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THE EFFECT OF ETHANOL ON INHIBITORY AND MOTOR RESPONSES IN THE RAT AND RABBIT ANOCOCCYGEUS AND THE BOVINE RETRACTOR PENIS MUSCLES

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1 Ethanol (200 mM) reduced the response to inhibitory nerve stimulation in the rat and rabbit anococcygeus and the bovine retractor penis (BRP) muscles. Ethanol also reduced the response to the inhibitory extract from the BRP consistent with the inhibitory factor in these extracts playing some part in the response to inhibitory nerve stimulation.

2 Ethanol's effect on the response to other inhibitory stimuli was examined in the rabbit anococcygeus and the BRP. In the anococcygeus the response to carbachol was reduced, to bradykinin and isobutylmethylxanthine (IBMX) unaltered, and to isoprenaline and adenosine 5′-triphosphate (ATP) potentiated. In the BRP responses to IBMX and sodium nitroprusside were unaltered but in this tissue the response to isoprenaline was reduced. Ethanol's ability to reduce inhibitory responses is, therefore, selective and confined to inhibitory nerve stimulation, inhibitory extract, carbachol and, in the BRP, isoprenaline.

3 Ethanol reduced the rate of development of inhibition even where the magnitude of the inhibitory response was unaltered.

4 In the rat anococcygeus, ethanol (200 mM) potentiated the response to motor nerve stimulation and to noradrenaline (NA) at low frequencies and low concentrations respectively. Higher ethanol concentrations (400 mM) reduced the response to both motor nerve stimulation and NA. The motor response to carbachol was also reduced.

5 Ethanol (200 mM) itself caused an easily reversible contraction in all three tissues. This was not due to the release of NA but was highly sensitive to the removal of external calcium from the medium.

6 A unified explanation of these varied effects of ethanol based on a reduction in membrane binding of calcium and a reduced efficiency of receptor coupling is suggested.

Introduction

In a previous paper (Gillespie & McKnight, 1978) we showed that 1–2% ethanol reduced the response to stimulation of the non-adrenergic non-cholinergic inhibitory nerves in the rat and rabbit anococcygeus muscles. We have since reported some properties of a smooth muscle inhibitory factor in extracts from both the bovine retractor penis (BRP) and the rat anococcygeus muscles (Gillespie & Martin, 1980). This inhibitory factor is not adenosine 5′-triphosphate (ATP) and might be the neurotransmitter of these inhibitory nerves (Bowman, Gillespie & Martin, 1979). If this is so and if ethanol reduces the response to inhibitory nerve stimulation by a postsynaptic action, then ethanol should also reduce the response to the inhibitory extract. This paper describes the results of experiments testing this possibility. We have also tested the selectivity of the action of ethanol both against other inhibitory stimuli and against the response to motor nerve stimulation and to the transmitter of these nerves, noradrenaline. Finally, ethanol itself causes muscle contraction and we have examined the dependence of this on external calcium in comparison with the motor response to noradrenaline and barium chloride. Some of these results have been reported to the Pharmacological Society (Gillespie, Hunter & McKnight, 1981).

Methods

Rat and rabbit anococcygeus muscles were isolated as previously described (Gillespie, 1972; Creed, Gillespie & McCaffery, 1977) and suspended under a tension of 1 g in Ag/AgCl ring electrodes in 10 ml baths containing Krebs saline at 36°C and gassed with a mixture of 95% O_2 and 5% CO_2. The saline

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composition was (mM): NaCl 118; KCl 4.7, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and dextrose 11. Strips of bovine retractor penis muscle 3–4 cm long by 2 mm thick were cut from abbattoir material and similarly suspended with a resting tension of 1–2 g. In all three preparations tension was measured with a Grass FTO3 tension transducer and displayed on a Grass Polygraph. Tone was raised in the rat and rabbit anococcygeus muscles by adding guanethidine to the bath to produce a concentration of 2×10^{-5} M. In the BRP, phentolamine (5×10^{-6} M) and propranolol (5×10^{-6} M) were added to abolish the effect of simultaneous motor nerve stimulation. Intramural nerve fibres were stimulated with 1 ms pulses at supramaximal voltage and frequencies varying from 0.5 to 20 Hz. Usually a fixed number of either 50 or 100 pulses were delivered irrespective of frequency. Drugs, with the exception of ethanol, were added to the bath in volumes not exceeding 0.3 ml. Ethanol was made up to the appropriate concentration in the Krebs saline in one of two reservoirs, the other containing ethanol-free Krebs solution. These were arranged so that the organ bath could be washed and replaced with either.

The following drugs were used and doses refer to the base: atropine sulphate (BDH); propranolol hydrochloride (ICI); phentolamine mesylate (CIBA); mepyramine maleate (May & Baker); noradrenaline bitartrate (Koch-Light); sodium nitroprusside (BDH); IBMX (Aldrich); isoprenaline sulphate (Burroughs-Wellcome); carbachol (L. Light & Co.); bradykinin triacetate (Sigma); ATP (Sigma); ethanol (Analar); barium chloride (Analar); 1, 2, bis, 2 aminoethoxyethane-NNN'N'-tetra acetic acid (EGTA) (Sigma).

Results

Effect of ethanol on motor and inhibitory nerve responses

Figure 1 illustrates the effect of ethanol 200 mM on the response to inhibitory nerve stimulation in the rat

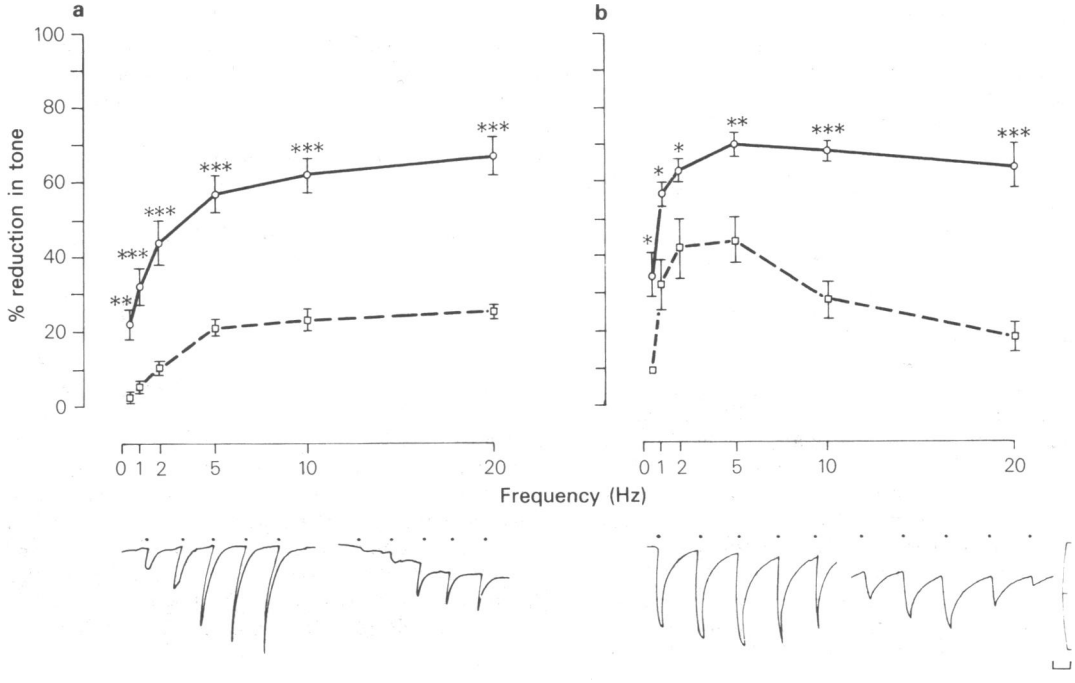

Figure 1 The inhibitory effect of ethanol on the response of (a) the rat anococcygeus and (b) the bovine retractor penis (BRP) muscles to field stimulation of their inhibitory nerves. The graphs show the mean responses from 5 experiments: (○) control; (□) in the presence of ethanol (200 mM). The experimental records below each graph illustrate representative experiments for each tissue. In each instance the five responses in the first panel are the control responses to stimulation at 1.0, 2.0, 5.0, 10.0 and 20 Hz and the second panel shows the responses to the same frequencies of stimulation in the presence of ethanol (200 mM). Tone in the rat anococcygeus was induced with guanethidine 2×10^{-5} M, tone in the BRP was spontaneous. Time marker 2 min, tension calibration bar band 12 g for the BRP and 2.5 and 5 g for the rat anococcygeus. Statistical significance: $^{**}P < 0.01$; $^{***}P < 0.001$.

anococcygeus and bovine retractor penis muscles. Similar rests were obtained in the rabbit anococcygeus. The nerve-mediated responses were reduced at all frequencies to between 10–40% in the rat and 35–70% in the BRP. This reduction was not accompanied by any fall in tone whether this was spontaneous as in the BRP and rabbit anococcygeus or induced by guanethidine. Similar, though smaller, reductions were produced by 100 mM ethanol but 20 mM had no effect.

Ethanol is known to be able to reduce the effects of nerves by a local anaesthetic action (Göthert, Dührsen & Rieckesmann, 1979). To test whether this was the cause of the reduction in response to inhibitory nerve stimulation, we examined in the rat anococcygeus the effect of ethanol on motor response to field stimulation of the adrenergic nerves, their transmitter noradrenaline and to carbachol. The results varied with the ethanol concentration and with the frequency of stimulation and are shown in Figures 2 and 3. Ethanol, at a concentration below 200 mM, usually potentiated the response to both noradrenaline and motor nerve stimulation. At 200 mM and higher the effect was variable. Usually both nerve and noradrenaline responses were reduced about 25% and equally (Figure 2) though, occasionally, potentiation was still present. At higher concentrations of 400 mM, the response to both stimuli was reduced, the nerve response more than the response to noradrenaline (Figure 2). The effect of ethanol 200 mM also varied with the frequency of motor nerve stimulation and with the dose of noradrenaline. As Figure 3 shows, low frequencies of stimulation of 2 Hz and less were potentiated as were low concentrations of noradrenaline, while higher frequencies or concentrations producing larger contractions were reduced. In the rat anococcygeus muscle both noradrenaline and carbachol produce contractions though acting through different receptors. As Figure 2 shows, ethanol was even more effective in reducing the motor response to carbachol than that to noradrenaline. These results suggest that only at high concentrations of 400 mM or more does ethanol exert any selective local anaesthetic action on

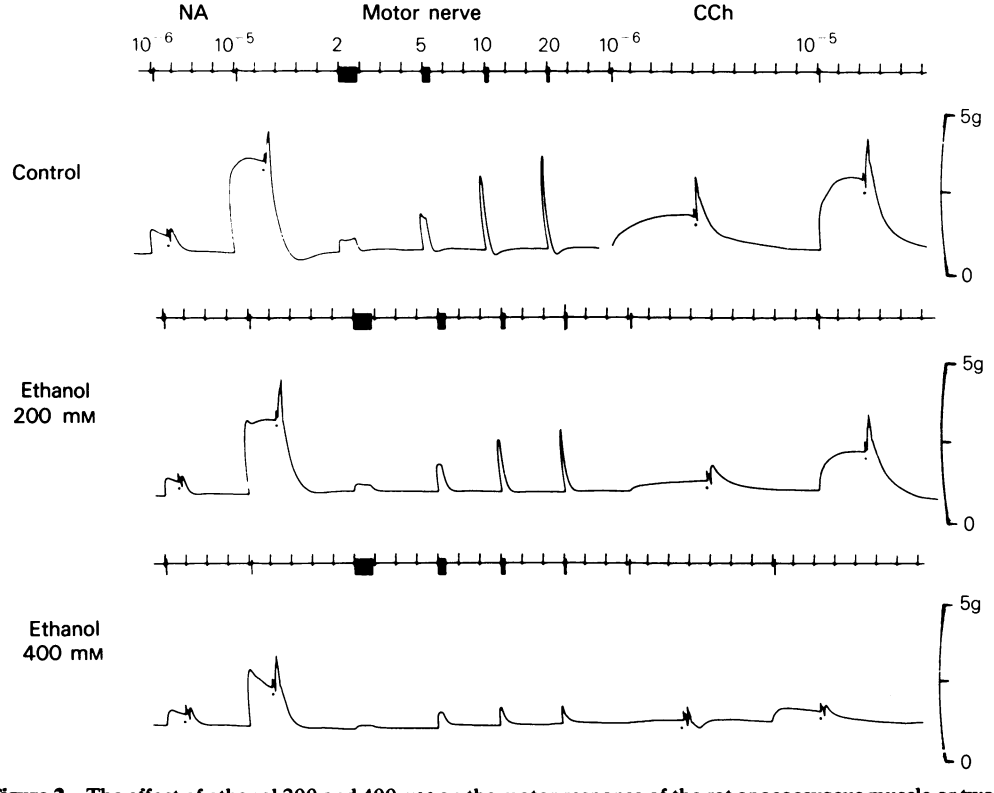

Figure 2 The effect of ethanol 200 and 400 mM on the motor response of the rat anococcygeus muscle or two dose levels of norodrenaline (NA), field stimulation of the motor nerves with 100 pulses at the frequencies shown and two dose levels of carbachol (CCh). Ethanol 200 mM reduces the response to all three stimuli about equally but at 400 mM the reduction to nerve stimulation is greater than that to NA.

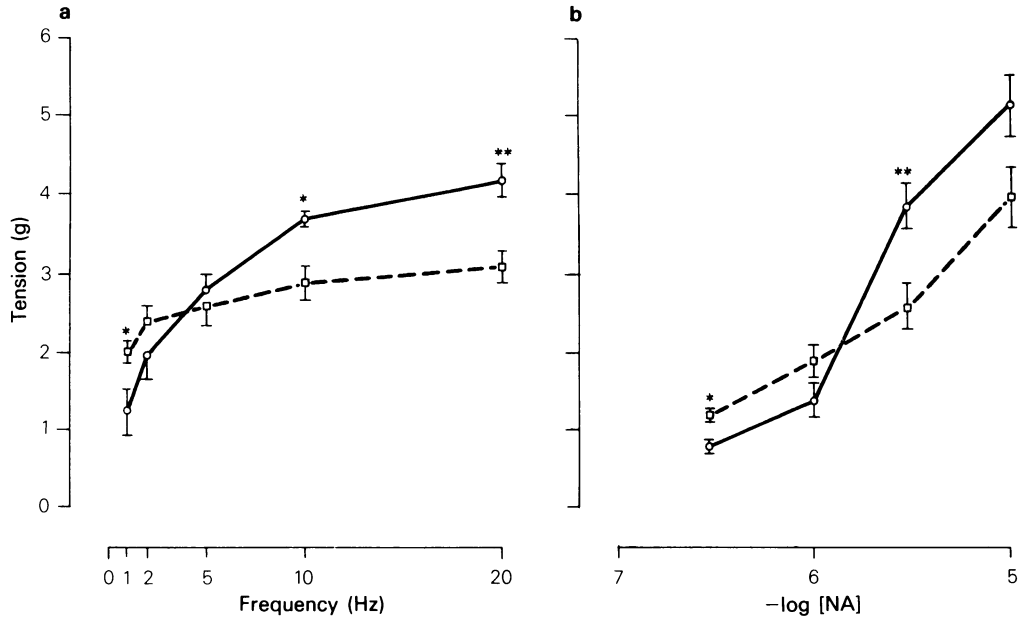

Figure 3 The effect of the frequency of motor nerve stimulation (a) and the dose of noradrenaline (NA) (b) on the effect of ethanol 200 mM in the rat anococcygeus muscle. At low frequencies of stimulation and low concentrations of NA, ethanol potentiated motor responses and at high frequencies and high NA concentrations inhibited responses. Each point in both graphs is the mean of between 5 and 7 experiments with the exception of 3×10^{-7} M NA which has only four results. Statistical comparison of these means showed a significant difference at the level of $P = 0.05$* and $P = 0.01$**.

nerve fibres and that the equal reduction or potentiation of the response to noradrenaline and nerve stimulation at 200 mM ethanol indicates a postsynaptic site of action.

Effect of ethanol on the response to other inhibitory stimuli

For these experiments the rabbit anococcygeus and BRP muscles were used since the rat anococcygeus is relaxed by few stimuli. In the rabbit the effect of ethanol (200 mM) was tested on the inhibitory response to isoprenaline, carbachol, bradykinin, isobutylmethylxanthine (IBMX) and ATP. On the BRP isoprenaline, IBMX and sodium nitroprusside were tested. Figures 4 and 5 illustrate some of these results. In the rabbit, carbachol inhibition like the response to inhibitory nerve stimulation was greatly reduced by between 50 and 90% depending on the carbachol dose. In contrast, the inhibition produced by bradykinin or IBMX was unaltered though its rate of development was slowed and the inhibitory effect of isoprenaline and ATP was actually potentiated (Figure 4). In the BRP somewhat similar effects were obtained, the inhibitory response to IBMX or sodium nitroprusside though slow to develop was unaltered

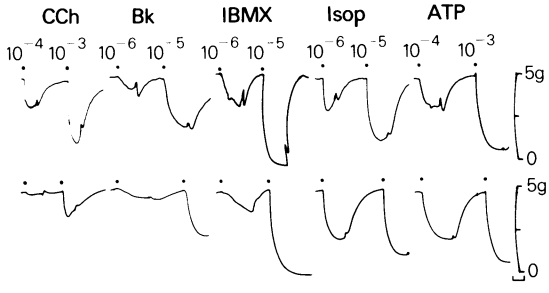

Figure 4 The effect of ethanol (200 mM) on the inhibitory responses of the rabbit anococcygeus muscle to two dose levels of carbachol (CCh), bradykinin (Bk), isobutylmethylxanthine (IBMX), isoprenaline (Isop) and ATP. The upper row of records are the control responses, the lower the responses in the presence of ethanol. Ethanol reduced the magnitude of the inhibitory response to carbachol, had no effect on that to bradykinin or IBMX and potentiated at least the low dose of isoprenaline and ATP. The rate of development of inhibition with all responses was slowed, this is particularly clear for the low doses of bradykinin and IBMX. Time marker, 2 min.

in magnitude. The response to isoprenaline, however, was reduced in this muscle (Figure 5). A consistent observation was that ethanol, irrespective of its effect on the final amplitude of inhibitory responses, did reduce their rate of development. This was observed in both tissues and at all points on the dose-response curve. The magnitude of this effect was estimated by measuring, for drug concentrations producing about 80% of the maximum response, the time taken for the inhibitory response to reach half its final amplitude. Figure 6 illustrates the effect of 200 mM ethanol on this half time for inhibitory nerve stimulation, the inhibitory extract, isoprenaline, IBMX and sodium nitroprusside. The half time is lengthened for each drug response but the effect is greater with sodium nitroprusside and IBMX whose site of action is intracellular.

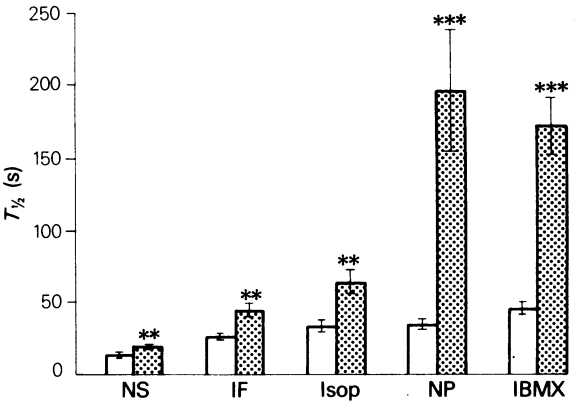

Figure 6 The effect of ethanol (200 mM) on the half time of relaxation of the bovine retractor penis muscle (BRP) to inhibitory nerve stimulation (NS, $n=9$), the inhibitory factor in extracts from the BRP (IF, $n=8$), isoprenaline (Isop, $n=5$), sodium nitroprusside (NP, $n=5$) and isobutylmethylxanthine (IBMX, $n=6$). Open columns represent the half-times in controls and stippled columns the half-times in the presence of ethanol. Asterisks indicate the statistical significance of the difference between means: $^{**}P<0.01$; $^{***}P<0.001$.

Effect of ethanol on the response to inhibitory extract

The experiments in the last two sections demonstrated a selective action of ethanol on inhibitory responses. Only carbachol suffered a reduction comparable to that of inhibitory nerve stimulation. It was particularly interesting, therefore, to see what effect, if any, ethanol would have on the response to the inhibitory factor in our extracts. The results are illustrated in Figure 7. In 12 out of 16 experiments, ethanol 200 mM reduced the response by between 30 and 70%. In the remaining 4 experiments the response was unaltered (Figure 7). Since ethanol slowed the rate of development of the responses it was possible that with a substance such as the inhibitory factor, which is rapidly inactivated at 37°C, there would be insufficient time for the complete development of mechanical inhibition in the presence of

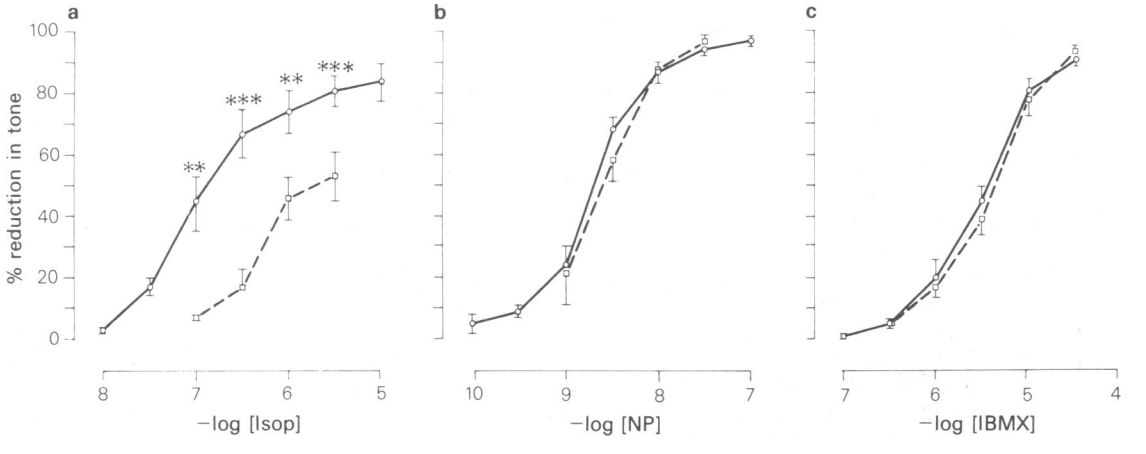

Figure 5 The effect of ethanol (200 mM) on the dose-response curves in the bovine retractor penis muscle (BRP) to (a) isoprenaline (Isop), (b) sodium nitroprusside (NP) and (c) isobutylmethylxanthine (IBMX). In each panel the control dose-response curve is shown in solid lines and the dose-response curve in the presence of ethanol as a dashed line. Each point is the mean of between 4 and 6 observations with the exception of those without error bars where only 3 or less points were available. Asterisks indicate the statistical significance of the difference between means: $^{**}P<0.01$; $^{***}P<0.001$. Ethanol displaced the dose-response curve to isoprenaline but had no effect on that to NP or IBMX.

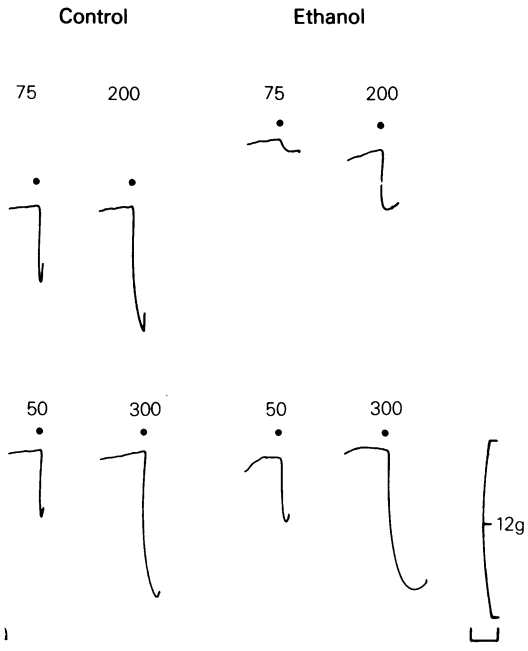

Figure 7 The effect of ethanol (200 mM) on the response of the bovine retractor penis muscle (BRP) to two dose levels of inhibitory extract. The figures above each response are the volume of extract in μl added to the 10 ml bath. The left hand pair of responses are the controls, the right hand pair the response to the same dose of extract in the presence of ethanol. The upper row represents the usual effect of ethanol, the response to inhibitory factor is reduced. The lower row is from the less frequent experiments in which ethanol did not alter the magnitude of the inhibitory effect. Time marker, 2 min.

ethanol before the inhibitory factor was destroyed. This would give an erroneous impression of inhibition of the response by ethanol. To exclude this possibility several BRP strips were superfused in series with Krebs saline in a closed cascade. Inhibitory extract was continuously infused into the superfusing solution by means of a syringe driven by a stepping motor. The steady infusion of extract was continued for between 5 and 8 min and produced a steady state of inhibition, the degree of which varied with the position of the strip in the cascade. The first preparation was exposed to the highest concentration with a correspondingly high degree of inhibition; the time taken to reach the second tissue permitted sufficient inactivation of inhibitory factor to produce a smaller response and the degree of inhibition was even less by the third preparation. The total time for perfusion fluid to pass over all three tissues was approximately 30 s. With this arrangement, the tissues are exposed to a steady level of inhibitory factor for a period long enough to allow full development of mechanical inhibition so that changes in the rate of development of the inhibitory response should not affect its magnitude. The results of one experiment representative of these experiments is shown in Figure 8. In the absence of ethanol, inhibition of the first preparation was complete and developed rapidly at both infusion rates; the second preparation was almost maximally inhibited but the rate of development of inhibition was less rapid. The last preparation was only partially inhibited especially at the low rate of infusion. In the presence of 200 mM ethanol the inhibitory responses were reduced in all three preparations. These inhibitory effects of ethanol were completely and rapidly reversed by washing as the last panel in Figure 8 illustrates.

The contractile effect of ethanol

In other smooth muscles, ethanol has been found to cause contraction and in the present experiments 200 mM and, to a lesser extent, 100 mM ethanol regularly caused contraction in all three muscles. The origin of this contraction was investigated in the BRP. Ethanol has been shown to increase the spontaneous release of noradrenaline in the rat vas deferens (Degani, Sellers & Kadzielawa, 1979) so that the contractions in the BRP could have been an indirect sympathomimetic effect. Phentolamine 10^{-6} M, which blocked matching doses of noradrenaline, had no effect on the response to ethanol so that this explanation is unlikely. Many of the effects of ethanol have been attributed to its ability to modify calcium movement in the cell and it seemed, therefore, useful to see whether this contractile effect was dependent on external calcium. Some agonists, such as high potassium or carbachol, open voltage-dependent channels which allows mainly extracellular calcium to enter the cell. Others, such as noradrenaline, may use both extracellular and a lightly bound membrane store of calcium, whereas others, such as barium chloride, can displace intracellular calcium and are relatively independent of extracellular calcium. We, therefore, compared the effect of omitting all calcium from the bathing medium and of combining this with the addition of EGTA (1 mM) to chelate loosely bound calcium on the contractile response to ethanol 200 mM, noradrenaline 10^{-5} M (a large but submaximal concentration) and barium chloride 10^{-2} M. The results are illustrated in Figure 9. Omission of calcium from the bathing medium completely abolished the response to ethanol, reduced that to noradrenaline by over 50% but, if anything, potentiated the response to barium chloride. The addition of EGTA to the calcium-free Krebs solution abolished the response to noradrenaline as well as to ethanol and greatly reduced that to barium chloride.

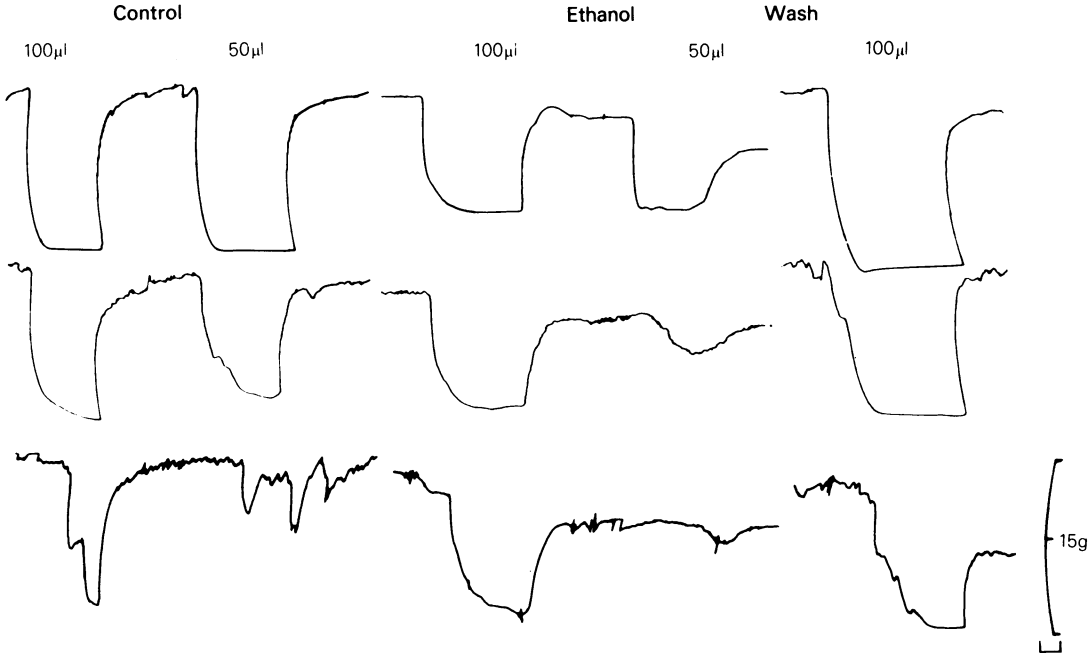

Figure 8 The effect of ethanol (200 mM) on the response to inhibitory factor of three preparations of the bovine retractor penis muscle (BRP) arranged as a cascade. The upper row of responses are from the first preparation in the cascade, the middle row from the second preparation and the bottom row from the last preparation. Inhibitory factor was infused as rates of either 50 μl or 100 μl/min for between 5 and 8 min into the physiological saline passing over the tissues. The first pairs of responses are controls, the second in the presence of ethanol and the final records, at the higher concentration of inhibitory factor only, show that the effects of ethanol are reversible. In the control both levels of infusion were sufficient to inhibit completely the spontaneous tone in the first preparation in the cascade. By the time the second preparation was reached destruction of the inhibitory factor was sufficient to reduce the response at 50 μl to a submaximal level and in the third preparation responses at both infusion levels were reduced. In the presence of ethanol all responses in each of the three preparations were reduced with the possible exception of 100 μl/min in the lower row. After washing the original responses were restored. Time marker, 2 min.

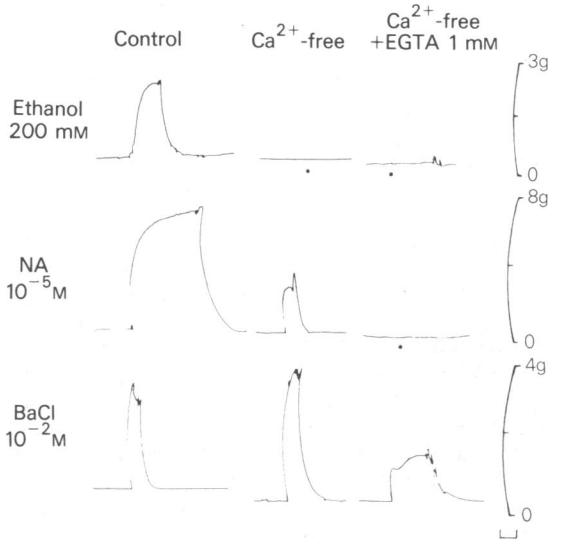

Figure 9 The effect of removal of calcium and removal of calcium plus the addition of EGTA on the contractile response of the bovine retractor penis muscle (BRP) to ethanol 200 mM, noradrenaline 10^{-5} M (NA) and barium chloride 10^{-2} M ($BaCl_2$). Removal of calcium alone abolished the response to ethanol, reduced that to NA and if anything potentiated the response to $BaCl_2$. Chelation with EGTA abolished, in addition, the response to NA and reduced that to $BaCl_2$. Time marker, 2 min.

Discussion

These results are of interest on two quite separate grounds. First, their relevance to the possible role of the inhibitory factor as the inhibitory neurotransmitter and, second, their implications for the pharmacological actions of ethanol. The response to inhibitory extract like that to inhibitory nerve stimulation is reduced by ethanol, consistent with a role as inhibitory transmitter. However, the degree of reduction is less than that of the response to inhibitory nerve stimulation and this, together with other work in this laboratory, does throw some doubt on this possibility. Nevertheless, the finding that, of a wide range of inhibitory drugs tested on the rabbit anococcygeus, only carbachol and the inhibitory extract were reduced by ethanol does support some involvement, possibly postsynaptically, of the inhibitory factor in the response to inhibitory nerve stimulation.

Ethanol's own pharmacological properties have been widely investigated. There seems to be general agreement that its actions are exerted principally on membranes in the lipids of which it selectively dissolves but its effects may be mediated through functional proteins incorporated in these membranes and influenced by changes in their lipid environment (Chin & Goldstein, 1977). In smooth muscle four effects have been reported. Most common is a reduced contraction by agonist drugs, which include acetylcholine, histamine, adrenaline, noradrenaline, angiotensin, vasopressin, prostaglandin E_1, high potassium and barium chloride (Guillot & Gwan, 1937; Hurwitz, Battle & Weiss, 1962; Altura, Edgarian & Altura, 1976; Altura & Edgarian, 1976; Altura, Ogunkoya, Gebrewald & Altura, 1979; Turlapaty, Altura & Altura, 1979b). Less frequently ethanol has been reported to potentiate the response to some of these same agonists though often only at low ethanol concentrations with a reduction in response at high concentrations (Kalsner, 1970; Altura & Edgarian, 1976; Altura *et al.*, 1976; Clement, 1980). Thirdly, ethanol itself can cause contraction in some smooth muscle (Carlström, 1926; Fewings, Hanna, Walsh & Whelan, 1966; Kalsner, 1970; Turlapaty *et al.*, 1979b) and, finally, ethanol can reduce both tone and rhythmic activity in some spontaneously active muscles (Carlström, 1926; Turlapaty *et al.*, 1979b). Curiously we have been unable to find any report on the effect of ethanol on the response to drugs that relax smooth muscle.

In the rat and rabbit anococcygeus, ethanol 200 mM regularly causes contraction unaffected by phentolamine, inhibits the motor response to adrenergic nerve stimulation, to noradrenaline and even more to carbachol (rat). Sometimes this and lower doses potentiate the contractile response especially at low frequencies of stimulation; in these circumstances increasing the ethanol concentration changes potentiation to inhibition. These results mirror those already quoted in the literature. New information in the rabbit anococcygeus and BRP is the remarkable reduction in the rate of development of mechanical inhibition mediated by a variety of relaxant drugs, isoprenaline, bradykinin, IBMX, sodium nitroprusside, carbachol, the inhibitory factor and inhibitory nerve stimulation. However, in the rabbit anococcygeus muscle the magnitude of the inhibitory response is reduced only for carbachol, inhibitory nerve stimulation and the inhibitory factor; in the BRP isoprenaline is also reduced. The mode of action of ethanol in producing these effects is unclear. Several possibilities can probably be excluded; for example, inhibition of drug receptor binding is unlikely since only one report, for adrenoceptors in the mouse brain, has found such a reduction (Ciofalo, 1980). Similar ligand binding experiments with dopamine or muscarinic receptors in the brain (Tabakoff & Hoffman, 1979; Ciofalo, 1980) or on the cholinoceptors in the electric organ of *Torpedo* (Sauter, Braswell & Miller, 1979) showed no reduction. This failure to reduce binding to cholinoceptors is particularly relevant to the present experiments since the response to carbachol acting on muscarinic receptors was particularly reduced by ethanol whether the effect was contraction (rat) or inhibition (rabbit). Nor is the action of ethanol likely to be on the contractile proteins since the drug itself can produce dose-related graded contractions up to the tissue maximum and in its presence a maximum inhibition was still possible with several relaxants. Finally, an action of ethanol on the inactivating pathways for these drugs is unlikely. Ethanol in the doses used has no effect on acetylcholinesterase (Gage, 1965), monoamine oxidase, catechol-*O*-methyltransferase or the uptake mechanism into adrenergic vesicles (Majchrowicz, 1973). The mode of action most favoured by recent workers is that ethanol alters the level of intracellular calcium at the contractile filaments (Hurwitz *et al.*, 1962; Kalsner, 1970; Seeman, 1972; Ross, 1976; Altura *et al.*, 1976; Turlapaty *et al.*, 1979a; Mayer, Khanna & Kalant, 1980). Calcium for smooth muscle contraction is derived from one of three sources, the extracellular fluid, a small labile store loosely bound to the external membrane and an intracellular store associated with endoplasmic reticulum and, perhaps, mitochondria and the inner surface of the plasma membrane (van Breemen, Aaronson, Loutzenhiser & Meisheri, 1980). Muscle contraction is ended by sequestering calcium within these organelles followed by a slower extrusion from the cell (Vallieres, Scarpa & Somlyo, 1975; van Breemen *et al.*, 1980). With this background we would like to propose a mode of action for ethanol which makes two assumptions. First, that

ethanol dissolved in membranes reduces their affinity for calcium and, secondly, the increased membrane disorder as a result of increased fluidity interferes with the coupling between plasma membrane receptors and the calcium pool on which these operate. On this hypothesis, ethanol-induced contractions are due to the rise of calcium levels in the cytoplasm. Since the effect is readily prevented by removing external calcium its source is unlikely to be the stable intracellular calcium store but is either direct entry of extracellular calcium from the bathing medium or release from the labile store in equilibrium with that medium. Since ethanol has no effect on resting calcium uptake or efflux (Harris & Hood, 1980) and reduces entry through the voltage dependent calcium channel (Hurwitz *et al.*, 1962; Göthert *et al.*, 1979; Harris & Hood, 1980) increased transmembrane transport from the external medium seems unlikely. This leaves release from the labile bound store as the most likely origin of the calcium responsible for ethanol contractions. Turlapaty *et al.* (1979b) came to a similar conclusion about vascular smooth muscle. On this hypothesis low concentrations of ethanol which potentiate the response to some agonists do so by reducing the calcium binding affinity so that the receptor-operated channels are more effective in releasing calcium. The decrease in response at higher concentrations of ethanol could be explained in two ways. First, by so disordering the membrane as to interfere with the link between the receptors and the calcium channel, and second, by reducing the amount of calcium available in the labile pool. Indirect evidence of ethanol decreasing coupling efficiency between presynaptic receptors and transmitter release has been found in adrenergic nerves (Göthert *et al.*, 1979) and between postsynaptic dopamine receptors and adenylate cyclase in the caudate mucleus (Tabakoff & Hoffman, 1979). Evidence for an ethanol-induced reduction in the labile calcium pool has also been reported for vascular smooth muscle (Turlapaty *et al.*, 1979a) and neuronal membranes (Ross, 1976). The decreased rate of relaxation produced by ethanol was observed with all the relaxant drugs tested, particularly those whose site of action is believed to be intracellular, suggesting a similar intracellular site for the action of ethanol. If ethanol acts on intracellular membranes to reduce their binding of calcium then the rate of transport of calcium into the organelles would be reduced with a reduced rate of relaxation but the capacity to sequester calcium would not necessarily be less so that the eventual degree of relaxation would be unimpaired. Since the magnitude of relaxation with carbachol, inhibitory nerve stimulation and inhibitory extract was reduced, this implies some difference in the site or mode of action of these agents in reducing intracellular calcium levels.

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