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2-Methoxyethanol inhibits gap junctional communication in rat myometrial myocytes

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Abstract

The glycol ethers 2-methoxyethanol (2-ME) and 2-ethoxyethanol (2-EE) prolong gestation in rodents. Because gap junctions in the myometrium likely facilitate parturition, the present study examined inhibition of gap junctional communication by 2-ME and 2-EE in myometrial smooth-muscle cell cultures. To measure gap junctional communication, the fluorescent dye Lucifer yellow was injected into cultured cells and the transfer of the dye to adjacent cells was scored with epifluorescence microscopy. The data are presented as the percentage of cells adjacent to the microinjected cell that exhibited dye following microinjection. A 30 min treatment with 32 or 63 mmol/L 2-ME decreased dye transfer to 71% and 63%, respectively ($p \leqslant 0.05$; control 90%). Similarly, 2-EE inhibited dye transfer, although myometrial cells were less sensitive to 2-EE compared to 2-ME. Dye transfer returned to control levels after 2 h in the continued presence of 2-ME. The primary metabolite of 2-ME, methoxyacetic acid (MAA), had no effect on dye transfer at concentrations equimolar to 2-ME. Because 2-ME and 2-EE inhibited gap junctional communication only at high concentrations and because the inhibition reversed in the continued presence of the compounds, it is suggested that glycol ethers delay parturition by a mechanism independent of a direct action on myometrial gap junctions.

Abbreviations: CMF-HBSS, calcium- and magnesium-free Hanks' balanced salt solution; 2-EE, 2-ethoxyethanol; MAA, methoxyacetic acid; 2-ME, 2-methoxyethanol; 4-MP, 4-methylpyrazole; PBSG, glucose-supplemented phosphate-buffered saline solution

Introduction

Several glycol ethers, including 2-methoxyethanol (2-ME), are reproductive and developmental toxicants. The toxicity of 2-ME is generally attributed to the active metabolite methoxyacetic acid (MAA). One interesting observation in female rodents is that 2-ME and a related glycol ether, 2-ethoxyethanol (2-EE), increase gestation length in both mice and rats when administered to pregnant dams (Nelson et al., 1981; Hardin, 1983; Toraason et al., 1986). In two of these studies (Nelson et al., 1981; Hardin, 1983), this response was observed in the absence of overt maternal toxicity or fetal malformations. With the

opportunity for widespread exposure to this class of solvents in the workplace, the mechanism by which glycol ethers affect gestation merits investigation.

One mechanism whereby glycol ethers may alter labor onset or progression is by altering gap junctional communication within the myometrium. Garfield and colleagues demonstrated that myometrial gap junctions are present at low levels throughout gestation, increase dramatically immediately prior to and during initial phases of labor, and decline rapidly postpartum (Garfield et al., 1977, 1978, 1989). This pattern is observed in all mammalian species examined to date. Additional experiments show that increased expression of the gap junction protein connexin 43 precedes the appearance of ultrastructurally visible gap junctions in the uterus (Risek et al., 1990). It has been hypothesized that gap junctions provide the coupling mechanism whereby pacemakers cells in the uterus transfer depolarizing signal to adjacent cells. This would allow for more rapid propagation of contraction-regulating signals and synchronization of uterine contractions for successful delivery of the fetus. Alternatively, gap junctions may be important as a means to metabolically couple cells during the energy-demanding process of parturition; hence, their disruption may affect successful progression of labor.

Previous studies showed that 2-ME, 2-EE, and several other glycol ethers inhibit gap junctional communication in rodent embryonic fibroblast cell lines (Loch-Caruso et al., 1984; Welsch and Stedman, 1984a,b). We hypothesized that glycol ethers may delay parturition by directly inhibiting myometrial gap junctional communication. To examine this hypothesis, the present study examined whether the glycol ethers 2-ME and 2-EE alter gap junctional communication among rat myometrial smooth-muscle cells in culture. These cultures, developed in our laboratory, exhibit gap junctional communication as measured by several assay procedures, and express the gap junction protein connexin 43, which appears to be most significant for the development of parturition (Caruso et al., 1990; Loch-Caruso et al., 1992). Using 2-ME as a model compound, additional experiments were performed to determine whether the decline in dye transfer was due to the parent compound or its primary metabolite, MAA.

Materials and methods

Chemicals

Cells were microinjected in Dulbecco's phosphate-buffered saline solution (2.68 mmol/L potassium chloride, 1.47 mmol/L potassium phosphate monobasic, 0.14 mol/L sodium chloride; 8.10 mmol/L sodium phosphate anhydrous, 0.90 mmol/L calcium chloride dihydrate, 0.49 mmol/L magnesium chloride hexahydrate) supplemented with 5.6 mmol/L glucose (PBSG). The fluorescent dyes Lucifer yellow and propidium iodide were from Molecular Probes (Eugene, OR, USA). The test chemical 2-ME and its primary metabolite MAA were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). 4-Methylpyrazole (4-MP) and 2-EE were obtained from Sigma Chemical (St Louis, MO, USA). Antibodies used to verify smooth-muscle character were anti-α-smooth muscle actin and anti-mouse IgG (whole molecule) FITC conjugate, both from Sigma Chemical.

Cell cultures

Rat uterine smooth-muscle cells were isolated according to a previously described procedure (Caruso et al., 1990). Basically, uteri were removed from pregnant Sprague–Dawley rats on gestation day 10 and embryos and excess adipose tissue were excised. Uterine tissue was minced and digested in an enzyme digestion

solution containing 100 μg/ml deoxyribonuclease I, 150 μg/ml type II collagenase, and 150 μg/ml type III trypsin at 37°C for 45 min. Cells were further dissociated by trituration and the resulting cell suspension was filtered, centrifuged, resuspended, and plated into 75 cm^2 flasks to establish cell lines, or into 60 mm dishes for microinjection experiments. Smooth-muscle character of the isolated cells was verified by immunofluorescence labeling with monoclonal anti-α-smooth-muscle actin antibody (raised in mouse) and anti-mouse IgG (whole molecule) FITC conjugate (raised in goat) according to a previously described protocol (Caruso et al., 1990). As reported by Skalli and colleagues (1986), α-smooth-muscle actin is specific for smooth muscle. Cell culture purity as determined by α-smooth-muscle actin staining was greater than 90% in all cases. Myometrial cells were grown in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% defined, iron-supplemented bovine calf serum (HyClone, Logan, UT, USA) and maintained at 37°C in a 5% CO_2 atmosphere. Cells were subcultured as needed to maintain a subconfluent state using 0.25% crude trypsin.

Exposure

To determine the concentration-response of myometrial cells to 2-ME or 2-EE, cells were treated with 16, 32, and 63 mmol/L 2-ME or 2-EE for 30 min by diluting 12.6 mol/L 2-ME or 10.2 mol/L 2-EE stock solutions in medium. In experiments to determine the time course for 2-ME effects on Lucifer yellow dye transfer, rat myometrial cells were treated with 63 mmol/L 2-ME for 15, 60, or 120 min. Control cells remained untreated. To determine the effects of MAA on intercellular dye transfer, the exposure solution was prepared by the method of Mebus and colleagues (1992) in order to maintain proper pH. Accordingly, 100 μl of 12.9 mol/L MAA was neutralized in 900 μl 10% sodium bicarbonate solution, followed by a 1:2 dilution in acidified water and dilution in medium as needed. Experiments using the alcohol dehydrogenase inhibitor 4-MP required a 1:1000 dilution with growth medium of a 100 mmol/L stock solution in ethanol. In additional experiments, cells were exposed to fresh medium containing 16, 32, or 63 mmol/L 2-ME every 15 min for a 60 min treatment period prior to dye injection. Results from these experiments were compared to those with cells that were exposed once only at the beginning of the 60 min treatment period.

Microinjection experiments

The microinjection procedure used in this study was the same as that previously described (Loch-Caruso et al., 1992). Basically, myometrial cells were plated at a density of 6×10^4 cells into 60-mm dishes 48 h prior to the onset of microinjection experiments. At the designated time, the medium was discarded and replaced with medium containing the test compound, and cells were allowed to incubate at 37°C for the required period of time. At the time of microinjection, the medium was discarded and replaced with PBSG containing the test compound at the corresponding concentration. Cells were injected with a dye solution containing 0.8% Lucifer yellow and 0.02% propidium iodide dissolved in PBS. Lucifer yellow is a low-molecular-mass dye capable of transfer through gap junctions (Stewart, 1978), whereas propidium iodide binds to nucleic acids within the injected cell, serving as a permanent marker of the injected cell (Goulet et al., 1988). Following microinjection, the PBSG solution was discarded to remove any dye that may have leaked into the medium and replaced with fresh PBSG containing the test compound. Injections required 4–8 min per dish and a minimum of 7 cells per dish were injected. Over a series of experiments, a mini-

mum of 6 plates per dose group and 71 cells per treatment group were injected. Dye transfer was scored as the percentage of dye transfer from injected cells to their nearest neighbor recipient cells. Microinjection experiments were performed using cells at passages 0 through 4. As reported by this laboratory (Loch-Caruso et al., 1992) and verified by control cells in these experiments, there is no significant difference in Lucifer yellow dye transfer levels following microinjection in primary and low-passage rat myometrial smooth-muscle cells.

Statistical analysis

Microinjection data were analyzed for significance by calculating the means of each treatment group, using angular transformation to normalize the data, then calculating a one-way analysis of variance (ANOVA) to compare these values. The time course experiments were analyzed by Student's *t*-test. A two-way ANOVA was used to analyze the experiment with 4-MP treatment and repeated exposures to 2-ME (Figure 4). If significant differences were found, pairwise comparisons of means were made to identify the significantly different treatment group(s). Bonferroni's correction was used as needed to ensure that the overall *p*-value was less than or equal to 0.05. *p*-Values meeting this criterion were considered significant. All statistical analyses were performed with the SYSTAT software (Wilkinson, 1990).

Results

The effects of 2-ME on gap junctional communication in rat myometrial smooth-muscle cell cultures were assessed by Lucifer yellow dye transfer following microinjection. Figure 1 shows an example of dye transfer among myometrial cells following microinjection. Treatment of myometrial cells for 30 min with 2-ME produced a concentration-dependent decrease in dye transfer (Figure 2). Lucifer yellow dye transferred to 90.18% ± 3.36% (mean ± SEM) of directly adjacent cells in control cultures. Dye transfer decreased to 80.83% ± 4.49%, 71.27% ± 3.53%, and 63.39% ± 3.27% in the 16, 32, and 63 mmol/L 2-ME-treated groups, respectively. The dye transfer values at 32 and 63 mmol/L 2-ME are significantly different from control values ($p \leq 0.05$). A similar inhibition of dye transfer was seen with 2-EE (Figure 2); again, significant inhibition of dye transfer was observed at 32 and 63 mmol/L 2-EE (77.68% ± 3.23% and 73.14 ± 2.50%, respectively) compared to controls ($p \leq 0.05$).

The time course of 2-ME inhibition of gap junctional communication was examined by evaluating dye transfer in myometrial cells after 15, 60, or 120 min of exposure to 63 mmol/L 2-ME (Figure 3). 2-ME inhibition of dye transfer was significant at all time points tested. After 15 min of exposure, Lucifer yellow dye transfer decreased to 82.86% ± 1.70% compared to control values of 93.44% ± 1.78% ($p \leq 0.05$). After 60 and 120 min of exposure to 63 mmol/L 2-ME, dye transfer was significantly ($p \leq 0.05$) decreased to 63.43% ± 2.49% and 81.71% ± 3.31% of adjacent cells, respectively, compared to untreated control cells that had dye transfer levels of approximately 93% at these time points.

Because dye transfer substantially recovered by 120 min in the continued presence of 2-ME, it was hypothesized that either there was a loss of available 2-ME (through evaporation, metabolism, or other means), or the cells adapted to 2-ME exposure by exhibiting less gap junctional communication changes with time. To begin examining these possibilities, the medium was exchanged every 15 min for 1 h to provide repeated exposures to fresh 16, 32, or 63 mmol/L 2-ME prior to microinjection. Results obtained with repeated exposures were compared with results from exposures to a

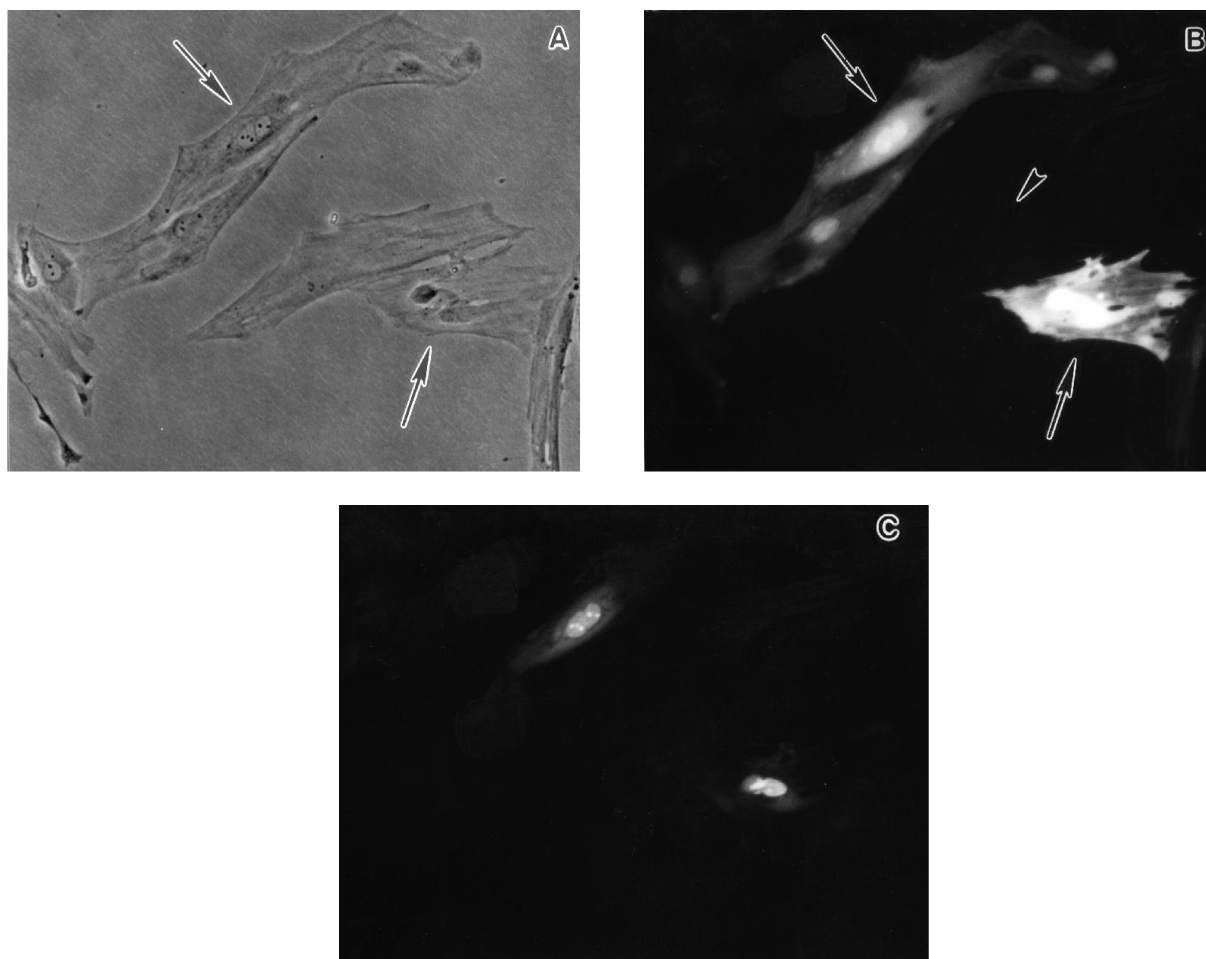

Figure 1. Lucifer yellow dye transfer from two microinjected rat myometrial cells to their neighboring recipient cells. (A) Phase contrast; arrows indicate the two injected cells. (B) Epifluorescence; Lucifer yellow has transferred from injected cells (arrow) to neighboring cells, except in one case (arrowhead). (C) Epifluorescence; the microinjected donor cells contain the permanent marker, propidium iodide. ($\times 150$).

single dose of 16, 32, or 63 mmol/L 2-ME for 60 min. As shown in Figure 4, decreases in dye transfer were significantly greater ($p \leqslant 0.05$) at 16 and 32 mmol/L 2-ME in cultures exposed to repeated doses of 2-ME ($71.13\% \pm 2.64\%$ and $65.57\% \pm 2.52\%$, respectively) compared to cultures exposed to a single dose of 2-ME ($91.86\% \pm 1.68\%$ and $79.81\% \pm 1.63\%$, respectively). Repeated exposures to 63 mmol/L 2-ME did not further inhibit dye transfer beyond that observed with a single treatment of 63 mmol/L 2-ME ($64.64\% \pm 2.79\%$ compared to $64.84\% \pm 1.78\%$). Dye transfer values for control cells did not differ, regardless of whether the medium was changed four times throughout the one-hour exposure period or once at the onset of exposure (Figure 4). Under these conditions, dye transfer levels were $87.23\% \pm 2.19\%$ and $90.63\% \pm 2.35\%$, respectively.

To examine the role of metabolism in contributing to 2-ME loss or inactivation, cells

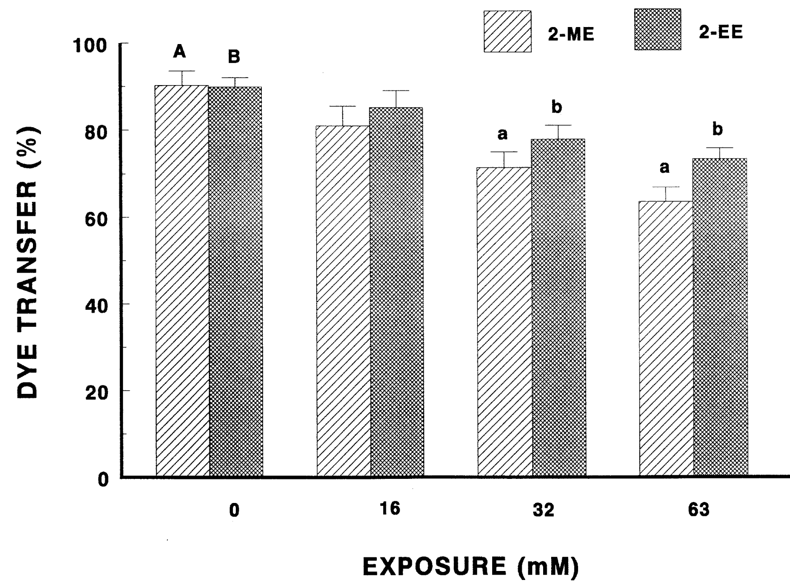

Figure 2. Exposure of rat myometrial cells to various concentrations of 2-methoxyethanol (2-ME) and 2-ethoxyethanol (2-EE) for 30 min resulted in inhibition of Lucifer yellow dye transfer following microinjection. Lowercase letters denote values significantly different from unexposed controls (corresponding uppercase letters) ($p \leqslant 0.05$). Values are expressed as mean percentage dye transfer ± SEM ($n \geqslant 6$ dishes for each exposure group).

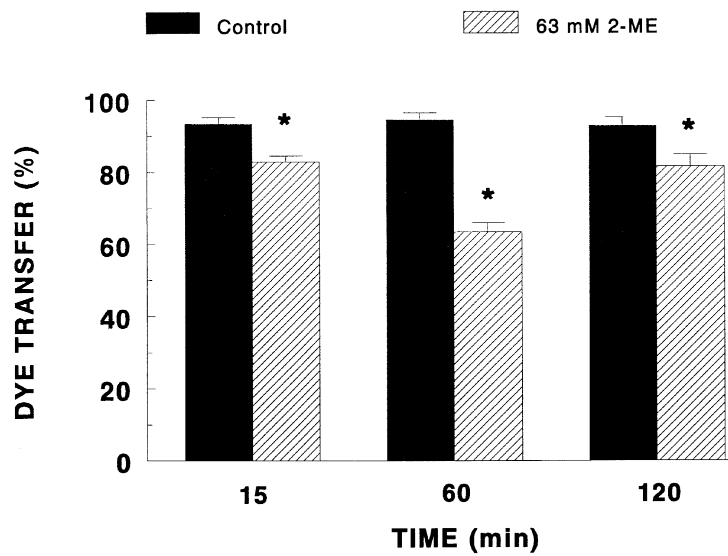

Figure 3. Rat myometrial cells recovered from inhibition of Lucifer yellow dye transfer by 2-methoxyethanol (2-ME) during continuous exposure. Asterisks (*) denote values significantly different from controls at $p \leqslant 0.05$. Values are expressed as mean ± SEM with a minimum of 6 dishes per exposure group per time point.

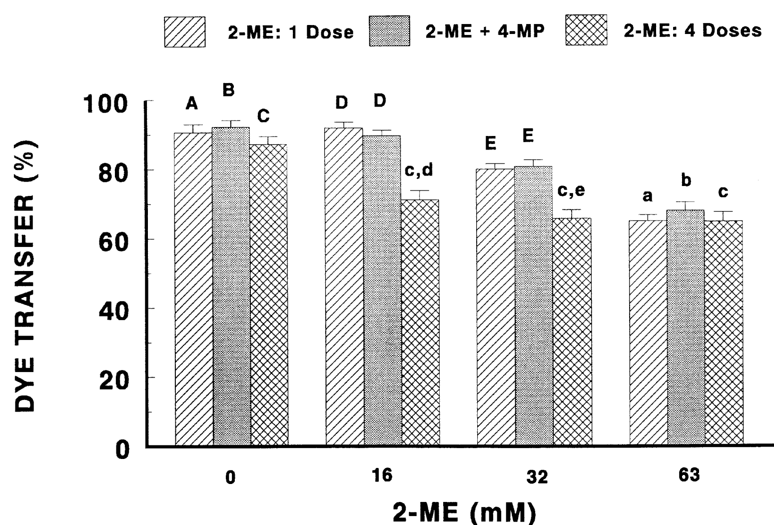

Figure 4. The effect of coincubation with 4-methylpyrazole (4-MP) or medium replacement on inhibition of Lucifer yellow dye transfer by 2-methoxyethanol (2-ME). Rat myometrial cells were exposed to 16, 32, or 63 mmol/L 2-ME for 60 min as a single exposure alone (hatched bar), or a single exposure in the presence of 100 μmol/L 4-MP (stippled bar), or received four media replacements at 15 min intervals in order to replenish 2-ME (cross-hatched bar). Exposure to 4-MP, an inhibitor of alcohol dehydrogenase, did not alter 2-ME-induced inhibition of dye transfer, but cells replenished with 2-ME at 15 min intervals exhibited significant reductions in dye transfer at all concentrations. Lowercase letters indicate values that differ significantly from values with corresponding uppercase letters at $p \leqslant 0.05$. Bars represent mean ± SEM ($n \geqslant 6$ plates per exposure group).

were treated concurrently with 16, 32, or 63 mmol/L 2-ME and 100 μmol/L 4-MP for 60 min. 4-MP is an inhibitor of alcohol dehydrogenase (Chou and Richardson, 1978; Blomstrand et al., 1979; Carter and Wands, 1988) and impairs the primary metabolic pathway of 2-ME. If metabolism by alcohol dehydrogenase was the initial step in a detoxification pathway, it was hypothesized that 4-MP would block this pathway and enhance the effects of 2-ME. Results from this experiment, illustrated in Figure 4, show that treatment with 4-MP did not significantly alter the inhibition seen with 2-ME alone (single dose) at any concentration of 2-ME. Dye transfer for cells treated with 2-ME with and without 4-MP were 89.65% ± 1.53% and 91.86% ± 1.68% at 16 mmol/L; 80.59% ± 1.92% and 79.81% ± 1.63% at 32 mmol/L; and 67.85% ± 2.39% and 64.84% ± 1.78% at 63 mmol/L, respectively. Control cells treated with 4-MP alone for 60 min had dye transfer levels of 92.24% ± 1.87%, which did not differ from untreated control cells (Figure 4). These data indicate that significant 2-ME metabolism by the alcohol dehydrogenase pathway has not occurred by 60 min and that MAA production does not appear to be necessary for 2-ME-induced inhibition of gap junctional communication.

To confirm this finding that MAA is not responsible for altered gap junctional communication, myometrial cells were treated for 30 min with 16, 32, or 63 mmol/L MAA, the primary metabolite of 2-ME. MAA did not produce any significant changes in gap junctional communication when utilizing the pH buffering solution described by Mebus and colleagues (1992) (data not shown). Dye trans-

ferred to 88.73% ± 2.01% of adjacent cells in control cultures, and cells treated with 16, 32 or 63 mmol/L MAA exhibited similar extents of dye transfer (87.65% ± 2.01%, 90.64% ± 3.03%, and 86.95% ± 2.18%, respectively). Positive controls of 63 mmol/L 2-ME-treated cells transferred dye to 51.83% ± 2.69% of adjacent cells. These data, in conjunction with the experiments with 4-MP, support the conclusion that MAA is not the likely proximate toxicant mediating 2-ME inhibition of myometrial gap junctions.

Discussion

Glycol ethers extend gestation length in rodents (Nelson et al., 1981; Hardin, 1983; Toraason et al., 1986), but the mechanism for this effect has not been elucidated. Considering the likely role of myometrial gap junctions in parturition (Garfield et al., 1977, 1978, 1989), this study characterized the response of rat myometrial gap junctions to 2-ME treatment *in vitro*.

Exposure to 2-ME produced a concentration-dependent decline in gap junctional communication between myometrial cells at 32 and 63 mmol/L concentrations. A second glycol ether, 2-EE, also inhibited Lucifer yellow dye transfer in myometrial cell cultures; however, 2-ME decreased dye transfer to a greater extent than 2-EE at equimolar doses. Other *in vivo* toxicity studies also reported a reduced response to 2-EE relative to 2-ME (Foster et al., 1983; Nagano et al., 1979).

Previous studies showed that glycol ethers inhibit gap junctional communication in embryo-derived fibroblast cell lines, although at concentrations higher than those used in the present study. Welsch and Stedman (1984a) observed inhibition at 25 mmol/L 2-ME in human embryonal palatal mesenchyme (HEPM) cells using the metabolic cooperation assay to measure gap junctional communication. However, this effect was attributed to cell cytotoxicity, resulting in failure to form gap junctions between cells. In a second study by Welsch and Stedman (1984b), using Chinese hamster V79 cells, inhibition of gap junctional communication was seen following a 3 day exposure to 130 mmol/L 2-ME. No adverse morphological effects were observed. Similarly, two additional studies using Chinese hamster V79 cells confirmed a decline in gap junctional communication at noncytotoxic concentrations of 2-ME (Loch-Caruso et al., 1984; Vitek, 1993). In the present study, cell cytotoxicity was considered minimal because cell morphology appeared normal, the cells recovered despite the continued presence of 2-ME, and cell leakage of the hydrophilic dye Lucifer yellow was not observed following injection. The previously published studies differ from the current work in that different cell types, glycol ether concentrations, and assays were used to study gap junctional communication.

Chemicals alter gap junctional communication by altering the number of gap junction channels available for communication or by altering the permeability of existing channels. In rat neonatal cardiac myocytes, the half-life of the 43 kDa gap junction protein is estimated to be approximatly 1 to 2 h (Laird et al., 1991). This suggests that the turnover of myometrial gap junctions may be sufficiently rapid for decline in protein synthesis to decrease gap junctional communication. Lateral diffusion of individual gap junction proteins within the plasma membrane (Lane and Swales, 1980) or endocytosis of gap junction-containing membrane (Larsen and Risinger, 1985) could decrease gap junction number, also. In addition, second messenger systems, such as those involving cAMP, protein kinase C, and tyrosine kinase, may mediate rapid changes in channel permeability. Because of the relatively rapid and transient nature of 2-ME effects on gap junctions, we suggest that the rapid inhibition of gap junctions was due to gating of gap

junctional channels to a closed, impermeable state, and/or an alteration of the gap junction environment which affects the functionality of the channels.

When 2-ME-containing medium was exchanged every 15 min during a 1 h treatment, greater inhibition of Lucifer yellow dye transfer was observed at 16 and 32 mmol/L 2-ME compared to cultures exposed to only a single dose of 2-ME. These results suggest that 2-ME may be removed from an active site, either by metabolism or by intracellular redistribution, or that the cells are compensating in some fashion for the continued presence of the compound. In any case, increased exposure frequency may compromise the mechanism by which myometrial cells cope with 2-ME insult. Although evaporation is also possible, it is unlikely because the temperature of vaporization of 2-ME is significantly higher than that of water. At 63 mmol/L 2-ME, repeated exposure did not enhance the decrease in gap junctional communication; thus, this degree of inhibition may represent a maximum response. If 2-ME is lost due to evaporation and/or metabolism, the amount lost at this concentration was inconsequential to producing a maximal effect. Furthermore, 63 mmol/L 2-ME may be a concentration at which conpensatory or detoxication mechanisms are ineffective or saturated.

The major route of metabolism of 2-ME *in vivo* involves conversion by alcohol dehydrogenase to MAA (Miller et al., 1983; Moss et al., 1985; Ritter et al., 1985; Sleet et al., 1988). Treatment of myometrial cells with 4-MP, an inhibitor of alcohol dehydrogenase (Ritter et al., 1985), had no effect on 2-ME inhibition of gap junctional communication. These data suggest that 2-ME metabolism by alcohol dehydrogenase does not explain the loss of inhibition observed in the continued presence of 2-ME. Additionally, these data suggest that MAA is not likely to be the proximate toxicant inhibiting gap junctional communication because MAA is the product of alcohol dehydrogenase. Although the enzyme isoforms present in a given system can alter 4-MP efficacy (Keung and Yip, 1989; Kassam et al., 1989; Nusrallah et al., 1989), the concentration of 4-MP used in this study is within the broad range of reported values for the dissociation constant of the enzyme–inhibitor complex (K_i). In these studies, K_i values range from 0.11 ± 0.03 μmol/L in rat liver (Plapp et al., 1984) to 560 μmol/L in rat microsomes (Feierman and Cederbaum, 1986). Although its specificity is relatively good, 4-MP has also been shown to alter cytochrome P450 activity in rats (Feierman and Cederbaum, 1986; Palakodety et al., 1988).

Several developmental toxicity studies (Miller et al., 1982, 1983; Brown et al., 1984; Yonemoto et al., 1984; Clarke et al., 1992) as well as male reproductive toxicity studies (Foster et al., 1983; Beattie et al., 1984) have implicated MAA, the primary metabolite of 2-ME, as the proximate metabolite responsible for 2-ME toxicity. To further verify that MAA was not responsible for decreasing dye transfer in myometrial cell cultures, cells were treated with concentrations of MAA equimolar to those used with 2-ME. Previous studies using teratogenic endpoints determined that the toxic effects produced by MAA *in vivo* are unlikely to be due to altered pH; therefore, the exposure medium was modified according to the method of Mebus et al. (1992) in order to prevent MAA-induced decrease in medium pH. Neither 32 nor 63 mmol/L MAA treatment produced a decline in Lucifer yellow dye transfer, strongly suggesting that MAA was not responsible for 2-ME inhibition of gap junctions.

The concentrations required to decrease dye transfer in myometrial cells were higher than doses reported to prolong gestation *in vivo*, which were 50 mg/kg per day in rats on gestation days 6–12 (Toraason et al., 1986) and 125 mg/kg per day in mice on gestation days 6–15 (Hardin, 1983). Likewise, concentra-

tions of 2-EE that inhibited myometrial cell dye transfer were higher than the *in vivo* exposure required to increase gestation length, which was 100 ppm (inhalation) on gestation days 14–20 (Nelson et al., 1981). It is possible that the *in vitro* environment may have contributed to the need for higher glycol ether concentrations to inhibit myometrial gap junctions in cell cultures. This possibility is supported by reports that the number and size of gap junctions rapidly increase in cultured uterine segments from midgestation rats, progressing to levels 2-fold greater than those seen during parturition (Garfield et al., 1978, 1980; Cole et al., 1985). The *in vitro* conditions of the myometrial cell cultures may have allowed upregulation of gap junctions beyond those present *in vivo* prior to parturition, thereby requiring higher concentrations of glycol ethers to inhibit Lucifer yellow dye transfer in the cell cultures. Nonetheless, because relatively high concentrations were required to produce a modest and transient inhibition of gap junctional intercellular inhibition, it seems most likely that direct inhibition of myometrial gap junctions is not the primary mechanism whereby 2-ME prolongs gestation.

Despite the ineffectiveness of low concentrations of 2-ME and 2-EE in this study, altered gap junctional communication cannot be ruled out entirely as a mechanism for glycol ether-induced delays in parturition. It remains to be investigated whether glycol ethers might affect myometrial gap junctional communication indirectly. For example, because gap junctions in the uterus are hormonally regulated (Garfield et al., 1989), glycol ethers might prevent the increase in gap junction number normally seen at parturition by altering the hormone signals. Alternatively, glycol ethers may affect uterine signals from cells other than myometrial muscle cells. If these signals are involved in regulating myometrial myocyte gap junctions, glycol ether effects by this mechanism would be missed in the cell cultures used in these experiments.

Aside from altered gap junctional communication, other hypotheses have been proposed for the mechanism by which glycol ethers prolong gestation. Hardin (1983) has previously suggested that 2-ME may alter uterine smooth-muscle contractility directly and, thereby, increase gestation length. Data collected in this laboratory using rat longitudinal uterine smooth-muscle strips in a contraction bath apparatus verified that the 63 mmol/L 2-ME dose was capable of significantly decreasing the frequency of basal spontaneous contractions (Pahl and Caruso, 1992); however, lower doses of 2-ME were not tested. This hypothesis is further supported by Von Oettingen and Jirouch (1931), who reported that 2-EE and butoxyethanol depressed rabbit intestinal smooth-muscle contraction capacity. Further, these authors reported that 0.5% butoxyethanol also inhibited contractions in perfused frog hearts and decreased blood pressure in rabbits following intravenous injections of small amounts of 50% glycol ether solutions. Alternatively, 2-ME is teratogenic (Nagano et al., 1981; Yonemoto et al., 1984; Sleet et al., 1988), suggesting that 2-ME could possibly modify gestation length by altering fetal development. The inhibition of onset of labor observed in sheep that ingested the plant *Veratrum californium* was attributed to severe fetal head malformations that, presumably, prevented the release of the fetal hypothalamic signal for initiating labor (Kennedy, 1971). However, 2-ME and 2-EE prolong gestation in rats and mice in the absence of overt physical malformations.

The mechanism by which the glycol ethers 2-ME and 2-EE prolong gestation in rodents remains uncertain. Because these glycol ethers only partially and transiently inhibit gap junctional communication of cultured myometrial myocytes, the present study suggests that prolonged gestation may not be explained by direct inhibition of myometrial muscle cell gap junctions. Furthermore, the data strongly

suggest that the metabolite MAA does not mediate 2-ME inhibition of myometrial gap junctional communication.

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