

Selective Actions of a Detergent on Ligand-Gated Ion Channels Expressed in *Xenopus* Oocytes¹

TINA K. MACHU, S. JOHN MIHIC and J. E. DILDY-MAYFIELD

Department of Pharmacology (T.K.M.), Texas Tech University Health Sciences Center, Lubbock, Texas, and Department of Pharmacology, University of Colorado Health Sciences Center (S.J.M., J.E.D.-M.) and VA Medical Center (J.E.D.-M.), Denver, Colorado

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ABSTRACT

Cytoclean, a commercially available detergent, has selective actions on ligand-gated ion channels. Cytoclean (0.0005–0.01% v/v) potentiated 50 μ M glycine responses in oocytes expressing *alpha*-2 glycine receptors by $23 \pm 7\%$ to $342 \pm 43\%$. Cytoclean is composed of five components dissolved in water, but only one reagent, Bio-Soft D-62, modulated responses of oocytes expressing *alpha*-2 glycine receptors. Bio-Soft D-62 (0.00005–0.001% w/v), potentiated 50 μ M glycine responses by $13 \pm 1\%$ to $474 \pm 50\%$. Bio-Soft D-62 is composed of linear alkylbenzene sulfonate (>95% C12 chain). The effects of Cytoclean or Bio-Soft D-62 were examined on *alpha*-1 *beta*-2 and *alpha*-1 *beta*-2 *gamma*-2L γ -aminobutyric

acid_A, γ -aminobutyric acid _{ρ 1}, DL- α -amino-3-hydroxy-5-methyl-4-isoxalonepropionic acid, kainate and 5-hydroxytryptamine₃ receptors expressed in *Xenopus laevis* oocytes. Enhancement of γ -aminobutyric acid_A receptor function ranged from $\approx 21\%$ to 458% with Cytoclean (0.0001–0.01%), respectively. Bio-Soft D-62 (0.001%) inhibited GABA _{ρ 1} receptor function by $\approx 72\%$. Cytoclean had no effect on 5-hydroxytryptamine₃ or GluR6 function, but Cytoclean (0.005% and 0.01%) inhibited GluR3-mediated currents by $\approx 21\%$ and $\approx 41\%$, respectively. These results suggest that trace amounts of Cytoclean, such as amounts adhering to glassware, may modulate ion channel function and potentially confound experimental results.

Ligand-gated ion channels are targets for a variety of compounds that act at agonist or allosteric binding sites on the receptor complexes. Although pharmacological studies of ligand-gated ion channels typically focus on drugs or drug-related compounds, it is important to note that common laboratory products contain chemicals that may have marked effects on these receptors. For instance, Papke *et al.* (1994) recently reported that tinuvin 770, a plasticizer used in syringes, inhibited the function of nicotinic acetylcholine receptors in a use-dependent manner. This plasticizer, which can leach from the walls of the syringes, also potently blocked L-type Ca²⁺ channels (Glossman *et al.*, 1993). In another study, three plasticizers were reported as contaminants in a lot of ethanol that had an unusually strong disordering action on biomembranes (Goldstein *et al.*, 1987). Finally, a tissue culture tube contaminant inhibited recombinant nicotinic and NMDA receptors expressed in *Xenopus laevis* oocytes (Reuhl *et al.*, 1990).

We examined the actions of Cytoclean, a commercial de-

tergent, on ligand-gated ion channels. This study was prompted by the observation of an unusually large enhancement of glycine receptor function by ethanol solutions prepared in glassware that had been washed with Cytoclean. This stimulation could be prevented by rinsing the glassware with ethanol and water before preparing solutions, suggesting that ethanol solutions solubilized Cytoclean from the walls of glassware. Furthermore, we characterized Cytoclean and its components and demonstrated potent actions on the majority of ion channels tested.

Materials and Methods

X. laevis female frogs were obtained from Xenopus I (Ann Arbor, MI). The mCAP RNA capping kit was from Stratagene (La Jolla, CA). 5-HT, GABA, kainic acid, glycine, Triton X-100, tetrapotassium pyrophosphate, sodium xylene sulfonate and dodecanol were purchased from Sigma Chemical (St. Louis, MO). Cytoclean was bought from Isolab (Akron, OH), and Bio-Soft D-62 was a generous gift of Stepan Chemical (Northfield, IL).

Transcription of cDNA to cRNA. Synthesis of cRNA was carried out with the mCAP RNA capping kit (Stratagene) with 5-HT₃ (provided by D. Julius, University of California at San Francisco) or GluR3 (flop) (provided by J. Boulter and S. Heinemann, Salk Insti-

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ABBREVIATIONS: AMPA, DL- α -amino-3-hydroxy-5-methyl-4-isoxalonepropionic acid; ANOVA, analysis of variance; DEPC, diethylpyrocarbonate, GABA, γ -aminobutyric acid; 5-HT, 5-hydroxytryptamine; MBS, modified Barth's solution; NMDA, N-methyl-D-aspartate; SDBS, sodium salt of dodecylbenzene sulfonate; SNK, Student-Newman-Keuls *post-hoc* test; GluR, glutamate receptor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

tute, La Jolla, CA) receptor cDNA as templates. Two μg of NCB-20 5-HT₃ receptor cDNA subcloned in pBluescript KS⁻ or GluR3 cDNA subcloned in pBluescript SK⁻ was linearized with *NotI* and *XhoI* endonucleases, respectively, extracted with phenol-chloroform and precipitated overnight with Na acetate and ethanol. The cDNA was transcribed to cRNA in 50 μl of reaction mixture according to the mCAP RNA capping kit protocol, with the following minor modifications: 5 μl of rNTPS, 60 units of RNase inhibitor and 40 units of T3 polymerase were used. The mixture was incubated at 37°C for 45 min, after which 40 units of T3 was added. After 30 min, the reaction was terminated with 10 units of DNase. The cRNA was extracted with phenol-chloroform, precipitated with sodium acetate and ethanol and stored at -70°C.

Isolation of *X. laevis* oocytes. *X. laevis* frogs were kept in tanks on a 12-hr light/dark cycle at 19°C and fed a diet of mealworms and beef heart three times per week. Frogs were anesthetized by immersion in ice water for 45 min and were kept on ice during surgery. After removal, ovarian lobes were placed in MBS containing (in mM) NaCl 88, KCl 1, NaHCO₃ 2.4, HEPES 10, MgSO₄ 0.82, Ca(NO₃)₂ 0.33 and CaCl₂ 0.91, pH 7.5.

Before dissection, ovarian lobes were placed in hypertonic isolation media containing (in mM) NaCl 108, KCl 2, EDTA 1 and HEPES 10, pH 7.5. Stage V and VI oocytes were dissected with fine surgical forceps according to the method of Dildy-Mayfield and Harris (1992) and placed in MBS. Dissected oocytes were incubated for 10 min in buffer containing 0.5 mg/ml collagenase Type IA and (in mM) NaCl 83, KCl 2, MgCl₂ 1 and HEPES 10, pH 7.5, to remove the follicular cell layer. After several rinses with MBS, oocytes were placed in sterile filtered incubation media (MBS containing 10 mg/liter streptomycin, 50 mg/liter gentamicin, 10,000 units penicillin/liter, 0.5 mM theophylline and 2 mM sodium pyruvate).

Microinjection of oocytes with receptor cDNA or cRNA.

Oocyte nuclei were microinjected with cDNAs encoding *alpha-1 beta-2* or *alpha-1 beta-2 gamma-2L* GABA_A (provided by P. Whiting, Merck Sharp and Dohme Research Laboratories, Harlow, Essex, UK), GABA_{ρ1} (provided by G. Uhl, National Institute on Drug Abuse, Baltimore, MD), glycine *alpha-1* or *alpha-2* (provided by H. Betz, Max-Planck-Institut für Hirnforschung, Frankfurt, Germany) or GluR6 (provided by S. Heinemann, Max-Planck-Institut für Hirnforschung, Frankfurt, Germany) subunits. The "blind" method of Colman (1984) was used to inject 50 pg to 5 ng of receptor cDNA dissolved in 30 nl of DEPC-treated water/oocyte. For cRNA injections, an aliquot of cRNA to be injected was centrifuged at 15,000 × *g*, and the ethanol was removed with a tuberculin syringe. After air-drying the pellet, it was reconstituted in a volume of DEPC-treated water to yield a concentration of 15 to 80 ng of cRNA/50 nl. Fifty nanoliters of cRNA was injected into the animal/vegetal boundary of each oocyte. Oocytes were stored in incubation media in Corning cell wells (Corning Glass Works, Corning, NY) at 18°C. Incubation media was changed daily. Oocytes were recorded from days 1 through 7 after injection.

Electrophysiological recording of receptor responses. Oocytes were perfused with MBS (2 ml/min) in a 100- μl volume recording chamber *via* a roller pump (Cole-Parmer Instrument, Chicago, IL). Oocytes were impaled with two glass electrodes (1–10 M Ω) filled with 3 M KCl. Oocytes were voltage-clamped at -70 mV using a Warner Instruments OC-725A (Hamden, CT) oocyte clamp. Clamping currents were plotted on a chart recorder (Cole-Parmer Instrument).

Neurotransmitters were dissolved in MBS and applied to the oocytes. CytoClean or its components were coapplied with the neurotransmitters through the roller pump. In addition, to block desensitization of GluR6s (Egebjerg *et al.*, 1991), oocytes were preexposed to conoavalin A (10 μM) in MBS for 5 min.

Data analysis. GraphPAD Prism or InStat Software (GraphPAD Software, San Diego, CA) was used to perform one- or two-way ANOVAs, SNK or Student's *t* test.

Results

CytoClean markedly enhanced glycine-mediated responses in oocytes expressing *alpha-2* receptors, as shown in the representative tracings in figure 1A. Concentration-response curves were generated for oocytes expressing *alpha-1* or *alpha-2* glycine receptors (fig. 1B). Concentrations of 15 or 50 μM glycine were used in *alpha-1* and *alpha-2* subunit-expressing oocytes, respectively, and represent approximately EC₁–EC₄ for both constructs. Currents produced by these concentrations of glycine ranged from 75 to 1800 nA. Poten-

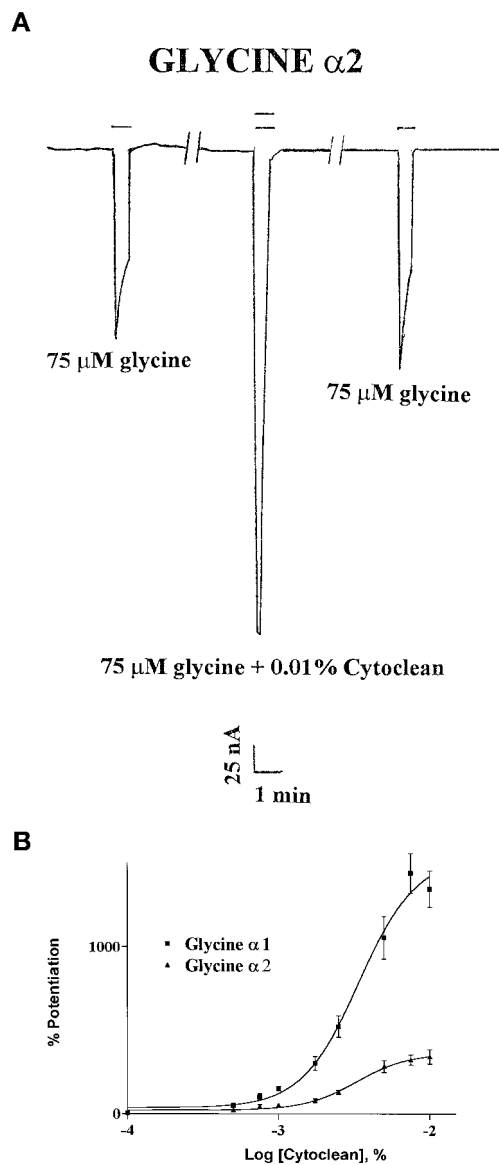


Fig. 1. CytoClean enhancement of glycine receptor function. A, Electrophysiological recordings of inward currents produced in the absence and presence of CytoClean in a representative oocyte expressing the *alpha-2* subunit of the glycine receptor. The oocyte was perfused with glycine (75 μM) with or without CytoClean (0.01%) for 30 sec. B, Concentration-response curves were generated for CytoClean in oocytes expressing *alpha-1* or *alpha-2* subunits ($n = 5-9$). CytoClean EC₅₀ values for *alpha-1* and *alpha-2* subunit-expressing oocytes are $0.0034 \pm 0.00095\%$ and $0.0033 \pm 0.00065\%$, respectively. Hill coefficients of 2.28 and 2.5 were obtained for *alpha-1* and *alpha-2* subunit-expressing oocytes. Two-way ANOVA revealed that the curves obtained were significantly different. Maximal potentiation in *alpha-1* and *alpha-2* subunit-expressing oocytes was 1543% and 364%, respectively.

tiation was observed in both receptor constructs at concentrations ranging from 0.0005% to 0.01% (v/v) and plateaued at 0.0075% to 0.01%. Concentrations higher than 0.01% were not tested because they were toxic to the oocytes. The efficacy of CytoClean was much greater in *alpha*-1-containing subunits, but the potency of the compound was very similar in the two constructs, given that the concentrations producing 50% of the observed stimulation (EC_{50} values) were $0.0034 \pm 0.00095\%$ and $0.0033 \pm 0.00065\%$ for *alpha*-1 and *alpha*-2 subunit-expressing oocytes, respectively.

Each component of CytoClean was tested for its contribution to enhancement of *alpha*-2 glycine receptor function (fig. 2A). CytoClean is an aqueous solution of Bio-Soft D-62 ($\approx 10\%$), tetrapotassium pyrophosphate ($\approx 2\%$), sodium xylene sulfonate ($\approx 3\%$), Triton X-100 ($\approx 2\%$) and ethanol ($\approx 29\%$). Each reagent was tested at a concentration that was equivalent to that found in CytoClean, 0.01%, the highest concentration used in this study. Bio-Soft D-62 produced approximately the same stimulation of glycine currents as CytoClean, but the other components of CytoClean were without effect.

Bio-Soft D-62 is a mixture of water and the sodium salt of linear alkylbenzene sulfonate ($\approx 58\text{--}62\%$). Linear sodium alkylbenzene sulfonate (fig. 2B) is composed of $\approx 95\%$ C12 chain (SDBS) and $\approx 5\%$ C13–C16 chain hydrocarbons. Dodecanol is used in the synthesis of these aromatic hydrocarbons and can represent up to 1.5% of the volume of Bio-Soft D-62. Dodecanol ($0.66 \mu\text{M}$), the concentration present in 0.01% CytoClean, had no effect on glycine-induced currents. Thus, linear alkylbenzene sulfonate sodium is responsible for enhancement of glycine receptor function by Bio-Soft D-62.

The potentiative action of Bio-Soft D-62 on glycine-mediated currents in oocytes expressing *alpha*-2 receptors was evaluated (fig. 3A). The Bio-Soft D-62 concentration-response

curve mirrored that of CytoClean (fig. 1B), although at 10-fold lower concentrations as expected, given that CytoClean is composed of $\approx 10\%$ Bio-Soft D-62. The EC_{50} of $0.00039 \pm 0.00012\%$ is equivalent to 9 nM SDBS.

The ability of Bio-Soft D-62 to enhance glycine-mediated currents was decreased as the glycine concentration was increased to that which produces a maximal response, 500 to 1000 μM (fig. 3B). Only currents produced by glycine (25–100 μM) were enhanced by the detergent, suggesting that Bio-Soft D-62 enhances the potency and not the efficacy of glycine at its receptor. The shift in potency was verified by the finding that the EC_{50} value for glycine was $58 \pm 1.0 \mu\text{M}$ in the presence of Bio-Soft D-62 (0.001%) compared with $116 \pm 5 \mu\text{M}$ in its absence (data not shown).

We next examined whether the actions of CytoClean or Bio-Soft D-62 generalized to other ligand-gated ion channels. Oocytes expressing GABA_A receptors composed of *alpha*-1 *beta*-2 or *alpha*-1 *beta*-2 *gamma*-2L receptors were perfused with GABA (5 μM) in the absence or presence of CytoClean (0.0001–0.01%) (fig. 4A) for 20 sec. A concentration-dependent increase in GABA-mediated currents was produced by CytoClean. However, there were no significant differences between the two constructs, suggesting that the *gamma*-2L

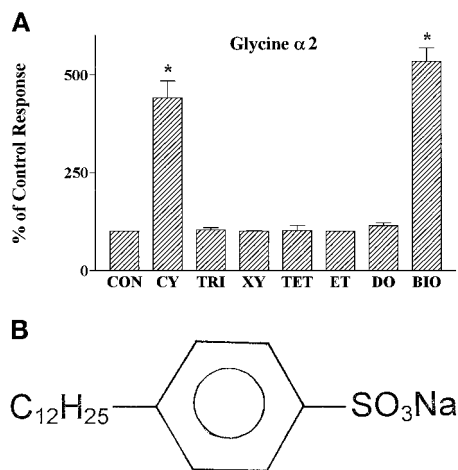


Fig. 2. The individual components of CytoClean (CY) were tested for their abilities to potentiate 50 μM glycine-mediated currents in oocytes expressing the *alpha*-2 subunit. Concentrations of these components were the same as that found in 0.01% CytoClean. Triton X (TRI), sodium xylene sulfonate (XY), tetrapotassium pyrophosphate (TET), ethanol (ET) and dodecanol (DO) had no effect, but Bio-Soft D-62 produced approximately the same potentiation as CytoClean ($n = 4\text{--}6$) (A). One-way ANOVA was significant. * $P < .05$ (SNK) compared with the control response. The components of Bio-Soft D-62 are the sodium salt of linear alkylbenzene sulfonate, predominately the C12 chain, and dodecanol, its precursor. Because dodecanol was eliminated as a candidate, the responsible reagent for enhancing glycine receptor function is linear alkylbenzene sulfonate. The predominate C12 chain form is shown in B.

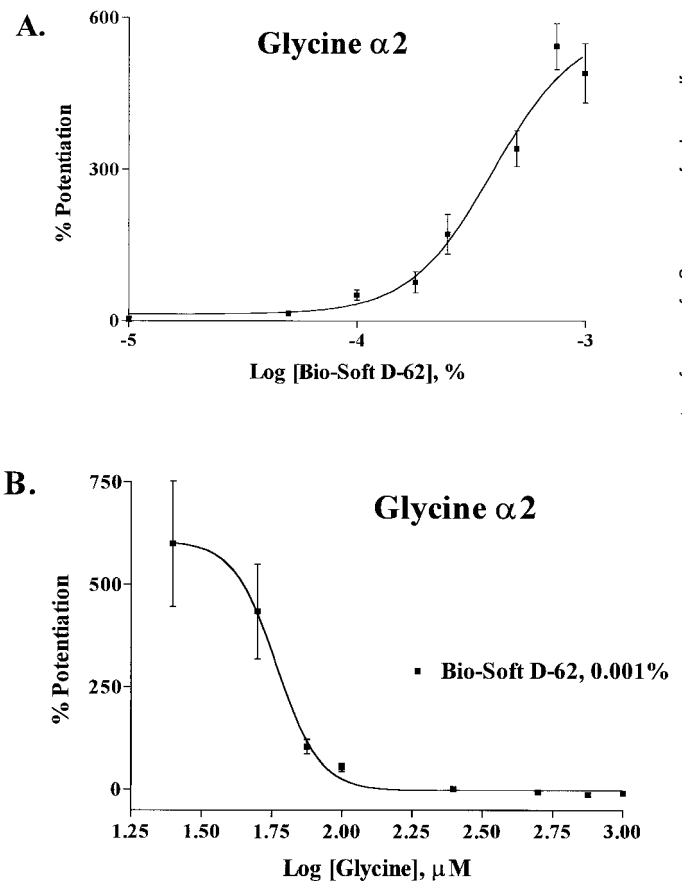


Fig. 3. A concentration-response curve for Bio-Soft D-62 (A) was generated for oocytes expressing the *alpha*-2 subunit of the glycine receptor ($n = 6\text{--}10$). Oocytes were perfused with glycine (50 μM) with or without Bio-Soft D-62 for 30 sec. An EC_{50} value of 0.00039% and a Hill slope of 2.39 were obtained. A concentration-response curve for glycine (B) in the presence of 0.001% Bio-Soft D-62 was generated ($n = 6\text{--}11$). Oocytes were perfused with glycine with or without 0.001% Bio-Soft D-62 for 30 sec. An EC_{50} value of 58 μM was obtained, which is significantly less than the EC_{50} value of 116 μM obtained in the absence of Bio-Soft D-62.

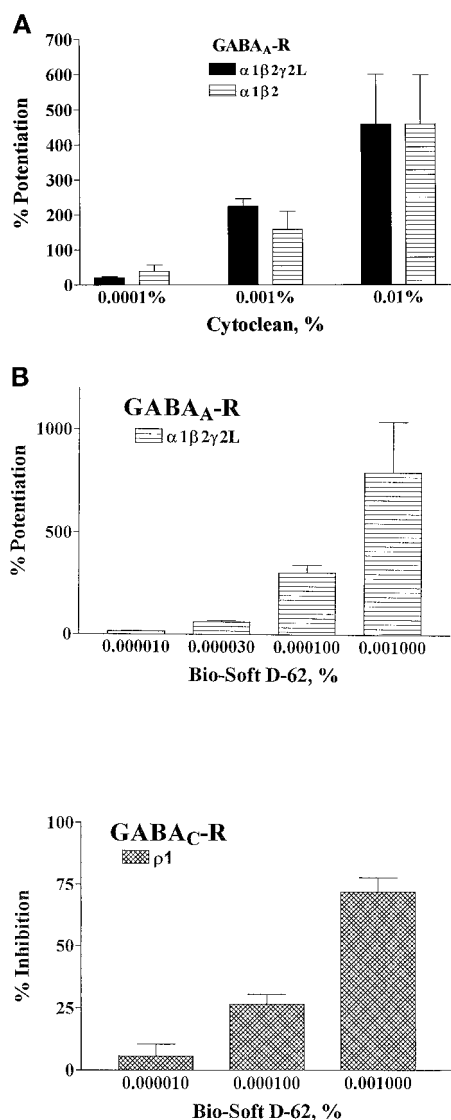


Fig. 4. A comparison of the abilities of CytoClean and Bio-Soft D-62 ability to potentiate GABA_A and GABA_{p1} receptor constructs. Oocytes expressing *alpha-1 beta-2* or *alpha-1 beta-2 gamma-2L* subunits were perfused with GABA (5 μM) in the presence or absence of CytoClean (0.0001–0.01%) for 20 sec (A). No significant difference was found between the two constructs by two-way ANOVA. Bio-Soft D-62 potentiated GABA_A receptors (B) composed of *alpha-1 beta-2 gamma-2L* subunits in a concentration-dependent manner. Oocytes were perfused with GABA (5 μM) in the absence or presence of Bio-Soft D-62 for 20 sec ($n = 4-6$). In contrast, Bio-Soft D-62 inhibited the GABA_{p1} receptor (C). Oocytes were perfused with GABA (500 nM) in the presence or absence of Bio-Soft D-62 for 3 min ($n = 4$ or 5).

subunit plays no role in determining the sensitivity of the receptor. Bio-Soft D-62 similarly enhanced GABA_A receptors composed of *alpha-1 beta-2 gamma-2L* subunits (fig. 4B). Finally, the response of GABA_{p1} receptors to Bio-Soft D-62 was measured (fig. 4C). Oocytes were perfused with GABA (500 nM) in the absence or presence of Bio-Soft D-62 for 3 min. In marked contrast to GABA_A receptors, an inhibition of ~6% to 70% was observed over the range of concentrations tested.

Within the glutamate family of receptors, the sensitivities of GluR3 and GluR6 to CytoClean or Bio-Soft D-62 were compared. CytoClean (fig. 5) and Bio-Soft D-62 (data not shown) inhibited GluR3, function although the inhibition

observed was less than that observed with GABA_{p1} receptors at comparable concentrations. CytoClean had no effect on GluR6-mediated currents (fig. 5).

The sensitivity of the 5-HT₃ receptor to CytoClean and Bio-Soft D-62 was evaluated as a function of 5-HT concentration (data not shown). Concentrations of 5-HT used were 0.25, 0.5 and 10 μM, which represent ~EC₂, ~EC₁₀ and ~EC₁₀₀ values, respectively. Neither compound, at the highest concentration used in these studies, had any effect on 5-HT-mediated currents.

Discussion

In the present report, we characterized the selective actions of the laboratory detergent CytoClean and one of its components, Bio-Soft D-62, on ligand-gated ion channels expressed in *X. laevis* oocytes. CytoClean and Bio-Soft D-62 potentiated glycine and GABA_A receptors but inhibited GABA_{p1} receptors. The opposite modulation of the two GABA receptors is not unexpected, given their differing responses to other compounds such as barbiturates, benzodiazepines and ethanol (Mihic and Harris, 1996; Shimada *et al.*, 1992). GluR3s were inhibited by CytoClean and Bio-Soft D-62, but GluR6s were insensitive. These results provide yet another example of differential modulation of AMPA/kainate and kainate receptors by amphiphilic compounds. Volatile anesthetics inhibit the function of GluR1–3s yet enhance that of GluR6s (Dildy-Mayfield *et al.*, 1996).

Detergents are commonly used to solubilize receptors from membrane preparations and to reconstitute receptors for biochemical analyses (Neugebauer, 1990), but they may influence the parameter being studied. For instance, solubilizing concentrations (1%) of Triton X-100, Tween 20 and octyl glucoside stabilized the desensitized form of *Torpedo* nicotinic receptor and reduced the labeling of all four of its subunits by 3-(trifluoromethyl)-3-(*m*-[¹²⁵I]iodophenyl) diazine (McCarthy and Moore, 1992). In contrast, solubiliza-

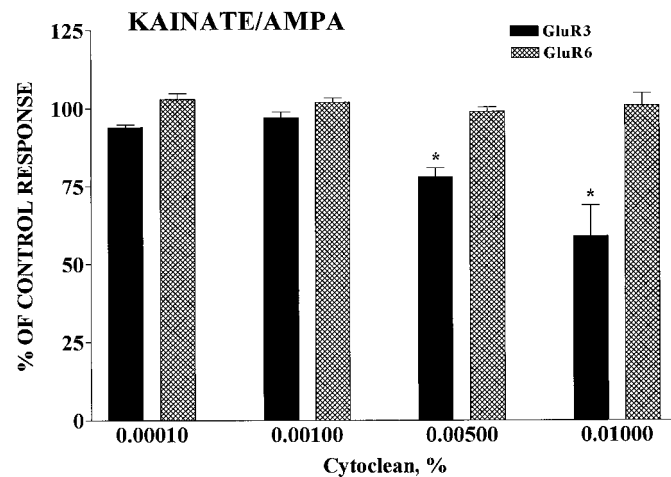


Fig. 5. CytoClean distinguished between the AMPA receptor GluR3 and the kainate receptor GluR6. Oocytes were perfused with 400 μM kainate (GluR3) or 10 μM kainate (GluR6) in the absence or presence of CytoClean for 30 sec. GluR6-expressing oocytes were also perfused with concanavalin A (10 μM) for 5 min before drug treatment to prevent receptor desensitization. Maximal concentrations of CytoClean had no effect on GluR6 receptor function but significantly reduced GluR3-mediated currents (two-way ANOVA, SNK, $P < .05$, compared to control response, $n = 4$ or 5).

tion in 1% sodium cholate appeared to produce a conformation of the nicotinic receptor that was similar to the resting state (McCarthy and Moore, 1992). Furthermore, Triton X-100 increased ³H-strychnine binding to glycine receptors at 1 to 10 μ l/mg of protein and decreased binding at >10 μ l/mg of protein (Galli *et al.*, 1983).

Lower concentrations of detergents (<1%) have also been reported to alter radioligand binding activity at receptors. Subsolubilizing concentrations of Triton X-100 were demonstrated to increase ³H-flunitrazepam binding (B_{max}) in forebrain synaptosomal membranes of chickens that were stressed, but not in chickens that had not been stressed (Martijena *et al.*, 1992). Triton X-100 increases radioligand binding to GABA_A (Flores *et al.*, 1986) and NMDA (Ogita *et al.*, 1990) receptors, presumably by removing membrane proteins not related to the receptor or by eliminating endogenous neurotransmitters or cofactors. Finally, Triton X-100 is a high affinity noncompetitive antagonist ($K_d \approx 0.001\%$) at *Torpedo* nicotinic receptors and shifts the conformation of the receptor to a desensitized state, with a high affinity for acetylcholine (Heidmann *et al.*, 1983). Thus, it is not surprising that the detergent Bio-Soft D-62 modulates ligand-gated ion channel function.

The primary component of Bio-Soft D-62, SDBS, is an amphiphilic aromatic hydrocarbon with a hydrophilic sulfonate group and a hydrophobic C12 chain. Apart from the fact that it is a charged molecule, SDBS bears structural similarity to alcohols and anesthetics, which also are amphiphilic compounds. The lipophilicity profile of anesthetics parallels their anesthetic potencies (Meyer, 1901; Overton, 1896), but it should be noted that the hydroxyls of alcohols and the hydroxyls, ether groups and amines of many anesthetics confer a polar character to these compounds. As has been hypothesized for alcohols and anesthetics, SDBS could modulate ligand-gated ion channel function through a lipid-disordering phenomenon or through a discrete site in the receptor complexes. The latter hypothesis is favored, given that fluidization of neuronal membranes does not always produce intoxication or anesthesia (Buck *et al.*, 1989). Furthermore, anesthetics inhibit highly purified firefly luciferase (Franks and Lieb, 1984). Last, preliminary work with a chimeric construct of the 5-HT₃ and *alpha-7* nicotinic receptor, which are modulated in opposite directions by ethanol, suggests that the amino terminus is the site of ethanol action (Yu *et al.*, 1996).

In summary, we have found that Bio-Soft D-62, a reagent in the laboratory detergent CytoClean, has marked effects on the function of a number of different ligand-gated ion channels. These results emphasize the importance of not assuming that common laboratory cleaning products and plasticware are inert. Furthermore, the possibility that unexpected

experimental results may be a result of this type of "contamination" should be considered.

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Send reprint requests to: Tina K. Machu, Ph.D., Department of Pharmacology, Texas Tech University Health Sciences Center, 3601 Fourth Street, Lubbock, TX 79430. E-mail: phrtkm@ttuhsc.edu