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The effect of continuous versus intermittent treatment with transdermal nitroglycerin on pacing-induced preconditioning in conscious rabbits

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1 Tolerance to the hypotensive effect of nitroglycerin (NG) blocks preconditioning induced by rapid ventricular pacing (RVP) in rabbits. In the present work the effect of continuous versus intermittent treatment with transdermal nitroglycerin on the pacing-induced preconditioning phenomenon was studied in conscious rabbits.

2 RVP (500 beats min^{-1} over 5 min) increased left ventricular end-diastolic pressure (LVEDP) from baseline 4.1 ± 0.9 to postpacing 13.8 ± 2.9 mmHg ($P < 0.001$) with a right intraventricular ST-segment elevation of 1.25 ± 0.13 mV, two indicators of myocardial ischaemia. These changes were significantly attenuated when the RVP period was preceded by a preconditioning pacing of the same rate and duration with an interpacing interval of 5 min.

3 Protection by preconditioning was abolished when the animals had been made tolerant to the vasodilator effect of 30 $\mu g\ kg^{-1}$ NG by the application of transdermal NG (approx. 0.07 mg $kg^{-1}\ h^{-1}$) over 7 days. Furthermore, transdermal NG *per se* attenuated both RVP-induced ST-segment elevation and LVEDP-increase over the 7 day period.

4 With intermittent transdermal NG treatment (12 h 'patch on' vs 'patch off'), neither development of vascular tolerance nor attenuation of the NG- or preconditioning-induced anti-ischaemic effects were observed. However, the severity of pacing-induced myocardial ischaemia was significantly increased during the 'patch off' periods.

5 In a second set of experiments, postpacing changes in cardiac cyclic GMP and cyclic AMP levels were determined by means of radioimmunoassay in chronically instrumented anaesthetized open-chest rabbits with the same NG-treatment protocols. Preconditioning reduced postpacing increase in cyclic AMP with an increase in cyclic GMP concentrations in hearts of the untreated animals and in those given patches intermittently during both 'patch on' and 'patch off' periods. However, the preconditioning effect on either cyclic nucleotide was blocked in the tolerant animals.

6 Transdermal NG increased resting levels of both cardiac cyclic nucleotides in the non-tolerant but not in the tolerant state. The postpacing increase in cyclic AMP content was inhibited by transdermal NG, independent of vascular tolerance development, whereas, an increase in cyclic GMP content was exclusively seen in the non-tolerant animals.

7 We conclude that the anti-ischaemic effect of NG is independent of the cyclic GMP mechanism in the tolerant state. While intermittent NG therapy prevents development of vascular tolerance and preserves preconditioning, the nitrate-free periods yield an increased susceptibility of the heart to ischaemic challenges.

Keywords: Ischaemic preconditioning; nitrate tolerance; rapid pacing; cyclic GMP; intermittent nitrate therapy

Introduction

Organic nitrates constitute the mainstay of acute therapy of angina pectoris. Transdermal nitroglycerin (NG)-delivery systems were developed to provide a sustained anti-ischaemic effect. However, the resulting steady plasma concentration was found to induce haemodynamic tolerance with an attenuation of the therapeutic effect (Parker & Fung, 1984; Abrams, 1984; Luke *et al*., 1987). Moreover, vascular nitrate tolerance has been shown to abolish protection conferred by ischaemic preconditioning (Szilvassy *et al*., 1994a). To avoid tolerance development, intermittent transdermal NG therapy is recommended to allow for an approximately 10 to 12 h daily nitrate-free period (Luke *et al*., 1987). The disadvantage of this latter regime is that the nitrate-free period due to intermittent application of patches leaves patients unprotected during this period. Furthermore, a decrease in anginal threshold has been shown during the nitrate-free interval in patients with stable angina pectoris (Parker *et al*., 1995).

Recently, Ferdinandy *et al*. (1995) have suggested that the anti-ischaemic effect of NG involves a direct myocardial mechanism and have shown that the rapidly developing vascular tolerance to NG does not affect its cardioprotective effect in isolated working hearts of the rat. Therefore, we studied whether one week continuous or intermittent transdermal NG treatment (i) produced tolerance to the vasodilator and/or anti-ischaemic effect of NG, and (ii) affected the ability of the heart to adapt to repetitive ischaemic insults, i.e. the rapid pacing-induced preconditioning phenomenon in conscious rabbits.

Methods

Animals

All experiments performed in this study conform to the guiding principles of the American Physiological Society. Seventy adult, male New Zealand white rabbits, weighing 3–3.5 kg,

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housed in an animal room (12 h light/dark periods a day, temperature of 22–25°C, humidity of 50–70%) with one animal per pen, fed commercial laboratory chow and tap water *ad libitum*, were used throughout. The animals underwent surgery after a two-week acclimatization period.

Surgical procedure

Surgery was performed under aseptic conditions as described previously (Szilvassy *et al*., 1994a,b,c; 1995). Briefly, the rabbits were anaesthetized with an intravenous bolus of 10 mg kg^{-1} diazepam (Sigma Chemicals Co, St Louis, MO) and 5 mg kg^{-1} ketamine (EGIS Pharmaceuticals Co, Budapest, Hungary). Lidocaine (EGIS Pharmaceuticals Co, Budapest, Hungary) was given subcutaneously for local pain relief. A bipolar French 4 electrode catheter (Cordis, Erkrath, Germany) was introduced into the apex of the right ventricle through the right jugular vein for cardiac pacing and recording the intracavitary electrogram. A polyethylene cannula was inserted into the left ventricular cavity through the right external carotid artery to measure intraventricular pressure. Another cannula was inserted into the distal third of the central ear artery for recording the mean arterial blood pressure (MABP) as described by Szilvassy *et al*. (1994b). During the first 24 h after surgery, the mean body temperature increased by 0.5°C, and a 10% to 15% weight loss occurred. One week later, the body weight had returned to the presurgery value.

Preconditioning with rapid ventricular pacing (RVP)

One week after surgery, two consecutive 5 min periods of RVP at a rate of 500 beats min^{-1} were performed with an interpacing interval of 5 min. The resulting intracavital ST-segment elevation and increase in left ventricular end-diastolic pressure (LVEDP), indicators of pacing-induced myocardial ischaemia were measured after both pacing periods as detailed elsewhere (Szilvassy *et al*., 1994a,b,c). The first RVP served to estimate ST-segment elevation produced by a single RVP and to perform a preconditioning challenge. The second RVP was to test the preconditioning effect.

Determination of cardiac cyclic nucleotides in anaesthetized rabbit

Electrode catheter-instrumented rabbits were anaesthetized and artificially ventilated (Szilvassy *et al*., 1993; 1994). The chests were opened and left ventricular samples were rapidly taken and put into liquid nitrogen within 5 s (Hearse, 1983) for determination of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and guanosine 3′:5′-cyclic monophosphate (cyclic GMP) contents by use of Amersham Biotrak radioimmunoassay systems (Little Chalfont, Buckinghamshire, U.K.) as described by Szilvassy *et al*. (1993, 1994b). Sampling was done (i) under resting conditions (without pacing), (ii) immediately after cessation of a single RVP period (500 beats min^{-1} over 5 min) and (iii) after two consecutive RVPs divided by an interpacing interval of 5 min. Four animals were included in each group.

Drug study design

Experiments with chronically instrumented conscious rabbits In this set of experiments, the same six rabbits underwent four different treatment protocols detailed below (Figure 1). The animals received transdermal patches that released approx. 0.07 mg kg^{-1} h^{-1} NG (Nitroderm TTS 5, Ciba Hungaria, Budapest, Hungary) continuously over 7 days (each patch was replaced daily with a new one). RVP was performed before treatment, 6 h following 'patch on' and 6 h before and after removal of the last patch (patch off). After a two-day intertreatment interval, the same rabbits received matching placebo patches over a subsequent 7-day period and the pacing protocol was repeated at the corresponding time points. After another 2-day intertreatment interval, active patches were applied at 8 h 00 min each day and removed at 20 h 00 min each evening. The pacing protocol was repeated on the 6th

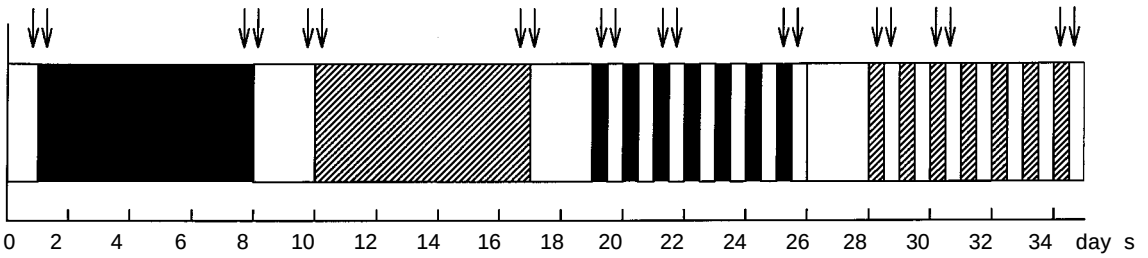

Figure 1 Schematic representation of the experimental protocol used to study the effect of continuous versus intermittent treatment with transdermal nitroglycerin on the rapid pacing-induced preconditioning phenomenon in conscious rabbits. The arrows indicate time points when the two consecutive periods of RVP (500 beats min^{-1} for 5 min with an interpacing interval of 5 min) were commenced. Open areas, patch off; solid areas, active patch on; hatched areas, placebo patch on.

Table 1 Effect of intravenous nitroglycerin (NG, 30 $\mu g\,kg^{-1}$) on MABP (mmHg) in concious rabbits

	Before TTS		*3rd day*		*7th day*		*Final patch off*	
Groups	*Before NG*	*After NG*	*Before NG*	*After NG*	*Before NG*	*After NG*	*Before NG*	*After NG, i.v.*
Active patches continuously	88±5.3	69±5.0*	91±6.2	87±6.4	94±5.8	93±4.4	93±3.9	92±4.6
Placebo continuously	90±6.2	69±4.4*	89±4.8	70±5.1*	92±4.9	68±5.9*	90±4.2	68±6.6*
Active 'patch on'	89±4.7	64±5.8*	85±3.9	62±2.6*	83±4.0	61±3.9*	93±4.0	61±2.7*
Active 'patch off'	91±3.9	67±4.1*	88±4.5	64±5.0*	87±5.2	70±5.8	87±4.4	66±5.2*
Placebo 'patch on'	90±5.8	71±3.3*	87±4.9	67±4.8*	91±6.3	69±5.5*	91±4.7	67±4.1*
Placebo 'patch off'	92±5.4	70±5.8*	90±4.4	69±4.1*	88±5.2	72±3.9*	89±5.5	72±5.1*

Data are means±s.d. obtained from 6 animals. *Indicates a statistically significant difference between corresponding values obtained before and after the intravenous administration of nitroglycerin (NG, 30 $\mu g\,kg^{-1}$) in 1 ml volume at $P<0.05$. 'After NG' values indicate maximum changes following NG injection.

hour of both 'patch on' and 'patch off' periods on the first, third and the seventh day. In the fourth period, placebo patches were applied in the same way with the same pacing protocol. NG 30 μg kg^{-1} (Pohl-Boskamp, Hochenlockstedt, Germany) and/or its vehicle, ethanol, were infused intravenously in a 1 ml volume over 1 min by means of a Braun (Melsungen, Germany) infusion pump to test the development of vascular tolerance to NG as described previously (Szilvassy *et al*., 1994a) at time points as follows: (i) before treatments with patches, (ii) on the 8th hour of the third day, and (iii) seventh day of each treatment period and (iv) 8 h following the final 'patch off' of each period.

Experiments with anaesthetized rabbits In the second set of experiments, resting and postpacing (with and without preconditioning) cyclic AMP and cyclic GMP levels were determined after 6 h and 7 day periods of continuous 'patch on'. 'Patch off' values also were determined 6 h following 12 h and 7 day continuous treatment periods with NG patches. The controls for this group of rabbits were those that had received placebo patches over 6 h. Four animals were included in each group.

Statistical analysis

The data expressed as means $\pm$ s.d of the mean were analysed with ANOVA followed by a modified *t* test for paired data, or a modified *t* test for simultaneous multiple comparison (Wallenstein *et al*., 1980).

Exclusions

Four animals had to be excluded from the study. Two animals were lost because of postsurgery pulmonary embolisms, one rabbit was excluded because of the mechanical damage of the implanted catheter and another one because of the inappropriate electrode catheter position.

Results

Vascular tolerance to nitroglycerin

NG (30 μg kg^{-1}, i.v.) significantly decreased MABP in animals intermittently treated with either active or placebo patches or when they were continuously treated with placebo patches. However, this intravenous 'test' dose of NG failed to elicit any decrease in blood pressure when the rabbits were exposed to continuous active 'patch on' for at least three days (Table 1). The vehicle for NG was without effect (data not shown).

Interaction between chronic treatment with transdermal nitroglycerin and the anti-ischaemic effect of preconditioning

ST-segment elevation Pacing-induced ST-segment elevation was significantly reduced by preconditioning in the untreated animals (Figure 2a), and in those intermittently treated with active patches (Figure 2b) during either the 'patch on' or 'patch off' period. 'Patch off' ST-segment elevations produced by the first RVP were significantly higher, whereas the 'patch on' values were lower, than those seen in the untreated animals. (Figure 2b). The continuous transdermal NG treatment resulted in an attenuation of ST-segment elevation produced by the 1st RVP. However, the preconditioning effect was found blocked after the one-week treatment period. The RVP-induced ST-segment elevation was significantly increased 6 h following removal of the last patch in this group with loss of the preconditioning effect (Figure 2a). Placebo patches applied either intermittently or continuously were without effect (data not shown).

LVEDP-increase The rapid pacing-induced increase in LVEDP was significantly attenuated by preconditioning, an effect which was blocked in the animals made tolerant to the hypotensive effect of NG (Figure 3a). In contrast, the protective effect of preconditioning on RVP-induced LVEDP-increase was preserved during the time course of the intermittent treatment schedule (Figure 3b). Placebo patches were without effect (data not presented).

Effect of transdermal nitroglycerin on resting cardiac cyclic nucleotide content

Six-hour exposure to active patches significantly increased resting levels of both cyclic AMP and cyclic GMP (Figure 4a

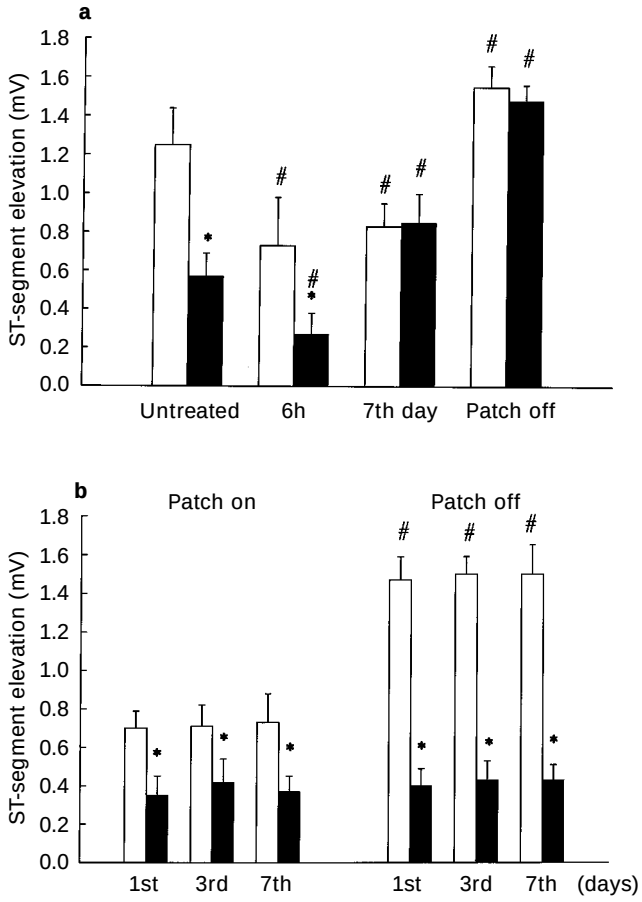

Figure 2 Effect of preconditioning of RVP-induced intraventricular ST-segment elevation in conscious rabbits. The 1st pacing (500 beats min^{-1} for 5 min, open columns) preceded the 2nd pacing (solid columns) of the same rate and duration with an interpacing interval of 5 min. The 1st pacing served as a preconditioning challenge, whereas the second pacing was to test the preconditioning effect. Postpacing ST-segment elevation was determined 40 ms after the intracavity 'J' point as described by Szilvassy *et al*. (1995). Average ST-segment elevation of the first five cardiac cycles at sinus rhythm following cessation of the periods of RVP produced the data for evaluation. (a) The effect of continuous treatment with transdermal nitroglycerin patches over 7 days. 'Untreated' refers to 'prepatch' values; 6 h data were obtained 6 h following 'patch on'; 7th day data were collected in the last 6 h of the 7-day period of continuous 'patch on'; 'patch off' values indicate the RVP-induced ST segment elevation 6 h after removal of the last patch. (b) The effect of intermittent (12 h 'patch on' vs 12 h 'patch off') treatment with transdermal nitroglycerin over 7 days. 'Patch on' ST-segment elevations were determined on the 1st, 3rd and 7th days 6 h after 'patch on', whereas 'patch off' values were measured 6 h following patch removal on these days. The data are means $\pm$ s.d. obtained with 6 animals. $^{*}P<0.05$, significant difference between 1st pacing and 2nd pacing in both (a) and (b). $^{\#}P<0.05$ represents a significant difference from corresponding untreated values in (a). In (b), $^{\#}P<0.05$ denotes a significant difference between corresponding 'patch on' and 'patch off' values.

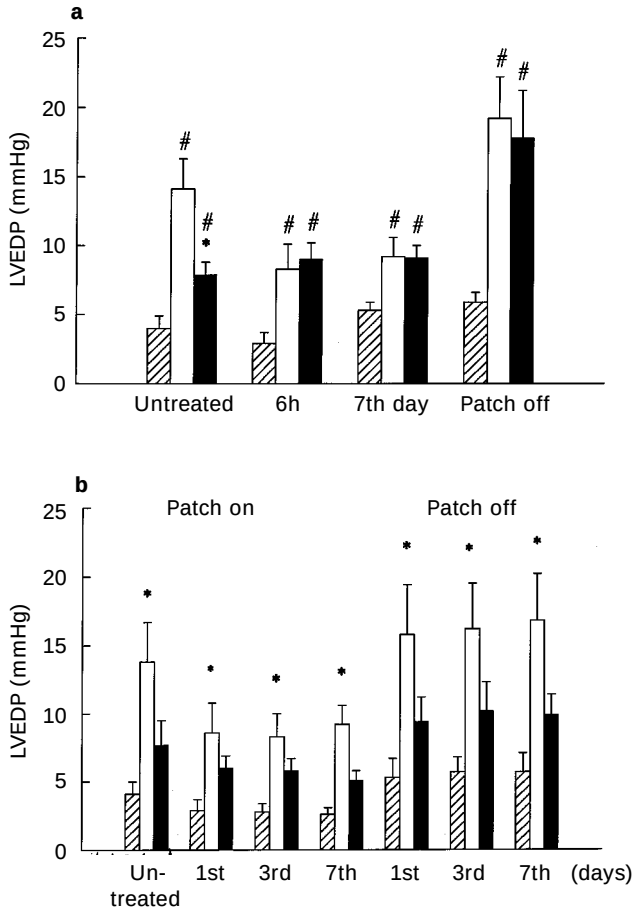

Figure 3 Effect of preconditioning on RVP-induced increase in left ventricular end-diastolic pressure (LVEDP) in conscious rabbits. The 1st pacing (500 beats min^{-1} over 5 min, open columns) preceded the 2nd pacing (solid columns) of the same rate and duration with an interpacing interval of 5 min. Average LVEDP values of the first five cardiac cycles at sinus rhythm following cessation of the periods of RVP produced the data for evaluation. (a) The effect of continuous treatment with transdermal nitroglycerin patches over 7 days. (b) The effect of intermittent (12 h 'patch on' vs 12 h 'patch off') treatment with transdermal nitroglycerin over 7 days. The data are means ± s.d. obtained with 6 animals. $^{*}P<0.05$, significant difference between 1st pacing and 2nd pacing. $^{\#}P<0.05$ represents significant difference as compared to the corresponding resting values (hatched columns) in both (a) and (b).

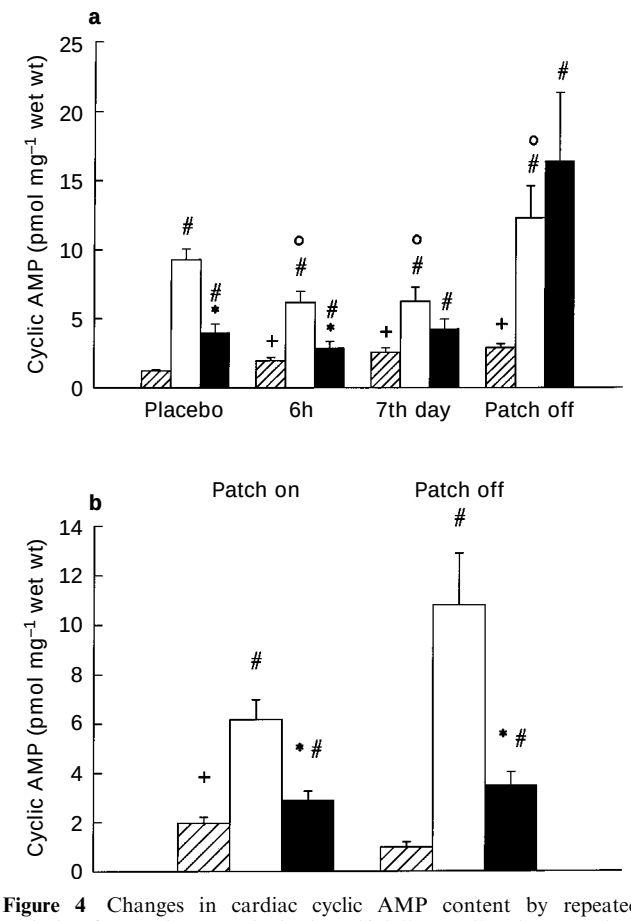

Figure 4 Changes in cardiac cyclic AMP content by repeated periods of RVP in anaesthetized, artificially ventilated, open chest rabbits. Values were determined in samples excised from the apical part of the left ventricles under resting conditions (without RVP); 1st pacing (open columns): immediately after cessation of a single RVP (500 beats min^{-1} over 5 min); 2nd pacing (solid columns): after cessation of RVP preceded by a preconditioning RVP of the same rate and duration (500 beats min^{-1} over 5 min) with an interpacing interval of 5 min. In (a): the effect of continuous treatment with transdermal nitroglycerin patches over 7 days. (b) The effect of nitroglycerin patches left on the skin of the rabbits for 6 h (patch on). 'Patch off' values indicate those determined 6 h after a 12 h patch on period (i.e. 6 h after patch removal). The data are means ± s.d. obtained with 4 rabbits in each group. $^{*}P<0.05$, significant difference between 1st pacing and 2nd pacing in both (a) and (b). $^{\#}P<0.05$, represents difference between corresponding resting versus postpacing values in both (a) and (b). $^{+}P<0.05$, designates difference in means of resting (hatched columns) placebo versus resting transdermal nitroglycerin-treated in (a), in (b) $^{+}P<0.5$ indicates a significant difference between 'patch on' and 'patch off' resting values (hatched columns). In (a), $^{\circ}P<0.05$ shows differences between 1st pacing values versus 1st pacing placebo.

and Figure 5a). These effects were preserved during the intermittent treatment schedule (Figure 4b and 5b) i.e. the 'patch on' values on the 1st, 3rd and the 7th day were significantly higher (to the same extent) than their corresponding 'patch off' values (data not shown). In animals with vascular tolerance to NG due to 7-day treatment with continuous active 'patch on', there was no change in the level of cyclic GMP (Figure 5a). However, the level of cyclic AMP continuously increased over the one-week continuous treatment period. Surprisingly, the 'patch off' resting cyclic AMP levels of this latter treatment schedule exceeded any of the corresponding values during 'patch on' (Figure 4a).

Effect of transdermal nitroglycerin on RVP-induced changes in cardiac cyclic nucleotides

A single period of RVP produced a significant increase in both cyclic AMP and cyclic GMP contents in hearts of rabbits with placebo patches (Figure 4a,b and Figure 5a,b). Exposure to transdermal NG for 6 h significantly attenuated the RVP-induced increase in cyclic AMP content. Nevertheless, the postpacing cyclic GMP values did not differ from those measured under resting conditions (Figures 4 and 5). Continuous treatment with transdermal NG over 7 days produced similar attenuation of postpacing cyclic AMP-increase to that seen after a 6 h period of 'patch on', whereas a single RVP failed to elicit any cyclic GMP-increase in hearts of these tolerant (Figure 4a and 5a) animals. 'Patch off' postpacing cyclic AMP values were higher than those measured in hearts of the placebo-treated rabbits. However, the difference did not prove to be statistically significant (Figures 4a and 5a). 'Patch off' values following a 12 h 'patch on' period (active patches) did not differ from those seen in the placebo-treated animals (Figures 4b and 5b).

Interaction between chronic treatment with transdermal nitroglycerin and preconditioning on the level of cardiac cyclic nucleotides

The RVP-induced cyclic GMP-increase was significantly amplified, whereas the RVP-induced increase in cyclic AMP

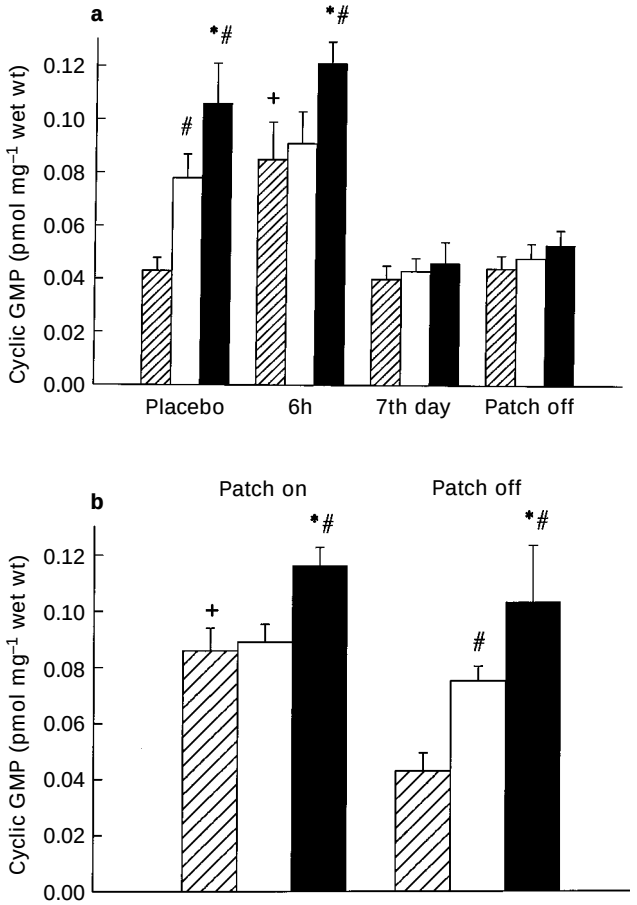

Figure 5 Changes in cardiac cyclic GMP content by repeated periods of RVP in anaesthetized, artificially ventilated, open chest rabbits. Values were determined simultaneously with cyclic AMP. The data are means ± s.d. obtained with 4 rabbits in each group. $^{*}P<0.05$, significant difference between 1st pacing (open columns) and 2nd pacing (solid columns) in both (a) and (b). $^{\#}P<0.05$ represents difference between corresponding resting (hatched columns) and postpacing values in (a) and (b). $^{+}P<0.05$ designates difference between resting placebo and resting transdermal nitroglycerin-treated in (a); in (b), $^{+}$indicates a significant difference between 'patch on' and 'patch off' resting values (hatched columns).

content was strongly attenuated, in the placebo-treated preconditioned animals (Figures 4 and 5). Either preconditioning effect was preserved in the 6th hour of active 'patch on' and 6 h following 'patch off' subsequent to a 12 h active 'patch on'. Development of vascular tolerance to NG due to continuous 7-day 'patch on' blocked these preconditioning effects. Moreover, the preconditioning effects were absent following 6 h 'patch off' subsequent to a 7 day treatment period with active patches (Figures 4 and 5).

Discussion

These results confirm that a preceding brief RVP protects the heart from global myocardial ischaemia induced by a subsequent RVP in parallel with an increase in cardiac cyclic GMP content, and that this protection is lost when tolerance to the vasodilator effect of NG develops (Szilvassy *et al*., 1994a). However, in the present study, the anti-ischaemic effect of transdermal NG was preserved when the hypotensive effect of the drug was seriously impaired. Similar to several clinical findings (Parker & Fung, 1984; Luke *et al*., 1987; Cowan *et al*., 1987), intermittent application of transdermal NG protected against the development of vascular tolerance in the conscious rabbit. Moreover, our present results demonstrate that intermittent NG treatment protected against the loss of the preconditioning phenomenon. However, RVP produced more severe ischaemic changes during the nitrate-free intervals of intermittent NG-treatment than in untreated controls.

The present results further support the hypothesis that a reduced myocardial generation of cyclic AMP during ischaemia might be related to the preconditioning phenomenon (Szilvassy *et al*., 1994b; Sandhu *et al*., 1996). However, the attenuation of RVP-induced increases in cardiac cyclic AMP levels in the preconditioned state has always been accompanied by an increase in cardiac cyclic GMP content in our model (Szilvassy *et al*., 1994a,b). Moreover, drugs that increase cardiac cyclic GMP level by inhibition of cardiovascular cyclic GMP phosphodiesterases have been found to confer protection on the ischaemic heart (Szilvassy *et al*., 1993; 1994c), with attenuation of ischaemia-induced increases in cardiac cyclic AMP content (Szilvassy *et al*., 1993). Therefore, it is suggested that an increased formation of cyclic GMP may be involved in the mechanism of preconditioning induced by rapid pacing.

The mechanism underlying preconditioning is believed to involve the increased formation/release of endogenous protective substances. Some of these substances can stimulate cyclic GMP formation, either directly, such as nitric oxide (NO) and acetylcholine, or indirectly, such as bradykinin through NO release (Parratt, 1995; Linz *et al*., 1996). There is substantial evidence that NO-generating systems are activated during the process of preconditioning (Parratt, 1995). It is also widely accepted that the majority of the cardiovascular effects of NO are mediated through cyclic GMP (Moncada & Higgs, 1995). An increased formation of cyclic GMP may therefore be considered an important step in the signal transduction pathways involved in the mechanisms of preconditioning. This is in accord with out previous finding that an increase in cardiac cyclic GMP content parallels the time course of the anti-ischaemic effect of RVP-induced preconditioning (Szilvassy *et al*., 1994b). Consequently, when preconditioning is blocked by vascular nitrate tolerance, the *in situ* rabbit heart does not produce increased amounts of cyclic GMP in response to pacing. This finding is similar to that described by others who have demonstrated that pharmacological inhibition of nitric oxide synthesis blocks the antiarrhythmic effect of ischaemic preconditioning induced by coronary artery occlusion (Vegh *et al*., 1992) in dogs, and blocks the anti-ischaemic effect of preconditioning induced by rapid pacing in rat hearts (Ferdinandy *et al*., 1996). Moreover, in diseased states such as hypercholesterolaemia and atherosclerosis, which are characterized by a reduced efficacy of the EDRF/nitric oxide system (Flavahan, 1992; Woditsch & Schror, 1994) the cardioprotective effect of preconditioning induced by RVP is lost (Szilvassy *et al*., 1995).

NG is believed to exert the majority of its pharmacological effects after metabolism into NO (Harrison & Bates, 1993). The therapeutic effect of NG in angina pectoris is conventionally thought to be due to a decrease in preload and afterload, improved collateral flow, dilatation of coronary arteries and inhibition of platelet aggregation. Nevertheless, we have recently shown in isolated working hearts of the rat that NG exerts a direct effect on the heart, possibly independent of any vascular effects (Ferdinandy *et al*., 1995). In the present study, we have shown that in the rabbit, another species deficient in coronary collaterals, the anti-ischaemic effect of transdermal NG is preserved in vascular NG-tolerance. However, this anti-ischaemic effect seems to be independent of cyclic GMP since, in the tolerant state, no increase in cyclic GMP was seen in response to either NG or preconditioning. It has been shown that both endogenously and exogenously applied NO reduce the activity of the constitutive and inducible NO synthases in several cell types and tissues at a post-transcription level (Moncada & Higgs, 1995). It is therefore suggested that prolonged continuous NG-treatment may block preconditioning through deterioration of the underlying signal transduction mechanisms at the level of the NO/cyclic GMP pathway. Moreover, the phenomenon of an unremitting *sui generis* anti-ischaemic effect of NG in hearts of the 'haemodynamically tolerant' animals indicates, that the development

of vascular nitrate tolerance may not necessarily be followed by an attenuation of the anti-anginal effect of NG. It is possible that a deficiency in cyclic GMP formation results in haemodynamic tolerance in animals continuously treated with NG, but the cyclic GMP-independent cardiac effect confers some protection on the ischaemic heart. This seems compatible with the assumption that in *in vivo* nitrate tolerance the biological activity of NO is significantly reduced in vascular tissue, with no change in the bioconversion of NG to NO (Laursen *et al*., 1995).

At present, the only method of preventing nitrate tolerance involves the use of dosing schedules that provide low or negligible plasma nitrate levels for a portion of the day. In our study, the intermittent NG-treatment schedule was found effective in preventing vascular tolerance development, to preserve preconditioning and obtain an anti-ischaemic effect during 'patch on' periods but a more severe ischaemic response to RVP was seen during the 'patch off' periods. The mechanisms of this particular rebound in nitrate-free intervals are obscure, although it may be due to counterregulatory vasoconstriction and reflex sympathetic activation which are unopposed when nitrate concentrations decrease rapidly (Parker *et al*., 1995; Freedman *et al*., 1995). In rabbits, coronary vasospasm was found to occur after abrupt withdrawal of NG similar to that seen in some clinical studies (Reeves *et al*., 1985).

In summary, the anti-ischaemic effect of NG may be preserved in the state of vascular NG tolerance possible due to a direct myocardial action that is independent of the cyclic GMP mechanism. We confirm that intermittent NG therapy prevents the development of vascular tolerance and preserves preconditioning. Nevertheless, the results draw attention to nitrate-free periods of intermittent nitrate treatment as 'risk periods' for ischaemic heart attacks.

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