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Selective anti-platelet aggregation synergism between a prostacyclin-mimetic, RS93427 and the nitrodilators sodium nitroprusside and glyceryl trinitrate

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1 Citrated platelet-rich plasma from human donors was used to examine turbidometrically the platelet aggregation response to collagen (2.5 $\mu g\,ml^{-1}$) and ADP (1.6 $\mu g\,ml^{-1}$).

2 With collagen as an aggregating agent, the limited (35% maximal inhibition) inhibitory effects of glyceryl trinitrate (GTN, 0.78–50 $\mu g\,ml^{-1}$) were markedly potentiated by threshold (3.3–10 $ng\,ml^{-1}$) concentrations of RS93427, an orally active prostacyclin-mimetic. Almost complete inhibition of aggregation could then be produced.

3 A threshold concentration of RS93427 (3.3 $ng\,ml^{-1}$) similarly potentiated the ability of sodium nitroprusside (NaNp, 0.78–10 $\mu g\,ml^{-1}$) to inhibit collagen-induced platelet aggregation. There was an 8 fold reduction in the IC_{25} concentration of NaNp.

4 Threshold concentrations of the nitrodilators were also able to potentiate the anti-aggregatory effects of RS93427 (0.03–30 $ng\,ml^{-1}$) on collagen-induced platelet aggregation. With threshold concentrations of either GTN (6.3–25 $\mu g\,ml^{-1}$) or NaNp (0.3–1.3 $\mu g\,ml^{-1}$), the mean IC_{50} concentration of RS93427 was reduced 4 or 6 fold, respectively, while the IC_{25} concentration was reduced 6 or 10 fold, respectively.

5 No similar synergistic interactions were seen between RS93427 and the nitrodilators when ADP was used as an aggregating agent.

6 In spontaneously hypertensive rats, the dose-response for the hypotensive response to bolus doses of RS93427 was not altered by concomitant steady state infusion of a threshold dose (1 $\mu g\,kg^{-1}\,min^{-1}$) of GTN.

7 Possible therapeutic implications of these findings are discussed.

Introduction

The stable, orally active prostacyclin-mimetic, RS93427-017, (hereafter called RS93427) is calcium *bis*-[Z-4-{(3'S,1S,2S,3R,6S)-2-(3'-cyclohexyl-3'-hydroxyprop-1-ynyl)-3-hydroxybicyclo[4.2.0]oct-7-ylidene}]butyrate. It is a potent inhibitor of aggregation of human platelets but is much less active on the platelets of most other species (Willis *et al.*, 1987). It shares with prostacyclin an ability to reduce systemic blood pressure via a marked fall in peripheral resistance (Willis *et al.*, 1987).

The nitrodilators, sodium nitroprusside and glyceryl trinitrate, are thought to act via the intracellular generation of nitric oxide (NO) which then elevates intracellular guanosine 3' : 5'-cyclic monophosphate (cyclic GMP) (see Waldman & Murad, 1987; Vane *et al.*, 1987; Sneddon *et al.*, 1988a). The activity of 'endothelium-derived relaxing factor' (EDRF), a previously uncharacterized unstable vasodilator derived from the vascular endothelium (Furchgott & Zawadski, 1980) has recently been attributed to NO (Ignarro *et al.*, 1987; Kahn & Furchgott, 1987; Palmer *et al.*, 1987).

The present studies were stimulated by a report by Radomski *et al.* (1987a) in which both natural EDRF and NO were found to work synergistically with prostacyclin in inhibiting aggregation of washed human platelets in response to collagen, thrombin, and adenosine diphosphate (ADP). This same group (Radomski *et al.*, 1987b) also found that NO inhibited adhesion of platelets to surfaces,

including collagen fibrils, an effect mediated by increased platelet cyclic GMP and not shared by physiological concentrations of prostacyclin, which only elevated cyclic AMP. Similar findings have been reported by Sneddon *et al.* (1988b) for platelet adhesion to vascular endothelium. Together, these data suggest existence of a homeostatic process by which vascular prostacyclin (PGI_2) and NO, released together by a coupled mechanism, may attenuate localized platelet reactivity (de Nucci *et al.*, 1988).

We now describe a study of mutual anti-aggregatory synergism between RS93427 (an orally active prostacyclin-mimetic) and two nitrodilators, sodium nitroprusside and glyceryl trinitrate. A preliminary account of these findings has been communicated to the British Pharmacological Society (Loveday *et al.*, 1988).

Methods

Platelet aggregation

Human whole blood was collected from drug-free volunteers (21–50 years old) of either sex via a 19 gauge butterfly infusion set into 0.1 vol of 3.8% (w/v) sodium citrate. Platelet-rich plasma (PRP) was then prepared by centrifugation at 750 *g* for 4 min. The remaining blood was centrifuged at 1470 *g* for 6 min to prepare platelet-poor plasma (PPP). Siliconised aggregation cuvettes (7.92 mm in diameter) containing 200 μl PRP (platelet count = 485,000–600,000 μl^{-1}) and 200 μl PPP were prepared; and four microlitres of a stock solution of RS93427 (final concentration: 0.033–30 ng ml^{-1}; 0.095–87 nM) in 0.01 M Tris buffer, pH 8.0, or Tris vehicle alone, was then added. Then 4 μl of GTN stock solution in 70% ethanol, or ethanol vehicle alone, was added to the appropriate cuvettes at a final minimal inhibitory concentration of GTN (see below). Alternatively, 4 μl of a stock solution of NaNp in water, or water vehicle alone, was added at a final minimal inhibitory concentration of NaNp (see below).

In other experiments, minimal inhibitory concentrations (see below) of RS93427 were added to similar cuvettes in which 4 μl of GTN or NaNp stock solution or corresponding vehicle had been added to the plasma-diluted PRP. Final concentrations of GTN were 0.78–50 μg ml^{-1} (7.5–480 μM), and final concentrations of NaNp were 0.08–10 μg ml^{-1} (0.3–38 μM).

The platelets were immediately stirred for 1.5 min (1,000 r.p.m., 37°C) in the light path of a Payton dual-channel aggregation module. Aggregation of the platelets was then induced by adding either collagen (1 μl; final concentration of 2.5 μg ml^{-1}) or ADP (4 μl; final concentration of 1.6 μg ml^{-1}, 3.75 μM). Aggregation of the platelets was recorded as the increase in light transmission for 5 min (with collagen as the aggregation stimulus) or for 3 min (with ADP as the stimulus), during which times full but submaximal aggregation responses were attained. Aggregation measurements were calculated as the percentage inhibition of maximum amplitude of aggregation obtained with control cuvettes to which only the appropriate vehicle solutions had been added.

Determination of threshold concentrations of glyceryl trinitrate and sodium nitroprusside For each aggregation inducer used (collagen or ADP), the minimal inhibitory concentrations of GTN or NaNp were chosen that gave <14% inhibition when added alone (pH 8 Tris buffered vehicle only). These concentrations, determined for each donor prior to their use in conjunction with RS93427, were 6.3–25 μg ml^{-1} (60–240 μM) and 0.78–1.6 μg ml^{-1} (7.5–15.4 μM) for GTN with collagen or ADP as the stimulus, respectively, and 0.3–1.3 μg ml^{-1} (1.1–5.0 μM) and 0.08 μg ml^{-1} (0.3 μM) for NaNp with collagen or ADP as the stimulus, respectively.

Determination of threshold concentrations of RS93427 Similarly, minimal inhibitory concentrations of RS93427 were chosen that gave <14% inhibition when added alone (vehicle only). These concentrations, determined for each donor prior to use in conjunction with nitrodilators, were 3.3–10 ng ml^{-1} (9.5–29 nM) with collagen as inducer and 0.33 ng ml^{-1} (0.95 nM) in all cases with ADP as inducer.

Blood pressure studies

Male spontaneously hypertensive rats (SHR) of 250–350 g were used throughout. The animals were initially anaesthetized with pentobarbitone sodium (70 mg kg^{-1}, i.p.) before surgical preparation. The left femoral artery was cannulated with PE50 polyethlyene tubing for blood pressure measurement, and both femoral veins were similarly cannulated for drug administration. Blood pressure and heart rate were monitored on a Grass polygraph via Statham 23Gb transducers.

Following surgery, anaesthesia was maintained by intravenous infusion of pentobarbitone (0.018 ml min^{-1}) at a rate of 20 mg kg^{-1} h^{-1} and the animal preparation allowed to stabilize for at least 20 min before administration of RS93427 or GTN was started.

Mean blood pressure was calculated as the diastolic blood pressure plus one-third of the pulse pressure. The ED_{25} was defined as the dose that resulted in a lowering of mean blood pressure of

25 mmHg or an increase in heart rate of 25 beats min^{-1}. The values were obtained graphically from the dose-response curves.

Dose-response curves were first obtained for injections of GTN and RS93427 administered as bolus doses in 1 ml kg^{-1} dose volumes, washed in through the catheter with 0.2 ml of 0.9% w/v NaCl solution. Eight rats were treated with cumulative i.v. doses of 0.3, 1, 3, 10 and 30 $\mu g\,kg^{-1}$ of RS93427 at intervals of 15 min (allowing baseline blood pressure to return to normal). Similarly, another 8 rats were injected with GTN at 0.1, 0.3, 1, 3, 10, 100 and 300 $\mu g\,kg^{-1}$. Repeated administration (16 sequential doses) of GTN (3 $\mu g\,kg^{-1}$, i.v.) at intervals of 15 min did not result in a diminished response ($P < 0.05$, $n = 4$).

Using this dose-response information, a threshold dose (1 $\mu g\,kg^{-1}\,min^{-1}$) of GTN was continuously infused in another group and the dose-response curve to ascending bolus doses of RS93427 was again examined; parallel studies were performed in which only the vehicle (0.9% saline) for GTN was infused at 0.018 ml min^{-1}.

Materials

SHR were obtained from Charles River Breeding Laboratories, Kingston, N.Y., U.S.A. Platelet aggregation studies were performed in a dual channel aggregation module (Payton Associates, Buffalo, N.Y., U.S.A.) in which PPP anti-coagulated with sodium citrate (Sigma Chemical Co., St. Louis, MO, U.S.A.) was used. Aggregation was induced by adenosine diphosphate (ADP, Sigma) or collagen (Chrono-par, Chronolog, Havertown, PA, U.S.A.). Tris buffer used for dissolving drug was also obtained from Sigma. NaNp was obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A. and GTN used in platelet aggregation studies was manufactured by Taylor Pharmaceutical Co. (Decatur, IL, U.S.A.) and supplied by Dey Laboratories (Napa, CA, U.S.A.). GTN used in blood pressure studies was obtained from Parke-Davis (Morris Plains, NJ, U.S.A.).

Statistical analysis

All data are presented graphically as means ± standard errors of the mean (s.e.mean). IC_{50} and IC_{25} are defined as the concentration of aggregation inhibitory agent required for 50% inhibition or 25% inhibition, respectively, of aggregation. Two approaches were used to analyse the data: comparison of IC_{50} and IC_{25} values for each pair of aggregation inhibition curves, and concentration-to-concentration comparisons between each pair of aggregation inhibition curves.

Regression curves were fitted from the data of each donor by least squares techniques, plotting percentage inhibition of aggregation versus ln(dose), as the logarithm provided a more consistently linear fit. These individual regression curves were then used to calculate an IC_{25} and IC_{50} value for each donor. Mean $\ln(IC_{25})$ and mean $\ln(IC_{50})$ values were then analyzed by one-way analysis of variance, accounting for subjects (paired t test).

Inhibition of aggregation due to each combination of agents also was compared with the effect of the single agent on a concentration-by-concentration basis by one-way analysis of variance (ANOVA) at each dose, accounting for subjects (paired t test).

For blood pressure studies, a two-way repeated measures analysis of variance was used, followed by pairwise comparison between time points or between treatment groups. The comparisons were adjusted by Fisher's LSD strategy and Dunn's procedure.

In all cases, statistical significance was assumed at $P < 0.05$.

Results

Figure 1a shows that RS93427, at a threshold anti-aggregatory concentration (3.3 $\mu g\,ml^{-1}$, confirmed for each donor) potentiated the ability of NaNp to inhibit aggregation induced by collagen (2.5 $\mu g\,ml^{-1}$). A clear leftward shift in the NaNp dose-response curve was produced (Figure 1a). A statistically significant 8 fold reduction in IC_{25} concentration (from 2.3 ± 1.3 $\mu g\,ml^{-1}$ to 0.3 ± 0.1 $\mu g\,ml^{-1}$) was seen. Significant enhancement of the activity of NaNp by RS93427 was seen at 0.6 and 1.25 $\mu g\,ml^{-1}$ of NaNp (Figure 1a).

By contrast, when ADP (1.6 $\mu g\,ml^{-1}$) was used to induce aggregation (Figure 1b), no such potentiation of the activity of NaNp by the threshold concentration (0.33 ng ml^{-1}) of RS93427 was observed.

Figure 2 shows the effects of similar studies with glyceryl trinitrate (GTN), which had only a weak intrinsic inhibitory effect on platelet aggregation. (The ethanolic vehicle had no effect alone on collagen-induced platelet aggregation: −0.4 ± 2.6% inhibition with 0.7% ethanol.) Again, threshold concentrations of RS93427 (3.3–10 ng ml^{-1}, determined empirically for each donor) markedly potentiated the inhibitory effects of GTN on collagen-induced aggregation, as seen by a clear leftward shift in the GTN dose-response curve (Figure 2a). Indeed, GTN was now rendered capable of producing an 87% maximal inhibition, compared to only 35% in the absence of RS93427. Although it was not possible in all donors to estimate quantitatively the IC_{50} value for GTN in the absence of RS93427 or the IC_{25} value in the presence of RS93427, significant enhancement of the

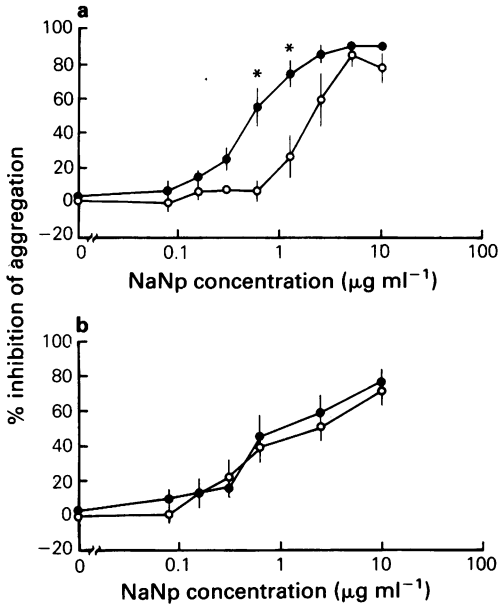

Figure 1 Effects of threshold concentrations of RS93427 on inhibition of human platelet aggregation by ascending concentrations of sodium nitroprusside (NaNp). (a) Effects on aggregation induced by collagen. Human citrated platelet-rich plasma (PRP) was induced to aggregate for 4 min by addition of 1 μl of a microfibrillar suspension of collagen (final concentration of 2.5 μg ml^{-1}). Ascending concentrations of NaNp together with a threshold concentration (3.3 ng ml^{-1}; confirmed empirically for each donor) of RS93427 were added to 400 μl aliquots of plasma-diluted PRP stirred (1,000 r.p.m., 37°C) in the light path of a Payton dual channel aggregation module. (○) NaNp + vehicle ($n = 4$); (●) NaNp + RS93427 ($n = 4$). * $P < 0.05$ vs vehicle; one-way ANOVA at each dose. (b) Effects on aggregation induced by ADP. Conditions were as described in (a), except that 4 μl of ADP (final concentration of 1.6 μg ml^{-1}) was used to induce aggregation and the threshold concentration of RS93427 was 0.33 ng ml^{-1}. (○) NaNp + vehicle ($n = 5$); (●) NaNp + RS93427 ($n = 5$). It can be seen that RS93427 markedly potentiates the effects of NaNp on collagen-induced aggregation, but not upon ADP aggregation.

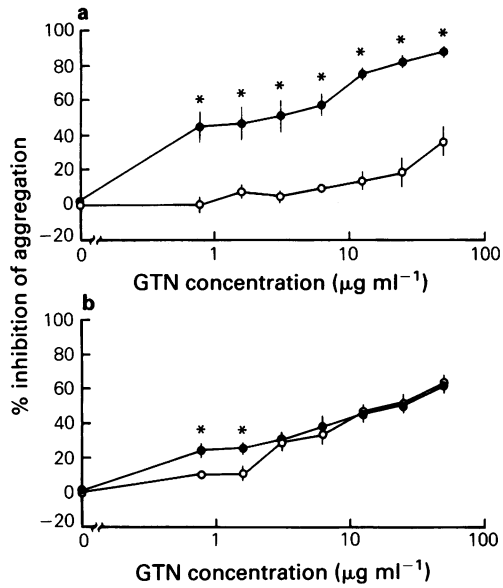

Figure 2 Effects of threshold concentrations of RS93427 on inhibition of human platelet aggregation by ascending concentrations of glyceryl trinitrate (GTN). Conditions are as described in the legend to Figure 1, except that ascending concentrations of GTN were added with a threshold concentration of RS93427. (a) Effects on aggregation induced by collagen suspension (2.5 μg ml^{-1}). Threshold concentrations of RS93427 were 3.3–10 ng ml^{-1}, chosen empirically for each donor. (○) GTN + vehicle ($n = 6$); (●) GTN + RS93427 ($n = 6$). * $P < 0.05$ vs vehicle; one-way ANOVA at each dose. (b) Effects on aggregation induced by ADP (1.6 μg ml^{-1}). The threshold concentration of RS93427, confirmed empirically for each donor, was 0.33 ng ml^{-1}. (○) GTN + vehicle ($n = 5$); (●) GTN + RS93427 ($n = 5$). * $P < 0.05$ vs vehicle; one-way ANOVA at each dose. GTN was able to inhibit only partially aggregation induced by ADP, with an even more limited effect on collagen-induced aggregation. However, in the presence of threshold concentrations of RS93427, a marked inhibition of collagen-induced aggregation was produced; the effects of GTN on ADP-induced aggregation were not similarly potentiated.

activity of GTN by RS93427 was seen at all GTN concentrations from 0.78 to 50 μg ml^{-1} (Figure 2a).

As previously described for NaNp, a threshold concentration (0.33 ng ml^{-1}) of RS93427 had no meaningful effect on inhibition of ADP-induced aggregation by GTN (Figure 2b).

With collagen as the aggregating agent, threshold concentrations of NaNp (0.3–1.3 μg ml^{-1}) and GTN (6.3–25 μg ml^{-1}) also potentiated the ability of RS93427 to inhibit platelet aggregation (Figure 3a and b). The IC_{25} of RS93427 was reduced 10.5 fold (from 6.7 ± 1.2 to 0.6 ± 0.1 ng ml^{-1}) by NaNp and 6 fold (from 4.8 ± 0.7 to 0.8 ± 0.2 ng ml^{-1}) by GTN. There was a similar, but less marked, reduction in IC_{50} by 6 fold (from 13.1 ± 2.8 to 2.1 ± 0.3 ng ml^{-1}) and 3.5 fold (from 9.0 ± 1.2 to 2.6 ± 0.7 ng ml^{-1}) by NaNp and GTN, respectively. NaNp significantly enhanced the activity of RS93427 at concentrations of 3.3 and 10 ng ml^{-1} (Figure 3a). Similarly, GTN significantly enhanced the activity of RS93427 at concentrations of 0.033, 0.33, 3.3 and 10 ng ml^{-1} (Figure 3b).

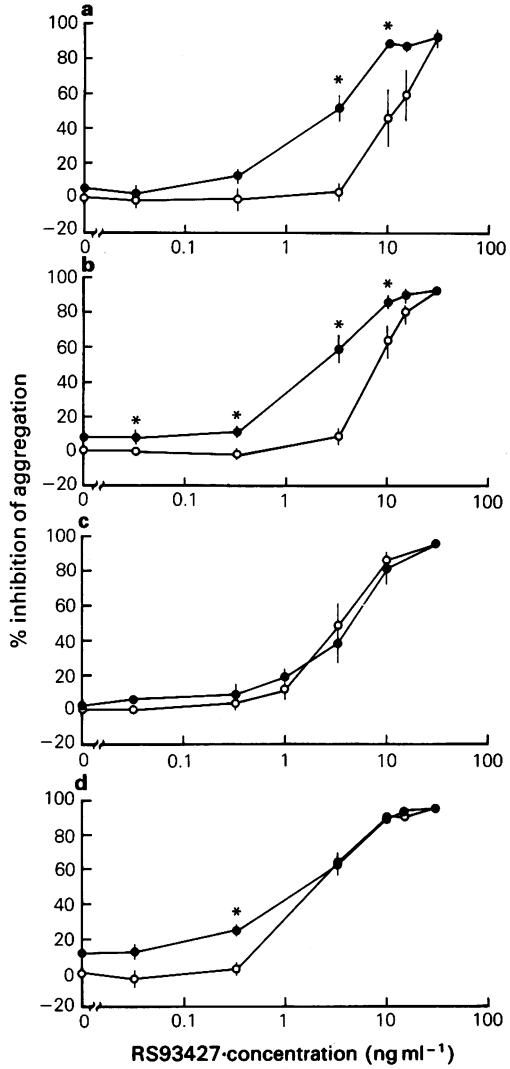

Figure 3 Effects of threshold concentrations of sodium nitroprusside (NaNp) and glycerol trinitrate (GTN) on aggregation response to ascending concentrations of RS93427. Conditions were as described in Figure 1, except that ascending concentrations of RS93427 were examined in the presence of threshold concentrations of NaNp or GTN. (a) Collagen-induced aggregation: marked potentiation by NaNp of the anti-aggregatory effects of RS93427. Threshold concentrations of NaNp were 0.3–1.3 $\mu g\,ml^{-1}$, chosen empirically for each donor. (○) RS93427 + vehicle ($n = 6$); (●) RS93427 + NaNp ($n = 6$). * $P < 0.05$ vs vehicle; one-way ANOVA at each dose. (b) Collagen-induced aggregation: marked potentiation by GTN of the anti-aggregatory effects of RS93427. Threshold concentrations of GTN were 6.3–25 $\mu g\,ml^{-1}$, chosen empirically for each donor. (○) RS93427 + vehicle ($n = 12$); (●) RS93427 + GTN ($n = 12$). * $P < 0.05$ vs vehicle; one-way ANOVA at each dose. (c) ADP-induced aggregation: lack of potentiation by NaNp of the anti-aggregatory effects of RS93427. The threshold concentration of NaNp, confirmed empirically for each donor, was 0.08 $\mu g\,ml^{-1}$. (○) RS93427 + vehicle ($n = 5$); (● RS93427 + NaNp ($n = 5$). (d) ADP-induced aggregation: lack of potentiation by GTN of the anti-aggregatory effects of RS93427. Threshold concentrations of GTN were 0.78–1.56 $\mu g\,ml^{-1}$, chosen empirically for each donor. (○) RS93427 + vehicle ($n = 5$); (●) RS93427 + GTN ($n = 5$). * $P < 0.05$ vs vehicle; one-way ANOVA at each dose.

By contrast, when ADP was used as the aggregating agent, threshold concentrations of neither NaNp (0.08 $\mu g\,ml^{-1}$; Figure 3c) nor GTN (0.78–1.56 $\mu g\,ml^{-1}$; Figure 3d) meaningfully altered the aggregation-inhibitory activity of RS93427.

Results for blood pressure and heart rate are shown in Figure 4. Effects on blood pressure of ascending doses of GTN are shown in Figure 4a ($ED_{25} = 2\,\mu g\,ml^{-1}$, i.v.). It can also be seen that the hypotensive dose-response for RS93427 ($ED_{25} = 4\,\mu g\,ml^{-1}$, i.v.) and the tachycardic dose-response ($ED_{25} = 6\,\mu g\,ml^{-1}$, i.v.) were not altered by infusion of a threshold concentration of GTN ($1\,\mu g\,kg^{-1}\,min^{-1}$) (Figure 4b and c).

Discussion

Our results complement those of Radomski *et al.* (1987a) and of MacDonald *et al.* (1988) who reported upon the synergistic interactions between NO (or EDRF) and PGI_2 in saline suspensions of washed human platelets. These effects were seen with collagen, ADP, platelet activating factor (PAF) and thrombin as aggregating agents. By contrast, we have examined platelets suspended in citrated platelet-rich plasma and have used the stable PGI_2-mimetic RS93427 together with two nitrodilators (NaNp and GTN) that may act via release of endogenous NO. We observed strong synergistic interactions for aggregation induced by collagen but not by ADP.

We have also examined blood pressure interactions in one species (the rat) and failed to show synergistic effects between RS93427 and GTN in the vasculature *in vivo*.

A similar lack of synergistic effects on vascular smooth muscle has been seen *in vitro* with another PGI_2-mimetic (iloprost) examined in combination with NaNp (Antunes *et al.*, 1988; P.S. Lidbury, personal communication). However, the same authors found that the synergistic anti-aggregatory effects of iloprost with NaNp were only weak (about two fold above the expected additive effect), although not spe-

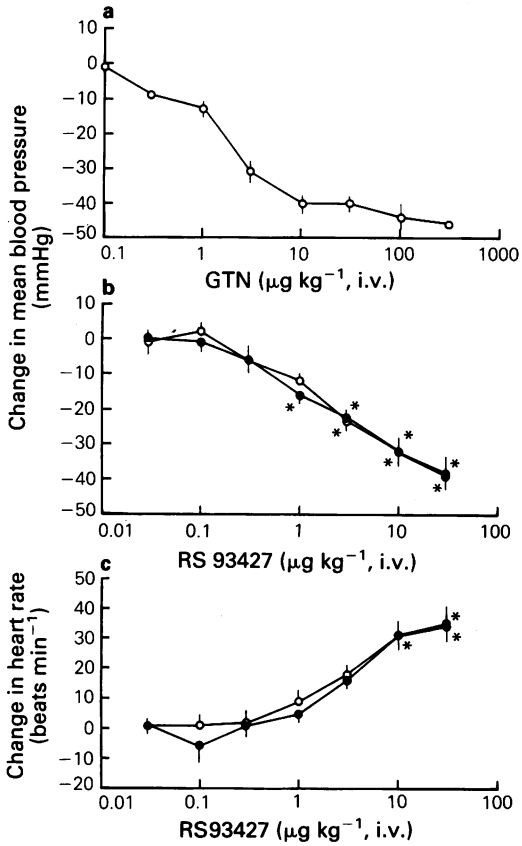

Figure 4 Effects on blood pressure and heart rate of glyceryl trinitrate (GTN) and RS93427. (a) Effect of GTN on blood pressure in anaesthetized spontaneously hypertensive rats. The predose mean blood pressure of the rats was 96 ± 4 mmHg ($n = 3$). (b) Inability of threshold concentrations of GTN to potentiate the hypotensive response to RS93427 in anaesthetized spontaneously hypertensive rats. The baseline blood pressure responses of the groups receiving saline (○) or GTN (○) were 82 ± 3 and 78 ± 8 mmHg, respectively ($n = 8$ each). * $P < 0.05$, compared to pre-dose controls (c) Effect of RS93427 on heart rate in anaesthetized spontaneously hypertensive rats. The baseline heart rate responses of the groups receiving saline (○) or GTN (○) were 264 ± 12 and 274 ± 8 beats min^{-1} ($n = 8$ each). * $P < 0.05$ compared to pre-dose levels. RS93427 was injected in cumulative bolus doses of 0.3 to 140 μg kg^{-1} (1 ml kg^{-1}) during infusions of either saline (0.18 ml min^{-1}) or a threshold dose (1 μg kg^{-1}) of GTN. There were no significant pairwise differences between RS93427 and RS93427 + GTN for either the hypotensive or the tachycardic responses.

cific to the agent used to induce platelet aggregation. These results are reminiscent of earlier findings by Levin *et al.* (1982) who showed some synergism between the anti-aggregatory effects of PGI_2 and NaNp for collagen or adrenaline-induced aggregation examined using platelet-rich plasma.

Differences in prostanoid activity are possibly responsible for these differences between aggregating agents. It is known, for instance, that RS93427 differs from iloprost in having a marked specificity for inhibition of human rather than animal platelets, an activity partially reminiscent of prostaglandin D_2 (Willis *et al.*, 1987 and unpublished data).

Perhaps the interactive effects between RS93427 and the nitrodilators represent a functional synergism only. Thus, RS93427 (via elevation of platelet cyclic AMP; see Mills & MacFarlane, 1977; Moncada & Vane, 1979) prevents the platelet-platelet aggregation process that amplifies the initial stimulus of adhesion of platelets to collagen. This adhesion process would itself be inhibited by GTN and NaNp via prior conversion to NO (Radomski *et al.*, 1987b; Sneddon *et al.*, 1988b) which then elevates platelet cyclic GMP, also resulting in inhibition of platelet aggregation *per se*.

This dual action on adhesion and aggregation is then a likely explanation for the apparent synergistic interactions of RS93427 with the nitrodilators. If this hypothesis were true, then similar synergistic effects should not be seen when platelet aggregation is induced directly without prior adhesion of the platelets to a surface.

Indeed, no synergistic response between RS93427 and the nitrodilators was seen when ADP was used as the aggregating agent. ADP is the archetypal soluble aggregating agent (Born & Cross, 1962). In addition to directly inducing the platelets to stick to one another (i.e., aggregate), it also induces a secondary aggregation response due to release of aggregation-inducing arachidonate metabolites and platelet granular contents (Willis, 1978).

Similarly, in the spontaneously hypertensive rat, no potentiating effects of GTN were seen on the hypotensive response to RS93427 (and its compensatory increase in heart rate). The fall in blood pressure is due to the vasodilator response to relaxation of vascular smooth muscle that can be produced by increases in cyclic AMP or cyclic GMP (Vane *et al.*, 1987). Therefore, by producing the same effect, it is unlikely that RS93427 or the nitrodilators could act synergistically. Correspondingly, the effects of the PGI_2-mimetic iloprost and NaNp seem no more than additive in ability to relax precontracted strips of rabbit aorta (Antunes *et al.*, 1988; P.S. Lidbury, personal communication).

Upon rupture of an atherosclerotic plaque, platelets adhere to exposed collagen and other material and then clump, releasing materials that induce further clumping, coagulation and vasospasm. Such processes are thought to occur in sudden death produced by coronary thrombosis (Wissler, 1984).

Thus, co-administration of RS93427 with a nitrodilator may be an important new therapeutic approach. Vasodepressor effects of the compound might be minimised in comparison with the enhancement of its inhibitory effects on adhesion-stimulated platelet aggregation.

In addition, possible new usefulness of nitrodilator compounds as anti-thrombotic agents is suggested. Daily oral administration of RS93427 in doses insufficient to produce any noticeable effects on platelet aggregation or blood pressure may render a nitrodilator such as GTN able to produce significant anti-thrombotic effects *in vivo* in the coronary circulation, against a background of coronary artery vasodilatation.

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