Differential Effects of Ethanol on Prostaglandin Responses of Arterial and Venous Smooth Muscle

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Summary. The present results, using isolated rat aortic strips and portal vein segments, demonstrate that ethanol, depending upon concentration, can either enhance or attenuate the contractile actions of $PGF_{2\alpha}$ on at least 2 different types of vascular smooth muscle. At the very least, the present findings question the use of ethanol as a solvent when investigating the contractile actions of PG molecules on smooth muscles.

COLLIER et al.[2] have recently demonstrated that ethanol can stimulate biosynthesis of prostaglandin (PG) E and F compounds in rat stomach muscle. Interestingly, most studies involving PG molecules employ ethanol as a solvent[3]. Recently, we have demonstrated that low concentrations of this alcohol (i.e., < 0.8 mg/ml) can potentiate, while high concentrations of ethanol, similar to those associated with alcoholic intoxication and coma (> 5 mg/ml), can inhibit catecholamine, vasopressin and angiotensin-induced contractions on several types of isolated arterial and venous smooth muscles[4]. In view of such diverse actions of ethanol, and its possible importance in studying PG molecules, we thought it advisable to examine the effects this alcohol might exert on contractions of arterial and venous muscle induced by PG compounds.

Methods. Thoracic aortas and portal veins were obtained from male Wistar rats (275–425 g), cut helically and longitudinally, respectively, and set up isometrically in vitro essentially similar to that described previously[5]. The vascular preparations were equilibrated for 2 h in muscle chambers containing Krebs-Ringer bicarbonate solution (KRB), the composition of which has been reported previously[5]. The KRB solution was oxygenated continuously with a 95% O_2–5% CO_2 mixture and kept at 37 °C (pH 7.4–7.5). After the 2 h incubation period the vascular segments were exposed to $PGF_{2\alpha}$ in a concentration which elicited 50% of a maximum contractile response. These responses were then repeated 2 times, after washing and relaxation in KRB solution. The vascular segments were then exposed to ethanol (0.3–17 mg/ml) for a period of 5 min, since preliminary experiments indicated that 60 min of ethanol exposure yielded results essentially similar to those obtained with 5 min of alcohol exposure. The $PGF_{2\alpha}$ was dissolved in phosphate buffer (pH 7.40–7.45)[6]. The total volume of phosphate buffer used never exceeded 0.2 ml. This phosphate buffer vehicle itself did not attenuate or enhance baseline tension, amplitude or frequency of the spontaneous mechanical responses of the vascular preparations, even when administered in volumes 3-times that used with the PG compound. Where appropriate, means and standard errors of the means were calculated and compared for statistical significance by means of a paired *t*-test.

Results. The Table demonstrates that a low concentration of ethanol (i.e., 0.7 mg/ml) can significantly enhance the contractile action of $PGF_{2\alpha}$ on both rat aortic strips and portal veins. Although lower concentrations of ethanol (i.e., down to 0.3 mg/ml) can also potentiate $PGF_{2\alpha}$ contractions, in these two types of vascular muscle, these effects were seen in only 8 out of 16 preparations examined. Concentrations of ethanol associated with anesthesia (i.e., > 5 mg/ml)[7], attenuate PG responses on the vascular smooth muscles (Table). Concentrations of ethanol which produce coma and death in mammals (i.e., > 10 mg/ml)[7] markedly inhibit the contractile actions of $PGF_{2\alpha}$ on both rat aortic strips and portal veins (Table). Although not shown, all of these ethanol-induced effects are completely reversible upon washing of the tissues in normal KRB solution.

Discussion. Overall, the data presented herein indicate that, depending upon concentration, ethanol can potentiate or inhibit the contractile actions of a PG compoumd on at least 2 different types of vascular smooth

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Differential effects of ethanol on contractions induced by $PGF_{2\alpha}$ in rat aortic strips and portal veins[a]

Vascular segment	*N*	Control tension[b] (mg)	Tension (mg) with ethanol (mg/ml)[b]		
			0.7	7.0	17.0
Aorta	8	800 ± 37.6	986.3 ± 42.4[c]	530 ± 56.2[c]	137.5 ± 13.3[c]
Portal Vein	8	556.2 ± 27.6	756.3 ± 28.1[c]	643.8 ± 21.5[c]	143.8 ± 12.0[c]

[a] The test doses (i.e., ED_{50}'s) of $PGF_{2\alpha}$ used, in these experiments, for the aortic strips and portal veins were 0.25 and 1.0 μg/ml, respectively. [b] Mean values ± SEM. [c] Significantly different from control tension ($p < 0.02$).

muscles. Although ethanol is considered to be a central nervous system depressant[7], it can depolarize neurons[7]. In addition, it has been demonstrated that low concentrations of ethanol can increase activity of afferent nerves from primary and secondary endings in the muscle spindles and tendon organs of in situ rat caudal muscle[8] as well as cause singly excited cat soleus motor nerve terminals to fire repetitively[9]. Collectively, such reports suggest that ethanol may have both excitatory and depressant effects on other excitable tissues, in addition to vascular muscles.

An attractive hypothesis to explain ethanol enhancement and inhibition of $PGF_{2\alpha}$ responses might be related to the effects of this alcohol on the intracellular availability of free, ionized calcium (Ca^{++}). Ethanol has been demonstrated to affect the movement of Ca^{++} in several excitable tissues[10], including those used here[4]. Ethanol-induced enhanced uptake, or release, of intracellular Ca^{++} could result in a potentiation of PG contractions, since the action of all vasoactive stimulants, including $PGF_{2\alpha}$, are dependent on availability of Ca^{++}[11]. High concentrations of ethanol could inhibit PG responses by simply reducing the availability of free, ionized Ca^{++}. Interestingly, these latter high concentrations of ethanol, which are associated with anesthesia, death and coma[7], can inhibit the contractile effect of Ca^{++} in potassium-depolarized rat aortic and portal vein segments[4].

Although the differential effects of ethanol observed here may be linked to actions on cellular Ca^{++}[4], further work will be required to corroborate this tenet since other explanations for the observed differences could be invoked[12].

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Phenobarbital and SKF-525A on Vinblastine and Vincristine Toxicity in Mice[1]

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Summary. The effect of SKF-525A and phenobarbital on the LD_{50} values of vinblastine and vincristine suggests that the toxicity of these agents in mice does not arise from a toxic metabolite.

The vinca alkaloids, vinblastine (VLB) and vincristine (VCR) are clinically useful antitumor agents, yet little is known of their mechanism of action or mode of metabolism[3]. Interestingly, VLB and VCR exhibit different antitumor spectra and different toxic symptoms despite a high degree of structural similarity between the two compounds. Among the hypotheses suggested for these differences is the possibility that VLB and VCR may have different metabolic fates and give rise to different toxicities and/or antitumor activities[4,5]. It was therefore of interest to observe the effect of the metabolic stimulator, phenobarbital, and the metabolic inhibitor, SKF-525A, on the toxicities of these two antitumor agents.

Six-week-old DBA/2 male mice were given aqueous solutions of the drug i.p. One group received only VLB or VCR. A 2nd group was treated twice daily with 50 mg/kg sodium phenobarbital solution for 3 days prior to administration of VLB or VCR. A 3rd group of mice was treated with a single 50 mg/kg dose of SKF-525A 1 h before giving VLB or VCR. All deaths occurring within 1 week of drug administration were counted. LD_{50} values were calculated using a maximum likelihood probit analysis method programmed for digital computation. The results are displayed in the Table.

Pretreatment of the animals with phenobarbital increased the LD_{50} to more than double that of VLB alone but, in contrast, had considerably less effect on the LD_{50} of VCR. When the animals were pretreated with SKF-525A a much greater decrease was seen in the LD_{50} of VLB than in the LD_{50} of VCR.

These results suggest that the lethal toxicity of VLB and VCR in these animals is due primarily to the parent drugs and not to the formation of toxic metabolites. If toxic metabolites were responsible for the toxic lethal effects, then pretreatment with phenobarbital would be expected to decrease the LD_{50} of the drugs, while pre-

Effect of phenobarbital and SKF-525A pretreatment on vinblastine and vincristine toxicity in DBA/2 mice

	LD_{50} (mg/kg ± SE)		
	Alone	Pretreatment	
		Phenobarbital	SKF 525A
VLB	9.85 ± 1.38	26.5 ± 3.8	2.80 ± 0.97
VCR	1.83 ± 0.17	2.38 ± 0.69	1.23 ± 0.23

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