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# Effects of hypoxia on the vasodilator activity of nifedipine and evidence of secondary pharmacological properties

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**Abstract**

The effects of hypoxia on the vasodilator response of endothelium-denuded rat aortic rings to the calcium channel blocker, nifedipine, were examined. Under normoxic conditions, nifedipine ($10^{-8}$–$3\times10^{-6}$ M) attenuated the contractility of noradrenaline precontracted rings in a concentration-dependent manner, although the sensitivity was less than what occurs with $K^+$ precontracted tissues. Under hypoxic conditions there was no relaxation by nifedipine. When a concentration–response curve to noradrenaline was constructed before and in the presence of a high concentration of nifedipine ($10^{-5}$ M), the response to noradrenaline was unaffected in both normoxic and hypoxic conditions. When noradrenaline was replaced by phenylephrine ($10^{-8}$–$10^{-5}$ M), the maximum tension was reduced in the presence of nifedipine to 59±6% of the pre-nifedipine value. Repetition of the experiment in the presence of cocaine ($10^{-5}$ M) revealed the inhibitory effect of nifedipine on noradrenaline-induced contraction, the maximum contraction in the presence of nifedipine falling significantly ($P<0.005$) to 67±6% of the pre-nifedipine response. When propranolol ($10^{-7}$ M) was present in the bath, the maximum contraction to noradrenaline was significantly ($P<0.05$) reduced by nifedipine to 55±4% of its previous value. The fact that nifedipine was able to inhibit phenylephrine-induced contractions and relax noradrenaline-precontracted aortic rings confirms its calcium channel blocking activity. The failure to inhibit noradrenaline when added prior to the noradrenaline-induced contractions suggests an opposing effect in addition to calcium channel blockade, which cancels out the attenuation of noradrenaline — but not phenylephrine-induced contractions. When neuronal uptake of noradrenaline was blocked with cocaine or β-adrenoceptors were blocked with propranolol, the inhibitory effect of nifedipine against noradrenaline-induced contractions was revealed. This suggests that the additional property was due to blockade of neuronal reuptake or antagonism at β-adrenoceptors. This study also showed that nifedipine is ineffective as a vasodilator in the rat aorta under hypoxic conditions.


*Keywords:* Aorta; Nifedipine; Hypoxia; Noradrenaline; Phenylephrine

## 1. Introduction

Nifedipine is the prototype 1,4-dihydropyridine calcium channel blocker. By allosteric interference with the gating mechanism of L-type voltage activated calcium channels in smooth muscle, these drugs prevent the influx of extracellular calcium required to activate the contractile machinery of the cell (Godfraind, 1994; McDonald et al., 1994). Nifedipine exerts its clinical effects due to vasodilatation of arterial smooth muscle, leading to reduced peripheral resistance and improved coronary flow. It has little effect on cardiac tissue. Nifedipine is indicated for the prophylaxis of angina pectoris and in peripheral circulatory disorders such as Raynaud's syndrome (Godfraind, 1994).

Catecholamines such as noradrenaline and phenylephrine exert their contractile effects by their action on α-adrenoceptors. In the rat aorta, $\alpha_1$-adrenoreceptors are predominant (Timmermans and Thoolen, 1987), specifically the $\alpha_{1D}$ subtype (Lyles et al., 1998). When activated, these receptors initiate a classic PLC-$IP_3$ mechanism leading to the release of calcium from stores in the sarcoplasmic reticulum (Hoffman and Taylor, 2001). It is likely that this causes a depolarisation of the cell membrane sufficient to activate voltage operated calcium channels (VOCCs) allowing calcium influx (Timmermans and Thoolen, 1987; Chen and Suzuki, 1989; Morel and Godfraind, 1991; Orallo, 1996; Gibson et al., 1998; Lyles et al., 1998; Hoffman and Taylor, 2001; McFadzean and Gibson, 2002; Ghisdal et al., 2003).

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The stimulation of $\alpha_1$-adrenoceptors by noradrenaline (Godfraind, 1994) and phenylephrine (Delaflotte et al., 1989) therefore causes a biphasic contraction, comprising an initial fast component consistent with intracellular calcium release and a sustained tonic component when calcium enters the cell. Hence it is this tonic phase which is susceptible to perturbation by calcium channel blockers (Godfraind, 1976, 1988, 1994; Van Meel et al., 1981; Godfraind et al., 1982, 1986; Koch et al., 1990).

Some authorities have suggested a role for dihydropyridines in the acute treatment of ischaemic stroke. It has been demonstrated that the restoration of circulation to ischaemic areas is extremely important in minimising long term damage to areas of brain tissue and nifedipine may be expected to improve blood flow by its vasodilator properties. Such an action, however, would depend on nifedipine being effective under the hypoxic conditions prevailing in the circulation of the brain of the stroke victim (Kobayashi and Mori, 1998). Similarly nifedipine has been implicated for acute treatment in a number of other conditions involving impaired blood flow, these include peripheral vascular disease, intermittent claudication and the prevention of neurological damage during cardiac arrest (Triggle, 1997).

Several factors, however, limit the efficiency of calcium antagonists in increasing blood flow to ischaemic regions. For example, the hypotension caused by calcium channel antagonists may worsen the blood supply to the ischaemic area. Also, the blood vessels in the ischaemic region may already be dilated to their physiological maximum, hence the calcium antagonist dilates the blood vessels in other areas and the drug 'steals' blood flow to non-ischaemic regions.

The effectiveness of these proposed uses of calcium antagonists depends on the ability of these compounds to function under hypoxic or ischaemic conditions and surprisingly, there is very little published work on this topic. The evidence available is also conflicting. Herrera and Walker (1998) showed that in rat thoracic aortic rings contracted with phenylephrine or potassium, hypoxia causes relaxation, which is attributed to the blockade of $Ca^{2+}$ channels because it is attenuated by the $Ca^{2+}$ antagonist, nifedipine. Other evidence however shows that hypoxia causes $Ca^{2+}$ influx and overload via L-type $Ca^{2+}$ channels (Dixon et al., 1987). We have previously shown that the vasodilator response to adenosine is unaffected by hypoxia (Broadley and Maddock, 1996) and therefore it could not be predicted what effect hypoxia has on the actions of calcium channel antagonists.

The aim of this investigation was therefore to examine the effects of hypoxia on the vasodilator activity of nifedipine, with a view to assessing the likely usefulness of $Ca^{2+}$ antagonists in ischaemic stroke. These experiments yielded unexpected results which led us to conduct experiments to identify secondary pharmacological properties of nifedipine.

## 2. Materials and methods

### 2.1. Drugs

The following drugs were supplied by Sigma (Poole, Dorset, U.K.): cocaine hydrochloride, nifedipine, (−)-noradrenaline bitartrate and phenylephrine hydrochloride. Ascorbic acid (10 μM) (B.D.H., Poole, Dorset, U.K.) was added to solutions to prevent oxidation of noradrenaline. (±)-Propranolol hydrochloride (AstraZeneca, Macclesfield, UK) was supplied as Inderal® injection (1 mg/ml).

All drugs were dissolved in double-distilled water with the exception of nifedipine which was dissolved in acetone (0.69 mg/ml) purchased from Fisons Scientific Equipment (Loughborough, U.K.) to form a stock solution. This was then diluted further using double-distilled water.

### 2.2. Tissue preparation

Animals were maintained in accordance with the Animals (Scientific Procedures) Act 1986. Male Wistar rats (250–350 g) were killed by a blow to the head and the thoracic aorta was removed. The aorta was gently rotated on a needle in order to denude the tissue of endothelium. Connective tissue was also gently removed. Sections of between 5 and 9 mm in length were set-up as ring preparations by suspending between two stainless steel hooks. The preparations were placed in a 20 ml organ bath at 37 °C in Kreb's solution (composition in mM: NaCl 118.4; $MgSO_4$ 1.2; KCl 4.7; $CaCl_2 \cdot 6H_2O$ 2.5; $KH_2PO_4$ 1.2; $NaHCO_3$ 25.0 and glucose 11.7) and gassed with either 5% $CO_2$ in $O_2$ to simulate normoxic conditions or 5% $CO_2$ in $N_2$ to simulate hypoxia.

### 2.3. Measurement of contractile responses

The contractile tension of the tissue was measured via a Pioden dynamometer UF1 isometric transducer (range, ±55 g). The signal was amplified using Grass model 79D EEG polygraph data recording system (Grass instrument Co. Quincy, Mass., U.S.A.) / Maclab bridge amplifier and was converted from analogue to digital data and passed to a computer (Hardware: Powerlab 200 ADInstruments. Software: Chart v.4.1.1 sampling frequency 4 Hz). The apparatus was adjusted so that there was an initial resting tension of 1 g (±0.3 g). All preparations were left to equilibrate for 45 min and washed once with fresh Krebs' before any drugs were added to the bath.

### 2.4. Experimental protocols

#### 2.4.1. Effects of nifedipine on noradrenaline precontracted aorta under hypoxic and normoxic conditions

Two tissues were taken from each animal and set-up in separate organ baths. After initial equilibration, both were subjected to a concentration–response curve to noradrenaline to confirm the viability of the preparation and to ensure a submaximal concentration of noradrenaline was used. The baths were then washed out and one bath was switched to hypoxic conditions and allowed to reach a stable baseline. Noradrenaline was then added to each bath to produce a concentration of $3 \times 10^{-8}$ M and the tissues were left until the contractions had reached a stable plateau. A cumulative concentration–response curve to nifedipine was then obtained, commencing at $10^{-8}$ M and increasing the concentration in half-log intervals to a maximum of $3 \times 10^{-6}$ M. Due to nifedipine

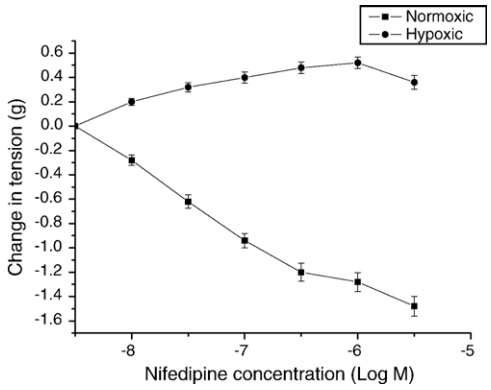


Fig. 1. Concentration–response curves for the relaxation of rat aortic rings to nifedipine in tissues precontracted with noradrenaline ($3\times10^{-8}$ M) under normoxic (■) or hypoxic (●) conditions. Mean responses (±S.E.M.) ($n=5$) are plotted as the change in tension from the pre-nifedipine tension induced by noradrenaline.

being liable to inactivation by photo-degradation, the organ baths and drug bottles in which the nifedipine solutions were prepared were wrapped in tin foil.

*2.4.2. Effects of nifedipine on noradrenaline and phenylephrine concentration–response curves*

Two preparations were taken from each animal and both were exposed to a cumulative concentration–response curve to noradrenaline which was initially added to the bath at a concentration of $10^{-9}$ M and then increased in half-log intervals to a maximum of $3\times10^{-5}$ M. The baths were then washed out three times. After the tension had returned to a stable baseline, $10^{-5}$ M nifedipine was added to the Krebs solution in one of the organ baths. In order to carry out a vehicle control, the same volume of acetone (100 μl) in which the nifedipine was dissolved was added to the other bath. The tissues were incubated in either nifedipine ($10^{-5}$ M) or acetone (100 μl) for ninety min. During the incubation period, both baths were drained and filled up with a new solution of Krebs and either nifedipine or acetone twice, at thirty and sixty min into the incubation time, this was in order to compensate for degradation of the drug over time. After the incubation, the concentration–response curve to noradrenaline was repeated in both baths in the presence of nifedipine or acetone. The procedure was then repeated using phenylephrine ($10^{-8}$–$3\times10^{-5}$ M) in place of noradrenaline.

The protocol involving two noradrenaline concentration–response curves obtained ninety min apart with incubation with either nifedipine or vehicle was repeated in the presence of the neuronal reuptake blocker, cocaine ($10^{-5}$ M) added 30 min prior to the first concentration–response curve and throughout the experiment, being replaced after each wash-out. The procedure was also repeated in the presence throughout of the non-selective β-adrenoceptor antagonist, propranolol ($10^{-7}$ M) and in the presence of cocaine ($10^{-5}$ M) and propranolol ($10^{-7}$ M).

*2.4.3. Effects of cocaine and propranolol on noradrenaline concentration–response curves*

Two preparations from each animal were exposed to a concentration–response curve to noradrenaline. After wash-out, the tissues were exposed either to propranolol ($10^{-7}$ M) or cocaine ($10^{-5}$ M) and the other to vehicle (distilled water) for ninety min, after which time, another concentration–response curve to noradrenaline was constructed.

*2.5. Measurement of responses and data analysis*

Responses were measured as the change in tension (g) from the baseline before commencing a concentration–response curve at each concentration of nifedipine, noradrenaline or phenylephrine. In the case of noradrenaline and phenylephrine concentration–response curves, in control experiments, the responses at each concentration on the second curve were expressed as a fraction of that on the first curve. The mean factors were then used to correct the corresponding individual test experiment. The responses on the first curve were multiplied by the corresponding correction factor at each concentration. The corrected first curve increases in tension and second curve values were then expressed as a percentage of the

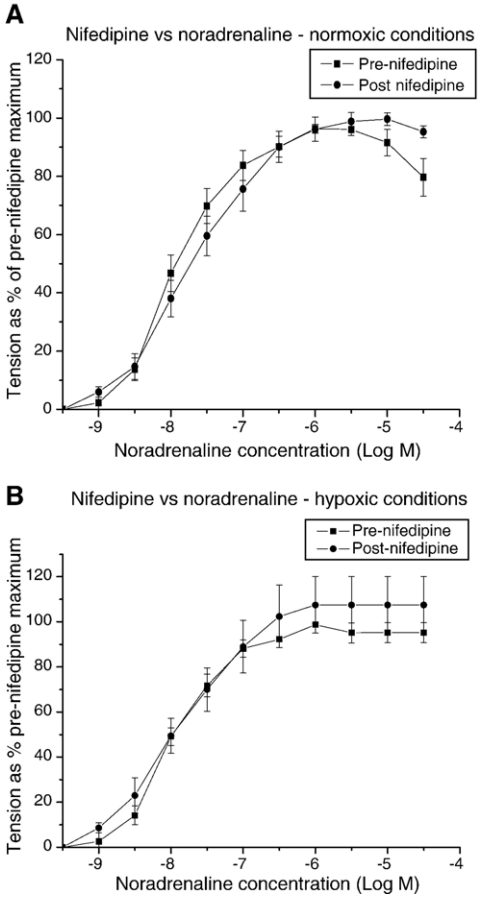


Fig. 2. Effect of nifedipine on concentration–response curves for the contraction of rat aortic rings to noradrenaline under normoxic (A) and hypoxic (B) conditions. Concentration–response curves were constructed before (■) and in the presence (●) of nifedipine ($10^{-5}$ M). The pre-nifedipine curves were corrected for changes in sensitivity from control experiments without nifedipine as described in the Methods. The changes in tension at each concentration of noradrenaline were expressed as a percentage of the corrected pre-nifedipine maximum and the mean values (±S.E.M.) ($n=5$) plotted.

Table 1
The mean maximum tensions (g) developed by noradrenaline or phenylephrine and the geometric mean $EC_{50}$ values (M) before and in the presence of nifedipine ($10^{-5}$ M)

| Constrictor | Inhibitor | Maximum constriction | | | $EC_{50}$ | |
|---|---|---|---|---|---|---|
| | | Pre-nifedipine (g) | Presence of nifedipine (g) | % pre-nifedipine max. | Pre-nifedipine | Presence of nifedipine |
| Noradrenaline | | 1.28±0.28 | 1.29±0.31 | 100±2 | 1.01 (0.72–1.39)×$10^{-8}$ | 1.91 (1.34–2.74)×$10^{-8}$ |
| Phenylephrine | | 0.75±0.11 | 0.42±0.04[a] | 59±6 | 3.15 (1.81–5.49)×$10^{-8}$ | 1.43 (1.08–1.89)×$10^{-7}$[a] |
| Noradrenaline | Cocaine | 0.91±0.17 | 0.65±0.15[a] | 67±6 | 8.16 (3.34–19.9)×$10^{-8}$ | 1.25 (0.67–2.36)×$10^{-7}$ |
| Noradrenaline | Propranolol | 1.36±0.42 | 0.78±0.29[a] | 55±4 | 5.37 (0.16–17.4)×$10^{-8}$ | 1.39 (0.58–33.7)×$10^{-7}$ |
| Noradrenaline | Cocaine + propranolol | 0.76±0.05 | 0.55±0.04[a] | 72±4 | 4.81 (2.80–8.25)×$10^{-8}$ | 1.15 (0.65–2.03)×$10^{-7}$ |

Geometric mean $EC_{50}$ values and their 95% confidence intervals and arithmetic maxima±S.E.M. were calculated from concentration–response curves in the presence of nifedipine and pre-nifedipine curves corrected from corresponding vehicle control experiments.
[a] Significant difference between pre- and post-nifedipine values ($P<0.05$).

corrected first curve maximum. Mean±S.E.M. responses were then calculated for each concentration of drug used for plotting concentration–response curves.

### 2.6. Statistics

Maxima are given in the text as arithmetic means±the standard error of the mean (S.E.M.). $EC_{50}$ values are presented as geometric means along with the 95% confidence intervals (CI). The percentage changes in maximum between two concentration–response curves are given as the mean±S.E.M. Points on graphs are also shown ±S.E.M.

Paired *t*-tests were carried out between the control and test maxima, measured in absolute units of change in tension (g) and the $Log_{10}$ of the $EC_{50}$ values for each set of experiments. Analyses were carried out using Microsoft® Excel 2002 (Microsoft® Corporation and Origin® 7 (OriginLab Corporation).

## 3. Results

### 3.1. Effects of nifedipine in noradrenaline precontracted aorta under hypoxic and normoxic conditions

Nifedipine relaxed rat endothelial denuded aortic rings previously contracted with noradrenaline in a concentration-dependent manner under normoxic conditions (Fig. 1). However, under hypoxic conditions, there was no relaxation by nifedipine. At the highest nifedipine concentration ($3\times10^{-6}$ M), the contraction was reduced by 1.48±0.17 g in the normoxic tissue, however under conditions of hypoxia, the contraction increased slightly by 0.36±0.12 g. The mean (±S.E.M.) initial contraction ($n=5$) to noradrenaline under normoxic conditions was 1.65±0.20 g and in hypoxic conditions ($n=5$) was 1.60±0.21 g. There was no significant difference between these values ($P>0.05$).

### 3.2. Effects of nifedipine on noradrenaline and phenylephrine concentration–response curves

In view of the low sensitivity to nifedipine against noradrenaline precontracted tissues, the effects when added prior to construction of a concentration–response curves to noradrenaline were examined with a high concentration of nifedipine ($10^{-5}$ M). However, under both normoxic and hypoxic conditions, this concentration of nifedipine ($10^{-5}$ M) did not alter the noradrenaline concentration–response curve (Fig. 2). Incubation with nifedipine did not significantly affect the $EC_{50}$ value or the maximum response to noradrenaline (Table 1).

However, nifedipine attenuated the concentration–response curves to phenylephrine under normoxic conditions (Fig. 3). In the presence of nifedipine, the mean ($n=6$) maximum response to phenylephrine was significantly ($P=0.01$) reduced to 59±6% of the pre-nifedipine value. The $EC_{50}$ was significantly ($P<0.005$) increased in the presence of nifedipine demonstrating a rightwards shift of the concentration–response curve (Fig. 3, Table 1).

### 3.3. Effects of nifedipine on noradrenaline concentration–response curves in the presence of cocaine and/or propranolol

When cocaine ($10^{-5}$ M) was present in the organ bath throughout, the relaxant effects of nifedipine on noradrenaline-induced contractions were revealed (Fig. 4A). The maximum response was found to be significantly ($P<0.005$) lower after

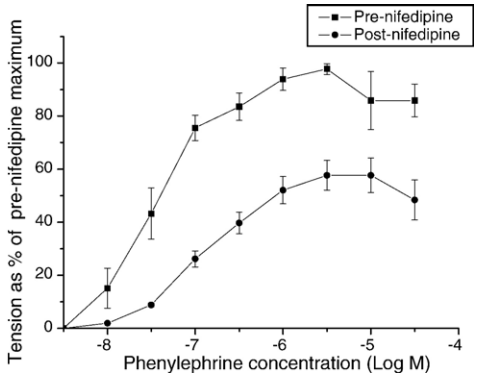


Fig. 3. Effect of nifedipine on concentration–response curves for the contraction of rat aortic rings to phenylephrine under normoxic conditions. Concentration–response curves were constructed before (■) and in the presence (●) of nifedipine ($10^{-5}$ M). The pre-nifedipine curves were corrected for changes in sensitivity from control experiments without nifedipine as described in the Methods. The changes in tension at each concentration of phenylephrine were expressed as a percentage of the corrected pre-nifedipine maximum and the mean values (±S.E.M.) ($n=5$) plotted.

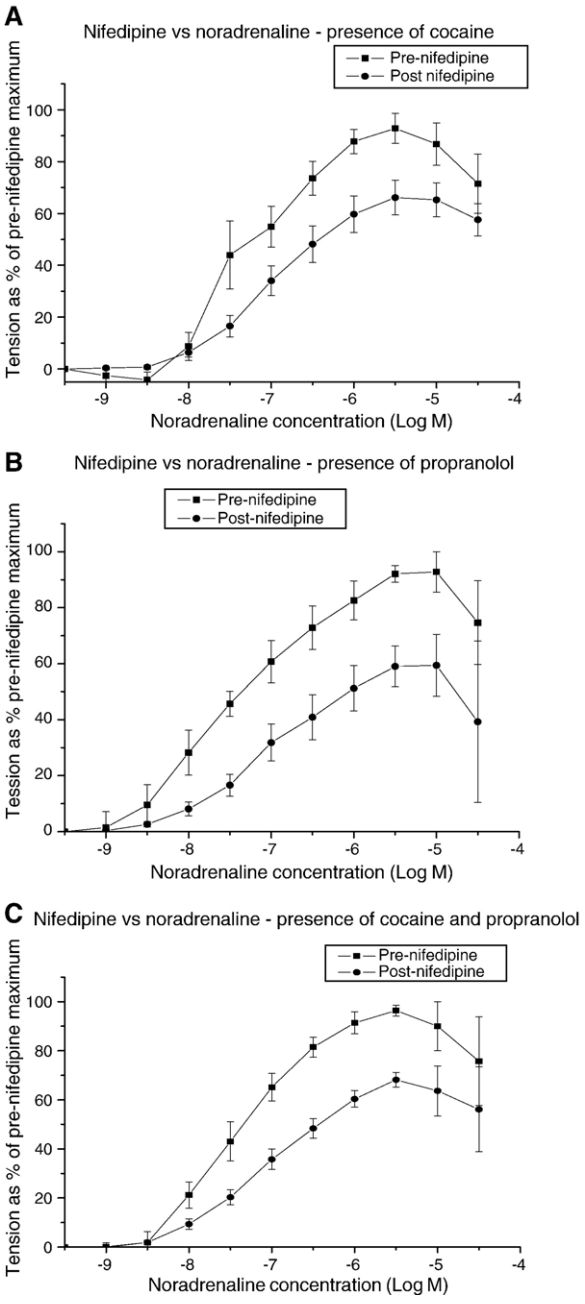


Fig. 4. Effects of nifedipine on concentration–response curves for the contraction of rat aortic rings to noradrenaline under normoxic conditions. Experiments were conducted in the presence of A. cocaine ($10^{-5}$ M) ($n=6$), B. propranolol ($10^{-7}$ M) ($n=5$) and C. cocaine ($10^{-5}$) and propranolol ($10^{-7}$ M) ($n=4$). Concentration–response curves were constructed before (■) and in the presence (●) of nifedipine ($10^{-5}$ M). The pre-nifedipine curves were corrected for changes in sensitivity from control experiments without nifedipine as described in the Methods. The changes in tension at each concentration of noradrenaline were expressed as a percentage of the corrected pre-nifedipine maximum and the mean values (±S.E.M.) plotted.

nifedipine incubation, the maximum falling to 67±6% of the pre-nifedipine response. There was no significant change in the $EC_{50}$ values ($P>0.05$) (Table 1).

Similarly, the presence of propranolol ($10^{-7}$ M) unmasked the inhibitory effects of nifedipine on noradrenaline-induced contractions. The concentration–response curve in the presence of nifedipine was suppressed, the maximum being significantly ($P<0.05$) reduced to 55±4% of the pre-nifedipine value (Fig. 4B). Nifedipine did not significantly affect the $EC_{50}$ under these circumstances ($P>0.05$) (Table 1).

When the tissue was incubated throughout the experiment with both cocaine ($10^{-5}$ M) and propranolol ($10^{-7}$ M), the maximum response to noradrenaline in the presence of nifedipine was significantly ($P=0.01$) depressed to 72±4% of the pre-nifedipine maximum. There was no significant difference in the $EC_{50}$ values of the curves before and after incubation with nifedipine (Fig. 4C, Table 1).

### 3.4. Effects of cocaine and propranolol on noradrenaline concentration–response curves

Concentration–response curves for noradrenaline were constructed before and repeated in the presence of either cocaine ($10^{-5}$ M) or propranolol ($10^{-7}$ M) alone. Neither inhibitor had any effect on the noradrenaline concentration–response curves (Fig. 5) and did not significantly affect the

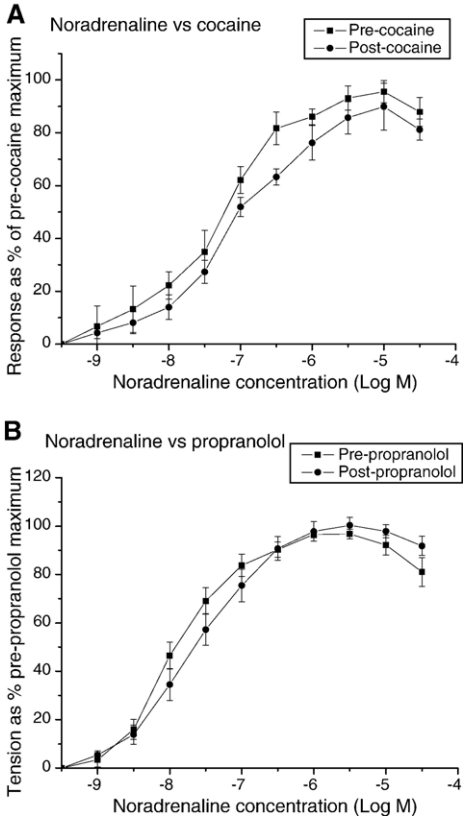


Fig. 5. Effects of A. cocaine ($10^{-5}$ M) and B. propranolol ($10^{-7}$ M) on concentration–response curves for the contraction of rat aortic rings to noradrenaline under normoxic conditions. Concentration–response curves were constructed before (■) and in the presence (●) of cocaine (A, $n=5$) or propranolol (B, $n=4$). The pre-inhibitor curves were corrected for changes in sensitivity from control experiments without cocaine or propranolol as described in the Methods. The changes in tension at each concentration of noradrenaline were expressed as a percentage of the corrected pre-inhibitor maximum and the mean values (±S.E.M.) plotted.

Table 2
The mean maximum tensions (g) developed by noradrenaline and geometric mean $EC_{50}$ values (M) before and in the presence of cocaine ($10^{-5}$ M, $n=5$) or propranolol ($10^{-7}$ M, $n=4$)

| Inhibitor | Maximum constriction | | $EC_{50}$ | |
|---|---|---|---|---|
| | Before | Presence | Before | Presence |
| Cocaine | 0.74±0.13 | 0.65±0.11 | 5.85 (3.39–10.1)×$10^{-8}$ | 6.88 (0.38–1.26)×$10^{-8}$ |
| Propranolol | 0.25±0.12 | 0.32±0.09 | 6.14 (0.69–54.8)×$10^{-8}$ | 7.02 (0.83–58.9)×$10^{-8}$ |

Geometric mean $EC_{50}$ values and their 95% confidence intervals and arithmetic mean maxima±S.E.M. were calculated from concentration–response curves in the presence of inhibitor and pre-inhibitor curves corrected from corresponding vehicle control experiments.

maximum response to noradrenaline or the $EC_{50}$ values (Table 2).

## 4. Discussion

Under normoxic conditions, nifedipine showed a characteristic vasodilator effect in the precontracted aortic tissue as a reduction in smooth muscle contraction. It can be assumed that this is due to blockade of calcium channels. In vivo, this effect is the basis of the therapeutic actions of nifedipine. The sensitivity to nifedipine was, however, much lower than reported in the literature for $K^+$ precontracted vascular tissues. A $pIC_{50}$ value of 7.78 was reported for human isolated small arteries (Angus et al., 2000) and concentrations from 1 nM caused inhibition of $K^+$-induced contractions of rat aortas (Godfraind, 1994). A similar reduced sensitivity of nifedipine against noradrenaline-induced contractions and $Ca^{2+}$ influx compared with $K^+$ has been reported previously (Godfraind, 1983; Mecca and Love, 1992; Godfraind, 1994).

The effect of nifedipine in reducing the noradrenaline-induced smooth muscle contraction, however, was abolished under hypoxic conditions. Although perhaps not surprising, this is a novel observation which could not have been predicted. We have shown previously that, in contrast, the vasorelaxation by adenosine was not inhibited by hypoxia (Broadley and Maddock, 1996). This finding means that whilst nifedipine is an effective vasodilator of blood vessels which are well supplied with oxygen, it is ineffective as a vasodilator during hypoxia. If this effect is extended to other blood vessels, nifedipine is unlikely to be a useful drug in the acute treatment of stroke and other acute ischaemic conditions as it would be unable to dilate blood vessels in ischaemic regions of tissues in order to restore blood flow. However it does not rule out its prophylactic use. To simulate the use of nifedipine as a prophylactic agent, it was next added prior to contraction with noradrenaline. A concentration ($10^{-5}$ M) of nifedipine at the maximum of the concentration–response curve obtained in noradrenaline-precontracted tissues was used for these experiments, to ensure that a substantial effect could be observed. However, under these conditions no effect of nifedipine was seen in either hypoxic or normoxic conditions.

The experiment using nifedipine added before induction of tissue contraction was repeated using phenylephrine as an alternative spasmogen. Phenylephrine and noradrenaline both exert their contractile effects in this tissue through the activation of $\alpha_1$-adrenoceptors with a consequent opening of calcium channels in the cell membrane. Thus, the two drugs would be expected to be inhibited to a similar degree by nifedipine. However, in contrast to the results obtained with noradrenaline, the concentration–response curves elicited by phenylephrine were attenuated significantly by nifedipine, implying that the contrasting actions of nifedipine against these two vasoconstrictors are due to differences in their properties.

Noradrenaline is removed from its site of action by neuronal uptake, whereas phenylephrine is not a substrate for this mechanism of removal (Trendelenburg, 1966; Yong and Chen, 1975; Matheny et al., 1977). Blockade of reuptake would therefore be expected to potentiate responses to noradrenaline but not phenylephrine. It was speculated that if nifedipine possessed some hitherto unobserved property of blocking neuronal reuptake, it would potentiate the vasoconstriction elicited by noradrenaline, by preventing its removal. This action would be expected to cause functional antagonism of the vasodilator effect of nifedipine. As phenylephrine is not subject to reuptake, only the inhibitory effects of nifedipine would be expected.

Another important difference between noradrenaline and phenylephrine is the fact that whilst phenylephrine is selective for α-adrenoceptors, noradrenaline also activates β-adrenoceptors (Broadley, 1996). In rat aorta, β-adrenoceptors mediate the relaxation of smooth muscle (Brawley et al., 2000). Since noradrenaline, but not phenylephrine, has some β-adrenoceptor activity there may be some underlying vasorelaxant action opposing the predominant contractile effects. Blockade of β-adrenoceptors might therefore be expected to potentiate the response to noradrenaline but not phenylephrine. It was therefore decided to investigate the possibility that the uncharacteristic action of nifedipine against noradrenaline-induced contractions was due to interference with either neuronal reuptake or β-adrenoceptor-mediated effects.

In order to test these hypotheses, the experiments were repeated in conditions designed to eliminate the effects of neuronal uptake and β-adrenoceptor stimulation by including cocaine and propranolol, respectively, throughout the experiment. Cocaine was able to reveal the inhibitory effects of nifedipine on noradrenaline-induced contractions, suggesting that a cocaine-sensitive pathway such as neuronal uptake of noradrenaline was masking the vasorelaxant action of nifedipine. The possibility arises that nifedipine perturbs neuronal reuptake. This hypothesis is interesting in light of previous work which has demonstrated by pharmacological evaluation and by use of radio-labelled noradrenaline that lidoflazine is able to inhibit neuronal reuptake in a concentration-dependent manner (Vanhoutte et al., 1980). Additionally, verapamil, gallopamil

and diltiazem have been demonstrated to affect neuronal uptake (Richardt et al., 1991). Whilst all of these drugs share properties as calcium antagonists, there is very little structural conservation between them; therefore it is difficult to understand why they should all share a second property of reuptake blockade in addition to their blockade of calcium channels. One possible explanation is that neuronal reuptake is a calcium-dependent process, in which case this property would be expected to be shared by all calcium antagonists, an idea which seems worthy of further investigation.

The presence of propranolol in the organ bath also revealed the inhibitory action by nifedipine on noradrenaline concentration–response curves. This also suggests that activation of β-adrenoceptors by noradrenaline masks the vasorelaxant actions of nifedipine and that nifedipine may perturb signalling initiated by β-adrenoceptors. Radioligand binding studies have demonstrated that nifedipine does not undergo significant binding to β-adrenoceptors (Feldman et al., 1985), a prerequisite to possessing any agonistic or antagonistic properties. This does not, however, rule out the possibility that nifedipine affects β-adrenoceptor-mediated effects by acting on a portion of the signal-transduction mechanism downstream of receptor activation (Briley et al., 1980).

If nifedipine does indeed interfere with physiological actions mediated by β-adrenoceptors, the consequences are potentially interesting from a clinical viewpoint. β-adrenoceptor antagonists and dihydropyridine calcium antagonists have an excellent record when used together in cardiovascular disorders. Their antihypertensive activity is additive, and the adverse effect profile is reduced due to the fact that β-blockers attenuate the reflex tachycardia produced by dihydropyridines. Similarly, calcium antagonists are able to dilate blood vessels in the peripheral circulation, reversing unwanted effects of β-blockers (Dargie, 1986; Nayler, 1988; Epstein, 1998). Compounds with both properties are currently actively being sought for these reasons (Setoguchi et al., 1995; Yeh et al., 2000; Liang et al., 2002). However it is not known whether the 'β-blocking' properties of nifedipine are present at clinically relevant concentrations in human tissues. Since nifedipine causes reflex tachycardia as an adverse effect, this seems unlikely.

The unmasking of the inhibitory effects of nifedipine on noradrenaline-induced contractions by blocking neuronal uptake with cocaine and β-adrenoceptor antagonism using propranolol was not additive. This probably reflects the fact that the effect of nifedipine on noradrenaline has a 'ceiling' dictated by the proportion of calcium utilised during the contraction that is derived from extracellular sources through VOCCs.

We have suggested that nifedipine may be acting to perturb neuronal reuptake and β-adrenoceptor signalling, thereby opposing its own vasodilator effect. If this is the case, it follows that β-adrenoceptor antagonists and blockers of neuronal uptake should potentiate noradrenaline responses. However, no significant potentiating effect of either cocaine or propranolol on noradrenaline-induced contractions was seen. Others have also observed that cocaine fails to enhance the contractions to adrenaline and noradrenaline in the rat aorta (Maling et al., 1971; Kuchii et al., 1973; Al-Sahli et al., 2001). This has been attributed to the sparse sympathetic innervation of the rat aorta and the fact that the neuronal endings are not located closely to the α-adrenoceptors that mediate the vasoconstriction (Maling et al., 1971; Kuchii et al., 1973; O'Donnell and Wanstall, 1984). The fact that indirectly acting sympathomimetic amines, such as tyramine, can exert vasoconstriction in this preparation (unpublished observations), suggests that there are neuronal uptake sites. Propranolol has also been shown by others not to potentiate the contractile effect of adrenaline (O'Donnell and Wanstall, 1984), an effect attributed to the dominance of α-adrenoceptors. Thus, we confirm that neither uptake inhibition nor β-adrenoceptor blockade potentiates the contractile actions of noradrenaline. This might appear to throw some doubt on the hypothesis that nifedipine inhibition of the noradrenaline-induced contractions was masked by neuronal uptake and β-adrenoceptor stimulation. It is possible however that the levels of neuronal uptake and β-adrenoceptor stimulation, although small and undetected by cocaine and propranolol, are sufficient to oppose the vasodilator actions of nifedipine. Whatever the explanation, it is clear that cocaine and propranolol were able to unmask the inhibitory action of nifedipine against noradrenaline to the same extent as against phenylephrine.

## 5. Conclusions

Nifedipine was effective as a vasodilator in noradrenaline-precontracted rat aorta under normoxic conditions, but was ineffective during hypoxia. When added before a noradrenaline concentration–response curve, however, nifedipine failed to shift the curve to the right or downwards, even in the presence of supramaximal concentrations. Thus, lower concentrations in the range predicted to cause vasodilatation would also be unlikely to inhibit noradrenaline. This high concentration of nifedipine was justified to ensure that sufficient drug was present to observe any relaxant effect, which indeed occurred with phenylephrine concentration–response curves. It might be expected that at high concentrations, nifedipine could have additional properties; however, these do not exert inhibitory effects upon the noradrenaline contractions. The failure to inhibit noradrenaline concentration–response curves does, however, appear to be due to additional properties of nifedipine which oppose its relaxant effect on noradrenaline-induced contractions. This effect may involve blockade of neuronal noradrenaline reuptake and perturbation of β-adrenoceptor signalling pathways. Other effects such as inhibition of adenosine transporters would not account for this opposing action since this would raise extracellular adenosine levels and relax blood vessels, thus potentiating not opposing the vasorelaxation by nifedipine.

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