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Catecholamine release and potentiation of thromboxane A_2 production by nicotine in the greyhound

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1 Thromboxane A_2 was generated by infusing arachidonic acid (2.5 $\mu g\,ml^{-1}$) into an extra-corporeal circuit of blood withdrawn from anaesthetized dogs, and assayed on a blood-bathed bioassay cascade of porcine and bovine coronary artery strips, chick rectum and rat stomach strip. All tissues except chick rectum were treated with phentolamine and propranolol to abolish direct effects of catecholamines.

2 The arachidonate-induced contractions of artery strips were abolished by a thromboxane synthetase inhibitor UK-38485 (3 $mg\,kg^{-1}$, i.v.), but were not altered by the 5-hydroxytryptamine antagonist ketanserin (10 μM) administered over the tissues.

3 Intravenous infusion of adrenaline (0.2 and 0.4 $\mu g\,kg^{-1}\,min^{-1}$) reversibly potentiated the coronary contractions produced by arachidonate, but did not alter contractions when applied directly over the bioassay tissues.

4 Intra-aortic infusion of nicotine (5 or 10 $\mu g\,kg^{-1}\,min^{-1}$) also increased the arachidonate-induced contractions of the bioassay tissues but only on those experiments where nicotine caused appreciable adrenaline release, as indicated by relaxation of chick rectum.

5 Phenoxybenzamine (2 $mg\,kg^{-1}$, i.v.) blocked the potentiation effect of adrenaline and nicotine on coronary contractions. The specific α_2-adrenoceptor antagonist, idazoxan (1 $mg\,kg^{-1}$, i.v.), also blocked nicotine-induced potentiation of the contractions.

6 These findings suggest that the ability of nicotine to potentiate thromboxane release from circulating platelets and blood cells is dependent upon the release of adrenaline, and probably involves an action on α-adrenoceptors of the circulating blood elements.

Introduction

Nicotine is an important ingredient of tobacco smoke, representing over 95% of its alkaloidal content. It is well established that the acute cardiovascular effects of nicotine infusions in man are similar to those produced by smoking, but these responses are elicited mainly by reflex stimulation of peripheral nicotine-sensitive sensory receptors, such as those in the carotid and aortic bodies (Comroe, 1964). Nicotine also stimulates release of catecholamines from the adrenal medullary chromaffin cells, and from sympathetic nerve endings and extra-medullary chromaffin cells in the heart (Watts, 1960; Kershbaum & Bellet, 1964; Rose, 1973). Cigarette smoking in man has also been shown to elevate circulating levels of catecholamines, particularly adrenaline (Cryer *et al.*, 1976; Seiss *et al.*, 1982). However, there is no clear evidence for a link between release of catecholamines and the adverse cardiovascular effects of smoking.

Recently in our laboratory it has been found that infusion of adrenaline and other catecholamines in rat isolated hearts, greatly potentiates the release of thromboxane A_2 from platelets perfused through the heart (Purchase *et al.*, 1985). We set out to study whether nicotine would also produce this effect via endogenous catecholamine release *in vivo*. We chose anaesthetized dogs for this study as thromboxane A_2 can be produced in an extracorporeal circuit of blood in this model (Mullane *et al.*, 1979).

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Methods

Greyhound dogs (22–23 kg) of either sex were anaesthetized with intravenous thiopentone (25–30 mg kg^{-1}) followed by α-cloralose (70 mg kg^{-1}, i.v.). Ventilation was artificially maintained with air plus additional oxygen to maintain arterial blood gases and pH in the following ranges: Po_2 150–225 mmHg; Pco_2 25–37 mmHg; pH 7.25–7.36. Systemic arterial pressure was measured from the left femoral artery using a Druck pressure transducer. A continuous measurement of heart rate was obtained from the pulse interval using a cardiotachometer coupler. A slow intravenous infusion of Hartmann's solution (Travenol) was maintained throughout the experiments. Polythene cannulae were tied into the right femoral artery and external jugular vein for removal and replacement of blood. Drugs were administered intravenously, via a femoral vein, or intra-arterially through a catheter passed up the right femoral artery with its tip resting above the origin of the adrenal arteries. Heparin (1,000 iu kg^{-1}) was injected intravenously.

Bioassay tissues

The bioassay tissues used were isolated chick rectum (CR), spiral strips of porcine and bovine coronary artery (PCA and BCA) as well as rat stomach strip (RSS). The tissues were superfused at 10 ml min^{-1} initially with Krebs solution containing indomethacin (3 μM) and 2 to 2.5 h later with blood from the dog. The blood passed through an incubation coil of tubing for 60 s before it reached the bioassay tissues. The blood was collected in a reservoir and returned continuously by gravity into the jugular vein. The tissues, with the exception of CR, were also superfused with a mixture of phentolamine (0.3 μM) and propranolol (1 μM) throughout the experiment to block the direct effects of catecholamines on these tissues.

Materials

The following drugs were used: adrenaline (David Bull), arachidonic acid (Sigma), (15S)-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 13E-dienoic acid (U46619, Upjohn), 5-hydroxytryptamine (Sigma), idazoxan (Reckitt & Coleman), ketanserin (Janssen), UK-38485 3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid, Pfizer), nicotine sulphate (Sigma), phenoxybenzamine hydrochloride (Smith, Kline and French), phentolamine mesylate (Ciba), propranolol hydrochloride (Sigma) and prostaglandin E_2 (Upjohn). Arachidonic acid was stored in *n*-hexane (10 mg ml^{-1}) under nitrogen at − 20°C. Immediately before use the solvent was evaporated under nitrogen and the arachidonic acid dissolved in 0.1 M sodium carbonate, making the sodium salt. All other drugs were diluted, when necessary, with 0.9% (w/v) NaCl solution (saline) immediately before use.

Statistical analysis

Differences between control responses and responses during or after drug treatment were compared by the paired *t* test.

Results

Responses to arachidonic acid infusion

Infusion of arachidonic acid (2.5 μg ml^{-1} for 4 min; total 100 μg) into the incubation coil (60 s) caused contraction of all tissues, but had no significant effects on the systemic blood pressure or heart rate of the dog (Figure 1). The contractile responses could be elicited repeatedly and were reproducible for several hours. U46619 (0.05–0.2 μg) and 5-hydroxytryptamine (5-HT; 1–2 μg), given as bolus doses directly over the blood bathed tissues, contracted all except CR, but 5-HT was much weaker in eliciting tissue contraction. Prostaglandin E_2 (PGE_2; 0.1 μg) caused contractions of CR and RSS only, and the response to arachidonate infusion was mimicked by the combination of U46619 and PGE_2.

Effects of UK-38485 and ketanserin on arachidonic acid responses

Intravenous administration of a specific thromboxane synthetase inhibitor, UK-38485 (3 mg kg^{-1}) abolished the arachidonate-induced contractions of PCA and BCA, but not those of CR and RSS ($n = 4$, Figure 1). The CR and RSS contractions were mimicked by PGE_2 (0.03–0.1 μg) given directly over the tissues. Similar effects were obtained by Mullane *et al.* (1979) using imidazole to block thromboxane synthesis. UK-38485 did not produce any significant cardiovascular effects on the dog. Ketanserin, (10 μM, superfused over the tissues) abolished the contractile responses of the tissues to 5-HT, but did not alter those elicited by arachidonic acid ($n = 6$, Figure 2).

Effects of adrenaline and nicotine on arachidonic acid responses

Intravenous administration of adrenaline (0.2 and 0.4 μg kg^{-1} min^{-1}) to the dog increased systemic blood pressure (Table 1) and this gradually returned to control level after the infusion was stopped. Adrenaline infusion always caused relaxation of the CR but it had no direct effect on the other tissues which were continuously superfused with phentolamine and

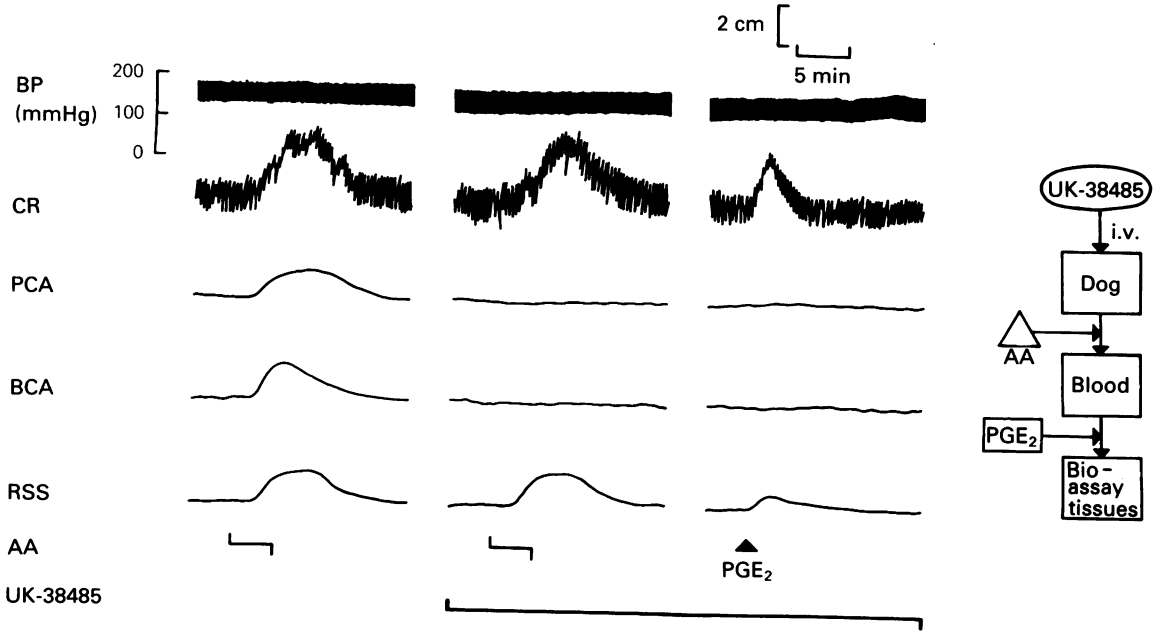

Figure 1 Infusion of arachidonic acid (AA; 2.5 $\mu g\,ml^{-1}$, indicated by the horizontal bars) into the incubation coil contracted the chick rectum (CR), porcine and bovine coronary artery strips (PCA and BCA) and rat stomach strip (RSS) without producing any significant effects on blood pressure (BP) in the anaesthetized dog (left panel). UK-38485 (3 $mg\,kg^{-1}$ i.v.), a specific thromboxane synthetase inhibitor, abolished the arachidonate-induced contractions of PCA and BCA, but not those of CR and RSS (middle panel). The CR and RSS responses were mimicked by prostaglandin E_2 (PGE_2; 100 ng) given directly over the tissues (right panel).

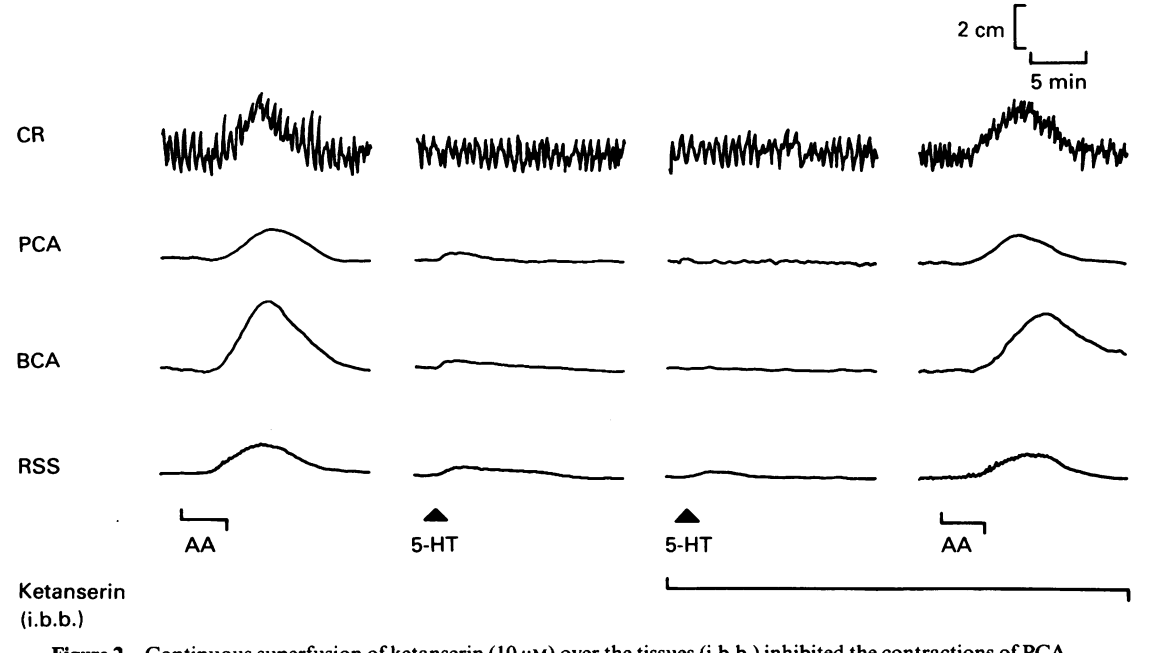

Figure 2 Continuous superfusion of ketanserin (10 μM) over the tissues (i.b.b.) inhibited the contractions of PCA, BCA and RSS induced by 5-hydroxytryptamine (5-HT; 1 μg) but not those elicited by infusion of arachidonic acid (AA; 2.5 $\mu g\,ml^{-1}$). For abbreviations see legend to Figure 1.

Table 1 Maximum effects on mean blood pressure and heart rate of adrenaline infused intravenously, and nicotine infused into the abdominal aorta

Infusion (rate $\mu g\,kg^{-1}\,min^{-1}$)	n	*Mean blood pressure* (mmHg)		*Heart rate* (beats min^{-1})	
		Control	*During infusion*	*Control*	*During infusion*
Adrenaline (0.2)	12	151 ± 4	163 ± 5**	121 ± 7	110 ± 9
Adrenaline (0.4)	5	159 ± 8	172 ± 9*	116 ± 3	109 ± 4
Nicotine (5)	7	144 ± 6	168 ± 7*	119 ± 6	119 ± 5
Nicotine 10	10	159 ± 7	188 ± 9*	126 ± 9	125 ± 7

* $P<0.05$, ** $P<0.001$, by the paired t test.

propranolol (Figure 3). Infusion of adrenaline also significantly enhanced the contractions of the bioassay tissues induced by arachidonic acid. The potentiation was reversible, and the tissue responses returned to control magnitude upon cessation of adrenaline infusion (Figures 3 and 4). The potentiation was not clearly dose-dependent over this narrow range of adrenaline concentrations, and therefore for quantification the responses for the 2 infusion rates were pooled (Figure 5).

Nicotine (5 and 10 $\mu g\,kg^{-1}\,min^{-1}$) infused into the abdominal aorta of the dog increased the blood pressure, but this returned to the control level despite continuous infusion of the drug (Table 1). In 9 out of 17 experiments, nicotine infusion caused relaxation of the CR, an effect very similar to that seen with adrenaline infusion. In these experiments, the arachidonate-induced contractions of the bioassay tissues, when pooled for the two nicotine infusions, were also significantly increased (Figures 4 and 5). The potentiation, like that induced by adrenaline, was reversible. On the other hand, in those experiments where nicotine infusion did not relax the CR, it failed to augment the tissue responses to arachidonic acid infusion (Figure 5).

Superfusion of adrenaline ($1-5\,ng\,kg^{-1}\,min^{-1}$; range $3-15\,ng\,ml^{-1}$) directly to the bioassay tissues caused marked relaxation of the CR but did not significantly alter the contractions induced by arachidonic acid infusion ($n=4$, Figure 3). Similarly, nicotine ($0.1-0.5\,\mu g\,kg^{-1}\,min^{-1}$; range $0.3-1.5\,\mu g\,ml^{-1}$) superfused directly onto the tissues had no effect on them and did not alter arachidonic acid-induced contractions ($n=3$).

Effect of phenoxybenzamine and idazoxan on action of adrenaline and nicotine

Intravenous administration of phenoxybenzamine ($2\,mg\,kg^{-1}$) markedly reduced the arterial blood pressure and heart rate of the dog, but did not exert any significant effect on the tissue responses to arachidonic acid ($n=6$, Figure 5). In dogs that had been treated with phenoxybenzamine, the arachidonate-induced contractions of PCA were not enhanced during intravenous infusion of adrenaline ($0.2\,\mu g\,kg^{-1}\,min^{-1}$). Similarly, in experiments where nicotine augmented initially the arachidonate-induced tissue responses, subsequent treatment with phenoxybenzamine abolished this potentiating effect of nicotine ($n=6$, Figure 6). Similar effects were observed on the contractions produced by arachidonate on the other tissues. After control responses to arachidonic acid and nicotine were studied, 5 dogs were given the specific α_2-adrenoceptor antagonist idazoxan ($1\,mg\,kg^{-1}$, i.v.). As with phenoxybenzamine, arachidonate-induced contractions of the tissues were not altered, but nicotine infusion no longer potentiated the contractions (Table 2).

Discussion

In the present study, thromboxane A_2 was generated by infusing arachidonic acid into the extracorporeal circulation so that it was incubated in the blood for 60 s before reaching the bioassay tissues. Mullane *et al.* (1979) characterized the thromboxane A_2 formed in a similar extracorporeal circuit in anaesthetized dogs. We confirmed that thromboxane A_2 was responsible for the contractile responses of the coronary artery strips, since UK-38485, a specific thromboxane synthetase inhibitor (Parry *et al.*, 1982), abolished the coronary artery contractions produced by arachidonic acid. Moreover, contractions of the coronary artery strips were mimicked by the thromboxane mimetic, U46619. It is unlikely that platelet-derived 5-HT contributed to the coronary contractions for ketanserin, a potent

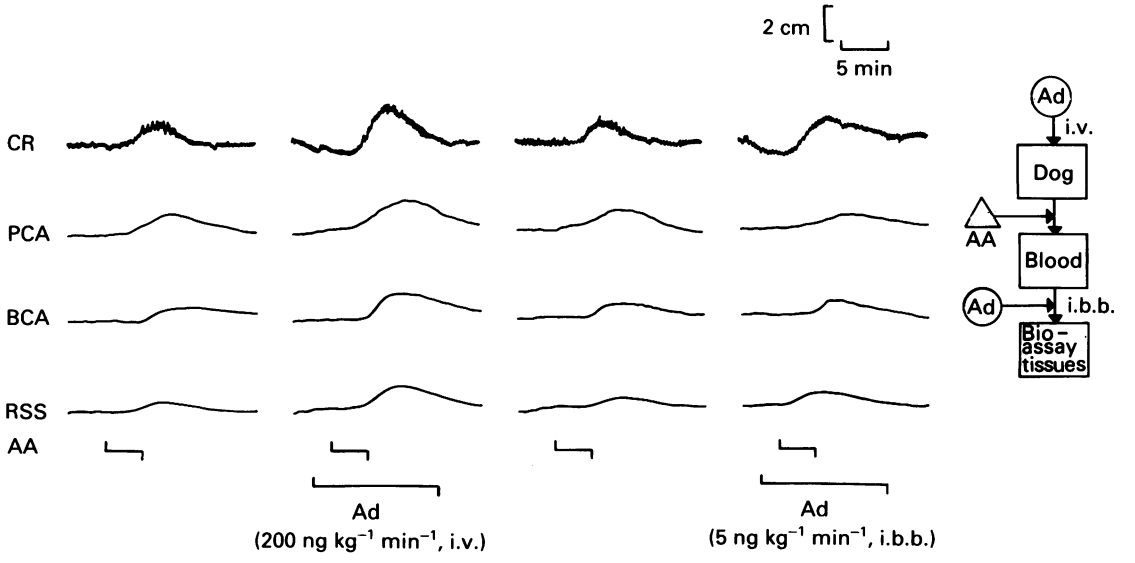

Figure 3 Intravenous infusion of adrenaline (Ad; 0.2 μg kg^{-1} min^{-1}) to the anaesthetized dog relaxed the CR and augmented the arachidonate-induced contractions of all bioassay tissues. The potentiation was reversible as shown in the third panel. Direct superfusion of adrenaline (5 ng kg^{-1} min^{-1}, equivalent to 15 ng ml^{-1}) over the tissues (i.b.b.) caused marked relaxation of the CR, but it did not augment the contractions elicited by arachidonic acid (AA; 2.5 μg ml^{-1}) infusion (fourth panel). For abbreviations see legend to Figure 1.

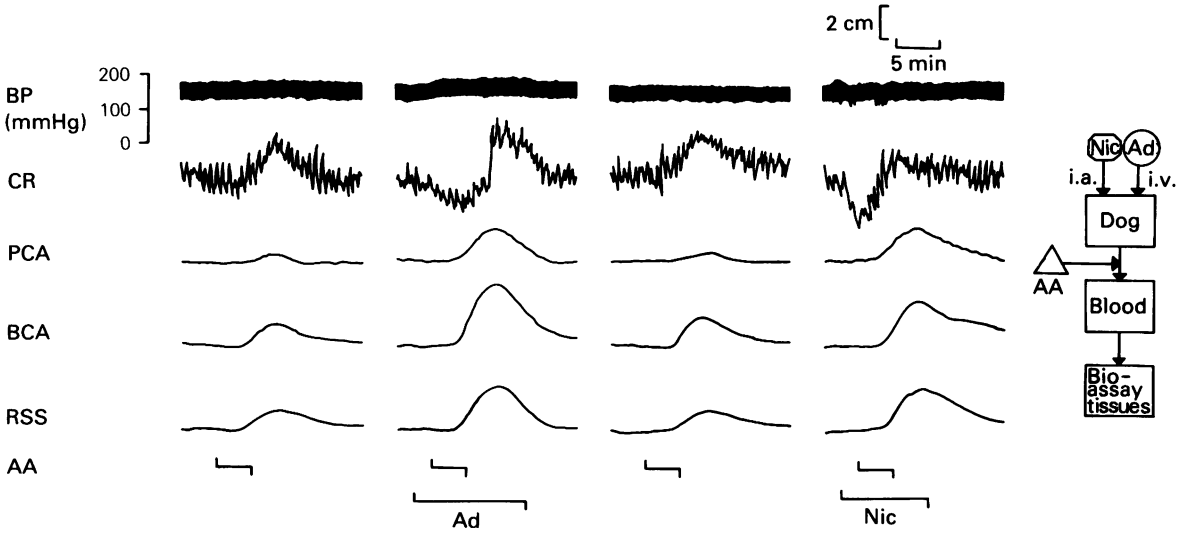

Figure 4 Adrenaline (Ad; 0.2 μg kg^{-1} min^{-1}) infused intravenously to the dog elevated the blood pressure, relaxed the CR and markedly enhanced contractions of the bioassay tissues in response to arachidonic acid (AA; 2.5 μg ml^{-1}) infusion (first two panels). In this experiment nicotine (Nic; 5 μg kg^{-1} min^{-1}) infused into the abdominal aorta (i.a.) had minor effects on the blood pressure, relaxed CR and potentiated the arachidonate-induced contractions of the bioassay tissues (last panel). For abbreviations see legend to Figure 1.

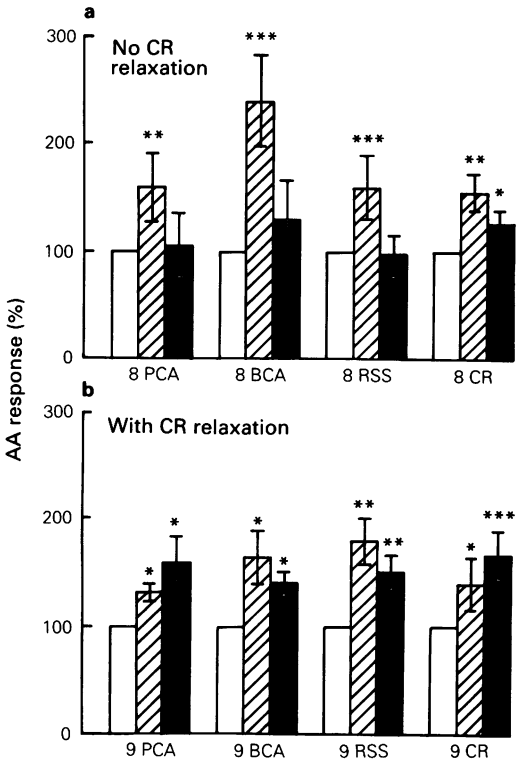

Figure 5 Histograms showing the effects of infusing adrenaline (0.2 or 0.4 $\mu g\,kg^{-1}\,min^{-1}$ i.v., hatched columns) and nicotine (5 or 10 $\mu g\,kg^{-1}\,min^{-1}$ i.a., closed columns) on the arachidonic acid (AA)-induced contractions of bioassay tissues. Contractions of the tissues are expressed as percentages of the control response (open columns) in each experiment and are pooled for the two doses of adrenaline and nicotine. The number beneath each column indicates the number of experiments and the vertical bars are the s.e.means. In experiments where nicotine infusion did not cause relaxations of the chick rectum (CR), it failed to potentiate the arachidonate-induced responses of PCA, BCA or RSS (a). In experiments where nicotine infusion caused CR relaxation, it significantly enhanced the arachidonate-induced contractions of bioassay tissues (b). Intravenous adrenaline always relaxed the CR and potentiated the tissue responses to arachidonic acid infusion. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, paired t test. For abbreviations see legend to Figure 1.

antagonist of 5-HT on coronary arteries (Brazenor & Angus, 1980), failed to alter the arachidonate-induced coronary contractions. Thromboxane A_2 is probably mainly derived from circulating platelets, but leucocytes may also contribute in this system.

The contractions of the bioassay tissues produced by arachidonic acid infusion were significantly enhanced by adrenaline infused intravenously to the dog,

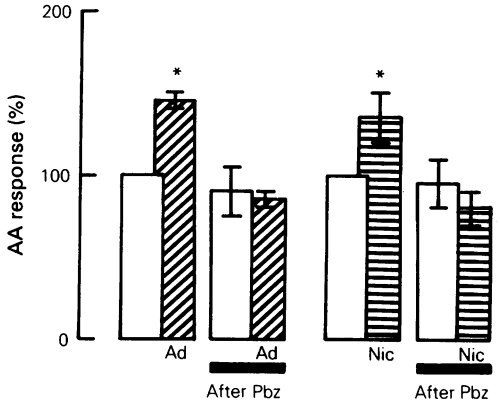

Figure 6 Inhibition by phenoxybenzamine (Pbz; 2 $mg\,kg^{-1}$, i.v.) of the potentiating effect of adrenaline (Ad; 0.2 $\mu g\,kg^{-1}\,min^{-1}$, i.v.) and nicotine (Nic; 10 $\mu g\,kg^{-1}\,min^{-1}$, i.a.) on arachidonate-induced contractions of blood-bathed porcine coronary artery strips (PCA). Responses are expressed as percentages of control (open columns) in 6 dogs as in Figure 3. Similar effects were observed on the other tissues.

suggesting that there was an increased production or release of thromboxane A_2 from the formed elements of the blood. We have recently found that extremely low concentrations of adrenaline and other catecholamines greatly increased the amount of thromboxane A_2 released by arachidonic acid from platelets

Table 2 Effect of intravenous administration of idazoxan (1 $mg\,kg^{-1}$) on the nicotine-induced potentiation of contractions elicited by infusion of arachidonic acid into the extracorporeal circulation

	Before idazoxan		*After idazoxan*	
Tissue	*Control*	*During nicotine infusion*	*Control*	*During nicotine infusion*
CR ($n=4$)	100	188 ± 28*	117 ± 3	118 ± 3
PCA ($n=5$)	100	152 ± 12*	85 ± 8	80 ± 8
BCA ($n=4$)	100	188 ± 38*	90 ± 13	94 ± 16
RSS ($n=5$)	100	144 ± 15*	95 ± 2	106 ± 7

Contractions of chick rectum (CR), porcine coronary artery (PCA), bovine coronary artery (BCA) and rat stomach strip (RSS) are expressed as percentages of the control responses elicited before administration of idazoxan.
* $P<0.05$, by the paired t test compared with 100%.

perfused through Langendorff preparations of rat and rabbit heart (Purchase *et al.*, 1985), although the precise mechanisms involved remain to be elucidated. We now show that this effect can be produced in circulating blood. It is clear that the amplifying effect of adrenaline on thromboxane-induced coronary vasoconstriction is not a direct effect on the bioassay tissues as adrenaline did not augment the responses to arachidonic acid when it was superfused, in appropriate concentrations, directly over the tissues. However, adrenaline is known to have a number of effects on platelets. In high concentrations (in the micromolar range) it induces direct aggregation of human platelets (O'Brien, 1963), although dog platelets in plasma appear to be refractory to this effect (MacMillan & Sim, 1970). At lower concentrations in human platelets adrenaline is known to have synergistic effects with other pro-aggregatory substances (ADP, vasopressin) (Ardlie *et al.*, 1966). Moreover, dog platelets that are refractory to arachidonic acid may be induced to aggregate and release 5-HT in the presence of adrenaline (Johnson *et al.*, 1979), but in this study thromboxane A_2 release was not measured. It is therefore possible that potentiation of platelet aggregation may account for the increased thromboxane A_2 synthesis in our experiments.

The major finding in this study was that infusion of nicotine also enhanced the release of thromboxane A_2 from circulating blood elements. Alster & Wennmalm (1981) found that nicotine does not directly influence platelet thromboxane synthesis in platelet rich plasma, nor does it affect conversion of ^{14}C-arachidonate in platelet microsomes. There is, however, some evidence that tobacco smoking may increase the ability of platelets to aggregate and the circulating level of the platelet-specific protein β-thromboglobulin (Levine, 1973; Schmidt & Rasmussen, 1984). It has also been reported that collagen-stimulated thromboxane B_2 formation and plasma thromboxane B_2 are increased marginally after the smoking of two cigarettes by normal subjects (Sturm *et al.*, 1984). These effects of smoking could be caused by nicotine.

A number of observations in our study suggest that nicotine-induced potentiation of thromboxane release was mediated indirectly via the release of endogenous catecholamines, particularly adrenaline. Firstly, potentiation of the contractile responses was observed only in those experiments where nicotine elicited relaxation of the chick rectum, which indicates release of adrenaline in the dog (Vane, 1969). In experiments where nicotine did not cause any relaxation of the chick rectum, thus indicating minimal release of endogenous catecholamine, it failed to increase the arachidonate-induced contractions of the bioassay tissues. Secondly, nicotine did not increase the arachidonate-induced contractions when superfused directly over the bioassay tissues. Thirdly, the potentiating effect of both adrenaline and nicotine was abolished by pretreating the dogs with phenoxybenzamine and idazoxan, indicating that stimulation of α-adrenoceptors was an intermediate step common to the mechanism of potentiation by both compounds.

Nicotine has very weak effects on isolated large coronary arteries from dogs, pigs and cattle (unpublished observations). However, *in vivo*, nicotine could markedly potentiate coronary vasoconstriction in atherosclerotic vessels by mobilizing adrenaline and thereby augmenting release of thromboxane A_2 from activated platelets. This effect might be of particular significance in vessels with deficient production of prostacyclin, since this prostanoid appears to modulate the effects of vasoconstrictor stimuli in large coronary vessels (Dusting & Angus, 1984). Folts & Bonebrake (1982) have also shown that inhalation of cigarette smoke and nicotine can potentiate platelet thrombus formation in stenosed coronary arteries in dogs. These effects were prevented by phentolamine, suggesting that they also involved an indirect action mediated via circulating catecholamines. Clearly these studies have implications for the possible mechanisms of coronary vasospasm and myocardial ischaemia in man.

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