EFFECTS OF ALCOHOLS ON RESPONSES EVOKED BY INOSITOL TRISPHOSPHATE IN XENOPUS OOCYTES

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SUMMARY

1. The effects of ethanol and other alcohols on inositol 1,4,5-trisphosphate (InsP$_3$) signalling were studied in Xenopus oocytes by the use of flash photolysis of caged InsP$_3$. Calcium liberation induced by InsP$_3$ was monitored by voltage-clamp recording of Ca$^{2+}$-activated membrane currents, and by fluorescence of the Ca$^{2+}$ indicator Fluo-3.

2. Membrane current and fluorescence Ca$^{2+}$ signals evoked by light flashes giving small responses were initially potentiated by bath application of ethanol (80–400 mM). However, the responses subsequently declined while ethanol was present and were strongly reduced or suppressed when it was removed.

3. These effects did not arise artificially from changes in photolysis of caged InsP$_3$, as similar results were seen with responses evoked by intracellular injections of InsP$_3$. Also, the effects on the membrane current did not arise primarily through actions on the Ca$^{2+}$-dependent Cl$^-$ channels, since currents evoked by intracellular injections of Ca$^{2+}$ were little changed by ethanol.

4. Ethanol reduced the threshold level of InsP$_3$ required to cause Ca$^{2+}$ liberation. Thus, potentiation was most prominent with small responses evoked by brief light flashes, whereas the predominant effect on larger responses was inhibitory.

5. The facilitatory and inhibitory actions of ethanol persisted after removing extracellular Ca$^{2+}$.

6. Intracellular injections of ethanol produced an initial inhibition of InsP$_3$ responses, followed, in some oocytes, by a potentiation.

7. Methanol had little effect on InsP$_3$ responses, whereas butanol and other long-chain alcohols produced strong inhibition, but little or no potentiation.

8. We conclude that extracellular application of ethanol produces a rapid potentiation of InsP$_3$-mediated Ca$^{2+}$ liberation, and a more slowly developing inhibition. The potentiation may arise through stimulation of InsP$_3$ formation at the plasma membrane, whereas the inhibition occurs more deeply in the cell. Both actions were evident at relatively low concentrations (a few tens of millimoles per litre), and might thus be important in the behavioural effects of ethanol intoxication.

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INTRODUCTION

Inositol 1,4,5-trisphosphate (InsP$_3$) functions as an intracellular messenger molecule in virtually every cell in the body, where it acts by liberating Ca$^{2+}$ from intracellular stores (for recent reviews see: Berridge & Irvine, 1989; Rana & Hokin, 1990). Because of this ubiquitous role, drugs acting on the intracellular InsP$_3$ receptor and other parts of the messenger pathway are expected to have widespread and varying effects on cellular function. We were thus motivated to look for pharmacological agents affecting the InsP$_3$ system. For this purpose, oocytes of *Xenopus laevis* offer a convenient experimental system, since they possess a messenger pathway whereby InsP$_3$-mediated Ca$^{2+}$ liberation leads to the generation of a Cl$^-$ membrane current (Miledi & Parker, 1984; Oron, Dascal, Nadler & Lupu, 1985; Parker & Miledi, 1986), and their large size greatly facilitates procedures including voltage-clamp recording and intracellular microinjection. Furthermore, flash photolysis of caged InsP$_3$ (McCray & Trentham, 1989) provides an elegant means by which to evoke repetitive, reproducible elevations of cytosolic InsP$_3$ concentration (Parker & Miledi, 1989). We had previously used these techniques to examine the influence of caffeine on InsP$_3$-evoked responses (Parker & Ivorra, 1991), and now use similar approaches to study the actions of alcohols.

The work presented here stems from an observation, made several years ago, that bath application of ethanol to oocytes could generate oscillatory currents (R. Miledi, unpublished data). This effect was seen also by Watford, Dunwiddie & Harris (1989), who further reported that oscillatory currents were induced by injection of ethanol into oocytes. These currents are carried by Cl$^-$ ions (Watford et al. 1989) and closely resemble those evoked by intracellular injections of InsP$_3$, suggesting that they arise through intracellular Ca$^{2+}$ mobilization. However, high concentrations (several hundred millimoles per litre) of ethanol are required to evoke appreciable oscillatory currents (Watford et al. 1989; the present paper), thus raising doubts as to whether actions on the InsP$_3$–Ca$^{2+}$ signalling pathway could be important in the behavioural effects of ethanol that become apparent at concentrations of a few tens of millimoles per litre. Nevertheless, we surmised that ethanol might modulate InsP$_3$-mediated signalling at concentrations lower than those which directly evoked responses, and looked for such effects on responses evoked by photorelease of InsP$_3$ in the oocyte. We describe here how ethanol (40–400 mM) causes rapid potentiation of InsP$_3$-evoked responses, together with a slowly developing depression. Because of this latter phenomenon, strong effects on InsP$_3$ signalling are apparent at concentrations of ethanol well below that required to directly evoke responses.

METHODS

Procedures for obtaining oocytes of *Xenopus laevis* for voltage-clamp recording and for light flash photolysis of caged InsP$_3$ were as previously described (Parker & Miledi, 1989; Parker 1991). Briefly, defolliculated oocytes were voltage clamped at a potential of −60 or −50 mV, and membrane currents were recorded while the oocytes were continuously superfused with frog Ringer solution at room temperature. Normal Ringer solution had the composition (in mM): NaCl, 120; KCl, 2; CaCl$_2$, 1.8; HEPES, 5; at about pH 7.0. Calcium-free Ringer was made by omitting CaCl$_2$ and adding 1 mM-EGTA and 5 mM-MgCl$_2$. In most experiments, oocytes were each loaded with 1–10 pmol of caged InsP$_3$ (myo-inositol 1,4,5-trisphosphate, P$_{45}^{[5]}$-1-(2-nitrophenyl)ethyl ester), and
were stimulated by brief flashes of near UV light to cause intracellular release of free \( \text{InsP}_3 \). The photolysis light was normally arranged as a square with sides of about 100 \( \mu \text{m} \), positioned on the vegetal hemisphere close to the equator. Bath applications of alcohols were accomplished by switching the intake of the superfusion system. The bars marked on the figures indicate the time at which the solution was changed, and do not correct for the dead time of 20–60 s (depending on flow rate) of the perfusion system. Solutions of alcohols were prepared by volumetric dilution, and the resulting molar concentrations calculated from their densities at room temperature. As a guide, a dilution of 0.1% ethanol corresponds to about 16 mm. Intracellular injections were made by applying pneumatic pressure pulses to glass micropipettes broken to a tip diameter of a few micrometres. \( \text{InsP}_3 \), caged \( \text{InsP}_3 \) and ethanol were injected at respective concentrations of 100 \( \mu \text{M} \), 1 mm and 1 mm, in aqueous solutions including 50 mm-EDTA and 5 mm-HEPES at pH 7.0. Recordings of intracellular Ca\( ^{2+} \) transients were made as described previously (Parker & Ivorra, 1990a,b; Parker, 1991), by use of the fluorescent indicator Fluo-3 (Minta, Kao & Tsien, 1989). \( \text{InsP}_3 \) and caged \( \text{InsP}_3 \) were obtained from Calbiochem (La Jolla, CA, USA), Fluo-3 from Molecular Probes Inc. (Eugene, OR, USA) and alcohols were obtained from Sigma Chemical Co. (St Louis, MO, USA).

RESULTS

Ethanol potentiates and inhibits caged \( \text{InsP}_3 \) responses

Figure 1 illustrates the basic phenomena with which this paper is concerned. An oocyte that had previously been loaded with caged \( \text{InsP}_3 \) was stimulated by a train of ultra-violet (UV) light flashes at 30 s intervals, so as to evoke a series of transient membrane current responses. These currents arose because the free \( \text{InsP}_3 \) that was formed by photolysis caused liberation of Ca\( ^{2+} \) from intracellular stores and that, in turn, activated Ca\( ^{2+} \)-dependent Cl\( ^{-} \) membrane currents (Parker & Miledi, 1989). Furthermore, because each oocyte was loaded with a large excess of caged \( \text{InsP}_3 \), it was possible to evoke hundreds of responses without depleting the reserve. The flash duration in this experiment was set slightly above the threshold necessary to evoke any response, so that a series of small and roughly constant currents were obtained, and ethanol was bath applied at a concentration of 400 mm for the time indicated by the bar. Initially, the light flash responses were greatly potentiated, but during continued application of ethanol they declined progressively. On washing out the ethanol, the currents were at first almost completely suppressed, but subsequently showed a partial recovery over several minutes.

Results like those illustrated were consistently observed in more than forty other oocytes examined although, as discussed later, the relative magnitude of the potentiation and inhibition varied with the concentration of ethanol and the duration of the stimulus light flash.

Facilitation and depression of responses to microinjected \( \text{InsP}_3 \)

The phenomena illustrated in Fig. 1 were observed with responses generated by photorelease of \( \text{InsP}_3 \). Before progressing further to investigate their mechanisms, it was first necessary to exclude the possibility that the effects arose artifactually from actions of ethanol upon the photolysis of caged \( \text{InsP}_3 \). We thus examined the action of ethanol on responses evoked by microinjection of free \( \text{InsP}_3 \) into oocytes.

Figure 2A shows responses evoked by injection of a low dose of \( \text{InsP}_3 \). With the oocyte bathed in normal Ringer solution this gave only a single small spike of current. However, after addition of 450 mm-ethanol the same amount of \( \text{InsP}_3 \) produced a much larger and more prolonged response. Results obtained in ten similar
Fig. 1. Potentiation and depression of InsP₃-evoked currents by bath application of ethanol. In this and other figures, traces show membrane currents recorded at a clamp potential of −60 mV; downward deflections correspond to inward membrane currents. The oocyte was loaded with about 0.8 pmol caged InsP₃ and was stimulated by repetitive light flasher (duration 24 ms) at 30 s intervals. During the time marked by the bar, the superfusate was switched to a solution including 400 mM-ethanol.

A

Fig. 2. A, potentiation of response to intracellular injections of low doses of InsP₃ by ethanol. Traces show successive responses to injections of about 0.2 fmol InsP₃, given at times indicated by the arrow-heads. The record on the left is a control response. That on the right was obtained a few minutes later, beginning about 70 s after bath applying 450 mM-ethanol. B, inhibition by 1-hexanol of responses evoked by greater amounts of InsP₃. Arrow-heads indicate when intracellular injections of roughly 40 fmol InsP₃ were made. The superfusion solution included 0.05% 1-hexanol when indicated by the bar. The third injection was made 5 min after washing out hexanol. Records in A and B are from different oocytes.

Experiments were quantified by measuring the charge displacements (i.e. area under the current traces) induced by InsP₃. The mean control response evoked by a near-threshold dose of InsP₃ was 3.4 ± 1.1 nC (± 1 s.e.m.), whereas corresponding injections
of InsP₃ gave a mean response of 15.3 ± 3.8 nC in the presence of ethanol (160–450 mM).

To investigate whether alcohol depressed – as well as facilitated – responses to injected InsP₃, larger doses of InsP₃ were used. Furthermore, we used hexanol in place of ethanol because, as described later, the actions of the higher alcohols were almost entirely inhibitory and thus simplified interpretation of the results. The oocyte in Fig. 2B was stimulated by repeated injections of about 40 fmol InsP₃. In

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**Fig. 3.** The effects of ethanol arise primarily from actions on intracellular Ca²⁺ release, and not on the Ca²⁺-activated chloride currents. **A.** Trace shows membrane currents evoked by alternate stimulation by light flashes (open arrow-heads) and by intracellular injections of about 7 fmol Ca²⁺ (filled arrow-heads). Ethanol (320 mM) was bath applied when indicated by the bar. **B.** Simultaneous records of intracellular Ca²⁺ transients and membrane currents evoked by repetitive light flashes (marked by •). The upper trace monitors fluorescence of the Ca²⁺ indicator dye Fluo-3 previously loaded into the oocytes together with caged InsP₃. Upward deflections correspond to increasing fluorescence and increasing free Ca²⁺ concentration; the magnitude of the trace is uncalibrated. Ethanol (400 mM) was bath applied during the time indicated by the bar. The unsteady baseline of the fluorescence following the solution change was the result of bubbles in the perfusion system.
control solution this evoked a large oscillatory current persisting for over 1 min. After addition of 0.05% 1-hexanol the response was greatly reduced and InsP₃ evoked only a single small spike of current, beginning after a latency of about 20 s. This inhibition was at least partly reversible, since a larger response was obtained to an injection of InsP₃ made 5 min after washing out the hexanol.

*Intracellular Ca²⁺ liberation is facilitated and inhibited by ethanol*

The InsP₃-evoked membrane currents arise because InsP₃ liberates Ca²⁺ from intracellular stores, and the resulting rise in cytoplasmic Ca²⁺ then activates Ca²⁺-dependent chloride channels in the plasma membrane (Miledi & Parker, 1984; Parker & Miledi, 1989). The effects of ethanol might thus arise through actions on InsP₃-mediated Ca²⁺ liberation, or through actions on the Ca²⁺-activated chloride channels. We used two approaches to discriminate between these possibilities.

In the first, ethanol was applied while currents were evoked alternately by light flashes (to release InsP₃) and by injections of Ca²⁺ (Fig. 3A). The light flash response was at first greatly (600%) facilitated by ethanol, but then declined during the application and was completely suppressed on washing. In contrast, the currents evoked by Ca²⁺ injection showed no facilitation. Instead they were reduced by about 15% while ethanol was present, but recovered rapidly to about the control level on washing. Results like those illustrated were obtained in six other experiments.

The second approach to determine whether ethanol affects InsP₃-mediated Ca²⁺ liberation utilized a fluorescent Ca²⁺ indicator dye (Minta et al. 1989) to more directly monitor intracellular free Ca²⁺ levels. Figure 3B illustrates an experiment in which Fluo-3 was used to monitor Ca²⁺ simultaneously with measurements of membrane currents. The oocyte was injected with a mixture of caged InsP₃ and Fluo-3 (Parker & Ivorra, 1990a), and repetitive flashes of UV light were used to evoke small current responses. Application of ethanol produced a large initial facilitation of the currents, but these subsequently declined during continued perfusion and were suppressed after washing. Similar to this, the fluorescent Ca²⁺ signals also showed a clear initial facilitation, with the responses subsequently declining during application of ethanol and being suppressed after washing. Some quantitative differences were, however, apparent between the fluorescence and current recording. Most notably, the maximal facilitation of the Fluo-3 signal was smaller (300%, as compared to 1900% for the current) and the decline during ethanol application was less (45% decline from peak response, compared to 84% for the current).

Eight trials in three oocytes gave similar results and, in all cases, the Ca²⁺ signal was potentiated during ethanol application and depressed after washing. Differences between the fluorescent and current signals were, however, sometimes more marked than in the record illustrated. For example, small fluorescent signals often remained after washing ethanol, even though the corresponding currents were undetectable.

*Potentiation is most evident with small InsP₃ responses*

Figure 4 shows the effects of various concentrations of ethanol on responses evoked by just-suprathreshold light flashes that evoked small (1–2 nA) currents, and on larger (about 100 nA) currents evoked by longer flashes. With the brief flashes, currents were potentiated during application of ethanol, and the extent of the
facilitation increased as the ethanol concentration was raised from 80 to 400 mM (Fig. 4A). Very different to this, the larger currents evoked by long flashes showed almost no facilitation at any concentration of ethanol tested (Fig. 4B). Instead, the currents were reduced by proportionally great extents with increasing concentrations of ethanol. A dramatic comparison of the differing effects of ethanol on small and large InsP₃-evoked currents is provided by the recordings at high (400 or 320 mM) concentrations of ethanol. The small response was potentiated by 1800%, whereas the larger response was reduced to 10%.

**Ethanol decreases the threshold amount of InsP₃ required to evoke a current**

We have previously shown (Parker & Miledi, 1989; Parker & Ivorra, 1990a, b) that a certain threshold level of InsP₃ is required in the oocyte before Ca²⁺ begins to be released from intracellular stores. It seemed, therefore, that the greater facilitation seen with small InsP₃-evoked responses might arise if ethanol reduced the threshold for InsP₃ action. This idea was examined more fully by measuring currents evoked by flashes of various durations, so as to obtain dose–response curves for InsP₃ in the absence and presence of ethanol. Figure 5 shows the results from one oocyte. In normal Ringer solution, a threshold flash duration of about 20 ms was required to evoke any detectable response, and the current then grew as the flash duration was lengthened until it reached a maximum with flashes longer than about 70 ms. A difficulty arose in obtaining similar data in the presence of ethanol, because of the progressive decline of the response during maintained application. Measurements
were made, therefore, by obtaining control responses to flashes of a particular duration, and then measuring the peak size of currents evoked by the same flash shortly after adding ethanol. To minimize errors resulting from slow recovery from the depressant effect of ethanol, applications were kept short (1 min or less) and the oocyte was washed for about 10 min between trials. The filled symbols in Fig. 5 show measurements in the presence of 250 mm-ethanol, and indicate that the predominant effect was a leftward shift of the InsP₃ dose–response relationship. Maximal responses evoked by longer flashes were increased little, whereas responses evoked by brief flashes were greatly potentiated. Unfortunately, measurements were not made at flash durations shorter than 20 ms, but extrapolation of the curve indicated that the threshold flash duration would have been reduced to about 5 ms in the presence of ethanol, as compared to the control value of about 20 ms.

**Dose dependence of ethanol actions**

The facilitatory effects of different concentrations of ethanol were estimated in experiments like that in Fig. 1A, in which ethanol was bath applied during repetitive stimulation by light flashes with durations that were set to evoke small (ca 5 nA) control responses. Peak currents were then measured following ethanol applications, and are expressed in Fig. 6A as the percentage potentiation of the corresponding control values. At a concentration of 40 mM the responses were potentiated by an
average of about 70%, and potentiation increased progressively to about 1500% as the concentration of ethanol was raised to 400 mM. On double-logarithmic co-ordinates the data fitted to a line with a slope of about 1.5, indicating that the facilitation varied as a steeper than linear function of ethanol concentration.

![Graph showing dose dependence of ethanol-induced facilitation and depression.](image)

Fig. 6. Dose dependence of ethanol-induced facilitation and depression. A, dose dependence of facilitation, determined in fifteen oocytes that were stimulated by light flashes with durations set to give small (5–10 nA) control current responses. The points show the maximal facilitation induced by various concentrations of ethanol as a percentage of the control response. The line is drawn by eye. B, dose dependence of depression estimated using longer light flashes that evoked control currents of about 100 nA. Data are from four oocytes, and show the size of the currents after 3–4 min exposure to ethanol, expressed as a percentage of the control currents (★). Error bars indicate ±1 S.E.M. from four to eight trials, except for the points at 80 mM-ethanol which are individual measurements. The curve was drawn by eye.

To examine the dose dependence of the ethanol-induced depression, ethanol was applied during repetitive stimulation by light flashes that evoked relatively large (ca 100 nA) currents. Measurements were made of responses recorded 3–4 min after applying ethanol, at which time the currents had fallen to a roughly steady level. Figure 6B shows these data, scaled as a percentage of the corresponding control responses. Half-maximal inhibition occurred at a concentration of about 150 mM-ethanol, and the responses were reduced to about 20% by 400 mM-ethanol.

**Effects of ethanol do not depend upon extracellular Ca**

Chloride current responses to photoreleased InsP₃ arise through liberation of intracellular Ca²⁺, and do not require the presence of extracellular Ca²⁺ (Parker & Miledi, 1989). Nevertheless, it remained possible that the facilitatory or inhibitory
effects of ethanol might require extracellular Ca$^{2+}$. To test this, we repeated the experiment of Fig. 1 in an oocyte bathed in a solution in which the free Ca$^{2+}$ concentration was reduced to very low levels by chelation with EGTA. As can be seen in Fig. 7, ethanol gave strong facilitatory and inhibitory effects, like those in normal (1·8 mm-Ca$^{2+}$) Ringer solution. Recovery of the responses after washing out ethanol was less obvious in this experiment as compared to Fig. 1, probably as a result of the smaller size of the control response and longer duration of ethanol application, rather than because of the absence of external Ca$^{2+}$.

**Intracellular injections of ethanol**

To determine whether ethanol acts at intracellular sites, we injected ethanol into the oocyte whilst evoking repetitive light flash responses. The amounts of ethanol injected were smaller, by factors of 10–100, than the doses used by Wafford et al. (1989) and usually did not themselves evoke oscillatory current responses. Nevertheless, as illustrated in Fig. 8, responses to the light flashes were strongly depressed shortly after injection of ethanol but subsequently became facilitated – the reverse sequence of events to that seen following bath application of ethanol. In a total of eleven trials in three oocytes, injections of ethanol always caused a rapid depression of the InsP$_3$ responses. The subsequent facilitation was more variable, and was clearly seen in only four trials.

Because intracellular injections of Ca$^{2+}$ also inhibit caged InsP$_3$ responses (Parker & Ivorra, 1990α), we were concerned that the depression seen with intracellular
ethanol might have arisen artificially because of contaminating Ca\(^{2+}\) in the injection solution. This, however, was unlikely for several reasons. Firstly, care was taken to avoid contamination by Ca\(^{2+}\). The fluid near the pipette tip (which becomes contaminated by Ca\(^{2+}\) from the bathing fluid) was expelled immediately before

![Graphs of ethanol, methanol, and 1-hexanol currents](image)

Fig. 9. Effects of various alcohols on caged InsP\(_3\) responses. Traces on the left show responses to just-suprathreshold light flashes that evoked control currents at about 10 nA; those on the right were obtained with longer light flashes that evoked control currents of 100–150 nA. Ethanol, methanol and 1-hexanol were bath applied at respective dilutions of 2, 2 and 0.05% during periods indicated by the bars. All data are from a single oocyte.

penetration of the oocyte, the injection solution included 50 μM-EDTA to chelate contaminating Ca\(^{2+}\), and injections of a control solution lacking ethanol failed to produce depression. Secondly, depression was still evident in experiments in which the injection itself evoked little or no current response (e.g. Fig. 8), whereas a Ca\(^{2+}\)-activated Cl\(^{-}\) current should have been evident if any appreciable Ca\(^{2+}\) were present in the injection solution (Parker & Ivorra, 1990a).

Other alcohols

Various alcohols were tested for actions on InsP\(_3\) signalling in the same way as described above for ethanol; that is to say, they were bath applied during stimulation by brief light flashes to look for potentiation and during stimulation by longer flashes to look for inhibition.

Figure 9 shows representative records obtained in a single oocyte that was exposed to methanol, ethanol and 1-hexanol. Ethanol produced the usual potentiation and depression. On the other hand, methanol was virtually without effect, even at a concentration of 500 mM. There was no detectable depression of responses to the longer flash and responses to the brief flash showed only a slight (about 50%)
potentiation, in marked contrast to the potentiation of 2100% seen with 400 mM
methanol. This relative ineffectiveness of methanol on caged InsP₃ responses was
confirmed in four other experiments. Furthermore, methanol (500 mM) produced no
obvious changes in the Fluo-3 signals evoked by light flashes, or in the currents
evoked by intracellular injections of Ca²⁺ (data not shown).

![Figure 10.](image)

**Fig. 10.** A, trace shows membrane currents evoked by alternate stimulation by light
flashes (brief responses) and by intracellular injections of Ca²⁺ (longer responses). During
the time indicated by the bar, 0.025% 1-heptanol was added to the superfusate. B,
simultaneous records of Fluo-3 Ca²⁺ transients and membrane currents evoked by
repetitive light flashes at 30 s intervals. Details are as in Fig. 3B. The superfusate included
0.025% 1-heptanol during the time marked by the bar.

Different again to ethanol, application of 1-hexanol produced a marked depression of
responses to both the brief and long light flashes, without any sign of potentiation.
Similar results were obtained with other long-chain alcohols tested, including iso-
butanol, heptanol and pentanol. Each of these was tested in at least three oocytes,
and produced strong inhibition but no detectable potentiation. We did not examine
the dose effectiveness of these alcohols in detail, but all depressed responses to 20%
or less of the control value at dilutions of 0.2%. The most potent appeared to be
1-heptanol, which almost completely suppressed large (100 nA) current responses at
a dilution of 0.025%. For comparison, the same dilution of ethanol (4 mM) would
not cause any measurable depression (Fig. 6B).

To examine whether the depressant effects of the longer chain alcohols arose in a
similar way to the ethanol-induced depression, we repeated the experiments of Fig.
3 using 0.025% 1-heptanol. The trace in Fig. 10A shows membrane currents evoked
by alternate light flashes and intracellular Ca²⁺ injections. During application of
heptanol the light flash responses were abolished, while the currents evoked by Ca²⁺
injections were reduced to about 36% of the control. Figure 10B shows simultaneous
records of Fluo-3 and membrane current signals evoked by light flashes during application of heptanol. The currents were suppressed by heptanol, and the Fluo-3 signals reduced to about 10% of the control. Both showed a partial recovery after washing for 3 min. Thus, it is clear that heptanol reduced InsP₃-mediated Ca²⁺ liberation. However, the appreciable reduction of the Ca²⁺-activated Cl⁻ current indicates that this mechanism may also play an important part in the depression of InsP₃-mediated currents by heptanol.

**DISCUSSION**

The major finding is that ethanol both potentiates and depresses membrane current responses evoked by InsP₃ in *Xenopus* oocytes. Among the alcohols, this combination of effects appears unique to ethanol; methanol produced no inhibition and only slight potentiation, whereas various longer chain alcohols produced strong depression but no detectable potentiation. Both the facilitatory and depressant effects appear to arise primarily through actions on InsP₃-mediated liberation of sequestered Ca²⁺, because they were evident in recordings of intracellular free Ca²⁺ made using the indicator dye Fluo-3, as well as in records where membrane Cl⁻ currents were used as a monitor of intracellular free Ca²⁺. However, a part of the depressant effects on the current may also arise from inhibitory effects on the Ca²⁺-activated Cl⁻ channels, as the Fluo-3 signals were reduced to a lesser extent than the current, and because currents evoked by intracellular injections of Ca²⁺ were depressed by ethanol and higher alcohols.

When ethanol was applied by bath superfusion, responses were first potentiated and then declined in the maintained presence of ethanol. In contrast, intracellular injection of ethanol produced an immediate depression followed, in some oocytes, by a potentiation. A possible explanation for this is that the potentiation arises at, or close to the cell membrane, whereas the depression arises more deeply into the cell. The slow time course of onset and recovery of the depression with bath applied ethanol may, therefore, reflect the time required for ethanol to equilibrate between the bathing solution and the cell interior. On this basis, the time course of records like that in Fig. 1 can be explained by a summation of facilitatory and inhibitory affects. Thus, the initial response to bath application of ethanol is a marked potentiation, because of the rapid onset of facilitation. During maintained application of ethanol the response then declines as the depressant effect grows and, after washing, the response is greatly reduced because the facilitation rapidly disappears whereas recovery from depression is slow.

Regarding the mechanism underlying the facilitation, a strong clue is provided by the observation that it arises through a reduction in the amount of InsP₃ required to evoke a threshold response. We have previously described a similar facilitation arising from procedures such as agonist activation and intracellular injection of low doses of InsP₃, that elevate the background level of InsP₃ in the oocyte (Parker & Miledi, 1989). Thus, the facilitatory effect of ethanol may arise if it elevates the intracellular level of InsP₃, possibly by stimulating its formation by the break-down of phosphatidylinositol bisphosphate in the plasma membrane. Such a mechanism is consistent with the localization of the facilitatory effect at the cell membrane.
discussed above. It is also consistent with findings that ethanol can evoke oscillatory
Cl− currents in the oocyte (Wafford et al. 1989), which almost certainly arise as a
result of InsP3 formation, and that ethanol mobilizes Ca2+ in hepatocytes by
activation of phospholipase C (Hoek, Thomas, Rubin & Rubin, 1987). However, the
contrary effect has been reported in platelets, where ethanol inhibits thrombin-
stimulated InsP3 formation (Rand, Vickers, Kinlough-Rathbone, Packham &
Mustard, 1988).

The depressant effects of ethanol and higher alcohols on InsP3-mediated Ca2+
release presumably arise within the cell at, or close to, the sites of Ca2+ release. At
present the mechanisms remain unclear, but we can consider two possibilities. One
is that the alcohols act directly to inhibit the binding of InsP3 to its intracellular
receptors, or to modulate the gating of the Ca2+ release channels. Against this,
ethanol at concentrations up to 500 mM has been found to have no effect on binding
to cerebellar InsP3 receptors (Smith, 1987). The second possibility is that alcohols
may raise the cytoplasmic free Ca2+ concentration, either through an InsP3-
independent mechanism or by increasing the efficacy of endogenous InsP3, and thus
cause a Ca2+-dependent inhibition of InsP3-mediated Ca2+ liberation (Parker &
Ivorra, 1990a; Payne, Flores & Fein, 1990). Since the depressant effect of ethanol
remains when oocytes are bathed in a Ca2+-free medium, the source of this calcium
would have to be intracellular. Evidence in support of this hypothesis is that ethanol
increases intracellular Ca2+ levels in various cells (Daniell, Brass & Harris, 1987;
Rabe & Weight, 1988; Davidson, Wilce & Shanley, 1988), including oocytes
(Cuthbertson, Whittingham & Cobbold, 1981), and that it is able to release Ca2+ from
InsP3-insensitive intracellular compartments (Machu, Woodward & Leslie, 1989).
We were therefore interested to determine whether the intracellular free Ca2+
concentration was raised during depression of the InsP3-mediated responses. The
results, however, were inconclusive. Recordings of Fluo-3 fluorescence failed to show
any clear rise during or after application of ethanol at concentrations sufficient to
almost completely suppress the light flash responses, although increases were
sometimes seen during superfusion with longer chain alcohols. Furthermore, the
small membrane currents evoked by ethanol application did not appear to arise
primarily through activation of the Ca2+-dependent Cl− conductance. The reversal
potential for Cl− in the oocyte is about −25 mV (Kusano, Miledi & Stimmakre, 1982),
whereas reversal potentials of the ethanol-induced currents varied widely in different
oocytes between about −10 mV to more negative than −60 mV. Also, the ethanol
currents subsided rapidly on washing, in contrast to the slow recovery of the InsP3-
mediated responses. However, it remains possible that if ethanol were to release
sequestered Ca2+ at sites close to the InsP3 receptors, the localized Ca2+ concentration
could be sufficient to inhibit InsP3 action without giving rise to detectable
fluorescence or membrane current signals.

Acute behavioural effects of ethanol are apparent at concentrations of a few tens
of millimoles per litre. For example, the drunk driving limit in many States in the
USA (0·1%) corresponds to a blood alcohol concentration of about 16 mM, and
ataxia is produced in mice at concentrations between 40 and 75 mM (Gallagher,
Parsons & Goldstein, 1982). The mechanisms underlying these effects are not
understood, but actions of ethanol on many different receptors, channels and
enzymes have been implicated (for reviews see: Gandhi & Ross, 1989; Ticku, 1989). Although the facilitatory and depressant effects that we describe were most prominent at high ethanol concentrations (a few hundred millimoles per litre), clear actions were apparent at physiologically relevant levels. They might, therefore, be important in the generation of some symptoms of ethanol intoxication, resulting from modulation of phosphoinositide-mediated synaptic transmission. In this respect it is interesting that the most pronounced effect during mild alcohol intoxication is ataxia, and that the cerebellum contains by far the highest density of InsP₃ receptors of any brain region (Worley, Baraban & Snyder, 1989).

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REFERENCES


