Voltage-activated calcium channel currents of rat dorsal root ganglion cells are reduced by trimethyl lead

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Abstract

Using the conventional whole-cell patch-clamp recording technique with cultured neurones of rat dorsal root ganglia (DRG), we analysed the effects of trimethyl lead (TML) on voltage-activated calcium channel currents. TML reduces voltage-activated calcium channel currents in a dose-dependent manner, with a threshold concentration below 0.5 μM and a total reduction of the current (≥80% of the control current) at concentrations above 50 μM. Half of the current is abolished at TML concentrations between 1 and 5 μM. The action is irreversible and is not voltage dependent. After application of TML the current decreases with each activation of the channel until a steady state is reached after 8–12 min, when the channel was activated every 10 s. The channel had to be in the open state for TML to act. TML is a potent compound for reducing voltage activated calcium channel currents. These effects of TML must be taken into account in explaining the neurotoxic effects of this organic metal compound. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

Lead is known to generate neurotoxic effects at very low concentrations. Trimethyl lead (TML) is a widely used organic lead compound. Many functions of the nervous system — including learning and memory — depend on the exact regulation of the intracellular calcium concentration. Lead reduces the generation and maintenance of a long-term potentiation in vivo and in vitro (Altmann et al., 1991; Hori et al., 1993). Voltage-activated calcium channels constitute one of the main routes by which calcium enters cells.

TML is a decomposition product of tetramethyl lead that is used as a chemical additive in a variety of chemical processes and in leaded gasoline. Tetra-alkyl derivatives of metals are
metabolised to trialkyl derivatives when incorpo-
rated into the body. They are lipophilic and once
ingested they persist in the nervous system for
prolonged periods (Grandjan and Nielsen, 1979).
Organic lead compounds interact with micro-
tubules (Zimmermann et al., 1985), enhance the
behavioural effects of dopaminergic agonists
(Walsh et al., 1986), change the activity of en-
vymes (Haeffner et al., 1984) and inhibit the
synaptosomal gamma-aminobutyric acid uptake
(Seidmann and Verity, 1987). Surprisingly, some
symptoms caused by the organic lead compound
TML are similar to those caused by Pb\(^{2+}\) and
result in deficits of cognitive functions
(Swartzwelder, 1986).

Calcium plays an important role in cell commu-
nication and synaptic plasticity. Therefore, the
intracellular calcium concentration is closely regu-
lated. Calcium enters the cell via membrane chan-
nels which are opened by ligands (receptor-activated calcium channels) or by depo-
larization (voltage-activated calcium channels). As
it has been shown in a variety of preparations,
some of the actions of different metal cations
result from their effects on voltage-gated calcium
channels (Audesirk and Audesirk, 1991;
Nachshen, 1984; Büsselberg et al., 1994a,b,c;
Leonhardt et al., 1994a,b; Pekel et al., 1993).
Although several types of voltage-gated calcium
channels have been described (Fox et al., 1987),
we only distinguish between low-voltage activated
calcium channel currents and high-voltage acti-
vated calcium channel currents in this investiga-
tion.

Here we examine the actions of TML on these
two groups of voltage-gated calcium channel cur-
rrents of cultured rat dorsal root ganglion cells.

2. Materials and methods

The preparation of dorsal root ganglion neu-
rones has been described in detail by Büsselberg
(Büsselberg et al., 1994a), as have the recording
techniques and data analysis used in the present
study. Briefly: Dorsal root ganglia were removed
from 2–4 day old rat pups and enzymatically
 treated with collagenase and trypsin. The re-
leased neurones were cultured in petri dishes (Fal-
con, 'easy grip') for up to 4 days. Calcium channel
currents were recorded using conventional,
'whole-cell patch-clamp' techniques with appro-
priate internal and extracellular solutions (intracell-
ular pipette solution: CsCl 140 mM, HEPES
10 mM, EGTA 10 mM, MgCl\(_2\) 4 mM, Na-ATP 2
mM, pH adjusted to 7.2; extracellular solution for
isolation of calcium channel currents: TEA 130
mM, glucose 10 mM, HEPES 10 mM, MgCl\(_2\) 1
mM, BaCl\(_2\) 10 mM, tetrodotoxin 0.2–2 \(\mu\)M, pH
adjusted to 7.3). Electrophysiological recordings
of neurones were performed with an EPC-9
patch-clamp amplifier. Cells were generally
clampped at a holding potential of \(-80\) mV. The
specific extracellular solution was applied after
formation of the gigaseal, and specific depolariza-
tions were used to separate various voltage-acti-
vated currents. Voltage-activated calcium channel
currents, carried by 10 mM barium, were elicited
by voltage steps from the holding potential to
0 mV for 75 ms. Leakage currents and capacitive
portions were corrected by a P/4 protocol (Chad
and Eckert, 1986). Extracellular media (with or
without TML) were exchanged by a bath perfu-
sion system. All experiments were carried out at
room temperature (\(\sim 22^\circ\)C).

3. Results

The effects of TML on voltage-activated cal-
ci um channel currents were examined on 64 dor-
sal root ganglion neurones. TML reduces both
low- and high-voltage activated calcium channel
currents. Fig. 1 shows control currents (before
application of the metal compound) and currents
recorded after application of TML for 10 min
when the current did not decline any further. Fig.
1A illustrates the action of 1 \(\mu\)M TML in a single
experiment. The currents were elicited by a
voltage step from the holding potential (\(-80\)
mV) to \(-30\) mV for 75 ms. In the example
shown, the peak current was reduced from 420–
130 pA (31%) and the sustained current from
90–45 pA (50%). In another neuron the same
concentration of TML reduced the peak of high-
voltage activated (HVA) calcium channel currents.
(Fig. 1B), activated by a depolarization to 0 mV, to 35% (from 1 nA to 350 pA) while the sustained current was reduced to 31% (from 650–200 pA).

Fig. 2A illustrates the time course of the reduction of peak currents, activated by depolarizations from the holding potential to 0 mV (HVA) every 10 s for 75 ms. Shown are averages of the data from applications of TML to 5 neurones. The control currents before application were set to 100%, the S.E.M. never exceeded 7%. The reduction of the peak channel current with 1 μM TML starts at the onset of application (0 min). A steady state was attained within 8–12 min with a total reduction of the peak current of 54%. The effect was never reversible by washing with TML-free solution (not shown).

The actions of TML depend on an open channel state. An example is shown in Fig. 2 B. The metal was applied at time = ‘0’. At this time the activation of the channels was interrupted for 3 min. The first calcium channel current measured after this period had approximately the same amplitude as the control currents. The amplitude of the following currents declined with a comparable time course as shown in part A of the same figure.

The reduction of the calcium channel currents by TML is concentration-dependent (Fig. 3). Values were taken after reaching a steady state (8–12

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Fig. 1. A concentration of 1 μM trimethyl lead (TML) reduces voltage-activated calcium channel currents. Calcium channel currents elicited by a depolarization from the holding potential of −80 mV to −30 mV (mainly activation of T-type calcium channel currents, lower trace) and the effect of TML on these currents (upper trace) is shown in part A. An example of currents activated by depolarising the cell from −80–0 mV (mainly L- and N-type calcium channel currents, lower trace), and the TML action (upper trace) is illustrated in part B.

Fig. 2. Time course of the action of 1 μM TML on peak calcium channel currents elicited by depolarizations from the resting membrane potential to 0 mV. TML was applied at time = ‘0’. (A) Averaged and normalized data of TML action on five neurones, about 9 min after the application of the substance, when a steady state was reached (activating the current every 10 s), the currents was reduced to 54% (± 12.8%) of the control value. (B) Example of the use dependence of the action of TML on peak calcium channel currents. The activation of the calcium channel current is interrupted for 3 min when TML was applied. When the activation of the channels was resumed the first current elicited was still 91% of the control value, the following currents decreased over the next 5–11 min.
min after application of TML). The threshold concentration of the effect is below 0.5 μM and the current is reduced more than 80% with TML concentrations of 50 μM or higher. Half of the peak current is abolished with TML concentrations between 1–5 μM.

The voltage-activated calcium channel currents are reduced over the entire voltage range tested. Fig. 4A shows the average current-voltage relation of six neurones. In control solution the maximal current was elicited with depolarizations to about 0 mV. With 1 μM TML the maximum current is still generated by stepping to 0 mV (from the holding potential of −80 mV). The relative reduction of the peak current over the entire voltage range is illustrated in Fig. 4B. There is no obvious voltage-dependence of the TML effect. The slight bending of the curve in the hyperpolarized range is most likely due to a low signal to noise ratio.

Application of TML changed the membrane currents in a number of cells. Nineteen neurones were tested for membrane currents, 13 showed a sustained outward current which developed a steady state within 5–10 min after TML application. In three of these 13 cells a smaller transient inward current (for about 1 min) was observed when applying TML (Fig. 5).

Fig. 3. Bar plot of the percentage reduction of the elicited currents by different concentrations of TML (means ± S.D.M.). A concentration of 0.5 μM TML reduced the currents by 21.6 ± 2.6%, n = 3; 1 μM TML reduced the currents by 49.9 ± 13.9%, n = 7; 5 μM TML reduced the currents by 58.7 ± 5.2%, n = 5; and 50 μM TML reduced the currents by 85.4 ± 2.8%, n = 4.

Fig. 4. The effect of TML is not voltage dependent in the range between −35 and +35 mV. (A) Averaged and normalized current voltage relations for peak calcium channel currents (means ± S.D.M.) without TML and after the application of 1 μM TML (n = 6). TML reduces the current over the entire voltage range tested. (B) The relative reduction of the peak calcium channel currents, calculated for voltages between −35 and +35 mV (means ± S.D.M.).

4. Discussion

TML reduces voltage-activated calcium channel currents in a dose dependent manner. The action is irreversible and not voltage-dependent. After application of TML the currents decreased with each activation of the channel until a steady state

Fig. 5. Changes of the resting membrane currents. TML generated a biphasic current in about 15% of the neurones. A transient inward current (about 1 min) was followed by a larger, persistent outward current. Such an outward current was obvious in two-thirds of the neurones tested.
was reached after 8–12 min at an activation rate of 10 min. TML acts only on open channel states. This is in contrast to the effects of the non metal organic calcium channel blocker diltiazem, which acts quickly and does not exhibit use dependence (Gandia et al., 1996).

The concentrations of TML used in this study had no effect on the current-voltage relation. Therefore, charge screening effects at the membrane do not play a major role and could not explain the reduction of the current through the calcium channels. This, together with the fact that an open channel state is needed for the action of TML, suggests that this organic metal cation binds within the channel. This hypothesis is also supported by the observation that the effect of TML is irreversible.

We do not know to which extent TML is incorporated into the plasma membrane or passes directly through the membrane as has been described for inorganic and organic mercury compounds (Gutknecht, 1981). We have demonstrated that an open channel is most likely needed for the TML action. This is not in agreement with the action of TML on an internal channel binding site. While our experiments cover a relatively short period of time compared to in vivo intoxications we cannot exclude, that TML enters the cell over a longer period of time and changes other cell functions.

While the focus of the present paper was to describe effects of TML on voltage activated calcium channel currents we also observed effects of this metal compound on general membrane conductance. We did not analyse these currents further but they might be important with regard to the neurotoxic effects of TML. Similar currents have been described by Arakawa and coworkers (Arakawa et al., 1991) after applying mercury to neurones. These authors suggested that mercury modulates non-specific cation channels.

The actions of TML on voltage-activated calcium channels are quite different to those of Pb\(^{2+}\). TML is less potent than Pb\(^{2+}\) in reducing calcium channel currents of rat DRG neurones. In addition, the effect of Pb\(^{2+}\) is faster, partly reversible and not dependent upon an open channel state. The manner in which TML reduces the currents through voltage-activated calcium channels is similar in several respects to the action of methyl-mercury (MeHg): both compounds are effective in the same concentration range (MeHg: IC\(_{50}\) = 2.6 \(\mu\)M), have a similar time course (to reach a steady state 5–15 min were needed), and show a similar use dependence (for both organic metal compounds an open channel state is necessary for action) (Leonhardt et al., 1996a,b).

The action of TML on Aplysia neurones is different: in neurones of this marine mollusc, TML generates a continuous run-down of voltage-activated calcium channel currents (Büsselberg et al., 1991) so that a dose-response curve could not be measured. To what extent this is due to the different alkyl residues (ethyl instead of methyl) or to the much higher concentration that was used to obtain the effect in Aplysia neurones is uncertain.

TML is a potent compound for reducing voltage activated calcium channel currents. These effects of TML must be taken into account in explaining the neurotoxic effects of this organic metal compound.

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