Bovine polymorphonuclear leukocytes increase sensitivity to noradrenaline in isolated mesenteric arteries

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1 The effects of polymorphonuclear leukocytes (PMN) on vascular function to (–)-noradrenaline were examined *in vitro*. Purified bovine PMN were incubated in siliconized organ baths containing rings of bovine mesenteric arteries, after which a concentration-effect curve in response to (–)-noradrenaline was obtained.

2 PMN-derived products induced a long lasting concentration-dependent contraction of the blood vessels generating $24.4 \pm 6.8\%$ of the maximal tension to (–)-noradrenaline at a cell concentration of $2.5 \times 10^6 \text{ ml}^{-1}$. The contractile response was also found in endothelium-denuded vascular rings.

3 PMN present in the organ bath caused an increase in the sensitivity of vascular rings to (–)-noradrenaline. At a cell number of $2.5 \times 10^6 \text{ PMN ml}^{-1}$ the pD_2 -value for (–)-noradrenaline was augmented 0.40 ± 0.05 ($P < 0.001$), while total contraction at the highest concentration (–)-noradrenaline was not affected. This increase in sensitivity was dependent on an intact endothelium.

4 The increase in sensitivity to (–)-noradrenaline by PMN was inhibited by superoxide dismutase, but not by catalase, dimethylthiourea, indomethacin or nordihydroguaiaretic acid. The non-stimulated bovine PMN produced oxygen radicals as measured by chemiluminescence.

5 Simultaneous incubation of PMN and (–)-noradrenaline with arterial rings induced an increase in the release of prostacyclin, measured by an elevated concentration of 6-keto-prostaglandin $\text{F}_{1\alpha}$ in the supernatant.

6 It is concluded that PMN can increase vascular tone directly or indirectly probably via the interaction of PMN-derived superoxide anions with endothelium-derived relaxing factor.

Keywords: Polymorphonuclear leukocytes; oxygen-derived radicals; mesenteric arteries; noradrenaline; endothelium

Introduction

Polymorphonuclear leukocytes (PMN) adhere to the vascular endothelium and accumulate at discrete sites within the circulation in many pathological states including acute inflammatory disorders, myocardial infarction, hypertension and haemorrhagic or endotoxin shock (Brigham & Meyrick, 1984; Chobanian *et al.*, 1984; Schmid-Schönbein & Engler, 1986; Carden *et al.*, 1990). The interaction of PMN and blood vessels may be important in the regulation of vascular function since activated granulocytes release a variety of factors that are capable of influencing vascular tone.

PMN-derived vasoactive compounds include reactive oxygen species, lipid metabolites, proteolytic enzymes and nitric oxide (Wright *et al.*, 1989). It has been suggested that superoxide anion may be a contracting factor (Katusic & Vanhoutte, 1989) and may also indirectly affect vascular tone by inactivating endothelium-derived relaxing factor (EDRF; Gryglewski *et al.*, 1986). Hydrogen peroxide induces vascular relaxation due to stimulation of guanylate cyclase (Burke & Wolin, 1987) and in addition, hydrogen peroxide enhances synthesis of prostacyclin (Harlan & Callahan, 1984). Lipid mediators, such as platelet-activating factor cause vascular relaxation (Siren & Feuerstein, 1989), while others, like leukotriene, constrict arteries (Feigen, 1983). Furthermore proteolytic enzymes are able to cleave vasoactive peptides (Wintroub *et al.*, 1984).

Endothelial cells play a critical role in maintaining vascular tone via generation of potent vasoconstrictor and dilator substances (Vanhoutte, 1989). The vasodilators prostacyclin and EDRF are released by a variety of neurohumoral substances. Endothelin is a potent vasoconstrictor.

Preliminary studies described in the literature show that neutrophils may either cause vasodilatation (Rimele *et al.*, 1988; Mehta *et al.*, 1989) or vasoconstriction *in vitro* (Gillespie

et al., 1986; Nishida *et al.*, 1990; Sessa & Mullane, 1990) and *in vivo* (Carden *et al.*, 1990). Besides direct responses on arteries, neutrophils have also been reported to augment platelet-induced contractions in rabbit aorta (Sessa & Mullane, 1990) and histamine-induced pig coronary vasoconstriction (Van Heuven-Nolsen *et al.*, 1990). The interaction of neutrophils with intact endothelium was necessary for some of these effects, but in most cases adhesion of neutrophils was not required.

In the present study we investigated the influence of bovine PMN *in vitro* on the response of bovine mesenteric arteries to (–)-noradrenaline. Adrenoceptors play a major role in the regulation of peripheral vascular resistance. In hypertension both sympathetic activity and contractile responsiveness of arteries to (–)-noradrenaline are enhanced (Michel *et al.*, 1990). We show that besides the ability of PMN to contract isolated arterial rings, PMN were also capable of increasing the sensitivity to (–)-noradrenaline.

Methods

Bovine PMN isolation (Henricks *et al.*, 1987)

Whole blood was obtained in heparinized tubes from cows. Plasma was withdrawn after centrifugation at 400 *g* for 10 min. Erythrocytes were lysed by hypotonic treatment with a phosphate buffer. The white blood cells were suspended in Krebs buffer. Krebs buffer consisted of (mM): NaCl 118, KCl 4.7, MgSO_4 1.1, CaCl_2 2.5, KH_2PO_4 1.2, NaHCO_3 25.0 and glucose 7.6 and was carbogenated with 95% O_2 and 5% CO_2 . The cell suspension was layered onto Percoll (1.077 g ml^{-1}) and centrifuged for 20 min at 400 *g* to remove mononuclear cells. The neutrophil-rich pellet was suspended in Krebs buffer and was washed three times with Krebs buffer, and centrifuged once at 400 *g* and twice at 100 *g* to reduce platelet contamination. The PMN suspension was kept on ice at a

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concentration of 1×10^7 to 2×10^7 cells ml^{-1} and was washed once more just before use in the organ bath experiments. The PMN suspension contained $74 \pm 10\%$ neutrophils, $18 \pm 11\%$ eosinophils and $7.8 \pm 0.9\%$ mononuclear cells (mean $\pm$ s.e.). Viability was always greater than 95% as assessed by trypan blue exclusion. Platelet contamination of the PMN suspension was less than one platelet per PMN.

Bovine mesenteric arteries

Bovine mesenteric arteries were obtained from a local slaughterhouse and transported to the laboratory in ice-cold Krebs solution. Adherent tissue was removed and in some experiments the arteries were kept overnight at 4°C under a continuous overflow of carbogenated Krebs buffer. Preliminary experiments revealed that this storage procedure did not affect the contractility of the preparation to (–)-noradrenaline. Arteries from the third or fourth branch of the mesenteric artery with a diameter of 2 to 2.5 mm were cut into rings of 2 mm length. In some experiments the endothelium was removed by careful rubbing of the intima with cotton wool. Destruction of endothelial cells was confirmed by histological study.

Organ bath experiments

The isolated rings were suspended between two stainless steel wire hooks in 12 ml siliconized water-jacketed organ baths which were filled with Krebs buffer which was gassed with 95% O_2 and 5% CO_2 . The temperature was maintained at 37°C . Tension was measured with a Harvard isometric transducer and registered by a computer. Preload of the mesenteric rings was adjusted during 30 min to 50 mN, which was optimal to generate a maximal contraction as determined with 50 mM KCl and (–)-noradrenaline (10^{-8} – 10^{-5} M). The isolated mesenteric arteries were allowed to stabilize for 50 min during which the tissue was washed several times. The rings were exposed initially to a single concentration of 10^{-5} M (–)-noradrenaline. Krebs buffer was refreshed several times after which (–)-noradrenaline (10^{-9} – 10^{-5} M) was added cumulatively to the organ bath. Again the arterial rings were washed several times. After a resting period the PMN suspension was added at several concentrations to the organ bath and incubated for 30 min. Thereafter a second cumulative concentration-effect curve was obtained with (–)-noradrenaline.

Some experiments were carried out in the presence of inhibitors of the arachidonic acid metabolism or oxygen-radical scavengers. The cyclo-oxygenase inhibitor indomethacin (1 μM) and the lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA, 10 μM) were used for inhibition of the arachidonic acid metabolism. Superoxide dismutase (SOD, 100 u ml^{-1}), catalase (1000 u ml^{-1}) or dimethylthiourea (10 mM) were used to scavenge respectively the oxygen-derived radicals superoxide anion, hydrogen peroxide or hydroxyl radical. These compounds were added to the organ bath 15 min before the incubation with PMN.

In one experiment, inhibition of EDRF synthesis was studied by adding N-monomethyl-L-arginine (L-NMMA; 100 μM) to the organ bath 15 min before the second cumulative concentration-effect curve with (–)-noradrenaline was obtained. In this experiment PMN were not added to the organ bath.

Radioimmunoassay experiments

Mesenteric arterial rings were prepared as described above. Arterial segments and PMN suspension (2.5×10^6 cells ml^{-1} Krebs buffer) were preincubated for 20 min at 37°C . Then (–)-noradrenaline (3×10^{-7} M) was added and the tissues were incubated for 10 min after which the rings were removed. Reaction tubes were stirred throughout the experiment. Incubation fluid was collected and centrifuged at 400 g for 5 min at

4°C to remove the PMN. The supernatant was stored at -80°C until assayed. The experiment was also performed in the presence of both the oxygen-radical scavengers SOD (100 u ml^{-1}) and catalase (1000 u ml^{-1}). 6-keto-prostaglandin $\text{F}_{1\alpha}$ and thromboxane B_2 , stable metabolites of prostacyclin and thromboxane A_2 respectively, were measured in the supernatant by means of a radioimmunoassay (Dupont de Nemour, NEN research products, Boston, U.S.A. and Amersham, Buckinghamshire, U.K. respectively).

Chemiluminescence

Superoxide anion production by PMN was measured by lucigenin enhanced chemiluminescence, with a LKB-Wallac 1251 luminometer (Finland). PMN suspension (final cell number $2.5 \times 10^6 \text{ ml}^{-1}$ Krebs buffer) and lucigenin (end concentration 0.5 mM) were added to Krebs buffer in a cuvette at a total volume of 0.5 ml. The cuvettes were placed in the luminometer and chemiluminescence was measured every minute during 1 h. The temperature was maintained at 37°C . To investigate the effect of arterial segments on the superoxide anion production by PMN, chemiluminescence was also determined in the presence of arterial rings with and without endothelium. The generation of superoxide radicals by PMN stimulated with phorbol myristate acetate (PMA; 100 ng ml^{-1}) was also determined according to the above described method.

Drugs

(–)-Noradrenaline (OPG, Utrecht, the Netherlands), superoxide dismutase (SOD; Sigma, St. Louis, U.S.A.), N-methyl-L-arginine (L-NMMA; Sigma, St. Louis, U.S.A.), catalase (Boehringer Mannheim, Germany), and dimethylthiourea (Merck, Suchardt, Germany) were dissolved in saline. Nordihydroguaiaretic acid (NDGA; 1 mM; Sigma, St. Louis, U.S.A.) was dissolved in 0.01 M NaOH. Indomethacin (10 mM; Merck Sharp & Dome, Haarlem, the Netherlands) was dissolved in 1 M Tris (2-amino-2-hydroxymethyl-propane-1,3-diol) buffer (pH = 7.8). All further dilutions were in saline. Phorbol myristate acetate (PMA) and lucigenin (both Sigma, St. Louis, U.S.A.) were dissolved and diluted in Krebs buffer.

Data analysis

All data are presented as mean $\pm$ s.e.mean. pD_2 -values ($-\log \text{EC}_{50}$) are used to indicate sensitivity to (–)-noradrenaline-induced vasoconstriction (Ariens & Van Rossum, 1957). Peak chemiluminescence and total chemiluminescence integrated over a period of 0 to 60 min were determined and presented as the percentage of the response of the PMN after PMA stimulation. Statistical analysis was performed using the two-tailed Student's *t* test for paired and unpaired observations, as appropriate.

Results

Effect of PMN incubation

The addition of PMN (2.5×10^5 – 5×10^6 ml^{-1}) to the organ bath elicited a sustained contraction of bovine mesenteric arteries at the highest cell concentrations (Figure 1). The vasoconstriction was endothelium-independent at the concentrations tested (2.5×10^5 – 2.5×10^6 PMN ml^{-1}). The contraction of intact bovine mesenteric arteries due to incubation with 2.5×10^6 PMN ml^{-1} was $24.4 \pm 6.8\%$ of the maximal (–)-noradrenaline contraction. This contractile effect was not significantly influenced by incubation with the oxygen-radical scavengers SOD (100 u ml^{-1}) and catalase (1000 u ml^{-1}) together ($12.5 \pm 7.8\%$), SOD alone ($32.2 \pm 13.5\%$), catalase

Figure 1 Contractions of bovine mesenteric arterial rings after 30 min incubation with different numbers of polymorphonuclear leukocytes (PMN). The open columns represent the response in intact arteries and the closed columns in endothelium-denuded rings. Data are expressed as the percentage of the maximal response to (–)-noradrenaline. Mean are given ($n \geq 7$) with s.e.mean shown by vertical bars.

alone ($43.7 \pm 8.8\%$) or dimethylthiourea (10 mM; $44.1 \pm 10.5\%$), or with the lipoxygenase inhibitor NDGA (10 μ M; $38.2 \pm 12.5\%$). By contrast, in the presence of the cyclo-oxygenase inhibitor indomethacin (1 μ M), PMN caused a contraction at a lower cell concentration (8×10^5 PMN ml^{-1} , $19.6 \pm 5.5\%$) and the contraction observed on incubation with 2.5×10^6 PMN ml^{-1} was significantly augmented ($55.0 \pm 10.3\%$, $P < 0.01$, $n = 8$).

Effect of PMN on (–)-noradrenaline-induced contraction

Different numbers of PMN in the organ bath increased the sensitivity of bovine mesenteric arteries to (–)-noradrenaline, as indicated by an enhanced pD_2 -value (Table 1). The pD_2 for (–)-noradrenaline was increased maximally (ΔpD_2 (PMN – control): 0.40 ± 0.05 , $P < 0.001$), when the concentration-effect curve was obtained in the presence of 2.5×10^6 PMN ml^{-1} (Figure 2). The maximal contraction to (–)-noradrenaline in control arteries was 252 ± 18 mN. The maximal response was not altered after incubation with 2.5×10^6 PMN ml^{-1} (257 ± 18 mN). Removal of the endothelium also increased the sensitivity to (–)-noradrenaline (pD_2 ; 6.77 ± 0.07 , $n = 32$ without endothelium vs 6.43 ± 0.03 , $n = 60$ with endothelium, $P < 0.001$). However, addition of PMN to endothelium-denuded vascular rings in the organ bath caused no further enhancement of the pD_2 value (Figure

Figure 2 Concentration-effect curves to (–)-noradrenaline in bovine mesenteric arteries in the absence (●) and presence of 2.5×10^6 polymorphonuclear leukocytes (PMN) ml^{-1} (■) are shown. The experiment was performed in intact arterial rings (a, $n = 18$) and in arteries denuded of their endothelium (b, $n = 8$). Data are expressed relative to the maximal increase in force by (–)-noradrenaline. Means are given with s.e.mean shown by vertical lines.

2). Only at a cell concentration of 2.5×10^5 PMN ml^{-1} was a small but significant enhancement observed (Table 1).

Effect of *N*-monomethyl-*L*-arginine on (–)-noradrenaline contraction

For comparison, the modulatory role of EDRF in the vasoconstriction induced by (–)-noradrenaline was investigated. Inhibition of EDRF synthesis by *L*-NMMA (100 μ M) resulted in an increase in the pD_2 -value for (–)-noradrenaline (ΔpD_2 (*L*-NMMA – control): 0.30 ± 0.08 , $P < 0.01$, $n = 12$), but had no effect on the maximal contraction. No differences in the contractile response to (–)-noradrenaline were observed after *L*-NMMA in endothelium-denuded preparations (ΔpD_2 (*L*-NMMA – control): 0.02 ± 0.18 , $n = 4$).

Table 1 Effect of polymorphonuclear leukocytes (PMN) on sensitivity to (–)-noradrenaline in bovine isolated mesenteric arteries

PMN (ml^{-1})	n	pD_2 (control)	pD_2 (PMN)	ΔpD_2	
<i>With endothelium</i>					
0	18	6.45 ± 0.06	6.50 ± 0.07	0.05 ± 0.03	
2.5×10^5	7	6.51 ± 0.12	6.65 ± 0.09	0.15 ± 0.05	$P < 0.05$
8×10^5	11	6.35 ± 0.07	6.52 ± 0.07	0.18 ± 0.06	$P < 0.01$
2.5×10^6	17	6.46 ± 0.06	6.86 ± 0.07	0.40 ± 0.05	$P < 0.001$
5×10^6	7	6.37 ± 0.07	6.60 ± 0.07	0.23 ± 0.09	$P < 0.01$
<i>Without endothelium</i>					
0	7	6.67 ± 0.14	6.58 ± 0.14	-0.08 ± 0.07	
2.5×10^5	7	6.72 ± 0.16	6.88 ± 0.18	0.15 ± 0.06	$P < 0.05$
8×10^5	10	6.83 ± 0.13	6.95 ± 0.15	0.12 ± 0.06	
2.5×10^6	8	6.83 ± 0.11	6.86 ± 0.12	0.03 ± 0.10	

Mean values $\pm$ s.e.mean are given. Two (–)-noradrenaline concentration-effect curves were obtained in bovine mesenteric arterial rings with and without endothelium. The control pD_2 -values were obtained from the first curve. The second concentration-effect curve was obtained after 30 min incubation of the tissues with different numbers of PMN (pD_2 (PMN)). The difference between the pD_2 -values of PMN-incubation and control curves is depicted as ΔpD_2 .

§ Significance was calculated by a Student's *t* test for paired results between PMN-incubation and control.

Table 2 Influence of inhibitors of the arachidonic acid pathway and oxygen radical scavengers on polymorphonuclear leukocyte (PMN)-induced increase in sensitivity to (–)-noradrenaline

Treatment ^b	$\Delta pD_2 \pm s.e.mean^a$		
	Without PMN	PMN $8 \times 10^5 \text{ ml}^{-1}$	PMN $2.5 \times 10^6 \text{ ml}^{-1}$
None	0.05 ± 0.03	$0.18 \pm 0.06\%$	$0.40 \pm 0.05\%$
<i>Inhibitors of arachidonic acid pathway</i>			
NDGA	-0.04 ± 0.04	$0.38 \pm 0.08\%$	$0.35 \pm 0.15\%$
Indomethacin	$0.28 \pm 0.07\uparrow$	$0.64 \pm 0.09\% \uparrow$	0.35 ± 0.11
<i>Oxygen-radical scavengers</i>			
SOD and catalase	-0.01 ± 0.05		$0.04 \pm 0.05\uparrow$
SOD	-0.09 ± 0.07		$-0.03 \pm 0.13\uparrow$
Catalase	-0.03 ± 0.07		$0.25 \pm 0.09\%$
Dimethylthiourea	-0.01 ± 0.08		0.31 ± 0.15

^a ΔpD_2 : difference between pD_2 -values of (–)-noradrenaline concentration-effect curves after and before incubation. For explanation see also Table 1.

^b $n \geq 6$. The concentrations used were $10 \mu\text{M}$ nordihydroguaiaretic acid (NDGA), $1 \mu\text{M}$ indomethacin, 100 u ml^{-1} superoxide dismutase (SOD), 1000 u ml^{-1} catalase and 10 mM dimethylthiourea.

$\%$ $P < 0.05$ in unpaired t test vs incubation with no PMN.

$\uparrow$ $P < 0.01$ in unpaired t test vs PMN-incubation without treatment.

Influence of inhibitors of arachidonic acid metabolism and oxygen radical scavengers

To examine the mechanism of action by PMN on the vasoconstriction to (–)-noradrenaline, the influence of inhibitors of the arachidonic acid pathway were investigated. The data are summarized in Table 2. The lipoxygenase inhibitor NDGA ($10 \mu\text{M}$) did not diminish the increase in pD_2 by PMN. In the absence of PMN the cyclo-oxygenase inhibitor indomethacin ($1 \mu\text{M}$) itself exerted an increase in pD_2 for (–)-noradrenaline. The pD_2 -value was further enhanced by incubation of the arterial rings with indomethacin and $8 \times 10^5 \text{ PMN ml}^{-1}$, but not with $2.5 \times 10^6 \text{ PMN ml}^{-1}$.

In order to examine a possible role of oxygen-derived radicals, free radical scavengers were added to the organ bath (Table 2). The increase in pD_2 for (–)-noradrenaline in bovine mesenteric arteries was entirely abolished by SOD (100 u ml^{-1}) and catalase (1000 u ml^{-1}) or SOD (100 u ml^{-1}) alone. However, the increase in the pD_2 -value for (–)-noradrenaline was not inhibited by catalase (1000 u ml^{-1}) alone and only partially inhibited by dimethylthiourea (10 mM). The used inhibitors and scavengers had no marked effect on the basal tone of the arterial rings or on the maximal contractions elicited by (–)-noradrenaline (data not shown).

Effect of PMN on 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 production

The ratio of prostacyclin and thromboxane A_2 (TxA_2) formation, two products of the cyclo-oxygenase pathway, is important for the regulation of vascular tone *in vivo*. Prostacyclin produced by vascular cells causes relaxation, while TxA_2

causes vasoconstriction. TxB_2 , a stable metabolite of TxA_2 , was not detected in the supernatants of any studied group (detection limit: 50 pg ml^{-1}). In contrast 6-keto-PGF $_{1\alpha}$ was already produced by non-treated arterial rings (Table 3). This basal level was not increased significantly by incubations with $2.5 \times 10^6 \text{ PMN ml}^{-1}$ or (–)-noradrenaline ($3 \times 10^{-7} \text{ M}$). However, simultaneous incubation of PMN and (–)-noradrenaline with arterial rings resulted in a twofold increase of the level of 6-keto-PGF $_{1\alpha}$ in the supernatant. This elevated production was inhibited by SOD (100 u ml^{-1}) and catalase (1000 u ml^{-1}). SOD and catalase had no effect on the 6-keto-PGF $_{1\alpha}$ levels produced by (–)-noradrenaline-treated vascular rings.

Chemiluminescence

Superoxide anions were produced by stimulated and non-stimulated PMN, as determined by lucigenin enhanced chemiluminescence which is selective for superoxide radicals. Peak time, maximal level and total chemiluminescence are listed in Table 4. Stimulation of $2.5 \times 10^6 \text{ PMN ml}^{-1}$ with PMA (100 ng ml^{-1}) resulted in a sharp rise of the chemiluminescence to a maximum of $565 \pm 71 \text{ mV}$ after $4.2 \pm 0.2 \text{ min}$. Maximal chemiluminescence by non-stimulated PMN was reached at $15.6 \pm 1.7 \text{ min}$ after which it gradually declined. Peak chemiluminescence was $9.6 \pm 3.1\%$ of the maximum by PMA-stimulated PMN. The chemiluminescence pattern, after addition of intact or endothelium-denuded arterial rings to the cuvette with PMN before measurement was started, showed no differences from that of non-stimulated PMN. No chemiluminescence could be detected in arterial rings in the absence of PMN.

Table 3 6-keto-Prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) (pg mg^{-1} tissue wet weight)

	No cells	PMN
Control	85 ± 15	101 ± 16
(–)-Noradrenaline ($3 \times 10^{-7} \text{ M}$)	111 ± 16	$244 \pm 57\%$
with SOD and catalase (100 u ml^{-1} and 1000 u ml^{-1})	111 ± 27	102 ± 23

Data represent mean $\pm$ s.e.mean ($n = 7$). Arterial rings were incubated with 2.5×10^6 polymorphonuclear leukocytes (PMN) ml^{-1} and (–)-noradrenaline. 6-keto-PGF $_{1\alpha}$ levels were determined in the supernatant by means of radioimmunoassay.

$\%$ $P < 0.05$ vs control, no cells or superoxide dismutase (SOD) and catalase treatment (unpaired t test).

Table 4 Oxygen-radical production by polymorphonuclear leukocytes (PMN): chemiluminescence measurements

	Peak (%)	Total (%)	t_{max} (min)
PMA-stimulated	100	100	4.2 ± 0.2
Non-stimulated	9.2 ± 3.1	15.0 ± 3.9	13.3 ± 1.1
With arterial rings			
intact	10.7 ± 3.1	18.3 ± 4.7	15.4 ± 1.7
denuded	8.2 ± 2.3	13.5 ± 2.9	13.6 ± 1.2

The cell number used was $2.5 \times 10^6 \text{ PMN ml}^{-1}$. Lucigenin-enhanced chemiluminescence was measured every min during 1 h. The response of PMN stimulated with phorbol myristate acetate (100 ng ml^{-1}) was used as reference (100%). Peak chemiluminescence, time to reach maximal chemiluminescence and total chemiluminescence integrated from 0 to 60 min are given as mean $\pm$ s.e.mean ($n = 7$).

Discussion

The present study demonstrates that addition of bovine PMN to organ baths containing segments of bovine mesenteric arteries provoked a concentration-dependent contraction and an increase in the sensitivity to (–)-noradrenaline induced contractions. The PMN-dependent increase in sensitivity to (–)-noradrenaline was apparently not mediated by eicosanoids or leukotrienes derived from PMN and vascular cells, because neither inhibitors of cyclo-oxygenase nor lipoxygenase attenuated the effect of PMN. On the other hand, oxygen radicals may play a role since the effect on (–)-noradrenaline responsiveness was inhibited by SOD.

The isolation of PMN by percoll procedure resulted in platelet contamination. It is well recognized that platelets can induce contractions by the release of vasoconstrictive mediators like TxA_2 and 5-hydroxytryptamine (Houston *et al.*, 1986). Consequently, the direct contracting effect of PMN might be due to platelet contamination. However, the number of platelets required to elicit contractions of a similar magnitude were reported to be 10 to 20 fold higher than those found in the PMN cell suspension (Sessa & Mullane, 1990). Moreover, removal of the endothelium did not modify the direct contractile effect which is in contrast to the platelet-mediated response (Houston *et al.*, 1989). The contractile effect can also not be attributed to the release of TxA_2 from platelets since the effect was not attenuated by inhibitors of cyclo-oxygenase and TxB_2 was not detected after incubation of PMN with arterial rings. Taken together, these observations do not support a role for platelets as a source of mediators for contractions of bovine mesenteric arteries.

A short review of the literature reveals several reports suggesting that PMN may influence vascular function. In isolated hearts of the rabbit perfused with artificial buffer containing neutrophils, activation of the cells by PMA produced an increase in coronary perfusion pressure, indicative of a vasoconstriction (Gillespie *et al.*, 1986). Sessa & Mullane (1990) found that supernatant from rabbit neutrophils stimulated with calcium ionophore release a factor which directly augments vascular tone. Rimele *et al.* (1988) showed that rat PMN can release a relaxing factor similar to that reported for the endothelium. Also in isolated lungs, neutrophils increased pulmonary arterial pressure and vascular permeability (Carpenter *et al.*, 1987). Our present finding that bovine neutrophils can directly contract isolated bovine mesenteric arteries again establishes the direct effects of neutrophils in influencing vascular smooth muscle tone.

In addition, we found that PMN increased the potency of (–)-noradrenaline contractions. It is important to define the mechanisms by which neutrophil suspensions can induce this effect. The effect of neutrophils on the sensitivity to (–)-noradrenaline is probably not due to an effect of neutrophils directly on smooth muscle. Addition of PMN to endothelium-denuded vascular rings did not influence the (–)-noradrenaline-induced contractions.

There is the possibility that PMN produce potent cytotoxic substances, such as active oxygen species and proteolytic enzymes, which may injure endothelial cells (Ward & Varani, 1990). Because endothelial cells release vasodilator substances, endothelial damage by PMN may result in an increase in sensitivity to (–)-noradrenaline. The contribution of the endothelium to the response to (–)-noradrenaline is demon-

strated by the fact that endothelium removal increased the sensitivity to (–)-noradrenaline. This indicates that (–)-noradrenaline can induce the release of a vasodilator factor from the endothelium.

The chemiluminescence studies of the present investigation show that superoxide anion radicals were produced by non-stimulated PMN. Since the chemiluminescence pattern was not altered in the presence of arterial rings, stimulation of PMN by allogenic tissue in the organ bath is not likely to occur. From the literature it is known that oxygen radicals may influence vasodilator substances from the endothelium, such as prostacyclin and EDRF (Harlan & Callahan, 1984; Rubanyi & Vanhoutte, 1986).

Measurement of 6-keto-PGF $_{1\alpha}$, the stable metabolite of prostacyclin, revealed that even under basal conditions arterial rings released prostacyclin. However, the production of prostacyclin was not influenced by (–)-noradrenaline at a concentration which causes half-maximal contraction in the organ bath experiments. The addition of PMN did not influence the basal level of 6-keto-PGF $_{1\alpha}$ in the supernatants. In contrast the release of prostacyclin was found to be increased by simultaneous incubation with PMN and (–)-noradrenaline. Therefore, the increase in (–)-noradrenaline sensitivity after addition of PMN is probably not due to an inhibition of prostacyclin production.

Endothelial cells can release a labile, local factor, EDRF that has chemical properties identical to those of nitric oxide (Palmer *et al.*, 1987). Cocks & Angus (1983) have shown that noradrenaline can induce endothelium-dependent relaxations. We confirmed in our experiments that inhibition of EDRF synthesis by L-NMMA resulted in a potentiation of the sensitivity to (–)-noradrenaline. EDRF has a short half-life and its activity is inhibited by agents which generate superoxide anion. Conversely, superoxide dismutase scavenges superoxide anion and increases the half-life of EDRF (Gryglewski *et al.*, 1986; Rubanyi & Vanhoutte, 1986). In the present study we found that the effect of PMN could be inhibited by superoxide dismutase, which suggests that oxygen radicals are involved in increasing the potency of (–)-noradrenaline. Since (–)-noradrenaline can release EDRF from the endothelium, it is suggested that oxygen radicals generated by the PMN inactivate EDRF, thereby increasing the sensitivity of (–)-noradrenaline contractions. Neither catalase nor dimethylthiourea diminished the augmented response after addition of the PMN, indicating that neither hydrogen peroxide nor hydroxyl radicals contribute to EDRF inactivation. Since it has been reported that hydrogen peroxide and hydroxyl radicals can induce or facilitate relaxations of vascular smooth muscle, it can be predicted that such an effect would have decreased the sensitivity of vascular rings to (–)-noradrenaline-induced contractions (Rubanyi & Vanhoutte, 1986). The fact that non-stimulated PMN were able to influence the contractions to (–)-noradrenaline supports the notion that a small amount of oxygen radicals is sufficient to inactivate EDRF.

In summary, the present study indicates that PMN can modulate vascular smooth muscle tone directly and indirectly, probably via EDRF inactivation.

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