Effects of aromatic solvents on acoustic reflexes

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INTRODUCTION

Chronic exposure to toluene (Tol) can impair the central nervous system (Yamanouchi et al. 1995; Greenberg 1997). Tol shares many effects with nervous system depressant compounds such as anesthetics (Evans & Balster 1991). In the past, the neuroactivity of anesthetics and related compounds was thought to be attributed to their ability to perturb the plasma membrane (Engelke et al. 1992). Today, clear evidence is emerging from the literature regarding actions of solvents on ion channels expressed in neurons. For instance, NMDA (Cruz et al. 1998), GABA (Krasowski & Harrison 2000) and Ach (Bale et al. 2002) receptors are important to nervous system and sensitive to Tol. Besides, Tol alters the function of several voltage-dependent ion channels including voltage-dependent Ca^{2+} channels (VDCCs) (Tillar et al. 2002; Shafer et al. 2005). Chronic exposure to Tol can impair the inner ear as well (Odkvist et al. 1982; Rybak 1986). The notion of ototoxicity stemming from organic compounds is important for people exposed at workplace (Morata & Campo 2001). Certain aromatic solvents are ototoxic and can even worsen the effects of noise (Lataye & Campo 1997; Lataye et al. 2000; Brandt-Lassen et al. 2000; Cappaert et al. 2001; Sliwinska-Kowalska et al. 2003; Chang et al. 2006).

The studies carried out in the rat showed that a co-exposure to noise and aromatic solvent can have synergistic adverse effects on hearing. The fair assumption proposed to explain these effects was that the solvents could weaken the outer hair cell (OHC) membranes and thereby increase their vulnerability to noise. But, in recent investigations performed with rats, it has been shown that Tol can inhibit Ach receptors (Lataye et al. 2007) and cancel the protective effect of the middle-ear reflex (MER) (Campo et al. 2007).

In the rat, motoneurons involved in the MER are mediated by Ach (Liu et al. 1998; Zaninetti et al. 1999), which is also the major neurotransmitter involved (1) in the synaptic network within the facial and trigeminal nuclei or integrator centers (Lee et al. 2006) and (2) in neuromuscular junctions connected to the MER muscles. In the nervous system, Ach exocytosis is mainly activated by P/Q-type Ca^{2+} channels (Wright & Angus 1996; Day et al. 1997) and to a lesser extent by N-type Ca^{2+} channels (Hamilton & Smith 1992; Rossoni et al. 1994). In contrast, the L-type Ca^{2+} channels are mainly dedicated to muscular contraction (Catterall et al. 1988; Patterson et al. 1995). There is therefore a dominating role of P/Q- and N-type Ca^{2+} channels at the level of motoneurons and a dominating role of L-type Ca^{2+} channels in muscles.

If Tol can inhibit the MER, the cellular sites of its action were still not completely elucidated. Could Tol interact with VDCCs in motoneurons, integrator centers or muscles? To better understand the Tol action at the level of the MER arc, two specific VDCCs blockers were used in the present study:

- the ω-conotoxin MVIIC (ω-Ctx), which is the only pharmacological tool inhibiting both P/Q- and N-type Ca^{2+} channels (Hillyard et al. 1992; McDonough et al. 1996) expressed in neurons,
- the verapamil, which inhibits the L-type Ca^{2+} channels, which are mainly expressed in muscular fibers (Almers et al. 1985).

These blockers have been administered in the rat by intra-carotidal injections to study their effects on the CMP, which is a good electrophysiological tool to record (1) the electro-activity of the OHCs (Withnell 2001) and (2) the trigger of the MER (Dancer and Franke 1980; Campo et al. 2007). The aim of this investigation was to study the prevailing action of Tol on the different elements of the MER arc. In this purpose, the effects of VDCC antagonists were compared to those of Tol administrated in the same experimental conditions.

METHODS

Adult rats were used throughout this investigation. Anesthesia was induced by *i.p.* injection of ketamine (50 mg/kg). Then, a platinum electrode was inserted in the bulla and placed on the round window, whereas the ground electrode was placed over the olfactory bulb. This technique allows auditory-evoked potentials to be recorded from the cochlea. A circular custom-made catheter was fitted into the carotid connected to the operated ear for administrating the tested substances.

The acoustic stimulus was a 2.6-s burst emitted every 12 s, its spectrum was a narrow BN centered at 4 kHz emitted at 85 dB SPL. The CMP (RMS) was amplified 5000X and filtered from 2 to 8 kHz.

Three concentrations of Tol (58.4, 116.2 and 229.5 mM) were tested with different groups of rats (n = 5).

A dose-response study was carried out for the two VDCC blockers:

- ω-Conotoxin MVIIC (ω-Ctx) (CAS 147794238), a snail neurotoxin which blocks both P/Q- and N-type VDCCs. Three concentrations of ω-Ctx: 83.5, 145.2 and 211.4 µM, were tested with different groups of rats (n = 2).
- Verapamil (CAS 152114), a drug which blocks L-type VDCCs. The effects of three increasing concentrations of blocker: 312.5, 625 and 1250 µM, were evaluated in different groups of rats (n = 3).

RESULTS

In each figure, curve represents the data obtained with one animal representative of the group.

Toluene effects on CMP

Figure 1 displays the CMP obtained with 85-dB SPL noise-stimulated rats before, during and after a 100-µL injection of Tol at 58.4, 116.2 and 229.5 mM. The Tol injections of 116.2 and 229.5 mM caused rapid and transient rises in CMP amplitude called P component. The mean amplitudes obtained with 0, 58.4, 116.2 and 229.5 mM were 0.0 ± 0.4 dB, 0.4 ± 0.9 dB, 4.3 ± 1.5 dB and 4.1 ± 1.7 dB respectively. The lowest concentration, 116.2 mM, causing a significant ($K = 13.59$; $p = 0.004$) increase in CMP was chosen as reference concentration in this experimental context.

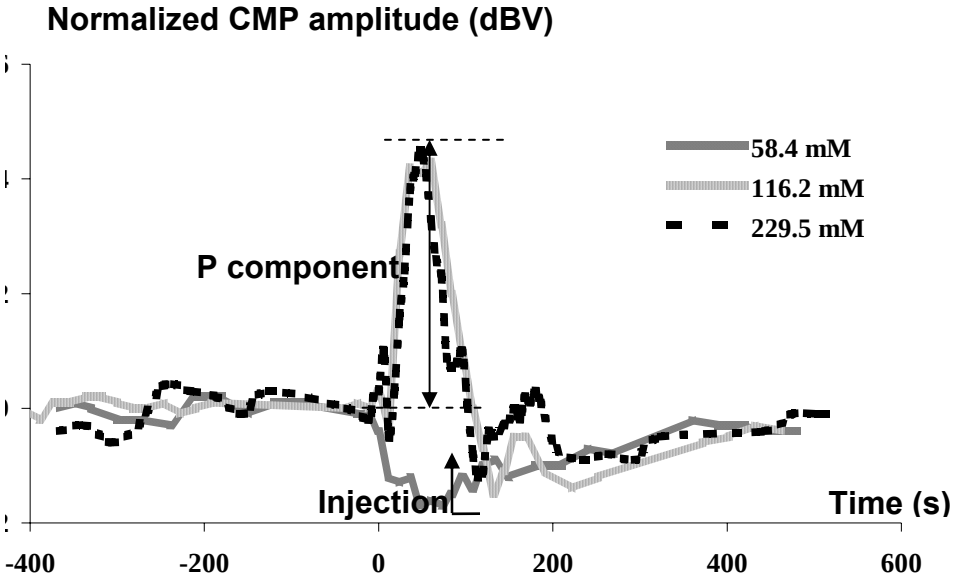

Figure 1: CMP (RMS) vs. Tol concentrations. 100-µl bolus of Tol were injected into the carotid. The acoustic stimulation was a 4 kHz-BN emitted at 85 dB SPL.

ω-Ctx effects on CMP

Figure 2 depicts the CMP obtained with 85-dB SPL noise-stimulated rats before, during and after a 100-µL injection of ω-Ctx at 83.5, 145.2 and 211.4 µM. Due to the expensive price along with a heavy administrative procedure to get the toxin from the supplier, we could afford only 2 experiments per concentration. Hopefully, the results were clear enough to draw conclusions. The injections of 145.2 and 211.4-µM of ω-Ctx provoked rapid and transient rises in CMP. The mean amplitudes obtained with 0, 83.5, 145.2 and 211.4 µM were 0.0 ± 0.4 dB, 0.3 ± 0.3 dB, 3.8 ± 0.8 dB and 7.3 ± 0.3 dB respectively. ω-Ctx-induced CMP rises were clearly concentration–dependent.

Verapamil effects on the CMP

Figure 3 illustrates the CMP obtained with 85-dB SPL noise-stimulated rats before, during and after a 100-µL injection of verapamil at 312.5, 625 and 1250 µM. The blocker induced rapid and transient CMP rises which the amplitude increases as a function of concentration. The mean amplitudes obtained with 0, 312.5, 625 and 1250 µM were 0.0 ± 0.4 dB, 1.4 ± 0.5 dB, 3.4 ± 0.7 dB and 4.1 ± 1.2 dB respectively. The verapamil effects on CMP were concentration-dependent (K=11.60; p=0.009).

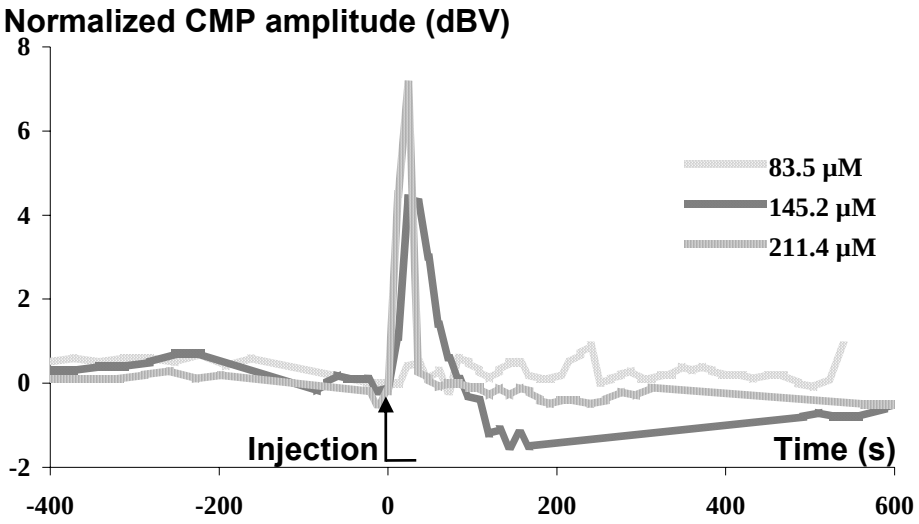

Figure 2: CMP (RMS) vs. ω-conotoxin concentration. 100-µl bolus of ω-Ctx were injected into the carotid. The acoustic stimulation was a 4 kHz-BN emitted at 85 dB SPL.

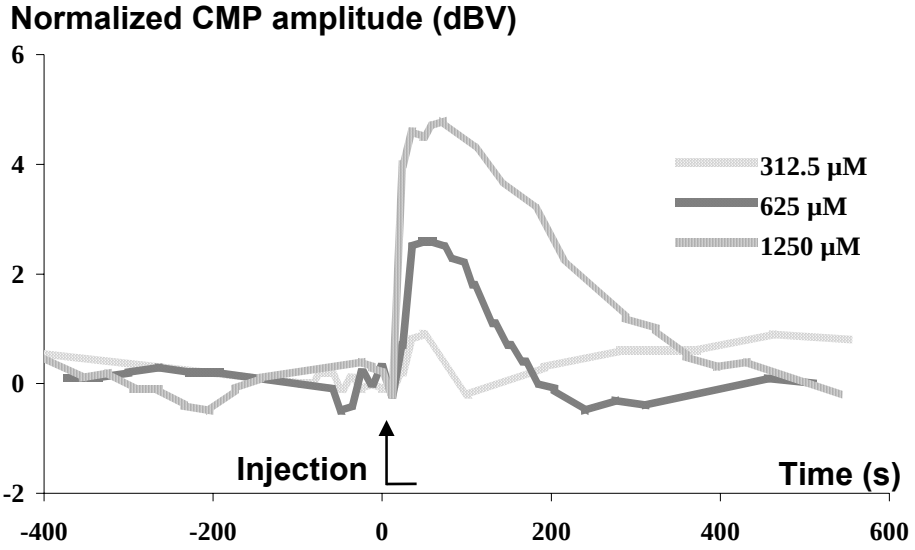

Figure 3: CMP (RMS) vs verapamil concentrations. 100-µL bolus of verapamil were injected into the carotid. The acoustic stimulation was a 4 kHz-BN emitted at 85 dB SPL.

Tol vs. VDCC blockers

Figure 4 allows the CMP obtained with blockers to be compared with that obtained with Tol. 145.2 µM of ω-Ctx and 1250 µM of verapamil were the required concentrations to induce a CMP change similar to that obtained with 116.2-mM of Tol (~4 dB). There were large differences of concentrations between the chemicals to induce the same amplitude of the P component: (Tol/ω-Ctx=800), (Tol/verapamil=93), (verapamil/ ω-Ctx=9). In the same way, there were also large differences of the area under curve (AUC) between the CMP obtained with Tol and blockers (Figures 1-3). These differences between AUC are well illustrated in Figure 4. For instance, the AUC ratios are (Tol/ω-Ctx=1.4), (Tol/verapamil=3.6), (verapamil/ω-Ctx=5.2). Therefore, verapamil had a long-lasting effect with respect to those of Tol and ω-Ctx.

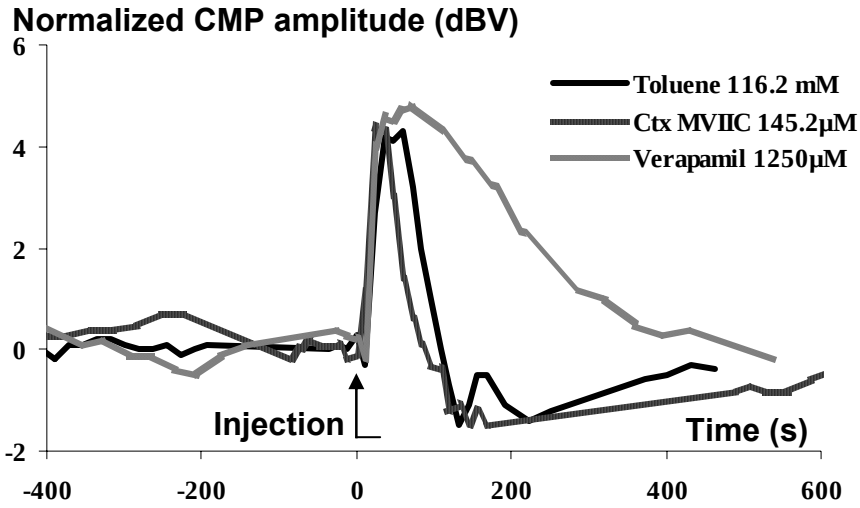

Figure 4: CMP (RMS) obtained with VDCC blockers and Tol. 100-µL bolus were injected into the carotid. The acoustic stimulation was a 4 kHz-BN emitted at 85 dB SPL.

CONCLUSIONS

Lataye et al. (2007) demonstrated in rats that Tol could induce a CMP rise by its anticholinergic-like effects and thereby confirmed Bale's results (Bale et al. 2002, 2005) obtained with *in vitro* preparations. Later on, Campo et al. (2007) demonstrated that this Tol-induced CMP rise corresponded to an inhibition to the MER. Unfortunately, the authors did not go further in their investigations and did not test others potential molecular targets for Tol. In the present *in vivo* study, ω-Ctx- and verapamil- induced CMPs were compared to that induced by Tol in order to (1) confirm the VDCCs as potential molecular targets, (2) localize the cellular sites perturbed by the solvent in the MER arc. As expected, the Tol dose-response study (Figure 1) showed a reversible MER inhibition from 116.2mM of Tol. Figures 2 and 3 show the reversible inhibition of the MER induced by neuronal and muscular VDCC blockers.

By comparing the curve patterns having the same amplitude (~4 dB), the ω-Ctx sensitivity was 9-fold higher with respect to that of verapamil (145.2, 1250 µM). The reversibility and the concentration-dependent responses were comparable with those previously obtained with Ach receptor antagonists. Figure 4 emphasizes the difference of concentration needed for Tol (116.2 mM) and for VDCC blockers (145.2-µM ω-Ctx; 1250-µm verapamil) to induce a 4-dB response. In our opinion, the concentration cannot be considered as a pertinent parameter because of the vehicle nature. Indeed, Tol needed a lipophilic vehicle (Intralipid) to be dissolved, but the efficiency of verapamil was deeply depressed by it (Tebbutt et al. 2006). Therefore, a saline solution was chosen as vehicle for both blockers. Because of this experimental bias, it seemed unrealistic to compare the concentrations to evaluate the relative affinity of Tol with regard to that of blockers. Indeed, the most striking drawback of Intralipid is that it can confine a part of the solvent, keeping the free-available part of the solvent low. Consequently, the Tol dose at the target structure is likely overestimated with respect to that inside the syringe. Actually, the most reliable approach was to compare the patterns of the responses having the same amplitude regardless of the difference of concentrations between solvent and blockers.

By comparing the patterns of the Tol-induced inhibition with those induced by 145.2-µM ω-Ctx and 1250-µM verapamil (Figure 4), it clearly appeared that the verapamil-

induced CMP lasted longer than those induced by Tol and ω-Ctx. Such a difference could be explained by the nature (muscular vs. neuronal) of the targets inhibited by the VDCC blockers. Since the verapamil inhibits mainly the muscular L-type VDCCs, it is likely that the duration required for reestablishing a normal muscular contraction was larger than that required for restoring a normal nervous conduction.

In case of a neuronal inhibition (Figure 2), the middle-ear muscles were forced in rest although functional. In fact, the nervous control of the middle-ear muscles was temporary interrupted but they kept the ability of contracting.

In case of verapamil injection (Figure 3), the L-type VDCCs were inhibited in the muscular T-tubules (invaginations) of the plasma membrane. T-tubules are the major sites for the coupling of excitation/contraction, which is the process whereby the spreading depolarization is converted into force production by muscle fibers. The L-type VDCCs are activated in response to nervous stimulation and this activation causes a mechanical interaction between L-type VDCCs and Ca^{2+}-release channels located on the adjacent sarcoplasmic reticulum membrane. This mechanical interaction is critical to trigger a proper skeletal muscle contraction (Tanabe et al. 1988; Nakai et al. 1998; Endo 2006). This all process lasted probably longer than the simple reestablishment of the nervous conduction.

Whatever the reasons on the origin of the different patterns recorded with both blockers, it appeared that Tol and ω-Ctx curves had a similar pattern (Figure 4). Therefore, Tol would act rather like a neuronal VDCC blocker, as suggested by Tillar et al. (2002) and Shafer et al. (2005) with *in vitro* experiments. In the present study, the *in vivo* findings confirmed the *in vitro* data and proved that VDCCs represent potential sensitive targets for Tol. In addition, since N-, P/Q-type channels constitute the major component of the Ca^{2+} channels expressed in the neuronal compartment of the MER arc (Plant et al. 1998; Hsiao et al. 2005), it seems therefore reasonable to claim that Tol can block the reflex by inhibiting the neuronal VDCCs at the level of its motoneurons and integrator centers. Inhibition of the transmitter receptors and associated Ca^{2+} channels would constitute the central mechanism responsible for the synergistic adverse effects on hearing of a co-exposure to noise and Tol: a higher acoustic energy penetrating into the cochlea would make the noise exposure more damaging.

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