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Nitroglycerin-inhibited whole blood aggregation is partially mediated by calcitonin gene-related peptide – a neurogenic mechanism

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1 The role of the vasculature and calcitonin gene-related peptide (CGRP) in nitroglycerin (NTG)-mediated platelet inhibition was studied.

2 *In vitro* incubations of CGRP in whole blood induced a dose-dependent inhibition of platelet aggregation with an IC_{50} of 62.1 nM.

3 The platelet inhibition induced by CGRP was blocked by co-incubation of 0.53 μM $CGRP_{8-37}$, as well as 30 μM N^G-nitro-monomethyl-L-arginine (L-NMMA).

4 In a separate group of experiments, 100 nM NTG in rat whole blood (WB) induced platelet inhibition of $6.0 \pm 1.3\%$ (mean $\pm$ s.d.), which was enhanced to $77.6 \pm 3.5\%$ by the addition of rat aortic tissue (AT) ($P < 0.001$). The inclusion of $CGRP_{8-37}$ with NTG and AT in WB reduced platelet inhibition to $31.6 \pm 6.8\%$ ($P < 0.01$). Incubation of WB and AT with 30 μM L-NMMA reduced NTG-induced inhibition of platelet aggregation to $26.4 \pm 4.2\%$ ($P < 0.001$).

5 It is concluded that vascular tissue contributes to the antiplatelet mechanism of action of NTG. Furthermore, NTG apparently evokes the release of CGRP from vascular tissue and this neuropeptide contributes to the antiplatelet actions of NTG.

6 The antiplatelet activity of CGRP in whole blood is mediated primarily through the activation of nitric oxide synthase.

Keywords: Nitroglycerin; neuropeptides; whole blood aggregation; aorta; endothelium-derived relaxing factor (EDRF); CGRP

Introduction

Platelets play a significant role in the aetiology of cardiovascular diseases such as unstable angina pectoris (Shah, 1991), myocardial infarction (Markovitz & Matthews, 1991) congestive heart failure (Mehta & Mehta, 1979) and restenosis of coronary arteries following angioplasty (Violaris *et al*., 1996). Because of their anti-ischaemic effects, nitric oxide donors (NODs), especially nitroglycerin (NTG), have been used to treat these diseases. However, scepticism exists with regard to the clinical usefulness of NTG as an antiplatelet agent (Stamler & Loscalzo, 1991), in part because no demonstrable antiplatelet effect could be discerned for NTG *ex vivo* (Mehta and Mehta, 1980) and because *in vitro* NTG studies of platelet inhibition have revealed a 100–1000 fold discrepancy between its *in vitro* potency and the plasma concentrations of NTG that are achieved therapeutically (Stamler & Loscalzo, 1991; Bauer & Fung 1991).

The lack of *in vivo* effect has been partially accounted for by technical difficulties associated with sample handling (Diodati *et al*., 1990), but the poor potency of NTG *in vitro* remains unexplained. We have hypothesized that the antiplatelet effects of NTG depend upon vascular tissue metabolism to produce sufficient NO to inhibit platelet aggregation. This hypothesis is based on the observation that therapeutic concentrations of NTG are incapable of stimulating elevations of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) in washed platelet preparations as well as in whole blood (Booth *et al*., 1996). This view is supported by recent *in vitro* aggregation studies of NTG, in which platelet inhibition was enhanced when different types of vascular cells were coincubated with this NOD (Benjamin *et al*., 1991; Feelisch *et al*., 1995). However, inhibition with therapeutic NTG concentrations could not be achieved, which suggests that the *in vivo* inhibition of aggregation by NTG may be mediated by other mechanisms that have not been duplicated *in vitro*. One potential mechanism may involve calcitonin gene-related peptide (CGRP), which NTG has been shown to release (Wei *et al*., 1992; Fanciullacci *et al*., 1995). CGRP is a neuropeptide with potent cardiovascular effects (Brain *et al*., 1985), as well as adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-elevating ability in platelets (Kitamura *et al*., 1992). These data have led us to test the second hypothesis that the inhibition of whole blood aggregation by NTG is partly induced by NTG-stimulated neurovascular release of CGRP.

Methods

Surgical procedures

All procedures were performed according to protocols approved by the SUNY Institutional Animal Care and Use Committee. Male Sprague Dawley Rats, 350 g (Harlan), were anaesthetized with thiobutabarbitone, 125 mg kg^{-1}, i.p., and a PE-50 cannula (Intramedic) was surgically implanted in the right femoral artery to facilitate blood sampling. In each experiment, a rat was maintained under anaesthesia for the duration of the experiment. At the end of the experiment, the rat was killed by an overdose of pentobarbitone.

Platelet aggregation

Whole blood (WB) impedance aggregometry was carried out on a Chronolog Model 500 Whole Blood Lumiaggregometer (Chronolog, Havertown, PA). Blood samples were placed in plastic cuvettes and the impedance electrode was inserted. Temperature was maintained at 37°C, and the samples were stirred at 900 r.p.m. The chosen endpoint was maximum ag-

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gregation in Ohms of resistance achieved within 5 min of initiating aggregation. % platelet inhibition was calculated as:

$$(1 - \text{aggregation}_{\text{Drug(s)}}/\text{aggregation}_{\text{Control}}) \times 100\%$$

Effect of CGRP on platelet aggregation in vitro

Blood samples of 590 μl were withdrawn into 10 μl of 3.8% acid-citrate-dextrose (ACD), mixed, and then 300 μl was added to 700 μl of physiological saline at 37°C. A maximum of 8 blood samples were withdrawn at 15–20 min intervals over a 160–180 min period. Platelet counts were monitored periodically with a Model Z_{BI} Coulter Counter (Hialeah, FL), to ensure that circulating platelets remained within the physiological range of the rat ($350-750 \times 10^6$ platelets ml^{-1}). Rat CGRP in concentrations ranging from 25 to 200 nM were added to the whole blood sample (WB) anticoagulated with ACD and incubated for 4 min. Aggregation was then initiated by the addition of 10 μl $CaCl_2$ (500 μM final concentration) and 5 μl adenosine 5′-diphosphate (ADP) (5 μM final concentration).

The mechanism of action of CGRP was studied by using $CGRP_{8-37}$, a competitive CGRP receptor antagonist (Mimeault *et al.*, 1992) and N^G-nitro-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase inhibitor (Radomski *et al.*, 1990a,b). WB samples anticoagulated with ACD were incubated for 3 min with 200 nM human $CGRP_{8-37}$, to block competitively the maximum CGRP concentration added, or 30 μM L-NMMA which has previously been shown to block platelet NOS (Radomski *et al.*, 1990a). Two hundred nanomolar CGRP was then added to the sample and incubated for a further 4 min. Aggregation was then initiated with $CaCl_2$ and ADP as described.

Effect of NTG on platelet aggregation in vitro

In these experiments, one rat was anaesthetized with pentobarbitone (100 mg kg^{-1}, i.p.), exsanguinated, and the aorta was excised and placed in ice-cold buffer consisting of (mM): NaCl 150.0, KCl 5.6, $CaCl_2$ 2.2, $MgCl_2$ 0.5, glucose 5.0 and $NaHCO_3$ 6.0, bovine serum albumin, 0.1%. The aorta was trimmed of extraneous tissue, and cut into 5 mm squares of aortic tissue (AT)

Blood samples of 250 μl were withdrawn from a second rat, which had been anaesthetized and prepared as described, and mixed with 50 μl of heparin-treated physiological saline (10 u ml^{-1}). The sample was then added to 700 μl of physiological saline at 37°C to measure aggregation. A maximum of 10 samples were taken at 25–30 min intervals over a 250–300 min period. Maximum blood loss was 11% of total blood volume (Creskoff *et al.*, 1963). In selected experiments, AT, AT + $CGRP_{8-37}$, or AT + 30 μM L-NMMA (Radomski *et al.*, 1990a) were added to the vials immediately before the addition of the WB. Since the concentration of CGRP that might be involved was unknown, 0.53 μM $CGRP_{8-37}$ was used to ensure complete blockade of the peptide. NTG at final concentrations of 50, 100 or 220 nM was injected as 10 μl aliquots into the reaction vial within 1 min and incubated for 15 min. Aggregation was started with 5 μl ADP (5 μM final concentration).

Drugs

Thiobutabarbitone and pentobarbitone were purchased from Research Biochemicals International (RBI, Natick, MA). Rat CGRP, $hCGRP_{8-37}$, L-NMMA and ADP were bought from the Sigma Chemical Co. (St. Louis, MO) and were dissolved in water. NTG was from Schwarz Pharma (Monheim, Germany).

Statistical analysis

Treatment effects were determined by one-way ANOVA with repeated measures, by use of a Newman Kuels *post-hoc* test for significance on untransformed data. Differences in platelet aggregation between control and $CGRP_{8-37} \pm$AT, or control and L-NMMA ± AT were determined by a two-tailed, paired *t* test. Data are presented as mean ± s.e.mean of 3–6 experiments.

Results

A representative tracing of ADP-induced platelet aggregation in whole blood anticoagulated with ACD is shown in Figure 1. No differences in rate or extent of aggregation were observed between 1 and 4 min control incubations (23.3 ± 1.6 vs 22.9 ± 0.8 Ω, respectively; $n = 11$; $P > 0.05$). CGRP 100 nM induced almost complete inhibition of platelet aggregation. The dose-effect relationship for CGRP-mediated inhibition of platelet aggregation in ACD-anticoagulated WB is shown in Figure 2.

Rat CGRP induced a dose-dependent inhibition of platelet aggregation, with an IC_{50} of 62.1 nM and a maximal effect at 200 nM (Figure 3; $n = 3-6$ experiments for each data point). Pre-incubation of 200 nM $CGRP_{8-37}$ in ACD-anticoagulated WB completely blocked the inhibition of platelet aggregation induced by 200 nM CGRP (Figure 3; $n = 3$; $P < 0.001$). Incubation of 30 μM L-NMMA for 3 min in ACD-anticoagulated WB had no significant effect on platelet aggregation (21.1 ± 1.3 vs 23.1 ± 3.9 Ω for control vs L-NMMA, respectively; $n = 3$; $P > 0.05$). However, L-NMMA did block CGRP-induced inhibition of platelet aggregation significantly (Figure 3; $n = 3$; $P < 0.001$).

A representative trace of the effect of NTG on platelet aggregation in heparin-treated whole blood is shown in Figure 4.

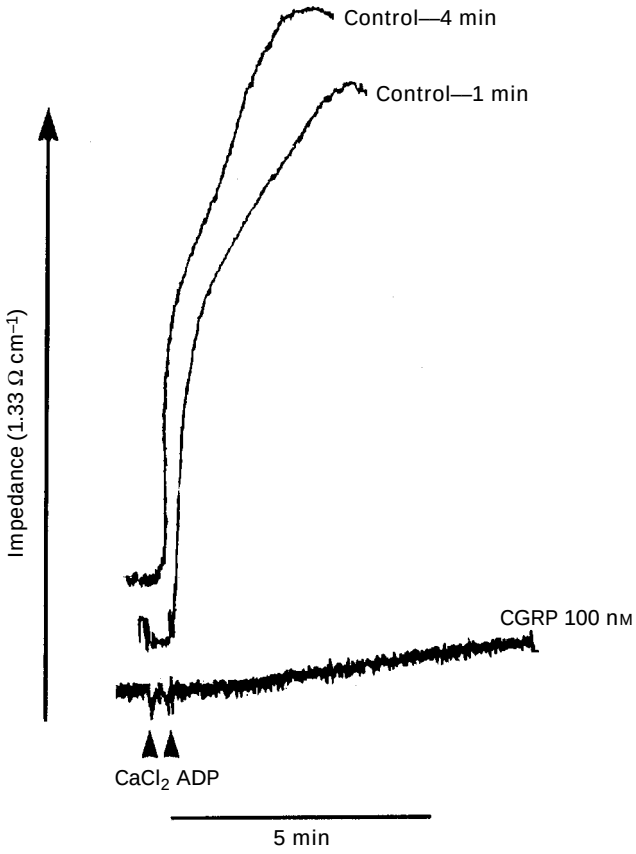

Figure 1 ADP-induced platelet aggregation in the presence and absence of CGRP. No difference was observed between control platelet aggregations incubated for 1 or 4 min, in response to 500 μM $CaCl_2$ and 5 μM ADP in ACD-anticoagulated whole blood. CGRP 100 nM induced almost complete inhibition of platelet aggregation.

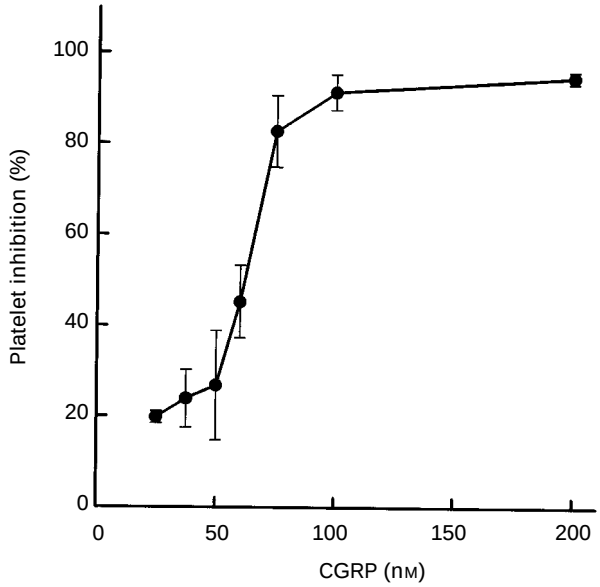

Figure 2 *In vitro* inhibition of platelet aggregation following 4 min incubations of rat CGRP, from 25 to 200 nM, in rat whole blood anticoagulated with ACD. Means are shown ($n=3-6$) and vertical lines indicate s.e.mean.

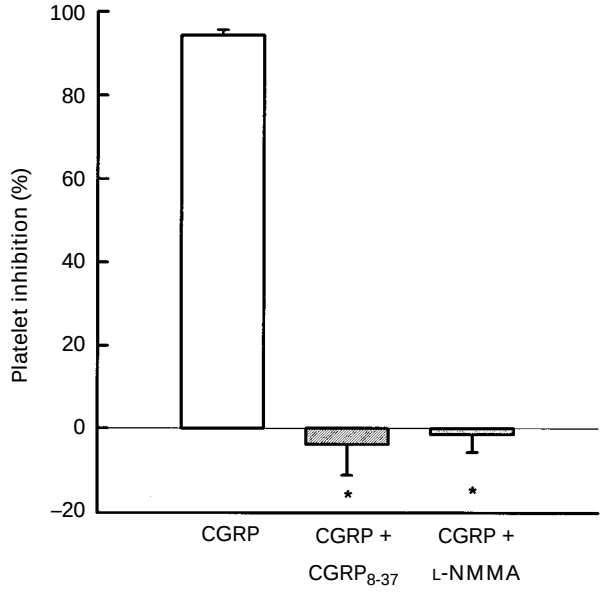

Figure 3 Inhibition of platelet aggregation by 200 nM CGRP in ACD-anticoagulated whole blood ($n=6$) was blocked by 200 nM $CGRP_{8-37}$ or 30 μM L-NMMA. *Indicates a significant difference from CGRP alone ($P<0.001$; $n=3$). Means $\pm$ s.e.mean are shown.

Control incubations of 1 min did not differ from those of 15 min (12.2 ± 0.6 ($n=7$) vs 13.1 ± 0.5 ($n=5$) Ω, respectively; $P>0.05$). NTG 50 nM alone had no discernible effect on platelet aggregation in heparin-treated WB, but addition of AT to the incubation significantly inhibited platelet aggregation. This platelet inhibition was partially reversed by the addition of $CGRP_{8-37}$ to the incubations. The results of these experiments are presented in Figure 5.

NTG alone, in concentrations of 50 to 220 nM, had no significant effect on platelet aggregation in heparin-treated whole blood (Figure 5; $n=4-6$; $P>0.05$). Addition of AT (mean 5.8 ± 0.3 mg per experiment; $n=12$) to the heparin-treated WB incubations had no effect on basal aggregation alone (8.4 ± 0.5 vs 8.7 ± 0.7 Ω, control vs added AT, respectively; $n=3$; $P>0.05$), but it significantly enhanced platelet inhibition by NTG at all concentrations tested ($n=4-6$;

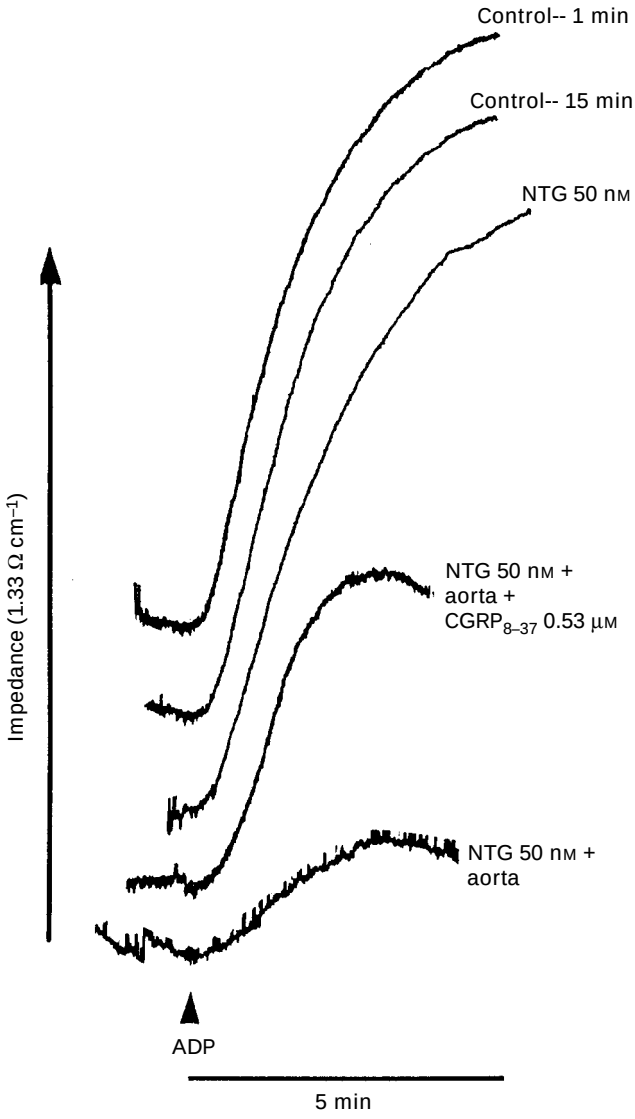

Figure 4 Inhibition of platelet aggregation in heparin-treated whole blood by nitroglycerin (NTG). No difference was observed between control platelet aggregations induced by 5 μM ADP following 1 or 15 min incubations. NTG 50 nM alone had no effect on platelet aggregation. Addition of aorta to the incubation with 50 nM NTG caused significant platelet inhibition which was partially reversed when 0.53 μM $CGRP_{8-37}$ was also included in the incubation.

$P<0.001$). Incubations of $CGRP_{8-37}$ with NTG and AT diminished the inhibition of heparin-treated WB aggregation obtained when compared to preparations tested without the CGRP antagonist ($n=4-6$; $P<0.001$). $CGRP_{8-37}$ was also inactive with regard to control platelet aggregation in heparin-treated WB (12.4 ± 1.2 vs 13.6 ± 3.6 Ω; $n=6$; $P>0.05$), as was $CGRP_{8-37}\pm$AT (9.6 ± 1.4 vs 9.5 ± 2.3 Ω; $n=3$; $P>0.05$).

Inclusion of 30 μM L-NMMA in WB incubations of 220 nM NTG + AT also significantly reversed NTG-inhibited platelet aggregation of heparin-treated WB (Figure 6; $n=3$; $P<0.001$). The effect of L-NMMA + AT on platelet aggregation was not different from the control (8.9 ± 1.0 vs 8.1 ± 1.3 Ω; $n=3$; $P>0.05$).

Discussion

The results of this study reveal two novel findings, namely, the contribution of the vascular tissue to NTG-mediated platelet inhibition and the involvement of CGRP in the antiplatelet actions of NTG. Several *in vitro* studies on the effects of NTG

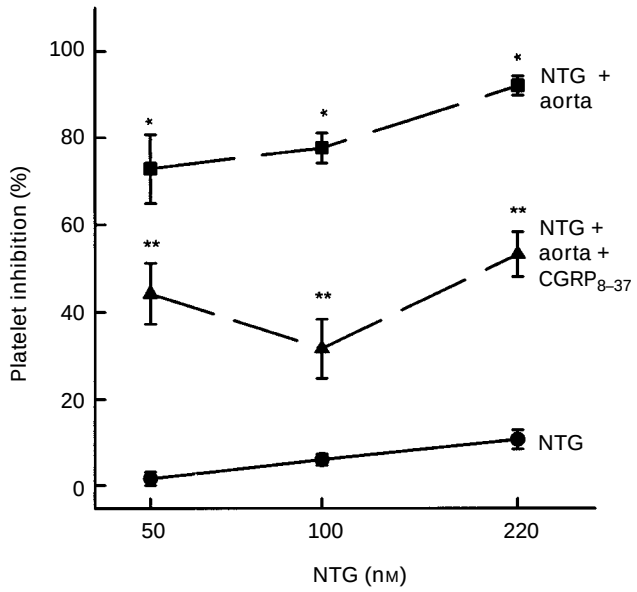

Figure 5 Platelet inhibition following incubation with 50, 100 or 200 nM concentrations of nitroglycerin (NTG) alone, NTG + aortic tissue (aorta), or NTG + aorta + 0.53 μM $CGRP_{8-37}$ in heparin-treated whole blood. *Indicates a significant difference compared to NTG alone, ($P<0.001$); **indicates a significant difference from NTG + aorta, and NTG alone ($P<0.001$; $n=4-6$). Means are shown and vertical lines indicate s.e.mean.

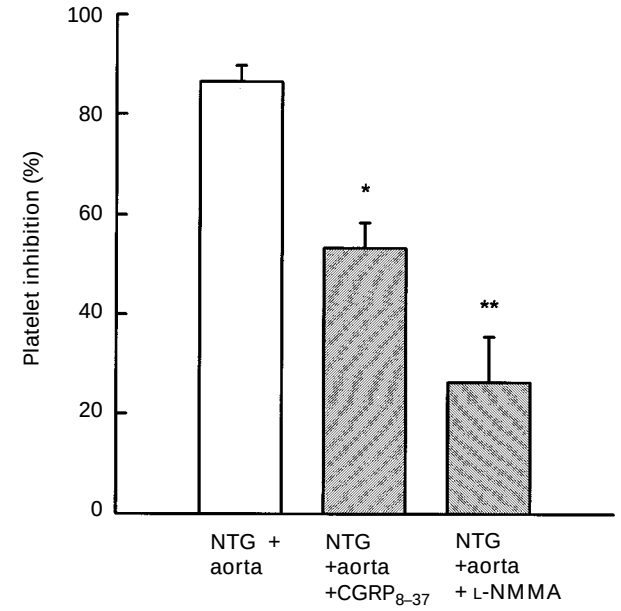

Figure 6 Reversal of platelet inhibition induced by 220 nM NTG + aortic tissue (aorta) by 0.53 μM $CGRP_{8-37}$ or 30 μM L-NMMA. *Indicates a significant difference from NTG + aorta ($P<0.01$; $n=5$). **Indicates a significant difference from NTG + aorta and NTG + aorta + $CGRP_{8-37}$ ($P<0.05$). Means ± s.e.mean are shown.

on platelet aggregation have shown that the minimally effective concentrations of NTG were consistently in the micromolar range (Schafer *et al*., 1980; Ahlner *et al*., 1985; Chirkov *et al*, 1992; Amano *et al*., 1994; Diodati *et al*., 1995). However, the plasma concentrations of NTG and other NODs that are achievable *in vivo* are in the nanomolar range (Armstrong *et al*., 1980; Stamler & Loscalzo, 1991; Bauer & Fung, 1991). The discrepancy between these potencies has cast doubt upon the physiological significance of platelet inhibition by NTG.

We have hypothesized that these results may be partly due to a limitation in the experimental design of these studies. NTG is a prodrug from which NO (or one of its redox forms) is released enzymatically in vascular tissue (Chung & Fung, 1990). The NO generated stimulates the production of cyclic GMP by guanylate cyclase, which leads to smooth muscle relaxation and inhibition of platelet aggregation (Mellion *et al*., 1981; Radomski *et al*., 1990b; Andrews *et al*., 1994). We have previously shown that cyclic GMP was not increased following incubations of whole blood or platelet suspensions with NTG until concentrations exceeded 40 μM (Booth *et al*., 1996), which indicated that the enzymatic capacity of platelets and whole blood to produce NO from NTG was limited. For this reason, it is unlikely that *in vitro* incubations of NTG with various platelet preparations could elicit significant platelet inhibition at therapeutic concentrations. By employing similar *in vitro* experimental conditions, as those used previously, in the present studies, we have observed similar results; i.e. poor antiplatelet potency of NTG. However, additions to the incubations of aortic tissue, which had no effect on platelet aggregation alone, vastly increased the anti-aggregating potency of NTG. These results suggest that the vascular tissue plays an important role in NTG-mediated platelet inhibition, most likely by providing the necessary metabolic activity to produce NO from therapeutic concentrations of NTG.

Experiments conducted by Feelisch *et al*. (1995) and Benjamin *et al*. (1991) support the present findings. In both cases, the inhibitory potency of NTG on platelet aggregation was significantly enhanced by adding vascular endothelial (Feelisch *et al*., 1995) and smooth muscle cells (Benjamin *et al*., 1991) to their *in vitro* preparations. Interestingly, neither group was able to obtain IC_{50}s in the therapeutic range of NTG, despite the addition of isolated vascular cells. This result might have arisen because more vascular tissue was needed, or that both endothelial and smooth muscle cells may be required. However, it is also possible that NTG-derived NO only accounts for a portion of NTG-induced platelet inhibition and that the remainder of this activity is accounted for by NTG-evoked release of CGRP.

The first suggestion that CGRP possessed platelet inhibitory characteristics arose in 1992 (Kitamura *et al*., 1992) when washed platelet suspensions were used as a bioassay to detect CGRP in crude thyroid fractions. The assay was based on the ability of CGRP to stimulate intraplatelet cyclic AMP elevation, which is known as an inhibitory second messenger in platelets (Hourani & Cusack, 1991). Bull *et al*. (1996) have since demonstrated that CGRP is capable of directly inhibiting aggregation of washed platelets *in vitro*, though the intracellular mechanism was not explored. In these *in vitro* experiments, we have determined the dose-response relationship of rat CGRP in whole blood. Inhibition of platelet aggregation was saturable and blocked by co-incubation with the competitive antagonist, $CGRP_{8-37}$ (Mimeault *et al*., 1992), which is indicative of receptor-mediated events. These data are consistent with previous studies that have shown CGRP to interact with a surface receptor, which is linked to adenylate cyclase by a G-protein, leading to intracellular elevations of cyclic AMP (Poyner, 1992). CGRP-dependent cyclic AMP increases can lead to vasodilatation by several different mechanisms, depending on the vascular bed. In cerebral arteries, the cyclic AMP produced by CGRP induced vasodilatation directly (Jansen *et al*., 1991), while in mesenteric arteries, an ATP-sensitive K^+ channel is activated resulting in hyperpolarization of the tissue (Nelson *et al*., 1990). It has also been demonstrated that CGRP induces endothelium-dependent vasodilatation in aorta, where it stimulates NOS activity to generate NO and subsequently cyclic GMP (Gray & Marshall, 1992a,b).

In these experiments, we showed that CGRP-induced inhibition of platelet aggregation was completely blocked by L-NMMA, which indicates that the most significant mechanism of platelet inhibition by CGRP in whole blood is NOS stimulation. NOS III (endothelial NOS) has been demonstrated

in platelets (Muruganadam & Mutus, 1994; Mehta *et al*, 1995) and these cells have been shown to produce cyclic AMP in response to CGRP (Kitamura *et al*., 1992), suggesting the existence of CGRP receptors on platelets. However, since incubations of CGRP were conducted in whole blood in these experiments, it is presently unknown whether the NOS activation leading to platelet inhibition occurred within platelets, or other blood cells, including lymphocytes and leukocytes, which are known to contain NOS (Miles *et al*., 1995; Reiling *et al*., 1996; Evans *et al*., 1996) and receptors for CGRP (McGillis *et al*., 1991; Richter *et al*., 1992).

Measurable inhibition of platelet aggregation by CGRP was observed from 25–200 nM and the IC_{50} was determined to be 62 nM, which is similar to the *in vivo* ED_{50} obtained for the reduction of mean arterial pressure in normotensive rats by CGRP (Marshall 1991). This observation suggests that both vasodilator and platelet inhibition activities may occur concurrently *in vivo* and that CGRP may play a role as a constitutive mediator of blood pressure and haemostasis control. This concept is supported by Lippe *et al*. (1993), who demonstrated that capsaicin treatment decreased bleeding time and increased aggregation in platelets from mesenteric arteries.

CGRP has been localized in peptidergic nerves around blood vessels (Mulderry *et al*., 1985) and its release has been shown to be a NO-dependent process (Hughes & Brain, 1994). Furthermore, Fanciullaci *et al*. (1995) have demonstrated that NTG administration stimulated significant increases in the cerebral plasma concentrations of CGRP, which implies that this NOD is capable of eliciting the release of this neuropeptide. Additionally, it has been determined that CGRP contributes to NTG-mediated vasodilatation of cerebral arterioles (Wei *et al*., 1992). We therefore hypothesized that NTG could evoke the release of CGRP in the peripheral circulation and that CGRP mediated a portion of the antiplatelet activity of NTG.

These hypotheses were tested by using $CGRP_{8-37}$, a specific, competitive inhibitor of CGRP-mediated activity, which possesses no apparent intrinsic activity itself (Mimeault *et al*., 1992). It has been shown in these studies that $CGRP_{8-37}$ is also an inactive, specific blocker of CGRP-inhibited platelet aggregation. Moreover, this CGRP antagonist blocked a significant amount of NTG-induced platelet inhibition in the presence of aortic tissue. Significant blockade was observed at the lowest concentration of NTG tested, namely 50 nM, which is a concentration that can be attained with current regimens of *in vivo* nitrate therapy. These observations suggest that NTG evokes the release of CGRP from the vasculature. This neuropeptide, in turn, mediates a portion of the anti-platelet effects of NTG *in vivo*.

Co-incubation of the NOS inhibitor, L-NMMA with NTG and aortic tissue also reduced the anti-platelet activity of NTG. This result is expected since we have shown previously (Figure 3) that the anti-platelet activity of CGRP itself is inhibited by L-NMMA. Thus, although NTG can release CGRP in the presence of innervated aortic tissue, the anti-platelet action of CGRP cannot be carried out when the NOS system is blocked by L-NMMA.

Interestingly, the extent of L-NMMA blockade appeared to be greater than that of $CGRP_{8-37}$ (Figure 6). Our control experiments indicated that L-NMMA by itself had no discernible effect on platelet aggregation, with or without aortic tissue, which suggests that the basal activity of platelet NOS was very low in this preparation. Radomski *et al*. (1990b) have previously demonstrated that one-third of the L-NMMA concentrations we used could significantly attenuate platelet NOS and enhance platelet aggregation. Since we used excess concentrations of both L-NMMA and $CGRP_{8-37}$, it may be assumed that both inhibitors have exerted their maximal effects. Thus, if the stimulation of NOS was mediated exclusively through the action of CGRP, then the extent of inhibition with L-NMMA would be expected to be similar to that of $CGRP_{8-37}$.

A possible explanation for this observation is that NTG may release other activators of NOS besides CGRP. It is well recognized that other neuropeptides, such as substance P and vasoactive intestinal peptide (VIP) are co-localized with CGRP (Sasaki *et al*., 1984; Gulbenkian *et al*., 1986). The release of VIP has also been shown to be mediated by NO (Allescher *et al*., 1996) and substance P has been shown to stimulate endothelial NOS in coronary endothelial cells (Kuroiwa *et al*., 1995). Thus, it is possible that the anti-platelet action of NTG is mediated additionally by other neuropeptides besides CGRP, although the action of CGRP appears to be predominant.

Finally, one point regarding the experimental procedures requires clarification. Preliminary studies on the inhibitory dose-effect relationship of platelet aggregation by CGRP revealed an interference by heparin, which occurred in an unpredictable fashion. This interference appeared to result from heparin binding (Salmivirta *et al*., 1996) to CGRP, and varied from one batch of heparin to the next. This necessitated the use of ACD as an anticoagulant in these experiments. In the experiments with NTG and aorta, heparin did not appear to affect the activity of CGRP, presumably as a result of greater heparin-protein binding to aortic proteins. Regardless of the anticoagulant used, these experiments demonstrated the same phenomenon: CGRP dose-dependently inhibits platelet aggregation in whole blood and this inhibition was blocked by $CGRP_{8-37}$ and L-NMMA.

In summary, we have demonstrated that the *in vitro* platelet inhibitory activity of NTG can be enhanced substantially by co-incubation with intact vascular tissue. The vasculature appears to enhance the antiplatelet activity of NTG by two mechanisms. Firstly, vascular tissue acts as an important site for the metabolism of NTG to produce NO. Secondly, NTG elicits the release of CGRP, possibly as a result of NO formation, presumably from perivascular nerves. The released CGRP then stimulates NOS and the EDNO produced contributes to NTG-mediated platelet inhibition.

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