Cervo, L., Rochat, C., Romandini, S., Samanin, R. (1981) Psychopharmacology 74: 271–274

Garattini, S., Buczko, W., Jori, A., Samanin, R. (1975) Postgrad. Med. J. 51 suppl. 1: 27–35

Gibson, R. D., Tingstad, J. E. (1970) J. Pharm. Sci. 59: 426–427

Gold, M. S., Redmond, D. E. Jr., Kleber, H. D. (1978) Lancet 2: 599–602

Lal, H., Numan, R. (1975) Life Sci. 18: 163–168

Meyer, D. R., Sbarber, S. B. (1976) Pharmacologist 18: 236

Samanin, R., Mennini, T., Ferraris, A., Bendotti, C., Borsini, F., Garattini, S. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 308: 159–163

Samanin, R., Cervo, L., Rochat, C., Poggesi, E., Mennini, T. (1980) Life Sci. 27: 1141–1146

Tseng, L.-F., Loh, H. H., Wei, E. T. (1975) Eur. J. Pharmacol. 30: 93–99

Vetulani, J., Bednarczyk, B. (1977) J. Pharm. Pharmacol. 29: 567–569

Wei, E., Loh, H., Way, E. L. (1973) J. Pharmacol. Exp. Ther. 184: 398–403

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The effects of ethanol dependence on drug responsiveness of mouse isolated vas deferens

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The effects of ethanol dependence on the responsiveness of the mouse vas deferens to noradrenaline (NA), carbachol, barium and calcium were studied. Ethanol dependence increases the maximum responses to NA and carbachol whereas responsiveness to barium remains unaltered. The concentration-effect curve to calcium was shifted to the left (3·0-fold at the EC50 level). It is concluded that in vas deferens isolated from ethanol-dependent mice the increased responsiveness to NA, carbachol and calcium is a consequence of an enhanced calcium entry through voltage-independent calcium channels, as it has been reported for brain tissue.

It has been proposed that ethanol dependence could involve adaptive alterations of neuronal membrane structure and physiological properties (Chin & Goldstein 1977a; Johnson et al 1979). The work presented here was an attempt to examine the drug responsiveness of vasa deferentia isolated from ethanol dependent mice. We reasoned that if ethanol increases calcium permeability, as a consequence of its action on the smooth muscle cell membranes, then a long-term administration of ethanol should enhance the responsiveness of the mouse vas deferens to drug stimulation.

Method

Swiss-Webster mice, 30 to 40 g, at the beginning of the experiments, were housed in groups of ten, in a well-ventilated room, kept at 22–24 °C and under a reversed 12-h light/dark cycle (light on at 12.00 noon). Dependence on ethanol was produced according to Goldstein (1972). Briefly, groups of 10 mice were transferred to a glass chamber (52 × 34 × 27 cm), with food and water freely available. An infusion pump delivered ethanol, at a rate of 30 mg min^{-1}, onto a filter paper wick in a flask. A continuously variable respirator pump delivered air through the flask into the chamber at a rate giving a nominal ethanol flow of 30 mg min^{-1}. At the start of the experiments all mice received a priming dose of ethanol (1·25 g kg^{-1} i.p.) and thereafter were exposed to ethanol vapour over five days. To all mice, including the control group, a daily injection of the alcohol-dehydrogenase inhibitor, pyrazole (1·0 mmol kg^{-1} i.p.) was given at 10.00 am. Groups of 10 control mice were also placed in the glass chamber over five days, with only air flowing through it. After five days of ethanol exposure the infusion pump was stopped and air allowed to flow through the chamber. Ethanol withdrawal was assessed using the single signal called 'convulsion on handling' and quantitatively evaluated using a previously reported scoring system of 1 to 4 (Goldstein 1972). Over 80% of the mice exposed to

Table 1. The effect of ethanol dependence on sensitivity and maximum response of the vas deferens of the mouse to noradrenaline, carbachol, barium and calcium.

	n[a]	EC50 × 10^{-6} M (95% C.I.)[b]	Ratio of EC50's[c]	Maximum response (± s.e.)[d]
Noradrenaline				
Control	6	16·8 (12·1–23·2)	—	1074·8 (115·9)
Ethanol	6	15·4 (10·9–21·9)	1·09	1447·3 (113·7)*
Carbachol				
Control	10	5·2 (3·2–7·1)	—	873·9 (49·8)
Ethanol	10	2·8 (0·8–4·7)	1·85	1106·4 (74·3)*
		EC50 × 10^{-3} M		
Barium				
Control	6	3·2 (1·6–5·6)	—	1382·3 (74·7)
Ethanol	6	2·2 (1·5–3·3)	1·45	1344·2 (95·2)
Calcium				
Control	8	14·5 (8·2–25·8)	—	538·3 (83·2)
Ethanol	8	4·8 (2·5–9·3)*	3·0	542·8 (60·4)

[a] Number of experiments.
[b] Geometric mean with 95% Confidence Intervals.
[c] EC50 control/EC50 ethanol.
[d] Mg of tension/10 mg of wet weight of tissue.
* Significantly different from control ($P < 0·01$).

* Correspondence.

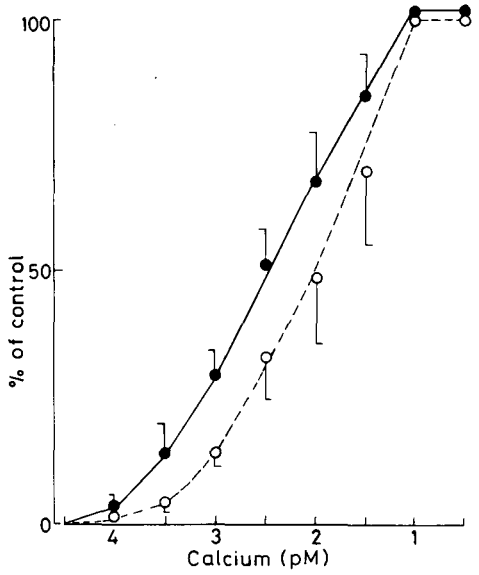

FIG. 1. Mean concentration-effect curves for calcium obtained in vasa deferentia isolated from control (○) and ethanol-dependent (●) mice. Vertical bars indicated s.e. and each point is the mean of 8 experiments.

ethanol vapour reached score 3, 10 h after withdrawal, confirming the development of dependence on ethanol.

Mice were killed by a blow on the head and both vasa deferentia, from the epididymis to the seminal vesicle, were quickly dissected and the surrounding connective tissue removed. Tissues were set up for isometric recording in 10 ml organ-baths containing (mM): NaCl, 130·0; KCl, 5·6; $CaCl_2$, 2·1; $NaHCO_3$, 25·0; glucose, 11·0 sacharose, 13·1 and ascorbic acid 0·11. The bathing solution was kept at 36·5 °C and continuously gassed with 95% O_2–5% CO_2. A resting tension of 0·25 g was applied to the tissues and after 1 h of equilibration period (the bathing medium being changed every 15 min), full concentration-effect curves to noradrenaline (NA), carbachol or barium were obtained by stepwise increases in the agonist concentration, each concentration being washed out before the next higher concentration was added. To prevent carbachol-induced release of endogenous NA (Lindamood et al 1978), concentration-effect curves to the cholinergic agent were obtained in the presence of 1·0 μM phentolamine. Cumulative concentration-effect curves to Ca^{2+} were obtained in vasa deferentia depolarized in a KCl-Ringer solution of the following composition (mM): KCl, 164·2; $KHCO_3$, 3·5 and glucose, 5·4, gassed with 95% O_2–5% CO_2, pH, 7·2. The EC50 values (the agonist molar concentration producing an effect which is 50% of maximum response) were determined and mean EC50's presented as geometric means with 95% confidence intervals (Fleming et al 1972). At the end of the organ-bath experiments all vasa deferentia were weighed and the protein content determined (Lowry et al 1951). Statistical difference between two means was determined using Student's *t*-test for unpaired samples.

Results

During exposure to ethanol vapour, mice were depressed, ataxic, tremulous and fell asleep in abnormal postures. However, they did not lose weight significantly during ethanol exposure or treatment with pyrazole (control group: 33·1 ± 0·9 g, at the beginning of the experiments and 34·2 ± 0·8 g, after five days of pyrazol treatment; $P > 0.05$ and ethanol-exposed group: 35·5 ± 1·3 g, at the beginning of ethanol exposure and 34·6 ± 1·0 g, after five days of ethanol inhalation; $P > 0.05$). The wet weight of vasa deferentia isolated from control and ethanol-dependent mice also did not differ statistically (control group: 10·3 ± 0·4 mg and ethanol-exposed group: 9·5 ± 0·5 mg; $P > 0.05$). The protein contents of vasa deferentia isolated from control and ethanol-dependent mice were 0·180 ± 0·060 mg of protein mg^{-1} of wet weight of tissue and 0·186 ± 0·009 mg of protein mg^{-1} of wet weight of tissue, respectively, $P > 0.05$).

Table 1 shows the effects of ethanol dependence on the responsiveness of the mouse isolated vas deferens to NA, carbachol, barium and calcium. There was a significant augmentation of the maximum response ($P < 0.01$) without a significant alteration of the sensitivity to NA and carbachol, measured at the EC50 level ($P > 0.05$), whilst responsiveness to barium remained unaffected ($P > 0.05$). However, ethanol dependence induced a parallel shift to the left of the concentration-effect curve to calcium (3·0-fold, at the EC50 level, $P < 0.01$). This effect of ethanol dependence is depicted in Fig. 1.

Discussion

It is generally accepted that tension generation in smooth muscle is brought about by a rise in the concentration of internal ionized calcium. Two other stores could be sources of ionized calcium. One is the extracellular compartment and the other is loosely bound to the cell membrane (Chang & Triggle 1973; Bolton 1979). Our results show that ethanol dependence increased the maximum response to NA and carbachol and shifted the concentration-effect curve to calcium to the left. Therefore, it seems plausible to assume that an increased calcium entry or a decreased sequestration of calcium into subcellular components could, mechanistically, explain ethanol dependence-induced increase in drug responsiveness of the vas deferens. Submaximal concentrations of agonists, such as NA and carbachol, probably occupy their own receptors thereby generating action potentials which in turn increase the concentration of internal ionized calcium through the activation of voltage-dependent calcium channels. However, the maximum response of several smooth muscles to NA or carbachol can still be

produced in high-potassium solutions, suggesting that the maximum response is independent of the activation of voltage-dependent calcium channels (Edman & Schild 1962; Jenkinson & Norton 1967; Freeman & Daniel 1973; Bülbring & Szurszewski 1974; De Moraes 1976). Recently, it has been reported that in brain slices taken from rats made tolerant to ethanol, the release of [^{3}H]dopamine was increased by a mechanism involving enhanced sensitivity of the nerve terminal to calcium entry (Linch & Littleton 1983). The present report strongly suggests that in the vas deferens isolated from ethanol-dependent mice the enhanced responsiveness to calcium and to drug stimulation is a consequence of an increased entry of calcium through voltage-independent calcium channels since only the maximum responses to NA and carbachol were enhanced and the responsiveness to calcium was assessed in a high-potassium solution. However, the responsiveness of the vas deferens to barium was unaltered. Probably, the contracting effect of this ion is independent of cellular calcium stores (Jurkiewicz et al 1975) and/or barium enters smooth muscle cells using mechanism(s) unaffected by induction of ethanol dependence. Another possible mechanism for ethanol dependence-induced increase in drug responsiveness of the vas deferens is that ethanol consistently depresses serum testosterone levels in male of every species thus far examined (Cicero 1981) which in turn could alter the mass of the vas deferens. It is also well known that a prolonged decrease in testosterone levels enhances the contractility of the vas deferens. However, the present results showed that neither the wet weights or the protein contents of vasa deferentia isolated from control and ethanol-dependent mice differed, apparently ruling out any testosterone-mediated effect of ethanol on the responsiveness of the vas deferens to NA, carbachol and calcium.

It has been reported that prolonged administration of ethanol to mice decreases the viscosity of neuronal membranes (Chin & Goldstein 1977a) and produces changes in protein conformation (Chin & Goldstein 1977b; Johnson et al 1979) and lipid composition (Chin et al 1978; Littleton et al 1979). Probably, the increased entry of calcium in smooth muscle cells of the vas deferens isolated from ethanol dependent mice could be a consequence of a structural alteration of the cell membrane, as it has been reported for brain tissues. However, the present report, which apparently is the first observation of a relationship between ethanol dependence and increased responsiveness to drug stimulation in a peripheral tissue, is not extensive enough to shed light on the molecular mechanism(s) of ethanol dependence. Nevertheless, the mouse isolated vas deferens could be a relatively simple and adequate experimental model to study some aspects of the cellular basis of ethanol dependence.

REFERENCES

Bolton, T. B. (1979) Physiol. Rev. 59: 606–718

Bülbring, E., Szurszewski, J. H. (1974) Proc. R. Soc. London Ser. B 185: 225–262

Chang, K. J., Triggle, D. J. (1973) J. Theor. Biol. 40: 125–154

Chin, J. H., Goldstein, D. B. (1977a) Science 196: 684–685

Chin, J. H., Goldstein, D. B. (1977b) Mol. Pharmacol. 13: 435–441

Chin, J. H., Parsons, L. M., Goldstein, D. B. (1978) Biochim. Biophys. Acta 513: 358–363

Cicero, T. J. (1981) Ann. Rev. Med. 31: 123–142

De Moraes, S. (1976) Eur. J. Pharmacol. 37: 13–22

Edman, K. A. P., Schild, H. O. (1962) J. Physiol. (London) 161: 424–441

Fleming, W. W., Westfall, D. P., De La Lande, I. S., Jellett, L. B. (1972) J. Pharmacol. Exp. Ther. 181: 339–345

Freeman, D. J., Daniel, E. E. (1973) Can. J. Physiol. Pharmacol. 51: 900–913

Goldstein, D. B. (1972) J. Pharmacol. Exp. Ther. 180: 203–215

Jenkinson, D. H., Norton, I. K. M. (1967) J. Physiol. (London) 188: 373–386

Johnson, D. A., Lee, M. N., Cooke, R., Loh, H. H. (1979) Mol. Pharmacol. 15: 739–746

Jurkiewicz, A., Markus, R. P., Picarelli, Z. P. (1975) Eur. J. Pharmacol. 31: 292–304.

Linch, M. A., Littleton, J. M. (1983) Nature (London) 303: 175–176

Lindamood, C. III, Johnson, S. M., Fleming, W. W. (1978) Proc. Soc. Exp. Biol. Med. 157: 200–201

Littleton, J. M., John, G. R., Grieve, S. J. (1979) J. Alcohol Clin. Exp. Res. 3: 50–56

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275