weaned guinea-pigs are similar to those in adult animals and include mucosal ulceration, inflammatory cell infiltration, dilatation of glands, and crypt abscesses, features which are also seen in human ulcerative colitis (Watt & Marcus 1971).

The assessment of the effect of degraded carrageenan on guinea-pigs, and its use as an animal model in the screening of drugs, must take into consideration factors such as weight loss, alteration of bowel function, as well as damage within the large bowel. The method of assessment of damage to the bowel may be on an arbitrary basis according to the grading system which we have used because of its practical value; this also includes the extent of involvement from caecum to rectum. Alternatively a more strict numerical assessment of ulceration, with or without histology, may be preferred,

An experimental model of this kind, with a relatively rapid onset of ulceration, provides a convenient and economic method for evaluating drugs of potential therapeutic value in the management of human ulcerative colitis. Newly-weaned guinea-pigs are less expensive than adult animals, drug administration is reduced and the work load much less in screening investigations that need last only four days.

This work was kindly supported by a research grant from the Mersey Regional Health Authority. For the photography, we wish to thank Mr A. J. Williams of the Department of Pathology, University of Liverpool.

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Communicated November 18, 1988

Hydroxylamine dilates resistance blood vessels of the perfused rat kidney and mesentery

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Abstract—Hydroxylamine (ED50 values, 47 ± 8·9 nmol and 320 ± 39 nmol) dilates resistance arterioles of the perfused noradrenaline-preconstricted rat kidney and mesentery. In this respect hydroxylamine was approximately 63 × and 320 × less potent than acetylcholine (ACh) and 15 × and 128 × less potent than nitroprusside in the two perfused organs studied. The vasodilator effect of hydroxylamine (unlike that of ACh) was unaffected by CHAPS de-endothelialization suggesting that its effect is independent of endothelium-derived relaxing factor (EDRF).

In recent years considerable interest has been shown in the mechanism of action of nitrovasodilators such as nitroprusside on vascular smooth muscle. This research has received added impetus following the recent discovery that endothelium derived relaxing factor (EDRF) is identical with nitric oxide (NO) (Palmer et al 1987) which may thus be considered the body's 'endogenous nitrovasodilator'. Hydroxylamine is both a natural product of mammalian cells (Gross 1985) and a potential source of NO under appropriate experimental conditions (see Waldman & Murad 1987 for review). In addition, hydroxylamine has been reported to cause dose-related relaxation of the mouse isolated anococcygeus preparation (Gibson & Mirzazadeh 1988). For this reason we decided to investigate the potential vasodilator effect of this substance on resistance blood vessels of the perfused rat kidney and mesentery.

Materials and methods

The procedures used have been described in detail elsewhere (Bhardwaj & Moore 1988). Briefly, rats (male, Sprague-Dawley, 250–350g) were killed by a blow to the head and exsanguinated. The left kidney and mesentery from each animal were removed and perfused (7 mL min^{-1}) with warmed (37°C), oxygenated (95% O_2:5% CO_2) Krebs solution (composition, mM: NaCl 118, $NaHCO_3$ 25, $CaCl_2$ 1·9, $MgSO_4$ 1·19, KCl 4·75, KH_2PO_3 1·19, glucose 11·1, pH 7·2) via cannulae inserted into the aorta and superior mesenteric artery respectively. Indomethacin (7 μM) was routinely added to the perfusing Krebs solution to inhibit vascular prostacyclin (PGI_2) biosynthesis. Perfusion pressure was constantly monitored by means of a Bell & Howell pressure transducer connected to a Devices pen recorder. Drugs were injected in small volumes (< 10 μL) via a self-sealing port. (−) Noradrenaline (NA) was routinely added to the perfusing Krebs solution at a concentration (0·1–0·5 mM, kidney; 100–250 μM, mesentery) which produced approximately 60–80% of the maximum response. Kidneys and mesenteries were weighed before and at the end of each experiment to determine the extent of oedema formation. In some experiments, endothelial cells lining resistance blood vessels were removed by infusion (30 s) of 4·7 mg mL^{-1} 3,3 cholamidopropyl dimethylammonio 1-propanesulphonate (CHAPS).

All drugs were purchased from Sigma Ltd, dissolved in saline (0·9% w/v NaCl) and kept on ice throughout the experiment. Drug solutions were prepared fresh each day. Results show mean ± s.e. mean with the number of observations indicated in parentheses. Statistical significance of differences between groups was determined using unpaired Student's *t*-test.

Results

Basal perfusion pressure of kidney and mesentery preparations

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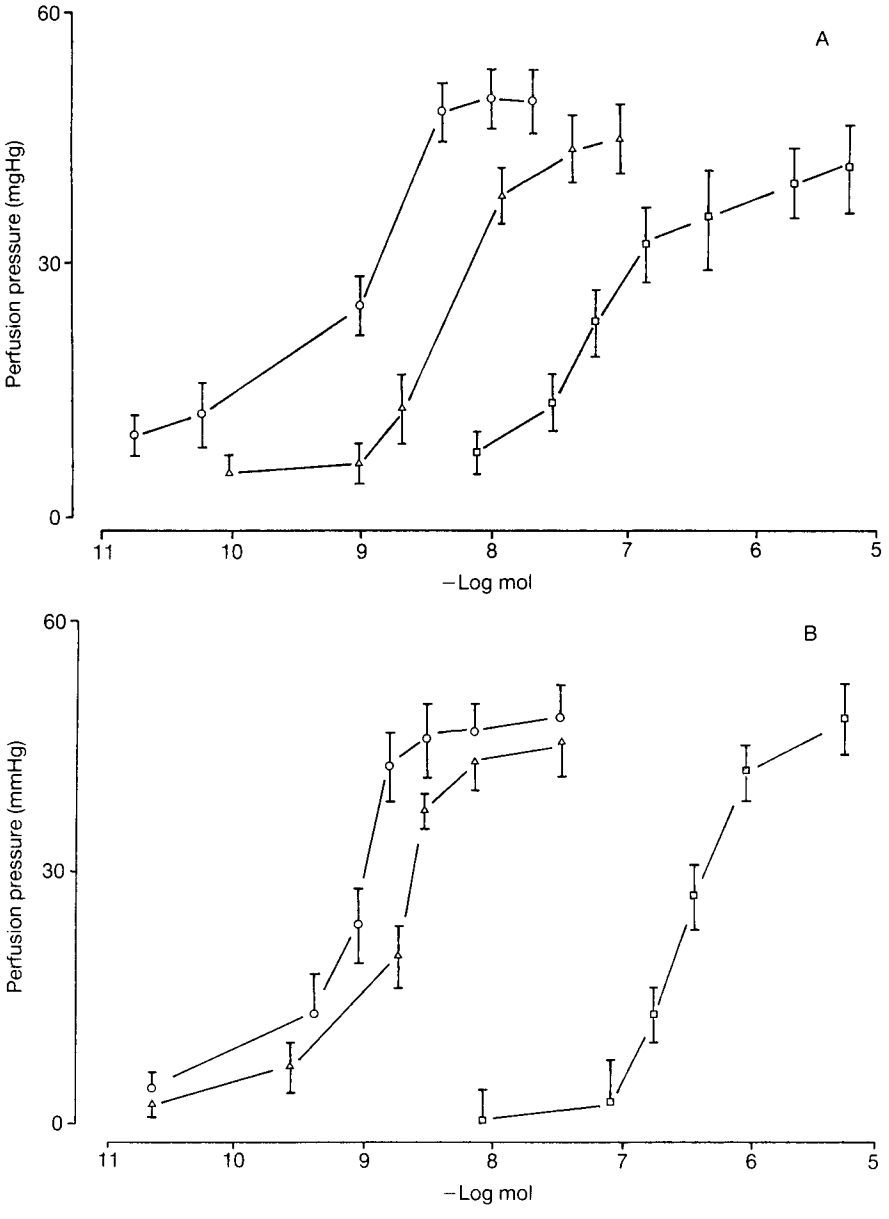

FIG. 1. Decrease in perfusion pressure (mm Hg) following bolus injection of ACh (○), nitroprusside (△) and hydroxylamine (□) in perfused 'high tone' rat kidney (A) and mesentery (B) preparations. Results show mean ± s.e. mean, n = 12.

used in this study were 154·0 ± 9·8 mm Hg, (n = 16) and 58·6 ± 7·7 mm Hg, (n = 14) respectively. Addition of NA to the perfusing Krebs solution increased perfusion pressure of both isolated organs by approximately 75–100 mm Hg. No change in perfusion pressure of such 'high tone' preparations was observed for up to 4 h. Kidneys and mesenteries gained an average 3% of their starting weight after 4 h perfusion suggesting the absence of significant oedema formation over this period.

Bolus injection of acetylcholine (ACh) produced transient (duration approximately 1 min) and dose-related falls in perfusion pressure in both kidney and mesentery. The doses required for half maximal effect (ED50) were 0·75 ± 0·3 nmol, n = 8 (kidney) and 1·0 ± 0·6 nmol, n = 6 (mesentery). Similarly, nitroprusside exhibited similar vasodilator activity (duration approximately 3 min) in the perfused 'high tone' rat kidney and mesentery with ED50 values of 3·2 ± 0·7 nmol, n = 6 and 2·5 ± 0·9 nmol, n = 5, respectively. Interestingly, hydroxylamine also dilated renal and mesenteric resistance arterioles. The duration of response and maximal effect were similar to those obtained with nitroprusside. ED50 values for hydroxylamine were 47 ± 8·9 nmol, n = 5 (kidney) and 320 ± 39 nmol, n = 5 (mesentery) respectively. Dose response curves showing the effect of all three drugs on the rat perfused kidney and mesentery are included in Fig. 1.

Administration of CHAPS to remove endothelial cells lining renal/mesenteric resistance blood vessels reduced (> 90%) the vasodilator response to ACh. The maximal vasodilator effect of nitroprusside in both the kidney (80 nmol) and mesentery (40 nmol) were reduced by 13·3 ± 3·2% and 11·6 ± 2·1%, (both n = 6, $P < 0·05$), respectively. In contrast, the renal/mesenteric vasodilator effect of hydroxylamine was not decreased following CHAPS-induced de-endothelialization. Indeed, the response to maximally effective doses of hydroxylamine in both organs were slightly, but not significantly, increased (e.g. kidney, 8 μmol, 10·0 ± 5·6%, mesentery, 6 μmol, 12·7 ± 6·7%, both n = 6, $P > 0·05$). Dose response curves to ACh, nitroprusside and

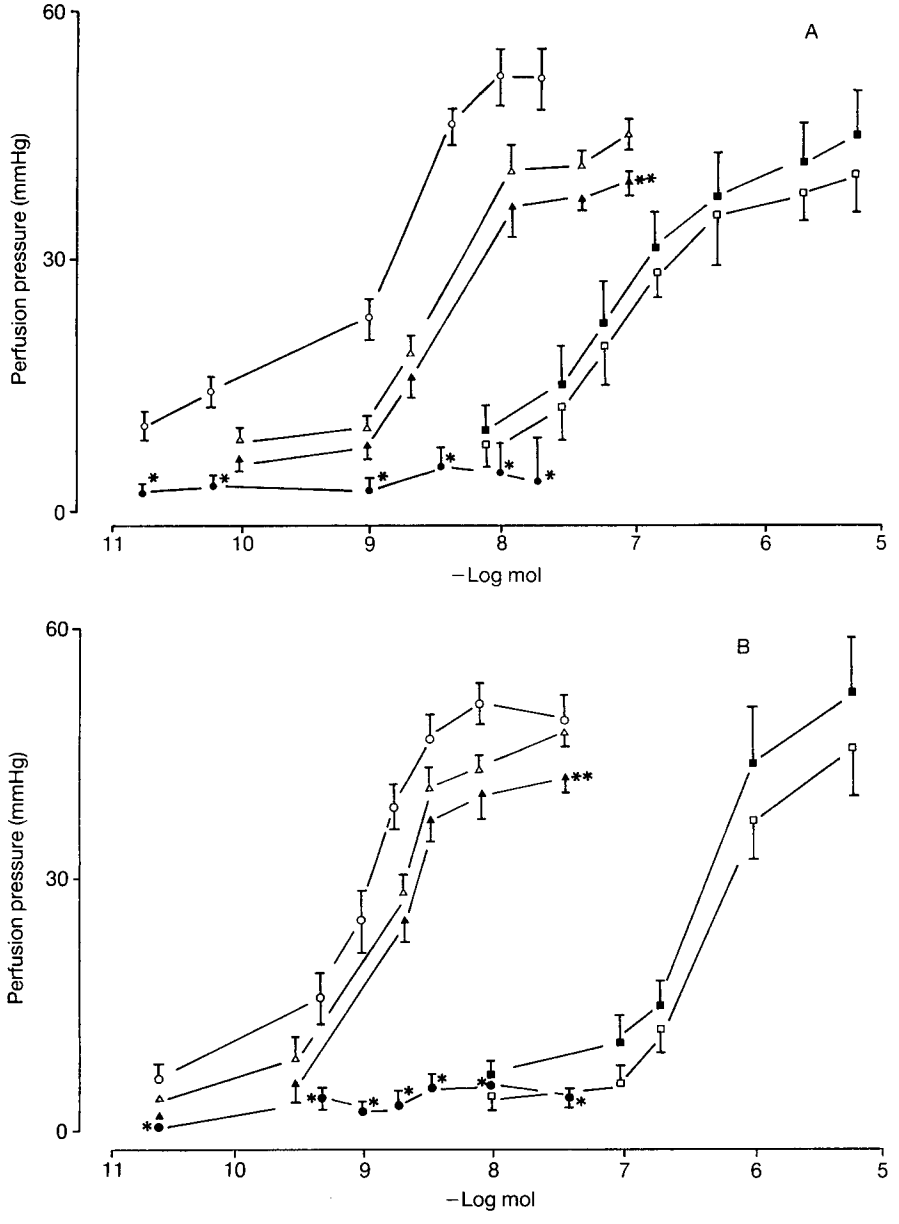

FIG. 2. Decrease in perfusion pressure (mm Hg) following bolus injection of ACh (○, ●), nitroprusside (△, ▲) and hydroxylamine (□, ■) in perfused 'high tone' rat kidney (A) and mesentery (B) preparations. Open symbols indicate responses to vasodilator drugs before and closed symbols indicate responses after CHAPS (4·7 mg mL^{-1}, 30 s) administration. Results show mean ± s.e. mean, n = 6, $*P < 0.001$, $**P < 0.05$.

hydroxylamine in the perfused kidney and mesentery before/after CHAPS de-endothelialization are shown in Fig. 2.

Discussion

ACh and nitroprusside are, respectively, endothelium-dependent and endothelium independent vasodilators of the rat perfused kidney and mesentery. These results, which are in accordance with data from a previously published study (Bhardwaj & Moore 1988), were obtained in preparations in which perfusion pressure was increased to approximately 250–300 mm Hg by NA. Exposure of the rat perfused kidney or mesentery to such high pressures might be expected to damage resistance blood vessels. However, this seems unlikely for two reasons. Firstly, the lack of weight gain over the experimental period suggests absence of significant oedema formation. Secondly, histological examination of renal resistance blood vessels from NA-preconstricted perfused rat kidneys revealed no evidence of damage to the endothelium or the underlying smooth muscle (Bhardwaj & Moore 1988).

A novel feature of the present research is the finding that hydroxylamine is also a potent vasodilator of rat renal and mesenteric resistance arterioles. Renal blood vessels were some 7 × more sensitive to hydroxylamine than were mesenteric blood vessels. Compared with acetylcholine, hydroxylamine is approximately 63 × and 320 × less potent in the kidney and mesentery respectively. That CHAPS de-endothelialization did not reduce the response to hydroxylamine in either vascular bed strongly suggests that this agent warrants classification as an endothelium-independent vasodilator. Interestingly, CHAPS administration produced a small but significant reduction in vasodilation due to a maximal dose of nitroprusside, which is in contrast to our previous study (Bhardwaj & Moore 1988) in which de-endothelialization did not influence the effect of nitroprusside.

Other authors have reported an augmented effect of sodium nitroprusside and sodium nitrite following mechanical removal of the endothelium of isolated arteries which was explained by increased accessibility to the underlying smooth muscle cells (Shirasaki & Su 1985). In the present study, CHAPS treatment may have resulted not only in removal of resistance vessel endothelial cells but also in minor damage to the smooth muscle layer. However, this conclusion is at odds with the marginally augmented vasodilator effect of hydroxylamine in both perfused organs.

The biological effects of hydroxylamine have received relatively little attention over the last few years. Hydroxylamine is a natural product both of mammalian cells (Gross 1985) and intestinal flora (Goldman 1975) although to the best of our knowledge its presence in the mammalian bloodstream has yet to be investigated. Pharmacologically, hydroxylamine increases cloride ion secretion in the dog colon (Rangachari & McWade 1987), prevents tolerance to morphine in mice (Reinis 1975) and inhibits human platelet aggregation (Iizuka & Kugawa 1972). The cellular mechanism of action of these effects as well as the vasodilator action described in the present investigation remains obscure. Hydroxylamine is known to stimulate Na^+, K^+ ATPase activity (Sachs et al 1971) and has the potential to inactivate a number of coenzymes and intermediates of cell metabolism by reacting chemically with carbonyl and ester groups (Budowsky 1976). However, the mechanism which is most likely to explain the vasodilator and possibly the platelet anti-aggregatory effect of hydroxylamine is activation of guanylate cyclase enzyme activity with resulting intracellular accumulation of cGMP (Katsuki et al 1977; Heim & Ruoff 1985). Such an effect is believed to underlie the relaxant effect of hydroxylamine in the isolated mouse anococcygeus muscle (Gibson & Mirzazadah 1988).

Clearly, an interesting parallel may be drawn between the pharmacological effects and mechanism of action of hydroxylamine and that of endothelium-derived nitric oxide (EDNO). Thus, like hydroxylamine, NO has potent vasodilator (Palmer et al 1987) and platelet anti-aggregatory (Radomski et al 1987) activity which in each case is dependent upon stimulation of the guanylate cyclase enzyme. Furthermore, superoxide anions which are known to inactivate EDNO also oxidize hydroxylamine to nitrite ion. Superoxide scavengers such as superoxide dismutase (SOD) prevent the breakdown of both EDNO and hydroxylamine (Grgylewski et al 1986; Kono 1975).

One simple explanation for these similarities may be that hydroxylamine is converted into nitric oxide (Keilin & Hartree 1954). In this context, it may be of interest that L-arginine, the recently discovered precursor to EDNO (Palmer et al 1988), is converted into nitrite and nitrate by cultured macrophages by an arginine deiminase-catalysed reaction possibly involving the formation of an arginine-hydroxylamine intermediate (Iyengar et al 1987).

In conclusion, the present results indicate a hitherto unrecognized vasodilator effect of hydroxylamine in resistance arterioles. In this context, hydroxylamine mimics the effect of NO.

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