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Development of tolerance to the inhibitory effects of ethanol in the rat isolated vas deferens: effect of acute and chronic ethanol administration *in vivo*

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1 Contractions of the rat vas deferens elicited by the addition of noradrenaline (NA), K^+-depolarizing solutions or by electrical stimulation were recorded before and after incubation with ethanol 181 mM.

2 In tissues from untreated rats, the contractions were inhibited 40–50% by such exposure.

3 Injection of ethanol ($2\,g\,kg^{-1}$) significantly attenuated ethanol's reduction of peak tension generated by the lowest concentration of NA (10^{-4} mM).

4 Chronic administration of ethanol, $18-14\,g\,kg^{-1}$ daily for two weeks, resulted in significant tolerance to ethanol. Tissues of treated animals demonstrated ethanol-induced decreases of roughly one-half those of the maltose dextrin (isocaloric) and water (fluid control) groups.

5 This tolerance persisted for at least 48 h after ethanol treatment had been terminated.

6 Overall, the data suggest that ethanol acts both pre- and postsynaptically to produce acute inhibition of smooth muscle contractions or tolerance to these actions upon chronic exposure.

Introduction

It has long been recognized that chronic exposure to ethanol is accompanied by an increased resistance to its intoxicating effects on the nervous system. Studies, both in man and experimental animals, have shown that tolerance can be induced fairly rapidly by appropriate doses of ethanol. Dillon *et al.* (1983) found no tolerance to the inhibitory *in vitro* effects of ethanol on contractions of vas deferens despite the presence of functional CNS tolerance (hypnosis and hypothermia) in mice treated with ethanol for 4 days. Tolerance to the inhibitory effects of ethanol *in vitro* on electrically-evoked contractions of the isolated, ileal longitudinal muscle/myenteric-plexus preparation has been reported by Mayer *et al.* (1980a,b) after 2 to 4 weeks of ethanol administration to guinea-pigs. It has also been found that vascular smooth muscle from rats dosed for 2 to 6 weeks became progressively tolerant to the inhibitory effect of ethanol on spontaneous contractions and on agonist and K^+-induced responses (Altura *et al.*, 1980).

Studies *in vitro* have shown that ethanol produces a dose-dependent reduction in the force of contraction of noradrenaline (NA)- and electrically-stimulated mouse vas deferens (Blum *et al.*, 1980a,b,c). However, the mechanism of this acute ethanol effect at the synaptic level is not clear. High ethanol concentrations (0.2 mM) reduce neuronal NA uptake and stimulate NA release from brain slices (Israel *et al.*, 1973; Roach *et al.*, 1973; Carmichael & Israel, 1975). In addition, an increase in spontaneous NA release from the rat vas deferens has more recently been reported by Degani *et al.* (1979) at lower (0.065 mM) ethanol concentrations. Since increased spontaneous exocytosis is thought to be due to an increased fluidization of the neuronal membrane (Seeman, 1972), ethanol may exert a non-specific effect on presynaptic and vesicular membranes of noradrenergic neurones. It has also been postulated, however, that postsynaptic events, such as an interference with Ca^{2+} mobility, may be involved in the attenuation of smooth muscle responses by ethanol (Clement, 1980; Altura & Altura, 1983).

The rat vas deferens preparation used in the present study receives a dense noradrenergic innervation. Each field impulse applied at low frequencies produces a discrete twitch response (Ambache *et al.*, 1972) and the cumulative addition of submaximal concentrations of NA evokes consistent step-wise increments in tension. Furthermore, contractile responses to high K^+ concentrations have been shown to involve presynaptic stimulation as well as a direct action

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postsynaptically (Shimodan & Sunamo, 1981). K^+ levels of 45 mM or less produce a gradual depolarization which results in a dose-dependent submaximal contraction, whereas concentrations greater than 50 mM cause a biphasic contraction (Syson & Huddart, 1973). With the selection of the various methods of both pre- and postsynaptic stimulation, the rat isolated vas deferens provides a useful experimental model to study the cellular and pharmacological mechanisms of the inhibitory action of ethanol *in vitro* and the *in vivo* production of tolerance to this effect.

The present paper is concerned with the effects of *in vitro* and *in vivo* ethanol administration on the contractions elicited in rat vas deferens by the addition of NA or K^+ or by electrical stimulation.

Methods

Animals and tissue preparation

Male Sprague-Dawley rats (Charles River Co.) weighing 200–300 g were housed individually in stainless steel hanging cages within a temperature controlled vivarium for one week before they were killed or underwent gastric catheterization surgery. All rats were maintained on a 12 h dark: 12 h light cycle (lights off from 09 h 00 min–21 h 00 min) with water and lab chow available *ad libitum*. After surgery, rats were kept in individual plexiglass cages and their gastric catheters were connected via liquid swivels to a peristaltic infusion pump for the administration of ethanol, maltose or water. Animals were decapitated (between 10 h 00 min and 11 h 00 min) and the entire length of vas deferens from the epididymis to the seminal vesicles was quickly dissected and washed in cold, modified Krebs-Ringer soluion of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $CaCl_2$ 2.1, $NaHCO_3$ 25, and glucose 10.0.

After the seminal contents were gently flushed out of the lumen, each tissue was suspended in an isolated organ bath of 25 ml capacity containing Krebs solution at 37°C and gassed with 95% O_2: 5% CO_2. The preparations were equilibrated for 2 h under 1.0 g resting tension with a solution change every 15–20 min. Contractions were subsequently elicited at 30 min intervals. Isometric longitudinal tension was recorded on a Grass Model 7A polygraph by means of Grass force-displacement transducers. A Datel Intersil integrator (DPP-Q7) was used to measure peak heights of the contractions.

For the construction of dose-response curves the peak tension development after each dose was regarded as the full response. Concentration-effect curves were constructed by cumulative NA administration (10^{-4} to 10^{-1} mM). NA bitartrate (Sigma) was dissolved in Krebs containing ascorbic acid 1 mM (Fisher) as an antioxidant. The high potassium in the medium for some experiments was achieved by replacing the bathing solution with high K^+-Krebs solution. K^+-Krebs solution (45 or 100 mM) was made in advance by mixing an appropriate volume of the normal K^+-Krebs solution. K^+-Krebs solution was made by replacing NaCl in the normal solution with equimolar KCl. Total exposure time of tissues was for 5 min. Lastly, maximal muscular contractions were elicited for 5 min by transmural stimulation via platinum ring electrodes with single supramaximal (80 V) pulses of 0.5 ms duration. All concentrations cited in the text represent final bath concentrations (mM) calculated for each compound.

Ethanol treatment in vitro

An initial group of tissues was exposed to increasing concentrations of ethanol to determine the IC_{50} for ethanol on NA-induced contractions (i.e., the ethanol concentration that produces approximately a 50% reduction in peak tension generated by NA administration). After collecting the initial dose-response data for NA-induced contractions, these preparations were challenged with graded concentrations of ethanol in buffer, separated by drug-free washouts to restore the original baseline response before each new ethanol exposure. The remaining preparations were first stimulated under control conditions, then exposed to ethanol 181 mM in Krebs and retested 15 min later.

Ethanol treatment in vivo

Acute: rats were injected i.p. with ethanol 2.0 g kg^{-1} (20% w/v) or saline 30 min before decapitation. Blood ethanol levels (BEL) at time of death were approximately 185 mg %.

Chronic: ethanol-dependence was induced by treating rats chronically with ethanol for a period of two weeks. Ethanol was administered in equal doses 4 times daily via surgically implanted, polyethylene gastric catheters. A total daily dose of 8 g kg^{-1} ethanol was given initially and thereafter the dose was increased 1 g kg^{-1} every two days. By the end of the two weeks, rats were receiving a total daily dose of 14 g kg^{-1} ethanol. Control rats received either isocaloric maltose dextrin (Dyets), or water, as controls for the volume of liquid, instead of ethanol. During the treatment period individual weight gains (means ± s.e.mean) were 38.2 ± 10 g for the ethanol-treated animals, 42.4 ± 12 g for maltose-treated rats, and 45 ± 13 g for the water group. There were no fatalities. Ethanol and control treatments were then discontinued, and the rats were sampled over a 3 to 48 h withdrawal period. During the withdrawal period the three treatment groups showed virtually identical

weight gains. Ethanol-treated subjects killed 3 h after the last dose of ethanol (dependent) exhibited behavioural signs of intoxication and demonstrated BEL in excess of 230 ± 25 mg %. Obvious signs of withdrawal were evident 12–18 h post treatment in the ethanol group, justifying use of the term dependent (Pohorecky, 1974).

Ethanol content of mixed arteriovenous, trunk blood was assayed by gas-liquid chromatography (Redmond & Cohen, 1972; Pohorecky & Brick, 1982).

Calculations and statistical analyses

Tissue responses were measured as the peak tension developed above baseline and were expressed in g of tension or as a percentage depression from the initial (reference) contraction induced by each particular stimulus. All results are expressed as means ± s.e.mean. Differences were compared employing a two-tailed Student's *t* test for paired or unpaired data, and were considered significant if *P* was less than 0.05.

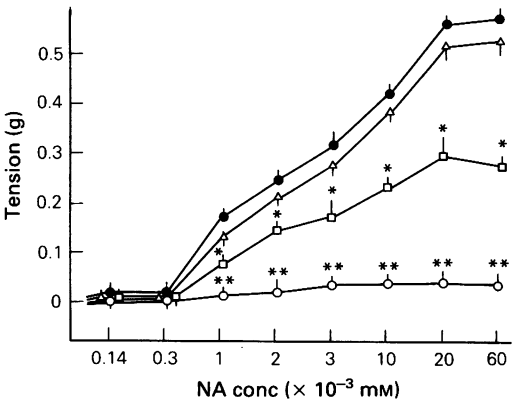

Figure 1 Noradrenaline (NA)-induced contractions of the rat vas deferens in presence of ethanol. NA ($0.14–60 \times 10^{-3}$ mM) was added cumulatively to tissues incubated in the absence of ethanol (●) or in the presence of 90 mM (△), 181 mM (□) or 362 mM (○) ethanol. Data are presented as mean tension in g for groups of 6 tissues; s.e.mean shown by vertical lines. $*P<0.05$ or less compared to tissues incubated in the absence of ethanol. $**P<0.001$ or less compared to tissues incubated in the absence of ethanol.

Results

Untreated animals

Cumulative NA application *in vitro* resulted in increasing tension development in the smooth muscle of the rat vas deferens (Figure 1). Exposure to ethanol alone had no effect on resting tissue tension but NA-induced responses were both smaller and demonstrated lower maximal response after the 15 min incubation period. Ethanol inhibited the muscular twitch induced by all concentrations of NA in a dose-dependent manner, with an IC_{50} equal to 181 mM. The ED_{50} for NA was 2.8×10^{-3} mM, 2.5×10^{-3} mM, 1.8×10^{-3} mM and 0.9×10^{-3} mM in the presence of 0, 90, 181 and 362 mM ethanol in the tissue bath. The highest concentration of ethanol almost completely inhibited contractions. Regardless of the applied stimulus, washing of the tissue completely reversed the inhibitory actions of ethanol *in vitro*.

Acute ethanol treatment

Acute ethanol administration *in vivo* had little effect on its inhibition *in vitro* of the NA-stimulated vas deferens (Table 1). Baseline tension and responses to NA alone were no altered. In comparison to the 58% depression produced by added ethanol in tissues from saline injected rats, tissues from rats given a single i.p. injection of 2.0 g kg^{-1} ethanol 30 min before they were killed showed significantly reduced ethanol attenuation of the peak tension generated in the preparation by the lowest concentration of NA (1.4×10^{-4} mM). Responses evoked by subsequent NA additions also tended to be greater, though not significantly so in tissues of the acutely intoxicated animals.

In tissues stimulated electrically or with KCl, the *in vitro* inhibitory effects of ethanol were comparable to those seen in tissues from rats treated acutely with ethanol and the two control groups (saline injected and uninjected rats) (Table 2). The overall depressant effect of ethanol ranged from 36 to 54%. The depressant effect tended to be larger in preparations stimulated with 100 mM KCl than with 45 mM KCl.

Chronic ethanol treatment

The chronic administration of ethanol to rats (8 to 14 g kg^{-1} daily for 14 days) altered the sensitivity of the vas deferens to various forms of stimulation. Tissues from ethanol-treated rats were less sensitive to NA stimulation (Figure 2). The ED_{50} for NA was 1.8×10^{-3} M in tissues from ethanol-treated rats versus 2.8×10^{-3} M from tissues from dextrin maltose-treated controls. The ED_{50} of the ethanol-treated rats did not differ from that of rats receiving water infusions (1.5×10^{-3} mM). However, these two tissues differed with respect to maximal tension developed with the highest concentration of NA. Maximal contractions were higher in tissues from ethanol-treated animals than those produced by tissues from either dextrin maltose or water infused animals, the latter two being

Table 1 Inhibitory effect of *in vitro* added ethanol on contractions of rat vas deferens induced by noradrenaline (NA)

In vivo *treatment*	*Concentration NA* (10^{-3} mM) 0.14	0.3	1.0	2.0	3.0	10.0	30.0	60.0
Saline	58.3 ± 3.5	49.5 ± 4.0	40.0 ± 2.9	33.1 ± 2.5	29.3 ± 2.0	32.7 ± 2.8	36.0 ± 3.0	41.0 ± 3.3
Ethanol 2 g kg^{-1}	42.1 ± 2.2*	43.3 ± 2.9	35.5 ± 2.7	30.1 ± 2.0	25.0 ± 1.5	27.5 ± 1.5	32.7 ± 1.9	38.6 ± 2.2
Water	49.9 ± 3.5	42.0 ± 3.9	41.1 ± 3.0	37.5 ± 2.8	40.2 ± 3.7	45.7 ± 3.2	51.6 ± 4.2	58.5 ± 4.3
Dextrin maltose	55.5 ± 4.9	54.4 ± 5.2	48.5 ± 4.0	45.3 ± 3.9	40.3 ± 3.5	44.2 ± 3.1	54.7 ± 4.1	65.0 ± 5.0
Chronic ethanol 3 h	18.0 ± 0.9*	17.5 ± 0.5*	17.3 ± 0.9*	16.5 ± 0.6*	15.0 ± 0.8*	25.0 ± 1.3*	20.0 ± 1.5*	25.5 ± 1.8*
Chronic ethanol 12 h	42.3 ± 3.5	50.0 ± 4.0	44.4 ± 3.7	46.5 ± 3.2	37.3 ± 2.5	42.2 ± 2.3	47.7 ± 2.5	54.0 ± 4.0
Chronic ethanol 24 h	20.0 ± 1.4*	20.4 ± 1.3*	26.8 ± 1.8*	23.3 ± 1.2*	31.1 ± 2.1*	33.0 ± 2.2*	42.4 ± 2.5*	52.7 ± 3.0*
Chronic ethanol 36 h	10.3 ± 0.8*	16.2 ± 1.1*	10.0 ± 0.7*	10.4 ± 0.7*	18.3 ± 1.5*	22.1 ± 1.1*	30.2 ± 1.9*	27.5 ± 1.9*
Chronic ethanol 48 h	25.3 ± 2.0*	22.6 ± 1.8*	20.4 ± 1.5*	20.3 ± 1.7*	18.1 ± 1.2*	9.5 ± 0.5*	8.7 ± 0.5*	18.3 ± 0.7*

Contractions in response to cumulative additions of NA were measured in tissues incubated in the presence or absence of 181 mM ethanol. Tissues were obtained from animals 30 min after an injection with either saline or 2 g kg^{-1} of ethanol, i.p., or from rats treated chronically with ethanol (8–14 g kg^{-1} daily, for 14 days), equicaloric dextrin maltose or water. In addition tissues from rats treated chronically with ethanol were also used 12, 24, 36 or 48 h after their last dose of ethanol. Results are expressed as the mean (± s.e.mean) % depression of the response in the presence of ethanol compared to the response of tissues incubated in its absence. Each mean represents at least 6 tissues.
*$P < 0.05$ or less compared to corresponding control group (saline, water or dextrin maltose).

Table 2 Inhibitory effect of *in vitro* ethanol on vas deferens contractions induced by high potassium and electrical stimulation

In vivo *treatment*	*% depression of contractions with ethanol* in vitro *KCl* 45 mM	*KCl* 100 mM	*Electrical*
Untreated	40.0 ± 3.5	48.0 ± 3.7	45.1 ± 2.9
Saline	43.2 ± 2.9	54.0 ± 4.1	50.0 ± 3.5
Ethanol, 2 g kg^{-1}	36.0 ± 2.5	51.0 ± 3.9	42.3 ± 3.1
Water	44.1 ± 3.5	56.3 ± 4.5	50.0 ± 4.3
Dextrin maltose	44.5 ± 3.3	50.0 ± 4.2	52.1 ± 4.0
Chronic ethanol – 3 h	38.2 ± 2.1	32.1 ± 2.2*	27.0 ± 1.9*
Water	47.5 ± 3.0	49.0 ± 3.1	52.3 ± 4.0
Dextrin maltose	51.2 ± 4.5	47.3 ± 2.8	52.8 ± 4.3
Chronic ethanol – 12 h	42.3 ± 3.1	37.7 ± 2.1	55.9 ± 4.4
Water	50.3 ± 4.3	57.3 ± 3.5	50.1 ± 4.3
Dextrin maltose	48.7 ± 4.4	61.1 ± 4.0	49.9 ± 3.5
Chronic ethanol – 24 h	33.8 ± 2.9*	43.5 ± 3.9*	34.0 ± 3.0*
Water	42.3 ± 3.3	50.6 ± 3.9	50.0 ± 3.7
Dextrin maltose	45.2 ± 3.7	53.7 ± 4.2	51.2 ± 4.2
Chronic ethanol – 36 h	28.5 ± 1.9*	32.3 ± 3.5*	32.2 ± 3.5*
Water	44.0 ± 4.0	54.7 ± 4.3	50.5 ± 3.9
Dextrin maltose	43.5 ± 3.5	53.2 ± 4.4	50.2 ± 4.3
Chronic ethanol – 48 h	30.4 ± 3.1*	34.3 ± 3.0*	33.4 ± 3.7*

Muscular contractions were measured in response to 45 or 100 mM KCl or to brief transmural electrical pulses in tissues incubated in the presence or absence of 181 mM ethanol. Tissues were obtained from animals 30 min after an injection with either saline or 2 g kg^{-1} of ethanol i.p., or from rats treated chronically with ethanol (8–14 g kg^{-1} daily for 14 days), equicaloric dextrin maltose or water. Groups of rats chronically ethanol-treated were also killed 12, 24, 36 or 48 h after their last dose of ethanol. Results are expressed as the mean (± s.e.mean) % depression of the response in the presence of ethanol compared to the response of tissues incubated in its absence. Each point is the mean of 6 determinations.
*$P < 0.05$ or less compared to corresponding control group (saline, water or dextrin maltose).

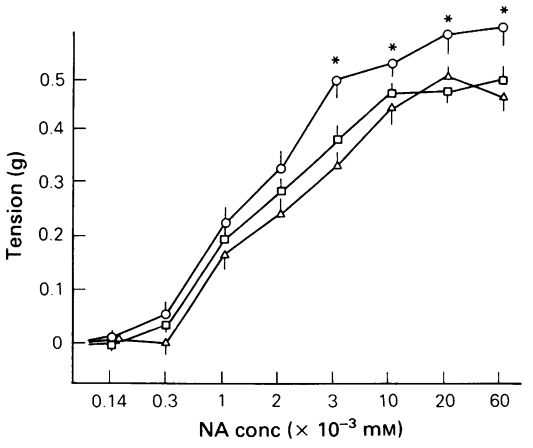

Figure 2 Noradrenaline (NA)-induced contractions in rat vas deferens tissues obtained from rats chronically treated with ethanol (○), dextrin maltose (△) or water (□). Tissues of chronic control (water- or dextrin maltose-treated animals) and chronic ethanol (8–14 g kg^{-1} daily for 14 days)-treated animals were stimulated by the cumulative addition of NA (0.14–60 × 10^{-3} mM). Results are presented as the mean tension in g for groups of 6 tissues; s.e.mean shown by vertical lines. *$P < 0.05$ or less compared to tissues from maltose-treated rats.

comparable. Tissues from ethanol-treated animals were completely tolerant to the depressant effects of ethanol added *in vitro* on NA-induced contractions (Table 1). In tissues from water- and maltose-treated animals the addition of 181 mM ethanol to the incubation medium produced on average a 45.5% and 50.6% inhibition of NA-induced contractions respectively. By contrast, in ethanol-treated animals, ethanol-induced inhibition was only about 19%. The observed differences between groups in contractile response in the presence of *in vitro* ethanol was greatest at the relatively low and high concentrations of NA. Thus chronic treatment with ethanol resulted in significant tolerance to the inhibitory effect of this drug on the muscular contractions elicited by NA.

However, there was little tolerance to the inhibitory effect of *in vitro* ethanol- on KCl-stimulated contractions (Table 2). The average depressant effect of ethanol on contractions induced with 45 and 100 mM KCl in control tissues was 44% and 53% respectively. Furthermore the inhibitory effect of *in vitro* ethanol on tissues from chronically treated rats was similar; i.e., 38% in media containing 45 mM KCl. But some tolerance to ethanol was evident in tissues stimulated with 100 mM KCl. Statistically significant tolerance was observed in tissues stimulated transmurally. The depressant effect of ethanol in these tissues *in vitro* was only 27% compared to the 51% inhibition shown in tissues from either the dextrin-maltose or water-infused control animals. That is tissues from ethanol-treated rats showed significant tolerance.

Changes in sensitivity to the three forms of stimulation of the vas deferens, and to the inhibitory effects of ethanol *in vitro*, were also examined at various times after termination of ethanol treatment; i.e., during withdrawal (Tables 1 and 2). At the earliest time tested, that is 12 h after the last administration of ethanol, the sensitivity to the *in vitro* effects of ethanol in vas deferens preparations stimulated by NA was similar to that of the two control tissues. By contrast, at all subsequent times tested tissues were again relatively tolerant to the *in vitro* addition of ethanol. Similarly tissues stimulated with KCl were not tolerant to ethanol at the 12 h period but showed statistically significant tolerance to ethanol at the later times. The greatest tolerance was seen with the 36 h group and this pattern of response was also seen in tissues stimulated electrically.

Discussion

The results clearly demonstrate that ethanol produces a reversible dose-dependent attenuation of smooth muscle contractions in the rat vas deferens preparation. Exposure to 181 mM ethanol, which had no effect on resting tension in the tissue, caused a significant reduction of the peak tension generated in response to NA, K^+ and electrical stimulation of the vas. Sensitivity of tissues to NA was increased in the presence of ethanol. The addition of NA initiates contraction presumably via its interaction with the α-adrenoceptor on the muscle membrane. Electrical stimulation, on the other hand, operates presynaptically by depolarizing the neuronal membrane. Finally, K^+-induced responses involve presynaptic depolarization in addition to direct postsynaptic activation. The present results indicate a comparable effect of 181 mM ethanol on responses to all three methods of stimulation. It therefore appears that moderate concentrations of ethanol act to a similar extent both pre- and postsynaptically to inhibit contractions of the rat vas deferens.

Consistent with previous work (Altura *et al.*, 1980; Mayer *et al.*, 1980a,b; Dillon *et al.*, 1983), the present findings also show that ethanol treatment *in vivo* can decrease its inhibitory actions *in vitro*. Acute *in vivo* treatment with ethanol did not alter sensitivity to *in vitro* ethanol, except at the lowest concentration of NA. After chronic ethanol administration, responses of the vas to all the stimulants tested were significantly greater in the presence of ethanol than those of control preparations. Since tolerance is defined as a reduced effectiveness that develops after repeated exposure (Tabakoff, 1977), a state of tolerance to the depressant actions of ethanol is, therefore, clearly demonstrated

in the vas deferens following chronic administration to rats. Furthermore, as NA-induced contractions appeared to be less attenuated by ethanol after only one *in vivo* exposure while K^+- and electrically-evoked responses remained inhibited, tolerance might first develop at the postjunctional level. This is also supported by the observation that the evidence of tolerance to ethanol was least in tissues stimulated with KCl.

A process might begin postsynaptically, during ethanol administration, which renders the α-adrenoceptor more susceptible to activation by neurotransmitter and/or increases the amount of intracellular Ca^{2+} available for contraction. This process might also help to explain the ethanol dependence-induced increase in tissue responsiveness to NA alone that we observed (Figure 2). Similarly using the mouse isolated vas deferens, DeMoraes & Capaz (1984) reported that ethanol dependence increased the maximum response to carbachol as well as NA. Furthermore, responsiveness to Ca^{2+} was also enhanced, shifting the concentration-effect curve to the left 3 fold at the EC_{50} level, although tissue responses to barium remained unaltered. The authors proposed that ethanol-dependence involved an increased sensitivity of the muscle to these agents as a result of an enhanced movement of Ca^{2+} across the neuronal membrane (DeMoraes & Capaz, 1984).

Another addictive drug, morphine, has similar effects to those of ethanol. *In vitro* it inhibits stimulated contractions of vas deferens preparations (Marshal *et al.*, 1981). With chronic treatment, tolerance develops to its inhibitory effects, and as we observed with ethanol, there was supersensitivity to NA (Marti, 1982; Contreras *et al.*, 1982). Furthermore local application of chlorpromazine to the hypogastric nerve *in vivo* produced results similar to those we observed with ethanol, i.e., there was supersensitivity to NA-induced contractions as well as an increase in maximal responsiveness (Goto, 1983). These effects were postulated to result from the membrane stabilizing effects of this drug or because of an inhibitory effect on the presynaptic intraneuronal calmodulin (Goto, 1983). Despite these similarities in sensitivity to NA of tissues from various drug-treated animals, differences have also been noted. For example, Saban *et al.* (1982) found a marked hypersensitivity to NA in vas preparations from animals chronically treated with barbitone during withdrawal from the drug.

The changes in sensitivity to ethanol observed in the vas deferens preparation in chronically treated animals during withdrawal is interesting. We have no explanation for the observed loss of tolerance to ethanol at the 12 h point. Subsequently tissues show tolerance to ethanol which persisted up to 48 h after the last treatment. Thus the tolerance to ethanol *in vitro* persists for at least 2 days after the last exposure to ethanol. This indicates that the structural and/or functional tissue changes induced by chronic treatment with ethanol persist a long time after disappearance of ethanol from the system. The disappearance to tolerance at 12 h may be due to hormonal, ionic or other disturbances occurring during the acute stages of withdrawal. Corticosterone levels are known to increase during the early part of withdrawal (Pohorecky, 1974), for example and adrenal cortical hormones play a role in the modulation of noradrenoceptors in the vas deferens (Maeda *et al.*, 1983). Furthermore changes in Na^+/K^+ATPase activity known to occur during withdrawal (Rangaraj & Kalant, 1978) may play a part in the temporary change in sensitivity of the vas deferens during withdrawal. Changes in vas deferens sensitivity occurring as a result of denervation of the organ are primarily the result of changes in Na^+/K^+ATPase activity (Gerthoffer *et al.*, 1979).

In summary, *in vitro* ethanol inhibits smooth muscle contractions produced by NA, K^+, and electrical stimulation. Prior *in vivo* treatment with ethanol reduces this inhibition, with significant tolerance developing upon chronic ethanol administration. Moreover, tolerance persists for at least two days after discontinuation of chronic treatment. Significant changes were seen across all concentrations of NA, thus they were not unique to a particular level of adrenoceptor stimulation.

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