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Ethanol- and isoprenaline-induced responses in pig parenchymal lung tissue

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Ethanol (8·7–34·8 mM; 0·5–0·20% v/v) had no significant effect on spontaneously developed tension in pig lung parenchyma strips, although higher cumulative concentrations caused relaxation (at 34·8–174 mM) and contraction (at 348–1392 mM). Ethanol-induced effects were not caused by activation of adrenoceptors or of cholinoceptors, since blockade of these receptors did not alter those effects. Single low concentrations of ethanol (8·7–34·8 mM) which can occur in-vivo in man, caused increases in the relaxant potency of isoprenaline in pig lung strips, although the sizes of these increases varied widely. In addition, ethanol-induced potentiation of isoprenaline was not concentration-related. This effect was not caused by inhibition of isoprenaline uptake since [^{3}H]isoprenaline transport was found not to be significantly altered in the presence of ethanol (8·7–34·8 mM).

Kalsner (1970) reported that ethanol (58–174 mM) progressively enhanced the amplitude of contractile responses of rabbit aortic strips to potassium, histamine, methoxamine and noradrenaline. Clement (1980) also observed potentiation of the neuromuscular actions of various cholinoceptor agonists in chick isolated biventer cervicis preparations in the presence of ethanol (145–290 mM). Ethanol (200 mM) also potentiated the response of the rabbit anococcygeus muscle to a range of inhibitory agonists (Gillespie et al 1982). Low concentrations of ethanol (4·3–43·4 mM), such as might be found in the blood in-vivo, markedly increased the metabolic coronary vasodilation (Sunahara & Talesnik 1974) produced during noradrenaline-stimulated inotropism in perfused rat heart (Talesnik et al 1980), and at 22–45 mM acts directly as a coronary vasodilator in heart-lung preparations from mongrel dogs (Abel 1980), showing that ethanol as a solvent should be avoided when preparing solutions of water-insoluble drugs for studies involving the measurement of smooth muscle reactivity.

Since blood and breath concentrations of ethanol in man have been shown to be virtually identical within 30 min of its oral administration (Stowell et al 1980), it was of interest to see whether low concentrations of ethanol altered the reactivity of an isolated airway preparation. Pig lung parenchyma strip is currently being evaluated in our laboratories and was therefore used in this study.

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Materials and methods

Pharmacological studies. Macroscopically normal specimens of central lung lobes were obtained from freshly slaughtered pigs. Strips of parenchyma were dissected from the marginal edges of the lung lobes and the pleural membranes removed. Longitudinal strips of tissue were cut to approximately 25 × 3 × 2 mm, and suspended under 500 mg tension in Krebs Henseleit solution aerated with 5% CO_2 in O_2 at 37 °C. Isometric changes in tension were measured with a Grass force-displacement transducer (FTO3C) coupled to a pre-amplifier and a Rikadenki pen recorder (Model 1328L). Preparations were left to equilibrate for 60–90 min with regular washing before any drug-induced effects were measured.

Agonist concentration-effect curves were constructed by cumulative drug administration and approximately 1 h was allowed to elapse between curves. Responses were measured as a % of the maximal response to an agonist (E_{max} = 100%). In some experiments, responses of lung parenchyma strips to ethanol (8·7–2784 mM) were recorded before and after a 45 min exposure to the β-adrenoceptor antagonist propranolol (0·5 μM), the α-adrenoceptor antagonist phentolamine

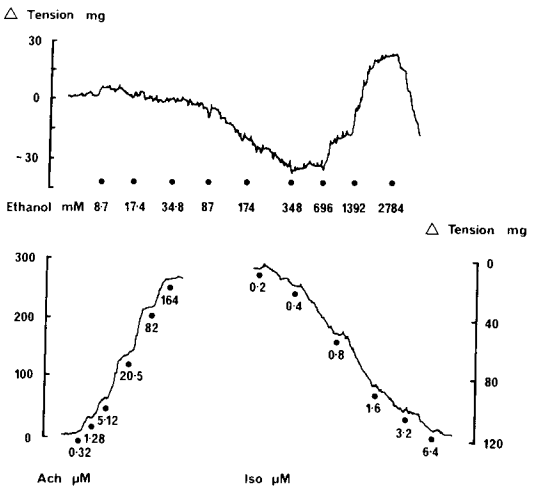

FIG. 1. Responses of individual preparations of pig lung parenchyma strip to cumulative additions of (a) ethanol, (b) ACh or (c) isoprenaline.

(1 μM) or the muscarinic cholinoceptor antagonist atropine (0·1 μM) or after simultaneous exposure to all three of these drugs. In other experiments, two consecutive concentration-effect curves to either acetylcholine (ACh) or isoprenaline were produced and the EC50 value derived from the second curve was taken as a measure of control agonist potency. Preparations used to examine responses to ACh were then exposed to atropine (0·02 μM) or to one concentration of ethanol (34·8 or 174 mM) for 45 min and a third concentration-effect curve produced. Where isoprenaline was the agonist, preparations were equilibrated for 45 min in the presence of one concentration of ethanol (8·7–34·8 mM) before a third curve was constructed. The ratio, $EC50_{control}$: $EC50_{test}$ was taken as a measure of the change in agonist potency.

Radio-tracer studies. Fine strips of pig lung parenchyma tissue, approximately 40 × 1 × 1 mm, were dissected out distal to major bronchioles and blood vessels. Strips were pooled to provide samples of 40–60 mg, tied to small silastic coated weights and individual preparations incubated in 3 ml of Krebs Henseleit solution aerated with 5% CO_2 in O_2 at 37 °C. They were allowed to equilibrate for 1 h after which some specimens were incubated in the presence of ethanol (8·7–34·8 mM) for 45 min. Control and ethanol pretreated strips were then exposed for 5 min to the extracellular fluid marker (+)-[^{3}H]sorbitol (33 μM, 100 nCi ml^{-1}), or to (±)-[^{3}H]isoprenaline (1·90 μM, 100 nCi ml^{-1}). Tissues were then blotted dry, weighed, placed in glass vials and digested in 1 ml NaOH (1 M) for 2 h at 75 °C. After cooling, 100 μl of hydrogen peroxide 27% w/w (8 M) was added to each vial as a decolourizing agent. After approximately 1 h, vials were reincubated at 75 °C to remove excess hydrogen peroxide. Fifteen ml of 2 : 1 toluene-Triton X-100 mixture containing 0·4% PPO was added to each vial followed by 100 μl of HCl (12 M). After this solution had clarified, radioactivity was measured in a Nuclear Chicago Isocap 300 liquid scintillation spectrometer. A 1 ml sample of each bath solution was also taken for estimation of radioactivity. Quench correction was employed using the external standards ratio facility. All estimates of tissue radioactivity derived from (±)-[^{3}H]isoprenaline were corrected for extracellular (±)-[^{3}H]isoprenaline.

Drugs used were acetylcholine chloride; (±)-isoprenaline hydrochloride (Sigma Chem. Co.); atropine sulphate (Drug Houses of Australia); ethanol (Colonial Sugar Refineries; redistilled); phentolamine mesylate (Ciba); propranolol hydrochloride (ICI Aust.); (±)-[7-^{3}H]isoprenaline, (+)-[1-^{3}H]sorbitol (New England Nuclear).

Results

Effects of ethanol on resting lung strip tension. Ethanol (8·7–25 mM) had no significant effect on spontaneously developed tension in pig lung parenchymal strip. However at 34·8–174 mM it caused concentration-dependent relaxations and at 348–1392 mM contractions (Fig. 1a), which were neither altered in the presence of propranolol (0·5 μM), phentolamine (1 μM) or atropine (0·1 μM) alone or by all three together. Ethanol (2748 mM) invariably caused complete relaxation of lung strips. The mean maximal ethanol-induced (8·7–174 mM) decrease in tension was 35 ± 4 mg s.e.m. (n = 6) while the mean maximal increase in tension from the relaxed state was 56 ± 16 mg (n = 6).

Table 1. Effect of ethanol on the relaxant potency of isoprenaline in pig lung parenchyma strip.

Ethanol (mM)			
8·7	17·4	26·1	34·8
	Isoprenaline $EC50_{control}$ / Isoprenaline $EC50_{ethanol}$		
22·7	24·6	14·6	42·7
14·3	18·1	12·9	5·6
11·6	13·8	11·1	2·6
2·8	13·1	10·6	0·5
1·0	9·7	2·5	
1·0	8·7		
	1·6		
	1·4		
	mean, x̄ ± s.e.m.		
8·9	11·4	10·4	12·9
3·6	2·8	2·1	10·0

Effects of ethanol on responses to ACh or isoprenaline. ACh caused concentration-dependent contractions of pig lung parenchyma strip (Fig. 1b). The mean maximal increase in tension was 246 ± 6 mg (n = 8). These contractions were inhibited by atropine (0·02 μM) (K_B = 1·00 ± 0·14 × 10^{-9} M, n = 4) but were not affected by ethanol (34·8 or 174 mM). The means for the ratio ACh $EC50_{control}$: ACh $EC50_{ethanol}$, were 1·35 ± 0·09 (n = 4), and 1·51 ± 0·16 (n = 4), for ethanol 34·8 and 174 mM respectively.

Isoprenaline caused concentration-dependent decreases in spontaneously developed tension in lung strips. The mean maximal decrease in tension in a random sample of preparations was 116 ± 10 mg (n = 8) (Fig. 1c). Responses to isoprenaline were usually markedly potentiated in the presence of low concentrations of ethanol (8·7–34·8 mM) as indicated by parallel shifts to the left in concentration-effect curves, although considerable variability was observed in the size of these potentiations. Mean ethanol-induced (8·7–34·8 mM) increases in the relaxant potency of isoprenaline varied between about 9 and 13 fold, although greater variability was observed when individual results were examined (Table 1). The control potency of isoprenaline varied between lung strips in the range 0·02–0·50 μM (mean ± s.e.m. isoprenaline EC50 = 0·11 ± 0·02 μM; n = 23). Regression analysis showed no significant correlation between control isoprenaline potency and the size of ethanol-induced increases in isoprenaline potency (r = 0·11; $P > 0·6$).

Effect of ethanol on the accumulation of radioactivity derived from [^{3}H]sorbitol or [^{3}H]isoprenaline. Following exposure of pig lung strips to [^{3}H]sorbitol for 5 min, the apparent extracellular fluid volume (EFV) was 440 ± 12 μl g^{-1} wet wt tissue. Ethanol (8·7–34·8 mM) had no significant effect on the apparent EFV. Accumulation of cellular radioactivity equivalent to (±)-[^{3}H]isoprenaline was 278 ± 11 ng g^{-1} wet wt tissue. Ethanol (8·7–34·8 mM) also failed to alter this value significantly.

Discussion

Ethanol (8·7–34·8 mM), which had little effect on resting tension in pig lung parenchyma strip, caused selective potentiation of isoprenaline-induced relaxation, in that ACh-induced contraction was not markedly altered. Ethanol (34·8–174 mM; 0·2–1·0% v/v) alone, caused relaxation of the preparation, while 348–1392 mM caused contractions. Blockade of adrenoceptors or cholinoceptors did not alter ethanol-induced effects. Similar results have been reported in canine coronary arteries (Altura et al 1983). Kalsner (1970) reported that ethanol (348–696 mM) caused phenoxybenzamine-insensitive contractions of rabbit aortic strip, and concluded that high concentrations of ethanol directly mobilized tightly bound calcium utilized for contractions, since these responses were reduced by 88% in calcium-free Krebs solution containing disodium EDTA (0·1 mM).

Kalsner (1970) also showed concentrations of ethanol (58–174 mM) that had little effect on the resting tension in rabbit aortic strip, caused significant potentiation of noradrenaline-induced contractions, suggesting that ethanol interfered with the rebinding of calcium released by noradrenaline near the contractile elements. Results of radio-tracer experiments in the present study demonstrate that ethanol-induced increases in isoprenaline potency were not due to inhibition of that drug's transport into cells. Furthermore, it has been shown that ethanol had no effect on monoamine oxidase, catechol-*O*-methyl transferase or catecholamine uptake into neuronal (Majchrowicz 1973) or extraneuronal uptake sites (Graefe & Trendelenburg 1974; Bönisch 1978; Major et al 1978).

Anaesthetics and related lipid-soluble drugs, as well as low concentrations of ethanol, increased the fluidity of lipid bilayers,erythrocyte, synaptosomal and mitochondrial membranes sufficient to modulate the function of membrane proteins (Jain et al 1975; Chin & Goldstein 1977; Johnson et al 1979, 1980). Furthermore, increasing the fluidity of cell membranes increased the activity of adenyl cyclase probably by increasing the likelihood of collision between β-adrenoceptors and adenyl cyclase (Rimon et al 1978; Bakardjieva et al 1979). Alternatively, ethanol may increase adenyl cyclase activity directly (Rabin & Molinoff 1981). Gillespie et al (1982) have postulated that ethanol modifies responses to agonists by altering the coupling between membrane receptors and the calcium pools with which they are associated, by changing membrane fluidity.

In the present study, the size of the ethanol-induced increase in the potency of isoprenaline was apparently not concentration-dependent in the range 8·7–34·8 mM. However, variation in the size of these increases was apparent at each ethanol concentration tested, and might be expected, given that responses of lung strips to isoprenaline probably resulted from activation of β-adrenoceptors in both bronchiolar smooth muscle and in alveolar contractile elements (Goldie et al 1982; Bertram et al 1983), and that the relative proportions of these components are known to vary between lung strips (Bertram et al 1983). Isoprenaline's potency in pig bronchial smooth muscle was not affected by ethanol (17·4 mM) (Foster et al 1983) and so may be less affected by ethanol in the bronchiolar component in lung strips than in alveolar contractile elements. Variation in the relative proportions of responding elements between preparations could explain the variability in ethanol-induced potentiations.

Although lung parenchyma from pigs was used in these experiments, the possibility exists that concentrations of ethanol occurring in the respired air and blood in man, may significantly enhance increases in peripheral airways calibre produced in response to isoprenaline. Furthermore, ethanol (>26 mM; 0·15% v/v) alone may induce relaxations of fine airways in man. Such an effect may facilitate ethanol exchange between the pulmonary blood and ventilating air.

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Effect of food, fluid and dosage form on the absorption of 52-522, a potential antianxiety agent, in the dog

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The absorption of 52-522 in the dog was studied by measuring blood concentrations of radioactivity after single oral doses of [^{14}C] 52-522 in a capsule with and without water, also as a food-drug mixture, and a solution in polyethylene glycol 400. Absorption was rapid, and its rate moderate with no significant differences in peak times among treatments. The extent of absorption was lowest after the capsulated [^{14}C] 52-522. The solution dose gave elevated blood concentrations, that were statistically significantly different when compared with the capsules. Hence, it appears that the absorption of [^{14}C] 52-522 is governed by the degree of dispersion of drug in the dosage form.

3-(α-Iminobenzyl)-4-hydroxy-6-phenyl-1-methyl-2(1H)-pyridinone [Sandoz compound number 52-522] has been investigated as a potential antianxiety agent. Preliminary animal pharmacokinetic studies showed consistent and efficient absorption from oral doses in the rat but erratic absorption in the dog, with approximately 10-fold differences in circulating levels of unchanged drug following equal doses in the same animal (unpublished data). In those dog studies, drug capsules were administered without water, although fluid intake was not controlled and the animals were allowed free access to food and water before and during the experiments. The present study was conducted to examine the effect of concomitant administration of food, vehicle, or water volume on the absorption of radiolabelled drug after a single oral dose in the dog.

Materials and methods

Capsule doses. Four male beagle dogs of ca 10 kg were used. Radioactive 52-522 (labelled with ^{14}C at the 6-position of the pyridinone ring, specific activity 0·15 μCi mg^{-1}) was supplied by the Synthetic Tracer Laboratory, Sandoz, Inc. The dose, 40 mg kg^{-1} based on individual weights, was well mixed with an equal mass of lactose and placed in a gelatin capsule.

The dogs were fasted overnight before each experiment. At 1 h before dosing, each dog received thiethylperazine maleate (Boehringer Ingelheim, 3 mg) intravenously to minimize the possibility of emesis. Dogs 1 and 2 then received the [^{14}C] 52-522 capsules, followed immediately by 100 ml of water, while dogs 3 and 4 each received a capsule without water. Thereafter, both groups of dogs were not allowed access to water for 2 h postdosing. Venous blood samples were collected in heparinized syringes immediately before and at 0·25, 0·5, 1, 2, 3, 4, 6, 8, 12, 24 and 32 h after drug administration, and aliquots pipetted for assay. After a washout period of 2 weeks, the experiment was repeated in a crossover fashion so that each animal received a dose with and without water during the study.

Dose with food. Dogs 1, 2 and 4 were used after allowing 2–3 weeks for drug washout, Dog 3 which died of non-drug-related causes, was replaced by Dog 5. Each individual dose of [^{14}C] 52-522 (40 mg kg^{-1}) was wetted with ca 0·5 ml of polyethylene glycol 400 (PEG 400) and blended into 50 g of moist dog food (Cadillac Pet Foods, Pennsauken, New Jersey, U.S.A.). The dogs were fasted overnight for approximately 20 h so that the drug-food mixture, given 1 h after thiethylperazine maleate (3 mg i.v.) was consumed within 15 min. An additional 100 g of moist dog food was then made available, and serial venous blood sampling began and continued for 32 h. No water or additional food was allowed for 2 h post-dosing.

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