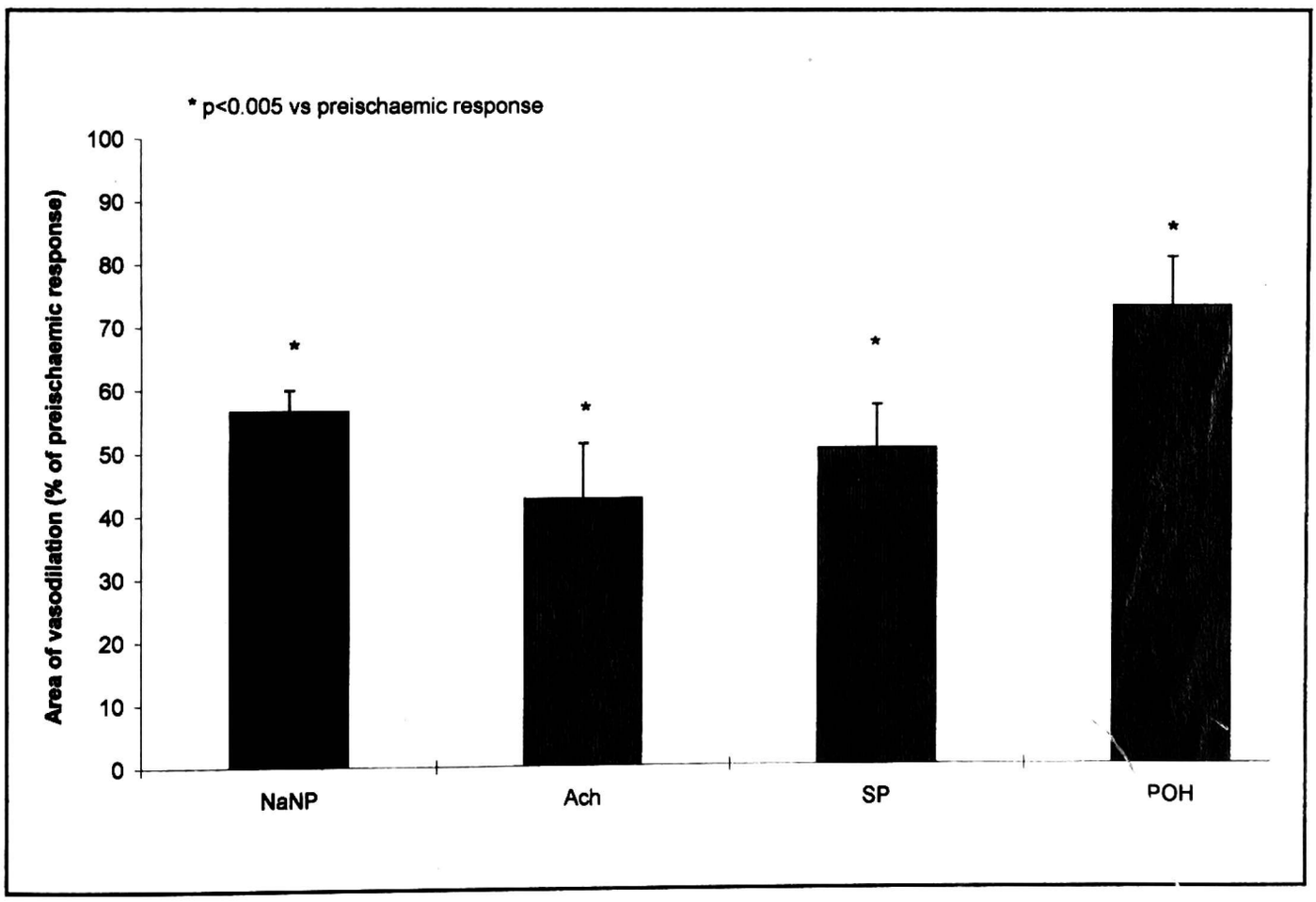

Fig. 3. Impairment of various endothelium-dependent and endothelium-independent vasodilatory responses induced by 30-min ischaemia. All responses were suppressed approximately to 50÷70% of preischaemic responses at 45 min of reperfusion. Data represent mean ± SEM for at least 6 experiments.

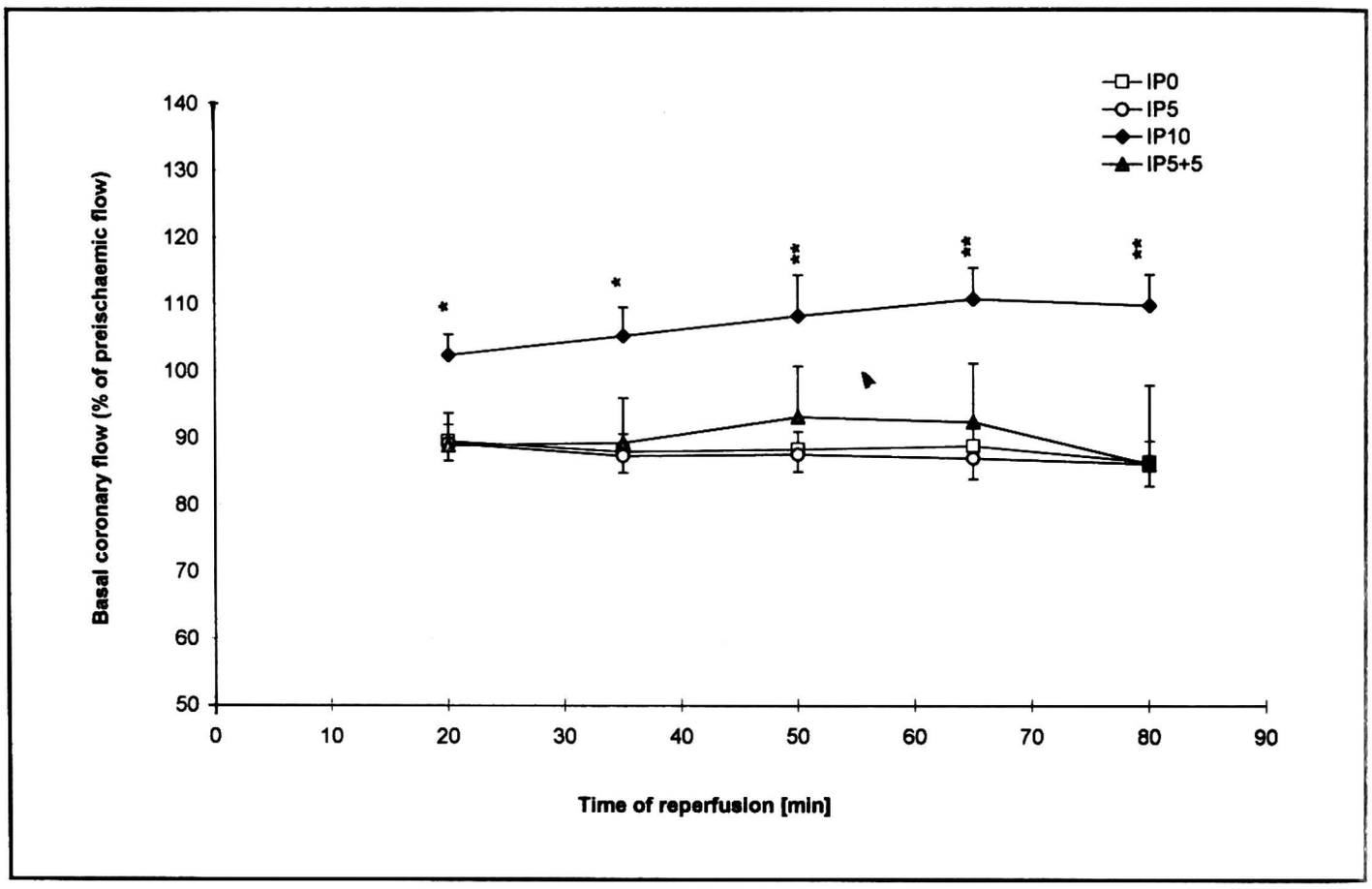

Fig. 4. Effect of various preconditioning protocols on basal coronary flow during reperfusion period. Neither IP5 nor IP5+5 influenced postischaemic suppression of coronary flow. In contrast IP10 reverted postischaemic suppression of basal coronary flow. Data represent mean ± SEM for at least 6 experiments.

Systolic contractility after 30-min ischaemia was slightly and not significantly impaired. Postischaemic contractility amounted to 94 ± 3%, 96 ± 4% and 94 ± 5% of preischaemic value after 20, 35 and 50 min of reperfusion, respectively (n = 27, ns).

Effect of various preconditioning protocols on ischaemia-induced suppression of vascular function

Short lasting single IP (IP5 group) was not effective in protecting against ischaemia induced suppression of endothelium-dependent vasodilator responses. A 10-min period of IP (IP10 group) was required to produce protection of endothelial function (*Fig. 5*).

On the other hand, endothelium-independent vasodilator responses to NaNP (*Fig. 5*), and to PGE_1 (81 ± 6% of preischaemic responses vs 52 ± 9%, n = 5, $p < 0.05$) were protected in the IP5 group.

Extension of IP to 10 min (IP10 group) further increased efficacy of IP. Responses to NaNP were enhanced up to 127 ± 10% of the preischaemic value ($p < 0.001$, n = 11). On the contrary, in the IP5+5 group, preconditioning was lost in the case of endothelium-depenndent vasodilators and in the case of CF, but not in the case of NaNP (*Fig. 4, Fig. 5*).

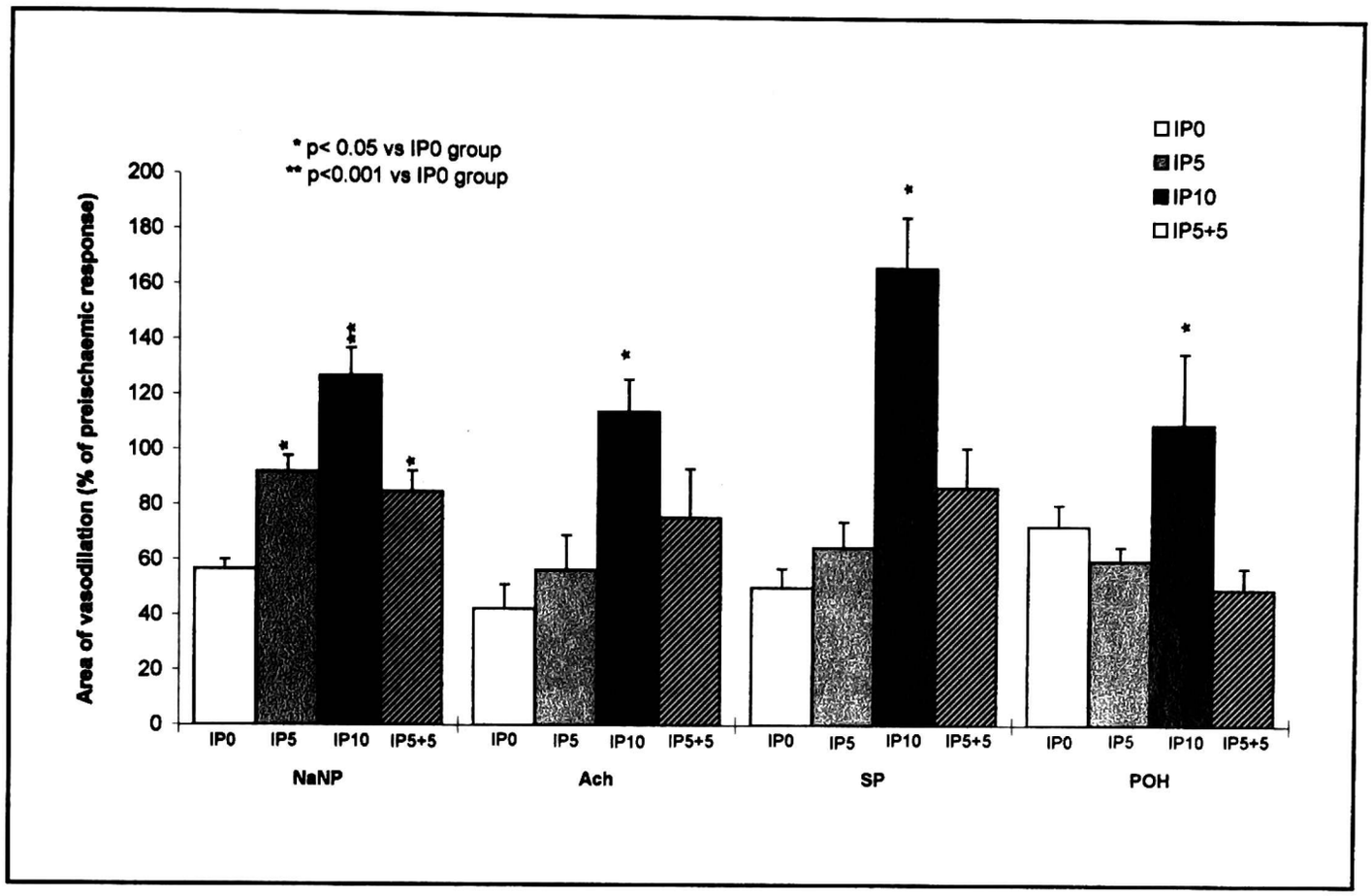

Fig. 5. Protection of endothelium-independent (NaNP) and endothelium-dependent (ACh, SP and POH) responses by various preconditioning protocols. Endothelium-independent (NaNP 3 μM) responses were protected against postischaemic injury by IP5 and IP5+5, whereas endothelium-dependent (ACh 100 nM, SP 10 nM and POH after 10 sec occlusion of CF) responses were protected only by IP10. Data represent mean ± SEM for at least 6 experiments.

In the control IP5C or IP10C hearts neither endothelium-independent responses to NaNP (91 ± 7% of preischaemic response, ns) nor endothelium-dependent responses to ACh (88 ± 13% of preischaemic response, ns) were diminished. Only in the IP5+5C group responses to ACh (68 ± 6% of preischaemic response, $p = 0.001$) were modestly suppressed.

Effect of various preconditioning protocols on ischaemia-induced suppression of systolic contractility

The suppression of myocardial contractility in the postischaemic hearts was faint in our model (see above) and the effect of IP on this parameter did not show statistical significance (data not shown).

DISCUSSION

It is well known that ischaemic preconditioning (IP) protects cardiomyocytes from ischaemia/reperfusion damage (1). This study was designed to answer the question whether coronary vasculature is or is not protected by various types of protocols of IP (5, 8, 10, 11).

Presently, we report that in guinea pig heart the ischaemia/reperfusion injury to coronary vascular bed encroached both endothelium-dependent and endothelium-independent mechanisms. Contrary to our findings many reports in species less sensitive to ischaemia/reperfusion damage (18) showed suppression of endothelium-dependent mechanisms only (5, 8, 19—21). Responses to endothelial NO that was released by ACh, SP or by postocclusive hyperaemia (POH), as well as endothelium-independent responses elicited by guanylate cyclase (NaNP) or adenylate cyclase (PGE_1) activators were suppressed approximately by half following subglobal ischaemia of 30 min of duration. It may well be that a damage to smooth muscle is solely responsible for postischaemic impairment of functioning of coronary circulation. However, such explanation is not satisfactory because single or double IP of 5 min duration (IP5 or IP5+5) did offer a protection against postischaemic suppression of NaNP-induced responses whereas endothelium-dependent responses remain still impaired. Therefore, it may be suspected that ischaemia infers damage to endothelial and smooth muscle cells in coronary vascular bed through two distinct mechanisms.

We used purposely two different endothelium-dependent vasodilators (ACh and SP) and POH response that was also endothelium-mediated (16), as well as two endothelium-independent vasodilators (NaNP and PGE_1) to study the effectiveness of various IP protocols against ischaemic damage to coronary vascular bed. It appears that endothelial cells require IP stimulus of longer duration than smooth muscle cells for self-protection. To our knowledge the present study is the first that unequivocally proves that various protocols of ischaemic preconditioning may lead to the preferential protection of vascular smooth muscle or endothelial cells. Moreover, we demonstrate that protection of endothelium afforded by IP10 could not be replaced by IP5+5. Indeed, multiple cycles of short ischaemia/reperfusion were demonstrated to have a detrimental effect on endothelial function (22) and repetitive preconditioning was found to be less protective than a single IP (13). It seems that endothelial protective mechanisms require at least 10 min of IP to be put in motion while protective mechanisms in smooth muscle cells are triggered even by 5 min of IP.

Protective effect of IP against ischaemic injury to the coronary circulation is a contradictory phenomenon (23). In canine heart IP 4×5 min failed to protect against postischaemic suppression of endothelium-dependent responses (10) whereas IP10 was effective (5). In rat hearts IP 3×3 min did not protect endothelium-independent responses (11), but IP 3×5 min fully restored them (8). Here we show that coronary circulation of guinea pig heart reacts more like canine rather than rat heart in terms of IP efficacy. Although different protocols of IP were used in above reports, it seems that in all three species the protection of endothelial cells required longer IP than protection of smooth muscle cells. Rat coronary circulation seems to be relatively more resistant to

reperfusion injury as compared with those of guinea pig or dog (18). Perhaps it explains why interrupted type of IP in rats but not in guinea pigs is additive in nature as far as the protection of endothelium is concerned.

So far most authors focus their attention on endothelial injury without paying much attention to the possible damage of smooth muscle cells by ischaemia/reperfusion. Interestingly, Kolocassides et al. (11) found that NaNP-induced responses were protected by IP whereas histamine-induced responses were still impaired. This finding again corraborates with our present conception that smooth muscle function can be preconditioned independently of preconditioning of endothelial cells.

Accordingly, our suggestion is that in guinea pig heart there occur two independent IP mechanisms in coronary vascular bed: one operating in endothelial cells and another in smooth muscle cells.

Myocardial injury by ischaemia/reperfusion seems to be associated with depletion of high-energy phosphates, accumulation of metabolites (24), and activation of polyADP-ribose synthetase (PARS) (25). There is still no consensus on mediators being responsible for protective effect of IP against myocardial infarction. Adenosine (26), bradykinin (27), catecholamines (28) and, recently, opioids were proposed (29). However, most of studies support an idea of opening of ATP-sensitive potassium channels as the end-point of IP mechanisms in cardiomyocytes (23).

Endothelial injury by ischaemia/reperfusion involves generation of oxygen free radicals (21). Indeed, substances which act as free radical scavengers, like SOD (20), pyruvate (30) or dimethylthiourea (31) protect against it. However, mechanisms of protection against endothelial damage by IP still remain unknown. Bradykinin and adenosine were proposed as mediators of protective effect of IP in endothelial cells (9, 27) but not accepted by others (32).

In our model the impairment of contractility by ischaemia/reperfusion procedure was feeble (by ca 10%) as it was also reported by others (33). This is the reason why effects of various IP protocols on postischaemic suppression of contractility show no statistical significance.

Summing up, in guinea pig heart ischaemic preconditioning may protect against impairment of endothelium-dependent and endothelium-independent responses induced by ischaemia/reperfusion injury. However, endothelial cells require longer preconditioning ischaemia than smooth muscle cells to be protected against ischaemic injury.

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