J. Pharm. Pharmacol. 1997, 49: 1102–1108
Received March 24, 1997
Accepted July 23, 1997

Twenty-four-hour Variations in the Effect of Nitrodilators in Rat Aorta: Lack of Influence of the Endothelium

UGUR HODOGLUGIL, CANAN ULUOGLU, H. ZAFER GÜNEY, C. ZAFER GÖRGÜN, Z. SEVIM ERCAN, NURETTIN ABACIOGLU* AND HAKAN ZENGIL

*Department of Pharmacology, Faculty of Medicine and *Pharmacy, Gazi University, Ankara, Turkey*

Abstract

Time-dependent variations of the vasodilator effects of sodium nitroprusside and glyceryl trinitrate on isolated smooth muscle have been studied on rings of rat thoracic aorta, both endothelium-intact and endothelium-denuded.

For most of the concentrations of sodium nitroprusside used the induced relaxations were significantly dependent on the time the tissues were obtained. However, significant temporal differences were obtained for glyceryl trinitrate-induced relaxations at lower concentrations only for both endothelium-intact and endothelium-denuded preparations. EC50 values of sodium nitroprusside and glyceryl trinitrate (i.e. the concentrations inducing half the maximum response) were also significantly different and they had quite similar rhythmic features both in endothelium-intact and in endothelium-denuded preparations.

These results clearly show that the in-vitro sensitivity of rat thoracic aorta to nitrodilator agents varies over a 24-h period and thus depends on when the animals were killed; the presence of endothelium does not change the rhythm of nitrodilator activity. These variations might be a result of circadian rhythm in the guanylate cyclase-cGMP system which mediates responses to nitrodilator agents.

Accumulated evidence suggests that several unfavourable cardiovascular events occur more frequently in the morning at the beginning of diurnal activity, from wakefulness to noon. This has been shown to be true for myocardial ischaemia (Nademanee et al 1987), myocardial infarction (Muller et al 1985), sudden cardiac death (Muller et al 1987), acute pulmonary embolism (Gallerani et al 1992; Manfredini et al 1993) and cerebrovascular disease (Gallerani et al 1993). Moreover, blood fibronolytic activity is lowest in the morning (Rosing et al 1970; Andreotti et al 1988) whereas platelet aggregability (Tofler et al 1987; Brezinski et al 1988), plasma adrenaline and noradrenaline levels (Linsell et al 1985), plasma renin activity (Gordon et al 1966), and cortisol secretion (Weitzman et al 1971) all increase.

Isolated vascular smooth muscle preparations have provided an important and convenient tool for understanding the properties of action of many vasoactive drugs because they reduce the complexity of the reaction between the drug and the organism. Recent reports have shown that there are time-dependent variations in the sensitivity of such preparations to various vasoactive agents. In-vitro 24-h rhythms have been reported for adrenergic agonistic activity (Gohar et al 1992; Keskil et al 1996), and endothelium-dependent or endothelium-independent relaxant agents (Keskil et al 1996) in rat aorta.

Although it is still unknown whether endothelium-derived relaxing factor (EDRF) is a single substance, it has been suggested that at least one EDRF is nitric oxide (NO) (Ignarro 1989). The stimulation of soluble guanylate cyclase and the subsequent increase in 3′,5′-cyclic monophosphate (cGMP) in smooth muscle is the common mechanism promoting nitrovasodilator- and EDRF (NO)-induced vascular relaxation (Ignarro 1989). It has been shown that there are circadian variations in the activity of guanylate cyclase and the levels of cGMP (Spessert et al 1992; Witte et al 1995; Faillace et al 1996).

It has been demonstrated that de-endothelization or use of NO synthase inhibitors enhances the response to nitrovasodilator agents in vascular smooth muscle (Shirasaki & Su 1985; Pohl & Busse 1987; Lüscher et al 1989; Ralevic et al 1991). Therefore, it is probable that the temporal effect of nitrates in vascular preparations is altered by the endothelium.

The aim of this study was to determine whether there is time-dependent variation in the relaxant effects of two different nitrovasodilators in rat aorta and whether the endothelium effects the process.

Materials and Methods

Animals

Experiments were performed on local-bred male albino rats, 2·5–3 months, obtained from our colony, which has been inbred for more than 15 generations to improve genetic homogeneity and thus ensure little variability in the circadian clock. They were housed under controlled environmental conditions (light, temperature, feeding time, etc.) throughout their life span, and food and water were freely available. The animals were acclimatized to a 12-h light (intensity approximately 100 lux) 12-h dark schedule (lights on from 0800 h to 2000 h). Illumination was provided by cool fluorescent bulbs controlled by an automatic timer. Photosafe red bulbs were used to facilitate the injection of rats during the dark span. To avoid seasonal variations, all experiments were undertaken in spring, during April and May.

Correspondence: U. Hodoglugil, Gazi University, Department of Pharmacology, Faculty of Medicine, P.O. Box 49, Bahcelievler, 06502 Ankara, Turkey.
E-mail: zengil@tr-net.net.tr

Tension measurement

Animals were anaesthetized with sodium pentobarbital (30 mg kg^{-1}) and killed by exsanguination from the common iliac arteries at six different times of day (0900, 1300, 1700, 2100, 0100 and 0500 h). The thoracic aortas were excized immediately, cleaned of adhering fat and connective tissue, and cut into transverse rings of approximately 2·5–3 mm length, with special care being taken not to damage the luminal surface. Four aortic rings were obtained from each rat and two were de-endothelized by gently rubbing the intimal surface with a roughened stainless steel rod ($n = 10$–14 preparations from 6–7 rats killed at any one time). The rings were mounted in 10-mL organ baths filled with Krebs solution of composition (mM): NaCl 118·1; KCl 4·7; $CaCl_2$ 1·3; $NaHCO_3$ 25; $MgCl_26H_2O$ 0·5; $NaH_2PO_42H_2O$ 0·9; and anhydrous glucose 11·0; oxygenated with 95% O_2 and 5% CO_2 and kept at 37°C. Tension was measured isometrically with a transducer (Grass, MA; FT 03) and recorded continuously with a transducer amplifier and recorder (Grass model 7E). Before experiments the rings were left to equilibrate for 1 h under a resting tension of 2·0 g. During this period, the Krebs solution in the tissue baths was replaced every 15 min. The absence of functional endothelium was assessed by the lack of vasodilation in response to 10^{-6} M acetylcholine in submaximally (70–80% of maximum contraction) phenylephrine-contracted rings.

Experimental protocol

After the rings had been pre-contracted submaximally with phenylephrine ($3–8 \times 10^{-7}$ M), sodium nitroprusside was added cumulatively from 10^{-10} to 10^{-6} M. After washing and recovery, the rings were again pre-contracted with phenylephrine and then cumulative concentration-response curves to glyceryl trinitrate (10^{-9}–3×10^{-6} M) were obtained. The same protocol was applied to six different time series, for both endothelium-intact and endothelium-denuded preparations.

Because in-vitro 24-h rhythms have been reported for both contractile and relaxant agents in rat aorta (Gohar et al 1992; Keskil et al 1996), it might be expected that the level of pre-contraction affects the magnitude of vasodilation. In a recent study in our laboratory, concentration–response curves for phenylephrine were obtained for de-endothelized rat aortic rings obtained at six different times of the day (the times the rats were killed were identical with those in this study). Values of percent maximum responses for some concentrations and EC50 values (the concentrations inducing half the maximum response) were significantly different for different time series. Although maximum responses were not statistically different, they varied by 20–25% (Görgün et al 1997). To indicate whether the magnitude of pre-contraction affected the relaxant responses, endothelium-intact and endothelium-denuded aortic rings dissected at 1200 were pre-contracted with phenylephrine to levels of 55–60%, 70–75% and 85–90% of maximum contraction (in the same preparation) and then used to obtain cumulative response curves to acetylcholine (10^{-8}–10^{-6} M) and sodium nitroprusside (10^{-10}–10^{-6} M).

All drugs except glyceryl trinitrate were dissolved in distilled water. In a different series of experiments the effect of anhydrous glucose and of propylene glycol (glyceryl trinitrate excipients) were studied and shown to have no effect on the observed responses (data not shown).

Drugs

All drugs were obtained from Sigma (St Louis, MO) except glyceryl trinitrate (Perlinganit, Schwarz Pharma AG).

Data analysis

Data are presented as means ± s.e.m. Sodium nitroprusside-, glyceryl trinitrate- and acetylcholine-induced relaxant responses were calculated as a percentage of the phenylephrine-induced submaximum contraction. EC50 values were calculated individually after non-linear regression of the transformed data of every concentration–response curve and expressed as means ± s.e.m. Statistical significance was examined by Kruskal–Wallis analysis of variance then post hoc Dunn test. A P value of $< 0·05$ was considered as indicative of statistical significance.

Results

Effects of pre-contraction level on acetylcholine- and sodium nitroprusside-induced relaxations

In endothelium-intact aortic rings, the magnitude of phenylephrine pre-contraction had no effect on relaxation induced by acetylcholine (10^{-8}–10^{-6} M) (Fig. 1a). For pre-contraction to 55–60%, 70–75% and 85–90% of maximum contraction, EC50 values were found to be $2·1 \pm 0·5$, $2·4 \pm 0·6$ and $2·7 \pm 0·3 \times 10^{-8}$ M, respectively; these differences were not statistically significant.

Sodium nitroprusside (10^{-10}–10^{-6} M) induced concentration-dependent relaxation of pre-contracted deendothelized aortic rings and neither pre-contraction levels nor EC50 values of relaxant responses were found to be statistically different among the three groups (Fig. 1b).

Time-dependent differences in the sodium nitroprusside-induced relaxations

Addition of sodium nitroprusside (10^{-10}–10^{-6} M) induced concentration-dependent relaxation of endothelium-intact and denuded aortic rings pre-contracted submaximally with phenylephrine. The relaxations were significantly different depending on the time the animals were killed. Statistically significant time-dependent variations at each concentration used were obtained with endothelium-intact preparations. The statistical results are summarized in the legend of Fig. 2a. Sodium nitroprusside induced greater relaxation when the preparations were obtained at 1700 h rather than at other times of the day; relaxation was least at 0500 and 1300 h (Fig. 2a, b). Results obtained at different times of day were plotted as chronograms (Fig. 2b). For the sake of clarity, only two consecutive concentrations were chosen for chronograms from the linear part (20%–80% of maximum) of the concentration–response curves.

With endothelium-denuded preparations, for most of the concentrations used there were statistically significant time-dependent differences (Fig. 3a). With sodium nitroprusside relaxation was greatest at 0900, 1700 and 0100 and least at 1300 (Fig. 3a, b). Chronograms of two consecutive concentrations are seen in Fig. 3b.

EC50 values for sodium nitroprusside were calculated to be highest at 1300 and 0500 h, and lowest at 1700 and 0100 h. The difference between EC50 values obtained from different time series were highly significant for both endothelium-intact

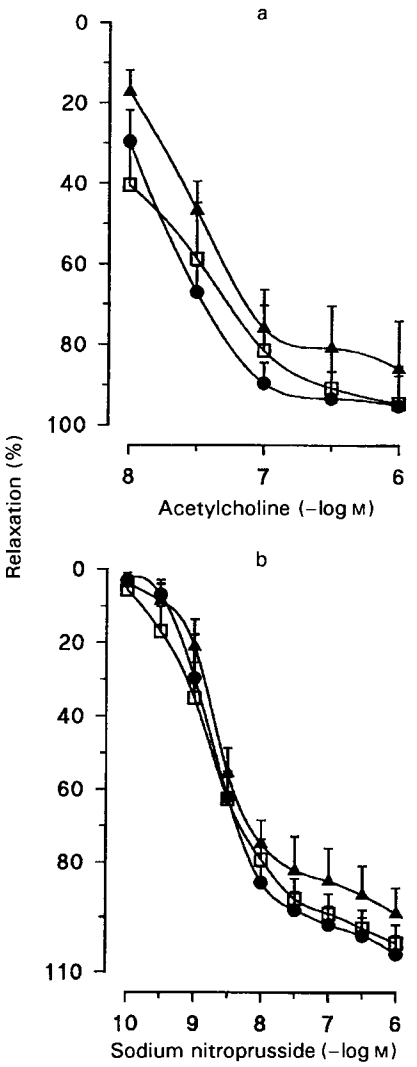

FIG. 1. Percentage relaxation to (a) acetylcholine in endothelium-intact and (b) sodium nitroprusside in endothelium-denuded rat thoracic aorta rings pre-contracted with phenylephrine at 55–60% (●), 70–75% (▲) or 85–90% (□) of maximum contraction. Each point represents the mean of results from 5–6 or 8–9 experiments for acetylcholine or sodium nitroprusside, respectively; vertical bars show s.e.m.

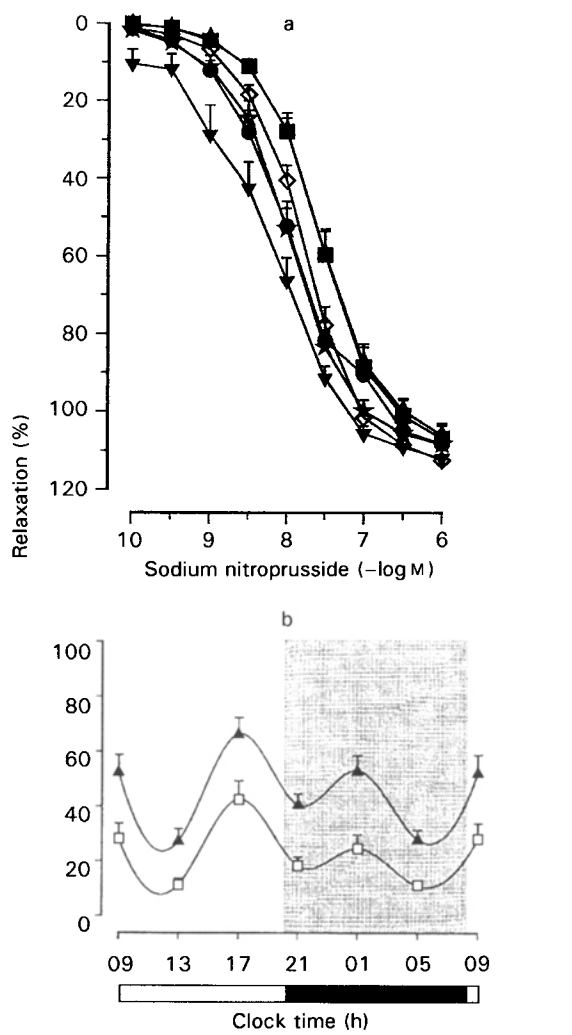

FIG. 2. (a) Relaxation to sodium nitroprusside in endothelium-intact rat thoracic aorta rings obtained at six different times of day. Each point represents the mean of results from 10–14 experiments for each time point; vertical bars show s.e.m. (●) 0900, (△) 1300, (▼) 1700, (◇) 2100, (★) 0100, (■) 0500 h. 1×10^{-10}, 3×10^{-10} M, $P < 0.05$; 1×10^{-9}, 3×10^{-9}, 1×10^{-8}, 3×10^{-8} M, $P < 0.001$; 1×10^{-7} M, $P < 0.01$; 3×10^{-7} M, $P < 0.05$. (b) Significant daily rhythms in the effect of the same concentrations of sodium nitroprusside: (■) 3×10^{-9} M, (▲) 10^{-8} M. Curves are non-linear least-squared fitted sine curves as percentages of the pre-contraction. Shaded area represents dark span.

and endothelium-denuded rings ($P < 0.001$, Table 1). De-endothelization augmented the relaxant responses and reduced the EC50 values at every time point (Table 1).

Time-dependent differences in the glyceryl trinitrate-induced relaxations

Addition of glyceryl trinitrate (10^{-9}–3×10^{-6} M) to sub-maximally pre-contracted aortic rings resulted in concentration-dependent relaxation (Figs 4 and 5). Depending on the time the tissues were obtained, these relaxations were significantly different at lower glyceryl trinitrate concentrations both for endothelium-intact (10^{-9}–3×10^{-8} M) and endothelium-denuded (10^{-8}–3×10^{-7} M) rings. No differences were found at higher concentrations.

Glyceryl trinitrate induced greatest relaxation at 1700 and 0100, and least relaxation at 1300 and 0500, than the other times of day, in both endothelium-intact and endothelium-denuded preparations (Figs 4a and 5a). Chronograms of two consecutive concentrations are seen in Figs 4b and 5b.

EC50 values for glyceryl trinitrate were found to be highest at 1300 and lowest at 0100. There were statistically significant ($P < 0.001$, Table 1) differences between EC50 values for the different time series for both endothelium-intact and endothelium-denuded rings. Chronograms of EC50 values are seen in Fig. 6b.

Discussion

Inorganic nitrates (e.g. sodium nitrite, 1,3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1) and sodium nitroprusside) generate NO non-enzymatically; NO can also be generated

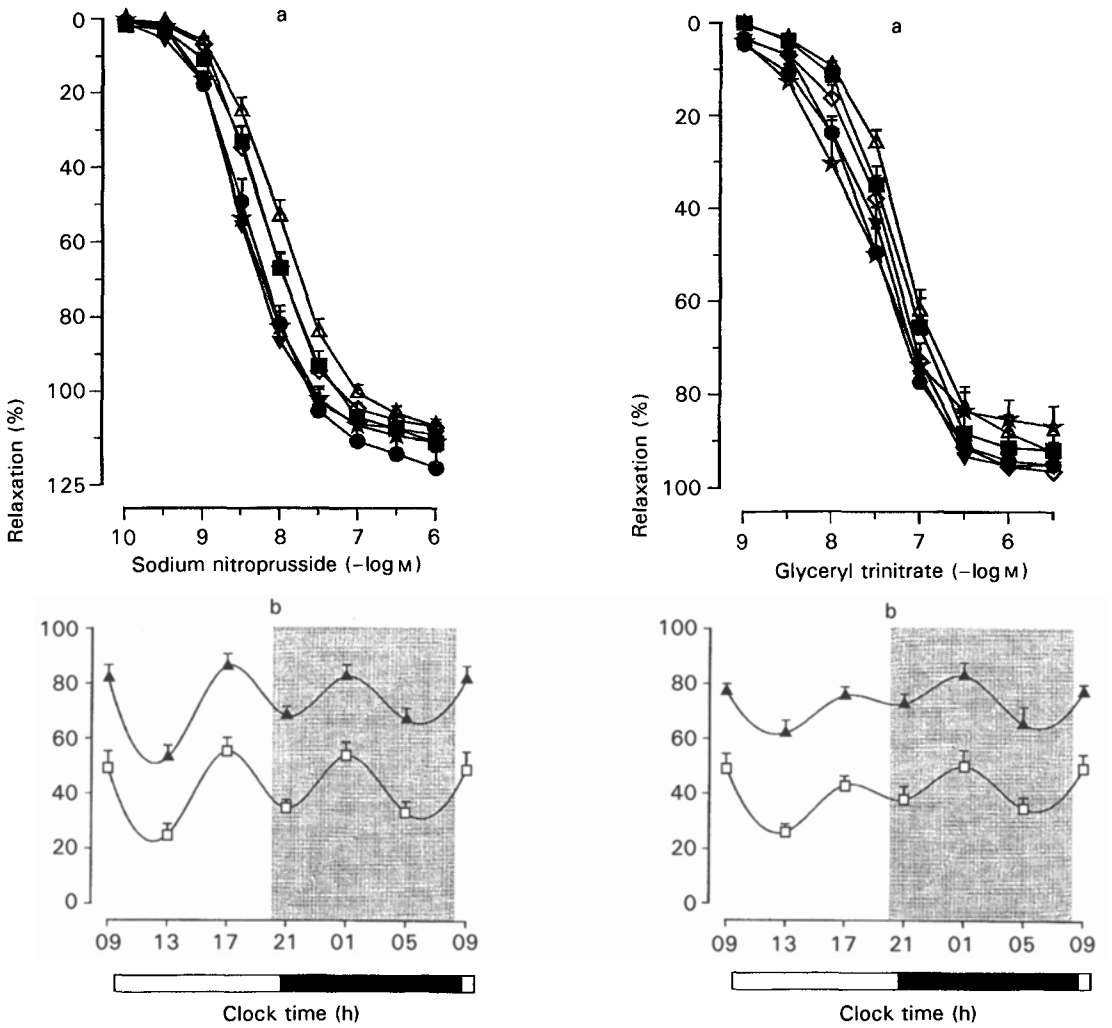

FIG. 3. (a) Relaxation to sodium nitroprusside in endothelium-denuded rat thoracic aorta rings obtained at six different times of day. Each point represents the mean of results from 10–14 experiments for each time point and vertical bars show s.e.m. (●) 0900; (△) 1300, (▼) 1700; (◇) 2100; (★) 0100; (■) 0500 h. 3×10^{-10} M, $P < 0.01$; 1×10^{-9}, 3×10^{-9}, 1×10^{-8}, 3×10^{-8} M, $P < 0.001$. (b) Significant daily rhythms in the effect of the same concentrations of sodium nitroprusside: (□) 3×10^{-9} M, (▲) 10^{-8} M. Curves are non-linear least-squared fitted sine curves as percentages of the pre-contraction. Shaded area represents dark span.

FIG. 4. (a) Relaxation to glyceryl trinitrate in endothelium-intact rat thoracic aorta rings obtained at six different times of day. Each point represents the mean value of 10–14 experiments for each time point and vertical bars show s.e.m. (●) 0900; (△) 1300; (▼) 1700; (◇) 2100; (★) 0100; (■) 0500 h. 1×10^{-9} M, $P < 0.005$; 1×10^{-8} M, $P < 0.001$; 3×10^{-8} M, $P < 0.005$. (b) Significant daily rhythms in the effect of the same concentrations of glyceryl trinitrate: (□) 3×10^{-8} M, (▲) 10^{-7} M. Curves are non-linear least-squared fitted sine curves as percentages of the pre-contraction. Shaded area represents dark span.

Table 1. EC50 values of sodium nitroprusside and glyceryl trinitrate for both endothelium-intact and endothelium-denuded rat aorta at six different times of the day.

Time	Sodium nitroprusside ($\times 10^{-8}$ M)		Glyceryl trinitrate ($\times 10^{-8}$ M)	
(h)	Endothelium-intact	Endothelium-denuded	Endothelium-intact	Endothelium-denuded
0100	1·1 ± 0·2	0·44 ± 0·08	2·7 ± 0·6	4·0 ± 1·3
0500	3·3 ± 0·8	0·76 ± 0·10	5·2 ± 0·7	7·3 ± 1·4
0900	1·3 ± 0·3	0·56 ± 0·10	3·2 ± 0·5	4·5 ± 0·7
1300	3·2 ± 0·5	1·10 ± 0·10	6·1 ± 0·6	8·0 ± 0·9
1700	0·8 ± 0·2	0·38 ± 0·04	3·6 ± 0·4	4·2 ± 0·4
2100	1·8 ± 0·2	0·68 ± 0·07	4·7 ± 0·6	4·9 ± 0·6
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Each value represents the mean ± s.e.m. of results from 10–14 experiments.

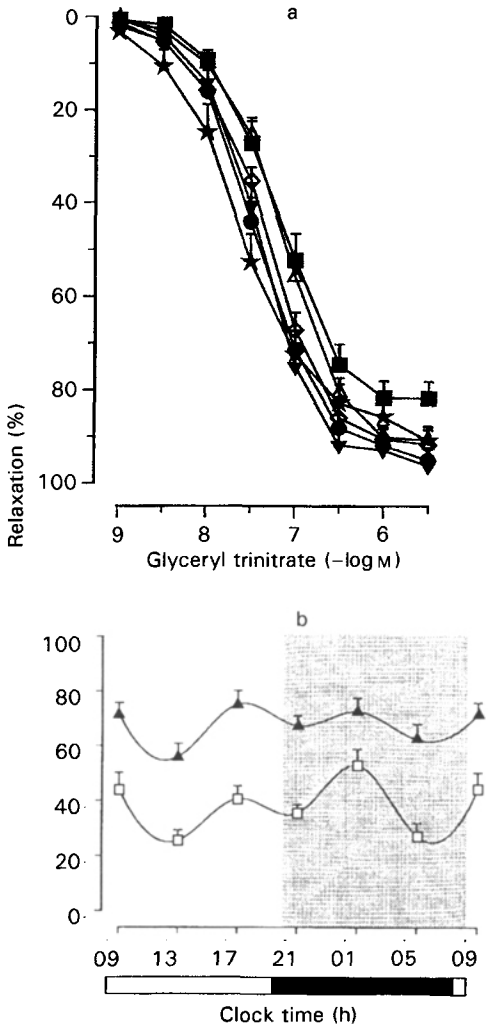

FIG. 5. (a) Relaxation to glyceryl trinitrate in endothelium-denuded rat thoracic aorta rings obtained at six different times of day. Each point represents the mean of results from 10–14 experiments for each time point; vertical bars show s.e.m. (●) 0900; (△) 1300; (▼) 1700; (◇) 2100; (★) 0100; (■) 0500 h. 1×10^{-8} M, $P < 0{\cdot}02$; 3×10^{-8} M, $P < 0{\cdot}002$; 1×10^{-7} M, $P < 0{\cdot}02$; 3×10^{-7} M, $P < 0{\cdot}05$. (b) Significant daily rhythms in the effect of the same concentrations of glyceryl trinitrate: (□) 3×10^{-8} M, (▲) 10^{-7} M. Curves are non-linear least-squared fitted sine curves as percentages of the pre-contraction. Shaded area represents dark span.

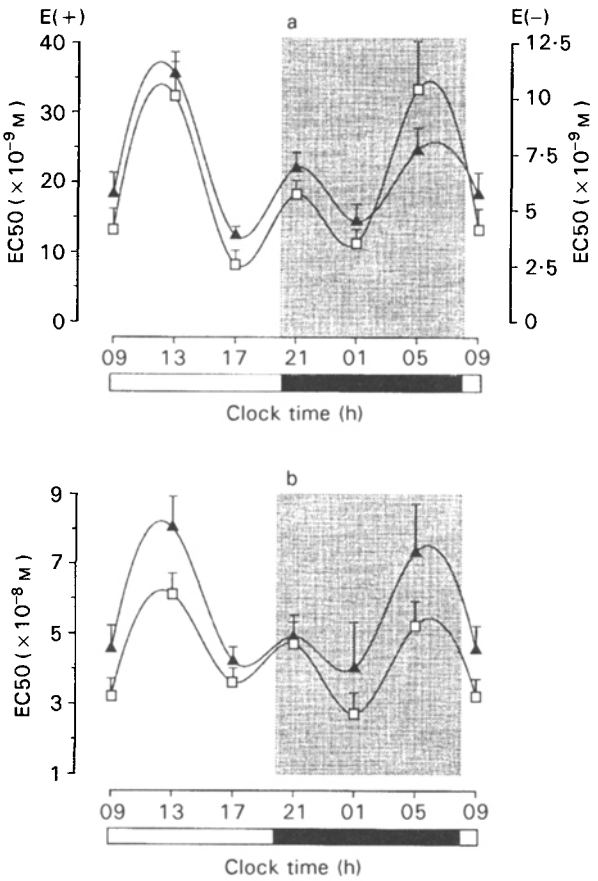

FIG. 6. Significant daily rhythm of EC50 values of sodium nitroprusside and glyceryl trinitrate. (a) Results obtained from endothelium-intact (□) and endothelium-denuded (▲) rings for sodium nitroprusside. The Y-axes were adjusted to show the similarities between ryhthms of endothelium-intact and endothelium-denuded groups for sodium nitroprusside (Left Y-axis for endothelium-intact values (E(+)) and right for endothelium-denuded (E(−)). (b) Results obtained from endothelium-intact (□) and endothelium-denuded (▲) rings for glyceryl trinitrate. Each point represents the mean of results from 10–14 experiments for each time point; vertical bars show s.e.m. Curves are non-linear least-squared fitted sine curves. Shaded area represents dark span.

from organic nitrates in the metabolic activation process in both endothelial and vascular smooth-muscle cells (Ignarro 1989; Salvemini et al 1992, 1993; Bennet et al 1994). Nitrovasodilators and NO elicit vascular relaxation by direct action on the smooth muscle via a mechanism involving increased production of cGMP, resulting from stimulation of soluble guanylate cyclase in the muscle cell (Ignarro 1989).

Circadian rhythms in basal and stimulated cGMP formation have been shown in aortic tissues of both Wistar-Kyoto (Witte et al 1995) and spontaneously hypertensive (Witte & Lemmer 1996) rats. Basal and stimulated cGMP levels peaked in the animals' daily resting period. In the current study we found two peaks in the EC50 values of both sodium nitroprusside and glyceryl trinitrate (Fig. 6), one in the middle of the day and the other in the last part of the night period. Our data are not in accordance with the studies mentioned above (Witte et al 1995; Witte & Lemmer 1996) which revealed only one peak in 24 h. On the other hand, in aortic tissues from Sprague–Dawley and transgenic hypertensive TGR (mRen-2)27 rats there was no significant circadian variation in either basal or nitric oxide-stimulated soluble guanylate cyclase (Witte & Lemmer 1996). To explain this discrepancy in the reported results, it is important to remember that circadian variation of a biological process can be completely different in animals of different strains or ages. It has even been shown that the specific binding of one ligand in a defined region of the rat brain might show circadian rhythm in one Wistar line but not in another (Jenni-Eiermann et al 1986; Witte & Lemmer 1991).

In a study using rat pineal gland, when the activities of cytosolic and particulate forms of guanylate cyclase were determined it was found that cGMP accumulation and guanylate cyclase activity showed 24-h variation with two peaks, one approximately 7 h after lights 'on' and the other approximately 7 h after lights 'off'. The activity of cytosolic

guanylate cyclase remains unchanged in the presence of N^G-L-arginine methyl ester (L-NAME, NO synthase inhibitor),-indicating that 24-h variations in the activity do not reflect changes in the synthesis of the guanylate cyclase stimulator NO (Spessert et al 1992). It has been reported that acute administration of L-NAME increased blood pressure dose-dependently and these effects were more pronounced after injection in the morning than in the evening, leading to an inverse blood pressure profile. These results indicated an involvement of the NO-guanylate cyclase system in circadian blood-pressure regulation (Witte et al 1995). Circadian rhythms in guanylate cyclase activity and cGMP formation were shown in these studies, but the role of NO in these circadian rhythms is still controversial. However, in the current study, responses to sodium nitroprusside and glyceryl trinitrate showed 24-h variations depending on the time the tissues were dissected, and these rhythms and their profiles were not influenced by the presence of endothelium. In this respect, it can be assumed that these temporal rhythms in the effect of nitrodilator agents depend on the 24-h variations in the sensitivity of soluble guanylate cyclase activity. Because guanylate cyclase activity was not measured in this study, the possible effect of other mechanism(s) on these rhythms cannot be discounted. It might be thought that circadian variations in cGMP levels occur as a result of rhythmic changes in its degradation, but recently Faillace et al (1996) have reported significant variations in cGMP content and guanylate cyclase activity through the 24-h cycle in the golden hamster retina whereas phosphodiesterase activity was unchanged. Whether or not there is a circadian pattern in cGMP-phosphodiesterase activity in vascular smooth muscle remains to be clarified.

Several reports have indicated that deendothelization or use of NO synthase inhibitors enhance response to nitrovasodilator agents (i.e. SIN-1, teopranitol, sodium nitroprusside, sodium nitrite) in various vascular smooth muscle preparations including rat aorta (Shirasaki & Su 1985; Pohl & Busse 1987; Lüscher et al 1989; Ralevic et al 1991). Because nitro compounds act by the same mechanism as NO, which is constantly released in basal amounts (Lüscher et al 1989; Ralevic et al 1991), NO might compete with the nitro compounds, thus reducing their vasodilator potency. We have recently reported 24-h variations in response to the endothelium-dependent and endothelium-independent relaxant agents acetylcholine and sodium nitroprusside, respectively, in endothelium-intact rat aorta (Keskil et al 1996). Thus, one might infer that endothelium plays a role on the rhythm of nitrodilator-induced relaxations. In the current study sodium nitroprusside-induced responses in denuded preparations increased at every time point, whereas there were no significant changes in glyceryl trinitrate-induced relaxations. Because these nitrodilator-induced rhythmic relaxations and their profiles were quite similar in both endothelium-intact and endothelium-denuded aortas, it can be concluded that endothelium does not play any role in these 24-h variations.

The chronopharmacology of oral nitrates has been investigated in healthy volunteers in several studies by Lemmer and co-workers. The pharmacokinetic and the haemodynamic effects of isosorbidedinitrate and two formulations (immediate and sustained release) of isosorbide-5-mononitrate were measured simultaneously. It was reported that there were circadian changes both in cardiovascular effects and pharmacokinetic parameters of organic nitrates, and that pharmacokinetic changes were not a prerequisite for exerting temporal variations in drug-induced haemodynamic effects (Blume et al 1986; Lemmer et al 1986, 1989; Scheidel et al 1989). It has also been reported that glyceryl trinitrate was more effective in the morning than in the afternoon in patients suffering from Prinzmetal's angina (Yasue et al 1979).

The mechanism(s) of variations in the in-vitro sensitivity of aortic smooth muscle to various vasoactive agents is speculative at present, though current evidence suggests that circadian changes in enzyme activity and receptor affinity or density (or both) play an important role. It has been reported that in rat brain there are circadian variations in the number of α- and β-adrenergic, muscarinic, dopaminergic, opiate and benzodiazepine receptors (Kafka et al 1983). The adenylate cyclase-cAMP-phosphodiesterase system (Lemmer et al 1987a, b; Prosser & Gillette 1991) and cGMP content and guanylate cyclase activity also showed temporal variation (Spessert et al 1992; Witte et al 1995; Faillace et al 1996). It can also be suggested that the probable time-dependent variations in the endogenous concentrations of a neurotransmitter or a second messenger within the tissue might provoke such up/down regulation of receptors.

Our results clearly show 24-h variations in the in-vitro sensitivity of rat thoracic aorta to nitrovasodilators, the sensitivity depending on the time the tissues were obtained, and that the endothelium does not play any role in these rhythmic circadian variations. The main limitations of using organic nitrates are tolerance development and unwanted side-effects. Although further studies are required to define the underlying mechanism(s) of time-dependent variations in the effect of organic nitrates on the cardiovascular system, these studies might help to elucidate the optimum time(s) and related dosage adjustments to enable better treatment with organic nitrates.

References

Andreotti, F., Davies, G. J., Hackett, D. R., Khan, M. I., DeBart, A. C., Aber, V. R., Maseri, A., Kluft, C. (1988) Major circadian fluctuations in fibrinolytic factors and possible relevance to time of onset of myocardial infarction, sudden cardiac death and stroke. Am. J. Cardiol. 62: 635–637

Bennet, B. M., McDonald, B. J., Nigam, R., Simon, W. C. (1994) Biotransformation of organic nitrates and vascular smooth muscle cell function. Trends. Pharmacol. Sci. 15: 245–249

Blume, H., Scheidel, B., Becker, H. J., Renczes, J., Lemmer, B. (1986) Chronopharmacology of oral isosorbide dinitrate in healthy subjects. Naunyn Schmiedebergs Arch. Pharmacol. 332: R57–R63

Brezinski, D. A., Tofler, G. H., Muller, J. E., Pohjola-Sistonen, S., Willich, S. N., Schafer, A. I., Czeisler, C. A., Williams, G. H. (1988) Morning increase in platelet aggregability: association with assumption of the upright posture. Circulation 78: 35–40

Faillace, M. P., Keller-Sarmiento, M. I., Rosenstein, R. E. (1996) Daily variations in cGMP, guanylate cyclase and phosphodiesterase in the golden hamster retina. Vision Res. 36: 1365–1369

Gallerani, M., Manfredini, R., Ricci, L., Grandi, E., Cappote, R., Calo, G., Pareschi, P. L., Fersini, C. (1992) Sudden death from pulmonary thromboembolism: chronobiological aspects. Eur. Heart J. 13: 661–665

Gallerani, M., Manfredini, R., Ricci, L., Cocurullo, A., Goldoni, C., Bigoni, M., Fersini, C. (1993) Chronobiological aspects of acute cerebrovascular disease. Acta Neurol. Scand. 87: 482–487

Gohar, M., Deleau, P., Atkinson, J., Gargouil, Y. M. (1992) Ultradian variations in sensitivity of rat aorta rings to noradrenaline. Eur. J. Pharmacol. 229: 69–73

Gordon, R. D., Wolfe, L. K., Island, D. P., Liddle, G. W. (1966) A diurnal rhythm of plasma renin activity in man. J. Clin. Invest. 45: 1587–1592

Görgün, C. Z., Aktuna Keskil, Z., Hodoglugil, U., Ercan, Z. S., Abacioglu, N., Zengil, H. (1997) In-vitro evidence on the tissue susceptibility rhythms. The circadian variations in the effect of potassium chloride and phenylephrine in rat aorta. Chronobiol. Int. In press.

Ignarro, L. J. (1989) Endothelium-derived nitric oxide: pharmacology and relationship to actions of organic nitrate esters. Pharm. Res. 6: 651–659

Jenni-Eiermann, S., von Hahn, H. P., Honegger, C. G. (1986) Diurnal rhythms in neurotransmitter receptor binding and CAT activity: different patterns in two rat lines of Wistar origin. Brain Res. 370: 54–60

Kafka, M. S., Wirz-Justice, A., Naber, D., Ioore, R. X., Benedito, M. A. (1983) Circadian rhythms in rat brain neurotransmitter receptors. Fed. Proc. 42: 2796–2801

Keskil, Z., Görgün, C. Z., Hodoglugil, U., Zengil, H. (1996) Twenty-four-hour variations in the sensitivity of rat aorta to vasoactive agents. Chronobiol. Int. 13: 465–475

Lemmer, B:, Becker, H. J., Renczes, J., Scheidel, B., Blume, H. (1986) Circadian-phase-dependency in the pharmacokinetics and cardiovascular effects of oral isosorbide dinitrate in man. Annu. Rev. Chronopharmacology. 3: 339–342

Lemmer, B., Bärmeier, H., Schmidt, S., Lang, P. H. (1987a) On the daily variation in the beta receptor-adenylate cyclase-cAMP-phosphodiesterase system in rat forebrain. Chronobiol. Int. 4: 469–475

Lemmer, B., Lang, P. H., Schmidt, S., Bärmeier, H. (1987b) Evidence for circadian rhythmicity of the β-adrenoceptor-adenylate cyclase-cAMP-phosphodiesterase system in the rat. J. Cardiovasc. Pharmacol. 10 (Suppl 4): 138–140

Lemmer, B., Scheidel, B., Blume, H., Becker, H. J. (1989) Clinical chronopharmacology of oral nitrates. Z. Kardiol. 78 (suppl 2): 61–63

Linsell, C. R., Lightman, S. L., Mullen, P. E., Brown, M. J., Causon, R. C. (1985) Circadian rhythms of epinephrine and norepinephrine in man. J. Clin. Endocrinol. Metab. 60: 1210–1215

Lüscher, T. F., Richard, V., Yang, Z. (1989) Interaction between endothelium-derived nitric oxide and SIN-1 in human and porcine blood vessels. J. Cardiovasc. Pharmacol. 14 (Suppl 11): S70–S80

Manfredini, R., Gallerini, M., Salmi, R., Grandi, E., Fersini, C. (1993) Circadian rhythmicity in the onset of fatal pulmonary embolism in hospitalized subjects. Eur. Med. J. 2: 183–185

Muller, J. E., Stone, P. H., Turi, Z. G., The MILIS Study Group (1985) Circadian variation in the frequency of onset of acute myocardial infarction. N. Engl. J. Med. 313: 1315–1322

Muller, J. E., Ludmer, P. L., Willich, S. N., Tofler, G. H., Aylmer, G., Klangas, I., Stone, P. H. (1987) Circadian variation in the frequency of sudden cardiac death. Circulation 75: 131–138

Nademanee, K., Intarachot, V., Josephson, M. A., Singh, B. N. (1987) Circadian variation in occurrence of transient overt and silent myocardial ischaemia in chronic stable angina and comparison with Prinzmetal angina in man. Am. J. Cardiol. 60: 494–498

Pohl, U., Busse, R. (1987) Endothelium-derived relaxant factor inhibits effects of nitrocompounds in isolated arteries. Am. J. Physiol. 252: H307–H313

Prosser, R. A., Gillette, M. U. (1991) Cyclic changes in cAMP concentration and phosphodiesterase activity in a mammalian circadian clock studied in-vitro. Brain Res. 568: 185–192

Ralevic, V., Mathie, R. T., Alexander, B., Burnstock, G. (1991) N^G-Nitro-L-arginine methyl ester attenuates vasodilator responses to acetylcholine but enhances those to sodium nitroprusside. J. Pharm. Pharmacol. 43: 871–874

Rosing, D. R., Brakman, P., Redwood, D. R., Goldstein, R. E., Beiser, G. D., Astrup, T., Epstein, S. E. (1970) Blood fibrinolytic activity in man: diurnal variation and the response varying intensities of exercise. Circ. Res. 27: 171–184

Salvemini, D., Mollace, V., Pistelli, A., Anggard, E., Vane, J. (1992) Metabolism of glyceryl trinitrate to nitric oxide by endothelial cells and smooth muscle cells and its induction by *E. coli* lipopolysaccharide. Proc. Natl Acad. Sci. USA 89: 982–986

Salvemini, D., Pistelli, A., Vane, J. (1993) Conversion of glyceryl trinitrate to nitric oxide in tolerant and non-tolerant smooth muscle and endothelial cells. Br. J. Pharmacol. 108: 162–169

Scheidel, B., Lenhard, G., Blume, H., Becker, H. J., Lemmer, B. (1989) Chronopharmacology of isosorbide-5-mononitrate (immediate release, retard formulation) in healthy subjects. Eur. J. Clin. Pharmacol. 36 (suppl A): A177–A184

Shirasaki, Y., Su, C. (1985) Endothelium removal augments vasodilation by sodium nitroprusside and sodium nitrite. Eur. J. Pharmacol. 114: 93–96

Spessert, R., Heil, K., Gupta, B. B., Vollrath, L. (1992) Biphasic 24-hour variations in cyclic GMP accumulation in the rat pineal gland are due to corresponding changes in the activity of cytosolic and particulate guanylate cyclase. J. Neurochem. 59: 2081–2086

Tofler, G. H., Brezinski, D. A., Schafer, A. I., Czeisler, C. A., Rutherford, J. D., Willich, S. N., Gleason, R. E., Williams, G. H., Muller, J. E. (1987) Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. N. Engl. J. Med. 316: 1514–1518

Weitzman, E. D., Fukushima, D. B., Nogeire, C., Roffwarg, H., Gallagher, T. F., Hellman, L. (1971) Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. J. Clin. Endocrinol. Metab. 33: 14–22

Witte, K., Lemmer, B. (1991) Rhythms in second messenger mechanism. Pharmacol. Ther. 51: 231–237

Witte, K., Lemmer, B. (1996) Signal transduction in animals models of normotension and hypertension. Ann. NY Acad. Sci., 783: 71–83

Witte, K., Schnecko, A., Zuther, P., Lemmer, B. (1995) Contribution of the nitric oxide guanyl cyclase system to circadian regulation of blood pressure in normotensive Wistar–Kyoto rats. Cardiovasc. Res. 30: 682–688

Yasue, H., Omote, S., Takizawa, A., Nagao, M., Miwa, K., Tanaka, S. (1979) Circadian variation of exercise capacity in patients with Prinzmetal's variant angina: role of exercise-induced coronary arterial spasm. Circulation 59: 938–948