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FAILURE OF ACETALDEHYDE OR ACETATE TO MIMIC THE SPLANCHNIC ARTERIOLAR OR VENULAR DILATOR ACTIONS OF ETHANOL: DIRECT *in situ* STUDIES ON THE MICROCIRCULATION

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The effects of acetaldehyde and sodium acetate on rat mesenteric arterioles (17–25 μm i.d.) and muscular venules (35–50 μm i.d.) were examined *in situ*, at the microcirculatory level, by use of a high-resolution closed circuit television microscope recording system. Local, intravenous or intra-arterial administration of acetaldehyde (1.8–3600 μmol) and sodium acetate (0.25–250 μmol) to mesenteric arterioles and muscular venules of the anaesthetized rat induced dose-dependent vasoconstriction. Systemic administration of a variety of pharmacological antagonists (i.e., phentolamine, diphenhydramine, methysergide, atropine and indomethacin) did not attenuate or prevent the dose-dependent vasoconstrictor actions of these metabolites of ethanol. Our findings do not support the concept that some or all of the peripheral vasodilator actions of ethanol can be attributed to its metabolites, acetaldehyde and acetate.

Introduction Acute ingestion or administration of ethyl alcohol (ethanol) has long been known to exert peripheral vasodilator actions (Wood, 1847; McDowall, 1925; Smith, 1977). More recently, by the use of closed-circuit television microscopy, it has been demonstrated that ethanol can exert potent, dose-dependent dilator effects on splanchnic arterioles and venules, irrespective of whether the alcohol is administered locally, intravenously or intra-arterially (Altura, 1978; Altura, Ogunkoya, Gebrewold & Altura, 1979). Although the latter lends support to the idea that ethanol is a potent splanchnic vasodilator (McDowall, 1925; Horvath & Williard, 1962), it is often suggested that the metabolites of ethanol, i.e., acetaldehyde and acetate, may be the active vasodilator substances, rather than ethanol itself (Gailis, 1975; Rix, 1977). Since at least acetaldehyde can mimic some of the inhibitory actions of ethanol on isolated arterial and venous smooth muscles (Altura, Carella & Altura, 1978), such data could be used to support the latter contention. However, to our knowledge there are no direct, *in situ* studies on splanchnic microscopic resistance vessels (17–25 μm) or microscopic capacitance vessels (35–50 μm) which have evaluated the microcirculatory actions of either acetaldehyde or acetate. Such direct, *in situ* studies are crucial to answering whether or not either of these metabolites could be responsible for the splanchnic dilator actions of alcohol.

We now describe experiments which show that neither acetaldehyde nor sodium acetate can mimic the splanchnic vasodilator actions of ethanol. Surprisingly, both acetaldehyde and sodium acetate produced dose-dependent constriction of mesenteric terminal arterioles and muscular venules, irrespective of whether these metabolites were administered locally, intravenously or intra-arterially. In addition, the results demonstrated that the splanchnic vasoconstrictor actions of acetaldehyde and acetate cannot be attributed to actions on α-adrenoceptors, 5-hydroxytryptamine-receptors, histamine receptors or cholinoceptors, nor to a synthesis and release of prostaglandin-like substances.

Methods Male rats (Wistar strain, 120 to 180 g) were lightly anaesthetized with intramuscular pentobarbitone sodium (Nembutal, 25 mg/kg). After induction of anaesthesia, tracheostomies were performed and catheters were placed in femoral veins for intravenous injection. *In vivo* quantitative microscopic observations (up to 3,000×) were carried out on terminal arterioles and muscular venules in the mesenteric vasculature by means of an image-splitting television microscope recording system similar to that described previously for microvessels (Altura, 1971; Altura & Altura, 1974). The rat mesentery was prepared and kept under physiological conditions according to procedures described previously (Altura, 1971; 1978; Altura & Altura, 1974). The mesenteric tissues were superfused with a Ringer-gelatin bicarbonate-buffered solution, maintained at a temperature between 36 to 37.5°C and at a pH of 7.3 to 7.4. The exposed mesenteric tissues were kept close to 37.5°C and measured with thermistor probes. Measurements of changes in microvascular lumen size, similar to those described previously (Altura & Altura, 1974; Altura *et al.*, 1979), were made before (control), during and after administration (topical, intravenous, or intra-arterial) of various doses of acetaldehyde and sodium acetate. The doses of acetaldehyde and acetate were randomized. Topical doses of acetaldehyde and sodium acetate (made up in Ringer-gelatin solution) were applied to the mesenteric vessels for periods of 3–10 min. Separate groups of animals were used for

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Table 1 Comparative effects of the local administration of acetaldehyde and sodium acetate on mesenteric terminal arteriolar and muscular venular lumen sizes in intact rats

		Arterioles			Muscular venules		
Agonist	*Dose* ($\times 10^{-6}$ mol)	*Control lumen size* (μm)	*Lumen size after agonist* (μm)	*% change*	*Control lumen size* (μm)	*Lumen size after agonist* (μm)	*% change*
Acetaldehyde	1.8	22.0 ± 0.54	21.0 ± 0.52	4.5	40.0 ± 0.10	37.8 ± 0.62	5.5*
	18	22.1 ± 0.52	19.6 ± 0.29	11.3**	40.2 ± 0.12	36.2 ± 0.75	10.0**
	36	22.0 ± 0.54	18.2 ± 0.37	17.3**	40.1 ± 0.11	34.0 ± 0.91	15.2**
	180	21.9 ± 0.53	15.8 ± 0.60	27.8**	40.0 ± 0.10	28.5 ± 0.28	31.2**
	900	22.1 ± 0.52	14.9 ± 0.74	32.5**	40.2 ± 0.12	26.6 ± 0.75	33.8**
	1800	22.0 ± 0.54	15.7 ± 1.23	28.6**	40.4 ± 0.14	29.0 ± 2.10	28.2**
Sodium acetate	0.025	21.1 ± 0.39	20.8 ± 0.38	1.4	40.2 ± 0.17	40.1 ± 0.19	0.2
	0.25	21.0 ± 0.40	18.6 ± 1.06	11.4*	40.2 ± 0.17	37.4 ± 0.41	7.0*
	2.5	21.2 ± 0.42	14.7 ± 1.18	30.7**	40.4 ± 0.20	31.6 ± 1.17	21.8**
	25	20.8 ± 0.44	11.1 ± 1.12	46.6**	40.0 ± 0.18	23.2 ± 0.92	42.0**
	250	20.8 ± 0.44	3.9 ± 0.97	81.3**	39.8 ± 0.22	3.4 ± 1.39	91.5**

Values are given as means ± s.e.mean; $n = 12$ different rats.
Significantly different from control $^*P < 0.05$; $^{**}P < 0.01$.

acetaldehyde and acetate. Intra-arterial (branch of ileo-colic artery) and intravenous infusions of ethanol were performed with a Model 600–900 Harvard pump, similar to that described previously (Altura *et al.*, 1979). In other experiments, we determined whether intravenous administration of specific pharmacological antagonists (i.e., phentolamine methanesulphonate, 1.0 mg/kg; methysergide maleate, 5 mg/kg; atropine sulphate, 5 mg/kg; diphenhydramine hydrochloride, 5 mg/kg; indomethacin, 1–5 mg/kg) in doses that antagonize their respective agonists (Altura, 1971; 1978; Altura *et al.*, 1979), would interfere with the arteriolar or venular actions of acetaldehyde and sodium actate. At least 6 animals were used for each pharmacological antagonist; each antagonist was administered systemically 15 min before challenge with acetaldehyde or sodium acetate.

Where appropriate, mean values (± s.e.mean) were calculated and the data analysed for statistical significance by using a paired *t* test.

Results The data shown in Table 1 clearly indicate that topical administration of acetaldehyde, in doses from 1.8 to 1800 μmol, produces dose-dependent constriction of both arterioles and muscular venules in the rat mesenteric vasculature. Local administration of sodium acetate, in doses from 0.25 to 250 μmol, also produced dose-dependent vasoconstriction of the microvessels (Table 1). However, sodium acetate is clearly more potent in producing constriction of these splanchnic microscopic resistance and capacitance vessels. Although not shown, intravenous administration of acetaldehyde (18 to 3600 μmol) produced dose-dependent constriction of the microvessels, ranging from a 17.2% decrease to a 69.4% decrease in lumen size ($n = 6$), whereas intravenous sodium acetate (2.5 to 250 μmol) resulted in constriction of arterioles and venules which reduced lumen size 4.2% to 41.4% ($n = 6$). Intra-arterial administration of similar doses of acetaldehyde and sodium acetate, similarly, produced degrees of constriction ranging from 5.0% to 60% ($n = 6$ each). Systemic administration of phentolamine, methysergide, diphenhydramine, atropine or indomethacin all failed to attenuate or prevent the vasoconstrictor actions of the metabolites of ethanol.

Discussion Although ethanol is a potent splanchnic vasodilator (McDowall, 1925; Horvath and Williard, 1962; Altura, 1978; Altura *et al.*, 1979), its major metabolites, i.e., acetaldehyde and acetate, cannot elicit vasodilatation of mesenteric arterioles and venules, at least in the rat. Our findings thus make it difficult, if not impossible, to implicate either acetal-

dehyde or acetate as mediators of ethanol-induced splanchnic vasodilatation. The present, direct *in situ* observations on splanchnic arterioles and venules, could thus, possibly, also be used to question, in general, the concept that acetaldehyde and acetate play roles in the peripheral vascular actions of ethanol.

The results described here which demonstrate that both acetaldehyde and sodium acetate can exert potent and dose-dependent constriction of microscopic resistance and capacitance vessels could, however, aid in explaining why experiments described in the literature, in which indirect methods have been used, indicate that ethanol can produce vasodilatation, vasoconstriction, biphasic responses, or no peripheral vascular effect (Dixon, 1907; Fewings, Hanna, Walsh & Whelan, 1966; Nakano & Kessinger, 1972). Formation of these substances via metabolism of ethanol could, collectively, counteract or oppose the vasodilator actions of alcohol, thus resulting in observations which might make one believe that ethanol induces vasoconstriction, biphasic responses or no peripheral vascular effect. In any event, the present findings with a variety of pharmacological antagonists suggest that neither acetaldehyde nor acetate induce splanchnic vasoconstriction via actions on α-adrenoceptors, 5-hydroxytryptamine receptors, histamine receptors, or cholinoceptors nor can the constrictor actions be attributed to a synthesis and release of prostaglandin-like substances.

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