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Ethanol-Prostaglandin Interactions in Contraction of Vascular Smooth Muscle[1] (39391)

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Many studies involving prostaglandin (PG) molecules employ ethanol as a solvent. Collier and his co-workers have recently demonstrated that ethanol can stimulate biosynthesis of PGE and F compounds in rat stomach smooth muscle (1). Two preliminary reports suggest that this alcohol can affect excitability and reactivity of rat aortic and portal venous muscles *in vitro* (2, 3). In view of these diverse actions of ethanol, and its possible importance in studying PG molecules, we examined the effects of ethanol on contractions of arterial and venous muscle induced by PGE_1, A_1, and B_1 molecules.

Methods. Thoracic aortas and portal veins were obtained from male rats (Wistar strain, 275–425 g) after sacrifice by decapitation. The aortas were cut helically into vascular strips (1.5–2 mm in width by 25 mm in length) and the force of contractions were recorded *in vitro*, essentially as described previously (4). Ten-millimeter segments of portal veins were tied at both ends with sutures and the force of contractions were recorded *in vitro*, as described previously (5). Both aortic strips and portal vein segments were equilibrated for 2 hr in 20-ml muscle chambers containing Krebs–Ringer bicarbonate (KRB) solution (4) at 37° oxygenated continuously with 95% O_2–5% CO_2 (pH 7.4–7.5). The recording equipment was identical to that described previously (6). After the 2-hr incubation period the vascular segments were exposed to one of the three different PG compounds (E_1, A_1, or B_1) in concentrations which were either (i) above threshold; or (ii) required for eliciting 50% of a maximum PG contractile response. These respective responses were then repeated two more times, after washing and relaxation in KRB solution. The vascular segments were exposed to ethanol concentrations of 17, 170, and 430 m*M* for 5 min. (Preliminary experiments had shown there was no difference in effects when ethanol exposure was as long as 60 min.) All of the PG compounds were dissolved in phosphate buffer (pH 7.40–7.45) (7). The total volume of phosphate buffer used never exceed 0.2 ml. At least six different preparations were examined with each ethanol-PG dose combination. Significance of changes in tension were calculated by Student's *t* test. Tension values for the rat portal vein were calculated in a manner similar to that described previously (5, 8).

Results. Tables I and II demonstrate that a low concentration of ethanol (i.e., 17 m*M*) can markedly inhibit the contractile action of PGE_1 on both rat aortic strips and portal veins. Although lower concentrations of ethanol (i.e., down to 5 m*M*) can also attenuate PGE_1 contractions, in these two types of vascular muscle these effects were seen in only about 50% of the preparations examined. Higher concentrations of ethanol, i.e., 170–430 m*M*, produced greater inhibition of PGE_1 responses which appeared to be dose-related; the greater the ethanol concentrations, the less the PGE_1 response (Tables I and II). Although not shown, these ethanol-induced effects as well as those described below are completely reversible upon washing of the tissues in normal KRB solution.

In contrast to what is seen for PGE_1, preincubation of aortic strips with 17 m*M* ethanol enhances contractions induced by PGB_1 (Tables 1 and 2). Even high concentrations of ethanol, i.e., 170 m*M*, can still

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TABLE I. DIFFERENTIAL EFFECTS OF ETHANOL ON CONTRACTIONS INDUCED BY PROSTAGLANDINS IN RAT AORTIC STRIPS.

PG compound (μg/ml)	N	Control tension (mg ± SE)	Tension (mg) with ethanol (mM)		
			17	170	430
PGE_1 (1)	8	572.8 ± 52.6	405 ± 36[a]	166.6 ± 22[a]	58.3 ± 12.6[a]
PGB_1 (0.25)	6	491.6 ± 84.7	925 ± 72.2[a]	683.3 ± 42.3[a]	133.3 ± 28.9[a]
PGA_1 (0.25)	8	825 ± 42.6	937.5 ± 20.5[a]	200 ± 34[a]	106.2 ± 38.2[a]

[a] Significantly different from control tension ($P < 0.02$).

TABLE II. DIFFERENTIAL EFFECTS OF ETHANOL ON CONTRACTIONS INDUCED BY PROSTAGLANDINS IN RAT PORTAL VEINS.

PG compound (μg/ml)	N	Control tension (mg ± SE)	Tension (mg) with ethanol (mM)		
			17	170	430
PGE_1 (1)	8	693.1 ± 23.1	531.2 ± 19.9[a]	475 ± 34.6[a]	43.7 ± 10.2[a]
PGB_1 (1)	6	700 ± 25	858.3 ± 29.9[a]	550 ± 22.6[a]	91.6 ± 10.2[a]
PGA_1 (1)	8	575 ± 28.3	750 ± 25[a]	518 ± 31	300 ± 10[a]

[a] Significantly different from control tension ($P < 0.02$).

effect potentiation of B_1 (Tables 1 and 2). Tables 1 and 2 demonstrate that a low concentration of ethanol (e.g., 17 mM) can also potentiate contractions induced by PGA_1. However, unlike that seen with B_1, higher concentrations of ethanol (i.e., 170 mM) markedly attenuate PGA_1 contractions (Tables 1 and 2). Although not shown, the phosphate buffer vehicle itself does not attenuate (or enhance) baseline tension, amplitude, or frequency of the spontaneous mechanical responses of the vascular preparations, even when administered in volumes at least three times that used with the doses of PG compounds.

Discussion. The data presented here indicate that low concentrations of ethanol can potentiate or inhibit the contractile actions of PG compounds on at least two different types of vascular smooth muscles.[3] Although both inhibition and potentiation by ethanol was seen with PGA_1 and PGB_1 compounds, only contractile inhibition was seen with the PGE_1 compound. The fact that the latter action of ethanol was demonstrated only with PGE_1 and on both arterial and venous smooth muscle suggests to us that some part(s) of the structure of a prostaglandin molecule may be quite important in ethanol-PG interactions in contractions of vascular muscle.

Although ethanol is considered to be a CNS depressant (10), it can depolarize neurons (10, 11). 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 rat caudal muscle (12) as well as cause singly excited cat soleus motor nerve terminals to fire repetitively (13). Such reports suggest that ethanol may have both excitatory and depressant effects on certain excitable tissues. In addition, ethanol has been demonstrated to increase the frequency of miniature endplate potentials of rat phrenic nerve diaphragm preparations and to concomitantly increase the force of contraction (14).

In the latter study, Gage reported that ethanol increased the quantal content of the end-plate potential and the frequency of minature endplate potentials. Such actions were used to explain the resultant increase in the force of contraction (14). An effect of ethanol on the resting membrane potentials of the rat aorta and portal vein could explain the potentiation of PGB_1 and A_1 contractions.

A second possibility is that ethanol can stimulate endogenous synthesis of PG com-

[3] Following the procedure recommended by The UpJohn Co. for preparation of stock solutions, one usually dissolves the PG compounds in 100% ethanol to yield 1 mg/ml (9). A dilution of 1 mg/ml to 1 μg/ml would result in yielding approximately 17 mM ethanol, or in other words, the concentration of ethanol which differentially affects the contractile actions of the several PG compounds used in the present study.

pounds (1). Since it has recently been demonstrated that synthesis of PG compounds can be stimulated in both arterial and venous smooth muscle by certain vasoactive substances (15), the latter hypothesis is plausible.

A third alternative hypothesis to explain ethanol enhancement of PG responses might be related to the effects of this alcohol on the intracellular availability of free, ionized calcium (Ca^{2+}). Ethanol has been demonstrated to affect the movement of Ca^{2+} in certain excitable tissues (16). Ethanol-induced enhanced uptake, or release of intracellular Ca^{2+} could result in a potentiation of PG contractions.

In this context, it is known that a wide concentration range of ethanol (i.e., 50–430 m*M*) can inhibit the contractile effect of Ca^{2+} in potassium-depolarized rat aortic strips and portal vein segments (2, 3, 17, 18). Since Ca^{2+} is undoubtedly the link between excitation and contraction for PG compounds in blood vessels (19, 20), high concentrations of ethanol could inhibit these contractions by simply reducing the availability of free, ionized Ca^{2+} (17, 18). Alternatively, one might be tempted to suggest that the inhibitory effects of ethanol on PG-induced contractions might be due to an affect of the alcohol on vascular smooth muscle cell metabolism and/or the contractile proteins. The present results, however, indicate that although different concentrations of ethanol can potentiate or inhibit PGA_1 or B_1 responses, the contractile responses elicited by PGE_1 are only inhibited. If ethanol exerted a general effect on metabolism and/or the contractile proteins, one might expect a particular concentration of the alcohol, e.g., 170 m*M*, to be inhibitory for all three PG compounds. The latter is, however, not the case.

Irrespective of the exact mechanism(s) by which ethanol enhances and attenuates PG contractions of aortic and venous smooth muscle, one must be cautious in the use of alcohol as a solvent for PG compounds when investigating vascular smooth muscle.

Summary. The present results, using isolated rat aortic strips and portal vein segments, demonstrate that ethanol (5–430 m*M*) can both enhance and attenuate the contractile actions of a variety of prostaglandin (PG) compounds on at least two 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 muscle.

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1. Collier, H. O. J., McDonald-Gibson, W. J., and Saeed, S. A., Lancet **i,** 702 (1975).
2. Edgarian, H., Altura, B. M., Altura, B. T., and Orkin, L. R., Pharmacologist **16,** 303 (1974).
3. Edgarian, H., Altura, B. M., Altura, B. T., Fed. Proc. **34,** 797 (1975).
4. Altura, B. M., and Altura, B. T., J. Pharmacol. Exp. Ther. **190,** 300 (1974).
5. Altura, B. T., and Altura, B. M., Anesthesiology **43,** 432 (1975).
6. Altura, B. M., and Altura, B. T., Amer. J. Physiol. **219,** 1698 (1970).
7. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *in* "Manometric Techniques," p. 133. Burgess Publishing Co., Minneapolis (1964).
8. Altura, B. T., Altura, B. M., and Baez, S., Blood Vessels **12,** 206 (1975).
9. Roseman, T. J., Sims, B., and Stehle, R. G., Adv. Biosci. **9,** 851 (1972).
10. Ritchie, J. M., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), p. 135. Macmillan, New York (1970).
11. Gallego, A., J. Cell Comp. Physiol. **31,** 97 (1948).
12. Kucera, A., and Smith, C. M., J. Pharmacol. Exp. Ther. **179,** 301 (1971).
13. Feng, T. P., and Li, T. H., Chin. J. Physiol. **16,** 317 (1941).
14. Gage, P. W., J. Pharmacol. Exp. Ther. **150,** 236 (1965).
15. Terragno, D. A., Crowshaw, K., Terragno, N. A., and McGiff, J. C., Circulation Res. **36** (Suppl. 1), 1-75 (1975).
16. Seeman, P., Pharmacol. Rev. **24,** 583 (1972).
17. Altura, B. M., Edgarian, H., and Altura, B. T., J. Pharmacol. Exp. Ther. **197,** 352 (1976).
18. Edgarian, H., and Altura, B. M., Anesthesiology **44,** 311 (1976).
19. Strong, C. G., and Bohr, D. F., Amer. J. Physiol. **213,** 725 (1967).
20. Greenberg, S., Kadowitz, P. J., Diecke, F. P. J., and Long, J. P., Arch. Intern. Pharmacodynamie Ther. **205,** 381 (1973).

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