The potentiating effects of ethanol on responses of aortic strips to stimulant drugs

The assumption has been made that ethanol itself has no discernible effects on smooth muscle contractility. In the experiments reported below, it was observed that as little as 0·05 ml of ethanol in a 15 ml muscle chamber (58 mM) enhanced the responses to a variety of agonists in aortic strips and in a higher concentration (348 mM) exerted direct contractile effects.

Rabbit aortic strips were prepared as described previously (Kalsner & Nickerson, 1968a) and suspended under 2 g tension in muscle chambers of 15 ml capacity. The bathing medium was Krebs-Henseleit solution (containing 0·03 mM disodium EDTA) which was maintained at 37°. Responses were recorded isotonically on a kymograph drum moving at 1·8 mm/min. Concentrations of noradrenaline bitartrate (Calbiochem), methoxamine hydrochloride (Burroughs Wellcome) and histamine dihydrochloride (Calbiochem) are referred to as the weight of the base in g/ml in the muscle chambers. Ethanol (absolute ethanol, Consolidated Alcohols, Toronto) and potassium chloride (British Drug Houses) are referred to in terms of molarity. Reserpine (Nutritional Biochemicals) was dissolved in 10% ascorbic acid and rabbits were injected intramuscularly with 1 mg/kg about 18–24 h before death. Mean values are reported with their standard errors.

In preliminary experiments it was found that 58, 116 and 174 mM ethanol progressively enhanced the amplitude of responses to a low concentration of noradrenaline (3×10^{-9} g/ml) without significantly increasing the resting tone of aortic strips (Fig. 1a). The potentiation was sustained during the period of exposure of strips to ethanol (usually 10 to 20 min) and was reproducible at 30 min intervals.

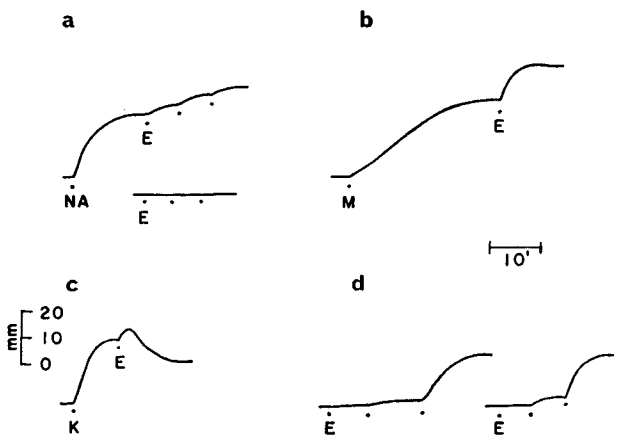

FIG. 1. Effects of ethanol on aortic strips. a. Upper trace shows effect of cumulative additions of ethanol (E) (58, 116 and 174 mM) on response to noradrenaline (NA) (3×10^{-9} g/ml); lower trace shows effect of ethanol alone. b. and c. Effects of ethanol (174 mM) on responses to methoxamine (M) (5×10^{-8} g/ml) and potassium (K) (20 mM). d. Responses of untreated (left) and reserpine-pretreated (right) strips to cumulative concentrations of ethanol (174, 348 and 696 mM).

In a more detailed set of experiments, strips of aorta were contracted by concentrations of noradrenaline, methoxamine, histamine and potassium, on the sharply rising portions of their dose-response curves, and after responses had reached stable plateau values they were exposed to ethanol (174 mM). The augmentation of responses to noradrenaline (3×10^{-9} g/ml), methoxamine (5×10^{-8} g/ml) and histamine (5×10^{-8}

g/ml) produced by ethanol was approximately equivalent to doubling the concentrations of the agonists in the muscle chambers (Table 1). In contrast, contractions induced by potassium (20 mM) were increased much less by ethanol, in terms of an equivalent concentration of potassium alone (Table 1), and the initial potentiation was followed by a depression of response amplitude. Typical traces obtained from several of the above experiments are shown in Fig. 1b and c. Pretreatment of aortic strips with reserpine, to deplete endogenous catecholamines, did not reduce the potentiating effects of ethanol. Ethanol alone in a concentration of 174 mM exerted a barely detectable effect on the tone of quiescent strips; an increase of $0{\cdot}9 \pm 0{\cdot}1$ mm in 17 strips.

Table 1. *Effects of ethanol on the amplitude of contractions produced by various agonists*

Agonist	Concn	No. of strips	Contraction amplitude (mm)	Ethanol increment		Final equiv. * concn of agonist
				mm	%	
Noradrenaline	3×10^{-9} g/ml	8	$28{\cdot}4 \pm 3{\cdot}9$	$9{\cdot}8 \pm 0{\cdot}5$	$39{\cdot}0 \pm 5{\cdot}0$	$8{\cdot}4 \times 10^{-9}$ g/ml
Histamine	5×10^{-8} g/ml	10	$6{\cdot}4 \pm 0{\cdot}7$	$7{\cdot}5 \pm 0{\cdot}5$	$126{\cdot}8 \pm 12{\cdot}7$	$9{\cdot}2 \times 10^{-8}$ g/ml
Methoxamine	5×10^{-8} g/ml	7	$14{\cdot}2 \pm 3{\cdot}8$	$15{\cdot}1 \pm 1{\cdot}3$	$176{\cdot}1 \pm 44{\cdot}9$	$9{\cdot}5 \times 10^{-8}$ g/ml
Potassium	2×10^{-2} M	11	$19{\cdot}3 \pm 2{\cdot}5$	$6{\cdot}2 \pm 0{\cdot}6$	$38{\cdot}9 \pm 6{\cdot}5$	$2{\cdot}3 \times 10^{-2}$ M

* Calculated from separately determined dose-response curves to each agonist as described previously (Kalsner & Nickerson, 1968b).
Ethanol was added to the muscle chambers after responses to agonists had reached plateau values.

In other experiments it was observed that higher concentrations of ethanol contracted aortic strips directly (Fig. 1d). Six strips responded to 348 and 696 mM ethanol with mean amplitudes of $2{\cdot}7 \pm 0{\cdot}4$ mm and $16{\cdot}3 \pm 1{\cdot}6$ mm. Contractions by ethanol could be obtained repeatedly in these strips at 30 min intervals. Neither pretreatment of rabbits with reserpine (Fig. 1d), nor treatment of strips with phenoxybenzamine (1×10^{-6} g/ml for 10 min) appeared to modify responses to ethanol. This concentration of phenoxybenzamine markedly reduced or eliminated responses to 1×10^{-6} g/ml of noradrenaline, histamine and 5-hydroxytryptamine.

To assess the role of extracellular and cellular bound calcium in the effects of ethanol, aortic strips were immersed for 30 min in a calcium-free Krebs solution containing disodium EDTA (0·1 mM). This procedure virtually eliminates extracellular and loosely bound calcium for contractions by agents such as potassium and materially reduces the tissue stores of tightly bound calcium utilized by compounds such as noradrenaline (Waugh, 1962; Hinke, 1965; Hudgins & Weiss, 1968). Under these conditions, responses to potassium (20–40 mM) were completely blocked and those to noradrenaline (3×10^{-9} g/ml) reduced to 12% of their amplitude in the standard Krebs solution. The potentiating effect of ethanol (174 mM) on responses to noradrenaline was proportionately the same as in the standard calcium solution; approximately equivalent to doubling the concentration of noradrenaline in the muscle chambers. The direct contractile effects of ethanol (696 mM) were decreased in calcium-free solution to about the same extent as were the responses to noradrenaline.

A possible explanation of the present results is that low concentrations of ethanol (58–174 mM) interfere with the rebinding of calcium released into the neighbourhood of the contractile filaments by stimulant drugs. This could adequately explain the potentiation of responses to all agonists tested, regardless of the source of calcium for contraction. Higher concentrations of ethanol (348–696 mM) appear to directly mobilize tightly bound calcium for contractions.

The depression of the amplitude of responses to potassium, which followed the initial potentiation may reflect an additional action of ethanol to impair the transmembrane flux of ions. Hurwitz, Battle & Weiss (1962) previously reported that ethanol depressed high potassium contractions in guinea-pig ileum. They suggested that ethanol impaired the inward movement of ionized calcium from membrane sites.

Gimeno, Gimeno & Webb (1962) observed that low concentrations of ethanol (24 to 192 mM) depressed the contractility of isolated rat atria and Fewings, Hannah & others (1966) found that ethanol administered into the brachial artery directly constricted the blood vessels of the forearm and hand. The present finding that as little as 58 mM ethanol enhanced responses to stimulant drugs in aortic strips indicates that it should be abandoned as a solvent in studies of the effects of water-insoluble drugs on smooth muscle reactivity. In addition, experiments in which it has been used should be re-interpreted with caution.

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