Effects of heptanol on the neurogenic and myogenic contractions of the guinea-pig *vas deferens*

¹K. Venkateswarlu, ¹Sumedha Y. Dange & ^{*,1}Rohit Manchanda

¹School of Biomedical Engineering, Indian Institute of Technology, Powai, Mumbai-400 076, India

1 The effects of the putative gap junction uncoupler, 1-heptanol, on the neurogenic and myogenic contractile responses of guinea-pig *vas deferens* were studied *in vitro*.

2 Superfusion of 2.0 mM heptanol for 20–30 min produced the following reversible changes in the biphasic neurogenic contractile response (8 trials): (i) suppression of both phases; (ii) delayed development of both the first as well as the second phase, accompanied by complete temporal separation of the two phases; (iii) prominent oscillations of force during the second (noradrenergic) phase only.

3 To eliminate prejunctional effects of heptanol, myogenic contractions were evoked by field stimulation of the *vas* in the presence of suramin (200 μ M) and prazosin (1 μ M). Heptanol (2.0 mM) abolished these contractions reversibly.

4 These results show that (i) heptanol inhibits both excitatory junction potential (EJP)-dependent and non EJP-dependent contractions of the *vas*; (ii) a postjunctional site of action of heptanol, probably intercellular uncoupling of smooth muscle cells, contributes to the inhibition of contraction.

Keywords: Neurogenic contraction; myogenic contraction; heptanol; *vas deferens*; smooth muscle; gap junctions

Abbreviations: ATP, adenosine 5'-triphosphate; EJP, excitatory junction potential; NA, noradrenaline; SEJP, spontaneous excitatory junction potential

Introduction

Smooth muscle cells form 'functional syncytia' (Tomita, 1975), in which biochemical or electrical changes originating in one cell are communicated to neighbouring cells through pathways of intercellular communication (Tomita, 1967; Christ *et al.*, 1996). These pathways, or 'gap junctions', are believed to be located at regions of close apposition of the surface membranes of neighbouring cells, in the form of intercellular ion channels connecting their interiors (Unwin & Zampighi, 1980; Loewenstein, 1981).

The contractile response of mammalian smooth muscle may depend upon the spread of excitation through gap junctions. In neurogenic contractions such as those evoked in the guinea-pig *vas deferens* by stimulation of the innervating hypogastric nerve, only a small proportion of cells are directly influenced by transmitter released from the sympathetic motor innervation, because of two reasons. (1) Only about a fifth of the cells receive direct innervation by close-contact axonal varicosities (Merrillees, 1968; Bennett, 1973); (2) Varicosities do not release transmitter in response to every invasion by the axonal action potential because of the low probability (≈ 0.01) of evoked transmitter release (Cunnane & Stjarne, 1984; Brock & Cunnane, 1988). Therefore, spread and co-ordination of excitation from the few directly activated cells to other cells probably requires the involvement of gap junctions.

Contraction of smooth muscle cells is triggered finally by an increase in concentration of cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$, Himpens & Somlyo, 1988). Activation leading up to elevation of $[\text{Ca}^{2+}]_i$ and the ensuing contraction in the guinea-pig *vas deferens* is of two kinds. The first is primarily electrical activation, mediated

by adenosine 5'-triphosphate (ATP) which is released as a co-transmitter from the sympathetic innervation along with the 'classical' sympathetic neurotransmitter noradrenaline (NA) (Burnstock, 1995; von Kugelgen & Starke, 1994). NA itself acts primarily *via* biochemical activation. Corresponding to its activation by the two transmitters, the contraction of the guinea-pig *vas* in response to tetanic (8–20 Hz) motor nerve stimulation is biphasic, consisting of a rapid twitch, mediated mainly by ATP and a slower tonic phase, mediated mainly by NA (Sneddon & Westfall, 1984; Kennedy *et al.*, 1996). The purinergic phase is triggered *via* P_{2x} purinoceptor-mediated excitatory junction potentials (EJPs) leading to regenerative muscle action potentials associated with Ca^{2+} influx (Blakeley *et al.*, 1981; Sneddon & Westfall, 1984; Sneddon, 1992; Driessen *et al.*, 1994), whereas the noradrenergic phase is mediated by elevation of $[\text{Ca}^{2+}]_i$ through the inositol phosphate (IP_3) second messenger pathway following α_1 adrenoceptor activation (Berridge, 1993).

The proposal that gap junctions modulate contractile activity has been explored, and strengthened, in vascular smooth muscle by the use of substances that appear selectively to block gap junctional communication, in particular the alkanol 1-heptanol (Christ, 1995; Christ *et al.*, 1996). These studies have concentrated on the effects of heptanol on contractions evoked by externally applied agonists. Neurogenic contractions, however, which may involve considerably different spatial patterns of directly excited and indirectly recruited cells, have not been similarly investigated.

The aim of the present investigation was to assess the contribution of cell-to-cell communication to the neurogenic contractile response of the guinea-pig *vas deferens*. It was found that both phases of the neurogenic contraction were reversibly suppressed by heptanol. To clarify the site of

*Author for correspondence at: University Department of Pharmacology, Mansfield Road, Oxford OX1 3QT.

heptanol action, we also explored its effects on myogenic contractions, and present evidence supporting a postjunctional mechanism of action of heptanol.

Methods

Contractile studies

Male guinea-pigs (350–450 g) were sacrificed by stunning and exsanguination. The abdomen was opened by a midline incision and the vasa on either side were exposed by pulling the testes out from the scrotal sacs. Both the vasa were dissected out along with the innervating hypogastric nerve. The isolated vas was suspended in a vertical organ bath of 25 ml capacity. The prostatic end was attached to an isometric force transducer using a silk suture. The epididymal end was anchored immovably to the bottom of the organ bath. The tissue was continuously superfused with carbogen (95% O₂, 5% CO₂) bubbled Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.7, MgCl₂ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, NaH₂PO₄ 0.4 and glucose 11.1. Experiments were conducted at room temperature (25–30°C).

The force transducer was connected to an amplifier (Polyrite, Biodevices, Chandigarh, India) whose output was directly displayed on a digital storage oscilloscope (1425, L&T Gould, Mumbai, India) and simultaneously stored on a digital tape recorder (DTR-1204, Biologic, Claix, France). The tissue was mounted under a resting tension of 0.1–0.5 g. The hypogastric nerve was stimulated *via* a pair of Ag/AgCl ring electrodes (separation about 3 mm), through which its proximal end was passed, using rectangular voltage pulses delivered by an electronic stimulator. A second pair of Ag/AgCl ring electrodes was placed surrounding the muscle, one electrode at the epididymal end and the other at the prostatic end, for direct stimulation of the muscle. Supramaximal tetanic nerve stimulation was carried out at 8 Hz, using a pulse amplitude of 10 V and pulse width 5 ms. A gap of 10 min was provided between consecutive tetani (~20 s duration each) to allow for recovery of the tissue. The muscle was stimulated selectively using pulses of amplitude 3 V and width 200 ms at 1 Hz, these parameters of stimulation being known to result in direct muscle stimulation (Holman *et al.*, 1977).

Electrophysiological studies

EJPs were recorded as described earlier (Manchanda & Venkateswarlu, 1997), using intracellular glass capillary microelectrodes with tip impedances of 40–60 M Ω interfaced with a high input impedance electrometer (IE 201, Warner Instruments, U.S.A.). The output of the amplifier was observed on an oscilloscope (TDS 310, Tektronix, U.S.A.) and stored simultaneously on the digital tape recorder.

Drugs

Salts used for the Krebs solution and 1-heptanol were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. Suramin sodium was a gift from Bayer plc, U.K. and prazosin HCl was a gift from Pfizer Ltd, Mumbai, India. Prazosin stock solution was prepared in N,N-dimethyl acetamide (LOBA Chemicals, Mumbai, India; see Brock & Cunnane, 1988) and suramin stock in distilled water. Experimental drug solutions were prepared by dilution of stock solutions in Krebs just before application. Solution of 1-heptanol were made by vigorous shaking with Krebs at the time of application.

Data collection and analysis

Signals played back on the tape recorder were amplified as required and digitized using an analogue to digital conversion card (PCL 209, Dynalog Microsystems, Mumbai, India) interfaced with an IBM compatible PC-AT 80486 using SCAN (part of Strathclyde Electrophysiology Software kindly supplied by Dr J. Dempster, Strathclyde University, Glasgow, U.K.).

All data are expressed as mean $\pm$ s.e. mean number of observations). Student's unpaired *t*-test was used to assess the statistical significance of the difference between means, a *P* value of <0.05 being taken to indicate a statistically significant difference.

Results

Effects of heptanol on neurogenic contractions

The contractile response of the guinea-pig *vas deferens* to tetanic stimulation of the hypogastric nerve was biphasic, as reported earlier (Sneddon & Westfall, 1984; Kennedy *et al.*, 1996) with the rising phase of the slow phase overlapping the decay of the fast phase (Figure 1). The response had the expected pharmacological profile, the fast phase being abolished selectively by the P_{2x} purinoceptor antagonist suramin (200 μ M; Sneddon, 1992), the slow phase selectively by the α_1 adrenoceptor antagonist prazosin (1 μ M) and the entire response by a combination of prazosin and suramin (see Figure 4A).

Suppression of force

The effects of heptanol on contractile force were investigated in most trials at a concentration of 2.0 mM. Effects at lower (0.5, 1.0 mM) or higher (4.0 mM) concentrations were qualitatively similar, though rates of onset were variable. 2.0 mM seems to be the optimal concentration of heptanol for the reversible abolition of EJPs in the organ under investigation, the guinea-pig *vas deferens* (see Discussion), hence this concentration was used so that effects on contractile and electrical activity could be compared.

In general, superfusion of 2.0 mM heptanol solution for about 20 min gradually suppressed both phases of the neurogenic contraction (Figure 1). In the majority of trials (seven out of nine), both phases were inhibited similarly. The peak force of the first phase was suppressed without change of shape by $83 \pm 7\%$ ($n=7$) on 20 min exposure to heptanol. Inhibition of the second phase was more complex, as it developed irregular fluctuations during its suppression, as described in greater detail below.

Figure 1A shows results of a trial in which the inhibition was complete for both phases at 20 min (Figure 1A, c). The effect was completely reversed on washout with normal Krebs for about 20 min (Figure 1A, e). In the other two trials the purinergic phase was partly resistant to the action of heptanol, its force being suppressed by only 50% following 20 min of heptanol exposure, whereas the adrenergic phase was completely abolished during this interval (see Figure 1B, c).

Increase in latency of force and separation of the phases

In control contractions the latency between the start of stimulation and the onset of the purinergic phase was 0.83 ± 0.11 s ($n=9$). Exposure to 2.0 mM heptanol for 20 min

Figure 1 Effect of 2.0 mM heptanol on neurogenic contractions of the guinea-pig *vas deferens* in two tissues. (A) Tissue showing complete suppression of two phases and (B) showing only partial suppression of purinergic phase a: Control; b: after 10 min exposure to heptanol; c: after 20 min exposure to heptanol; d,e: 10 and 20 min respectively after start of heptanol washout. Note the separation of two phases and oscillations in second phase in presence of heptanol. The thick line at the bottom indicates the period of hypogastric nerve stimulation (10-V, 5-ms pulses at 8 Hz).

increased the latency of the first phase significantly ($P < 0.05$) to 1.58 ± 0.30 s ($n = 7$). The change in latency is displayed in the superimposed and expanded traces of Figure 2A and B, which show responses recorded during the progressive inhibition of contractions by heptanol, before their complete abolition.

The onset of the noradrenergic phase was more strikingly delayed by heptanol (Figure 2A). The latency of the second phase in control contractions is difficult to determine with certainty because its onset is obscured by the preceding purinergic phase. However, the latency of the second phase can reasonably be assumed to be similar to that of the first since both ATP and NA, the two neurotransmitters mediating the phases, are known to be released simultaneously from the innervation following stimulation, though the noradrenergic phase is slower to develop (von Kugelgen & Starke, 1994; von

Kugelgen *et al.*, 1994; Stjarne & Stjarne, 1995). On the basis of this assumption the latency of the second phase was increased from 0.83 ± 0.11 s in controls to 14.93 ± 1.23 s ($n = 7$) following 20 min exposure to heptanol (Figure 2A).

Since the increase of latency of the second phase was much greater than that of the first phase, the induced suppression of contractile force was accompanied by a progressive temporal separation of the two phases. The extent of separation at 20 min exposure to heptanol was such that there was no longer any overlap between the phases (Figure 2A).

Since the first phase of contraction is EJP-dependent, the increase in latency of this phase suggests that EJPs inhibited by heptanol (Manchanda & Venkateswarlu, 1997) take longer to reach threshold for action potential generation. To test this, we recorded EJPs in the presence of heptanol at 8 Hz, the same frequency of stimulation as that employed in the contractile

studies. In control conditions, EJPs at ≥ 5 Hz readily summate to reach action potential threshold and produce contractions (Blakeley *et al.*, 1981). In the presence of heptanol, the development of threshold depolarization was considerably delayed, and in one trial EJPs at 8 Hz did not reach threshold in 8 s of stimulation (Figure 2C). When the frequency of stimulation was increased gradually beyond 8 Hz to about 70 Hz, an action potential and contraction were finally generated (Figure 2D). Threshold for regenerative excitation is achieved at higher frequencies of stimulation since a larger degree of summation results in a larger mean depolarization.

Figure 2 (A,B) Comparison of control neurogenic response with that in the presence of heptanol. Note the increase in latency, separation of the two phases and oscillations in the second phase induced by heptanol. Stimulation carried out at 8 Hz (10 V, 5 ms pulses). (B) Upper and lower traces of A expanded to show increase of latency of the first phase induced by heptanol. (C) Intracellularly recorded transmembrane potential in response to hypogastric nerve stimulation at 8 Hz (10 V, 0.1 ms pulses) in the presence of 2.0 mM heptanol. Note the slow development of depolarization and failure to reach threshold for action potential generation even after about 50 stimuli. (D) Transmembrane potential in response to hypogastric nerve stimulation in the presence of 2.0 mM heptanol at various frequencies from 0.7 to 70 Hz, indicated by arrows. At 70 Hz, the electrode was expelled, possibly due to contraction of the tissue. Spiky transients in C and D are stimulus artifacts.

Correspondingly in contractile studies, the heptanol inhibited neurogenic contraction, too, was partially restored by increasing the stimulus frequency to 70 Hz (not shown).

Oscillations in the noradrenergic phase

In addition to the remarkable increase of latency in the presence of heptanol, the second phase also developed, during its suppression, prominent, irregular oscillations in force, being split into an uncoordinated series of alternate contractions and relaxations. Examples of these oscillations are evident in Figures 1A, b,d and 2A. The extent of variation of force during these oscillations could be as much as $\sim 50\%$ of the peak force developed (Figure 1A, b), or just enough to cause a significant ripple during the second phase (e.g. in Figure 1B, d). The effect was specific to heptanol action, since control contractions either before or after application of heptanol were seen not to possess such pronounced fluctuations, though a slight, but regular ripple could occasionally be observed (Figures 1A, a,e and 2A).

A possible interpretation of the oscillations observed in the presence of heptanol is that they are the result of muscle action potentials triggered intermittently by residual purinergic EJPs which may not have been inhibited completely by heptanol. To confirm the nature of the transmitter responsible for the delayed response in the presence of heptanol, the *vas* was superfused with suramin (200 μ M) before the application of heptanol. Suramin inhibited powerfully the first phase of contraction and a monophasic contraction remained, corresponding to the second, noradrenergic phase of the normal biphasic neurogenic contraction (Figure 3). These suramin-resistant contractions were affected by heptanol in the same way as the second (noradrenergic) phase of the normal neurogenic contraction, i.e. in suppression of force, induction of oscillations in force during suppression, and marked increase of latency of the same order (Figure 3). Thus these oscillations are likely to be due to effects exerted specifically on noradrenergic mechanisms by heptanol.

Effect of heptanol on myogenic contractions

Suppression of neurogenic contractions by heptanol can conceivably be explained on the basis of purely neuroinhibitory effects of this agent. To determine whether heptanol indeed affects muscle function independent of neuronal elements, we investigated its effect on myogenic contractions,

Figure 3 Persistence of heptanol-induced contractile oscillations in the second phase in the presence of suramin (200 μ M). Exposure to suramin for 40 min inhibits profoundly the first (purinergic) phase of the neurogenic contraction, while leaving force developed in the second phase almost unaffected (middle trace). Vertical arrow denotes start of stimulation. Stimulation at 8 Hz (10 V, 5 ms pulses).

which originate in the muscle without neuronal contribution. Neurogenic contractions were first eliminated by applying a combination of the P_{2x} purinoceptor antagonist suramin ($200 \mu\text{M}$) together with α_1 adrenoceptor antagonist prazosin ($1 \mu\text{M}$). Figure 4A shows that this combination completely abolished neurogenic transmitter-mediated contractions.

To elicit myogenic contractions, stimulation was switched to the muscle stimulation electrodes using appropriate pulse

parameters (see Methods), which elicited contractions of the type illustrated in Figure 4B, a. These contractions, since they arise despite the presence of suramin and prazosin, are therefore likely to be of purely myogenic origin. To confirm this, the same pair of electrodes was used to deliver stimulation selective for nerve stimulation (10 V, 5-ms pulses at 8 Hz). Figure 4B, b shows that in the presence of the antagonists, this stimulation failed to generate any detectable force, indicating that neurogenic contributions were entirely eliminated. The latency of the myogenic response (0.32 ± 0.02 s, $n=6$) was about 60% less than that of the neurogenic response (0.83 ± 0.11 s, $n=9$) (see Figure 4B, a). This is expected from the elimination of neuronal conduction, synaptic delay and time taken for the summation of EJPs to reach threshold for generation of muscle action potential.

Following the application of 2.0 mM heptanol for 20–30 min, peak myogenic force was almost entirely suppressed in all trials, by $98 \pm 1\%$ ($n=5$) (Figure 5A–C). The reduction of force observed when using the long duration pulses required for myogenic contraction could possibly occur as a result of electrode polarization. However, the suppression of force persisted when stimulation was delivered after reversing electrode polarity (Figure 5D). This indicates that the suppression of the myogenic force was unrelated to electrode polarization and was indeed a heptanol mediated effect. The suppression was reversible on washout of heptanol (Figure 5E,F).

Discussion

The role of gap junctional communication in the generation and maintenance of smooth muscle contraction has received strong support in previous studies from the effects of heptanol on the contractions (Christ *et al.*, 1993; Christ, 1995). Heptanol at concentrations between 1.0 and 5.6 mM has been shown to selectively block gap junctions in various tissues including smooth muscle (Christ *et al.*, 1991; Bastide *et al.*, 1995; Blennerhassett & Garfield, 1991; Bukauskas *et al.*, 1992; Peracchia, 1991; Christ, 1995), and to inhibit agonist evoked contractions in smooth muscle such as rat aorta and corpus cavernosum (Christ *et al.*, 1991; 1993). However, neurogenic contractions have not yet been similarly investigated. In the present inquiry, heptanol has been used at a concentration of 2.0 mM. This concentration falls within the range mentioned above for specific action on gap junctional communication. Furthermore, in electrical recordings, heptanol at concentrations of 2.0 mM completely and reversibly abolished the EJP of the *vas deferens* (Manchanda & Venkateswarlu, 1997). Reversible suppression of EJPs was observed at concentrations greater than 2.0 mM (up to 10 mM) as well, while at concentrations below 2.0 mM there was a dose-dependent suppression, but not abolition, of the EJP (Manchanda & Venkateswarlu, 1997; unpublished observations). Since 2.0 mM was the lowest concentration required for complete, reversible abolition of the EJP in the absence of non-specific actions (Manchanda & Venkateswarlu, 1997), we used this concentration in these studies.

The results presented here show that 1-heptanol inhibits, profoundly and reversibly, the neurogenic contractions of the guinea-pig *vas deferens*. These findings may, therefore, throw additional light on the significance of intercellular coupling in the modulation of smooth muscle tone *in vivo*, to which neuronal input makes significant contributions. However the interpretation of the effects of heptanol on neurogenic contractions is complicated by the contribution to these

Figure 4 Generation of myogenic contractions of the guinea-pig *vas deferens*. (A) Suppression of neurogenic force, generated by hypogastric nerve stimulation at 8 Hz in the presence of suramin and prazosin (S+P). a: Control; b,c: 40 and 70 min respectively after the application of a mixture of S ($200 \mu\text{M}$) and P ($1 \mu\text{M}$). The calibration bar applies to all the traces. (B) a: Myogenic force generated by direct muscle stimulation after abolition of the neurogenic force by S+P, using 3-V, 200-ms pulses at 1 Hz with field stimulation electrodes. b: Absence of force generation in the presence of S+P by stimulation with 10-V, 5-ms pulses at 8 Hz (suitable for intramural nerve stimulation) with the field stimulation electrodes. Duration of stimulation indicated by thick line (common for (A)). The vertical arrow shows that traces (A)b,c and (B)a,b were all obtained in the presence of S+P.

contractions by prejunctional processes. This raises the possibility that the observed effects of heptanol arise from interference with neuronal mechanisms rather than with postjunctional cell-to-cell coupling.

Postjunctional site of action of heptanol

Previous work has already shown that the site of action of heptanol is probably located postjunctionally. For instance, Christ *et al.* (1993, 1996) have shown from various lines of evidence that heptanol blocks gap junctions, including its inhibition of α_1 -agonist induced contractions coupled with the

lack of effect on KCl-induced contractions in vascular smooth muscle. Furthermore, presynaptic effects on neuronal conduction have been ruled out by the observation that the *vas deferens* nerve action potential elicited by preganglionic stimulation remains unaffected in the presence of heptanol (Manchanda & Venkateswarlu, 1997).

Perhaps the most direct confirmation that heptanol affects selectively postjunctional mechanisms in the *vas deferens* stems from our observation that it abolishes the myogenic contractions, which do not include neuronal contribution. To place confidence in these results it is essential to be confident that the method used here for direct muscle stimulation excites only postjunctional smooth muscle, without involving prejunctional elements. This has been ensured in the present work by the choice of stimulation parameters for muscle excitation (Geddes & Baker, 1975; Holman *et al.*, 1977), and the concomitant use of suramin and prazosin at concentrations that completely abolished the neurogenic response (Figure 4).

The suppression of myogenic contractions by heptanol indicates strongly that this agent has a postjunctional, post-receptor site of action in the guinea-pig *vas deferens*. Such a site of action could be located in any of the processes leading from receptor activation to contraction, including receptor-mediated second messenger generation, activation of the contractile machinery by cytosolic Ca^{2+} (Somlyo & Somlyo, 1960; Somlyo *et al.*, 1985), and spread and co-ordination of contractile activity in smooth muscle through intercellular pathways. A large body of evidence gathered in studies on several smooth muscle organs indicates, however, that the mechanism lies probably in the specific inhibition of cell-to-cell coupling by this agent (Christ *et al.*, 1991; 1993; 1996).

Such an action would result in inhibition of contractions as follows. Electrically evoked smooth muscle contraction results from influx of Ca^{2+} into the cells through voltage gated calcium channels (Somlyo & Somlyo, 1960; Mekata, 1981). In the present experiments, action potentials during muscle stimulation would be directly evoked in only a narrow band of cells directly encircled by the cathode. A much larger number of cells is presumably recruited for contraction by the spread of the action potentials, or of elevated cytosolic $[\text{Ca}^{2+}]$, through gap junctions. If heptanol blocks gap junctional communication, the recruitment of cells for contraction would be reduced, resulting in inhibition of the myogenic response.

In view of the arguments presented above, we restrict attention below to blockade of gap junctional communication by heptanol as the mechanism underlying its effects on neurogenic contractions.

Inhibition of neurogenic contractions

The purinergic phase of contraction in the *vas deferens* depends upon the generation of regenerative action potentials in the smooth muscle cells, which in turn are triggered by ATP-mediated EJPs (Blakeley *et al.*, 1981). The sparseness of direct innervation (Merrillees, 1968; Bennett, 1973) and the low probability of release from the axonal varicosities (Cunnane & Stjärne, 1984) of the guinea-pig *vas deferens* suggest that only a small proportion of cells are directly acted upon by neurotransmitter, and hence develop EJPs, following each stimulus delivered to the nerve. In the uninnervated cells, EJPs are passively transmitted from the directly activated cells. Since this transmission most likely takes place through gap junctions, block of these junctions would suppress activation of uninnervated cells in the following ways. (1) EJPs would not be relayed to the uninnervated cells (Manchanda & Venkateswarlu, 1977), precluding the development of action

Figure 5 Reversible suppression of myogenic force by 2.0 mM heptanol. (A) Control; (B,C) 20 and 30 min respectively after application of heptanol. (D) absence of force generation following reversal of stimulation polarity using the same parameters as in (C). (E,F) 30 and 50 min respectively after start of heptanol washout. The thick line represents the duration of stimulation for each trace.

potentials in them. (2) Since cell-to-cell coupling is inhibited, relay of action potentials into these cells from the directly activated cells would also not occur. (3) Finally, any elevation of cytosolic $[Ca^{2+}]$ in the directly activated cells, associated either with the EJP or the action potential, would not be relayed to these cells. All these factors would contribute to the reduction in recruitment of cells activated for contraction, and resultant suppression of contractile force in the presence of heptanol.

The suppression of the noradrenergic phase by heptanol can be explained along lines similar to those mentioned for the purinergic phase above, and for its inhibition of agonist-induced contraction of vascular smooth muscle (Christ *et al.*, 1991, 1993; Christ, 1995). In many smooth muscle organs, including the *vas deferens*, noradrenaline evokes contraction not *via* direct electrical activation involving EJPs but *via* biochemical pathways involving second messengers such as IP_3 and Ca^{2+} (Somlyo *et al.*, 1985; Han *et al.*, 1988). Because of the features of neuronal activation mentioned above, NA, like ATP also activates directly a small fraction of smooth muscle cells. Contractile activation of the noninnervated cells would then depend upon the cell-to-cell diffusion of Ca^{2+} presumably through gap junctions (Christ *et al.*, 1992). Hence heptanol, if it blocks gap junctional communication, would profoundly reduce the number of cells recruited for contraction, inhibiting the noradrenergic phase.

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