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Original Article

Modulation of Smooth Muscle Relaxation by Short and Long Carbon Based Chemicals

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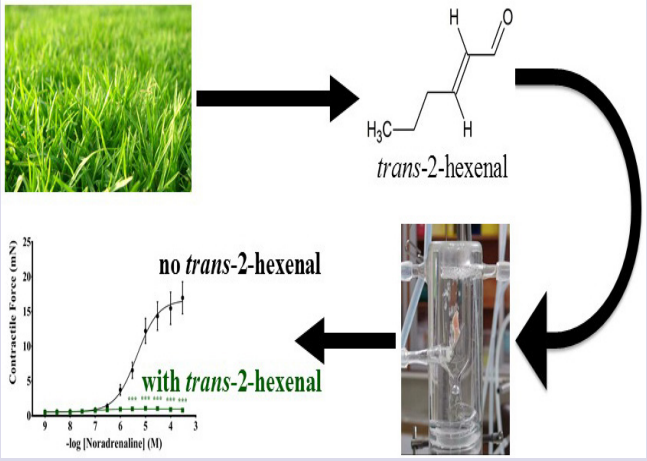
ABSTRACT

Introduction: Short and long-chained based carbon chemicals derived from plants are found in many household products and are widely used in complementary medicine. It has been suggested anecdotally that some of these chemicals have the ability to relax smooth muscle tissues, and to test this concept, this study examines the effects of 1,8-cineole, 1-heptanol, α-pinene, *cis*-3-hexen-1-ol and *trans*-2-hexenal on neurotransmission and contraction of mouse and rat vas deferens. **Methods:** Focal extracellular recordings from the surface of mouse vas deferens of electrically-evoked nerve terminal impulses (NTIs) and electrically-evoked excitatory junctional currents (EJCs) were examined in both the presence and the absence of each chemical. Likewise, noradrenaline-evoked contractile forces of rat vas deferens were examined in both the presence and the absence of each chemical. **Results:** Relative to the respective controls, 1,8-cineole, 1-heptanol and *trans*-2-hexenal each statistically significantly decreased both smooth muscle contractile forces and EJC amplitudes, with 1-heptanol and *trans*-2-hexenal also statistically significantly decreasing NTI amplitudes. On NTI and EJC amplitudes the effects of 1,8-cineole and 1-heptanol were reversible whereas the effects of *trans*-2-hexenal were irreversible. **Conclusion:** Our results demonstrate that 1,8-cineole and *trans*-2-hexenal decrease smooth muscle neurotransmission and contraction and thereby cause smooth muscle relaxation, thus suggesting that these chemicals may have clinical applications and health benefits.

Key words: 1,8-cineole, *cis*-3-hexen-1-ol, 1-heptanol, α–pinene, smooth muscle, relaxation, *trans*-2-hexenal.

SUMMARY

- 1,8-Cineole, 1-heptanol and *trans*-2-hexenal decrease nerve terminal impulse (NTI) amplitudes and smooth muscle contractile forces.
- 1,8-Cineole, 1-heptanol and *trans*-2-hexenal also decrease excitatory junction current (EJC) amplitudes.
- Reductions in NTIs and EJCs by 1-heptanol and 1,8-cineole were reversible, but reductions caused by *trans*-2-hexenal were irreversible.
- Minor structural differences between the chemicals can lead to significant changes in their effects on smooth muscle neurotransmission and contraction.



PICTORIAL ABSTRACT

Abbreviations used: ATP: Adenosine 5′-triphosphate, EJC: Excitatory junction current, NTI: Nerve terminal impulse.

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INTRODUCTION

Plant-derived chemicals are found in many household products and are widely used in complementary medicines for their fragrance and perceived soothing or calming effects.[1-3] Beyond these sensory and psychological benefits, the topical use of some plant-derived chemicals has been shown to cause smooth muscle relaxation.[4-7] The long-chained plant-derived carbon based chemicals 1,8-cineole (Figure 1a) and α-pinene (Figure 1b) and the short-chained plant-derived carbon based chemicals *cis*-3-hexen-1-ol (Figure 1c) and *trans*-2-hexenal (Figure 1d) may also have smooth muscle relaxing properties. Mechanisms by which organic chemicals may induce smooth muscle relaxation include impeding the propagation of NTIs, reducing EJCs or uncoupling the syncytium of smooth muscle cells.[8-13] 1-Heptanol (Figure 1e), for example, has been reported to reversibly decrease chemically and electrically-evoked smooth muscle contractions without affecting either NTI or EJC amplitudes under control conditions.[13-15]

In the present study, the concentration-dependent effects, reversibility and potential sites of action of 1,8-cineole, 1-heptanol, α-pinene, *cis*-3-hexen-1-ol and *trans*-2-hexenal on smooth muscle tissues were examined. This was done by measuring NTI and EJC amplitudes, and noradrenalin-evoked contractile forces of smooth muscle preparations in the absence and presence of these chemicals.

MATERIALS AND METHODS

Chemicals

1,8-Cineole (Cineole) (99% purity), 1-heptanol (≥ 99.9% purity), α-pinene (98% purity), *cis*-3-hexen-1-ol (98% purity), *trans*-2-hexenal (98% purity) and noradrenaline (L-(−)-Norepinephrine (+)-bitartrate salt monohydrate) (≥ 98% purity) were produced by Sigma-Aldrich Corp. (St Louis, Missouri, USA). The chemicals examined were each diluted in buffer to create buffer solutions containing the respective chemical at concentrations ranging from 3.77×10^{-5} M to 4.31×10^{-3} M. These solutions were mixed well prior to their application to tissues.

Animals

Inbred male C57BL/6J mice (*Mus musculus*, 6 weeks postnatal) were used for electrophysiological experimentation, and outbred male Wistar

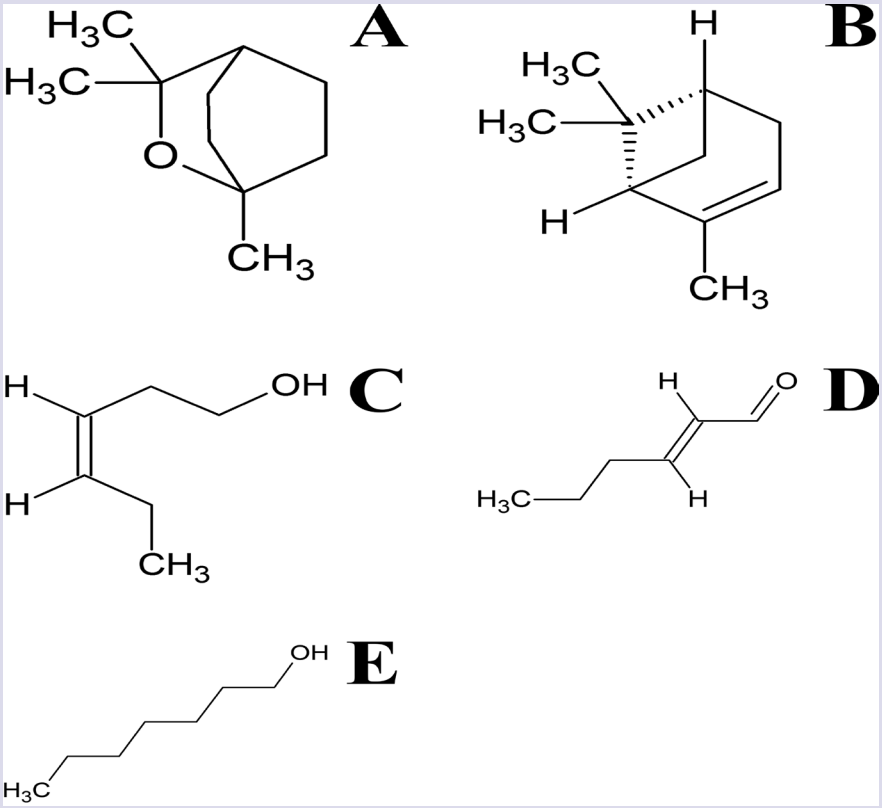

Figure 1: The chemical structures of 1,8-cineole (A), α-pinene (B), *cis*-3-hexen-1-ol (C), *trans*-2-hexenal (D) and 1-heptanol (E).

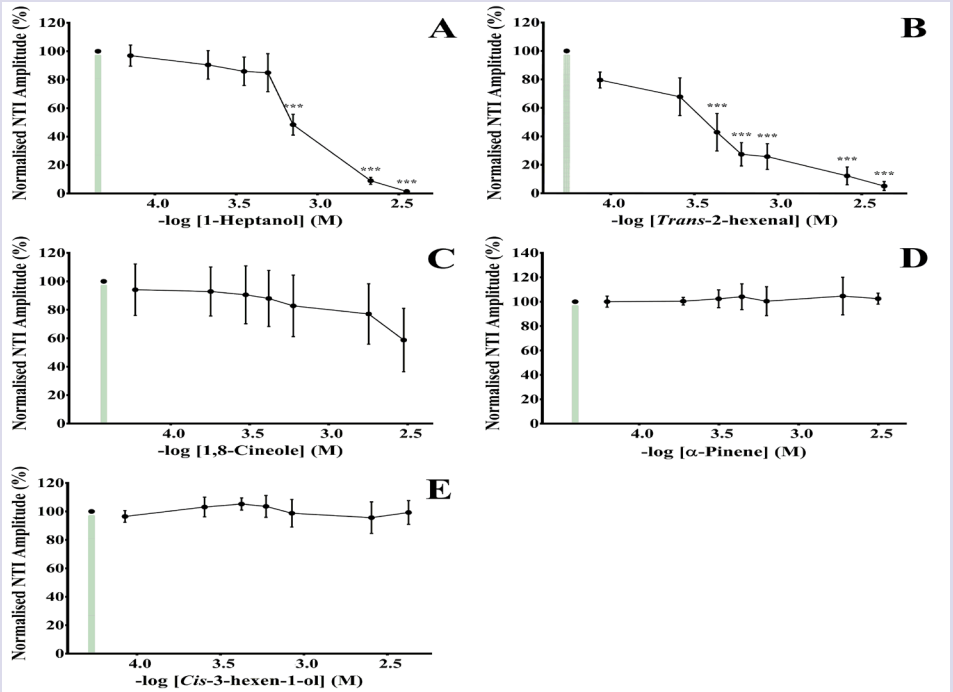

Figure 2: The effects of 1-heptanol (7.07×10^{-5} M - 3.54×10^{-3} M) (A), *trans*-2-hexenal (8.62×10^{-5} M - 4.31×10^{-3} M) (B), 1,8-cineole (5.97×10^{-5} M - 2.99×10^{-3} M) (C), α-pinene (6.30×10^{-5} M - 3.15×10^{-3} M) (D) and *cis*-3-hexen-1-ol (8.47×10-5 M - 4.23×10-3 M) (E) on NTI amplitudes of mouse vas deferens. All NTI amplitudes are normalised to control (100%) amplitudes (represented above bars).

Results are expressed as mean amplitudes ± SEM for six individual experiments. *** = $p<0.001$

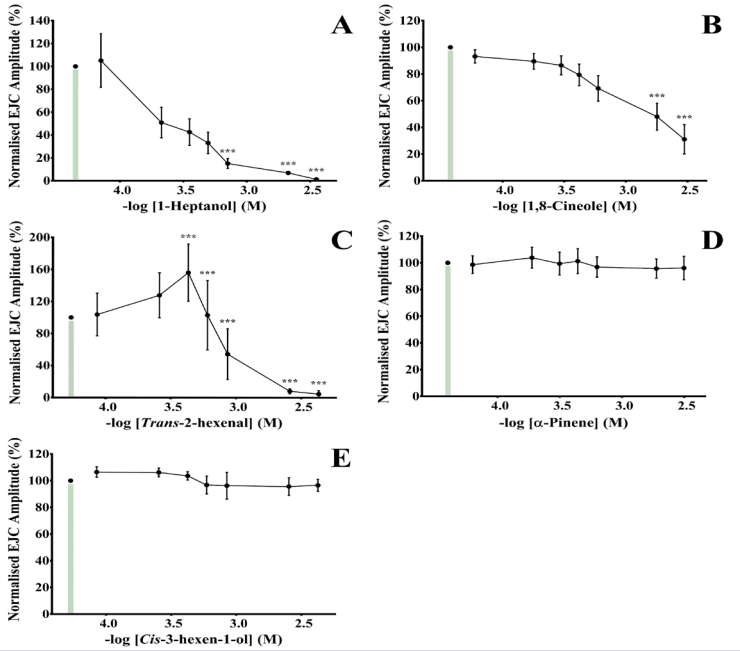

Figure 3: The effects of 1-heptanol (7.07×10^{-5} M - 3.54×10^{-3} M) (A), 1,8-cineole (5.97×10^{-5} M - 2.99×10^{-3} M) (B), *trans*-2-hexenal (8.62×10^{-5} M - 4.31×10^{-3} M) (C), α-pinene (6.30×10^{-5} M - 3.15×10^{-3} M) (D) and *cis*-3-hexen-1-ol (8.47×10^{-5} M - 4.23×10^{-3} M) (E) on EJC amplitudes of mouse vas deferens. All EJC amplitudes are normalised to control (100%) amplitudes (represented above bars).

Results are expressed as mean amplitudes ± SEM for six individual experiments.

*** = $p<0.001$.

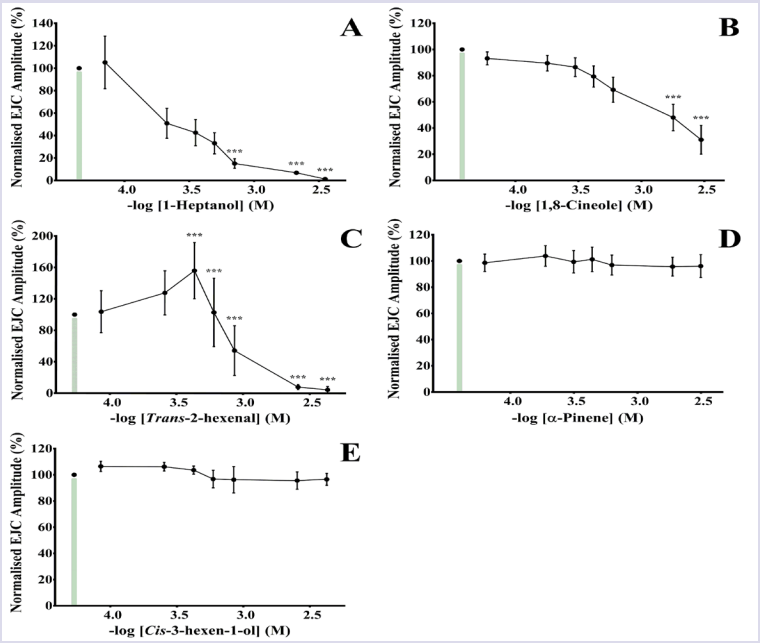

Figure 4: The effects of 1,8-cineole (1.79×10^{-3} M) (A), 1-heptanol (2.12×10^{-3} M) (B), *trans*-2-hexenal (2.59×10^{-3} M) (C), α-pinene (1.89×10^{-3} M) (D) and *cis*-3-hexen-1-ol (2.54×10^{-3} M) (E) on noradrenaline-evoked contractile forces of rat vas deferens. Circles symbolise contractile forces evoked in the absence of the chemicals (controls), while squares symbolise contractile forces evoked in the presence of the respective chemical.

Results are expressed as mean forces ± SEM for twelve (controls) or six (chemicals) individual experiments. *** = $p<0.001$

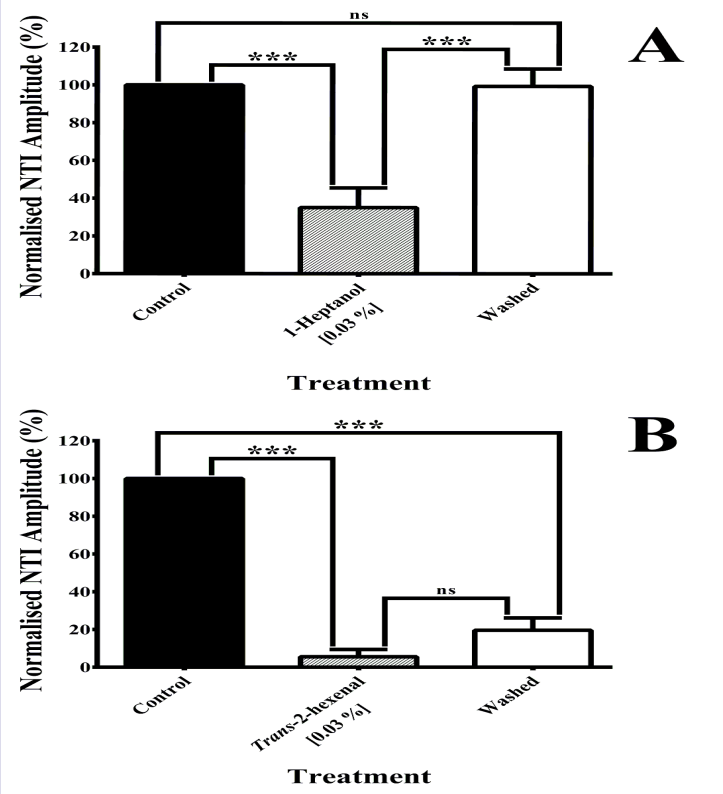

Figure 5: The reversibility of the effects of 1-heptanol (2.12×10^{-3} M) (A) and *trans*-2-hexenal (2.59×10^{-3} M) (B) on NTI amplitudes of mouse vas deferens. All NTI amplitudes are normalised to control (100%) amplitudes. NTI amplitudes were recorded in the absence (Control) and in the presence of 1-heptanol (A) or *trans*-2-hexenal (B), and then after thorough washing of the vas deferens with buffer solution (Washed). Results are expressed as mean amplitudes ± SEM for six individual experiments. *** = p<0.001, ns = not significant

rats (*Rattus norvegicus*, 11 weeks postnatal) were used for contractile experimentation. Rodents were sourced from the Central Animal Breeding House (Brisbane, Australia), and housed and randomly selected from the School of Chemistry and Molecular Biosciences Animal House (The University of Queensland, Brisbane, Australia). Rodents were housed with other rodents of the same species within rooms maintained on a 12 hour day/night cycle at room temperature, with food and water available *ad libitum*. Animal ethical clearance was granted by the Animal Ethics Committee of The University of Queensland (Australia).

Rodents were euthanised with CO_2 followed by cervical fracture. Both vas deferens were dissected out of the rodent, with adipose and connective tissue being removed. For contractile experimentation, the rat vasa deferentia were cut in half, with the epididymal halves discarded.

Electrophysiological and contractile experimentation were conducted as described previously.[7,16] For electrophysiological experimentation, 1,8-cineole (5.97×10^{-5} M to 2.99×10^{-3} M), α-pinene (6.30×10^{-5} M to 3.15×10^{-3} M), *cis*-3-hexen-1-ol (8.47×10^{-5} M to 4.23×10^{-3} M), *trans*-2-hexenal (8.62×10^{-5} M to 4.31×10^{-3} M) and 1-heptanol (7.07×10^{-5} M to 3.54×10^{-3} M) were examined. For contractile experimentation, noradrenaline (1×10^{-9} M to 3×10^{-4} M) was utilised to evoke contractions in the absence and presence of 1,8-cineole (1.79×10^{-3} M), α-pinene (1.89×10^{-3} M), cis-3-hexen-1-ol (2.54×10^{-3} M), *trans*-2-hexenal (2.59×10^{-3} M) and 1-heptanol (2.12×10^{-3} M).

Statistical Analyses

The effects of the examined chemicals on NTI and EJC amplitudes and noradrenaline-evoked contractile forces relative to the respective controls were determined using an unpaired two-tailed one-way analysis of variance with a Tukey's multiple comparison post-test.

RESULTS

NTI Amplitudes

The effects of 1,8-cineole, 1-heptanol, α-pinene, *cis*-3-hexen-1-ol and *trans*-2-hexenal on NTI amplitudes were examined. 1-Heptanol (7.07×10^{4} M to 3.54×10^{-3} M) and *trans*-2-hexenal (4.31×10^{-4} M to 4.31×10^{-3}M) caused statistically significant (P<0.05) concentration-dependent decreases of NTI amplitudes relative to the respective controls (Figures 2a and 2b). Conversely, 1,8-cineole, α-pinene and *cis*-3-hexen-1-ol did not statistically significantly (P>0.05) affect NTI amplitudes relative to the respective controls at any concentration examined (Figures 2c to 2e).

EJC Amplitudes

The effects of 1,8-cineole, 1-heptanol, α-pinene, *cis*-3-hexen-1-ol and *trans*-2-hexenal on EJC amplitudes were examined. 1,8-Cineole (1.79×10^{-3} M to 2.99×10^{-3}M), 1-heptanol (7.07×10^{-4} M to 3.54×10^{-3}M) and *trans*-2-hexenal (4.31×10^{-4} M to 4.31×10^{-3}M) caused statistically significant (P<0.05) concentration-dependent decreases of EJC ampli-

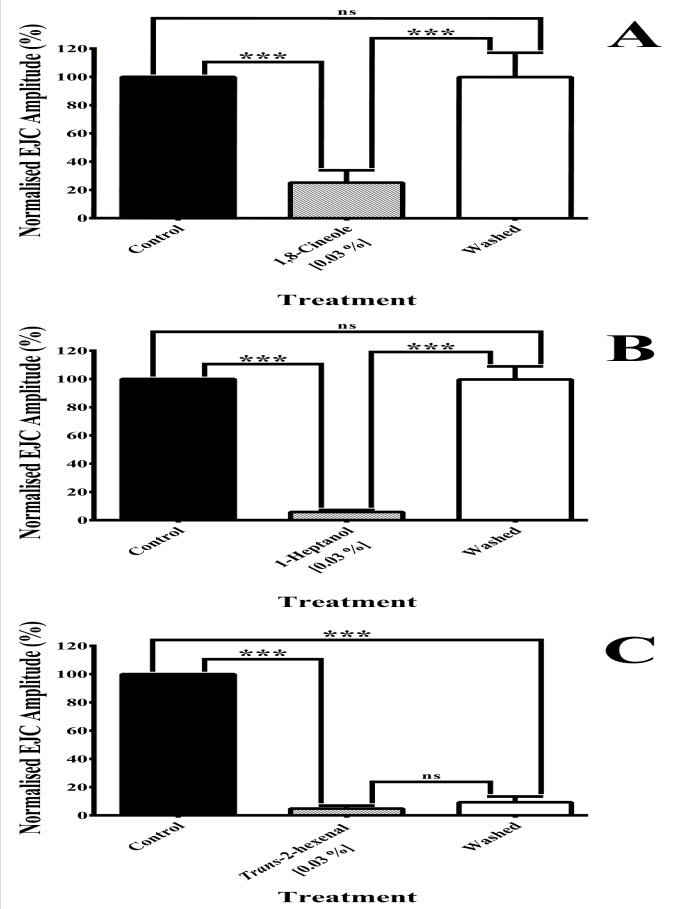

Figure 6: The reversibility of the effects of 1,8-cineole (1.79×10^{-3} M) (A), 1-heptanol (2.12×10^{-3} M) (B) and *trans*-2-hexenal (2.59×10^{-3} M) (C) on EJC amplitudes of mouse vas deferens. All EJC amplitudes are normalised to control (100%) amplitudes. EJC amplitudes were recorded in the absence (Control) and in the presence of 1,8-cineole (A), 1-heptanol (B) or *trans*-2-hexenal (C), and then after thorough washing of the vas deferens with buffer solution (Washed). Results are expressed as mean amplitudes ± SEM for six individual experiments. *** = p<0.001, ns = not significant.

tudes relative to the respective controls (Figures 3a to 3c). Conversely, α-pinene and *cis*-3-hexen-1-ol did not statistically significantly (P>0.05) affect EJC amplitudes relative to the respective controls at any concentration examined (Figures 3d and 3e).

Noradrenaline-evoked Contractile Forces

Increasing concentrations of noradrenaline caused concentration-dependent increases of contractile forces, resulting in an EC_{50} value of 4.53×10^{-6} M. The presence of 1,8-cineole (1.79×10^{-3} M), 1-heptanol (2.12×10^{-3} M) and *trans*-2-hexenal (2.59×10^{-3} M) caused statistically significant (P<0.05) decreases of noradrenaline-evoked contractile forces relative to the respective controls (Figures 4a to 4c). Conversely, α-pinene (1.89×10^{-3} M) and *cis*-3-hexen-1-ol (2.54×10^{-3} M) did not statistically significantly (P>0.05) affect noradrenaline-evoked contractile forces relative to the respective controls at any concentration examined (Figure 4d and 4e).

Reversibility of Chemical Effects

As the presence of 1-heptanol and *trans*-2-hexenal caused statistically significant decreases of NTI amplitudes relative to the respective controls, the reversibility of these effects was of interest, as such effects could be attributed to either chemical interactions or non-specific tissue damage. The statistically significant (P<0.05) inhibitory effects relative to the respective controls of 1-heptanol (2.12×10^{-3} M) on NTI amplitudes were statistically significantly (P<0.05) reversed by washing the vas deferens with chemical-free buffer (Figures 5a and 5b), whereas the statistically significant (P<0.05) inhibitory effects relative to the respective controls of *trans*-2-hexenal (2.59×10^{-3} M) on NTI amplitudes were not (P>0.05; Figure 5c).

Similarly, the statistically significant (P<0.05) inhibitory effects relative to the respective controls of 1-heptanol (2.12×10^{-3} M) and 1,8-cineole (2.54×10^{-3} M) on EJC amplitudes were statistically significantly (P<0.05) reversed by washing the vas deferens with chemical-free buffer (Figures 6a and 6b), whereas the statistically significant (P<0.05) inhibitory effects relative to the respective controls of *trans*-2-hexenal (2.59×10^{-3} M) on EJC amplitudes were not (P>0.05; Figure 6c).

DISCUSSION

This study has examined the effects of 1,8-cineole, 1-heptanol, α-pinene, *cis*-3-hexen-1-ol and *trans*-2-hexenal on smooth muscle neurotransmission and contraction and the reversibility of the effects of 1,8-cineole,

1-heptanol and *trans*-2-hexenal on smooth muscle neurotransmission. By examining the effects of these chemicals on smooth muscle we have been able to suggest sites and mechanisms of action of 1,8-cineole, 1-heptanol and *trans*-2-hexenal. We have also explored how the structure and molecular weight of chemicals may contribute to their effects on smooth muscle neurotransmission and contraction.

1-Heptanol and *trans*-2-hexenal caused statistically significant concentration-dependent decreases of NTI amplitudes relative to the respective controls. While not statistically significant, 1,8-cineole also caused concentration-dependent decreases of NTI amplitudes. 1,8-Cineole has been reported to cause statistically significant depressant effects upon compound action potential amplitudes.[17-19] Conversely, α-pinene and *cis*-3-hexen-1-ol did not statistically significantly affect NTI amplitudes relative to the respective controls. However, α-pinene (3×10^{-2} M) has been shown to abolish compound action potential amplitudes of frog sciatic nerves after 30 hours.[19]

NTI amplitudes are the depolarisations, or action potentials, caused by the opening of smooth muscle axonal voltage-gated sodium channels.[10] The opening of these axonal voltage-gated sodium channels mediates the propagation of action potentials through smooth muscle axons.[20] The most likely mechanism for the decreases of NTI amplitudes is by inhibition of axonal voltage-gated sodium channels. Lima-Accioly *et al.* (2006) suggested that 1,8-cineole caused concentration-dependent depressant effects on compound action potential amplitudes of rat sciatic nerves by affecting sodium channels.[17] Inhibition of axonal voltage-gated sodium channels by 1,8-cineole, 1-heptanol and *trans*-2-hexenal could be either a specific inhibition or a non-specific inhibition. Specific axonal voltage-gated sodium channel inhibitors are known to occur with compounds such as tetrodotoxin.[20] However, due to the widely contrasting differences in structure between 1,8-cineole, 1-heptanol and *trans*-2-hexenal, non-specific effects on axonal voltage-gated sodium channels appears more likely.

1,8-Cineole, 1-heptanol and *trans*-2-hexenal caused overall statistically significant concentration-dependent decreases of EJC amplitudes relative to the respective controls. EJCs are the postsynaptic local depolarisations that occur within smooth muscle cells when released adenosine 5'-triphosphate (ATP) from varicosities activates $P2X_1$ receptor ligand-gated cation channels localised within the cell membrane of these smooth muscle cells.[22-25] Conversely, α-pinene and *cis*-3-hexen-1-ol did not statistically significantly affect EJC amplitudes relative to the respective controls.

The decreases of EJC amplitudes caused by 1,8-cineole, 1-heptanol and *trans*-2-hexenal could be caused either by inhibition of $P2X_1$ receptor ligand-gated cation channels or by the decreases of NTI amplitudes. Inhibition of $P2X_1$ receptor ligand-gated cation channels could be either a specific inhibition or a non-specific inhibition. It is unlikely that these chemicals specifically inhibit $P2X_1$ receptor ligand-gated cation channels due to the differences in structure between 1,8-cineole, 1-heptanol and *trans*-2-hexenal and known specific $P2X_1$ receptor ligand-gated cation channel inhibitors, such as TNP-ATP.[26] However, the most likely mechanisms for the decreases of EJC amplitudes are a non-specific effect on axonal voltage-gated sodium channels or an effect on smooth muscle gap junctions. 1-Heptanol has been reported to effect smooth muscle gap junctions and thereby cause smooth muscle uncoupling.[13,27]

1,8-Cineole, 1-heptanol and *trans*-2-hexenal caused statistically significant decreases of noradrenaline-evoked contractile forces relative to the respective controls. Similarly, 1,8-cineole has been shown to cause concentration-dependent relaxation of phenylephrine pre-contracted rat aortae.[28] Furthermore, it has also been shown that the concentration-dependent relaxation of guinea pig tracheae with intact epithelia caused by 1,8-cineole is not affected by propranolol,[29] suggesting that 1,8-cineole does not cause relaxation via activation of β receptors. Conversely, α-pinene and *cis*-3-hexen-1-ol did not statistically significantly affect noradrenaline evoked contractile forces relative to the respective controls.

Noradrenaline evokes contractions of rat vas deferens primarily via the α_{1A} receptor, which is a G_q subfamily coupled receptor.[30-32] 1,8-Cineole, 1-heptanol and *trans*-2-hexenal most likely inhibit noradrenaline-evoked contractions of smooth muscle via inhibition of the G_q subfamily signalling pathway. It is unlikely that these chemicals inhibit noradrenaline evoked contractions of smooth muscle by acting as specific antagonists at the α_{1A} receptor due to the differences in structure between 1,8-cineole, 1-heptanol and *trans*-2-hexenal and known specific α_{1A} receptor antagonists, such as SNAP 5089.[33]

1,8-Cineole, 1-heptanol and *trans*-2-hexenal caused statistically significant decreases of EJC amplitudes relative to the respective controls. 1-Heptanol and *trans*-2-hexenal also caused statistically significant decreases of NTI amplitudes relative to the respective controls. However, after 1,8-cineole and 1-heptanol had been washed from the vas deferens there were no statistically significant decreases of EJC amplitudes, or NTI amplitudes for 1-heptanol, relative to the respective controls. Conversely, after *trans*-2-hexenal had been washed from the vas deferens there remained statistically significant decreases of NTI and EJC amplitudes relative to the respective controls. These results demonstrate that the decreases caused by 1,8-cineole and 1-heptanol are reversible whereas those caused by *trans*-2-hexenal are irreversible.

Due to differences in the structure and molecular weight of the chemicals examined within this study, it is possible to infer how the structure and molecular weight of chemicals may contribute to their effects on smooth muscle neurotransmission and contraction. *Trans*-2-hexenal ($C_6H_{10}O$) significantly affected smooth muscle neurotransmission and contraction relative to the respective controls, and its effects were irreversible, whereas *cis*-3-hexen-1-ol ($C_6H_{12}O$) did not significantly affect smooth muscle neurotransmission or contraction. The chemical structures of *trans*-2-hexenal ($C_6H_{10}O$) and *cis*-3-hexen-1-ol ($C_6H_{12}O$) are very similar with the only chemical structural differences being that *trans*-2-hexenal ($C_6H_{10}O$) possesses an additional double bond between carbons 1 and 2, and that the other double bond is between carbons 3 and 4 whereas it is between carbons 4 and 5 within *cis*-3-hexen-1-ol ($C_6H_{12}O$). These chemical structural differences result in very different effects on smooth muscle neurotransmission and contraction suggesting that minor differences between the structures of chemicals can result in significantly different effects of such chemicals on smooth muscle neurotransmission and contraction. However, these differences in the potency of a chemical do not appear to be due to the number of double bonds it possesses, as 1-heptanol ($C_7H_{16}O$) does not possess double bonds yet significantly affected smooth muscle neurotransmission and contraction (Figure 1E). In addition, the molecular weight of a chemical does not appear to correlate with its effects on smooth muscle neurotransmission and contraction. *Cis*-3-hexen-1-ol (100.16 g/mol) and α-pinene (136.23 g/mol) did not affect smooth muscle neurotransmission and contraction whereas *trans*-2-hexenal (98.14 g/mol), 1-heptanol (116.20 g/mol) and 1,8-cineole (154.25 g/mol) did. Furthermore, the molecular weight of a chemical does not appear to correlate with the reversibility of its effects, as the effects of *trans*-2-hexenal (98.14 g/mol) were irreversible (and the effects of linalool (154.25 g/mol) have previously been demonstrated to be irreversible),[7] whereas the effects of 1-heptanol (116.20 g/mol) and 1,8-cineole (154.25 g/mol) were reversible. It is possible that the number of double bonds a chemical possesses does correlate with the reversibility of its effects on smooth muscle neurotransmission and contraction, as *trans*-2-hexenal and linalool both possess two double bonds and their effects were irreversible,[7] whereas the effects of 1,8-cineole, which possesses one double bond, and 1-heptanol, which possesses no double bonds, were reversible.

Our results indicate that 1,8-cineole, 1-heptanol and *trans*-2-hexenal have non-specific effects on smooth muscle neurotransmission and

contraction. Our results also suggest that minor differences between the structures of chemicals can result in significantly different effects of such chemicals on smooth muscle neurotransmission and contraction. In addition, we have demonstrated that 1,8-cineole and *trans*-2-hexenal decrease smooth muscle neurotransmission and contraction, thereby causing smooth muscle relaxation, suggesting that these chemicals may have clinical applications for health benefits.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

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