Free-radical scavengers, thiol-containing reagents and endothelium-dependent relaxation in isolated rat and human resistance arteries

W. SUNMAN, A.D. HUGHES and P.S. SEVER
Department of Clinical Pharmacology, St Mary's Hospital, Imperial College of Science Technology and Medicine, London, U.K.

(Received 14 September/11 November 1992; accepted 17 November 1992)

1. Small arteries were isolated from either rat mesentery or human subcutaneous fat, and mounted in a myograph for the measurement of isometric force.
2. Superoxide dismutase, either in the presence or absence of catalase, relaxed noradrenaline-induced tone. This effect was abolished by removal of the endothelium or incubation with an inhibitor of NO synthase, N-o-nitro-L-arginine methyl ester. Catalase alone had a negligible effect on noradrenaline-induced tone.
3. Captopril, an angiotensin-converting enzyme inhibitor and putative free-radical scavenger, did not relax pre-contracted isolated vessels. N-Acetylcysteine caused an endothelium-independent relaxation of rat vessels. Similar effects were observed in human vessels.
4. Acetylcholine induced a concentration-dependent relaxation of isolated resistance arteries, which was inhibited by removal of the endothelium or N-o-nitro-L-arginine methyl ester, but unaffected by indomethacin. Preincubation with captopril, N-acetylcysteine or catalase alone did not alter the acetylcholine concentration–response relationship, but superoxide dismutase in combination with catalase enhanced responses to acetylcholine, causing a six-fold increase in potency.
5. Superoxide dismutase causes endothelium-dependent relaxation of resistance arteries and potentiates responses to acetylcholine. This action is probably due to the ability of the enzyme to scavenge superoxide anions which inhibit endothelium-dependent relaxation.
6. N-Acetylcysteine causes an endothelium-independent relaxation of resistance arteries which is probably unrelated to the putative ability of this compound to scavenge superoxide radicals and may reflect a direct action on vascular smooth muscle.

INTRODUCTION

The vascular endothelium exerts an important influence on the tone of underlying vascular smooth muscle. A number of different mediators have been identified as being released by the endothelium and acting on vascular muscle [1]. One such mediator is the labile factor, endothelium-derived relaxing factor (EDRF) [2]. EDRF is released by the vascular endothelium in response to a number of stimuli, including increases in flow [3], and several vasodilators, most classically acetylcholine [4]. Recent work has indicated that EDRF is either NO [5, 6] or a labile nitroso compound derived from NO [7, 8]. Under most experimental conditions EDRF(NO) has a short half-life (around 3–30s) [9], and undergoes rapid inactivation probably by oxidation of NO to the nitrite and nitrate anions [10, 11]. It is known that such inactivation of EDRF(NO) may be brought about by reactive oxygen species and agents which generate reactive oxygen species, especially the superoxide anion (O₂⁻) [12–15].

Free radicals, such as reactive oxygen species, have recently been implicated in a number of pathological conditions, including reperfusion injury [16], atheroma formation [17] and the vascular complications of diabetes mellitus [18]. Superoxide dismutase (SOD, EC 1.15.1.1.) is an endogenous enzyme which is responsible in part for tissue defence against free radical (in particular O₂⁻) induced damage in vivo [19]. Two isoenzymes of SOD exist in cells; both catalyse the conversion of O₂⁻ into H₂O₂ [20]. However, in the presence of trace amounts of transition metals, H₂O₂ can combine with O₂⁻ forming, among other things, the hydroxyl radical (OH⁻), which is possibly more toxic than O₂⁻. In addition, it is known that the OH⁻ radical is capable of causing smooth muscle relaxation by activating soluble guanylate cyclase [21], as does NO [11]. For these reasons the action of SOD may be obscured unless H₂O₂ is also converted into stable, less reactive products. Catalase (CAT), a naturally occurring metalloprotein, catalyses the formation of water and oxygen from H₂O₂ and acts in combination with SOD in vivo. Therefore confound-

Key words: endothelium, free radicals, nitric oxide, superoxide dismutase, vascular smooth muscle.
Abbreviations: ACE, angiotensin-converting enzyme; CAT, catalase; EDRF, endothelium-derived relaxing factor; KPSS, high-potassium physiological salt solution (see the text for composition); NA, noradrenaline; NAC, N-acetylcysteine; L-NAME, N-o-nitro-L-arginine methyl ester; PSS, physiological salt solution (see the text for composition); SOD, superoxide dismutase.
Correspondence: Dr. A.D. Hughes, Department of Clinical Pharmacology, St. Mary’s Hospital, Imperial College of Science Technology and Medicine, London W2 1NY, U.K.
ing effects due to SOD-induced accumulation of \( \text{H}_2\text{O}_2 \) can be prevented by studying the action of SOD in the presence of CAT.

Captopril is an angiotensin-converting enzyme (ACE, EC 3.4.15.1) inhibitor containing a thiol group. Recently, it has been suggested that it may cause vasodilatation by a non-ACE mechanism either through enhancing the action of EDRF [22, 23] or by blockade of calcium channels [24, 25].

\( \text{N-Acetylcysteine (NAC)} \) is also a thiol-containing compound used clinically to treat paracetamol overdose. Thiol-containing compounds similar to captopril or NAC have been reported to combine with NO to produce more stable nitroso-thiol compounds which are potent relaxants of vascular smooth muscle [26]. In addition it has been suggested that thiol-containing compounds may also interact with reactive oxygen species, so sparing EDRF(NO) and enhancing its action, a 'free-radical scavenging' effect [27]. Both NAC [28] and captopril [22, 29] have been proposed to act in this way.

In this study we have investigated whether captopril or NAC have any effect on the vascular tone of isolated arteries. The arteries studied were small enough to contribute significantly to peripheral vascular resistance in vivo and have been termed resistance arteries [30]. In addition to investigating the possible existence of direct effects of these agents on resistance arteries, we have examined whether they affect basal and stimulated release of EDRF, and compared their actions with those of SOD and CAT, well-known scavengers of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \).

**METHODS**

**Resistance arteries**

Sprague-Dawley rats of either sex (approximately 250 g) were killed by cervical dislocation and small mesenteric arteries (approximately 200 \( \mu \)m normalized internal diameter) were isolated. Human resistance vessels (132–448 \( \mu \)m normalized internal diameter) were isolated from subcutaneous fat obtained during surgery. Local ethical committee approval was obtained for this procedure. Vessels were mounted on a myograph for the measurement of isometric tension as previously described [31]. Arteries were bathed in physiological salt solution (PSS), pH 7.42–7.45, and gassed with 95% \( \text{H}_2\text{O}_2 \) can be prevented by studying the action of SOD in the presence of CAT.

Captopril is an angiotensin-converting enzyme (ACE, EC 3.4.15.1) inhibitor containing a thiol group. Recently, it has been suggested that it may cause vasodilatation by a non-ACE mechanism either through enhancing the action of EDRF [22, 23] or by blockade of calcium channels [24, 25].

\( \text{N-Acetylcysteine (NAC)} \) is also a thiol-containing compound used clinically to treat paracetamol overdose. Thiol-containing compounds similar to captopril or NAC have been reported to combine with NO to produce more stable nitroso-thiol compounds which are potent relaxants of vascular smooth muscle [26]. In addition it has been suggested that thiol-containing compounds may also interact with reactive oxygen species, so sparing EDRF(NO) and enhancing its action, a 'free-radical scavenging' effect [27]. Both NAC [28] and captopril [22, 29] have been proposed to act in this way.

In this study we have investigated whether captopril or NAC have any effect on the vascular tone of isolated arteries. The arteries studied were small enough to contribute significantly to peripheral vascular resistance in vivo and have been termed resistance arteries [30]. In addition to investigating the possible existence of direct effects of these agents on resistance arteries, we have examined whether they affect basal and stimulated release of EDRF, and compared their actions with those of SOD and CAT, well-known scavengers of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \).

**METHODS**

**Resistance arteries**

Sprague-Dawley rats of either sex (approximately 250 g) were killed by cervical dislocation and small mesenteric arteries (approximately 200 \( \mu \)m normalized internal diameter) were isolated. Human resistance vessels (132–448 \( \mu \)m normalized internal diameter) were isolated from subcutaneous fat obtained during surgery. Local ethical committee approval was obtained for this procedure. Vessels were mounted on a myograph for the measurement of isometric tension as previously described [31]. Arteries were bathed in physiological salt solution (PSS), pH 7.42–7.45, and gassed with 95% \( \text{O}_2 \)/5% \( \text{CO}_2 \) at 37°C. After a 1 h equilibration period the vessels were set to a normalized internal circumference, estimated to be 90% of the circumference they would maintain when exposed to a transmural pressure of 13.3 kPa [31]. Before the experiment vessels were contracted by exposure to a sequence of high-potassium PSS (KPSS, PSS with 120 mmol/l \( \text{K}^+ \) replacing an equimolar concentration of \( \text{Na}^+ \)), 10 \( \mu \)mol/l noradrenaline (NA) and 10 \( \mu \)mol/l NA followed by 1 \( \mu \)mol/l acetylcholine, with intervening washouts. Vessels were discarded if their maximal contraction in response to KPSS did not exceed a tension equivalent to a transmural pressure of 13.3 kPa or if acetylcholine failed to produce >50% relaxation of NA-induced tone. In some studies the vascular endothelium was mechanically removed by passing a coarse bristle several times through the lumen of the vessel while it was mounted on the myograph. The lack of functional endothelium was verified by the lack of relaxation in response to 1 \( \mu \)mol/l acetylcholine.

**Vasorelaxant effect of drugs and effect on EDRF**

The effects of relaxants were examined in vessels with stable tone induced by 10 \( \mu \)mol/l NA, or in some cases KPSS. In these studies NA induced a tension equivalent to a pressure of 23 ±1 kPa and 31 ±11 kPa, and KPSS induced a tension equivalent to a pressure of 20 ±1 kPa and 30 ±1 kPa in human \((n=14)\) and rat \((n=42)\) vessels, respectively. Responses to a relaxant were calculated as a percentage reduction of pre-contracted tone. In addition to direct vasorelaxant effects, the possibility that drugs enhanced stimulated release of EDRF(NO) was investigated by examining the effects of pre-exposure to the drugs on the concentration–response relationship for acetylcholine, a well-described endothelium-dependent relaxant. Concentration–response relationships for acetylcholine were constructed in vessels pre-constricted with 10 \( \mu \)mol/l NA. After washout and recovery for 30 min a second concentration–response relationship was constructed 5–10 min after the addition of drug to the pre-contracted artery (1 \( \mu \)mol/l captopril, 1 mmol/l NAC, or 150 units/ml SOD and 1200 units/ml CAT in combination). In the case of indomethacin, indomethacin (1 \( \mu \)mol/l) was preincubated for 30 min before pre-contraction of the artery. Cumulative addition of acetylcholine was commenced once vessels had regained stable contraction after any relaxation due to the drug under study. In a preliminary study (data not shown) the concentration–response relationship for acetylcholine was shown to be reproducible on repetition in the absence of any added drug.

**Drugs and solutions**

The composition of PSS was (mmol/l): \( \text{NaCl}, 119; \text{KCl}, 4.7; \text{CaCl}_2, 2.5; \text{MgSO}_4, 7\text{H}_2\text{O}, 1.17; \text{NaHCO}_3, 25; \text{KH}_2\text{PO}_4, 1.18; \text{EDTA} \) (di-sodium salt), 0.026; glucose, 5.5. Acetylcholine, bovine erythrocyte SOD, bovine hepatic CAT, captopril, N\( \text{-}\)o-nitro-L-arginine methyl ester (l-NAME) and NA bitartrate were obtained from Sigma, Poole, Dorset, U.K. NAC (Parvolex) was obtained from Duncan Flockart. The drugs were freshly prepared in distilled water.

**Statistics and data analysis**

Concentration–response relationships were fitted to a logistic function \( \frac{\text{E}}{\text{E}_{\text{max}}} = \frac{\text{C}}{\left( \text{EC}_{\text{50}} + \text{C} \right) } \), where
RESULTS

Effects of SOD and CAT on resistance artery tone

Addition of SOD (150 units/ml) consistently relaxed isolated rat resistance arteries (Fig. 1a). SOD-induced relaxation was seen both in the absence and presence of CAT (1200 units/ml). Addition of CAT alone, before or after SOD, generally had no effect on vessel tone (Fig. 1b), although occasionally CAT induced a very small transient relaxation (Fig. 2). Preincubation with CAT did not significantly alter the relaxation induced by SOD ($E_{\text{max}} = 46 \pm 11\%$, $n = 5$ by SOD alone; $E_{\text{max}} = 49 \pm 13\%$, $n = 5$ by SOD in the presence of CAT). SOD-induced relaxation in the presence or absence of CAT was abolished by physical removal of the endothelium (Fig. 1a) and blocked by the addition of L-NAME ($10-100 \mu\text{mol/l}$) (Fig. 1b).

Effects of NAC and captopril on resistance artery tone

Addition of NAC to rat resistance arteries pre-contracted with NA caused a concentration-dependent relaxation: $pD_2 = 2.5 \pm 0.3$, $E_{\text{max}} = 69 \pm 8$, $n = 10$ (Fig. 3). The effect of NAC was not noticeably altered by removal of the endothelium (Fig. 3) or pretreatment with $100 \mu\text{mol/l}$ L-NAME ($pD_2 = 2.7 \pm 0.2$, $E_{\text{max}} = 61 \pm 8\%$, $n = 4$). Similar responses to NAC were also seen in human subcutaneous resistance arteries with intact endothelium ($pD_2 = 2.7 \pm 0.4$, $E_{\text{max}} = 51 \pm 9\%$, $n = 3$).

Captopril ($10 \text{nmol/l}$ to $100 \mu\text{mol/l}$) failed to relax four rat and three human vessels contracted with $10 \mu\text{mol/l}$ NA or four rat and four human vessels contracted with KPSS (Fig. 4).

Effect of acetylcholine on resistance artery tone and of drugs on acetylcholine-induced relaxation

Acetylcholine caused a concentration-dependent relaxation of rat resistance arteries pre-contracted with NA. This effect of acetylcholine was completely abolished by mechanical removal of the endothelium (Fig. 1a) and was unaffected by preincubation of the tissues with the cyclo-oxygenase inhibitor indomethacin ($1 \mu\text{mol/l}$) (Fig. 5, Table 1).
Fig. 2. Effect of SOD and CAT on responses to acetylcholine (ACh) in rat resistance arteries. Representative traces of the effect of ACh on the tone of an artery pre-contrasted by NA in the presence and absence of SOD (150 units/ml) and CAT (1200 units/ml) are shown above. Drug concentrations are shown as the log molar concentrations. The concentration-response relationship derived from studies in six vessels is shown below: ○, ACh alone; ●, ACh after SOD and CAT. Points represent means; SEMs are indicated by the vertical bars. The pD₂ values for the two curves differed significantly (P < 0.05).

Fig. 3. Effect of NAC on resistance arteries. The upper panel contains representative traces showing the effect of NAC on an artery before and after mechanical removal of the endothelium. NAC was added as indicated and the concentrations are shown as the log molar concentrations. Abbreviation: w/o, washout. The concentration-response relationship derived from studies in 10 vessels is shown below: □, NAC, endothelium intact; ■, NAC, endothelium removed. Points represents means; SEMs are indicated by the vertical bars.
Fig. 4. Effect of captopril on responses to acetylcholine (ACh) in rat resistance arteries. Representative traces of the effect of ACh on the tone of an artery pre-contracted by NA in the presence and absence of captopril (10 μmol/l) are shown above. Drug concentrations are shown as the log molar concentrations. The concentration-response relationship derived from studies in eight vessels is shown below: ○, ACh alone; ●, ACh in the presence of captopril. Points represent means; SEMs are indicated by the vertical bars.

Fig. 5. Effect of indomethacin on responses to acetylcholine (ACh) in rat resistance arteries. Representative traces of the effect of ACh on the tone of an artery pre-contracted by NA in the presence and absence of indomethacin (1 μmol/l) are shown above. Indomethacin was preincubated for 30 min before addition of NA. Drug concentrations are shown as the log molar concentrations. Abbreviation: w/o, washout. The concentration-response relationship derived from studies in six vessels is shown below: ○, ACh alone; □, ACh in the presence of indomethacin. Points represent means; SEMs are indicated by the vertical bars.
After addition of CAT and SOD the potency of acetylcholine-induced relaxation was significantly enhanced (Fig. 2, Table I). Preincubation with CAT alone had no significant effect on the subsequent responses to acetylcholine (Fig. 6, Table I).

Addition of NAC (1 mmol/l) before addition of acetylcholine caused a small relaxation, but did not significantly affect the concentration–response relationship for acetylcholine (Fig. 7, Table I). Similarly, preincubation with captopril (10 μmol/l) did not alter the concentration–response relationship for acetylcholine (Fig. 8, Table I).

**DISCUSSION**

Addition of SOD to pre-contraction arteries causes an endothelium-dependent relaxation which is inhibited by an NO synthase (EC 1.14.13.39) antagonist. This may indicate that SOD releases EDRF(NO), but we consider that the more likely interpretation of these findings is that SOD inactivates endogenous O$_2^\cdot$, thereby enhancing the effect of basally released EDRF. Similar effects of SOD in increasing the half-life of EDRF(NO) have previously been reported in in vitro bioassay systems using perfused vessels or cultured cells [13, 32, 33]. However, in large arteries studied in conventional organ bath systems SOD has not been consistently shown to cause relaxation itself or to enhance the effects of endothelium-dependent relaxants [13, 34, 35]. Our observations in resistance arteries may indicate that there is increased inactivation of EDRF(NO) by O$_2^\cdot$ in resistance arteries in vitro. This could reflect increased generation of O$_2^\cdot$ by resistance arteries and may relate to the relatively high oxygen partial pressure used to aerate the preparations. We did not study whether the use of lower oxygen partial pressures influences the responses to the agents; however, the use of lower oxygen partial pressures has been reported previously to alter the responsiveness of isolated blood vessels to a variety of agents [4, 36].

An alternative explanation for the marked effect of SOD on resistance arteries is that there is a decreased amount of intrinsic SOD in these vessels. Extracellular SOD in the vasculature is probably largely bound to extracellular matrix elements, such as heparin [37]. In rabbit arteries SOD is largely associated with the endothelium, with lesser amounts in the media [38]. The amount of extracellular SOD which binds to heparin (extracellular SOD type C) is known to vary between species, with little or none being present in rat vasculature [39]. Moreover, the effectiveness of endogenous SOD as a free-radical scavenger may vary between cell types [40]. At present it is not known whether large arteries and resistance arteries differ with respect to the amounts of endogenous SOD present.

Since CAT alone was without effect and the action of SOD was not affected by CAT it seems that generation of H$_2$O$_2$ and OH$,^\cdot$, either basally or as a result of the action of SOD, does not contribute significantly to tone under these conditions. In addition to causing endothelium-dependent relaxation itself, SOD also enhances relaxation induced by another endothelium-dependent relaxant, acetylcholine. This result appears to indicate that O$_2^\cdot$ also inactivates EDRF(NO) released by acetylcholine in these arteries and that in the absence of SOD this attenuates the response to acetylcholine.

Unlike SOD, NAC, a thiol-containing reagent, causes an endothelium-independent relaxation. Since removal of the endothelium did not alter the response to NAC there is little evidence to indicate participation of EDRF(NO) in the response to NAC. Captopril, another thiol-containing reagent, had no effect on tone induced by NA or KPSS, even at high concentrations (>100 μmol/l); the therapeutic level of captopril in plasma is between 0.2 and 0.4 μmol/l [41]. Previous studies in platelets [23] and myocardial cells [24] have suggested that captopril may block calcium channels. We can find no evidence for such an effect in these tissues. Neither is there any evidence that captopril can release, or in some way enhance, the action of basally released EDRF(NO), since captopril, unlike SOD, does not cause any endothelium-dependent relaxation in these arteries. This finding appears to be in contrast with that of Vanhoutte et al. [32], who reported that captopril relaxed perfused canine coronary arteries in an endothelium-dependent manner. A possible explanation of this apparent discrepancy is suggested by the recent observation that endothelial cells may generate bradykinin, an endothelium-dependent relaxant [42]. A number of vasodilators, including ATP, acetylcholine and substance P, have been reported to be released from endothelial cells by increased flow [43]. It is possible that bradykinin is also released locally by the endothelium of perfused arteries. ACE inhibitors cause accumulation of kinins by blocking their inactivation, so ACE-inhibitor-induced relaxation in a perfused artery could be due to enhancement of locally released bradykinin. Although such a sugges-

---

**Table I. Effect of drugs on acetylcholine-induced relaxation of resistance arteries.** The Table shows pD$_2$ and E$_{max}$ values derived from the analysis of concentration-response data for acetylcholine-induced relaxation in the presence or absence of the specified drug (Indo, indomethacin (1 μmol/l); SOD, superoxide dismutase (150 units/ml); CAT, catalase (1200 units/ml); NAC, N-acetylcysteine (1 mmol/l); Capt, captopril (10 μmol/l)). Statistical significance: *p < 0.05 compared with respective control data. Values are means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>pD$_2$</th>
<th>E$_{max}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
</tr>
<tr>
<td>Indo</td>
<td>7.7 ± 0.2</td>
<td>7.8 ± 0.1</td>
</tr>
<tr>
<td>SOD/CAT</td>
<td>7.9 ± 0.2</td>
<td>8.8 ± 0.3 *</td>
</tr>
<tr>
<td>CAT</td>
<td>7.5 ± 0.1</td>
<td>7.7 ± 0.1</td>
</tr>
<tr>
<td>NAC</td>
<td>7.5 ± 0.1</td>
<td>7.6 ± 0.1</td>
</tr>
<tr>
<td>Capt</td>
<td>7.5 ± 0.3</td>
<td>7.7 ± 0.2</td>
</tr>
</tbody>
</table>
Free radicals and resistance arteries

Fig. 6. Effect of CAT on responses to acetylcholine (ACh) in rat resistance arteries. Representative traces of the effect of ACh on the tone of an artery pre-contracted by NA in the presence and absence of CAT (1,200 units/ml) are shown above. Drug concentrations are shown as the log molar concentrations. Abbreviation: w/o, washout. The concentration-response relationship derived from studies in seven vessels is shown below: ○, ACh alone; ●, ACh after CAT. Points represent means; SEMs are indicated by the vertical bars.

Fig. 7. Effect of NAC on responses to acetylcholine (ACh) in rat resistance arteries. Representative traces of the effect of ACh on the tone of an artery pre-contracted by NA in the presence and absence of NAC (1 mmol/l) are shown above. Drug concentrations are shown as the log molar concentrations. Abbreviation: w/o, washout. The concentration-response relationship derived from studies in seven vessels is shown below: ○, ACh alone; ●, ACh after NAC. Points represent means; SEMs are indicated by the vertical bars.
cation is speculative, it is interesting that Feletou et al. [44] reported that ACE inhibitors had little effect on bradykinin-induced endothelium-dependent relaxation in organ bath studies, but did enhance release of EDRF(NO) by bradykinin in perfused vascular segments.

In these studies neither NAC nor captopril had any effect on the concentration–response relationship for acetylcholine, which is also in contrast to the effect of SOD and argues against a significant effect of NAC or captopril on EDRF. These results are similar to the findings of Lawson et al. [45], who found no evidence for captopril-induced enhancement of endothelium-dependent relaxation in rat aorta, but conflict with the findings of Goldschmidt and Tallarida [21], who found that captopril relaxed some, but not all, rabbit aortic rings by an endothelium-dependent action. It is unclear whether such discrepancies relate to species or methodological differences. Since thiol-containing reagents have been shown to reverse tolerance to nitrovasodilators [45, 46], perhaps these agents are only effective when a significant degree of depletion of intracellular thiols has occurred. Nevertheless, our findings do not support the hypothesis that these thiol-containing reagents interact with EDRF(NO) to form a more stable intermediate or that they may scavenge $O_2^-$ to a significant effect in resistance arteries.

Although Bagchi et al. [47] reported that captopril was a powerful scavenger of many free radicals, including $O_2^-$; recent studies do not generally support this conclusion; Aruoma et al. [48] reported that captopril did react rapidly with $H\cdot$OCl but showed little interaction with $H_2O_2$ or $O_2^\cdot$. Mehta et al. [49] also concluded that ACE inhibitors, including captopril, did not scavenge $O_2^-$; although captopril did affect the spectrophotometric absorbance of ferricytochrome. Kukreja et al. [50] concluded that captopril was a non-specific antioxidant and did not interact specifically with $O_2^-$; and Jay et al. [51] suggested that the action of captopril in some free-radical-generating systems was more likely to be due to its ability to chelate copper than to an interaction with $O_2^\cdot$. Similarly, the evidence for NAC being a scavenger of $O_2^\cdot$ appears to be weak. There is better evidence that NAC is a non-specific antioxidant and can interact with $H\cdot$OCl and OH· radicals rather than $O_2^\cdot$ [52, 53].

Although NAC does not appear to cause endothelium-dependent relaxation, the direct vasorelaxant action of NAC is of interest. NAC is known to cause an 'anaphylactoid' response when given intravenously to prevent paracetamol toxicity, characterized in part by flushing and hypotension. Unlike some other adverse effects of NAC, this reaction appears to be dose-dependent, with rapid resolution on withdrawal of NAC, suggesting a reproducible and predictable pharmacological effect rather than an immunologically triggered anaphylaxis [54, 55]. Plasma levels of NAC rise to 2 and 5 mmol/l initially during the standard intravenous treatment regimen for paracetamol overdose [56]. Relaxation of isolated large canine arteries by NAC at concentrations in excess of 10 mmol/l has been noted before [57] and it has also been reported to reduce peripheral vascular resistance in animals [27] at plasma levels around 100 $\mu$mol/l [58]. It therefore seems likely that a direct vasorelaxant action of NAC as seen in this study may account for some of the hypotensive reactions associated with its use.

ACKNOWLEDGMENT

A.D.H. is a British Heart Foundation Intermediate Fellow.

REFERENCES

Free radicals and resistance arteries


12. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production of peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990; 87: 1620-4.


