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Ionic mechanisms contributing to the vasorelaxant properties of iodinated contrast media: a comparison of iohexol and iodixanol in the rabbit isolated aorta

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1 We have used rings of rabbit thoracic aorta to investigate the vasorelaxant properties of two different classes of non-ionic iodinated radiographic contrast media (IRCM) and the mechanisms, underlying their mode of action. Iohexol (a triiodinated monomer) was compared with iodixanol (a hexaiodinated dimer).

2 Iohexol and iodixanol both relaxed phenylephrine (0.3 μM) constricted rabbit aorta in a concentration-dependent manner that did not depend on the presence of an intact endothelium. When expressed as a function of iodine concentration, iodixanol caused significantly less relaxation than iohexol. However, the extent of relaxation was similar for both IRCM when expressed on a molar basis. Furthermore, increasing the molarity of the buffer to comparable levels with mannitol evoked only a small ($\sim$15%) relaxation of phenylephrine-induced tone.

3 Ouabain (10 μM) significantly inhibited both iohexol- and iodixanol-induced relaxations by $\sim$30%. 5-(N-Ethyl-N-isopropyl)-amiloride (EIPA, 100 nM) significantly inhibited iohexol-induced relaxation to the same extent as ouabain, but did not alter the vasorelaxant effect of iodixanol. Co-incubation with ouabain and EIPA had an additive effect in the case of iohexol, increasing inhibition of relaxation to $\sim$60%, whereas inhibition of iodixanol-induced relaxation by the combination of ouabain plus EIPA did not differ from that of ouabain alone.

4 Replacing NaCl with N-methyl-D-glucamine (NMDG) to lower extracellular [Na^+] and thereby inhibit Na^+-Ca^{2+} exchange, attenuated the relaxation evoked by iohexol or by iodixanol (by $\sim$25%) in each case.

5 We conclude that iohexol- and iodixanol-induced vasorelaxation in rabbit aorta is mediated through a direct action on vascular smooth muscle that is not simply a consequence of altered osmolality. It involves modulation of the Na^+-K^+ ATPase and, in the case of iohexol, Na^+-H^+ exchange. Both agents also appear to modulate Na^+-Ca^{2+} exchange, through direct and/or indirect mechanisms. This is the first study to show specific pharmacological differences between monomeric and dimeric contrast media in vascular smooth muscle.

Keywords: Contrast media; vasorelaxation; ion exchange; iohexol; iodixanol

Introduction

Iodinated radiographic contrast media (IRCM) have been in routine clinical use for several decades and it is well known that their intra-arterial administration may cause a variety of clinical side effects (Bush & Swanson, 1991).One of the most common of these is a sensation of warmth or even pain attributable to peripheral vasodilatation, which is more pronounced with the older, high osmolality ionic contrast media (e.g. sodium metrizoate), than the newer low osmolality non-ionic monomeric contrast media such as iohexol and iopamidol (Almen, 1987). It has been speculated that high osmolality (in comparison to blood) is the main factor contributing to this phenomenon. Indeed, recent studies have demonstrated that peripheral vasodilatation and patient discomfort during aorto-femoral angiography is significantly greater with nonionic monomers than the new iso-osmolar dimeric contrast agent iodixanol, at concentrations providing equivalent radiographic contrast (Pugh *et al.*, 1993; Verow *et al.*, 1995). Nevertheless, formulations of iodixanol that are iso-osmotic with blood still cause significant vasodilatation both in animal models and clinically (Almen 1987; Pugh *et al.*, 1992; 1993; 1995b). The exact mechanisms underlying IRCM-induced vasodilatation consequently remain incompletely understood.

Studies with isolated blood vessels have shown that hyperosmolar solutions of 'inert' compounds such as mannitol and sucrose also cause vasorelaxation which can be inhibited by ouabain, a specific inhibitor of Na^+-K^+ ATPase, and amiloride, a relatively non-specific inhibitor of Na^+-H^+ and Na^+-Ca^+ exchange (Toda *et al.*, 1992). Previous experiments have shown a potential interaction of Na^+-Ca^{+2} exchange with both Na^+-K^+ ATPase and Na^+-H^+ exchange in the regulation of vascular tone (Ozaki *et al.*, 1978; Blaustein, 1988). The homeostatic balance between multiple ion exchange mechanisms and fluxes may therefore be altered by hyperosmolar solutions and thereby contribute to relaxation. In the present study we have used rabbit isolated aortic ring preparations, both with and without an intact endothelium, to compare two differing contrast agents, iohexol and iodixanol, and to evaluate the relative contributions of Na^+-K^+ ATPase, Na^+-H^+ exchange and Na^+-Ca^{2+} exchange to their vasorelaxant activity. We have also compared their relaxant activity with that of mannitol at similar molar concentrations. In previous experiments from this laboratory we employed 'ready-to-use' formulations of IRCM (Pugh *et al.*, 1995b), whereas in the present study pure compounds were dissolved directly into physiological buffer to eliminate secondary effects due to alterations in the net ionic composition of the incubation medium.

Methods

Isolated ring preparations

Male, New Zealand White rabbits (2.0-2.5 kg) were killed by an overdose of sodium pentobarbitone (120 mg kg^{-1},

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i.v.) and the thoracic aortae quickly removed and placed in Holman's solution, kept at room temperature (composition in mM: NaCl 120, KCl 5.0, $CaCl_2$ 2.5, NaH_2PO_4 1.3, $NaHCO_3$ 25, glucose 11 and sucrose 10). Adventitial fat was removed and the tissue cut into ring segments 3–4 mm wide. Each preparation was mounted in a 10 ml organ bath, and subsequently allowed to equilibrate in oxygenated (95% O_2/5% CO_2) Holman's buffer, at 37°C for 60–90 min with frequent adjustments of tension until stress relaxation no longer occurred and a resting tension of 1.5–2.0 g was obtained. Changes in tension were measured via a pressure transducer connected to a Maclab/4e (ADInstruments, NSW, Australia). Rings were preconstricted with 0.3 μM phenylphrine (PhE) and at steady state, cumulative concentration-response curves for relaxation to iohexol and iodixanol (final organ bath concentrations 1–100 mg iodine ml^{-1}) were constructed. The concentrations of the stock solutions were 300 and 320 mg iodine ml^{-1}, respectively, which were found to be close to the maximum concentration attainable in Holman's buffer. In some preparations the endothelium was removed by gentle abrasion, and the lack of a functional endothelium confirmed by the absence of a relaxant response following application of 1 μM acetylcholine (ACh).

Na^+-K^+ ATPase and Na^+-H^+ exchanger

To assess the role that Na^+-K^+ ATPase and Na^+-H^+ exchange play in IRCM induced vasorelaxation, ouabain (a selective inhibitor of Na^+-K^+ ATPase), (10 μM) and 5-(N-ethyl-N-isopropyl)-amiloride (EIPA, a selective inhibitor of the Na^+-H^+ antiporter) (100 nM) either alone or in combination, were added to the organ bath 40 min before the application of PhE and maintained throughout the experiment. In some experiments ouabain alone was incubated with the preparation, in the absence of PhE, and cumulative concentration-response curves to iohexol and iodixanol constructed at steady state.

Na^+-Ca^{2+} exchange

The contribution of the Na^+-Ca^{2+} exchanger to IRCM-induced vasodilatation was investigated by reducing the concentration of extracellular Na^+ to ~25 mM, which results in inhibition of the exchanger, by replacing NaCl in the Holman's buffer with an equimolar concentration of N-methyl-D-glucamine (NMDG).

Mannitol

In a further set of experiments mannitol was used in place of iohexol or iodixanol as a control to study the possible effects of hyperosmolality.

Drugs

Pure iohexol and iodixanol were provided by Nycomed Imaging AS, Norway and were dissolved directly into Holman's solution. EIPA was obtained from Research Biochemicals International (RBI) (St. Albans, U.K.) and was dissolved in dimethylsulphoxide (DMSO) and diluted before use so that the final concentration of DMSO was less than 0.001%. This concentration of vehicle had no significant effect on vascular responses. Phenylephrine, ACh, ouabain, NMDG and mannitol were obtained from Sigma Ltd (Poole, U.K.) and dissolved directly into Holman's solution.

Statistical analysis

Results of the experiments are expressed as mean ± s.e.mean. Statistical analysis was with ANOVA (analysis of variance) followed by Bonferroni's multiple comparison test and Student's t test, where appropriate. $P<0.05$ was accepted as significant.

Results

IRCM induced relaxations of phenylephrine constricted rabbit aorta: role of the endothelium and hyperosmolality

Rabbit aortic rings, preconstricted with PhE (0.3 μM), were relaxed by both iohexol and iodixanol in a concentration-dependent manner that did not depend on the presence of an intact endothelium (Figure 1a). At iodine concentrations of 100 mg ml^{-1} relaxation of endothelium denuded preparations to iohexol (58.1 ± 6.2%; $n=12$) was significantly higher than to iodixanol (46.1 ± 2.9%; $n=10$; $P<0.05$). Similarly, in endothelium-intact rings iohexol-induced relaxation (65.9 ± 6.0%; $n=12$) was significantly greater than that induced by iodixanol (36.1 ± 5.7%; $n=10$; $P<0.05$) (Figure 1a). However, when relaxation was plotted as a function of molarity, the concentration-response curves for iohexol and iodixanol were statistically similar (Figure 1b). Absolute tension developed in response to PhE was increased, but not significantly, in preparations denuded of endothelium (2.7 ± 0.2 g; $n=34$), compared with rings with an intact endothelium (2.4 ± 0.2 g; $n=22$). All further experiments were performed with endothelium-denuded preparations.

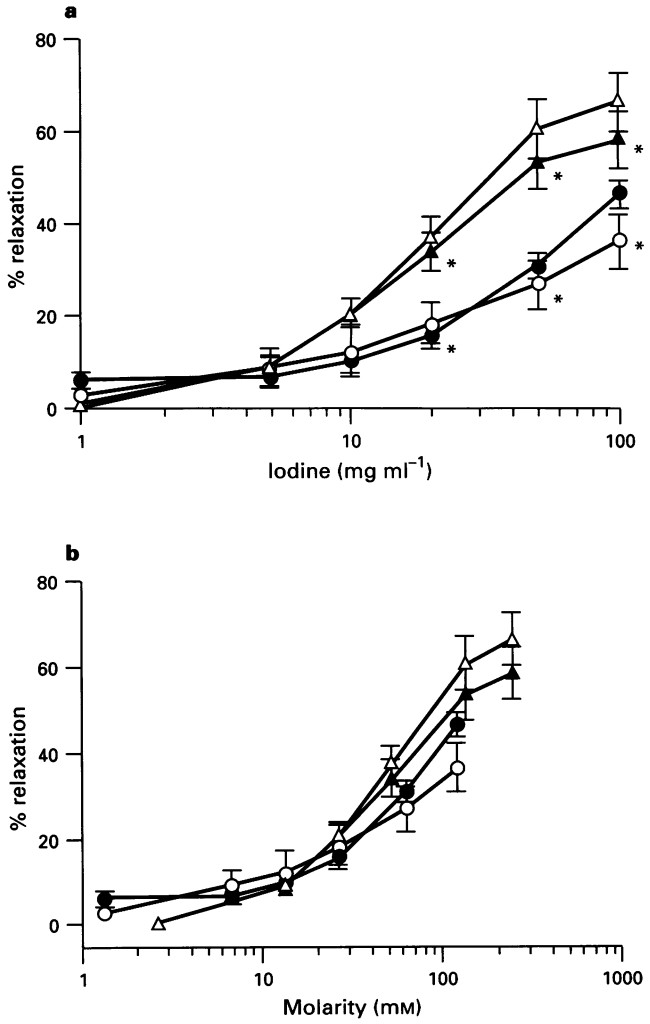

Figure 1 Concentration-response curves for relaxation of phenylephrine (0.3 μM) constricted rabbit aorta by iohexol (triangles) and iodixanol (circles) plotted as a function of (a) iodine concentration and (b) molarity. Open symbols (△,○) denote the presence of an intact endothelium, closed symbols (▲,●) denote denuded preparations. Results are the mean of 10–12 separate experiments, where vertical line represent the s.e.mean. Statistically significant difference from corresponding control values is shown by $*P<0.05$.

Effects of ouabain and EIPA

Following preincubation with 10 μM ouabain before the application of PhE relaxations of endothelium-denuded preparations by iohexol (maximum relaxation of final tone 39.2±6.8%; $n=10$) and iodixanol (maximum relaxation of final tone 28.6±3.5%; $n=8$) were significantly attenuated by 32% and 38%, respectively ($P<0.05$; Figure 2a,b). Ouabain 10 μM caused a small increase in resting tone (0.7±0.1 g) so that the absolute tension subsequently developed following PhE was significantly greater than in PhE-only controls (3.3±0.2 g and 2.4±0.2 g, respectively; $P<0.05$). Preincubation with EIPA (100 nM) had no effect on resting tension or absolute tension developed but caused a 38% reduction in the relaxant effect of iohexol (maximum relaxation of final tone 35.8±4.9%; $n=7$; $P<0.05$) (Figure 2a). However, EIPA had no effect on the relaxant effects of iodixanol (maximum relaxation of final tone 43.2±6.5%; $n=7$) (Figure 2b). In the case of iohexol, preincubation with EIPA and ouabain for 40 min had an additive effect, inhibition of relaxation at the highest concentration of iohexol being increased to 68% (maximum relaxation of final tone 18.6±3.4%; $n=7$; $P<0.001$) (Figure 2a). In marked contrast, in the case of iodixanol the combination of ouabain and EIPA did not increase the inhibition of relaxation above that obtained with ouabain alone (maximum relaxation of final tone 28.5±0.4%; $n=6$) (Figure 2b). The rise in resting tension (0.9±0.2 g) observed during preincubation with ouabain and EIPA and the absolute tension subsequently developed following application of PhE (3.6±0.4 g) were similar to the values found with ouabain alone.

Relaxation of ouabain constricted preparations

Iohexol caused concentration-dependent relaxation of rabbit aorta constricted by 100 and 200 μM ouabain; maximum relaxation of final tone 36.4±8.9%; $n=5$; and 28.9±5.1%; $n=4$, respectively (Figure 3a), which were significantly smaller than the corresponding relaxation of PhE-induced tone at the $P<0.01$ and $P<0.001$ levels. Iodixanol also relaxed ouabain (100 and 200 μM) constricted aorta (maximum relaxation of final tone 35.2±4.6 and 28.1±4.8%; $n=4$ respectively) and this was significantly lower than relaxation of PhE-induced tone in the case of 200 μM ouabain ($P<0.05$) (Figure 3b). Application of ouabain at either 100 or 200 μM, gave rise to a developed tension (2.3±0.3 and 2.4±0.4 g respectively) that was not significantly different from the developed tension due to PhE alone (2.4±0.2 g).

Reduced external [Na^+]

Replacing the normal incubation medium with Holman's buffer in which the Na^+ concentration was reduced to

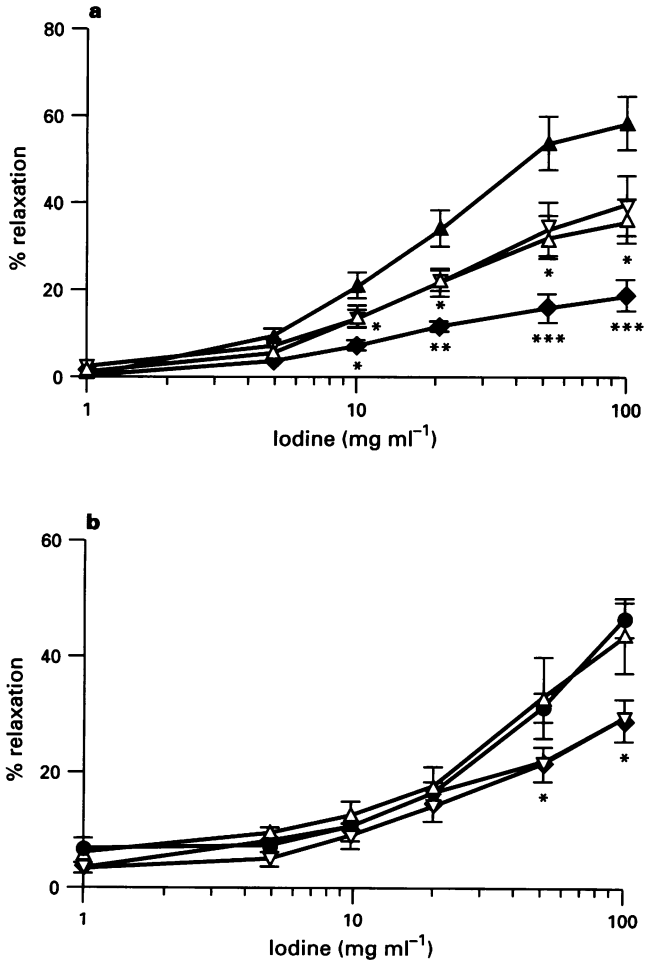

Figure 2 Concentration-response curves for (a) iohexol-induced (▲) and (b) iodixanol-induced (●) relaxation of endothelium denuded rabbit aorta, pre-constricted with phenylephrine, either alone or in the presence of 10 μM ouabain (▽), 100 nM 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) (△) or 10 μM ouabain + 100 nM EIPA (◆). Results are the mean of 6–10 separate experiments, where vertical lines represent s.e.mean. Statistically significant difference from control is shown by $*P<0.05$; $**P<0.01$; $***P<0.001$.

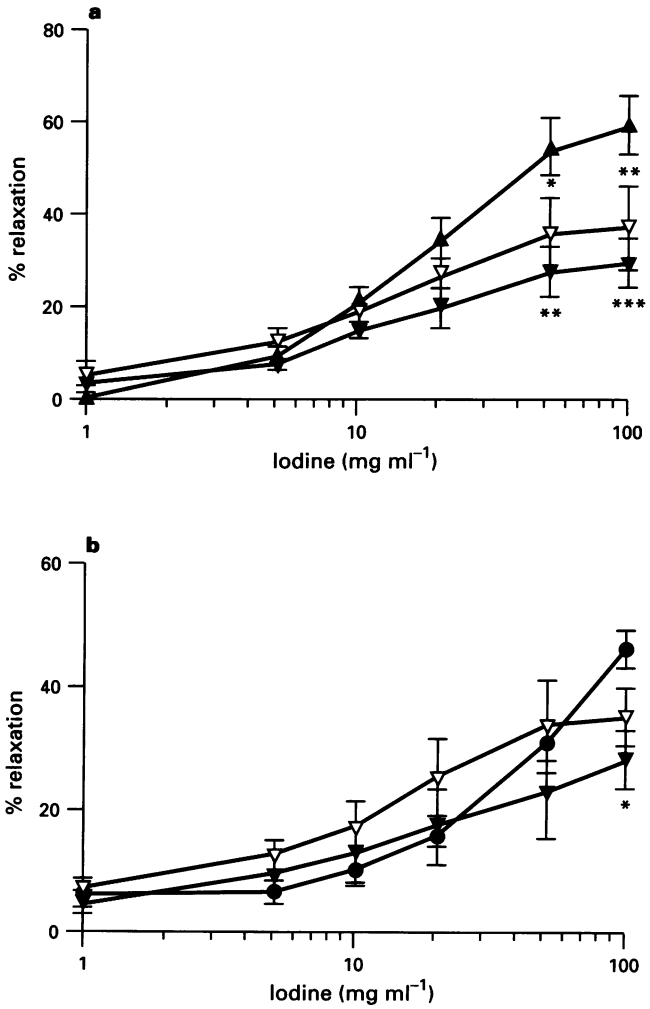

Figure 3 Concentration-response curves for (a) iohexol-induced (▲) and (b) iodixanol-induced (●) relaxation of endothelium denuded rabbit aorta rings preconstricted with phenylephrine, compared to relaxations of ouabain 100 μM (▽) and 200 μM (▼) contracted aorta. Results are the mean of 4–5 separate experiments, where vertical lines represent s.e.mean. Statistically significant difference from control is shown by $*P<0.05$; $**P<0.01$; $***P<0.001$.

~25 mM, inhibited the vasorelaxant effect of both iohexon (maximum relaxation of final tone 43.4±5.1%; $n=8$; $P<0.05$) and iodixanol (maximum relaxation of final tone 32.7±5.2%; $n=5$; $P<0.05$) by 29 and 25%, respectively (Figure 4). Absolute steady state tension development under these conditions was not different from controls (2.0±0.3; $n=13$ and 2.4±0.2 g; $n=10$ respectively; NS).

Mannitol

Altering molarity by the addition of increasing volumes (0.1–30% v/v) of a solution of 300 mM mannitol (final concentrations 0.3–180 mM) caused only a small (~15%) relaxation of PhE-constricted aortic ring preparations (Figure 5). Relaxation to mannitol was not affected by incubation with either EIPA, ouabain or the combination of the two drugs (Figure 5).

Discussion

We have confirmed previous observations that relaxation of isolated arterial rings by iodinated radiographic contrast media (IRCM) is an endothelium-independent phenomenon (Pugh *et al.*, 1995a,b). The present data also show that iodixanol causes a smaller degree of relaxation than iohexol at equivalent iodine concentrations consistent with *in vivo* studies demonstrating that iodixanol (320 mg I ml^{-1}) is a less potent vasodilator than either iohexol (320 mg I ml^{-1}) or iopromide (300 mg I ml^{-1}) in femoral arteries of dogs (Almen, 1987) and man (Pugh *et al.*, 1993). However, when relaxation of the isolated rings was plotted as a function of molarity, the concentration-relaxation curves for both agents could be superimposed, analogous to the findings of Pugh *et al.* (1995b). By comparison, the concentration-relaxation curve for mannitol was shifted downwards and to the right, and maximal relaxation was only 25–30% of that produced by equivalent molar concentrations of the contrast agents. As a consequence of the effects of molecular aggregation, which is more pronounced with dimeric than monomeric IRCM, the rank order of osmolarity would be expected to be mannitol>iohexol>iodixanol at equivalent molarities (Eivindvik & Siogren, 1995). As the relaxation observed experimentally was iodixanol=iohexol>mannitol our findings suggest that hyperosmolality *per se* is not the dominant factor causing vasorelaxation to either contrast agent. Earlier work by Lardini *et al.* (1982) analogously demonstrated that the vasodilator action of diatrizoate against K^+-evoked contraction of canine isolated coronary arteries could not be reproduced by increasing osmolarity to equivalent levels with sucrose, relaxation always being <20% of that produced by the contrast agent. Jansen *et al.* (1987) demonstrated a maximum relaxation of 40% to mannitol (at a concentration of 480 mM cf. 180 mM in the present study) in rabbit basilar artery contracted by high K^+. However, at equivalent osmolalities (determined by a direct freezing point method), the relative relaxation evoked by mannitol was only ~30% of that observed with iohexol over the range 270–400 mOsm kg^{-1}. In contrast to these findings, Toda *et al.* (1992) obtained an almost complete relaxation with 100 mM mannitol in endothelium-denuded monkey cerebral arteries activated by elevated $[K^+]_o$.

There may thus be considerable variability in the ability of mannitol to evoke vascular relaxation. Indeed hypertonic solutions of mannitol and IRCM may also evoke endothelium-independent vasoconstriction of isolated arteries in the absence of exogenously-induced tone (Gomi, 1992; Low *et al.*, 1994). Preincubation of rat tail artery with mannitol and sucrose (both at 100 mM) also amplifies the constriction induced by subsequent administration of PhE through direct effects on smooth muscle and moderate depression of endothelial NO synthesis (Rocha *et al.*, 1995). It thus appears that the resultant effect of mannitol or IRCM on vascular smooth muscle tone depends on the balance between competing constrictor and dilator mechanisms which may vary considerably between species and tissues. It may also depend on the absolute level of tone and the method by which contraction is induced (i.e. agonist or high $[K^+]_o$).

'Inert' agents such as mannitol are known to modulate the electrogenic Na^+-K^+ ATPase (Sasaki *et al.*, 1994). In the present study we found that ouabain inhibited the vasodilator action of both iohexol and iodixanol in PhE-constricted preparations by ca. 30%, suggesting that these agents partially

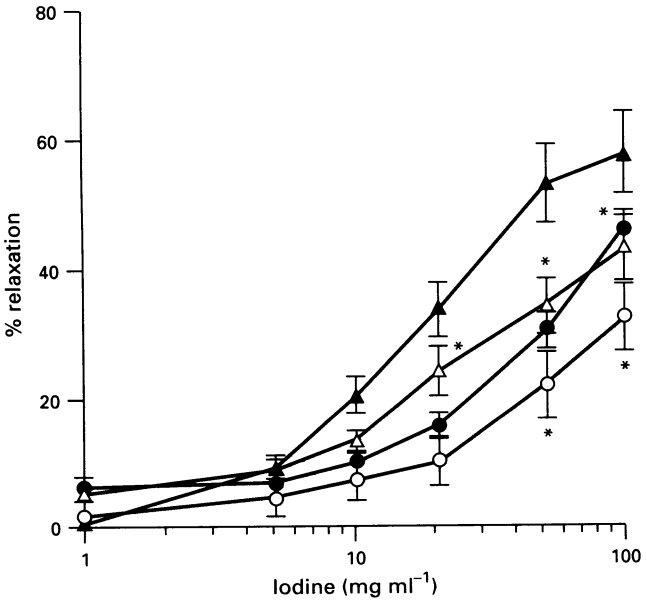

Figure 4 Concentration-response curves for iohexol-induced (triangles) and iodixanol (circles)-induced relaxation of phenylephrine constricted endothelium denuded rabbit aorta in normal (▲/●) and low sodium (△/○) Holman's buffer. Results are the mean of 5–8 separate experiments, where vertical lines represent s.e.mean. Statistically significant difference from normal Holman's is shown by *$P<0.05$.

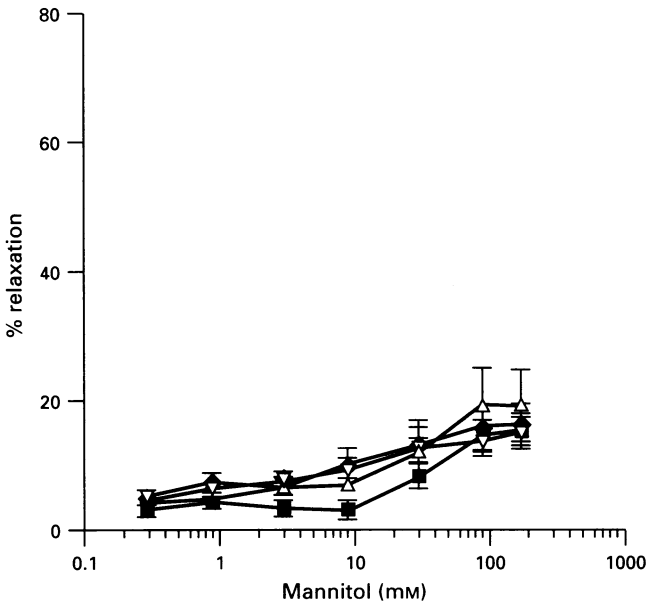

Figure 5 Concentration-response curves for mannitol-induced relaxation of endothelium denuded rabbit aorta, pre-constricted with phenylephrine, either alone (■) or in the presence of 10 μM ouabain (▽), 100 nM 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) (△) or 10 μM ouabain + 100 nM EIPA (◆). Results are the mean of 6–12 separate experiments, where vertical lines represent s.e.mean.

mediate relaxation through direct and/or indirect stimulation of the Na^+-K^+ pump. Furthermore, relaxation of PhE-constricted aorta was significantly greater than in preparations contracted by ouabain at concentrations (100 and 200 μM) selected to cause a similar increase in tension to that of PhE. The attenuation of IRCM-induced vasodilatation by ouabain cannot therefore be attributed to functional antagonism (i.e. different levels of initial tone). Inhibition of the Na^+-K^+ ATPase will inevitably exert secondary effects on the movement of other ions. Administration of ouabain thus increases intracellular $[Na^+]$ causing the Na^+-Ca^{2+} exchanger to operate in reverse mode and elevate cytosolic $[Ca^{2+}]$ (Ozaki *et al.*, 1978; Blaustein, 1988). This rise in Ca^{2+} is subsequently amplified by Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum, further enhancing tension development (Blaustein, 1988). Experiments with human umbilical arteries have also shown that the contractions to ouabain consist of discrete early and late phases that respectively involve Ca^{2+} entry via voltage-operated Ca^{2+} channels and via Na^+-Ca^{2+} exchange (Sato & Aoki 1998; 1991).

Further experiments were therefore performed to examine the role of Na^+-Ca^{2+} exchange in relaxation to IRCM. Previous studies have shown that reductions in external Na^+ concentration to 20–30 mM, as in the present study, cause ~50% inhibition of the Na^+-Ca^{2+} exchanger (Ashida & Blaustein, 1987). At the highest concentrations employed iohexol- and iodixanol-induced relaxations were both inhibited by ~25% in low Na^+ buffer, suggesting that both compounds modulate Na^+-Ca^{2+} exchange. However, the present protocol does not allow differentiation between a direct action on the exchanger or a secondary homeostatic change in ionic balance. Indeed, Moore *et al.* (1993) have shown that in toad stomach smooth muscle cells there is a high degree of co-localization of the Na^+-K^+ pump and Na^+-Ca^{2+} exchanger within specific domains of the cell membrane. This spatial clustering may facilitate interactions between the Na^+-K^+ pump and Na^+-Ca^{2+} exchanger.

Several studies have demonstrated that high concentrations of mannitol and sucrose also activate Na^+-H^+ exchange (Whalley *et al.*, 1991; Dascalu *et al.*, 1992; Toda *et al.*, 1992; Soleimani *et al.*, 1995). In rat aorta EIPA blocks the Na^+-H^+ antiporter with an IC_{50} of 19 nM and also inhibits Na^+-Ca^{2+} exchange with an IC_{50} of 261 μM (Bingham-Smith *et al.*, 1991). At the concentrations employed in the present study it is therefore highly selective for Na^+-H^+ exchange. EIPA was found to inhibit iohexol-induced vasorelaxation of rabbit aorta to a similar degree as ouabain. Nevertheless, two lines of evidence indicate that these two inhibitors block IRCM-induced vasodilatation via different mechanisms: (i) iodixanol-induced relaxation was unaffected by the presence of EIPA and (ii) combined incubation with both inhibitors synergistically attenuated iohexol-induced relaxation, whereas the effects of ouabain against iodixanol-induced vasodilatation were not enhanced by EIPA. Stimulation of Na^+-H^+ exchange elevates intracellular pH ($[pH]_i$), which mediates relaxation of rat aorta precontracted with PhE or K^+ (Danthuluri & Deth, 1989) and in cardiac myocytes causes a fall in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by 60% (Kim *et al.*, 1987). Levitsky and Benevolensky (1986) showed that elevated $[pH]_i$ enhanced Ca^{2+} sequestration by cardiac sarcoplasmic reticulum. This potentially provides an explanation for one component of the relaxation induced by iohexol in rabbit aorta. The differences in the ability of EIPA to alter the vasorelaxant properties of iohexol and iodixanol is a novel finding that remains to be clarified fully.

Several aspects of our findings with IRCM are analogous to those of Toda *et al.* (1992) who found that mannitol-induced relaxation of monkey cerebral arteries was inhibited (40–50%) in the presence of ouabain or amiloride (a relatively non-selective inhibitor of Na^+-H^+ exchange) and almost abolished by incubation in Na^+ free buffer. Clearly, therefore, there are major differences between artery types as in the present study with rabbit aorta ouabain and EIPA exerted no significant inhibitory effect on mannitol-induced relaxation, and the degree of relaxation elicited by mannitol was markedly different from that obtained by Toda *et al.* (100% cf. ~15%).

In conclusion, interactions between multiple ion transport systems contribute to the vasorelaxant properties of iohexol and iodixanol in rabbit aorta. Both agents appear to stimulate the Na^+-K^+ ATPase pump which hyperpolarizes the vascular smooth muscle and hence diminishes Ca^{2+} influx. IRCM may also cause relaxation by lowering $[Ca^{2+}]_i$ as a consequence of altered Na^+-Ca^{2+} exchange. In the case of iohexol, modulation of Na^+-H^+ exchange additionally participates in relaxation of this tissue. This is the first study to show that the effects of monomeric and dimeric contrast media on vascular smooth muscle exhibit specific pharmacological differences.

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