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Short communication

Lack of inhibition of glyceryl trinitrate by diphenyleneiodonium in bovine coronary artery

Ivan S. De la Lande *, Theron Philp, Irene Stafford, John D. Horowitz

Cardiology Unit, The Queen Elizabeth Hospital, University of Adelaide, 28 Woodville Road, Woodville South, Adelaide, South Australia 5011, Australia

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Abstract

Recent studies indicate that diphenyleneiodonium is a potent inhibitor of glyceryl trinitrate-induced relaxation in rat aorta precontracted with phenylephrine. We have explored the generality of this action in bovine coronary artery precontracted with the thromboxane A_2 mimetic, 9,11-dideoxy-11α,9α-epoxy-methano-prostaglandin $F_{2\alpha}$ (U46619). Diphenyleneiodonium 0.3 μM was without effect (endothelium absent) or caused mild potentiation (0.3 μM or 10 μM; endothelium present) of the relaxant response to glyceryl trinitrate. Lack of inhibition was not due to U46619, since inhibition was still prominent in rat aorta precontracted with this agent. It is concluded that diphenyleneiodonium distinguishes between cellular mechanisms mediating vasodilator responses to glyceryl trinitrate in rat aorta and bovine coronary artery.

Keywords: Diphenyleneiodonium; Glyceryl trinitrate; Artery, bovine coronary; Aorta, rat

1. Introduction

Diphenyleneiodonium sulphate, an inhibitor of the NADPH oxidase system (Holland et al., 1973; Cross and Jones, 1986; Doussiere and Vignais, 1992), was shown recently to inhibit the relaxant response of the rat aorta to glyceryl trinitrate (Bennett et al., 1994; McGuire et al., 1994). The effect of diphenyleneiodonium was accompanied by inhibition of cyclic GMP formation and inhibition of the metabolism of glyceryl trinitrate, and hence constitutes evidence for a major role of the cytochrome P_{450}-NADPH cytochrome P_{450} reductase system in the biotransformation of glyceryl trinitrate to its active metabolite in rat aorta. In view of the potential value of such an inhibitor in analysing the mechanism of action of glyceryl trinitrate in the coronary vasculature, we have explored the action of diphenyleneiodonium on the bovine isolated coronary artery. Earlier studies on glyceryl trinitrate biotransformation in this vessel have focussed on the mu isozyme of glutathione-*S*-transferase with evidence both for (Lau et al., 1992) and against (Fung et al., 1992) a role of this enzyme.

2. Materials and methods

2.1. Materials

Acetylcholine hydrochloride, bradykinin acetate, indomethacin, *N*-ω-nitro-L-arginine (NOLA), L-phenylephrine hydrochloride and 9,11-dideoxy-11α,9α-epoxy-methano-prostaglandin $F_{2\alpha}$ (U46619) were purchased from Sigma (St. Louis, MO, USA). Glyceryl trinitrate was purchased from Fisons, Australia. Diphenyleneiodonium sulphate was purchased from Colour Your Enzyme (Ontario, Canada). Stock solutions were made up in either ethanol (glyceryl trinitrate, indomethacin, U46619) or distilled water (acetylcholine, bradykinin, diphenyleneiodonium, NOLA, phenylephrine) and stored at −20°C. Dilutions of the stock solutions were made in distilled water and maintained on ice. Krebs-bicarbonate solution was of the following composition (mM): NaCl (118), KCl (3.89), $NaHCO_3$ (25), KH_2PO_4 (1.18), $MgCl_2$ (1.05), $CaCl_2$ (2.52), EDTA (0.01) and glucose (5.55), pH 7.4. Krebs solution in which the NaCl was replaced with iso-osmolar

* Corresponding author. Tel.: (61-8) 8222-7635; Fax: (61-8) 8222-6030.

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KCl is referred to as potassium physiological salt solution (KPSS).

2.2. Isolated artery ring preparations

Rats were exsanguinated under halothane anaesthesia and thoracic aorta removed. Segments (3 mm) were mounted in organ baths (15 ml volume). Tension was recorded isometrically via two luminally placed wires one of which was fixed and the other attached to a Grass FTO3 transducer. Baseline tension was 2 g. Vessels were dissected immediately and stored in ice-cold Krebs solution.

Bovine hearts were obtained from an abattoir. Segments (3 mm) of the epicardial left anterior descending artery were set up in a similar fashion to the rat aorta segments. Baseline tension in one series of experiments was 3.3 g but was increased to 4.5 g after separate studies in the laboratory indicated that the latter tension corresponded more closely to the IC_{90}, i.e., the circumference that was 90% of the value when the intraluminal distending pressure was 100 mmHg.

In some experiments the endothelium was removed by inserting a cotton bud on a wooden stem through the lumen.

2.3. Measurements of relaxation

After equilibration for approximately 1 h when the baseline tension was stable, the preparation was contracted with KPSS to assess viability. A concentration-response curve was then elicited to either phenylephrine (in one series of experiments in rat aorta) or to the thromboxane A_2 mimetic, U46619 (in all other experiments) in order to establish a concentration eliciting a steady-state response in a high but submaximal range (60–90%). Acetylcholine (0.3–1.0 μM, in rat aorta) or bradykinin (0.01–0.1 μM, in bovine artery) was then applied to assess the functional integrity of the endothelium. After bath washout of the agents and application of the contractile agent, concentration-response curves to the relaxant effects of glyceryl trinitrate, commencing with 1.0 nM, were elicited. With one exception, diphenyleneiodonium 0.3 μM was added 15 min before the glyceryl trinitrate. The exception was in experiments where NOLA (100 μM) and indomethacin (10 μM) were present throughout, and diphenyleneiodonium (10 μM) was added 30–40 min before the glyceryl trinitrate. In all experiments, pairs of arteries were used, one of which was exposed to diphenyleneiodonium.

2.4. Data analysis

EC_{50} and E_{max} values were calculated from the concentration-response curves to glyceryl trinitrate. The curves were fitted to a sigmoidal relationship by the non-linear regression program Graph Pad Prism 1.01. EC_{50} refers to the concentration of glyceryl trinitrate eliciting 50% of the maximum relaxation. Significance ($P < 0.05$) of the effect of diphenyleneiodonium was assessed by Student's paired t-test. n refers to the number of animals.

3. Results

3.1. Rat aorta

In vessels precontracted with phenylephrine, diphenyleneiodonium (0.3 μM) caused a marked (approximately 15-fold) rightward shift of the glyceryl trinitrate concentration-response curve (Fig. 1A), in agreement with the results of McGuire et al. (1994). In arteries precontracted with U46619, interpretation of diphenyleneiodonium's action was complicated by tendencies for responses to glyceryl trinitrate in the control but not in the diphenyleneiodonium-treated aortas to fade from peak values before attaining steady-state levels (particularly evident in the 0.3–3 μM range). In addition the concentration-response curve in the control vessels appeared to reach a maximum in the vicinity of 1.0 μM, although it was probable that this was a plateau rather than a true maximum since vessels relaxed further to glyceryl trinitrate at high concentrations (30, 100 μM) (Fig. 1B). In the presence of diphenyleneiodonium (0.3 μM), there was no indication of a plateau in the concentration-response curve and responses in the upper region of the curve

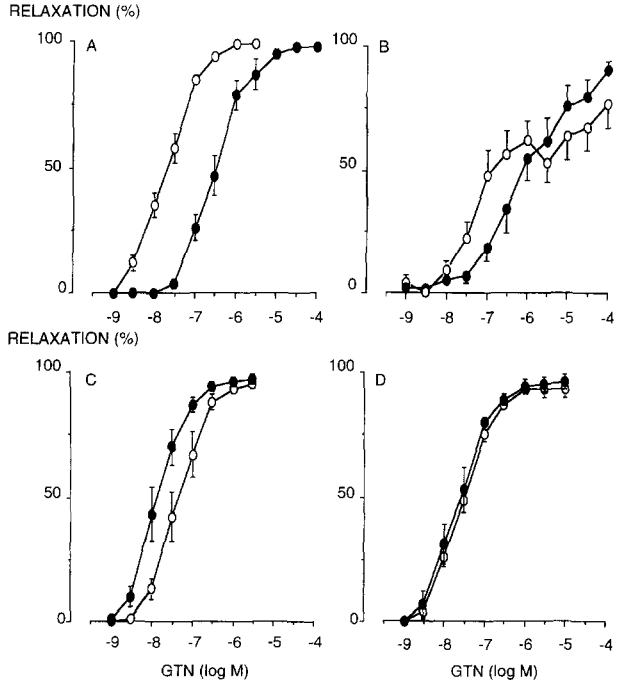

Fig. 1. Concentration-relaxant response curves to glyceryl trinitrate (GTN) in control (○) and diphenyleneiodonium-treated (0.3 μM; ●) arteries. (A) Rat aorta contracted with phenylephrine. (B) Rat aorta contracted with U46619. (C) Bovine coronary artery contracted with U46619. (D) Bovine coronary artery, endothelium denuded, contracted with U46619.

Table 1
Effect of diphenyleneiodonium [a] on responses to glyceryl trinitrate

Artery	n	Contractile agent	Endothelium	EC_{50} (nM)		E_{max} (%)	
				Control	DPI [a]	Control	DPI [a]
Rat aorta	5	PE [c]	+	23 (13–41)	31 [d] (119–809)	98 ± 1	97 ± 1
Rat aorta	8	U46619	+	50 (29–86)	597 [d] (189–1886)	60 ± 9	82 ± 7 [d]
Bovine coronary	6	U46619	+	38 (20–69)	13 [e] (4–38)	99 ± 1	97 ± 2
Bovine coronary	5	U46619	−	23 (13–41)	21 (13–34)	93 ± 3	96 ± 3
Bovine coronary [b]	5	U46619	+	17 (12–26)	9 [d] (7–11)	87 ± 2	94 ± 1 [d]

The EC_{50} is expressed as the mean (with 95% confidence limits). The E_{max} is the percentage maximum effect expressed as mean ± S.E.M.
[a] Diphenyleneiodonium (DPI) is 0.3 μM in all experiments except (b) where the concentration is 10 μM.
[b] NOLA (100 μM) plus indomethacin (10 μM) present in these experiments only.
[c] Phenylephrine (PE).
[d] Effect of diphenyleneiodonium significant, $P < 0.05$, paired t-test.
[e] $P = 0.08$. n = number of animals.

tended to exceed those on the control vessels (significant at 30 μM). However, diphenyleneiodonium depressed responses in the lower region of the curve (significant at 30, 100 and 300 nM). The extent of inhibition, when based on a comparison of EC_{50} values where the value in the control vessel corresponded to 50% of the plateau response, appeared to be comparable with inhibition in the vessels contracted with phenylephrine (Table 1).

3.2. Bovine coronary artery

The bovine arteries were insensitive to phenylephrine and hence were contracted only with U46619. In endothelium-intact vessels, 0.3 μM diphenyleneiodonium did not inhibit responses to glyceryl trinitrate but instead tended to shift the glyceryl trinitrate concentration-response curve to the left (Fig. 1C). The same tendency was evident when the concentration of diphenyleneiodonium was 10 μM and NOLA (100 μM) and indomethacin (10 μM) were present (Table 1). In endothelium-denuded arteries, this leftward shift was not evident, the concentration-response curves in the absence and presence of diphenyleneiodonium (0.3 μM) being indistinguishable (Fig. 1D, Table 1).

4. Discussion

The results confirm the marked inhibition by diphenyleneiodonium (0.3 μM) of the relaxant response to glyceryl trinitrate in the rat aorta, but fail to demonstrate a similar effect in the bovine coronary artery. Although this concentration (0.3 μM) was supramaximal for inhibition of relaxation in the rat aorta in the experiments of McGuire et al. (1994), the possibility that it was suboptimal in the bovine coronary artery is rendered unlikely by absence of inhibition when the concentration was increased 33-fold (to 10 μM). It has been shown in other vessels that procedures which depress or eliminate formation of endothelium-derived nitric oxide enhance the vasodilator effects of nitric oxide donors (Pohl and Busse, 1987). Although diphenyleneiodonium exerts an inhibitory action on nitric oxide-synthase (Stuehr et al., 1991; Rand and Li, 1993; Wang et al., 1993) the possibility that this action may have potentiated the responses to glyceryl trinitrate to an extent which masked concomitant inhibition can be excluded on the grounds that diphenyleneiodonium failed to inhibit responses either in the presence of an inhibitor of nitric oxide-synthase (in the experiments with 10 μM diphenyleneiodonium) or following endothelium removal.

In case the absence of inhibition was related to the use of U46619 as a contractile agent, the effects of diphenyleneiodonium were also examined in rat aorta contracted with this agent. Although the rat aorta was less responsive to glyceryl trinitrate under these conditions and displayed evidence of a potentiating effect of diphenyleneiodonium at the highest concentration of glyceryl trinitrate, inhibition was still evident in the lower region of the glyceryl trinitrate curve. The result does not exclude a contributary influence of the contractile agent but implies that presence or absence of an inhibitory effect of diphenyleneiodonium is related primarily to the type of vessel.

Diphenyleneiodonium also exerted a mild potentiating effect on responses to glyceryl trinitrate in the bovine vessels. Although this effect was endothelium dependent, it appeared unrelated to the inhibitory action of diphenyleneiodonium on nitric oxide-synthase since it persisted in the vessels exposed to a combination of a nitric oxide-synthase and a cyclo-oxygenase inhibitor (which also excludes an effect of diphenyleneiodonium mediated by prostanoids). It is conceivable that the potentiation is related to inhibition of superoxide free radical (O_2^-) generation by diphenyleneiodonium. Cells or tissues in which the inhibition has been documented include the bovine

coronary endothelium (Mohazzab et al., 1994), rabbit aorta (Pagano et al., 1993; Munzel et al., 1995) and leukocytes (Cross and Jones, 1986). In the rabbit aorta, conditions which reduced O_2^- generation, namely elevation of intracellular superoxide dismutase levels, caused an endothelium-dependent mild increase in the vasorelaxant response to glyceryl trinitrate (Munzel et al., 1995), in accord with other evidence that O_2^- interacts chemically with nitric oxide (Lipton et al., 1993). Despite the potential for multisites of action of diphenyleneiodonium, the complete absence of an inhibitory effect on relaxation means that there is a strong argument against an important role of the NADPH oxidase-cytochrome P_{450} reductase system in the biotransformation of glyceryl trinitrate to nitric oxide in the bovine coronary artery.

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