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Halothane inhibits the pressor effect of diphenyleneiodonium

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1 We have recently found that diphenyleneiodonium (DPI), a novel inhibitor of nitric oxide (NO) synthase, causes pressor and tachycardic responses in pentobarbitone- but not halothane-anaesthetized rats. The present study investigated the mechanism by which halothane suppresses the pressor response of DPI. The effects of halothane on the pressor response of DPI were also compared with those of other anaesthetic agents.

2 In conscious rats, i.v. bolus injections of DPI (0.025– 1.6 mg kg^{-1}) caused dose-dependent increases in mean arterial pressure (MAP), with ED_{50} of 0.07 ± 0.01 mg kg^{-1} and maximal rise of MAP (E_{max}) of 59 ± 2 mmHg. While ketamine potentiated E_{max} without altering the ED_{50} and pentobarbitone increased the ED_{50} without changing E_{max} of the pressor response to DPI, chloralose, urethane and ethanol displaced the curve to the right and potentiated E_{max}. In contrast, halothane (0.5–1.25%) dose-dependently and non-competitively reduced the pressor responses to DPI.

3 Intravenous bolus injection of a single dose of DPI (1.6 mg kg^{-1}) caused immediate and large increases in plasma noradrenaline and adrenaline, as well as MAP in conscious rats. Halothane (1.25%) almost completely inhibited these increases.

4 The results suggest that DPI causes a pressor response in conscious rats by activating the sympathetic nervous system and halothane abolishes this pressor response by inhibiting activities of the sympathetic nervous system. The results also show that influences of anaesthetics must be taken into consideration when evaluating pressor response of vasoactive agents.

Keywords: Diphenyleneiodonium (DPI); nitric oxide synthase inhibitor; anaesthetics; halothane; pentobarbitone; ketamine; urethane; ethanol; chloralose; pressor; sympathetic nervous system; noradrenaline; adrenaline; endothelium-dependent vasodilatation

Introduction

Diphenyleneiodonium (DPI), a bivalent iodine compound, was first reported to be a potent hypoglycaemic agent (Stewart & Hanly, 1969; Gatley & Martin, 1979). It was later shown to suppress the activities of neutrophil and macrophage NADPH-dependent oxidase (Cross & Jones, 1986), as well as macrophage nitric oxide (NO) synthase (Stuehr *et al.*, 1991), probably via the inhibition of a flavoprotein (Hancock & Jones, 1987; Ellis *et al.*, 1989; Stuehr *et al.*, 1990). DPI was also found to inhibit endothelium-dependent vasodilatation *in vitro* (Stuehr *et al.*, 1991; Poon *et al.*, 1993) and *in vivo* (Poon *et al.*, 1993). Recently, we found that DPI caused pressor and tachycardic effects in pentobarbitone-anaesthetized rats; the cardiovascular effects were due to the modulation of sympathetic nerve activities (Wang & Pang, 1993). In our preliminary experiments, we also found that halothane markedly suppressed the pressor effect of DPI.

Halothane has been shown to inhibit sympathetic nervous transmission at several levels (Seagard *et al.*, 1982; Larach *et al.*, 1987; Rorie *et al.*, 1990). Halothane was also found to alter endothelium-dependent vasodilatation (Muldoon *et al.*, 1988; Blaise, 1991), and to abolish the pressor responses of other NO synthase inhibitors, namely N^G-nitro-L-arginine (L-NNA) and its methyl ester (L-NAME) (Wang *et al.*, 1991a; Pang *et al.*, 1992). The aim of this study was to (1) investigate the characteristics of the inhibitory effect of halothane on the pressor response to DPI; (2) to examine whether halothane suppresses the pressor response of DPI by inhibiting activities of the sympathetic nervous system; (3) to compare the effect of halothane with those of intravenous and inhalation anaesthetic agents on the pressor response of DPI.

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Methods

Surgical preparation

Sprague-Dawley rats (300–360 g) were used in this study. Cannulae (PE50) were inserted into the left iliac vein for the administration of drugs, and into the left iliac artery for the recordings of mean arterial pressure (MAP) by a P23DB pressure transducer (Gould Statham, CA, U.S.A.) and heart rate which was determined electronically from the upstroke of the arterial pulse pressure using a tachograph (Grass, Model 7P4G). In some rats, another PE50 cannula was also inserted into the right iliac artery to collect blood samples. The body temperature of the anaesthetized rats was maintained at 37°C with a heating lamp connected to a thermostat (73A, Yellow Springs Instruments). In halothane-anaesthetized rats in Protocol 2 and 3 (see later), tracheostomy was also performed with a PE160 catheter. All anaesthetized rats were equilibrated for 20 min at the appropriate anaesthetic doses before the commencement of studies. The conscious rats in all the protocols were first anaesthetized with halothane (1.5% in air), to allow surgical preparation, and were allowed to recover for at least 6 h from the effects of anaesthesia before further use. The catheters were tunnelled subcutaneously and exteriorized at the back of the neck.

Measurement of plasma catecholamines

Plasma catecholamines levels were determined by a catecholamine Assay kit (Amersham Canada Ltd., Ont., Canada) (Passon & Peuler, 1974). The radioactivity (^3H) was detected by a 1600 TR liquid scintillation analyzer (Packard Instrument Co., CT, U.S.A.). Blood samples (0.5 ml) were immediately placed in prechilled tubes containing EGTA and reduced glutathione and centrifuged at 1,200 *g* at 4°C. Afterwards, the plasma was removed and stored at – 70°C until assayed within two weeks. Duplicate assays were run for the

standard (in blank plasma) and plasma samples (50 μl each) using distilled water as a blank control for each run. The sensitivity of the catecholamine assay was 0.005 ng ml^{-1} for each catecholamine.

Drugs

Halothane and diphenyleneiodonium (DPI) sulphate were obtained from Ayerst Lab. (Quebec, Canada) and Colour Your Enzyme Ltd. (Ont., Canada), respectively. Urethane ethyl carbamate and ketamine hydrochloride were from Sigma Chemical Co. (MO, U.S.A.). The following anaesthetics were also used: α-chloralose (BDH Chemical Ltd., Poole, England), sodium pentobarbitone (M.T.C. Pharmaceuticals, Cambridge, Ontario, Canada) and anhydrous ethyl alcohol (Stanchem Co., Quebec, Canada). The powder drugs were dissolved in normal saline (0.9% NaCl) except for DPI which was solubilized in 5% glucose solution by 10 min sonication.

Experimental protocol

The rats ($n = 6$ each group) were randomly assigned into groups. MAP and HR were continuously monitored throughout the experiments.

(1) *Dose-MAP response curves to DPI in conscious and anaesthetized rats* Six groups of rats were used: conscious rats and rats anaesthetized with sodium pentobarbitone (65 mg kg^{-1}), ketamine (140 mg kg^{-1}), urethane (2 g kg^{-1}), ethanol (4 g kg^{-1}) and chloralose (150 mg kg^{-1}). Dose-MAP response curves to i.v. bolus injections of DPI (0.025–1.6 mg kg^{-1}) were constructed at dose-intervals of 3–6 min, the time required to recover from the effects of the previous dose.

(2) *Dose-MAP response curves of DPI in conscious and halothane-anaesthetized rats* Five groups of rats were used: conscious rat and rats anaesthetized with different concentrations of halothane (0.5, 0.75, 1 and 1.25% in air) at flow rates of 1500 ml min^{-1}. The dose-MAP response curves of i.v. bolus injections of DPI (0.025–6.4 mg kg^{-1}) were constructed at dose-intervals of 3–6 min as above.

(3) *Effects of DPI on plasma catecholamine levels in conscious and halothane-anaesthetized rats* One group of conscious rats and one group of halothane (1.25%)-anaesthetized rats were injected i.v. with a single bolus dose of DPI (1.6 mg kg^{-1}). Blood samples were collected 20 min prior to and 30 s after the injections of DPI. MAP was continuously monitored.

Calculation and statistical analysis

Maximum effect (E_{max}), half-effective dose (ED_{50}) and Hill coefficient (n), from individual dose-response curves were fitted by a computer programme using non-linear least-squares to the relation $Y = a + bx$, where Y = response and $x = [D]^n/(ED_{50}^n + [D]^n)$ (Quastel & Saint, 1988; Wang & Pang, 1993). All results were expressed as mean ± standard error (s.e.mean) and analyzed by the analysis of variance followed by Duncan's multiple range test with $P < 0.05$ selected as the criterion for statistical significance.

Results

Effects of anaesthetics on DPI-induced MAP response

Baseline MAP in rats anaesthetized with urethane, ethanol and chloralose, but not pentobarbitone or ketamine, was less than that in conscious rats (Table 1).

In conscious rats, DPI caused an immediate (approximately 15 s in onset) and transient (1–2 min in duration) pressor response. The pressure response was dose-dependent (Figure 1) with ED_{50} of 0.07 ± 0.01 mg kg^{-1} and maximal MAP reached at 59 ± 2 mmHg, based on the best-fitted calculations (Table 1). The Hill coefficient n of 3.3 ± 0.5 was significantly different from 1, 2 and 5 but not from 3 or 4. DPI also caused tachycardia at lower doses (0.025–0.1 mg kg^{-1}), bradycardia at higher doses (0.2–1.6 mg kg^{-1}) and movements following the onset of the pressor response (data not shown).

Pentobarbitone, chloralose, urethane and ethanol but not ketamine displaced the dose-MAP curve of DPI to the right (Figure 1) by increasing ED_{50}s (Table 1). On the other hand, ketamine, chloralose, urethane and ethanol but not pentobarbitone potentiated the maximal MAP response to DPI (Figure 1 and Table 2). None of the anaesthetic agents significantly altered the n value of the dose-response curves of DPI (Table 1). Under the influence of all anaesthetics, the hindlimbs and occasionally the forelimbs displayed kicking motion immediately following injections of DPI.

Inhibitory effect of halothane on DPI pressor response

Halothane (0.5–1.25%) reduced baseline MAP in a dose-dependent manner (Table 1). In conscious rats, i.v. bolus injections of DPI also caused similar pressor responses as in protocol (1) (Figure 2 and Table 1). Halothane dose-dependently reduced the maximal effect of DPI and shifted the DPI curve to the right (Figure 2). ED_{50} values were linearly correlated while E_{max} values were inversely correlated with the

Table 1 Values of baseline mean arterial pressure (MAP), as well as parameters (Hill coefficient n, ED_{50} and E_{max}) of the dose-pressor response curves of diphenyleneiodonium (DPI) in conscious rats and rats anaesthetized with sodium pentobarbitone (65 mg kg^{-1}), ketamine (140 mg kg^{-1}), urethane (2 g kg^{-1}), ethanol (4 g kg^{-1}), chloralose (150 mg kg^{-1}) and halothane (0.5–1.25%)

Anaesthetics	*Baseline MAP* mmHg	*Dose-response curve to DPI* *n*	ED_{50} (mg kg^{-1})	E_{max} (mmHg)
Protocol 1				
Conscious	118 ± 2	3.3 ± 0.5	0.07 ± 0.01	59 ± 2
Ketamine	107 ± 7	3.2 ± 0.4	0.08 ± 0.01	72 ± 2*
Pentobarbitone	111 ± 6	3.3 ± 0.7	0.22 ± 0.04*	64 ± 4
Chloralose	89 ± 3*	3.7 ± 0.4	0.12 ± 0.01*	90 ± 4*
Urethane	72 ± 7*	2.9 ± 0.6	0.20 ± 0.03*	71 ± 4*
Ethanol	57 ± 6*	2.5 ± 0.4	0.26 ± 0.03*	78 ± 4*
Protocol 2				
Conscious	113 ± 4	2.9 ± 0.5	0.07 ± 0.01	59 ± 2
0.5% Halothane	96 ± 10	2.6 ± 0.2	0.48 ± 0.09*	62 ± 4
0.75% Halothane	83 ± 4*	2.7 ± 0.5	0.57 ± 0.08*	47 ± 4*
1% Halothane	80 ± 7*	3.8 ± 0.7	0.77 ± 0.15*	34 ± 3*
1.25% Halothane	69 ± 2*	–	–	13 ± 4*,[a]

Values are mean ± s.e.mean $n = 6$ for each group
*denotes significant difference from the conscious rat groups ($P < 0.05$). [a]represents observed data.

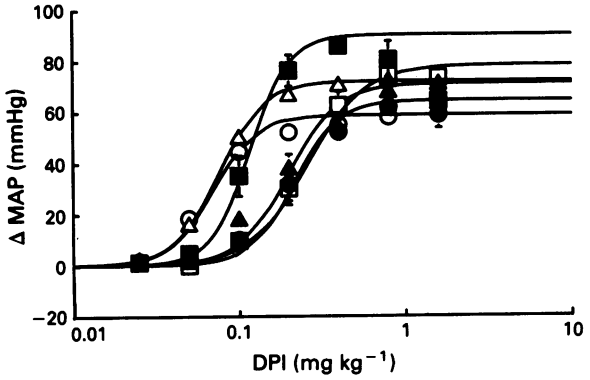

Figure 1 Dose-response (mean ± s.e.mean) curves of i.v. bolus injections of diphenyleneiodonium (DPI) on mean arterial pressure (MAP) in conscious rats (○), and rats anaesthetized with pentobarbitone (65 mg kg^{-1}) (●), ketamine (140 mg kg^{-1}) (△), urethane (2 g kg^{-1}) (▲), ethanol (4 g kg^{-1}) (□) and chloralose (150 mg kg^{-1}) (■). $n = 6$ each group. The lines represent theoretical curves using the formula $Y = E_{max}([D]^n/ED_{50}^n + [D]^n)$ and best-fitted n, ED_{50} and E_{max} shown in Table 1.

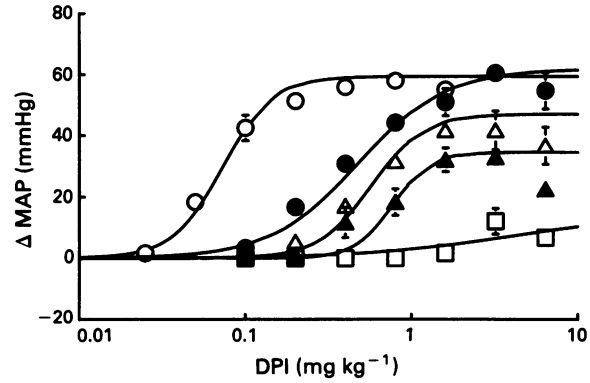

Figure 2 Dose-response (mean ± s.e.mean) curves of i.v. bolus injections of diphenyleneiodonium (DPI) on mean arterial pressure (MAP) in conscious rats and halothane (0.5–1.25%)-anaesthetized rats ($n = 6$ each group). Halothane: 0 (○); 0.5% (●); 0.75% (△); 1% (▲); 1.25% (□). The lines represent theoretical curves using the formula $Y = E_{max}([D]^n/ED_{50}^n + [D]^n)$ and best-fitted n, ED_{50} and E_{max} shown in Table 1.

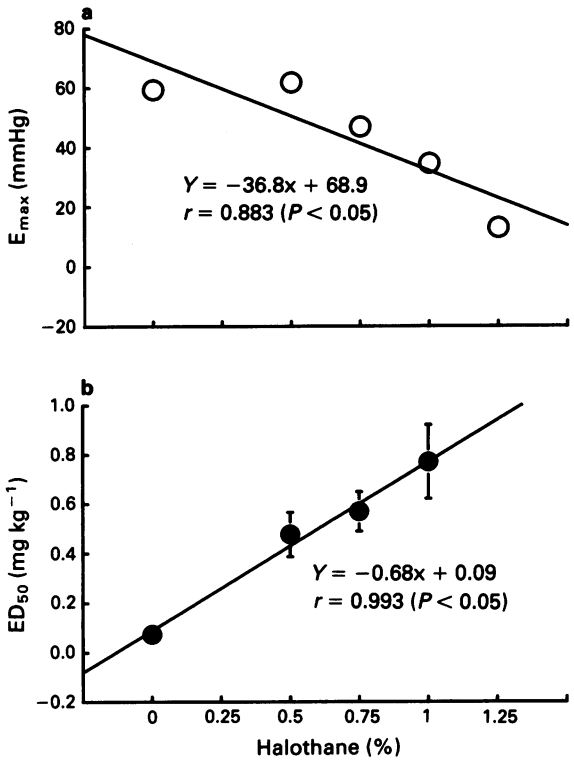

Figure 3 Effects of halothane (0.5–1.25%) on the maximal effect (E_{max}, a) and half-effective dose (ED_{50}, b) of the dose-mean arterial pressure response curves of i.v. bolus injections of diphenyleneiodonium (0.025–6.4 mg kg^{-1}) in rats ($n = 6$ each group).

Table 2 Baseline values of plasma catecholamines and mean arterial pressure (MAP) in conscious and halothane (1.25%)-anaesthetized rats

	Plasma catacholamine (ng ml^{-1})			
	Noradrenaline	*Adrenaline*	*Dopamine*	*MAP*
Conscious	0.26 ± 0.05	0.12 ± 0.01	0.10 ± 0.01	119 ± 4
Halothane	0.25 ± 0.03	0.06 ± 0.01*	0.10 ± 0.01	76 ± 2*

Values are mean ± s.e.mean. $n = 6$ each group.
*denotes significant difference from conscious rats ($P < 0.05$).

concentration of halothane (Figure 3). None of the doses of halothane affected the n value of the curves (Table 1). Halothane also inhibited DPI-induced tachycardia, bradycardia as well as movements (data not shown).

Effect of halothane on DPI-induced catecholamine release

Baseline MAP in halothane (1.25%)-anaesthetized rats was lower than that in conscious rats (Table 2). Baseline plasma level of adrenaline but not noradrenaline or dopamine in halothane-anaesthetized rats was also significantly lower than that in conscious rats (Table 2).

In conscious rats, i.v. bolus injection of DPI (1.6 mg kg^{-1}) caused immediate and large increases (more than 1 ng ml^{-1}) in plasma noradrenaline and adrenaline and a smaller increase (0.1 ng ml^{-1}) in plasma dopamine (Figure 4a), as well as immediate pressor response (Figure 4b). Halothane markedly attenuated DPI-induced increases in plasma catecholamines (Figure 4a) and in MAP (Figure 4b); the reductions of plasma noradrenaline, adrenaline and MAP were 86%, 81% and 95%, respectively.

Discussion

As in previous experiments with pentobarbitone-anaesthetized rats (Wang & Pang, 1993), DPI caused transient and dose-dependent increases in MAP in conscious rats with a Hill coefficient not significantly different from 3 or 4. Moreover, none of the anaesthetics affected the Hill coefficient value. The results suggest that the pressor effects of DPI in conscious and anaesthetized rats are due to the positive cooperation of 3 or 4 molecules of DPI with the corresponding 'receptors' (see Rang, 1971; see discussion of Pennefather & Quastel, 1982; Wang & Pang, 1993).

Although DPI inhibits endothelium-dependent vasodilatation *in vitro* and *in vivo* (Stuehr *et al.*, 1991; Poon *et al.*, 1993), the pressor response to DPI is not a consequence of this inhibition. In a previous study, we found that DPI caused pressor and tachycardic responses by sympathetic stimulation as reflected by concurrent elevations of blood pressure and plasma noradrenaline and adrenaline (Wang & Pang, 1993). Moreover, the pressor response of DPI was attenuated by manoeuvres which impair sympathetic nerve transmission,

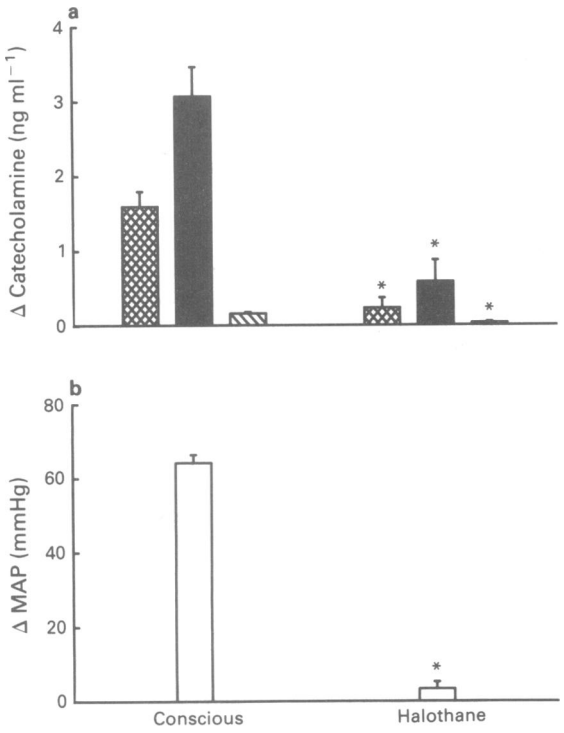

Figure 4 Effects (mean ± s.e.mean) of i.v. bolus injection of diphenyleneiodonium (1.6 mg kg^{-1}) on plasma catecholamines (a) and mean aterial pressure (MAP, b) in conscious and halothane (1.25%)-anaesthetized rats ($n = 6$ each group). In (a), noradrenaline: cross hatched columns; adrenaline: solid columns; dopamine; hatched columns.

namely, pithing, spinal cord transection, tetrodotoxin, mecamylamine, guanethidine, reserpine and phentolamine (Wang & Pang, 1993). In accordance with our previous results, the pressor responses of DPI in conscious rats in the present study were also accompanied by large increases in plasma noradrenaline and adrenaline. Although it is not clear how DPI activates sympathetic nerve activities, the following mechanisms are unlikely to be involved. Firstly, the effect of DPI on sympathetic nerve activity is unrelated to its hypoglycaemic action since the blood pressure and sympathetic stimulatory effects of DPI are immediate and transient (present study) while its hypoglycaemic effect is slow in onset (plateau at 4 h after i.p. administration) and prolonged in action (Gatley & Martin, 1979). Secondly, the pressor response to DPI is unlikely due to the activation of 'pain receptors' as pretreatment with capsaicin (100 mg kg^{-1} s.c. for 2 d) blocked DPI-induced limb kicking but not pressor responses in pentobarbitone-anaesthetized rats ($n = 6$, unpublished observations). Thirdly, the possible inhibitory effect of DPI on brain NO synthesis is unlikely to be responsible for the increase in sympathetic outflow (see discussion of Wang & Pang, 1993).

DPI caused tachycardia in pentobarbitone-anaesthetized rats, with the same time course and pharmacodynamics (ED_{50} and Hill coefficient) as the pressor response (Wang & Pang, 1993). It should be pointed out that the pressor and tachycardic responses of DPI are not interdependent, as propranolol blocked tachycardia but not the pressor response while phentolamine attenuated the pressor but potentiated the tachycardic response (Wang & Pang, 1993). In conscious rats in the present study, DPI produced tachycardia at low doses and bradycardia at high doses. In pentobarbitone-anaesthetized rats, the latter response was absent (Wang & Pang, 1993). The abilities of pentobarbitone anaesthesia to inhibit bradycardic responses to high doses of DPI and phentolamine to potentiate tachycardic responses suggest that bradycardia in response to high doses of DPI in conscious rats was secondary to hypertension-induced baroreflex activation. Different anaesthetic agents have variable influences on baroreflex activity. Due to the difficulty in separating variable direct and indirect effects of DPI on heart rate in different anaesthetic conditions, detailed kinetic analyses of the heart rate effect on DPI were not performed.

Our results also show that halothane dose-dependently and non-competitively inhibited the pressor responses of DPI. It should be emphasized that the inhibitory effect of halothane is not due to its hypotensive action as DPI caused greater pressor responses in rats anaesthetized with chloralose, urethane or ethanol where baseline blood pressures were either similar or lower than those in halothane-anaesthetized rats. Therefore, anaesthetics, at standard anaesthetic doses, have differential effects on the potency and/or efficacy of the pressor response to DPI, and this can be classified as follows: (1) no change in ED_{50} but potentiation of E_{max} of the pressor response to DPI – ketamine; (2) increase in ED_{50} and no change in E_{max}-pentobarbitone; (3) increase in ED_{50} and potentiation of E_{max} - urethane, ethanol and chloralose; (4) increase in ED_{50} and reduction of E_{max} - halothane. The results suggest that the variable influence of anaesthetic agents must be taken into consideration when evaluating the pressor effects of DPI and other pressor agents (Wang *et al.*, 1991a; Abdelrahman *et al.*, 1992).

Halothane has been shown to potentiate (Blaise, 1991) or reduce (Muldoon *et al.*, 1988) endothelium-dependent vasodilatation in different vascular preparations. We have also found that halothane (1.25%) abolishes the pressor effects of L-NNA and L-NAME but not those of noradrenaline or angiotensin II (Wang *et al.*, 1991a; Pang *et al.*, 1992). Although it appears that halothane has a selective inhibitory effect on NO synthase inhibitors, the mechanism by which it suppresses pressor responses to L-NNA and L-NAME is different from that of DPI. L-NNA and L-NAME cause pressor responses by inhibiting NO synthase and subsequent endothelium-dependent relaxation (Wang *et al.*, 1993a; see Moncada *et al.*, 1991). The pressor responses of L-NNA and L-NAME are L-arginine-sensitive (Wang & Pang, 1990; Wang *et al.*, 1991b; 1992; see Moncada *et al.*, 1991) and are independent of the integrity of the central and sympathetic nervous systems (Wang & Pang, 1991; Wang & Pang, 1993). The mechanism by which halothane inhibits pressor responses to L-NNA may involve selective increase in NO synthesis and/or release, or potentiation of the effect of NO (Wang *et al.*, 1991b; Pang *et al.*, 1992) and, this may be analogous to the inhibitory effect of sodium nitroprusside on the pressor effect of L-NNA (Wang *et al.*, 1993b).

Halothane markedly inhibited DPI-induced increases in both plasma catecholamines and MAP. The results suggest that the inhibitory effect of halothane on the pressor response to DPI is primarily due to the suppression of sympathetic activation rather than inhibition of endothelium-dependent vasodilatation. Halothane has been shown to depress activities of the sympathetic nervous system at different levels (1) areas of the central nervous system controlling sympathetic nerve activity (Price *et al.*, 1963; Millar *et al.*, 1969; Larach *et al.*, 1987; Bazil & Minneman, 1989), (2) sympathetic ganglia (Skovsted *et al.*, 1969; Christ, 1977; Bosnjak *et al.*, 1982; Seagard *et al.*, 1982), and (3) sympathetic nerve endings located in the walls of blood vessels (Muldoon *et al.*, 1975; Lunn & Rorie, 1984; Rorie *et al.*, 1990). In addition, a small component of non-specific inhibition by halothane may also be responsible for its effect on the pressor response to DPI. Halothane, at 1.25%, inhibited the maximal pressor response to DPI by 95%, and the increases in plasma noradrenaline and adrenaline by 86% and 81%, respectively. These results are consistent with those of our other study in which the same concentration of halothane reduced the pressor response produced by exogenous noradrenaline or angiotensin II by 18% (Pang *et al.*, 1992). Therefore, the inhibition by halothane of the pressor response to DPI is primarily (approximately 80%)

attributable to the inhibition of sympathetic transmission and secondarily (approximately 20%) due to non-specific inhibition of vascular smooth muscle contraction.

The influence of ketamine and pentobarbitone on sympathetic transmission may also account for their effects on the pressor response to DPI. Ketamine activates the sympathetic nervous system (Traber & Wilson, 1969) by inhibiting neuronal noradrenaline uptake (Nedergaard, 1973; Clanachan & McGrath, 1976). The blockade of uptake$_1$ by ketamine may cause potentiation of the pressor effect of DPI. This interpretation is consisent with our unpublished observation that the uptake$_1$ inhibitor, cocaine also potentiates pressor and tachycardic responses of DPI ($n = 6$). The inhibitory effect of pentobarbitone may involve the suppression of catecholamine release since pentobarbitone has been shown to inhibit the release of noradrenaline from the peripheral sympathetic nerve terminals in rabbit and chicken isolated hearts (Gothert & Rieckesmann, 1978; see Richter & Holtman, 1982), as well as releases of noradrenaline and adrenaline from the perfused cow adrenal glands (see Richter & Holtman, 1982).

In summary, i.v. bolus injections of DPI caused immediate and transient pressor responses in conscious rats; the pressor response was accompanied by large increases in plasma noradrenaline and adrenaline. Among the various anaesthetics (ketamine, urethane, ethanol, chloralose) studied, halothane is the only one which dose-dependently and non-competitively inhibits DPI-induced pressor response and the rises in plasma noradrenaline and adrenaline. The results suggest that (1) DPI causes a pressor response by stimulating sympathetic nerve activities in conscious rats; (2) halothane inhibits the pressor response of DPI by suppressing sympathetic nerve activities rather than interfering with endothelium-dependent vasodilatation; (3) the variable effects of anaesthetics must be taken into consideration when assessing the pressor and heart rate responses of DPI and other vasoactive agents.

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References

ABDELRAHMAN, A., WANG, Y.-X. & PANG, C.C.Y. (1992). Effects of anaesthetics on pressor response to β-blockers in the rat. *J. Pharm. Pharmacol.*, **44**, 34–38.

BAZIL, C.W. & MINNEMAN, K.P. (1989). Clinical concentrations of volatile anesthetics reduce depolarization-evoked release of [^{3}H] norepinephrine, but not [^{3}H] acetylcholine, from rat cerebral cortex. *J. Neurochem.*, **53**, 962–965.

BLAISE, G.A. (1991). Effect of volatile anaesthetic agents on endothelium-dependent relaxation. *Adv. Exp. Med. Biol.*, **301**, 229–235.

BOSNJAK, Z.J., SEAGARD, J.L., WU, A. & KAMPINE, J.P. (1982). The effects of halothane on sympathetic ganglionic transmission. *Anesthesiology*, **57**, 473–479.

CHRIST, D. (1977). Effects of halothane on ganglionic discharges. *J. Pharmacol. Exp. Ther.*, **200**, 336–342.

CLANACHAN, A.S. & MCGRATH, J.C. (1976). Effects of ketamine on the peripheral autonomic nervous system of the rat. *Br. J. Pharmacol.*, **58**, 247–252.

CROSS, A.R. & JONES, O.T.G. (1986). The effect of the inhibitor diphenylene iodonium on the superoxide-generating system of neutrophils. *Biochem. J.*, **237**, 111–116.

ELLIS, J.A., CROSS, A.R. & JONES, T.G. (1989). Studies on the electron-transfer mechanism of the human neutrophil NADPH oxidase. *Biochem J.*, **262**, 575–579.

GATLEY, S.J. & MARTIN, J.L. (1979). Some aspects of the pharmacology of diphenyleneiodonium, a bivalent iodine compound. *Xenobiotica*, **9**, 539–546.

GOTHERT, M. & RIECKESMANN, J.-M. (1978). Inhibition of noradrenaline release from sympathetic nerves by pentobarbital. *Experientia*, **15**, 382–384.

HANCOCK, J.T. & JONES, T.G. (1987). The inhibition by diphenyleneiodonium and its analogues of superoxide generation by macrophages. *Biochem. J.*, **242**, 103–107.

LARACH, D.R., GREGG SCHULER, H., DERR, J.A., LARACH, M.G., HENSLEY, F.A. & ZELIS, R. (1987). Halothane selectively attenuates α_2-adrenoceptor mediated vasoconstriction *in vivo* and *in vitro*. *Anesthesiology*, **66**, 781–789.

LUNN, J.J. & RORIE, D.K. (1984). Halothane-induced changes in the release and disposition of norepinephrine at adrenergic nerve endings in dog saphenous vein. *Anesthesiology*, **61**, 377–384.

MILLAR, R.A., WARDEN, J.C., COOPERMAN, L.H. & PRICE, L.H. (1969). Central sympathetic discharge and mean arterial pressure during halothane anaesthesia. *Br. J. Anaesth.*, **41**, 918–928.

MONCADA, S., PALMER, R.M.J. & HIGGS, E. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

MULDOON, S.M., HART, J.L., BROWN, K.A. & FFREAS, W. (1988). Attenuation of endothelium-mediated vasodilation by halothane. *Anesthesiology*, **68**, 31–37.

NEDERGAARD, O.A. (1973). Cocaine-like effect of ketamine on vascular adrenergic neurones. *Eur. J. Pharmacol.*, **23**, 153–161.

PANG, C.C.Y., WANG, Y.-X. & ABDELRAHMAN, A. (1992). Halothane attenuates pressor and hemodynamic effects of N^G-nitro-L-arginine (L-NNA) in rats (abstract). *FASEB. J.*, **6**, A1298.

PASSON, P.G. & PEULER, J.D. (1973). A simple radiometric assay for plasma norepinephrine and epinephrine. *Anal. Biochem.*, **51**, 618–631.

PENNEFATHER, P. & QUASTEL, D.M.J. (1982). Desensitization of the nicotinic receptor at the mouse neuromuscular junction. *Br. J. Pharmacol.*, **77**, 395–404.

POON, C.I., WANG, Y.-X., POON, K.S. & PANG, C.C.Y. (1993). Inhibitory effects of diphenyleneiodonium on endothelium-dependent vasodilatation *in vitro* and *in vivo* (abstract). *FASEB. J.*, (in press).

PRICE, H.L., PRICE, M.L. & MORSE, H.T. (1969). Central nervous actions of halothane affecting systemic circulation. *Anesthesiology*, **24**, 770–778.

QUASTEL, D.M.J. & SAINT, D.A. (1988). Transmitter release at mouse motor nerve terminals mediated by temporary accumulation of intracellular barium. *J. Physiol.*, **406**, 55–73.

RANG, H.P. (1972). Drug receptors and their function. *Nature*, **231**, 91–96.

RICHTER, J.A. & HOLTMAN, J.R. Jr. (1982). Barbiturates: their in vivo effects and potential biochemical mechanism. *Prog. Neurobiol.*, **18**, 275–319.

RORIE, D.K., HUNTER, L.W. & LUNN, J.J. (1990). Halothane decreases the release of neuropeptide Y and 3,4-dihydroxyphenylglycol from superfused segments of dog pulmonary artery. *Anesthesiology*, **73**, 722–30.

SEAGARD, J.L., HOPP, F.A., DONERAN, J.H., KALBFLEISCH, J.H. & KAMPINE, J.P. (1982). Halothane and carotid sinus reflex: evidence for multiple sites of action. *Anesthesiology*, **57**, 191–202.

SKOVSTED, P., PRICE, M.L. & PRICE, H.L. (1969). The effects of halothane on arterial pressure, preganglionic sympathetic activity and barostatic reflexes. *Anesthesiology*, **31**, 507–514.

STEWART, G.A. & HANLEY, T. (1969). Other oral hypoglycemic agents. In *Oral Hypoglycemia Agents:* Pharmacology and Therapeutics. ed. Campbell, G.D. pp. 398–407. London and New York: Acadmic Press.

STUEHR, D.J., FASEHUM, O.A., KWON, N.S., GROSS, S.S., FONZALEZ, J.A., LEVI, R. & NATHAN, C.F. (1991). Inhibition of macrophage and endothelial cell nitric oxide synthase by diphenyleneiodonium and its analogs. *FASEB. J.*, **5**, 98–103.

STUEHR, D.J., FASEHUM, O.A., KWON, N.S. & NATHAN, C.F. (1990). FAD and GSH participate in macrophage synthesis of nitric oxide. *Biochem. Biophys. Res. Commun.*, **168**, 558–565.

TRABER, D.L. & WILLSON, R.D. (1969). Involvement of the sympathetic nervous system in the pressor response to ketamine. *Anaesth. Analg.*, **48**, 248–252.

WANG, Y.-X. & PANG, C.C.Y. (1990). Pressor effect of N^G-nitro-L-arginine in pentobarbital-anesthetized rats. *Life Sci.*, **47**, 2217–2224.

WANG, Y.-X. & PANG, C.C.Y. (1991). Possible dependance of pressor and heart rate effects of N^G-nitro-L-arginine on autonomic nerve activity. *Br. J. Pharmacol.*, **103**, 2004–2008.

WANG, Y.-X. & PANG, C.C.Y. (1993). Functional integrity of the central and sympathetic nervous systems is a prerequisite for pressor and tachycardic effects of diphenyleneiodonium, a novel inhibitor of nitric oxide synthase. *J. Pharmacol. Exp. Ther.*, (in press).

WANG, Y.-X., POON, C.I. & PANG, C.C.Y. (1993a). *In vitro* and *ex vivo* inhibitory effects of L and D enantiomers of N^G-nitro-arginine of endothelium-dependent relaxation of rat aorta. *J. Pharmacol. Exp. Ther.*, (in press).

WANG, Y.-X., POON, C.I., YAU, L.C.Y. & PANG, C.C.Y. (1992). The *in vivo* and *in vitro* vasoconstrictor effects of L and D enantiomers of N^G-nitro-arginine methyl ester (NAME) (abstract). *The 35th Annual Meeting of Canadian Federation of Biological Societies*, Victoria, Canada. p.80.

WANG, Y.-X., ZHOU, T., CHUA, T.C. & PANG, C.C.Y. (1991a). Effects of inhalation and intravenous anaesthetic agents on pressor response to N^G-nitro-L-arginine. *Eur. J. Pharmacol.*, **198**, 180–185.

WANG, Y.-X., ZHOU, T. & PANG, C.C.Y. (1991b). Pressor effects of L and D enantiomers of N^G-nitro-arginine in conscious rats are antagonized by L- but not D-arginine. *Eur. J. Pharmacol.*, **200**, 77–81.

WANG, Y.-X., ZHOU, T. & PANG, C.C.Y. (1993b). A comparison of the inhibitory effects of sodium nitroprusside, pinacidal and nifedipine on pressor response to N^G-nitro-L-arginine. *Br. J. Pharmacol.*, **108**, 398–404.

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