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Thromboxane-mimetic U46619 causes depressor responses in anaesthetized rats

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Abstract—Intravenous (i.v.) or intra-arterial injections of U46619, a thromboxane A_2 (TxA_2)-mimetic agent, into chloralose-anaesthetized rats dose-dependently decreased the arterial blood pressure. Indomethacin (8 mg kg^{-1}) or atropine (1 mg kg^{-1}), given i.v. 30 min beforehand, attenuated the hypotensive effect of U46619 i.v. whereas methysergide pretreatment (5 mg kg^{-1} i.v.) was without action. Pretreatment with AH23848 (5 mg kg^{-1} i.v.), a specific TxA_2-receptor antagonist, completely abolished the depressor responses to U46619. The findings suggest that the vasodepressor effect of U46619 appears to be mediated via TxA_2-receptor activation, with the release of prostacyclin and/or acetylcholine both of which produce vasodilatation.

Thromboxane A_2 (TxA_2) is a vasoactive metabolite of arachidonic acid. It is a potent vasoconstrictor and inducer of platelet aggregation. Stable analogues of TxA_2 have been developed and used as tools to study its effects; U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin A_2) a stable endoperoxide analogue, has been shown to be a selective TxA_2-like agonist (Coleman et al 1980, 1981).

Numerous studies, employing in-vitro preparations, have demonstrated the vasoconstricting and pro-aggregatory properties of U46619 (Burke et al 1983; Bove et al 1986; Quilley et al 1989). Investigations in our laboratory using U46619, injected intravenously (i.v.) into anaesthetized rats, have yielded the surprising observation that it causes vasodepressor responses.

The present study was, therefore, undertaken to examine the vasodepressor effect of U46619 in anaesthetized rats.

Materials and methods

Male Sprague-Dawley rats, 230–250 g, were housed in a room with controlled temperature (22 ± 1°C) and humidity (65–70%). At the start of experiments, anaesthesia was induced with diethyl ether (BDH), and maintained with intravenous (i.v.) injections of chloralose (BDH, 80 mg kg^{-1}), supplemented when necessary with further doses of 10 mg kg^{-1}. The rats were kept warm with a heating lamp; the trachea was then cannulated. Drugs were administered via a cannulated right jugular vein. Arterial blood pressure was recorded by a Statham P23ID pressure transducer connected to the left common carotid artery, and was displayed on a physiograph (MK-IV, Narco Bio-Systems). For the intra-arterial (i.a.) injection of drugs, a cannula was inserted into the left carotid artery until it reached the aortic arch in order to bypass the lungs; in these experimental animals, the right femoral artery was cannulated for blood pressure recordings.

Rats were randomly divided into groups. A logarithmic series of doses of U46619 (Upjohn) was injected i.v. or i.a, into two separate groups of rats. In a third batch of rats, after obtaining in each animal dose-dependent responses to U46619 i.v., methysergide (Sandoz) (5 μg kg^{-1} i.v.) was given before the same doses of U46619 were repeated 30 min later. Similar experiments were carried out in the fourth, fifth and sixth groups of rats, using a different i.v.-administered antagonist for each group, namely indomethacin (Sigma, 8 mg kg^{-1}); atropine (Sigma, 1 mg kg^{-1}) and AH23848, (1α(z),2β,5α)-7-(5-((1,1-biphenyl)-4-yl)methoxy)-2-(4-morpholinyl)-3-oxocyclopentyl)-4-heptenoic acid) (Glaxo, 5 mg kg^{-1}).

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All drugs were freshly dissolved in 0·9% w/v NaCl (saline) before use and their weights expressed in terms of their salts.

The data were analysed for significance of differences by Student's *t*-test.

Results

The initial resting systolic and diastolic blood pressures (mean ± s.e.m.) were 128 ± 5 and 95 ± 4 mmHg, respectively. Injections i.v. of methysergide (5 mg kg^{-1}), indomethacin (8 mg kg^{-1}), atropine (1 mg kg^{-1}) or AH23848 (5 mg kg^{-1}) did not significantly influence the resting blood pressure.

When injected i.v. or i.a., U46619 elicited depressor responses in a dose-dependent manner. The magnitude of the hypotensive effect induced by the lower i.a. doses of U46619 (2·5–5 μg kg^{-1}) was significantly greater than that produced by i.v. injection of the drug (Table 1). However, the depressor response to the higher dose of U46619 (10 μg kg^{-1}) given via the i.a. route was not significantly different from that of a same dose administered i.v.

Table 1. Fall in diastolic blood pressure (mm Hg) after intravenous or intra-arterial administration of U46619.

Route of injection	U46619 (μg kg^{-1}) 2·5	5	10
Intravenous	6·6 ± 2·3	12·8 ± 2·4	50·1 ± 5·3
Intra-arterial	15·1 ± 3·1**	24·0 ± 7·5*	51·4 ± 8·7

Each value represents the mean ± s.e.m. of 8 rats. In comparison with the corresponding intravenous response, * $P < 0.05$, ** $P < 0.01$.

Methysergide (5 mg kg^{-1} i.v.) pretreatment did not have any significant effect on the depressor responses to all doses of U46619 given i.v. (Fig. 1a). However, pretreatment with AH23848 (5 mg kg^{-1} i.v.) completely abolished the hypotensive action of U46619 (2·5–10 μg kg^{-1} i.v.) (Fig. 1d). Indomethacin (8 mg kg^{-1} i.v.) or atropine (1 mg kg^{-1} i.v.) pretreatment produced partial blockade of the depressor responses to U46619 10 μg kg^{-1} i.v. (Fig. 1b, c); the fall in diastolic pressure following U46619 (10 μg kg^{-1}) was attenuated by only 50% (indomethacin) and 41% (atropine) of their control values. AH23848 (5 mg kg^{-1}), injected after indomethacin or atropine pretreatment, completely abolished the residual responses to U46619 which were seen after indomethacin or atropine.

Discussion

As found previously (unpublished results), U46619 injected i.v. or i.a. into chloralose-anaesthetized rats evoked vasodepressor responses instead of the pressor effect usually produced by a vasoconstrictor. The present observation that the depressor responses to the lower doses of U46619 given directly into the aortic arch were significantly greater than those induced by similar i.v. doses suggests that U46619 may be partly removed by the lungs. The complete inhibition of this hypotensive effect of U46619 by pretreatment with AH23848, a potent and specific TxA_2-receptor antagonist (Brittain et al 1984; Humphrey & Lumley 1984), points to the likelihood that the vasodepressor

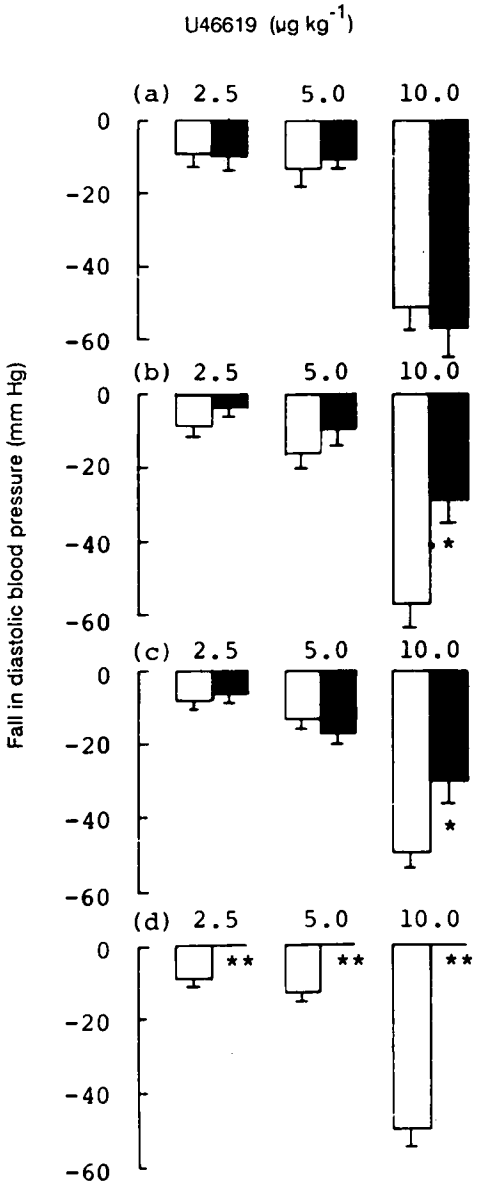

FIG. 1. Vasodepressor effects of U46619 injected i.v. before □ and after ■ 30 min pretreatment with (a) methysergide (5 mg kg^{-1} i.v.), (b) indomethacin (8 mg kg^{-1} i.v.), (c) atropine (1 mg kg^{-1} i.v.) or (d) AH23848 (5 mg kg^{-1} i.v.). Each column represents the mean of 10 rats, bars indicate s.e.m. $*P<0.05$, $**P<0.001$, compared with values of the corresponding doses of U46619 before antagonist pretreatment.

effect was due to an action of the agonist on TxA_2-receptors. Partial abolition of the depressor responses to U46619 by indomethacin indicates that the effect may be due, at least partly, to the release of a cyclo-oxygenase product, most probably prostacyclin (PGI_2) which is a potent vasodilator and the major metabolite of arachidonic acid in vasculature (Moncada & Vane 1979). Indeed, the possible release of PGI_2 by the endoperoxide analogue U46619 has already been reported. Mehta et al (1984) have shown that infusion of U46619 in anaesthetized dogs causes the release of PGI_2 which they consider to have an autoregulatory function. Others (Nicholson et al 1984) have demonstrated a dose-dependent increase in PGI_2 formation by incubating U46619 with cultured bovine endothelial cells.

The finding that the depressor effect of U46619 was partly inhibited by pretreatment with atropine but not by methysergide suggests that acetylcholine may be released by U46619, and not 5-hydroxytryptamine. This possibility is in accord with a report that U46619 induces prejunctional release of acetylcholine in canine bronchial smooth muscle (Chung et al 1985).

In summary, the findings from this study suggest that the depressor responses to U46619 in anaesthetized rats, possibly mediated via TxA_2-receptor stimulation, could partly be due to the resulting release of vasodilators, i.e. PGI_2 and/or acetylcholine. Other factors, such as changes in cardiac output, and in venous or pulmonary pressure, may also contribute to the fall in arterial pressure. Further studies are needed.

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