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Trichloroethanol impairs NMDA receptor function in rat mesencephalic and cortical neurones

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Abstract

The effects of 2,2,2-trichloroethanol, the active compound of the sedative–hypnotic chloral hydrate, were investigated on *N*-methyl-D-aspartate (NMDA)-induced increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultured mesencephalic and cortical neurones by means of the fura-2 method. Trichloroethanol inhibited the NMDA response in a concentration-dependent manner in cortical ($IC_{50} = 2.76$ mM) and mesencephalic neurones ($IC_{50} = 1.12$ mM), with a maximum effect of approximately 85 and 94%, respectively. Ethanol was considerably less potent than trichloroethanol. In conclusion, the trichloroethanol-induced impairment of NMDA receptor function may contribute to the sedative–hypnotic properties of chloral hydrate.

Keywords: NMDA receptor; 2,2,2-Trichloroethanol; Ca^{2+} concentration; Intracellular

Orally administered chloral hydrate is rapidly metabolised to 2,2,2-trichloroethanol which is presumably responsible for the depressant effect of this sedative/hypnotic compound on the central nervous system (Breimer, 1977). Trichloroethanol has previously been shown to potentiate γ-aminobutyric $acid_A$ receptor-mediated synaptic transmission in vitro (Lovinger et al., 1993). In the present study, a possible effect of trichloroethanol on *N*-methyl-D-aspartate (NMDA) receptor function was investigated in primary cultures of rat cortical and mesencephalic neurones using the fura-2 method.

For details of cell culture conditions see Shimoda et al. (1992) and Hansson and Rönnbäck (1989) for mesencephalic and cortical neurones, respectively. In brief, rat (Wistar) embryonic tissue sections of ventral mesencephalon (gestational day 14) or cerebral cortex (gestational day 16) were dissociated enzymatically (0.25% trypsin followed by DNAse treatment) and by careful trituration. Subsequently, the cells were plated on glass coverslips coated with poly-L-lysine in a 1:1 mixture of Dulbecco's modified Eagle's medium and Nutrient F12 (supplemented with 20% foetal calf serum, 2 mM L-glutamine, 50 μg/ml gentamycin, and 36 mM D-glucose) at a density of 250,000 (mesencephalic neurones) or 100,000 cells/cm^2 (cortical neurones).

Mesencephalic and cortical neurones, 8 and 12 days in vitro, respectively, were loaded with fura-2 acetoxymethyl ester (5 μM; Sigma) for 30 min at 37°C, then left to equilibrate in magnesium-free HEPES buffer (composition in mM: NaCl 133, KCl 4.8, $CaCl_2$ 1.3, KH_2PO_4 1.2, HEPES 10; D-glucose 10; pH 7.4 adjusted with NaOH). Fura-2 fluorescence (excitation wavelengths: 340 and 380 nm; emission wavelengths 510–520 nm) was measured under an inverted microscope by means of a photomultiplier-based detection system (PTI, Wedel, Germany). The Δ fluorescence ratio F340/F380 (i.e., the fluorescence ratio subsequent to NMDA application − the basal fluorescence ratio) was used for evaluation of drug effects on NMDA-induced changes in $[Ca^{2+}]_i$. NMDA (Sigma; plus 10 μM glycine) was applied for 1 min up to three times, separated by 15-min intervals. Calibration was performed according to Grynkiewicz et al. (1985).

Basal $[Ca^{2+}]_i$ determined in the cell bodies of rat cortical and mesencephalic neurones averaged 118.6 ± 1.3 nM ($n = 4$) and 95.6 ± 13.4 nM ($n = 11$), respectively. NMDA, 30 μM (cortical neurones) and 100 μM (mesencephalic neurones) induced a maximal increase in Δ fluorescence ratios of 1.02 ± 0.02 ($n = 4$; cortical neurones) and 0.70 ± 0.04 nM ($n = 13$; mesencephalic neurones) corresponding to an increase in $[Ca^{2+}]_i$ to 399.2 ± 54.3

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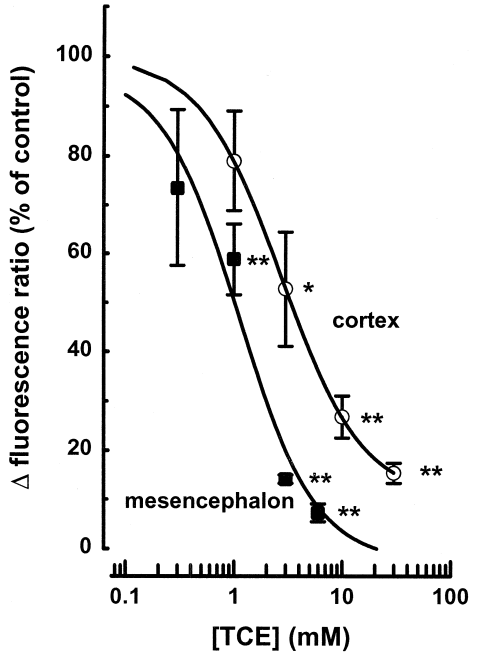

Fig. 1. Effects of 2,2,2-trichloroethanol (TCE) on the NMDA-induced enhancement of intracellular calcium in individual rat cortical (open circles) and mesencephalic (filled squares) neurones. Cultured cortical and mesencephalic neurones were superfused and stimulated twice with 30 μM and 100 μM NMDA (plus 10 μM glycine), respectively. The effects of 2,2,2-trichloroethanol added 10 min before the second stimulation were evaluated by calculating the ratio between the second and the first NMDA response, and are expressed as a percentage of the mean NMDA control ratio in the absence of 2,2,2-trichloroethanol (0.98 ± 0.07 ($n = 6$) cortical neurones; 0.96 ± 0.06 ($n = 4$) mesencephalic neurones). Means ± S.E.M. of 3–6 observations are given. Significant differences vs. respective control (Student's *t*-test): $* P < 0.05$; $** P < 0.01$.

nM and 320.5 ± 28.6 nM, respectively. As previously demonstrated, voltage-dependent Na^+ or Ca^{2+} channels are not involved in this effect (Scheibler et al., 1998). Application of 2,2,2-trichloroethanol attenuated the NMDA-induced enhancement of $[Ca^{2+}]_i$ in a concentration-dependent manner in both cortical and mesencephalic neurones (Fig. 1) with IC_{50} values of 2.76 ± 0.04 and 1.12 ± 0.38 mM, respectively (4-parameter logistic function; SigmaPlot). In cortical neurones, 30 mM 2,2,2-trichloroethanol caused a maximum inhibition of approximately 85%, whereas in mesencephalic neurones, 6 mM 2,2,2-trichloroethanol virtually abolished the NMDA response (i.e., 94% inhibition; Fig. 1). Ethanol was considerably less potent than 2,2,2-trichloroethanol. Ethanol (100 mM) inhibited the NMDA-induced increase in $[Ca^{2+}]_i$ by 32.2 ± 4.3 ($n = 4$; $P < 0.05$) and $43.9 \pm 8.9\%$ ($n = 6$; $P < 0.01$) in cortical and mesencephalic neurones, respectively.

Together, the results are the first demonstration that 2,2,2-trichloroethanol counteracts NMDA-induced increases in $[Ca^{2+}]_i$ in neurones. Since subsequent to chloral hydrate administration 2,2,2-trichloroethanol concentrations may reach the low millimolar range (Owen and Taverner, 1980), the inhibition of NMDA receptor function may be of therapeutic significance. The higher potency of 2,2,2-trichloroethanol in comparison to that of ethanol is possibly associated with the increased hydrophobicity of the halogenated alcohol. Currently, it has been demonstrated that 2,2,2-trichloroethanol also affects excitatory amino acid-activated currents in mouse hippocampal neurones at slightly higher concentrations (Peoples and Weight, 1998).

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