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Effects of heptanol on electrical activity in the guinea-pig vas deferens

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1 The effects of the putative intercellular uncoupling agent 1-heptanol on electrical activity in the guinea-pig vas deferens were studied by use of intracellular and extracellular recording techniques.

2 At concentrations of 0.5, 1 and 2 mM, heptanol rapidly, monotonically and reversibly attenuated intracellularly recorded excitatory junction potential (e.j.p.) amplitude without affecting its time course, while spontaneous excitatory junction potentials (s.e.j.ps) were left unaffected.

3 Heptanol did not affect either the extracellularly recorded evoked excitatory junction current (e.j.c.), or the nerve terminal impulse that preceded it. These observations indicate that heptanol does not affect nerve impulse conduction, neurotransmitter release, or the postjunctional receptors involved in the production of the e.j.p.

4 E.j.ps appear to be suppressed by heptanol due to its intercellular uncoupling effects. Therefore, functional intercellular coupling may be necessary for the generation of the e.j.p. in smooth muscle.

Keywords: Excitatory junction potential; heptanol; vas deferens; smooth muscle; cell-to-cell communication

Introduction

Smooth muscle cells are electrically interconnected to one another to form a functional syncytium (Bennett, 1973; Bennett & Gibson, 1995; Tomita, 1975). In the smooth muscle cells of the vas deferens, transient depolarizations known as excitatory junction potentials (e.j.ps) are produced following stimulation of the innervating hypogastric nerve. E.j.ps are thought to reflect not just the depolarization of the cell being recorded from, but the summed activity of several cells in the neighbourhood, by virtue of intercellular electrical coupling (Tomita, 1967; Purves, 1976; Cunnane & Manchanda, 1990; Manchanda, 1995). The aliphatic alcohols heptanol and octanol have been shown to disrupt specifically intercellular electrical coupling at concentrations $\leqslant 2$ mM in a variety of cell types (Huizinga *et al*., 1988; Blennerhassett & Garfield, 1991; Lazrak & Peracchia, 1993; Bastide *et al*., 1995; Christ, 1995). Therefore these agents might be expected to affect junction potentials in smooth muscle, a question that has not yet been examined. We decided to use a combination of intracellular and focal extracellular recording to investigate the effects of heptanol on junction potentials in the guinea-pig vas deferens.

Methods

Male Hartley guinea-pigs (350–450 g) were stunned and exsanguinated, and the vasa deferentia were removed along with the innervating branch of the hypogastric nerve. The vas was pinned out on the silicone rubber (Reliance Silicones Limited, Mumbai, India) base of a Perspex organ bath in which the recordings were carried out. The tissue was continuously superfused with Krebs solution at 2–3 ml min^{-1} (composition in mM: NaCl 118.4, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, NaH_2PO_4 0.4 and glucose 11.1), bubbled with 95% O_2 and 5% CO_2 to maintain pH between 7.3 and 7.5. The solution was heated indirectly to 35–37°C. Solutions of 1-heptanol (S.D. Fine Chemicals, Mumbai, India) were made up by vigorous shaking with Krebs at the time of the experiment. Solutions were applied from reservoirs by gravity feed, and were changed by switching between reservoirs with a stopcock. Intracellular recordings of membrane potential change were obtained by use of glass microelectrodes filled with 3 M KCl (tip resistance 20–60 MΩ). Signals were led to an electrometer (IE-201, Warner Instrument Corp., Hamden, U.S.A.) and were low pass filtered (−3 dB cutoff at 1 kHz). Focal extracellular recordings were obtained by using microelectrodes with tips broken back to diameters of 50–100 μm and filled with normal Krebs (Brock & Cunnane, 1988; Cunnane & Manchanda, 1989; Cheung, 1990). The electrodes were applied to the surface of the vas with slight suction and the signals were led to an a.c. amplifier (−3dB high pass cutoff 0.1 Hz and low pass cutoff 2 kHz). The hypogastric nerve was stimulated 1–3 cm proximal to its point of entry at the prostatic end of the vas by Ag/AgCl ring electrodes with rectangular pulses of 2–10 V amplitude, 0.05 to 0.5 ms pulse duration at a frequency of 0.7 Hz. Signals were displayed on a storage oscilloscope (TDS 310, Tektronix Inc., Beaverton, U.S.A.). They were collected either directly on an IBM PC-AT compatible with an analogue to digital conversion card (PCL 209, Dynalog Microsystems, Mumbai, India) or stored on tape (DTR-1204, Bio-logic Science Instruments, Claix, France) for subsequent collection and analysis. Data collection and analysis on computer was done with the help of the Strathclyde Electrophysiology Software (Dempster, 1993) kindly supplied by Dr J. Dempster, Strathclyde University, Glasgow.

Results

In intracellular recordings, superfusion of heptanol solution (0.5, 1 and 2 mM) resulted in a rapid reduction of the amplitude of the fully facilitated e.j.p. of the guinea-pig vas deferens without a change in resting membrane potential. Following application of heptanol for 1.5–2 min, e.j.ps were profoundly suppressed in all the cells sampled ($n = 30$, in 5 preparations) without any noticeable change in time course. The effects of heptanol were most strikingly demonstrated in recordings from single cells where it was possible to maintain the insertion and observe e.j.ps before, during and after the application of heptanol (Figure 1). In one such cell, superfusion of 1 mM heptanol caused e.j.ps to decline to 50% of control amplitude in about 115 s after switching to the heptanol solution, and to be nearly abolished in about 300 s (Figure 1b). Resting membrane potential did not change, indicating that heptanol did not block e.j.ps by a non-specific effect on basal electrical

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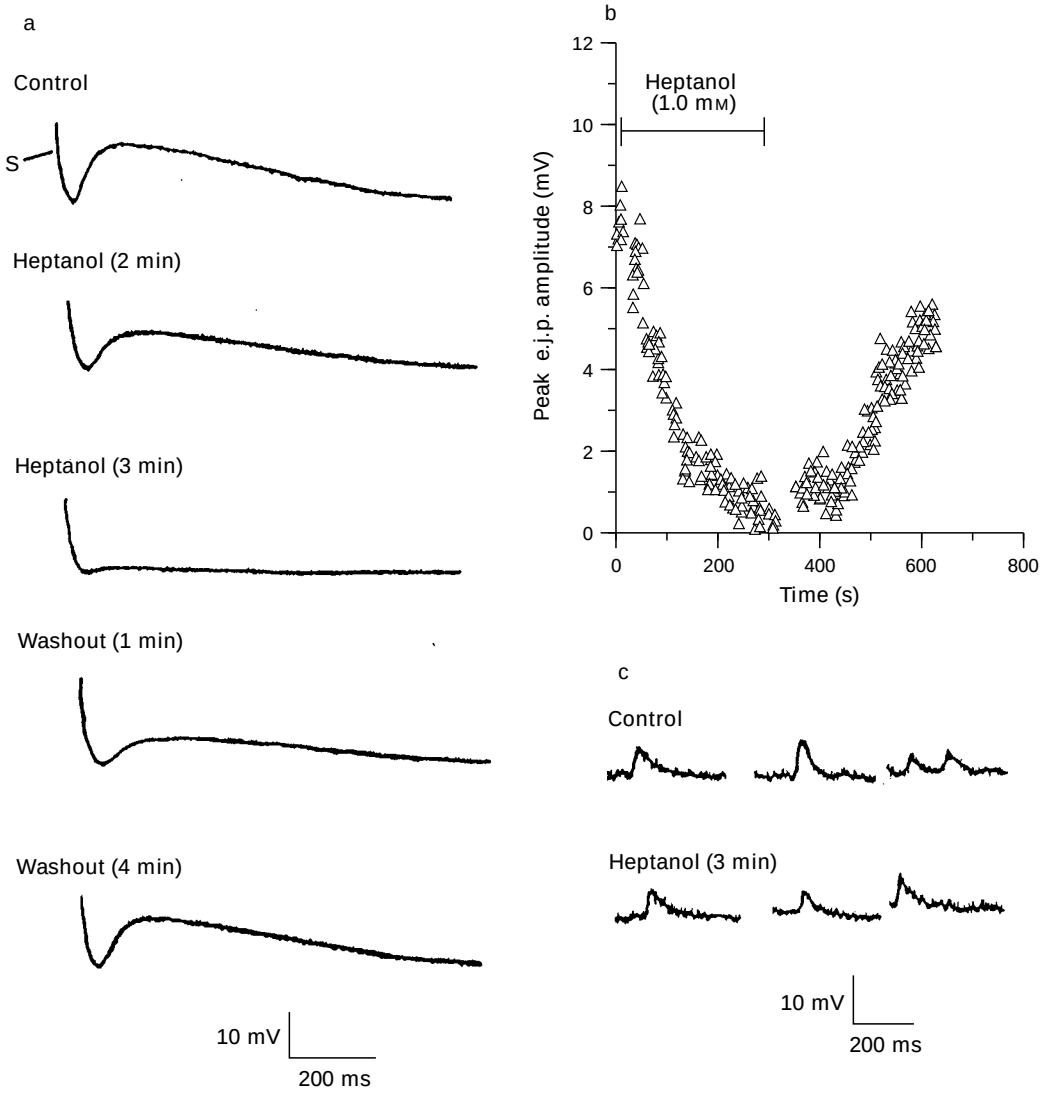

Figure 1 Effect of 1.0 mM heptanol on smooth muscle junction potentials (a) E.j.ps in a single cell before, during and after the application of heptanol. Each record is the average of 10 successive e.j.ps. The latency of the e.j.p. from the time of stimulation (~40 ms) is not apparent in these records because of the smearing of the stimulation artifact into the beginning of the depolarization. The vertical line (S) preceding the e.j.p. is the stimulus artifact. (b) Time course of effect of heptanol on peak amplitude of e.j.p. (same experiment as in a). Each open triangle represents the peak amplitude of an individual e.j.p. Note that heptanol solution was applied from a reservoir by gravity, and took about 30 s to gain uniform access to the tissue (estimation done by separate superfusion with dyes). Thus the effect of e.j.ps may be considerably more rapid than is apparent in this figure. (c) Lack of effect of heptanol on spontaneous e.j.ps (recorded in a cell different from a and b). The s.e.j.ps shown before and after the application of heptanol are from the same cell.

properties of the cell membrane. The suppression of e.j.ps was monotonic, and they were restored to 80% of control amplitude following reintroduction of normal Krebs solution for about 4 min (Figure 1a,b). In contrast to the effect on e.j.ps, application of heptanol for as long as 3 min did not detectably affect the generation of spontaneous e.j.ps even though e.j.ps had been fully inhibited (Figure 1c).

In order to explore the mechanism by which heptanol inhibited the e.j.p., focal extracellular recordings were made of the spontaneous and evoked electrical activity underlying the membrane potential change (Brock & Cunnane, 1988; Cunnane & Manchanda, 1989). In 10 trials, exposure to heptanol for up to 4 min (either 1 or 2 mM) did not significantly suppress or alter the shape of either the nerve impulse or the impulse-locked negative going excitatory junction currents (e.j.cs) elicited by hypogastric nerve stimulation (Figure 2a). Spontaneous excitatory junction currents (s.e.j.cs) were also left unaffected by heptanol (Figure 2b).

Discussion

Heptanol, a putative gap junction blocker, has been shown to block intercellular coupling in a variety of cell types at concentrations between 1 and 5.6 mM (Huizinga *et al*., 1988; Lazrak & Peracchia, 1993; Watts *et al*., 1994; Bastide *et al*., 1995). In smooth muscle, it seems to affect contractility by a specific effect on uncoupling at concentrations $\leqslant$2 mM (Christ, 1995). The syncytial properties of smooth muscle determine not only its contractile but also its electrical properties (Tomita, 1967; Purves, 1976; Bywater & Taylor, 1980; Cunnane & Manchanda, 1989; Huizinga *et al*., 1992). Our results showed that heptanol abolished e.j.ps in the vas deferens, presumably by disrupting cell-to-cell coupling. However, since e.j.ps were elicited by preganglionic stimulation of the hypogastric nerve, heptanol could have affected their generation by interference with processes that precede smooth muscle depolarization, namely, (i) conduction in the hypogastric nerve; (ii) ganglionic

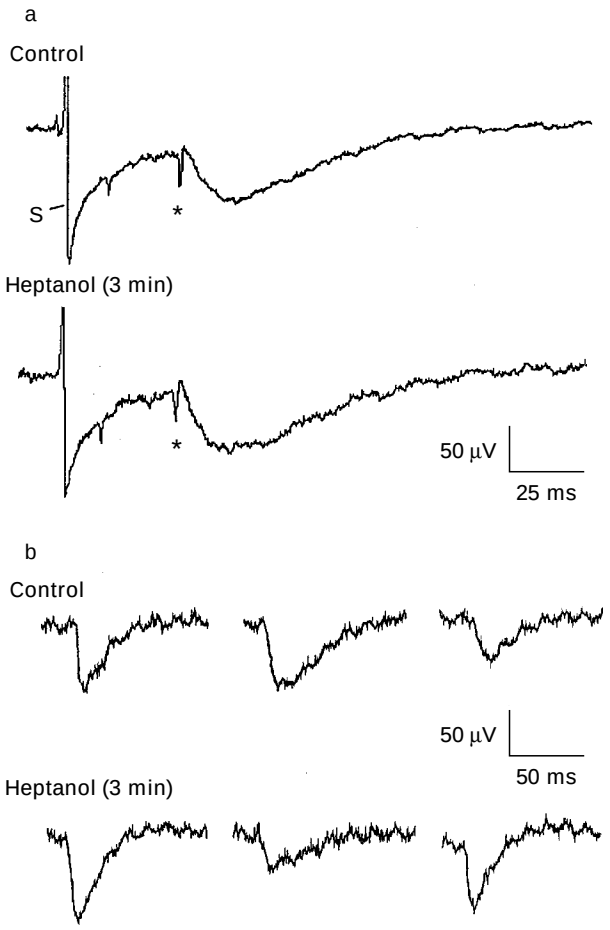

Figure 2 Focal extracellular potentials recorded from the surface of the vas deferens. (a) Shows that heptanol (1.0 mM) does not affect either the excitatory junction current (e.j.c.) or the nerve terminal impulse (*) preceding it. The control record is an average of 35 e.j.cs and the one in the presence of heptanol is an average of 20 e.j.cs. S, stimulus artifact. (b) Shows that heptanol does not alter spontaneous e.j.cs. Each record is an individual s.e.j.c. All the records in this figure are from the same extracellular attachment.

relay from hypogastric to vas deferens nerve; (iii) conduction in vas deferens nerve axons; (iv) neurotransmitter release; and (v) neurotransmitter action at postjunctional receptors. The first three possibilities can be dismissed because the extracellularly recorded nerve terminal impluse preceding the e.j.c. was not altereed by heptanol, indicating that heptanol did not affect conduction or transmission at any location proximal to the terminal axon.

The excitatory junction currents recorded extracellularly in smooth muscle are direct indications of neurotransmitter release and its action at the postjunctional receptors (Brock & Cunnane, 1988; Cunnane & Manchanda, 1989; 1990). In the present experiments, since the nerve impulse-locked e.j.cs were not blocked by heptanol, it may be concluded that the alkanol does not inferfere with evoked transmitter release at the concentrations used here. Finally, the observation that both s.e.j.cs and e.j.cs continued to be recorded in the presence of heptanol indicates that the postjunctional receptors mediating the e.j.p. of the vas deferens, probably P_{2x} purinoceptors (Sneddon *et al*., 1982; Sneddon & Westfall, 1984; Cunnane & Manchanda, 1988; Sneddon, 1992; Kennedy, 1993; Bültmann *et al*., 1994) were left unaffected by this agent. This is also supported by the persistence of s.e.j.ps following exposure to heptanol even while e.j.ps were fully inhibited.

Our observations indicate that heptanol probably does not abolish e.j.ps by mechanisms unrelated to its proposed action of uncoupling the smooth muscle cells (Christ, 1995), e.g. block of nerve action potential conduction, interference with neurotransmitter action or alteration of resting electrical properties of smooth muscle cells. Several other studies have also shown that the alkanols heptanol and octanol interfere specifically with intercellular coupling, mediated probably by gap junctions, leaving unaltered other membrane properties (Peracchia, 1991; Blennerhasset & Garfield, 1991; Bukauskas *et al*., 1992; Bastide *et al*., 1995; Christ, 1995). This suggests that the suppression of e.j.ps by heptanol may be due directly to the uncoupling of smooth muscle cells. The e.j.p. in the vas deferens and in blood vessels is thought to be composed of transmitter-activated depolarization produced not only in the cell being recorded from but also that produced in neighbouring cells, the latter being recorded by passive spread of depolarization to the site of recording (Tomita, 1967; Cunnane & Stjärne, 1984; Manchanda, 1995; Bennett & Gibson, 1995). It is possible therefore that heptanol may suppress the e.j.p. by preventing the passive propagation of depolarization between smooth muscle cells. This would imply further that functional intercellular coupling is necessary for the generation of the e.j.p. in smooth muscle. However, direct evidence for the uncoupling actions of heptanol in this preparation will have to be obtained in separate studies.

Our results also have implications on the inhibition of smooth muscle contractility by heptanol. This has been postulated to occur due to block of the spread of activating second messengers between cells resulting from cell-to-cell uncoupling (Christ, 1995). However, our observations indicate that heptanol may abolish contractile activity by effects on processes that precede excitation-contraction coupling, in particular, the generation of the e.j.p. At the sympathetic neuroeffector junction in smooth muscle, such as that of the vas deferens and arterial vessels, adenosine 5′-triphosphate (ATP) is thought to mediate the rapid part of the biphasic neurogenic contractile response, probably via the generation of the e.j.p. and, subsequently, smooth muscle action potentials. The second, slower part is believed to be mediated by noradrenaline (NA) by mechanisms independent of rapid depolarization (Cheung, 1982; Sneddon & Westfall, 1984; Burnstock, 1985; Cunnane & Manchanda, 1988; Bültmann *et al*., 1991; Sneddon, 1992; Morris, 1994; von Kügelgen & Starke, 1994). The suppression by heptanol of the intercellular spread of activating second messengers that mediate 'pharmacomechanical coupling' (Christ, 1995) would presumably interfere mainly with the noradrenaline-mediated contraction. In addition, we suggest that the suppression of the syncytial depolarization produced by ATP, i.e. the e.j.p., may also affect the purinergic component of contraction in the vas deferens and other sympathetically innervated smooth muscle organs. This should therefore be taken into account when the effects of uncoupling agents on contractions are being investigated.

In conclusion, our observations on the effects of heptanol on electrical activity in the guinea-pig vas deferens indicate that heptanol suppresses e.j.ps probably by blocking electrotonic spread of depolarization between smooth muscle cells, apparently leaving the conduction of the nerve action potential and neurotransmitter release and action unaffected.

The precise site and mode of action of heptanol remains to be confirmed and how a reduction in electrical coupling should result in a decrease of e.j.p. amplitude needs to be investigated, in the framework of existing electrical models of smooth muscle function (Bennett *et al*., 1993; Bennett & Gibson, 1995).

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