British Journal of Pharmacology (1996) 118, 93-104

Baroreflex resetting but no vascular tolerance in response to transdermal glyceryl trinitrate in conscious rabbits

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1 We investigated whether acute (5 h) and chronic (3 days) transdermal glyceryl trinitrate (GTN) patches could cause the development of tolerance in terms of haemodynamics and vascular reactivity in the conscious rabbit. The effects of haemodynamic tolerance were assessed on arterial pressure, heart rate and the baroreflex control of heart rate, while hindquarter vascular reactivity in response to dilator and constrictor drugs and reactive hyperaemia were used to assess vascular tolerance.

2 Seven days prior to experiments, an inflatable cuff, a pulsed Doppler flow probe and an indwelling intra-aortic catheter (for i.a. agonist infusions) were implanted around the lower abdominal aorta.

3 In acute experiments, the effects of 0-5 h treatment with transdermal GTN (0 Sham), 10 or 20 mg 24 h^{-1}) on MAP, HR and the baroreflex were examined. Chronic experiments were performed on three separate days (days 0 - before, 4 - with GTN patch and 8 - recovery). On each day, the baroreflex, reactive hyperaemic responses and hindquarter vascular dose-response curves to i.a. GTN, adenosine, acetylcholine, *S*-nitroso-N-acetylpenicillamine (SNAP) and methoxamine were assessed. On days 1-4, GTN was administered transdermally via a patch(es) (10 mg 24 h^{-1} (low dose) or 20 mg 24 h^{-1} (high dose); renewed every 24 h).

4 Acute treatment with 20 mg GTN 24 h^{-1}, but not with 0 ($n=4$) or 10 mg GTN 24 h^{-1} ($n=4$), caused a significant fall in MAP (-8 ± 1 mmHg; $n=4$) and resetting of the baroreflex by 5 h. Chronic GTN caused a significant fall in MAP of 8 ± 2 and 8 ± 2 mmHg on day 4 with low ($n=8$) and high dose ($n=8$), respectively, with no change in HR. There was no significant change to hindquarter vascular reactivity to i.a. infusion of GTN, nor were there any significant differences in the reactivity to i.a. adenosine, acetylcholine, SNAP or methoxamine with either low or high doses of GTN.

5 Chronic GTN treatment with low and high dose patches caused a parallel leftward shift ('resetting') of the baroreflex on day 4. By day 8, the baroreflex had still not recovered from this leftward shift.

6 In the rabbit, chronic exposure to clinical nitrate patches caused haemodynamic compensation and baroreflex resetting but no evidence of vascular reactivity tolerance. Novel NO donor drugs and delivery regimens which provide intermittent dosing may prevent the development of haemodynamic resetting rather than preventing vascular tolerance, a commonly perceived difficulty in chronic nitrate therapy.

Keywords: Nitrate tolerance; glyceryl trinitrate; baroreceptor reflex resetting; reactive hyperaemia; vascular reactivity

Introduction

The phenomenon of nitrate tolerance defined as 'increased drug requirement to produce the same effect or ... a state in which less effect is produced by the same amount of drug' (Kalant, 1971) has been well documented since it was first reported in the late nineteenth century (Laws, 1898) and later by others (Steward, 1905; Ebright, 1914). Armstrong & Moffat (1983) have divided the phenomenon into three settings: (a) clinical tolerance where there is still controversy concerning whether there is impairment of the *antianginal* benefit with chronic use of long-acting nitrates (for review see Ahlner *et al.*, 1991); (b) industrial exposure of glyceryl trinitrate (GTN)-based explosives where workers become 'immune' to GTN-induced headache during the week but suffer withdrawal symptoms, perhaps even coronary vasospasm, after a drug-free weekend (Ebright, 1914); and (c) laboratory experimentation where rabbits (Du *et al.*, 1991), rats (Bauer & Fung, 1990; Newman *et al.*, 1990; Boesgaard *et al.*, 1994) and sheep (Husain *et al.*, 1994) treated chronically with GTN show an attenuation of the hypotensive effects to intravenous doses of GTN. Moreover, vascular tissues isolated from these treated animals and challenged in organ baths have a reduction in range, and a decreased sensitivity, of GTN concentration-relaxation curves.

In isolated vascular tissue, the run down of sulphydryl moieties by the metabolism of GTN to release its inorganic nitrate had been thought to explain the tolerance to GTN (Needleman & Johnson, 1973). However, tolerance to other organic nitrates and nitric oxide (NO) donor drugs that interact with the bioactivation pathway of GTN at various stages has also been reported (Newman *et al.*, 1990; Shaffer *et al.*, 1992). This development of cross-tolerance has led to the suggestion that mechanisms other than reduced GTN metabolism may be involved in the development of nitrate tolerance.

From these settings it would seem important to differentiate the 'vascular tolerance' phenomenon, centred principally on isolated large artery experiments from the 'haemodynamic adjustment', assessed in intact animals or human subjects. Clearly, *in vivo*, many homeostatic mechanisms such as the renin-angiotensin system and baroreflex-induced changes in heart rate, myocardial contractility and vasoconstriction may buffer the direct vasodilation to GTN providing an explanation for clinical haemodynamic and antianginal tolerance, without having to evoke an argument for additional vascular tolerance. Reflex activation of the renin-angiotensin system and release of vasopressin may occur to maintain systemic blood pressure during chronic nitrate therapy. However, there is controversy whether angiotensin-converting enzyme inhibition attenuates (Katz *et al.*, 1991) or has no effect (Parker & Parker, 1993) on the development of nitrate tolerance. Another important homeostatic mechanism, the baroreflex, has not been studied in relation to the development of haemodynamic nitrate tolerance. Alterations in the function of this reflex have been reported to occur

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during pathological states such as hypertension. Under such conditions, the baroreceptors 'reset' in the direction of the prevailing arterial pressure (Koushanpour, 1991).

The goal of our study was to investigate the haemodynamic changes in conscious rabbits when chronically exposed to transdermal GTN. We studied the effects of chronic GTN treatment on arterial blood pressure, heart rate and the cardiac baroreflex and the haemodynamic response to removal of autonomic reflexes with the ganglion blocker, mecamylamine. We also used close intra-arterial infusion of GTN, as well as other vasodilator and vasoconstrictor drugs, and reactive hyperaemia to study vascular tolerance in the hindquarter vascular bed.

Methods

Animals

Male and female New Zealand white rabbits (mean 2.46 ± 0.03 kg, range 2.20–2.82 kg; $n=38$) were used in the study. Rabbits were kept on a 12 h light/dark cycle and had free access to food and water. This study was approved by the University of Melbourne Animal Ethics and Experimentation Committee in accordance with the guidelines of the National Health and Medical Research Council of Australia.

Surgery

At least 7 days prior to experimentation, rabbits underwent preliminary surgery under open-circuit halothane (Rhone Merieux, Melbourne, Victoria, Australia) anaesthesia following induction with i.v. alphaxalone and alphadolone (6 and 2 mg kg^{-1} respectively; Saffan, Pitman-Moore, North Ryde, NSW, Australia). Through a midline laparotomy, a Doppler ultrasonic flow transducer probe (3.2 mm i.d.) was placed around the lower abdominal aorta, immediately anterior to the iliac bifurcation, for measuring hindquarter blood flow by the pulsed Doppler technique (Haywood *et al.*, 1981; Wright *et al.*, 1987). Following the Barger method (Herd & Barger, 1964), a fine polyvinylchloride catheter was implanted in the aorta approximately 1 cm anterior to the flow probe, filled with heparin (1000 u ml^{-1}, Commonwealth Serum Laboratories, Melbourne, Victoria, Australia) and the end sealed. An inflatable balloon catheter (4 mm i.d., In Vivo Metric, Healdsburg, CA, U.S.A.) was implanted proximal to the aortic catheter. The catheters and transducer lead wires were tunnelled subcutaneously to the back of the neck and the midline incision closed. The rabbits received Tribrissen 0.2 ml kg^{-1} s.c. (trimethoprim 80 mg ml^{-1}, sulphadiazine 400 mg ml^{-1}; Coopers, North Ryde, NSW, Australia) at the start of surgery and Temgesic (buprenorphine 0.04 mg kg^{-1}, s.c.; Reckitt & Colman, West Ryde, NSW, Australia) at the end of the operation. Four rabbits underwent a sham operation where the lower abdominal aorta was exposed and cleared but not instrumented.

Experimental procedure

On the day of each experiment, lead wires from the flow probe, vascular balloon occluder and the end of the aortic catheter were retrieved under local anaesthesia (0.5% lignocaine hydrochloride, Xylocaine, Astra, North Ryde, NSW, Australia). The flow transducer was connected to a pulsed Doppler flowmeter (Model 545C-3; Bioengineering, University of Iowa, Iowa City, IA, U.S.A.), and the intra-arterial (i.a.) catheter was connected to a roller pump (Model MS-4-Reglo-100, Ismatech, Zurich, Switzerland) for infusion of vasoconstrictor and vasodilator agents at a constant flow rate of 1 ml min^{-1}. The central ear artery and marginal ear vein were cannulated under local anaesthesia. The ear artery catheter was connected to a pressure transducer (CDX, Cobe, Lakewood, CO, U.S.A.) for the measurement of phasic and mean arterial blood pressure (MAP). The blood pressure signal triggered a rate meter (Model 173, Baker Medical Research Institute, Melbourne, Victoria, Australia) for the measurement of heart rate (HR). The ear vein catheter was for administration of drugs. Following these minor operative procedures, rabbits rested quietly in comfortable polycarbonate restrainers (Nalgene, Nalge, Rochester, NY, U.S.A.) for approximately 30 min before the start of the experiment.

Experimental design

The acute effects of transdermal nitrate treatment on MAP, HR and the baroreflex were examined on a single experimental day. Rabbits received either a single transdermal glyceryl trinitrate (GTN) patch (Transiderm-Nitro 50, Ciba-Geigy, Pendill Hill, NSW, Australia) that delivered 10 mg GTN 24 h^{-1} (Single Patch group, $n=4$) or two GTN patches delivering a total dose of 20 mg GTN 24 h^{-1} (Double Patch group, $n=4$) applied to the inner surface of the rabbit's ear for the duration of the experiment (5 h). In the Sham group (no GTN, $n=4$), a piece of Sleek tape (Smith & Nephew, Hull, England) of the same size as the transdermal nitrate patch (sham patch) was applied as above.

The chronic study consisted of three groups in which rabbits received either no GTN (Sham), 10 mg GTN 24 h^{-1} (Single patch group) or 20 mg GTN 24 h^{-1} (Double patch group). Experiments of 6–8 h duration were performed on three experimental days. On Day 0, a control experiment was completed. On the day following the control experiment a 10 mg GTN 24 h^{-1} transdermal glyceryl trinitrate (GTN) patch was applied to the inner surface of the rabbit's ear ($n=13$) and replaced every 24 h for a period of three days (days 1–3). On day 4, prior to the experimental procedure outlined above, a new GTN patch was applied to the ear for the duration of the experiment. Rabbits were allowed three days to recover after removal of the patch (days 5–7) before the experiment was repeated on day 8. In the Sham group, a piece of Sleek tape (Smith & Nephew, Hull, England) of the same size as the transdermal nitrate patch(es) (sham patch) was applied to the inner surface of the rabbit's ear and renewed every 24 h as above.

In a second chronic study, a patch was applied to the inner surface of both ears of the rabbit (i.e. total dose of GTN, 20 mg 24 h^{-1}; $n=9$, Double patch study). Apart from the additional patch, the experimental design remained the same as in the Single patch study outlined above. In 6 of the 9 rabbits an additional experiment was performed after a further 4 GTN-free days (i.e. day 12) to determine the time period for recovery.

Experimental protocol

Acute GTN treatment The protocol on the acute GTN treatment day was as follows:

(i) Baroreflex measurement At the start of the experimental day, a baroreceptor-heart rate reflex curve was constructed by eliciting alternate incremental falls and rises in MAP (± 5–35 mmHg) and measuring reflex changes in HR. Phenylephrine (5–100 μl; 1 mg ml^{-1}, i.v.) was administered to increase MAP and sodium nitroprusside (25–300 μl; 1 mg ml^{-1}, i.v.) to reduce MAP.

(ii) Application of transdermal nitrate patch After completion of the baroreflex curve, a transdermal nitrate patch(es) or sham patch was applied and MAP and HR monitored for a period of 5 h.

(iii) Baroreflex measurement At the end of the 5 h GTN treatment period, a second baroreceptor-heart rate reflex curve was constructed in the presence of the patch(es).

(iv) Ganglion blockade Cardiovascular reflexes were then obviated by administration of the ganglion blocker, mecamy-

lamine (4 mg kg^{-1} i.v. bolus). A 20 min stabilization period was allowed before MAP and HR were measured.

Chronic GTN treatment The protocol on Days 0, 4 and 8 was as follows:

(i) Baroreflex measurement At the start of each experimental day, a baroreceptor-heart rate reflex curve was constructed as described above.

(ii) Ganglion blockade Cardiovascular reflexes were then obviated with the ganglion blocker mecamylamine, administered intravenously (4 mg kg^{-1} bolus, then 2.5 mg kg^{-1} h^{-1}) for the duration of the experiment. A 40 min stabilization period was allowed before further tests were performed.

(iii) Reactive hyperaemia In the Single patch study (GTN 10 mg 24 h^{-1}), the reactive hyperaemic response to periods of hindquarter ischaemia were measured as an indicator of local regulation of blood flow. The lower aortic balloon catheter was inflated to reduce hindquarter vascular bed blood flow to zero for periods of 5, 10, 20, 40 and 80 s. Upon release of the balloon cuff, the hyperaemic response was measured as the increase in hindquarter vascular conductance which was computed continuously from the ratio of hindquarter blood flow to MAP (conductance computer, Baker Medical Research Institute, Melbourne, Victoria, Australia). Hindquarter vascular conductance was allowed to return to the baseline value before applying subsequent periods of ischaemia. Periods of hindquarter ischaemia were performed in ascending order of duration.

(iv) Intra-arterial agonist dose-response curves Dose-response curves to a particular agonist were constructed by intra-aortic infusion of increasing doses at a constant rate of 1 ml min^{-1}. Each dose was infused until a steady state of MAP and hindquarter blood flow had been reached. After each dose-response curve had been completed, a recovery period of at least 30 min was allowed before the next curve was constructed. Curves to GTN (0.01–100 μg kg^{-1} min^{-1}), GTN vehicle (30% propylene glycol (v/v), 30% ethanol (v/v) in saline; 0.02–20 μl kg^{-1} min^{-1}), adenosine (1–1000 μg kg^{-1} min^{-1}), acetylcholine (0.1–10 μg kg^{-1} min^{-1}; Single patch study only) and *S*-nitroso-N-acetylpenicillamine (0.1–100 μg kg^{-1} min^{-1}) were constructed in random order. Curves to methoxamine (1–100 μg kg^{-1} min^{-1}; Single patch study only) were constructed last due to long-lasting effects of the highest doses of methoxamine in the hindquarter bed.

Drug

Drugs used were freshly prepared in 0.9% saline each day and included acetylcholine bromide (Sigma, St Louis, MO, U.S.A.), adenosine (Sigma), glyceryl trinitrate (GTN, David Bull Laboratories, Melbourne, Victoria, Australia), mecamylamine hydrochloride (Merck Sharp & Dohme, Rahway, NJ, U.S.A), methoxamine hydrochloride (Sigma), (−)-phenylephrine hydrochloride (Sigma), *S*-nitroso-N-acetylpenicillamine (SNAP, Sapphire Bioscience, Alexandria, NSW, Australia), and sodium nitroprusside (David Bull Laboratories). Immediately following dilution, acetylcholine and SNAP were stored on ice and shielded from direct light to minimize deterioration.

Analysis and statistical methods

Parameter measurement and agonist dose-responses curves Data are presented as mean ± 1 standard error of the mean (s.e.mean). Vascular resistance units are (mmHg kHz^{-1} Doppler shift) and vascular conductance units were calculated as 100 (kHz Doppler shift $mmHg^{-1}$). Values for MAP and HR presented in the text and tables have been rounded to the nearest whole number. Average s.e.mean within animals for each cardiovascular variable was calculated from two-ways analysis of variance (ANOVA) using the pooled estimate of error from the residual mean square as (error mean square/number of animals)$^{0.5}$ after subtracting the sums of squares between animals and between doses from the total sums of squares for each agonist (Wright *et al.*, 1987). This error bar (±1 average s.e.mean) was located on the average dose-response line in each case. Agonist resistance or conductance curves within animals were compared between treatment days by repeated measures ANOVA. The Greenhouse-Geisser estimate of epsilon was used as a correction for correlation (Ludbrook, 1994). Changes in MAP with maximum dose of each vasodilator agonist were compared between agonists by 1-way ANOVA with Bonferroni post-hoc test for multiple comparisons.

Sigmoid logistic dose-vascular response curves for each agonist were constructed using absolute values (Nakashima *et al.*, 1982). The responses were then expressed as a percentage of the maximum effect for each agonist, and values for the doses at effective dose 10% (ED_{10}), 30% (ED_{30}), 50% (ED_{50}), 70% (ED_{70}) and 90% (ED_{90}) were obtained from each fitted curve. Values for ED_{10-90} within animal were compared between treatment days by repeated measures ANOVA as above.

Between rabbit groups, comparison of resting cardiovascular parameters on day 0 was made by Student's *t* test for unpaired data. In the acute tolerance study, comparison between groups of resting MAP and HR before and after GTN patch-treatment, and after ganglion blockade, was made by 1-way ANOVA. Body weights were compared between Sham and Patch groups on day 0 and day 8 by 1-way ANOVA.

Baroreceptor-heart rate reflex curves Reflex changes in HR were measured as peak bradycardic and tachycardic responses

Table 1 Cardiovascular parameters before and after ganglion block on days 0–8 in conscious rabbits in the Single ($n=8$) and Double ($n=8$) transdermal GTN patch treatment groups

		Experimental day					
		0-*Control*		4-*Patch*		8-*Recovery*	
Parameter	Group	*Before*	*After*	*Before*	*After*	*Before*	*After*
Heart rate	Single	204±7	290±18*	209±4	280±12*	194±10	284±15*
(beats min^{-1})	Double	208±4	268±9*	208±3	246±6*	199±5	250±8*
MAP	Single	71±3	58±2*	63±2	48±2*	66±2	58±3*
(mmHg)	Double	73±2	56±3*	66±1	51±3*	67±1	59±3*
HQ	Single	5.7±0.5	5.5±0.4	4.8±0.4	4.4±0.3	4.7±0.5	5.0±0.5
(kHz)	Double†	5.8±0.6	5.8±0.6	5.7±0.6	5.8±0.6	4.7±0.6	5.0±0.5
HVR	Single	12.9±1.1	11.1±1.2*	14.3±1.4	11.5±0.9*	15.0±1.3	12.3±1.1*
(mmHg kHz^{-1})	Double†	13.7±1.5	10.4±1.4*	12.4±1.4	9.6±1.3*	15.7±2.6	12.8±2.0*

Values are mean ± 1 s.e.mean. GTN, glyceryl trinitrate. *n*, Number of rabbits. Values shown are before and 40 min after ganglion blockade with mecamylamine (4 mg kg^{-1} i.v. bolus + 2.5 mg kg^{-1} h^{-1}) on each experimental day. HQ = hindquarter blood flow velocity. HVR = hindquarter vascular resistance. *$P<0.05$ significant difference within group due to ganglion blockade; paired *t* test. †$n=6$.

following administration of bolus doses of phenylephrine and sodium nitroprusside respectively. The changes in MAP that were induced, together with the corresponding peak bradycardic or tachycardic responses, were fitted to a four parameter (P_1 to P_4) sigmoidal logistic equation; where P_1 = lower HR plateau, P_2 = HR range (difference between the two plateaus), P_3 = normalized gain, or a curvature coefficient which is independent of range, and $P_4 = MAP_{50}$, i.e. MAP at 50% of HR range. The average gain (units are beats min^{-1} $mmHg^{-1}$) is the slope of the linear portion of the curve between the upper and lower plateaus. The upper plateau is determined by P_1 plus P_2 (see Head and McCarty, 1987). Barocurve parameters P_1 to P_4 plus resting HR and MAP, were compared within group and between experimental days by repeated measures ANOVA with Greenhouse-Geisser correction for correlation. In the acute GTN treatment groups, barocurve parameters, plus resting HR and MAP, were compared within group by Student's t test for paired data.

Reactive hyperaemic response The hyperaemic responses to periods of hindquarter ischaemia were measured as the increases in hindquarter conductance above resting flow upon reperfusion. Area under the conductance curve for flow repayment was measured as the weight of a tracing of the response. Repayment area was compared by repeated measures ANOVA, with Greenhouse-Geisser correction for correlation. The linear portion of the repayment curve was tested for variations in slope by analysis of covariance.

In all cases, statistical significance was accepted when $P<0.05$.

Results

Resting cardiovascular parameters before and after ganglion blockade

In rabbits treated acutely for 5 h with double GTN patches, ganglion blockade caused MAP to fall markedly from 68±2 to 56±1 mmHg ($n=4$; $P=0.014$). There was no significant difference in resting MAP after ganglion blockade in either the single GTN patch-treated (69±5 to 62±3 mmHg; $n=4$) or sham (69±3 to 64±1 mmHg; $n=4$) groups. Ganglion blockade caused a significant tachycardia in all three groups, corresponding changes in HR were 184±10 to 297±6, 182±8 to 272±19 and 199±5 to 299±24 in the Sham, Single and Double GTN patch groups respectively.

In the main study, there were no significant differences in resting values of MAP and HR in the sham-operated group ($n=4$) over the three experimental days (76±0.4, 76±0.2, 79±2 mmHg and 196±6, 190±8, 200±8 beats min^{-1}, for days 0, 4 and 8 respectively). These values were similar to the corresponding day 0 values in the Single and Double GTN patch groups (Table 1). Treatment with single or double GTN

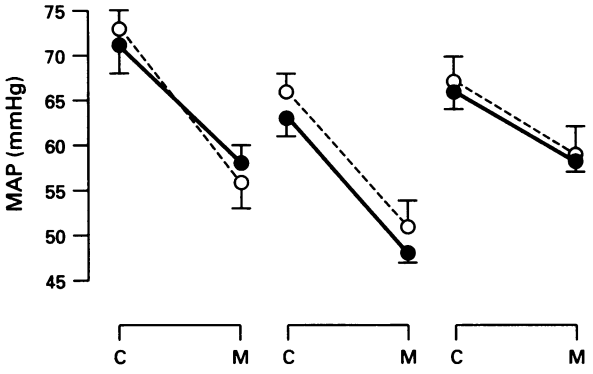

Figure 1 MAP before (C) and 40 min post-administration (M) of the ganglion blocker, mecamylamine in the Single (10 mg $24h^{-1}$, $n=8$; ●, solid line) and Double (20 mg $24h^{-1}$, $n=8$; ○, dashed line) GTN patch treatment groups on days 0, 4 and 8 (left, middle and right panels respectively). Values are mean±1 s.e.mean.

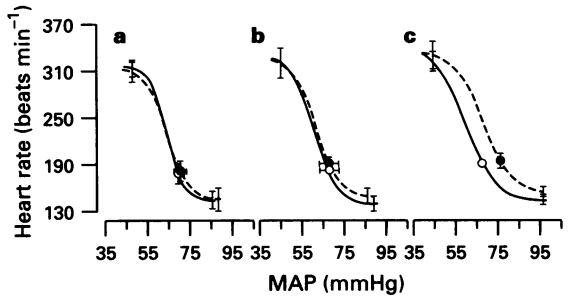

Figure 2 Average baroreceptor-heart rate reflex curves relating mean arterial pressure (MAP) to heart rate in conscious rabbits in the Sham (a, 0 mg $24h^{-1}$, $n=4$), Single (b, 10 mg $24h^{-1}$, $n=4$) and Double (c, 20 mg $24h^{-1}$, $n=4$) acute GTN patch groups. Curves shown are before (●, dashed line) and after (○) 5 h GTN or sham treatment. The symbols on the curves represent the average resting values for MAP and heart rate. Values are mean±1 s.e.mean (those not shown are contained within the symbol). The error bars on the curves represent the s.e.mean of the lower heart rate plateau (right) and heart rate range (left).

Table 2 Baroreflex curve parameters before and after 5 h GTN treatment in the Sham ($n=4$), Single ($n=4$) and Double ($n=4$) transdermal GTN patch treatment groups

Parameter		*Group* *Sham*	*Single patch*	*Double patch*
MAP (mmHg)	Before patch	70±3	68±5	76±2
	After patch	70±2	67±4	68±1*
HR (beats min^{-1})	Before patch	183±12	193±7	196±9
	After patch	179±10	184±6	192±5
HR range (beat min^{-1})	Before patch	171±13	178±19	183±17
	After patch	173±11	191±19	199±13
Average gain (beats min^{-1} $mmHg^{-1}$)	Before patch	7.6±0.3	7.6±0.7	6.1±0.7
	After patch	8.6±1.2	7.8±0.2	6.6±1.3
MAP_{50} (mmHg)	Before patch	64±3	62±5	68±2
	After patch	64±3	61±4	59±1*
Lower HR plateau (beats min^{-1})	Before patch	149±14	149±9	153±6
	After patch	145±11	140±9	144±8

Values are mean±s.e.mean. GTN, glyceryl trinitrate. n, Number of rabbits. HR, heart rate. MAP_{50}, MAP at half the HR range. Values shown are for baroreceptor-heart rate reflex curves in Sham, Single (10 mg GTN 24 h^{-1}) and Double (20 mg GTN 24 h^{-1}) transdermal GTN patch groups before and after 5 h patch treatment. *$P<0.05$, significant difference between corresponding values within group; Student's t test for paired data.

patches caused significant falls in baseline MAP on day 4 which remained at this lower level on day 8. MAP values on days 0, 4 and 8 were 71±3, 63±2 and 66±2 and 73±2, 66±1 and 67±1 mmHg in Single ($n=8$; $P=0.030$) and Double ($n=8$; $P=0.006$) patch groups, respectively. Corresponding baseline HR values were similar during the treatment period in both groups (Table 1).

Average values for resting cardiovascular parameters before and after ganglion blockade on days 0, 4 and 8 in the single GTN patch-treated group are shown in Table 1. Ganglion blockade caused marked falls in MAP on each experimental day (−18, −24 and −12% on days 0, 4 and 8 respectively, Figure 1). This fall in MAP was accompanied by increases in HR of 42, 34 and 46% on days 0, 4 and 8 respectively. Resting hindquarter vascular resistance (HVR) fell significantly following ganglion blockade on all three experimental days (−14, −20 and −18% on days 0, 4 and 8 respectively). Similarly, in the double GTN patch-treated group, ganglion blockade caused marked falls in MAP of 23, 23 and 12% (Figure 1) with corresponding increases in HR of 29, 18 and 26% on days 0, 4 and 8 respectively (Table 1). The tachycardia after ganglion block on day 4 was of a significantly lower magnitude in the double GTN patch-treated rabbits than in the single patch-treated group ($P=0.046$). Resting HVR fell significantly in the double GTN patch-treated group on days 0, 4 and 8 following ganglion blockade (−24, −23 and −18 on days 0, 4 and 8 respectively).

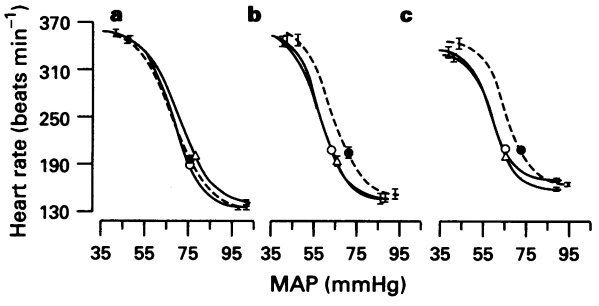

Figure 3 Average baroreceptor-heart rate reflex curves relating mean arterial pressure (MAP) to heart rate in conscious rabbits in the Sham (a, $n=4$), Single (b, $n=8$) and Double (c, $n=8$) GTN patch groups. Curves shown are day 0 (●, dashed line), day 4 (○) following 3 days treatment with GTN (0, 10 or 20 mg 24 h^{-1}, a, b and c respectively) and day 8 (△), after 3 days recovery from Sham or GTN patch treatment. The symbols on the curves represent the average resting values for MAP and heart rate. The values are mean±1 s.e.mean (those not shown are contained within the symbol). The error bars on the curves represent the s.e.mean of the lower heart rate plateau (right) and heart rate range (left).

Haemodynamics

Baroreceptor-heart rate reflex Intravenous administration of incremental doses of sodium nitroprusside caused graded falls in MAP, followed by reflex tachycardia in conscious rabbits. Conversely, phenylephrine administration caused increases in MAP that elicited reflex bradycardia. Excessive increases in MAP caused reflex bradycardic responses that lay obviously below the level of the lower plateau of the fitted baroreceptor-heart rate reflex curve (barocurve). These points were removed as outliers, indicative of cardiac reflex activation and not a component of the baroreceptor reflex (Ludbrook, 1984).

Acute treatment with transdermal GTN caused a significant leftward shift of the barocurves in the Double patch group only, due to a significant fall in MAP after 5 h (−8±1 mmHg; $n=4$; $P=0.006$; Figure 2). However, HR values were no different from the pretreatment baseline. Also within the Double

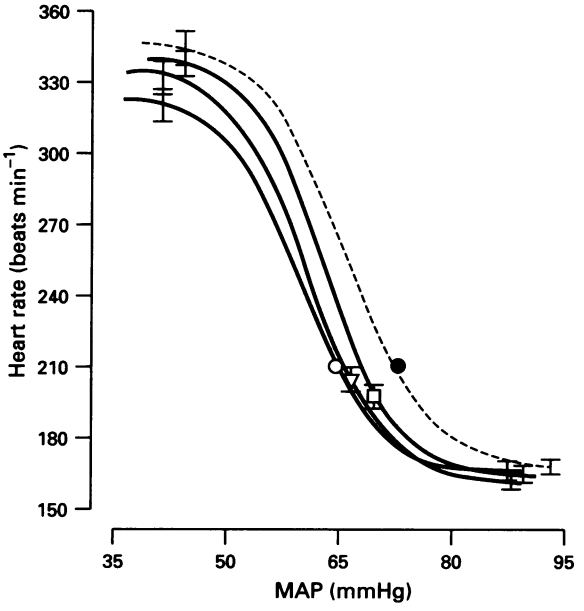

Figure 4 Average baroreceptor-heart rate reflex curves relating mean arterial pressure (MAP) to heart rate in conscious rabbits ($n=6$) on day 0 (●, dashed line), day 4 (○) following 3 days chronic treatment with Double transdermal GTN patches (20 mg 24 h^{-1}), day 8 (△), after 3 days recovery from GTN patch treatment and day 12 (□) after 7 days recovery. The symbols on the curves represent the average resting values for MAP and heart rate. The values are mean±1 s.e.mean (those not shown are contained within the symbol). The error bars on the curves represent the s.e.mean of the lower heart rate plateau (right) and heart rate range (left).

Table 3 Baroreflex curve parameters on days 0–8 in conscious rabbits in the Sham ($n=4$), Single ($n=8$) and Double ($n=8$) transdermal GTN patch treatment groups

Day	*Group*	*MAP* (mmHg)	*HR* (beats min^{-1})	*HR range* (beats min^{-1})	*Average gain* (beats min^{-1} mmHg^{-1})	*MAP$_{50}$* (mmHg)	*Lower HR plateau* (beats min^{-1})
0	Sham	76±0.4	196±6	225±4	6.6±0.7	69±1	133±3
	Single patch	71±3	204±7	214±10	7.1±0.5	64±3	150±8
	Double patch	73±2	208±4	180±7	7.1±0.6	67±2	164±3
4	Sham	76±0.2	190±8	226±6	7.6±0.5	69±2	133±5
	Single patch	63±2	208±4	212±6	7.3±0.8	58±2	147±5
	Double patch	66±1	208±3	159±6	6.6±0.5	60±1	165±3
8	Sham	79±2	200±8	219±6	6.1±0.5	71±2	139±6
	Single patch	66±2*	194±10	209±9	8.1±1.0	59±2	146±11
	Double patch	67±1*	199±5	176±7	7.3±0.6	60±1*	158±4

Values are mean±s.e.mean. GTN, glyceryl trinitrate. n, Number of rabbits. HR, heart rate. MAP$_{50}$, MAP at half the HR range. Values shown are for baroreceptor-heart rate reflex curves for Sham, Single (10 mg GTN 24 h^{-1}) and Double (20 mg GTN 24 h^{-1}) transdermal GTN patch groups on each experimental day. *$P<0.05$, significant difference between corresponding values within group on days 0–8; repeated measures ANOVA with Greenhouse-Geisser correction for correlation.

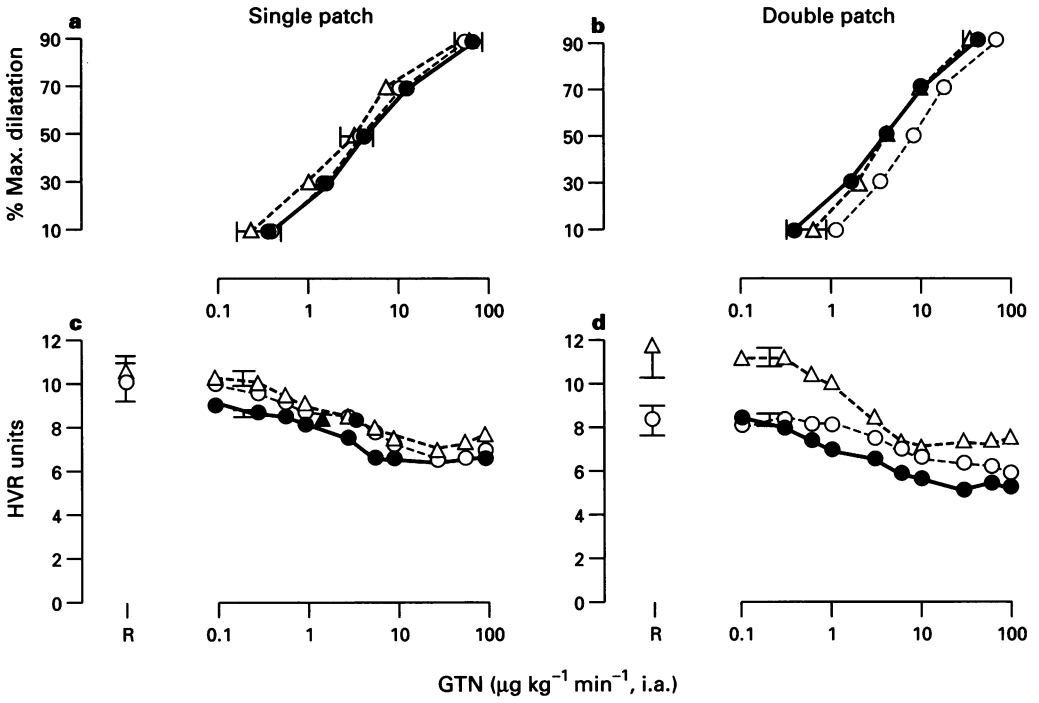

Figure 5 Cardiovascular parameters during infusion of glyceryl trinitrate (GTN) on experimental day 0 (●), day 4 (○) and day 8 (△) in the Single (10 mg 24 h^{-1}; a, c; $n=7$) and Double (20 mg 24 h^{-1}; b, d; $n=6$) transdermal GTN patch treatment groups. (a, b) are average ED_{10-90} values expressed as a percentage of the maximum hindquarter vasodilation. Error bars at ED_{10}; ED_{50} and ED_{90} are ±1 s.e.mean. Error bars not shown are contained within the symbol. (c, d) are hindquarter vascular resistance (HVR; units are [mmHg kHz^{-1}]). R is the resting value for each parameter. Error bars on R are ±1 s.e.mean and on the lines are average s.e.mean (see Methods).

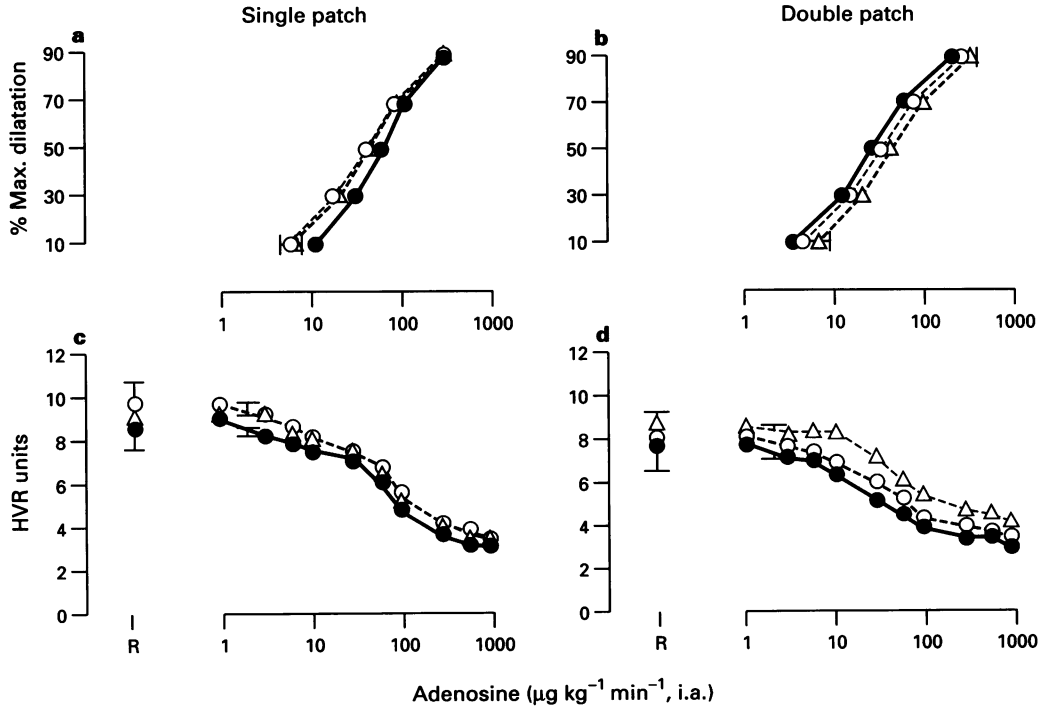

Figure 6 Cardiovascular parameters during infusion of adenosine on experimental day 0 (●), day 4 (○) and day 8 (△) in the Single (10 mg 24 h^{-1}; a, c,; $n=6$) and Double (20 mg 24 h^{-1}; b, d; $n=6$) transdermal GTN patch treatment groups. (a, b) are average ED_{10-90} values expressed as a percentage of the maximum hindquarter vasodilation. Error bars at ED_{10}, ED_{50} and ED_{90} are ±1 s.e.mean. Error bars not shown are contained within the symbol. (c, d) are hindquarter vascular resistance (HVR; units are [mmHg kHz^{-1}]). R is the resting value for each parameter. Error bars on R are ±1 s.e.mean and on the lines are average s.e.mean (see Methods).

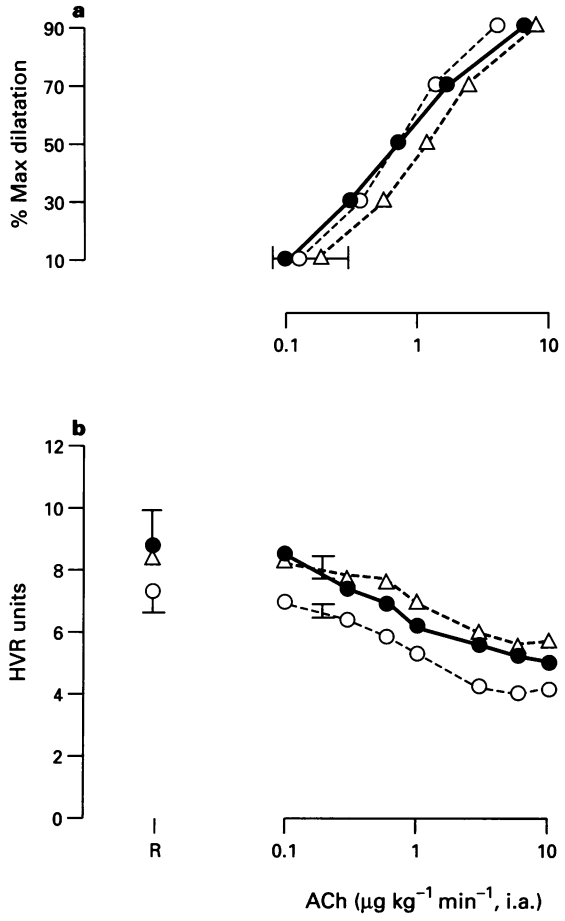

Figure 7 Cardiovascular parameters during infusion of acetylcholine (ACh) on experimental day 0 (●), day 4 (○) and day 8 (△) in the Single (10 mg 24 h^{-1}; $n=5$) transdermal GTN patch treatment group. (a) is average ED_{10-90} values expressed as a percentage of the maximum dilation and (b) is hindquarter vascular resistance (HVR; units are [mmHg kHz^{-1}]). Error bars at ED_{10}, ED_{50} and ED_{90} are ±1 s.e.mean. Error bars not shown are contained within the symbol. R is the resting value for HVR on each experimental day. Error bars on R are ±1 s.e.mean and on the lines are average s.e.mean (see Methods).

patch group, GTN patch treatment caused a fall in MAP_{50} ($P=0.04$). There were no significant differences in the other parameters of the barocurve (Table 2). There was no significant difference between any barocurve parameter after 5 h in either the group treated with a single transdermal GTN patch (10 mg 24 h^{-1}; $n=4$) or sham animals ($n=4$).

In the chronic study, the average barocurves for sham-operated rabbits are shown in Figure 3. There was no significant difference between any barocurve parameter on days 0 to 8 (Table 3). In the group treated with a single transdermal GTN patch (10 mg 24 h^{-1}; $n=8$), three days of continuous GTN treatment caused a significant leftward shift of the barocurve with a fall in resting MAP on day 4, (−8±2 mmHg; $P=0.03$; Figure 3). However, corresponding HR values were similar to day 0. There were no other changes in the parameters of the barocurve (Table 3). Surprisingly, after a three day GTN-free recovery period the barocurve on day 8 remained left-shifted with resting MAP being maintained at a level still significantly below control on day 0 (66±2 day 8 compared with 71±3 mmHg day 0, $n=8$; $P=0.03$). There was no significant difference between any other barocurve parameter over the three experimental days.

Treatment of rabbits with 20 mg 24 h^{-1} transdermal GTN (Double patch group; $n=8$), similarly caused the barocurves to be leftward-shifted (Figure 3). This shift was also accompanied by a significant fall in MAP on day 4 compared to the resting value on the control day 0 (66±1 and 73±2 mmHg, respectively; $P=0.007$), with no recovery of MAP by day 8 (67±1 mmHg). Within the Double patch group, the MAP at half the HR range (MAP_{50}) on day 4 was also significantly less than the control day 0 value, and remained lower on day 8 ($P=0.005$; Table 3). The magnitude of the fall in resting MAP was comparable to that which occurred in the Single patch group (−8±2 and −8±2 mmHg in Double and Single GTN patch groups, respectively). In the Double patch group, there was a trend for the upper plateau of the barocurve to be depressed on day 4, recovering slightly by day 8, but this was not statistically significant.

In some of the double GTN patch-treated rabbits ($n=6$), an additional experiment was performed after a further four GTN-free days (i.e., on day 12) to determine the time period for recovery of the barocurve. All parameters of the curves had fully recovered by day 12 (Figure 4).

Vascular reactivity

Intra-arterial agonist dose-response curves There were no significant differences in the dose-HVR curves to GTN, adenosine, ACh and SNAP, or methoxamine-vascular conductance curves, over the eight day experimental protocol in either the Single or Double GTN patch groups (Figures 5–9, lower panels). There was a difference between the GTN-HVR curves due to day in the Double GTN patch group ($P=0.020$) with a significantly higher resting resistance at the start of the GTN-HVR on day 8 (Figure 5). There was no change in the sensitivity (ED_{50}) to any of the agonists tested during the experimental period (Table 4; Figures 5–9, upper panels). Comparison of the ED_{50} values for GTN-HVR curves over the three experimental days in the Double patch group did, however, indicate a trend for a decrease in the sensitivity to GTN on day 4, but this failed to reach statistical significance after Greenhouse-Geisser correction for correlation ($P=0.069$).

A marked fall in MAP occurred during infusion of the highest doses of GTN and SNAP (GTN, Single patch study −14, −14 and −16 mmHg, Double patch study, −15, −10 and −19 mmHg; SNAP, Single patch study −20, − 13 and −19 mmHg, Double patch study −19, −18 and −26 mmHg; days 0, 4 and 8 respectively; $P<0.005$; data not shown). Reflex changes in HR were successfully obviated by ganglion block during GTN, adenosine and methoxamine infusions as indicated by no significant changes in HR with administration of each agonist (data not shown). Infusion of the maximum doses of ACh (Single patch group only) and SNAP did, however, elicit significant tachycardic responses (ACh, 2, 15 and 12%; SNAP, Single patch study 10, 7 and 7%, Double patch study 15, 21 and 17%; days 0, 4 and 8 respectively; $P<0.05$) which may have acted to buffer the falls in MAP that each agonist induced. Infusion of GTN vehicle caused no change in MAP, HR or HVR (data not shown).

In the Single patch study, three rabbits did not complete the 3 day protocol and their data were excluded from the group results; the flow probe failed in two rabbits and the i.a. catheter was non-patent in one animal.

Reactive hyperaemia The reactive hyperaemic responses to periods of aortic occlusion are shown in Figure 10. There was no significant difference between these responses over the three experimental days ($n=9$). The slopes of the linear portion of each curve (responses to periods of 20–80 s occlusion) were also similar over the experimental period. These data suggest that chronic GTN patch treatment (10 mg 24 h^{-1}) has no effect on this metabolic regulation of regional blood flow.

Rabbit body weight

There was no significant difference in the post-operative body weight of the rabbits on the first experimental day in single patch, sham and double patch treatment groups (2.49±0.06, $n=13$; 2.69±0.05, $n=4$ and 2.60±0.04 kg, $n=6$, respectively).

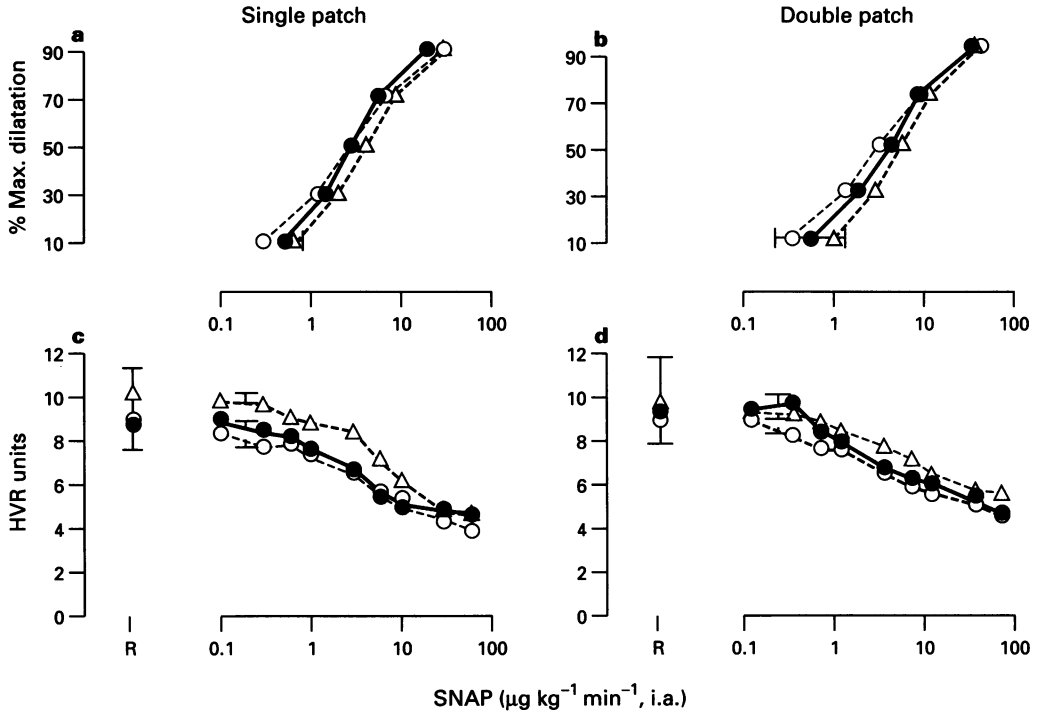

Figure 8 Cardiovascular parameters during infusion of *S*-nitroso-N-acetylpenicillamine (SNAP) on experimental day 0 (●), day 4 (○) and day 8 (△) in the Single (10 mg 24 h^{-1}; a, c; $n=5$) and Double (20 mg 24 h^{-1}; b, d; $n=6$) transdermal GTN patch treatment groups. (a, b) are average ED_{10-90} values expressed as a percentage of the maximum hindquarter vasodilation. Error bars at ED_{10}, ED_{50} and ED_{90} are ±1 s.e.mean. Error bars not shown are contained within the symbol. (c, d) are hindquarter vascular resistance (HVR; units are [mmHg kHz^{-1}]). R is the resting value for each parameter. Error bars on R are ±1 s.e.mean and on the lines are average s.e.mean (see Methods).

Rabbits in all three groups gained weight at approximately the same rate during the experimental period.

Discussion

This study showed that three day transdermal GTN treatment lowered blood pressure and reset the baroreceptor-heart rate reflex to the left in a parallel manner due to the fall in MAP. Despite the continued presence of the GTN patch there was no evidence of vascular tolerance or cross-tolerance in the hindquarter vasculature since local intra-arterial infusions of GTN or other vasodilator or vasoconstrictor drugs caused a similar range of reactivity changes and sensitivity as in GTN-deprived rabbits.

Transdermal nitrate patches that deliver a clinically effective dose of GTN (10 mg 24 h^{-1}) in 70 kg man was considered an appropriate method of chronic GTN administration in this study. These patches are commonly used for the prophylaxis of angina pectoris (Reynolds, 1989) and tolerance to the anti-anginal and haemodynamic effects of such patches has been reported to occur within 24 h of continuous application (Ahlner *et al.*, 1991). These 10 mg patches raise plasma GTN levels to 0.2–0.25 ng ml^{-1} (Curry *et al.*, 1984; Woodcock *et al.*, 1986; Torfgård & Ahlner, 1993). A weakness of the present study was that rabbit plasma GTN levels were not measured during acute or chronic transdermal application. However, if the GTN is absorbed from the patch at the same rate from rabbit ear skin as from human skin, then on a body weight basis (3 kg rabbit) GTN plasma levels should be at least 10–20 times higher than that required to show haemodynamic tolerance in human subjects (Hogan *et al.*, 1990; Levy *et al.*, 1991; Parker *et al.*, 1991; Parker & Parker, 1993; Cloarec-Blanchard *et al.*, 1994). It is not known whether transdermal absorption of GTN, or its metabolism, occurs at the same rate in rabbits as it does in human subjects. In our initial rabbit experiments the 10 mg 24 h^{-1} dose lowered the MAP by 8 mmHg but failed to cause any vascular tolerance. In a second protocol we doubled the dose to 20 mg 24 h^{-1}; however, the MAP did not fall any further nor again was there any evidence of vascular tolerance. Indeed, acute (5 h) treatment with transdermal GTN 20 mg 24 h^{-1} also caused a fall in MAP of 8 mmHg indicating that there had been no attenuation of the depressor affect of GTN during chronic treatment. It is worth considering whether a failure to show vascular tolerance in the rabbit *in vivo* from single or double patch treatment was due to a lack of sensitivity of rabbit blood vessels to GTN compared with human arteries, experiments best done in the organ chamber. In human isolated large internal mammary artery pEC_{50} (M) for GTN was 7.21±0.26 in thromboxane-mimetic (U46619)-contracted vessels and 7.27±0.45 in K^+ pre-contracted vessels (He *et al.*, 1989). This is a very similar sensitivity to that reported in rabbit aortic rings precontracted with phenylephrine, pEC_{50} (M) 7.23±0.48 (Du *et al.*, 1991), and in dog coronary artery precontracted with K^+ (pEC_{50} GTN 7.5) and U46619 (pEC_{50} GTN 7.43) (Angus & Brazenor, 1983). While these studies relate to large artery reactivity and show that GTN has very similar activity across human, dog and rabbit vessels, the same may not hold for resistance arteries well known to be less sensitive to GTN than large arteries studied *in vivo* and *in vitro* (Hintze & Vatner, 1983; Angus *et al.*, 1986). Intra-arterial infusion of GTN in human forearm of 0.5–2.0 μg min^{-1} (i.e. 7–29 ng kg^{-1} min^{-1}) reversed forearm vasoconstriction to noradrenaline infusion (Cheesman & Benjamin, 1994) while Cloarec-Blanchard *et al.* (1994) used 450 μg GTN infused over 1 min i.v., i.e. 6.4 μg kg^{-1} min^{-1}, to test haemodynamic response. Therefore, our dose-range of GTN 0.1–100 μg kg^{-1} min^{-1} i.a. certainly covers the range used in various protocols to test GTN sensitivity in rabbits and man.

Haemodynamic tolerance

Apart from the fall in MAP, the resetting of the baroreflex observed with acute (double patch) and chronic single or double GTN patch treatment was a significant pointer to alterations in at least one homeostatic mechanism. In anecdotal experiments, rabbits treated with a single (10 mg 24 h^{-1}; $n=1$) or double (20 mg 24 h^{-1}; $n=1$) GTN patch(es) were monitored before and during 3 days of chronic nitrate treatment. Within the first 24 h, MAP fell by 9 and 11 mmHg in single and double patch-treated rabbits respectively, with no change in HR. This lower baseline MAP was maintained for the remainder of the 3 day nitrate treatment period. In the main study, the delay in recovery of the barocurves after patch removal in both single and double patch chronic treatment groups was most striking. Curves remained left-shifted 4 days after removal of the GTN patch (day 8) in both groups. In a number of rabbits, after a further 4 days of recovery (day 12) curve parameters had returned to control values. However, the day 12 recovery experiments were only performed in the group treated with GTN 20 mg 24 h^{-1}. Additional experiments are necessary to determine at what stage the baroreflex recovers and whether this recovery time differs according to the dose of GTN. The slow recovery of the baroreflex may be indicative of baroreceptor 'resetting'. Resetting is suggested to occur when blood pressure changes are maintained for periods greater than 10 min (Eckberg & Sleight, 1992). In conscious and anaesthetized rabbits, Dorward *et al.* (1982) showed that sustained decreases in MAP caused by nitroprusside infusion or haemorrhage were sufficient to reset whole aortic nerve baroreceptor function, and baroreflex curves in the same direction as the fall in MAP.

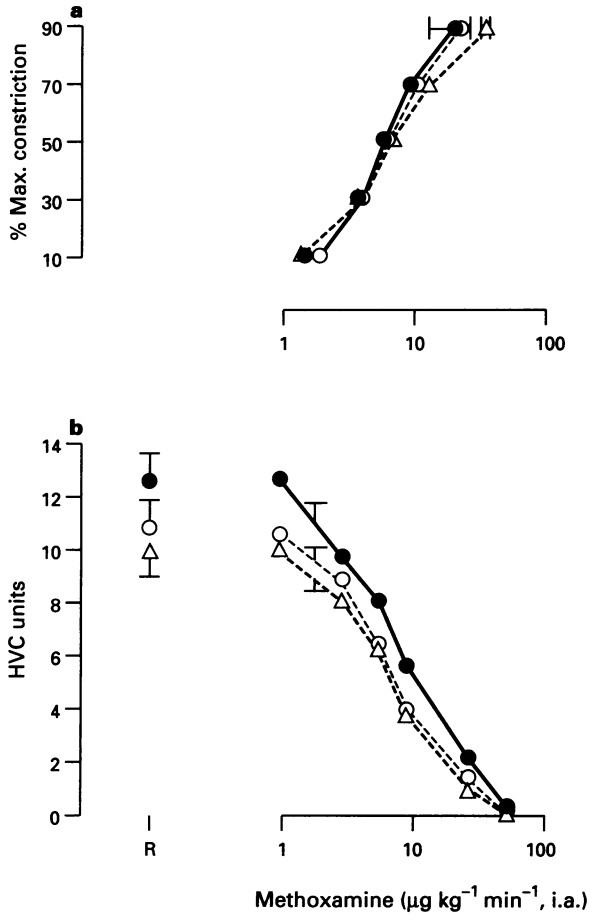

Figure 9 Cardiovascular parameters during infusion of methoxamine on experimental day 0 (●), day 4 (○) and day 8 (△) in the Single (10 mg 24 h^{-1}; $n=6$) transdermal GTN patch treatment group. (a) is average ED_{10-90} values expressed as a percentage of the maximum hindquarter vasoconstriction. Error bars at ED_{10}, ED_{50} and ED_{90} are ±1 s.e.mean. Error bars not shown are contained within the symbol. (b) is hindquarter vascular conductance (HVC; units are 100[kHz $mmHg^{-1}$]). R is the resting value for HVC on each experimental day. Error bars on R are ±1 s.e.mean and on the lines are average s.e.mean (see Methods).

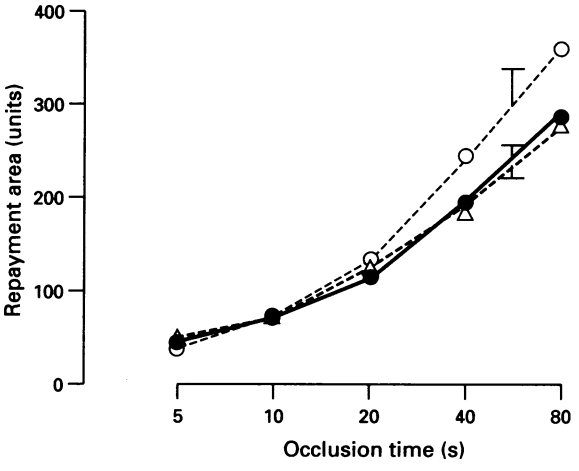

Figure 10 Reactive hyperaemic response to periods of hindquarter ischaemia (5–80 s of aortic occlusion) in the conscious rabbit. Responses were measured in the Single GTN patch treatment group ($n=9$) on day 0 (●), day 4 (○) and day 8 (△). Y-axis: hindquarter vascular conductance repayment area, see Methods; X-axis: aortic occlusion time. Error bars are average s.e.mean (see Methods).

Table 4 Hindquarter vascular sensitivity to agonists in the Single and Double GTN patch treatment groups, days 0–8

Agonist	*Group* (n)	*ED50* (log_{10}[μg kg^{-1} min^{-1}]) *Day 0*	*Day 4*	*Day 8*
GTN	Single (7)	0.60±0.10	0.54±0.11	0.46±0.13
	Double (6)	0.60±0.05	0.89±0.10	0.58±0.10
Adenosine	Single (6)	1.78±0.03	1.62±0.04	1.64±0.07
	Double (6)	1.47±0.03	1.52±0.08	1.65±0.07
ACh	Single (5)	−0.16±0.06	−0.15±0.04	0.05±0.07
SNAP	Single (5)	0.45±0.09	0.46±0.04	0.62±0.07
	Double (6)	0.48±0.12	0.46±0.06	0.70±0.06
Methoxamine	Single (6)	0.85±0.05	0.87±0.03	0.91±0.05

Values are mean ± 1 s.e.mean. *n*, Number of rabbits. Values shown are the averages of the individual hindquarter vascular resistance (or conductance in the case of methoxamine) curves to each agonist. ED_{50}, log_{10} (effective dose of agonist producing 50% of the maximum response in the hindquarter vascular bed). GTN, glyceryl trinitrate. ACh, acetylcholine. SNAP, *S*-nitroso-N-acetylpenicillamine.

Baroreceptors, present in the adventitia of conduit vessels such as the aorta and carotid arteries, may reset as a consequence of alterations in vessel distensibility (Koushanpour, 1991). GTN is known to increase arterial compliance (Sumimoto *et al.*, 1993), therefore stretch of the baroreceptors may be reduced during GTN treatment allowing them to rest at a lower threshold for activation. The baroreflex may then act to buffer pressure changes around the 'new' resting MAP. In this study, baroreflex resetting was seen within 5 h of transdermal GTN (20 mg 24 h^{-1}) treatment. But acute 5 h single patch (10 mg 24 h^{-1}) treatment did not affect the baroreflex, nor cause a fall in MAP. These data suggest that the resetting of the baroreflex is more likely to be a consequence of the depressor effect of GTN, rather than a direct effect of the nitrate on arterial compliance.

From the results of this study, however, it remains unclear to what extent other homeostatic systems such as the renin-angiotensin-aldosterone axis and elevation of circulating catecholamines may be buffering the depressor response to GTN observed following the acute and chronic 3 day treatment period. Parker *et al.* (1991) have shown that circulatory plasma levels of catecholamines, vasopressin and aldosterone, as well as plasma renin activity, remain significantly elevated in healthy human subjects undergoing continuous transdermal nitrate therapy, even after the depressor response to GTN had been attenuated. Thus the nitrate was still capable of exerting neurohumoral stimulation, even during a period of defined clinical depressor 'tolerance'. In the current work, activation of such neurohumoral mechanisms may explain the apparent dose-independence of the GTN patch-induced fall in blood pressure. If sympathoadrenal activation was supporting the circulation during chronic GTN treatment, we may have expected to see a greater fall in MAP following ganglion blockade in the double patch compared with single patch groups. However, there were similar falls in MAP following mecamylamine on day 4 (see Figure 1) which in part may be explained by elevated circulating levels of vasopressor hormones such as vasopressin and angiotensin II (Hiwatari *et al.*, 1985). Further studies are required to examine the involvement of these neurohumoral effectors in modulating the dose dependence of the depressor action of GTN.

Vascular tolerance

Vascular tolerance *in vitro* defined in isolated large arterial or venous tissue as the marked attenuation in range or loss of sensitivity (fall in pEC_{50}) of GTN concentration-relaxation (CR) curves has been firmly established. For example, in human saphenous vein a first CR curve to GTN from 0.1 nM up to 10 μM had a pEC_{50} of 8 and was followed by a 2 h drug-free wash period. On rechallenge, sensitivity to GTN was then more than one thousand fold less, pEC_{50} 4.5 (Armstrong & Moffat, 1983). Similar findings have been reported in isolated vessels from rabbit (Slack *et al.*, 1989; Förster *et al.*, 1991; Zimmermann *et al.*, 1991), pig (Kodja & Noack, 1993; Kodja *et al.*, 1994) and cow (Henry *et al.*, 1989a,b,c; 1990; Kukovetz & Holzmann, 1990). These high concentrations of GTN (10 μM) that are reached towards the end of the first CR curve *in vitro* contrast with the relatively low plasma concentration of 0.2 ng ml^{-1} measured in man ($\sim$1 nM) following patch delivery of GTN (Curry *et al.*, 1984).

The evidence for vascular tolerance from *in vivo* studies is not so clear. Studies in rabbits (Shaffer *et al.*, 1992), rats (Boesgaard *et al.*, 1994) and dogs (Berdeaux *et al.*, 1992) and human clinical studies (Hiremath *et al.*, 1989; Dupuis *et al.*, 1990; Katz *et al.*, 1991; Levy *et al.*, 1991; Ghio *et al.*, 1992; Cheesman & Benjamin, 1994) have provided evidence both for and against the existence of tolerance at the level of the local vasculature. In the conscious rabbit, Du *et al.* (1991) found significant attenuation of i.v. GTN-induced depressor responses after transdermal treatment with GTN (20 mg 48 h^{-1}), but no effect on reductions in arterial pressure induced by sodium nitroprusside or ACh. Cheesman & Benjamin (1994) demonstrated no evidence of venous or arterial tolerance in human subjects to i.a. infusion of GTN following 7 days of transdermal GTN administration. This work supports the current findings of a lack of vascular tolerance to GTN at the level of the local circulation within the hindquarter vascular bed of the conscious rabbit. An important feature of the present study was the use of arterial catheterisation to provide local i.a. infusions of both vasodilator and vasoconstrictor agonists. This permitted construction of full dose-response curves with assessment of maximum dilation or constriction, which is not possible when using the intravenous route of administration due to limiting effects on systemic MAP. The power of local i.a. agonist administration is that information may be gained on changes in sensitivity, as well as changes in range of the responses to an agonist (Wright *et al.*, 1987). However, there were no changes in response range nor sensitivity to any of the agonists tested in this study.

Several reports have suggested that development of cross-tolerance to agonists such as SNAP (a direct nitric oxide donor that acts at the level of the target enzyme for GTN soluble guanylate cyclase) occurs during development of tolerance to GTN (Henry *et al.*, 1989c; Kodja *et al.*, 1994; Shaffer *et al.*, 1992). In this study, we were unable to detect tolerance to the haemodynamic effects of SNAP (or ACh). This finding was not surprising given the absence of vascular tolerance to GTN. Furthermore, in contrast to previous studies that have shown an enhanced response to pressor agents in the presence of nitrate tolerance (Molina *et al.*, 1987; Du *et al.*, 1991), we were unable to demonstrate an augmentation of the vasoconstrictor response to the α_1-adrenoceptor-selective agonist, methoxamine. This concurs with Hiremath *et al.* (1989) who demonstrated a lack of tolerance to transdermal GTN application in human peripheral veins and no change in the sensitivity of noradrenaline-induced constriction.

Reactive hyperaemia, which occurs in response to periods of arterial occlusion, is the result of an autoregulatory mechanism involving both accumulation of local metabolic factors, such as adenosine and superoxide anions (Kaminski & Wolin, 1993), and autonomic nervous system control (Beaufort-Krol *et al.*, 1989). Recent studies in isolated hearts have indicated a possible role for nitric oxide in the reactive hyperaemic response to periods of both hypoxia and ischaemia (Park *et al.*, 1992; Brown *et al.*, 1993; Pohl *et al.*, 1994). In light of the suggested role for nitric oxide in such regulation, we evaluated the effect of chronic transdermal GTN treatment on the hyperaemic response to periods of hindquarter ischaemia. We found no evidence to suggest that prolonged periods of GTN treatment (albeit in the apparent absence of vascular tolerance) had any influence on the metabolic regulation of blood flow in the hindquarter of the conscious rabbit.

In conclusion, chronic transdermal GTN administration failed to cause vascular tolerance in the hindquarter vascular bed of the conscious rabbit. Reactive hyperaemic responses were not affected by chronic GTN treatment, nor was there any evidence of cross-tolerance to other vasodilator drugs or potentiation of α_1-adrenoceptor-mediated vasoconstriction. Chronic GTN treatment caused haemodynamic changes including a fall in MAP and long-lasting resetting of the baroreflex to this lower MAP. This shift in the baroreflex did not increase with administration of the larger dose of GTN possibly due to activation of neurohumoral pressor mechanisms such as the renin-angiotensin system which may have counteracted the fall in blood pressure.

Assessing tolerance to GTN at the level of the vascular bed requires careful consideration of haemodynamic compensatory changes and the use of protocols to allow assessment of range and sensitivity to nitrates. As novel NO donor drugs and delivery systems are introduced into therapy, the role of intermittent exposure may have benefit with regard to compensatory haemodynamic resetting rather than to preventing vascular tolerance, a commonly perceived difficulty in chronic nitrate therapy.

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(Received June 19, 1995
Revised January 9, 1996
Accepted January 16, 1996)