Bull. Environ. Contam. Toxicol. (2001) 67:476–482

DOI: 10.1007/s00128-001-0148-1

Bulletin of Environmental Contamination and Toxicology

Effect of 1,1,1-Trichloroethane on Calcium Current of Rat Dorsal Root Ganglion Neurons

M. Okuda, I. Kunitsugu, S. Kobayakawa, T. Hobara

Department of Public Health, Yamaguchi University School of Medicine, Kogushi, Ube 755-8505, Japan

Received: 13 February 2001/Accepted: 10 June 2001

Trichloroethane is used principally for cold-cleaning, dip-cleaning, and bucket-cleaning of metal for removal of grease, oil, and wax (Stewart, 1983). It has been recently replaced by other solvents because it destroys the ozone layers (Aoki et al., 1996; King et al., 1985; House et al., 1996).

Acute exposure produces transient peripheral neuropathy as well as central nervous system dysfuncition and liver or kidney injury (House et al., 1996). In the present study, we investigate how trichloroethane influences calcium currents of rat DRG neurons, sensory neurons, using patch-clamp methods. The Ca^{2+} channel plays an important role in the neural system: neurotransmitter release in the synapse and action potential conduction and bursting. There are two types of Ca^{2+} channels in their activating thresholds (Scroggs and Fox, 1992). Low-voltage-activated (LVA) current is activated at low voltage and can produce a large after-depolarization. High-voltage-activated (HVA) current is activated at high voltage and can release neurotransmitters or enlarge amplitude of action potential. Neurons have each pattern of Ca^{2+} channel subtype composition according to their role, and it is important in shaping the signals, which are transmitted through reflex arcs, and from the periphery to the central nervous system. We isolated biophysically these voltage-operated calcium channels by the use of different negative holding potentials, evaluated the effect of trichloroethane.

MATERIALS AND METHODS

Dorrsal Root Ganglion (DRG) neurons were taken from 2-4 day-old newborn rats. The spines were dissected and approximately 40 ganglions were taken from each rat under dissection microscope. The materials were digested with 2 mg collagenase and 1 mg trypsin in 2 ml Earle's balanced salt solution, and shaken in a 37°C bath for 30 min. Cells were plated onto a plastic dish on which was placed 5 mm square cover-glass coated with poly-L-lisine.

To isolate calcium currents, an external solution composed of choline Cl 136 mM, glucose 10, HEPES 10, $MgCl_2$ 1 and $CaCl_2$ or $BaCl_2$ 2with pH adjusted to 7.3 with tris. Pipette solution was composed of CsCl 135mM, $MgCl_2 \cdot 6H_2O$ 5, glucose 12, BAPTA 5, HEPES 10, ATP2Na 5, and adjusted with CsOH to pH 7.3. 1,1,1-trichloroethane was purchased from Katayama Chemical (Osaka, Japan). The cover glass with attached neurons was put in a chamber under an inverse microscope (IX-70 Olympus, Japan). All experiments were carried out at room temperature 20-25°C.

Correspondence to: M. Okuda

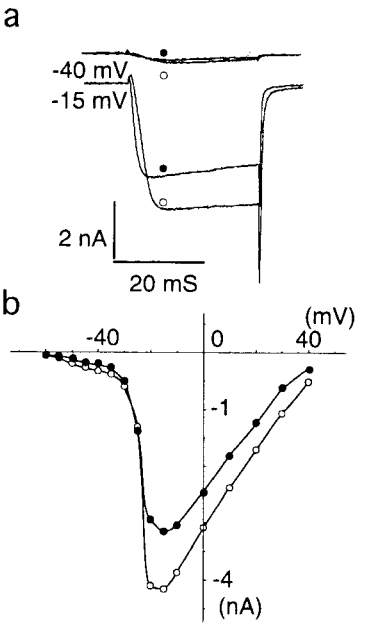

Figure 1. Calcium currents in response to voltage pulses of -40 and -15 mV from a holding potential of -80 mV (a). Currents recorded in control solution (open circle) and 5 x 10^{-3} M 1,1,1-trichloroethane (filled circle). Peak currents are plotted at each test potential and an interlaced line is drawn (b).

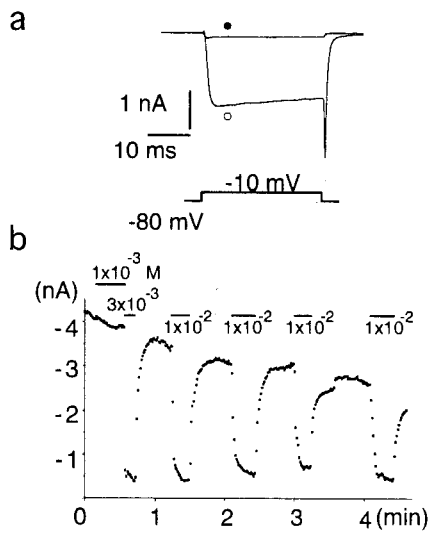

Figure 2. Calcium channel currents in response to voltage pulses of -10 mV with an interval of 10 sec. One pair of the recorded currents in control solution (open circle) and in 1 x 10^{-2} M 1,1,1-trichloroethane (filled circle) (a). Peak currents of each recording are plotted (b). Lines above the plotted data represent superfusions of 1,1,1-trichloroethane.

Glass pipette electrodes were used with a resistance of 2-3 megaohm. Electrical activity was recorded with a patch-clamp amplifier (Axopatch 200A, Axon Instruments, USA). Series resistance was compensated for using the Axopatch 200A series resistance compensation circuit. Electrical records were digitized at 10 K Hz and stored on personal computer (Power Macintosh 7200/80AV, Apple Japan, Japan) using Axodata v1.2.3 (Axon Instruments). Data were analyzed off-line with Axograph v3.0.3 (Axon Instruments). Curve fitting was done with Axograph 3.0.Calcium currents were recorded with step pulse from the holding potentials at -60~80mV. We certified these currents as Ca^{2+} currents, which reduced when external Ca^{2+} was replaced with La^{3+} and Mn^{2+}. Afterwards, we used Ba^{2+} instead of Ca^{2+} in the external solution and carried out voltage clamp experiments.

Acquired data were fitted to following equations. A Hill equation was fitted to the concentration-inhibition curve: $Y(X) = 1 / (1 + (X / Kd)^n)$; where Y is the ratio

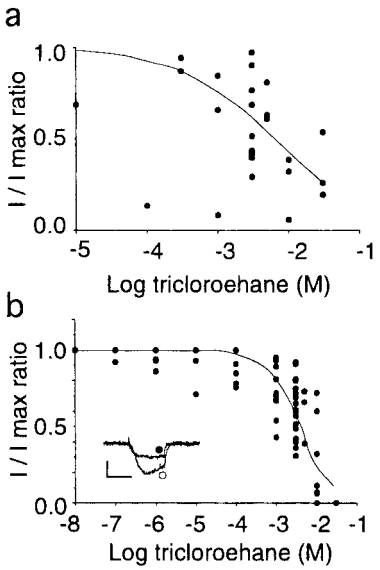

Figure 3. Dose-inhibition relationships are obtained from the peak currents of each neuron. Low-voltage activated currents in response to a voltage pulse of -4(mV (a), and high-voltage activated currents in response to a voltage pulse of -10 mV (b). Inhibition is presented in a ratio with the stable current before superfusion of 1,1,1-trichloroethane solution. Dose-dependence is described by the Hill equation, shown as a line in the plotted data.

of a current remaining to the maximal current, X is the trichloroethane concentration, Kd is the concentration that exhibits half-maximal inhibition, and n is the Hill coefficient. A Boltzmann equation was fitted to the normalized peak I-V curves (activation curve): $Y(X) = 1 / (1 + \exp[- (X - V50) / k])$; where Y is the observed fraction of maximal current, X is the test potential, V50 is the potential at which a current is half of maximal current, and k is the slope factor. An inactivation curve was fitted to the next Boltzmann equation: $Y(X) = 1 / (1 + \exp[(X - V50) / k])$; where Y is the observed fraction of the maximal current, X is the conditioning potential, V50 is the potential at which a current is reduced to 50%, and k is the slope factor.

Data are presented as mean ± standard error. Statistical significance of a trichloroethane effect was determined using Student's t test, with $p<0.05$ considered as significant.

RESULTS AND DISCUSSION

An inward current was observed with a step pulse from a holding potential of -80 mV (Figure 1). The properties of the inward current seen in these cells were identical to those reported by Kostyuk et al. (1989). During depolarizing steps from a holding potential of -80 mV, the major currents were transient inward currents seen at potentials from -60 mV to about -30 mV (LVA calcium current) and larger and more maintained currents seen at potentials more positive than -30mV (HVA calcium current) (as seen in Bean, 1989a). Trichloroethane reduced

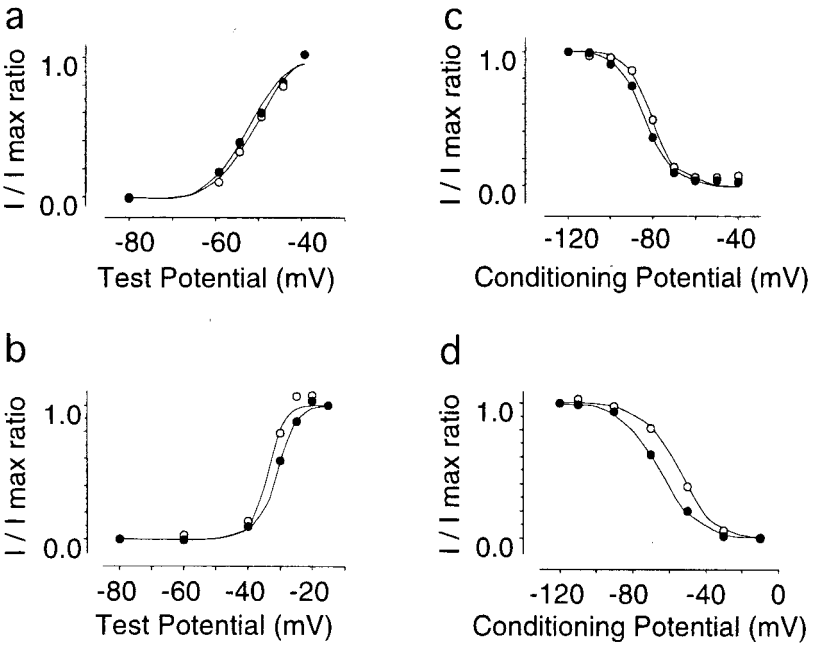

Figure 4. Voltage dependent activation and inactivation curves. Activation of the low-voltage-activated and high-voltage-activated currents (a and b, respectively). Inactivation of the low-voltage-activated and high-voltage-activated currents (c and d, respectively). Recorded peak currents are normalized. Data plotted in control (open circle) and 3 x 10^{-3} M 1,1,1-trichloroethane (filled circle) solutions are derived from the same cell, while data in a-d are derived from different cells. Voltage-dependence is described by the Boltzmann equation, shown as a line in the plotted data.

Ca^{2+} currents and reduction was seen at any voltage steps in current-voltage relation (Figure 1). Applying 40 msec step pulses with 10 sec interval, the effects of trichloroethane appeared rapidly within 10 sec after superfusion changed, and washing-out of trichloroethane recovers Ca^{2+} currents more than 3 min (Figure 2).

The concentration-inhibition relationship was obtained from the peak LVA current as a peak measured at a test potential of -40mV from a holding potential of -80mV, and the peak HVA current as a peak measured at -10mV from a holding potential of -80mV (Figure 3). LVA currents were seen in all experimental cells. We omitted cell data that had LVA current less than one-tenth of the HVA current from the analysis for the LVA current concentration-inhibition relationship.

Inhibitory effects on the LVA current and that on the HVA current were different in dependence of the concentration of trichloroethane. A Hill equation was fitted to the concentration-inhibition curve. Half-maximal inhibition of the LVA current was 5.76 x 10^{-3} M, and the Hill coefficient was 0.61. Half-maximal inhibition of the HVA current was 3.99 x 10^{-3} M and the Hill coefficient was 1.04. In the LVA current, the fitted curve was flat.

Inhibition of current raises the question whether trichloroethane reduces the calcium current by shifting the activation or inactivation curve. We therefore evaluated the voltage dependence of calcium current activation and inactivation (Figure 4). The voltage dependence of the peak LVA current appeared to be little affected by trichloroethane (Figure 4a). A Boltzmann equation for 7 activation curves was fitted to the normalized peak I-V curves. The half-maximal activation was not influenced by trichloroethane; in control solution, the mean half-maximal activation was -51.19 ± 0.95 mV (n = 7), whereas in 3 x 10^{-2} M trichloroethane the mean was -52.96 ± 2.55 mV (n = 7). The mean steepness was 2.30 ± 4.29 mV for control curves and 2.91 ± 1.05 mV in the presence of trichloroethane. In experiments with other concentrations of trichloroethane, the concentrations did not influence the voltage-dependent curve. Values obtained in the presence of trichloroethane did not differ from those in control solutions. These observations indicate that the voltage dependence of the peak LVA current is not shifted by trichloroethane, even though the amplitude is reduced.

The HVA current was isolated from the LVA calcium current for HVA calcium current evaluation. Prepulses of -40 mV with duration of 100 msec inactivated the LVA current. Test potentials with duration of 30 msec were applied after 3 msec interval. The voltage dependent activation of the peak HVA current showed the same pattern in between control and trichloroethane solution (Figure 4b). The half-maximal activation averaged from 8-neruon data was -51.19 ± 0.95 mV in control and the mean in 3 x 10^{-3} M trichloroethane was -52.96 ± 2.55 mV. The mean steepness was 4.76 ± 0.86 mV in control and the mean in trichloroethane was 4.95 ± 0.64 mV.

In studying voltage-dependent inactivation of the calcium current, conditioning potentials were applied with duration of 1 sec before the holding potential of -80 mV with duration of 15 msec. LVA and HVA currents were evaluated as currents which developed at a test pulse of -10 mV and -40 mV respectively (Figure 4c,d). An inactivation curve was also fitted to the Boltzmann equation. In the LVA inactivation curves of 6 neurons, the mean half-maximal inhibition was 86.75 ± 8.19 mV in control, while the mean was 84.99 ± 2.54 mV in 3 x 10^{-3} M trichloroethane. The mean steepness was 9.88 ± 3.39 mV in control, and the mean in trichloroethane was 10.91 ± 4.42 mV. The LVA inactivation curve was not influenced by trichloroethane.

Currents in response to the -10mV step pulse included both the LVA and HVA currents and were impossible of dissociation. The HVA currents were measured at a lapse of 40 msec in test pulse in order to minimize LVA current component. The HVA inactivation curve was influenced in the presence of 3 x 10^{-3} M trichloroethane. An inactivation curve fitted with a Boltzmann equation was shifted to the left (Figure 4d). The half-maximal inhibition averaged from 5-neruon data was 49.00 ± 6.18 mV in control, while the mean half-maximal inhibition in trichloroethane was 62.22 ± 5.44 mV (significant with p=0.008). The mean steepness was 10.27 ± 2.03 mV in control and 10.08 ± 1.04 mV in trichloroethane. The concentration used here was 3 x 10^{-3} M. We did not evaluate the concentration-dependent change in an inactivation curve.

Axons of DRG neurons transmit stimuli on skin nerve endings to the dorsal horn of the spinal cord. Scroggs and Fox (1992) classified DRG neurons into three types; small, medium, and large. The neuron types have not been clearly understood in terms of function. Small and medium sized neurons have relatively large LVA currents. Neurons used in the present study were categorized into a medium sized subpopulation in reference to size and calcium currents. We showed trichloroethane

reversibly inhibits calcium currents of this type of DRG neurons.

The inhibitory effects of trichloroethane had a half-inhibitory concentration (IC50) of 3.99 to 5.76 x 10^{-3} M in this study. You et al. (1994) reported that the threshold of behavioral inhibition for mice was 130 μ g/ml blood, about 0.97 x 10^{-3} M. Some neurons showed partially reduced LVA or HVA currents around 1 x 10^{-3} M of trichloroethane (Figure 3). DRG neuron activity may be inhibited at this concentration of trichloroethane. This suggests that peripheral neuropathy may be subclinically coincident with central nervous depression.

Recovery from the inhibition effects took more time than the appearing of the effects. The chamber in this study was so small enough that solutions were able to be rapidly changed. There were two possibilities indicating slow recovery and / or retained Ca^{2+} channel inhibition. First, calcium currents did not show complete recovery to the level before treatment. This phenomenon can be explained by run-down, in which Ca^{2+} channels need cytoplasmic materials. Takenoshita et al. (1991) reported reversible inhibition of calcium currents by halothane. In that report, calcium currents inhibited by halothane also showed run-down. Second, calcium currents recovered reluctantly. Trichloroethane diffused into the lipids does not directly react to proteins because lipid-protein interaction is so tight (Li et al, 1994). This suggests that trichloroethane diffuses in lipophilic substances like membrane lipids or hydrophobic proteins. In the human body peripheral neuropathy may remain for some time after exposure to trichloroethane.

Trichloroethane inhibited both LVA and HVA channels. In high concentrations of trichloroethane, there remained a relatively large fraction of the LVA currents in response to a -40 mV step pulse. The remaining current had a long-lasting character during step pulse, which was different from the normal LVA current. It raised the question of what type of calcium channels are activated in a high concentration of trichloroethane.

The concentration-inhibition curve for the LVA current and that for the HVA current were different, and the goodness of curve fitting is bad in LVA current. There are two possible explanations. First, LVA current could not be purely detected because other currents were activated in the step pulse of -40mV. Secondly, sensory neurons show heterogeneous expression of T-type calcium channels (Schoroeder et al., 1990). Response to trichloroethane might be heterogeneous for the LVA current in each DRG neuron. Relatively large currents seen at more than 10^{-2} M had longer decays (Figure 3 inset).

We can not evaluate the kinetic mechanism of trichloroethane for LVA channels. Voltage dependence for activation and inactivation of LVA current were not influenced by trichloroethane, but amplitudes of LVA were reduced. This is consistent with the idea either that some channels are completely removed from the activatable pool, or that single-channel conductance is reduced. (Takenoshita et al., 1991).

Voltage dependence for activation of the HVA current seemed not to be influenced. The method used to describe the voltage dependence of activation was not appropriate for full evaluation. Tail current measurement was necessary (Bean, 1989b). Voltage dependence for inactivation of the HVA currents was shifted to the left (Figure 4). This indicates that an open state of HVA channels easily shift to an inactivated or closed state at more negative potentials and therefore may be one of the mechanisms for reducing the HVA current.

There are two important things to be considered. First, the Ca^{2+} channels that we examined are membrane channels of cell bodies. Cell bodies have no involvement for the nerve impulse conduction and have not yet been implicated in neuronal modification. Second, nerve impulse consists of other ion channels: Na^+, K^+, and Cl^-. We did not evaluate these other ion channels. However, the axon of DRG has the same types of Ca^{2+} channels as the cell bodies. but different proportion of types, so this study indicates that trichloroethane could influence nerve endings and axons and modulate the nerve activity in dependence on Ca^{2+} channel subtypes population of neurons.

Acknowledgment. We thank Dr. Koji Okabe and Dr. Kenji Kitamura at Fukuoka Dental College for instruction in electrophysiological recordings. Miss Masako Yoshikuni kindly prepared the manuscript.

REFERENCES

Aoki N, Masuda T, Soma K, Kurosawa T, and Ohwada T (1996) Transient disturbance of pulmonary blood flow and subsequent pressure overloading in the right ventricle following the inhalation of a water-proof spray. J Japan Soc Intensive Care Med 3: 99-102

Bean BP. (1989a) Classes of calcium channels in vertebrate cells. Ann Rev Physiol 51: 367-384

Bean, BP (1989b) Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. Nature 340 153-156

House RA., Liss GM., Wills, MC, Holness DL (1996) Parenthesis and sensory neuropathy due to 1,1,1-trichloroethane. J Occup Environ Med 38 123-124

King GS., Smialek J.E., Troutman WG. (1985) Sudden death in adolescents resulting from the inhalation of typewriter correction fluid. J Am Med Associ 253: 1604-1606

Kostyuk PG, Shirokov RE (1989) Deactivation kinetics of different components of calcium inward current in the membrane of mice sensory neurons. J Physiol (Lond) 409: 343-355

Li C, Peoples RW, Weight FF (1994) Alcohol action on a neuronal membrane receptor: evidence for a direct interaction with the receptor protein. Proc Natl Acad Sci USA 91: 8200-8204

Schoroeder,JE Fischback PS McCleskey EW (1990) T-type calcium channels: heterogeneous expression in rat sensory neurons and selective modulation by phorbol esters. J Neurosci 10: 947-951

Scroggs RS, Fox AP (1992) Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size. J Physiol 445: 639-658

Takenoshita M, Steinbach JH (1991) Halothane blocks low-voltage-activated calcium current in rat sensory neurons. J Neurosci 11: 1404-1412

You L, Muralidhara S, Dallas CE (1994) Comparisons between operant response and 1,1,1-trichloroethane toxicokinetics in mouse blood and brain. Toxicology 93: 151-163