FUNDAMENTAL AND APPLIED TOXICOLOGY 18, 59–65 (1992)

Reversible Inhibition of Intercellular Communication among Cardiac Myocytes by Halogenated Hydrocarbons[1]

MARK TORAASON, MICHAEL J. BREITENSTEIN, AND HOWARD E. WEY

Cellular Toxicology Section, Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, Ohio 45226

Received December 3, 1990; accepted June 18, 1991

Reversible Inhibition of Intercellular Communication among Cardiac Myocytes by Halogenated Hydrocarbons. TORAASON, M., BREITENSTEIN, M. J., AND WEY, H. E. (1992). *Fundam. Appl. Toxicol.* **18**, 59–65.

We examined the effect of 11 aliphatic halogenated hydrocarbons on the transfer of microinjected dye among cardiac myocytes from neonatal rats. Myocytes were suffused with increasing concentrations of halocarbon added as a 0.2% solution of dimethyl sulfoxide to M199 containing 1.8 mM Ca and 5% serum. Single cells were microinjected with the fluorescent probe Lucifer yellow (5% in 0.1 mM LiCl) and dye coupling to adjacent cells was monitored. All of the halocarbons tested exhibited a concentration-dependent inhibitory effect on intercellular communication that was reversible following washout of the compounds. Intercellular communication was blocked within 1 min of exposure to an effective concentration, and recovery of communication occured even after 2 hr of exposure. Pretreatment of cells with SKF 525-A (25 μM) did not prevent the inhibition of intercellular communication by carbon tetrachloride suggesting an absence of P-450 involvement. EC50s were calculated for each chemical using probit analysis. A log–log comparison of the EC50s and the physicochemical properties of the chemicals demonstrated a high correlation ($R^2 = 0.933$) between the EC50s and the octanol/water partition coefficients of the halocarbons. This suggests that incorporation of halocarbons in the membrane may block intercellular communication though modification of the immediate environment of the gap junctions. The results are in agreement with the hypothesis that inhibition of gap junctional communication is a factor in the arrhythmogenic effects of acute halocarbon exposure.

Levy and Lewis (1911) first reported that cats anesthetized with chloroform were sensitized to epinephrine-induced cardiac arrhythmias which could result in sudden death. Since that time the depressant and sensitizing effects of various chlorinated, fluorinated, and brominated hydrocarbons on the myocardium have been thoroughly described (Katz and Epstein, 1968; Merin, 1977; Steffey, 1982; Zakhari and Aviado, 1982; Reynolds, 1984). The majority of information on the effects of halocarbons has come from investigations with humans or anesthetized animals. As a result, the underlying mechanism at the cellular or molecular level responsible for attenuated contractility and sensitization of the myocardium has not been well elucidated. A clearer understanding of these mechanisms would provide a more direct means of determining the potential hazard of untested compounds.

Recently, Spray and Burt (1990) proposed that the arrhythmogenicity of the halocarbon anesthetics, halothane and ethrane, may be related to the inhibition of intercellular communication by these compounds (Wojtczak, 1985; Burt and Spray, 1989). Intercellular communication in the heart occurs through gap junctions which are considered the molecular entity that constitutes the low resistance electrical pathway between cardiac myocytes that allows the heart to contract as a syncitium (De Mello, 1987; Deleze, 1987; Cole *et al.*, 1988). Blockade of junctional channels increases electrical resistance in the myocardium and may predispose the heart to arrhythmia (Spray and Burt, 1990). The action of halothane and ethrane on gap junctional communication in the heart indicates that other halocarbons exhibiting arrhythmogenicity could also inhibit intercellular communication in myocardium. The present investigation examined the effect of 10 aliphatic halogenated hydrocarbons in addition to halothane on intercellular communication in cultured heart cells. Since the ability of halocarbons to sensitize the myocardium to arrhythmias has been proposed to be correlated with their physicochemical properties (Clark and Tinston, 1973), the intercellular communication results were compared using least-squares linear regression to formula weight, boiling point, vapor pressure, density, and various partition coefficients of each compound.

MATERIALS AND METHODS

Heart cells. Hearts were harvested aseptically from 2- to 4-day-old Sprague–Dawley rats obtained from our own breeding colony as previously described (Toraason, *et al.*, 1989). In short, excised hearts were minced into 1-mm^3 pieces and refrigerated (4°C) overnight in Hanks' balanced salt so-

[1] Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

0272-0590/92 $3.00

lution (Hazelton, Lenexa, KS) containing 0.1% crude trypsin (Sigma, St. Louis, MO). The following morning minced hearts were washed and then incubated at 35°C for 25 min in M199 (Hazelton) containing 10% newborn calf serum (Hyclone, Logan, UT) and 100 U/ml penicillin–streptomycin (Hazelton). Following incubation, cardiac tissue was dispersed by rapid repipetting to produce a single cell suspension. Approximately 5×10^6 cells were obtained from each heart, and viability was consistently about 90% as determined by trypan blue exclusion. Enriched myocyte cultures were obtained by plating 40 to 60 ml of suspension containing 10^6 cells per milliliter in 175-cm^2 flasks for 1 hr. The 1-hr preplating removed the majority of fibroblasts from the heart cell suspension. Two milliliters of the enriched suspension was plated in 35-mm plastic culture dishes, and cells were used 2 days after plating. Myocytes were easily discernable from the small number of fibroblasts by their morphology, striations, and spontaneous beating.

Treatment. Cells were continuously suffused at room temperature with M199 containing 25 mM Hepes (Sigma) and 5% serum in open culture dishes using a peristaltic pump and aspirator adjusted to maintain 2 ml in the 35-mm culture dish and provide for a complete turnover of the medium in the dish within 2 min. Because of the volatility of halocarbons (certified grade, Fisher Scientific, Pittsburgh, PA) the perfusion reservoir was capped and replaced every 10 min with fresh medium containing the test concentration of halocarbon. Test compounds, prepared as V/V% stock solutions in dimethyl sulfoxide (DMSO), were added to medium and vortexed immediately prior to perfusion. Pilot studies were performed for each chemical to determine the effective concentration ranges. For each chemical, heart cells were exposed to four or five sequential concentrations selected from the following percentage concentrations: 0, 0.001, 0.0025, 0.005, 0.0075, 0.01, 0.025, 0.05, 0.075, 0.1, 0.2, 0.3. The final concentration of DMSO plus halocarbon was 0.2% except for the two highest concentrations of halocarbon where DMSO was 0.2 and 0.3% respectively. DMSO was included in all control perfusions and had no discernable effect on dye coupling. An additional exposure regimen was utilized with carbon tetrachloride to insure that sequential perfusions with increasing concentrations of halocarbon were not having a cumulative effect. In this design, control culture medium was removed from dishes and replaced with 2 ml of culture medium containing carbon tetrachloride at a designated concentration, and dye injection experiments were conducted immediately. Each dish was exposed to only a single concentration of halocarbon, and injection and scoring were performed without knowledge of treatment concentration. The possible involvement of P450 metabolism in the inhibition of intercellular communication by the halocarbons was assessed by pretreating cells with SKF 525-A (provided by Smith Kline & French, King of Prussia, PA). Cells were incubated in 25 μM SKF 525-A added to cultured medium 18–20 hr prior to exposure to carbon tetrachloride (Klaunig *et al.*, 1989).

Microinjection. Suffusion of cells was performed on the stage of an inverted microscope (Nikon Diaphot, 20× phase objective) equipped with epifluorescence (excitation band, 450–490 nm), high sensitivity video camera (Dage SIT66), video recorder (Panasonic), and video printer (Sony). Cells were suffused with control medium for 20 min for equilibration to room temperature in the opened-air-flow system. For concentration–response studies, cells were normally exposed for 3–5 min at each concentration prior to beginning injections. Injection pipettes were 1.5-mm single barrel glass pipets (World Precision Instruments, New Haven, CT) pulled to a diameter of 1 μm with a gravity pipet puller (Narishige, Japan). Pipets were back loaded first with 5% Lucifer yellow CH (Sigma, St. Louis, MO) in 0.1 M LiCl and then backfilled with 0.1 M LiCl. A Narashige M0103 micromanipulator was used to position pipets and impale cells. Injection of Lucifer yellow was accomplished iontophoretically, and injections were for 5–10 sec. Movement of dye from injected cells was monitored on a video display terminal and recorded on video tape. Transfer of dye to adjacent cells was evident within 10 sec and attained maximum transfer within 1 min.

Data analysis. Five heart cells were injected in each culture dish at each concentration of halocarbon, and the movement of dye to adjacent cells was monitored. If dye was detectable in any cell adjacent to the injected cell, the injected cell was considered to be communicating and was scored positive. The absence of detectable dye in any cell adjacent to the injected cell resulted in a negative score. Therefore, in each dish at each concentration a score of 100 (five of five injected cells communicated), 80, 60, 40, 20, or 0% communication could be calculated. A concentration–response curve was obtained by sequentially testing increasing concentrations of halocarbon in a single dish. Suffusion with halocarbon was terminated when there was a complete absence of dye coupling in five sequentially injected cells at a single concentration. The perfusion medium was then replaced with medium without halocarbon, and a minimum of five postexposure cells were injected to insure that recovery of communication among myocytes was complete. Probit analysis (Gad and Weil, 1988) was used to obtain an EC50 concentration (point at which 50% of injected cells transferred dye to at least one adjacent cell) for each dish. Four dishes were evaluated and the mean EC50 was calculated for each chemical. The log of EC50s for tested chemicals were compared to the logs of formula weight, boiling points, densities, vapor pressures, and various partition coefficients using least squares linear regression and analysis of variance performed on commercial microcomputer software (Statgraphics, STSC, Inc., Rockville, MD) Fishers's Exact test was used to compare SKF-525A cultures to controls.

RESULTS

Inhibition of Dye Coupling

Lucifer yellow, when injected into a cardiac myocyte, spread rapidly to adjacent cells and was detected two or three cells beyond the injected cell within 20 sec (see Imanaga *et al.*, 1987, for details on the cell-to-cell transfer of this dye). As cells were exposed to increasing concentrations of halocarbon, the number of cells that communicated with adjacent cells declined to zero. Inhibition of dye coupling was evident within 1 min of exposure to an effective concentration, and cells readily dye-coupled within 1 to 2 min of washout of the compounds. Although a reduction in the extent of dye transfer was evident between controls and cells exposed to marginally effective concentrations of halocarbon, communication was scored as present or absent to avoid subjectivity in the assessment of dye coupling. Using this "all-or-none" scoring technique, a concentration response was obtained for each halocarbon by exposing a single dish to sequentially increasing concentrations. Figure 1 illustrates probit plots obtained in a single dish for each halocarbon. Four such probits were obtained for every chemical, allowing calculation of the mean ± SD EC50 for each chemical. Sequential suffusion of a single dish with increasing concentrations of halocarbon reduced the number of dishes and time required to obtain a concentration–response curve. To ensure that this procedure did not compromise results, heart cell cultures were also exposed to single concentrations of carbon tetrachloride. The mean EC50 from four replicate experiments was 0.72 mM compared with 0.73 mM from sequential exposure (Table 1). This indicates that preexposure to carbon tetrachloride did not have a cumulative effect when dishes were perfused with sequentially increasing concentrations of halocarbon.

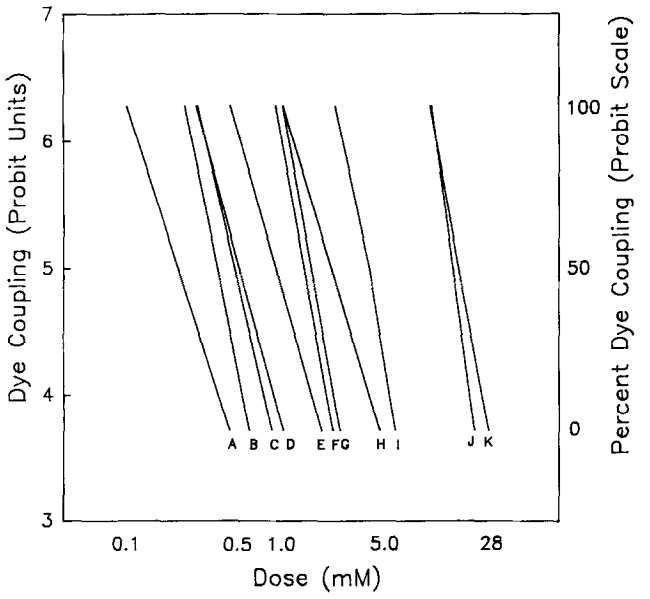

FIG. 1. Probit plots for inhibition of intercellular communication by halocarbons. Each plot was obtained from a single dish exposed to sequentially increasing concentrations of halocarbon. Five cells were injected at each concentration, and the EC50 was calculated for each dish. The mean and standard deviation of EC50s from four such experiments for each chemical tested are listed in Table 1. (A) pentachloroethane; (B) 1,1,1,2-tetrachloroethane; (C) tetrachloroethylene; (D) carbon tetrachloride; (E) halothane; (F) 1,1,1-trichloroethane; (G) trichloroethylene; (H) chloroform; (I) 1,1,2-trichloroethane; (J) 1,2-dichloroethane; K, methylene chloride.

Reversibility of the Halocarbon Effect

For all of the compounds tested, washout of medium containing halocarbon with halocarbon-free medium resulted in virtually 100% recovery of dye coupling (at least five sequentially injected cells communicated). However, 100% recovery could only be detected by injecting cells not previously injected. Cells previously injected and exhibiting blocked dye coupling (Fig. 2B) did not spontaneously dye couple upon washout of halocarbons. Lucifer yellow apparently binds to intracellular macromolecules and does not move out of cells when halocarbons are washed out. Cells previously injected must be reinjected with dye in order to demonstrate reestablishment of communication, but this is difficult to achieve. Prolonged exposure of cells loaded with Lucifer yellow to uv light appears to damage cells (granulation and bleb formation) possibly through the production of reactive intermediates. In addition, removing the injection pipet and reinjecting increases the potential of producing a calcium leak which will inhibit dye coupling (De Mello, 1975). Figure 2C shows recovery of dye coupling in a reinjected cell in which dye coupling was blocked during exposure to 1 mM carbon tetrachloride (Fig. 2B). Reversibility could be demonstrated in this fashion in only 1 out of 10 cells reinjected. Performance of this experiment in low calcium medium increased the success rate to 8 out of 10 cells, thereby supporting the role of a calcium leak in preventing recovery of dye coupling in cells previously injected with Lucifer yellow.

Cytotoxicity

Additional experiments demonstrated that the inhibition of intercellular communication was not the result of general cytotoxicity. Prolonged exposure to halocarbons did not appear to alter the reversibility of the halocarbon effect. Recovery of dye coupling occurred in 19 of 20 cells injected following a 2-hr exposure (longest duration attempted) to a concentration (1 mM) of carbon tetrachloride that completely blocked dye coupling within 1 min. Likewise, a 2-hr exposure to a subeffective concentration (0.07 mM) of 1,1,1-trichloroethane did not result in inhibition of dye coupling (19 of 20 cells injected communicated). Furthermore, cell cultures exposed for 2 hr to concentrations of carbon tetrachloride or 1,1,1-trichloroethane that inhibited intercellular communication could be returned to control culture medium and used 24 hr later to demonstrate the reversible inhibition of dye coupling by halocarbons. Other than inhibition of dye coupling, the only effect of halocarbon exposure was an apparent reduction in spontaneous beating activity of myocytes. Heart cells beat slower or stopped beating completely when exposed to halocarbons and resumed normal spontaneous beating activity when halocarbons were washed out. Variability within dishes and inconsistency among dishes thwarted efforts to quantify and subjectively evaluate this effect on contractility for the present study. An indicator of cytotoxicity related to membrane integrity such as leakage of lactate dehydrogenase (LDH) was not used to assess toxicity. From our experience (Toraason *et al.*, 1989) significant leakage of LDH is associated with nonreversible beating cessation, marked morphological changes (cell retraction, gran-

TABLE 1
EC50 Values for Inhibition of Intercellular Communication

Chemical	CAS No.	EC50 (mM)
Methylene chloride CH_2Cl_2	75-09-2	21.05 (4.47)
1,2-Dichloroethane CH_2ClCH_2Cl	107-06-2	18.61 (2.88)
1,1,2-Trichloroethane $CH_2ClCHCl_2$	79-00-5	4.59 (0.79)
Chloroform $CHCl_3$	67-66-3	2.99 (1.46)
Trichloroethylene $CHCl=CCl_2$	79-01-6	1.73 (0.36)
1,1,1-Trichloroethane CH_3CCl_3	71-55-6	1.56 (0.25)
Halothane $BrCH(Cl)CF_3$	151-67-7	0.96 (0.11)
Carbon tetrachloride CCl_4	56-23-5	0.73 (0.26)
Tetrachloroethylene $CCl_2=CCl_2$	127-18-4	0.49 (0.08)
1,1,1,2-Tetrachloroethane CH_2ClCCl_3	630-20-6	0.39 (0.03)
Pentachloroethane Cl_2CHCCl_3	76-01-7	0.2 (0.03)

Note. EC50 is the concentration inhibiting intercellular communication in 50% of cells as determined by probit analysis. In each dish, five cells were injected with Lucifer yellow and assessed for dye coupling with adjacent cells at each concentration evaluated. Values are the mean (SD) from four dishes.

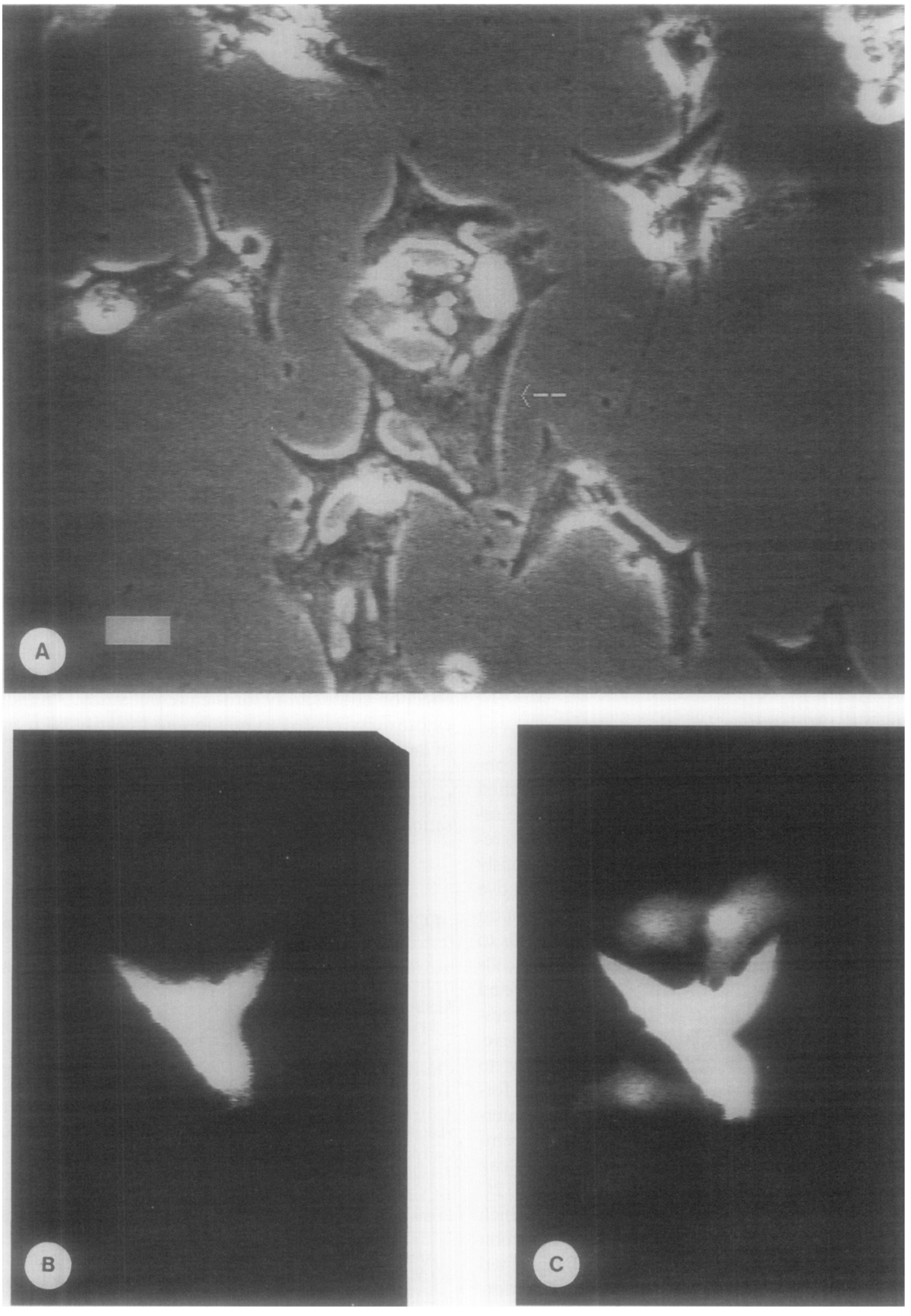

FIG. 2. Dye transfer in a cardiac myocyte microinjected with Lucifer yellow. Arrow in phase-contrast images (A) indicates injected cell. The corresponding fluorescent images show blocked communication (B) during exposure to 1 mM carbon tetrachloride and reestablishment of communication (C) after washout of carbon tetrachloride and reinjection of Lucifer yellow. Bar in (A) 20 μm.

ulation), and the sloughing of myocytes from the culture dish. None of these effects were evident during the present exposure conditions.

SKF 525-A

Pretreatment of cells with the P450 inhibitor SKF 525-A did not affect the inhibition of intercellular communication produced by exposure to 0.6 mM carbon tetrachloride. One hundred percent of cells (n = 20) treated with SKF 526-A alone communicated normally. Forty-five percent (9/20) of cells treated with carbon tetrachloride communicated, and fifty percent (10/20) of cells treated with SKF 525-A and carbon tetrachloride communicated. This small difference was not statistically significant.

Correlation of Inhibition of Dye Coupling With Octanol/Water Partition Coefficients

Comparison of EC50 values and the physicochemical properties of halocarbons was done according to the linear free energy method of investigating structure–activity relationships (Martin, 1983). The assumptions of this method are that (1) the physicochemical properties of the chemicals can be described mathematically, (2) the biological effect can be quantified, and (3) the relationship between the physicochemical property and the biological effect can be described mathematically. These assumptions were easily met with the present chemicals and data. The basic properties of formula weight; boiling point; density; vapor pressure; and saline/air, oil/air, oil/saline, and octanol/water partition coefficients (Table 2) were log transformed and compared to the log of the EC50 values using linear least-squares regression. The correlation coefficients and p values indicate that the highest correlation was between the EC50 value and the octanol/water partition coefficient (Fig. 3).

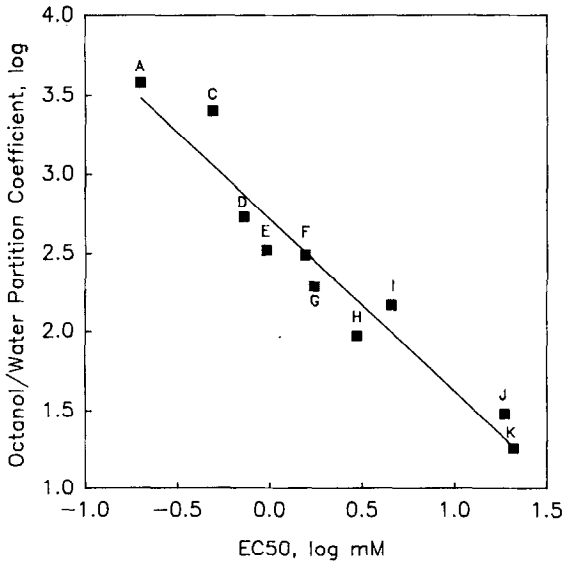

FIG. 3. Correlation of EC50s for inhibition of intercellular communication with octanol/water partition coefficients. Values are log transformations of mean EC50s and partition coefficients plotted on linear scales. (A) pentachloroethane; (C) tetrachloroethylene; (D) carbon tetrachloride; (E) halothane; (F) 1,1,1-trichloroethane; (G) trichloroethylene; (H) chloroform; (I) 1,1,2-trichloroethane; (J) 1,2-dichloroethane; (K) methylene chloride. Octanol partition coefficient for 1,1,1,2-tetrachloroethane was not obtained.

TABLE 2

Correlation Coefficients for Comparison of EC50 for Inhibition of Intercellular Communication vs Physicochemical Properties of Halocarbons

Chemical property	R^2 [a]	p values
Formula weight[b]	0.868	0.0003
Boiling point[b]	0.385	0.042
Density[b]	0.637	0.003
Vapor pressure[c]	0.359	0.051
Partition coefficients		
Blood/Air[d]	0.09	0.929
Saline/Air[d]	0.363	0.049
Oil/Air[d]	0.415	0.032
Oil/Saline[d]	0.925	0.00001
Octanol/Water[e]	0.933	0.00001

[a] Least-squares linear regression with log of EC50 for inhibition of intercellular communication vs log of the physicochemical properties of the 11 halocarbons listed in Table 1.

[b] Product information, Aldrich Chemical Co., Inc.

[c] CINFOdisc (90-2), Canadian Centre for Occupational Health and Safety.

[d] Gargas *et al.* (1989).

[e] Hansch and Leo (1979) except tetrachloroethylene (Hansch and Leo, 1985), pentachloroethane (De Wolf *et al.*, 1988). Octanol/water partition coefficient for 1,1,1,2-tetrachloroethane was not obtained.

DISCUSSION

This study demonstrates the reversible inhibition of intercellular communication among cultured cardiac myocytes produced by 11 halogenated hydrocarbons. The tested halocarbons act rapidly and recovery of intercellular communication is evident almost immediately upon washout of the compounds. Although the effective concentration for several of the halocarbons was greater than 1 mM, it does not appear that the inhibition of intercellular communication is the result of nonspecific cytotoxicity. Present exposures did not produce any overt signs of cytotoxicity, and recovery of intercellular communication occurred even after a 2-hr exposure to carbon tetrachloride or 1,1,1-trichloroethane.

With the exception of halothane, none of the tested halocarbons have previously been reported to inhibit intercellular communication in cardiac myocytes. The EC50 for halothane in the present study is 0.96 mM which is comparable to the effective range of 1–2 mM reported by Burt and Spray (1989). Burt and Spray (1989) attributed impaired

coupling by halothane and ethrane to a reduction in conducting channels rather than a decrease in unitary conductance. A reduction in junctional conductance would be expected to decrease synchrony of force development and reduce contractility. We have previously demonstrated reduction in contractility by carbon tetrachloride and 1,1,1-trichloroethane at concentrations similar those shown to be effective in inhibiting intercellular communication (Toraason *et al.,* 1990). EC50 concentrations for halothane, 1,1,1-trichloroethane, and carbon tetrachloride (Table 1) would be in equilibrium with air concentrations of 4.7, 5.1, and 5.1%, respectively, when saline/air partition coefficients of Gargas *et al.* (1989) are used to calculate them. Because blood/air partition coefficients are generally more than one order of magnitude greater than saline/air partition coefficients for the compounds tested here, blood levels comparable to EC50 concentrations in saline buffer would be in equilibrium with considerably lower air concentrations than those noted above.

Carbon tetrachloride, chloroform, and trichloroethylene have been shown to inhibit intercellular communication in rodent hepatocytes (Saez *et al.,* 1987; Klaunig *et al.,* 1989). A complete blockade of dye coupling by carbon tetrachloride in rat hepatocytes occurred at 430 μM, which is less than the EC50 of 0.73 mM reported here for cardiac myocytes. Likewise, trichloroethylene reduced dye coupling by approximately 50% among mouse hepatocytes at 100 μM. Although the exposure period was 3 hr, this concentration is more than one order of magnitude less than the EC50 of 1.73 mM for inhibition of dye coupling in heart cells by trichlorethylene. The lower effective concentrations may be due to metabolism of these compounds by hepatocytes to form reactive intermediates. The inhibition of dye coupling by trichloroethylene in mouse hepatocytes (Klaunig *et al.,* 1989), and carbon tetrachloride in rat hepatocytes (Saez *et al.,* 1987), was partially prevented by treatment of cells with P450 inhibitors. This suggested that reactive intermediates were partially responsible for the effect of these compounds on intercellular communication. In the case of carbon tetrachloride, hepatic P450 catalyzes the one-electron reduction of CCl_4 to the trichloromethyl radical and chloride anion (Cheeseman *et al.,* 1985). Despite the fact that P450 activity in myocytes is low, formation of free radicals has been detected in gerbil heart following exposure to CCl_4 (Ahmad *et al.,* 1987). Because of the effectiveness of SKF 525-A in attenuating the inhibition of intercellular communication by carbon tetrachloride and trichloroethylene in hepatocytes, we conducted a comparable experiment in cardiac myocytes. However, under present conditions SKF 525-A did not reduce the inhibition of intercellular communication induced by 0.6 mM carbon tetrachloride. If it is assumed from this result that cardiac myocytes lack the metabolic capability to produce reactive intermediates, this finding would explain the greater effective concentration needed to inhibit intercellular communication in myocytes compared to cultured hepatocytes. However, the lack of sensitivity of our method for assessing inhibition of intercellular communication may have masked any contribution of metabolism. For example, the effect of trichloroethylene on intercellular communication in hepatocytes was reduced only 15–20% by pretreatment with SKF 525-A (Klaunig *et al.,* 1989). Such a small change may be beyond the resolution of the present method used for quantifying intercellular communication in cultured cardiac myocytes. Hepatocytes take on a "cobble stone" morphology in culture which allows accurate counting of dye recipients. Myocytes overlap and individual cells are not readily discernable; therefore, the number of recipients cannot be accurately counted. As a consequence, each injection is given an all-or-none score. It is also possible that SKF 525-A was ineffective because the source of reactive intermediates in the myocardium is the mitochondrial metabolism of CCl_4 (Tomasi *et al.,* 1987). Therefore, a more extensive dosimetry of other metabolic inhibitors and free radical scavengers may reveal a role of reactive intermediates in the inhibition of intercellular communication in cardiac myocytes by halocarbons.

Clark and Tinston (1973) found that the ability of halocarbons to sensitize the myocardium to epinephrine-induced arrhythmias was closely correlated with the vapor pressure of the compounds. The same conclusion was reached by Aviado (1981), who reported that the lower the vapor pressure of a halogenated hydrocarbon the lower the threshold concentration required to affect the heart. Halocarbons with low vapor pressures would attain higher blood concentrations at a given airborne concentration and result in higher concentrations at the myocardium. In the present study, the concentration of halocarbons in the aqueous medium surrounding the myocardial cells was controlled and the effective concentrations correlated with oil/saline and octanol/water partition coefficients. After these two properties, the next highest correlation was observed with formula weight. However, it should be noted that the range of formula weights only doubled from smallest to largest molecule tested, whereas partition coefficients and EC50 values ranged over two orders of magnitude. Therefore, the magnitude of change in EC50 values does not correspond to a comparable magnitude of change in formula weight as it does with partition coefficients. This suggests that the ability to partition in the membrane is the critical physical property of the compounds in regard to inhibition of intercellular communication. Burt (1989) has proposed that it is the disordering effect produced by halothane between the C-9 and C-18 positions of the phospholipid membrane that perturbs the gap junctions and blocks intercellular communication. Spray and Burt (1990) further proposed that blocked gap junctional communication uncouples cells from one another and as a result action potentials become more and more dictated by the intrinsic membrane properties of individual cells. Such a condition

would predispose the heart to reentrant type arrhythmias, which could be brought on by a catecholamine challenge (Reynolds, 1984).

The present report demonstrates the inhibition of intercellular communication in cultured heart cells by 11 halogenated hydrocarbons. The immediate and completely reversible action of these chemicals on gap junctional communication parallels their ability to sensitize the myocardium to catecholamine-induced arrhythmias (Zahkari and Aviado, 1982). Although the role of reactive intermediates has not been ruled out, the inhibition of intercellular communication appears to be largely dependent on the ability of these compounds to partition in the cell membrane. This observation adds additional circumstantial evidence to the theory that the physicochemical properties of the halocarbons are a major factor in their acute affect on the heart.

REFERENCES

Ahmad, F. F., Cowan, D. L., and Sun, S. Y. (1987). Detection of free radical formation in various tissues after carbon tetrachloride administration in gerbil. *Life Sci.* **41,** 2469–2476.

Aviado, D. M. (1981). Comparative cardiotoxicity of fluorcarbons. In *Cardiac Toxicology* (T. Balazs, Ed.), Vol. 2, pp. 213–222. CRC Press, Boca Raton, FL.

Burt, J. M. (1989). Uncoupling of cardiac cells by doxyl stearic acids: Specificity and mechanism of action. *Am. J. Physiol.* **256,** C913–C924.

Burt, J. M., and Spray, D. C. (1989). Volatile anesthetics block intercellular communication between neonatal rat myocardial cells. *Circ. Res.* **65,** 829–837.

Cheeseman, K. H., Albano, E. F., Tomasi, A., and Slater, T. F. (1985). Biochemical studies on the metabolic activation of halogenated alkanes. *Environ. Health Perspect.* **64,** 85–101.

Clark, D., and Tinston, D. (1973). Correlation of the cardiac sensitizing potential of halogenated hydrocarbons with their physicochemical properties. *Br. J. Pharmacol.* **49,** 355–357.

Cole, W. C., Picone, J. B., and Sperelakis, N. (1988). Gap junction uncoupling and discontinuous propagation in the heart. A comparison of experimental data with computer simulations. *Biophys. J.* **53,** 809–818.

Deleze, J. (1987). Cell-to-cell communication in the heart: Structure–function correlations. *Experientia* **43,** 1068–1075.

De Mello, W. C. (1987). Modulation of junctional permeability. In *Cell-To-Cell Communication* (W. C. De Mello, Ed.), pp. 29–64. Plenum, New York.

De Mello, W. C. (1975). Effect of intracellular injection of calcium and strontium on cell communication in heart. *J. Physiol.* **250,** 231–245.

De Wolf, W., Canton, J. H., Deneer, J. W., Wegman, R. C. C., and Hermens, J. L. M. (1988). Quantitative structure–activity relationship and mixture–toxicity studies of alcohols and chlorohydrocarbons: Reproducibility of effects on growth and reproduction of *Daphnia magna*. *Aquat. Toxicol.* **12,** 39–49.

Gad, S., and Weil, C. S. (1988). *Statistics and Experimental Design for the Toxicologist,* second ed. Telford Press, Caldwell, NJ.

Gargas, M. L., Burgess, R. J., Voisard, D. E., Cason, G. H., and Andersen, M. E. (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* **98,** 87–99.

Hansch, C., and Leo, A. (1979). *Substituent Constants for Correlation Analysis in Chemistry and Biology.* Wiley, New York.

Hansch, C., and Leo, A. (1984). *The Log P Data Base.* Pomona College, Claremont, CA.

Imanaga, I., Kameyama, M., and Irisawa, H. (1987). Cell-to-cell diffusion of fluorescent dyes in paired ventricle cells. *Am. J. Physiol.* **252,** H223–H232.

Katz, R. L., and Epstein, R. A. (1968). Interaction of anesthetic agents and adrenergic drugs to produce cardiac arrhythmias. *Anesthesiology* **29,** 763–784.

Klaunig, J. E., Ruch, R. J., and Lin, E. L. C. (1989). Effects of trichloroethylene and its metabolites on rodent hepatocyte intercellular communication. *Toxicol. Appl. Pharmacol.* **99,** 454–465.

Levy, A. G., and Lewis, T. (1911). Heart irregularities, resulting from the inhalation of low percentage of chloroform and vapor, and their relationship to ventricular fibrillation. *Heart* **3,** 99–111.

Martin, Y. C. (1983). Studies of relationships between structural properties and biological activity by Hansch analysis. In *Structure–Activity Correlation as a Predictive Tool on Toxicology* (L. Goldberg, Ed.), pp. 77–92. Hemisphere, New York.

Merin, R. G. (1977). Effect of anesthetic drugs on myocardial performance in man. *Annu. Rev. Med.* **28,** 75–83.

Reynolds, A. K. (1984). On the mechanism of myocardial sensitization to catecholamines by hydrocarbon anesthetics. *Can. J. Physiol. Pharmacol.* **62,** 183–198.

Saez, J. C., Bennett, M. V. L., and Spray, D. C. (1987). Carbon tetrachloride at hepatotoxic levels blocks reversibly gap junctions between rat hepatocytes. *Science* **236,** 967–969.

Spray, D. C., and Burt, J. M. (1990). Structure–activity relations of the cardiac gap junction channel. *Am. J. Physiol.* C195–C205.

Steffey, E. P. (1982). Cardiovascular effects of inhalation anesthetics. In *Cardiovascular Toxicology* (E. W. Van Stee, Ed.), pp. 259–279. Raven Press, New York.

Tomasi, A., Albano, E., Banni, S., Botti, B., Corongiu, F., Dessi, M. A., Iannone, A., Vannini, V., and Dianzani, M. U. (1987). Free-radical metabolism of carbon tetrachloride in rat liver mitochondria. *Biochem. J.* **246,** 313–317.

Toraason, M., Breitenstein, M. J., and Swearingen, T. S. (1990). Carbon tetrachloride and 1,1,1-trichloroethane inhibit contractility in cultured cardiac myocytes from neonatal rat. *Toxicol. In Vitro* **4,** 363–368.

Toraason, M., Luken, M. E., Breitenstein, M., Krueger, J. A., and Biagini, R. E. (1989). Comparative toxicity of allylamine and acrolein in cultured myocytes and fibroblasts from neonatal rat heart. *Toxicology* **56,** 107–117.

Wojtczak, J. A. (1985). Electrical uncoupling induced by general anesthetics: A calcium-independent process? In *Gap Junctions* (M. V. L. Bennett and D. C. Spray, Eds.), pp. 167–175, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Zakhari, S., and Aviado, D. M. (1982). Cardiovascular toxicology of aerosol propellants, refrigerants, and related solvents. In *Cardiovascular Toxicology* (E. W. Van Stee, Ed.), pp. 281–314, Raven Press, New York.