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COMPARISON OF NEUROTRANSMISSION WITH NERVE TRUNK AND TRANSMURAL FIELD STIMULATION IN GUINEA-PIG MESENTERIC ARTERY

BY K. D. KEEF*, O. D. HOTTENSTEIN†, A. G. MEEHAN, T. L. ANTHONY AND D. L. KREULEN

From the Department of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724, USA

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SUMMARY

1. Intracellular electrical and contractile responses to sympathetic nerve trunk stimulation (NTS) and transmural electrical field stimulation (TMS) were compared in the guinea-pig mesenteric artery *in vitro*.

2. Step increases in voltage with NTS gave rise to excitatory junction potentials (EJPs) which reached a plateau amplitude of 5–10 mV, whereas with TMS larger amplitude EJPs and sometimes action potentials were obtained.

3. EJPs of equal amplitude (1–7 mV) elicited with TMS and NTS had the same rise time, duration and decay half-time.

4. Slow depolarization obtained with repetitive stimulation was significantly greater in amplitude with TMS than with NTS.

5. Equal amplitude EJPs were obtained throughout the preparation with NTS. With TMS, the amplitude of responses declined substantially with distance from the stimulating electrodes.

6. Tetrodotoxin (TTX) completely blocked EJPs, slow depolarization and contraction with NTS; however, with TMS a TTX-resistant component was observed. The TTX-resistant response to TMS was abolished in the presence of a low-Ca^{2+} superfusion solution but was not affected by endothelium removal.

7. Phentolamine or prazosin abolished slow depolarization but not EJPs with NTS or TMS. Prazosin abolished contraction with NTS and reduced but did not abolish contraction with TMS.

8. α,β-Methylene ATP abolished EJPs with NTS, whereas with TMS only EJPs obtained with low stimulus intensities were abolished. α,β-Methylene ATP did not block contraction with either NTS or TMS.

9. Combined TTX, prazosin and α,β-methylene ATP abolished EJPs initiated with TMS at all but the highest stimulus intensities (12–20 V, 0·3 ms duration).

10. It is concluded that responses obtained with NTS can be reliably attributed to

* Present address: Department of Physiology, School of Medicine, University of Nevada, Reno, NV 89557, USA.

† Present address: Department of Physiology, School of Medicine, University of Colorado, Denver, CO 80262, USA.

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the release of transmitter by the conduction of action potentials in paravascular nerves, whereas activation by TMS is a more complex phenomenon dependent upon stimulus strength and probably involving multiple forms of activation.

INTRODUCTION

Nervous excitation of vascular smooth muscle cells involves the invasion of action potentials into the autonomic ground plexus and consequent excitation of nerve terminals which mediates the release of transmitter. Several techniques for excitation of nerves in isolated blood vessels have been described (see: Bevan & Su, 1975; Moulds, 1983). The most commonly used technique employs electrical field stimulation of strips or rings isolated and mounted in tissue baths. Only a few studies in isolated blood vessels have reported the direct stimulation of sympathetic nerve trunks before they separate to form the final perivascular plexus (Bevan, 1962; de la Lande & Rand, 1965; Holman & Mclean, 1967; Holman & Surprenant, 1980*b*). The advantage of stimulating nerve trunks before they enter the vessel wall is that this method allows '... purely indirect (nerve) stimulation which reproduces the sequence of excitation as close to the physiological, *in vivo* conditions as can be obtained' (Bevan & Su, 1975, p. 443).

Several contractile studies which have compared transmural field stimulation (TMS) and nerve trunk stimulation (NTS) suggest that these two forms of excitation do not produce identical results. Small rings of the rabbit ear artery contract more at each frequency of stimulation when the rings are stimulated with field stimulation than when the postganglionic sympathetic nerves are stimulated (de la Lande & Rand, 1965). Similar differences between these modes of stimulation have also been described in sheep mesenteric veins (Holman & Mclean, 1967). The electrophysiological bases of these differences have not been studied.

The inferior mesenteric artery is well suited for studies comparing NTS with TMS because it can be removed intact in the mesocolon containing the inferior mesenteric ganglion and the lumbar colonic nerves, the postganglionic nerves innervating this vessel. Previous studies have shown that significant intracellular neural responses can be obtained with both TMS and NTS in this preparation (Kreulen, 1986; Hottenstein & Kreulen, 1987). The present study examined the intracellular and contractile responses to NTS and TMS in this mesenteric preparation.

The goals of the present study were to: (1) investigate the relative importance of action potentials in perivascular nerves for initiation of junction potentials and contraction with TMS and NTS, (2) compare the relative potency of adrenergic and purinergic antagonists commonly used to block the actions of sympathetic nerves on the contractile and electrical responses to TMS and NTS, and (3) estimate the extent to which non-neural mechanisms may be contributing to the depolarizing potentials and contractions obtained with TMS and NTS.

METHODS

The results in this study were obtained from fifty-nine animals. Male guinea-pigs (242 ± 21 g) were killed by cervical dislocation and exsanguinated. The abdomen was opened and the region along the abdominal aorta from the diaphragm to the bifurcation of the aorta was exposed. The

aorta, inferior mesenteric artery and ganglion (IMG) with intact nervous connections and colon were removed from the animal and placed in oxygenated Krebs solution at room temperature for further dissection. The final preparation was placed in an organ bath and the edges pinned out with fine tungsten pins (Fig. 1).

The bath compartment (5·5 ml) was superfused (8–10 ml/min) with Krebs solution of the following composition (mM): NaCl, 118·5; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 23·8; KH_2PO_4, 1·2; dextrose, 5·5. Low-calcium/high-magnesium solutions were prepared with substitution of 0·5 mM-$CaCl_2$ and 12 mM-$MgCl_2$ in the Krebs solution. The Krebs solution in the supply reservoir was gassed continuously with 95% O_2/5% CO_2 gas mixture (pH 7·4). Temperature was monitored with a thermistor probe (Yellow Springs Instruments, Inc.) near the recording site and maintained at 36·5–37 °C.

Electrical stimulation. Square-wave stimuli of 2–18 V measured at the electrodes and 0·1–0·5 ms duration were delivered from a Grass S88 stimulator through stimulus isolation units (Grass SIU-5). Stimulation rates and train lengths were controlled by a HP9836 computer and HP6940B multiprogrammer system which remotely operated the stimulator. Repetitive nerve stimulation of vessels included frequencies from 0·1 to 20 Hz and train lengths from 5 to 20 s for the electrical recordings, and train lengths up to 120 s for the diameter measurements.

Nerve trunk stimulation. The lumbar colonic nerves were placed on bipolar platinum hook electrodes close to where this nerve trunk exits from the IMG. This site was 1–2 cm from the recording sites in the vessels. The mesenteric artery was excluded from the stimulating electrodes to reduce direct stimulation of the perivascular nerves in the artery (Fig. 1).

Transmural stimulation. Electrodes were placed with a pole on each side of the mesenteric artery or its branches 10–15 mm from the nerve trunk electrodes (Fig. 1). The electrodes were insulated down to the final 2 mm; each pole was placed 1 mm from the arterial wall. Stimulation intensity was varied by altering either the duration (0·1–2 ms) or the voltage (2–18 V) of the applied pulses.

Intracellular measurements. Transmembrane potential recordings from arteries were made with fibre-filled glass micropipettes filled with 3 M-KCl. Microelectrodes had resistances of 70–120 MΩ with tip potentials of less than 5 mV. Membrane potentials were displayed on a digital storage oscilloscope (Nicolet 201) and recorded on an instrumentation tape-recorder (HP3964A) for subsequent reproduction on an *X–Y* plotter (Gould 3054) or strip chart recorder (Gould 2400). Vessels were impaled from the adventitial surface. Resting membrane potentials were taken as the voltage deflection upon withdrawal from the cell. All results were taken from cells that had stable membrane potentials for at least 5 min before the start of nerve stimulations. EJPs were stored and analysed for amplitude, rise time, duration and decay time constant by custom-design software. Decay time constants were determined by fitting a single exponential to the falling phase of the EJPs between 80 and 20% of peak amplitude.

Observation of vessel diameter. Diameter measurements were determined with a stereo dissecting microscope (Wild M7A) with a calibrated eyepiece reticle. The limits of resolution allowed for the discrimination of vessel changes of 15 μm or greater. Measurements were made at the position of the transmural stimulating electrodes. The vessels were stretched slightly by pinning out the mesentery but were not distended.

Removal of endothelium. In experiments where the endothelium was to be removed, the lumens of arteries were perfused with distilled water for 15 min. Removal of functional endothelium was confirmed at the end of experiments by the loss of the dilator effect of acetylcholine in noradrenaline-contracted vessels.

Statistical analysis. Results are expressed as means ± standard error, except where otherwise indicated. Statistical differences between treatment groups or preparations were determined by using Student's two-tailed *t* test. Within individual cells membrane potential measurements for control and experimental periods were analysed by a paired *t* test (Snedecor & Cochran, 1967). Where appropriate, analysis of variance procedures (ANOVA) were conducted for one-way and two-way comparison followed by post-tests for multiple comparisons with least significant differences or Duncan's new multiple range test (Duncan, 1955; Hicks, 1973).

Drugs. Drugs used were as follows: acetylcholine chloride, noradrenaline hydrochloride, α,β-methylene ATP (Sigma, USA), tetrodotoxin (Calbiochem-Behring), prazosin hydrochloride (Pfizer) and phentolamine mesylate (Ciba, Regitine).

RESULTS

Intracellular responses to a single stimulus

General characteristics of responses to transmural electric field stimulation and sympathetic nerve trunk stimulation

The mean membrane potential (E_m) for thirty-one cells studied with both types of stimulation was $-73{\cdot}7 \pm 1{\cdot}1$ mV (Table 1). In the same cells, excitatory junction

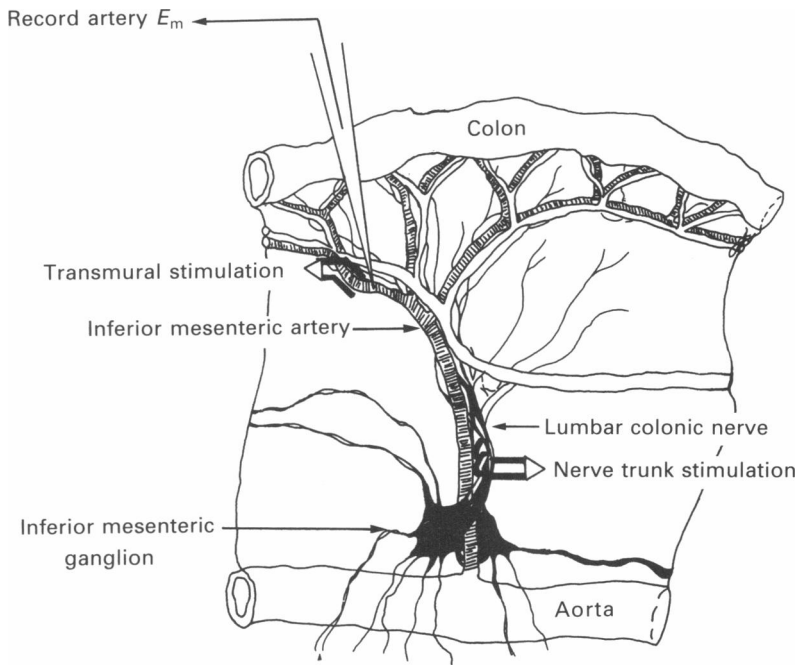

Fig. 1. Diagram of experimental preparation. The colon was removed before completing the dissection but is left in the figure for illustrative purposes. Arterial membrane potential recordings were made within 5 mm of the region of the bifurcation of the inferior mesenteric artery and the left colonic artery and the cranial rectal artery. The nerve trunk stimulating electrode was placed directly on the lumbar colonic nerve trunk. The transmural stimulation electrode and recording site were 5–15 mm away from the nerve trunk stimulating electrode.

potentials (EJPs) were elicited with NTS and with TMS. With both types of stimulation the amplitude of the EJP could be increased by increasing the stimulus voltage or duration. However, the relationship between EJP amplitude and stimulus voltage was different for the two kinds of stimulation. For NTS, the amplitude of the EJP increased steeply with stimulus strength (0·5 ms pulse duration) until a plateau was reached at approximately 9 mV (Fig. 2*B*). In the same cells, the amplitude of EJPs evoked with TMS did not plateau but rather there was a continued increase in EJP amplitude with greater stimulus intensities. Larger EJPs obtained with TMS were of sufficient amplitude to elicit variable amplitude action potentials (Fig. 2).

EJPs obtained with TMS which were greater than 8 mV in amplitude also were of significantly longer duration and faster rise time although the time constant of decay was not different from those evoked with NTS (Table 1). On the other hand, when equal amplitude EJPs of less than 7 mV were compared, the EJPs obtained with NTS and TMS were not significantly different in time course.

TABLE 1. Time course and magnitude of all EJPs obtained with TMS and NTS

E_m (mV)	Amplitude (mV)	Rise time (ms)	Duration (ms)	τ (ms)	Latency (ms)
		Nerve trunk stimulation			
−73·8	4·9	45·4	325	122	36·4
(1·2)	(0·3)	(2·5)	(17)	(7)	(1·6)
n.s.	*	*	*	n.s.	*
		Transmural electric field stimulation			
−73·6	9·0	39·8	354	116	11·6
(1·1)	(0·6)	(3·4)	(18)	(6)	(0·8)

* $P < 0{\cdot}05$; two-tailed paired t test.
$n = 31$ cells; NTS: 16–18 V, 0·3 ms duration. TMS. 6–10 V, 0·3 ms duration.
n.s. = not significant. Values in parentheses indicates S.E.M.

The latency for generation of EJPs with NTS was always greater than for TMS due to the conduction delay involved in propagation of the action potential from the point of stimulation to the point of recording (Table 1, and inset Fig. 2*A*).

Relationship between stimulating electrodes and the distance over which EJPs are recorded

One obvious difference between NTS and TMS is the relationship between the stimulating electrode and the recording site. In one case (NTS) the stimulating electrode is remote and in the other (TMS) it is at the same position as the recording electrode. In the next set of experiments we investigated how responses to TMS change as the recording electrode is moved away from the point of stimulation. We also recorded responses to NTS from the same sites to determine the extent to which responses to NTS may decrement over these distances.

The stimulating electrodes for these experiments were placed 10 mm from one another. First a cell in the artery was impaled at the location of the TMS electrodes (i.e. a distance of 10 mm from the NTS electrodes) and then the response to maximal NTS (16–18 V, 0·3 ms duration) was obtained. Thereafter the response to TMS was tested over three voltage ranges i.e. low (2–5 V), moderate (6–10 V) and maximum (10·5–18 V), with a stimulus duration of 0·3 ms. The electrode was then moved distal to the TMS electrodes in increments of 1·5 mm and the process was repeated. Finally, the electrode was moved in 1 mm increments proximal to the TMS electrodes and stimulation was repeated once more. The amplitudes of responses obtained with distance for NTS and TMS are shown in Fig. 3. Responses to TMS with all three stimulus ranges were greatest near the stimulating electrodes although the amount of decline differed. It is of interest that although action potentials were recorded with TMS near the stimulating electrodes using a stimulus duration of 0·5 ms (i.e. Fig. 2)

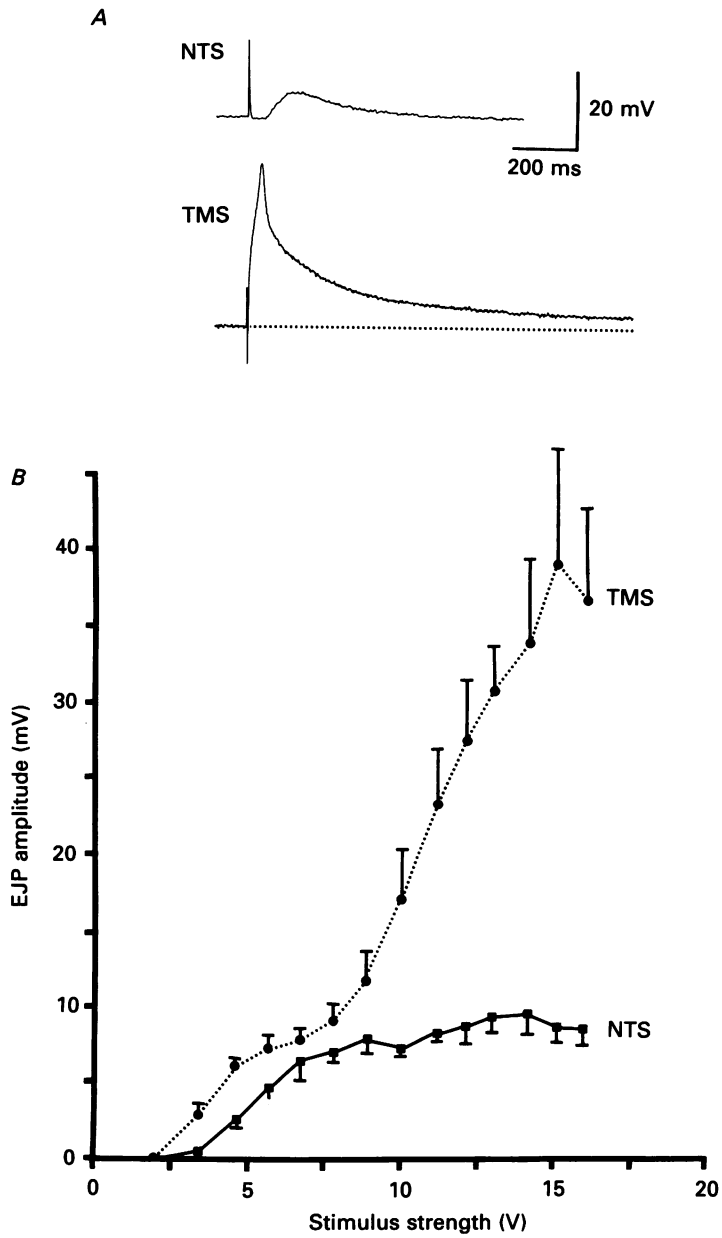

Fig. 2. Comparison of response amplitude with transmural stimulation (TMS) and nerve trunk stimulation (NTS) as a function of stimulus strength (0·5 ms duration pulses). *A*, example of the response to NTS and TMS in a single cell at maximum stimulus intensity (i.e. 16 V). *B*, increasing amplitude responses are observed with both TMS and NTS as stimulus strength is increased from 2 to 8 V. Above this stimulus strength responses with NTS begin to plateau, reaching a maximum of approximately 9 mV, whereas responses to TMS continue to increase in amplitude up to a maximum of approximately 40 mV. Larger amplitude EJPs with TMS give rise to an action potential of variable amplitude.

they were not observed with a stimulus duration of 0·3 ms (i.e. Fig. 3). Responses to maximum stimulus voltage (18 V, 0·3 ms duration) decreased sharply in amplitude in the distal direction to a level which was not significantly different from that observed with the lower stimulus voltage. Although not shown here, the action

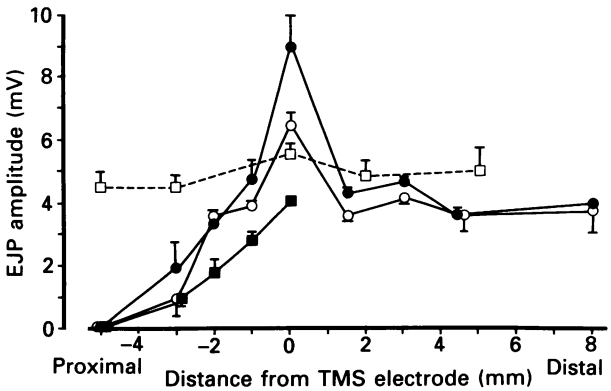

Fig. 3. Comparison of the amplitude of excitatory junction potentials (EJPs) with nerve trunk stimulation (NTS) and transmural stimulation (TMS) at varying distance from the stimulating electrodes. □---□, plot of the amplitude of EJPs elicited with NTS (16–18 V) at distances of 5–15 mm from the nerve trunk stimulating electrodes (shown in the graph as −5 mm to +5 mm from the TMS stimulating electrodes). There was no significant difference in the amplitude of EJPs over this distance. Continuous lines, plots of the amplitude of EJPs while recording at the position of the TMS electrode ('0 mm') and 8 mm distal and 5 mm proximal to the stimulating electrode. Three ranges of stimulus voltage were tested (i.e. 10·5–18 V (●), 6–10 V (○) and 2–5 V (■)). Values plotted are means ± S.E.M.

potential initiated with a longer pulse duration (i.e. 0·5 ms, 18 V) declined even more steeply with distance than the response obtained with a shorter pulse duration (0·3 ms, 18 V). In the proximal direction responses to all three stimulus ranges with TMS (0·3 ms duration) declined to zero at 5 mm distance from the stimulating electrodes even though responses to NTS were the same as at the position of the TMS electrodes.

Conduction velocities were also estimated from the latency between the stimulus artifact and the onset of the EJP for TMS and NTS with distances from 0·3 to 15 mm. In seven cells (two preparations) the conduction velocity for NTS (0·46 ± 0·03 mm/s) was significantly greater than for TMS (0·28 ± 0·04 m/s, $n = 6$ cells). There was no significant difference in the conduction velocity for TMS at different voltages (i.e. 6 *versus* 18 V).

Effect of tetrodotoxin

Vessel segments were initially exposed to TTX (0·3 μM) for 5 min and the stimulus voltage–response amplitude relationship with TMS or NTS measured (0·3 ms pulse duration) and compared to that obtained in control Krebs solution. TTX completely abolished the response to NTS over the entire range of voltages tested (i.e. 2–18 V;

$n = 5$) With TMS, TTX significantly shifted the voltage–response relationship to the right but did not entirely abolish EJPs. With longer pulse durations (i.e. 0·75–1·0 ms), TTX abolished responses to NTS but did not significantly reduce responses to TMS. Examples from a single cell are shown in Fig. 4. In Fig. 5 the entire voltage–response relationship is shown for five preparations.

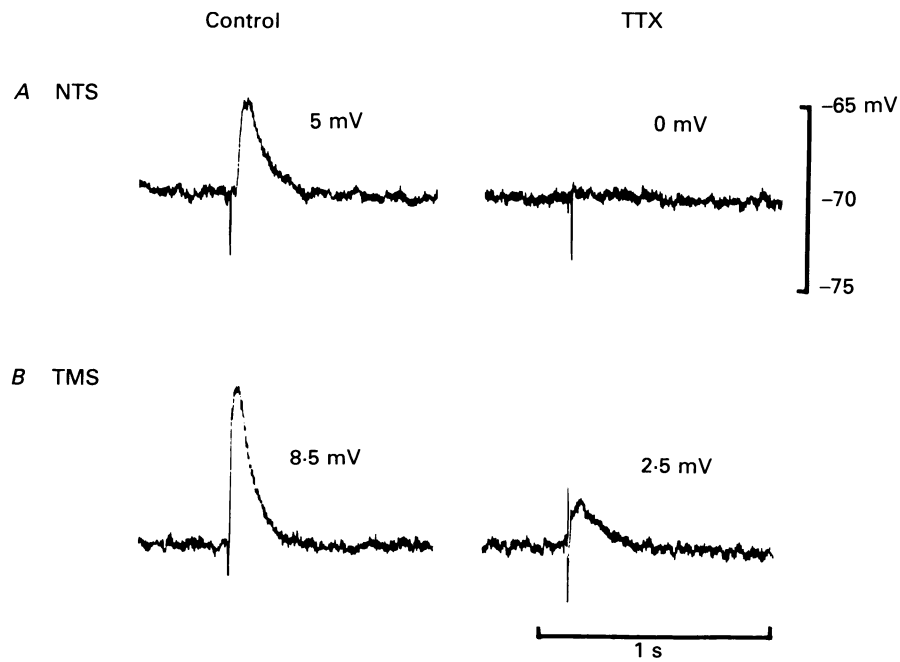

Fig. 4. Effect of tetrodotoxin (TTX) on the amplitude of EJPs elicited with nerve trunk stimulation (NTS) or transmural stimulation (TMS) in a single cell. Stimulus voltage with TMS was adjusted to produce the same amplitude EJP as with NTS. *A*, example of an EJP obtained with NTS (left). The response in this cell is completely abolished with 0·3 μM-TTX (right). *B*, same cell as *A*. Example of an EJP obtained with TMS (left). The response in this cell is reduced in the presence of TTX but not abolished (right).

Effect of phentolamine and prazosin

Facilitated EJPs (0·3 Hz) were potentiated by phentolamine (1 μM) with both TMS and NTS, suggesting the presence of presynaptic autoinhibition by noradrenaline as proposed by others (Duckles, 1979; Surprenant, 1980). In contrast, prazosin (0·5 μM) significantly reduced the amplitude of EJPs initiated with either TMS or NTS by $17 \pm 6\%$ after 8–15 min exposure time ($n = 7$ cells).

Effect of α,β-methylene ATP

Desensitization with α,β-methylene ATP was tested to determine the possible contribution of a purinergic mechanism to the generation of the EJP with TMS and NTS. Introduction of α,β-methylene ATP (0·5 μM) into the superfusion medium resulted in a large depolarization. Contraction and loss of the membrane potential

recording occurred on occasion. After 15 min the membrane potential had returned to within 2 mV of control. Following this period of superfusion with α,β-methylene ATP, EJPs with NTS were abolished. In two cells, EJPs of equal amplitude initiated with TMS were also abolished with α,β-methylene ATP. However, with larger

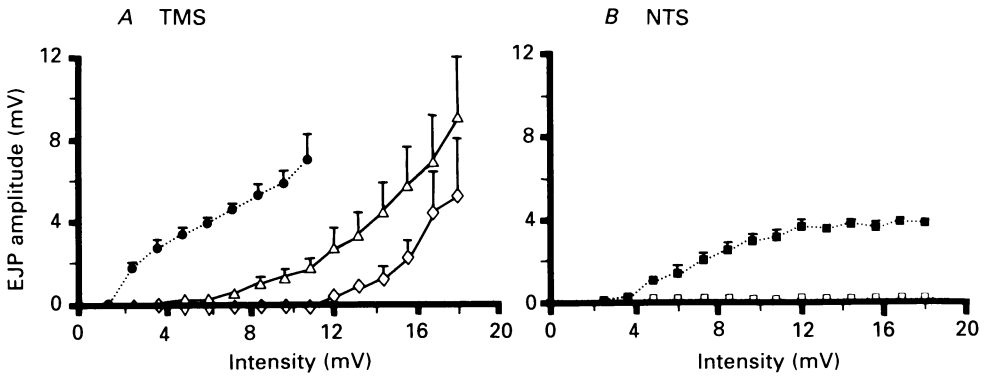

Fig. 5. Comparison of the amplitude of EJPs elicited with transmural stimulation (TMS) and nerve trunk stimulation (NTS) with increasing stimulus strengths (i.e. intensity) in regular Krebs solution and in the presence of either tetrodotoxin (0·3 μM-TTX) or mixed blockade (i.e. 0·3 μM-TTX, 0·5 μM-prazosin and 0·5 μM-α,β-methylene ATP. *A*: ●, control EJP amplitude as a function of stimulus intensity with TMS. △, EJP amplitude in the presence of TTX. ◇, EJP amplitude with mixed blockade. Note that even mixed blockade does not completely eliminate depolarizations elicited with TMS. *B*: ■, control EJP amplitude as a function of stimulus intensity with NTS. In the presence of either TTX or mixed blockade (□) depolarizing responses are completely eliminated. Shown are mean values ± S.E.M.

stimulus voltages (16 V, 0·3 ms duration) EJPs could still be obtained with TMS but not NTS in a manner similar to the mixed drug effects discussed in the next section.

Effect of mixed drugs

In the presence of combined TTX (0·3 μM), α,β-methylene ATP (0·5 μM) and prazosin (0·5 μM), the EJP with NTS was abolished at all stimulus voltages ($n = 3$ preparations), as was the case for TTX. EJPs elicited with TMS in the voltage range of 2–11 V were also abolished by this procedure; however with greater stimulus voltages EJPs could still be recorded (Fig. 5).

Effect of low Ca^{2+}/high Mg^{2+}

In twelve cells from five preparations TTX-resistant, TMS-evoked EJPs were abolished after a 5 min exposure to 0·5 mM-Ca^{2+}/12 mM-Mg^{+}.

Effect of endothelium removal

In the presence of 0·3 μM-TTX, the average amplitude of control TTX-resistant TMS-evoked EJPs (3·2 ± 0·3 mV, $n = 6$ cells) did not significantly differ from the average amplitude of TTX-resistant EJPs obtained following endothelium removal (3·4 ± 0·5 mV, $n = 5$ cells).

Slow depolarization responses to repetitive nerve stimulation

Repetitive TMS and NTS led to facilitation of EJPs at frequencies above 0·1 Hz and to summation above 1 Hz. There was no obvious difference in the degree to which these two characteristics occurred with TMS and NTS. At higher frequencies

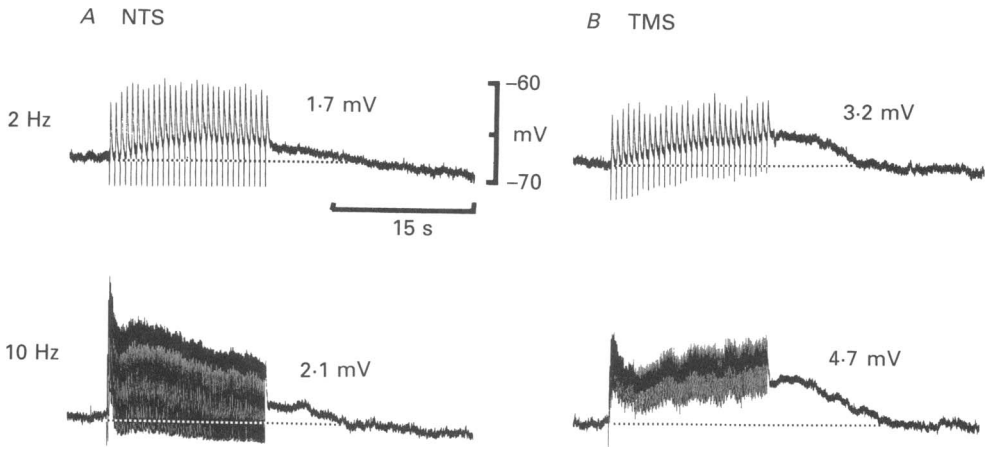

Fig. 6. Comparison of slow depolarizing responses to transmural stimulation (TMS, 6 V, 0·3 ms duration) and nerve trunk stimulation (NTS, 18 V, 0·3 ms duration) with repetitive stimulation in a single cell. Stimulus voltage for TMS was adjusted to produce the same amplitude EJP as with NTS. *A*, repetitive NTS at 2 Hz produced EJPs and a slow depolarization of 1·7 mV (upper recording). At 10 Hz the slow depolarization has increased to 2·1 mV (lower recording). *B*, repetitive TMS in the same cell at 2 Hz produced a significantly larger amplitude slow depolarization of 3·2 mV (upper recording). At 10 Hz the slow depolarization increased to 4·7 mV.

(2–10 Hz) a period of prolonged depolarization was observed lasting 5–10 s beyond the end of stimulation. The amplitude of this slow depolarization was compared in vessels at frequencies between 1 and 10 Hz (eight cells, five vessels). Stimulus parameters were adjusted to obtain equal amplitude EJPs with the two types of stimulation. The amplitude of slow depolarization was significantly greater at 2, 5 and 10 Hz with TMS than with NTS. Representative responses for one cell with both methods of stimulation are shown in Fig. 6 and the mean frequency–amplitude relationship for eight cells is shown in Fig. 7. Frequency had a significant effect on TMS-induced slow depolarization from 2 to 10 Hz; however, this effect was only apparent at 10 Hz for NTS (ANOVA).

Effect of phentolamine and prazosin

The adrenergic blockers phentolamine (1 μM) and prazosin (0·5 μM) produced nearly complete inhibition of slow depolarization induced with NTS (1–10 Hz), as demonstrated previously (Hottenstein & Kreulen, 1987; Suzuki, Ishikawa, Nagao, Komori, Ibegwe & Fujioka, 1987). Responses to TMS at these same frequencies (when voltage was adjusted for equal EJP amplitudes) were also completely abolished

($n = 3$). Responses obtained with TMS in one cell at a larger stimulus voltage (i.e. 10 V, 0·3 ms duration) were only reduced by 65 and 50% at 7·5 and 10 Hz, respectively.

Contractile responses to repetitive nerve stimulation

The contractile responses to NTS and TMS were compared by measuring the change in diameter of vessels with a calibrated eyepiece at the position of the

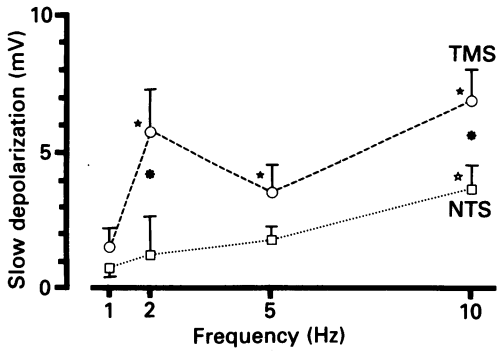

Fig. 7. Plot of the amplitude of slow depolarization with transmural stimulation (TMS) and nerve trunk stimulation (NTS) at frequencies between 1 and 10 Hz (5 s stimulation) for cells with equal amplitude EJPs. Slow depolarization with TMS is significantly greater than with NTS at 2 and 10 Hz (*). Slow depolarizations were significantly greater than zero for TMS at 2, 5 and 10 Hz (★) but only at 10 Hz for NTS (☆). These data indicate that the magnitude and onset frequency of slow depolarization differs between the two types of stimulation (ANOVA).

transmural stimulating electrodes in thirteen preparations (thirteen to nineteen segments for each frequency). Both percentage decrease in diameter and time to maximum contraction were measured. Vessels were stimulated repetitively at frequencies from 1 to 20 Hz. Stimulation was continued until peak contraction was obtained; thus, the duration of stimulation was not equivalent for all frequencies. Stimulus periods ranged from 10 to 120 s. Maximum voltage (18 V, 0·3 ms duration) was selected for NTS and 6 V (0·3 ms duration) was selected for TMS. Six volts with TMS gave an EJP comparable to that obtained with NTS. The extent of contraction with either NTS or TMS was dependent upon the frequency of nerve stimulation and TMS contractions were significantly greater than NTS for all frequencies tested (ANOVA and Duncan's test).

For stimulus parameters which gave equal amplitude EJPs with NTS or TMS, transmurally evoked contractions were significantly greater in amplitude than occurred with NTS at all frequencies tested (Fig. 8, $n = 10$ vessel segments). Interestingly, contraction was never observed at 2 Hz with NTS even when periods of stimulation as long as 2 min were tested suggesting that even at low frequencies of activation the mechanisms leading to contraction with these two types of stimulation may differ. Average time to peak contraction was significantly less

(ANOVA) for TMS than for NTS at all frequencies tested (i.e. NTS = 103±7 s at 2 Hz and 22±4·5 s at 20 Hz *versus* TMS = 83±10 s at 2 Hz and 15±2 s at 20 Hz; $n = 19$ vessels).

Effect of tetrodotoxin

Vessels were exposed to TTX (0·3 μM) 5 min prior to beginning nerve stimulation experiments. Contractile responses were obtained for frequencies from 2 to 20 Hz

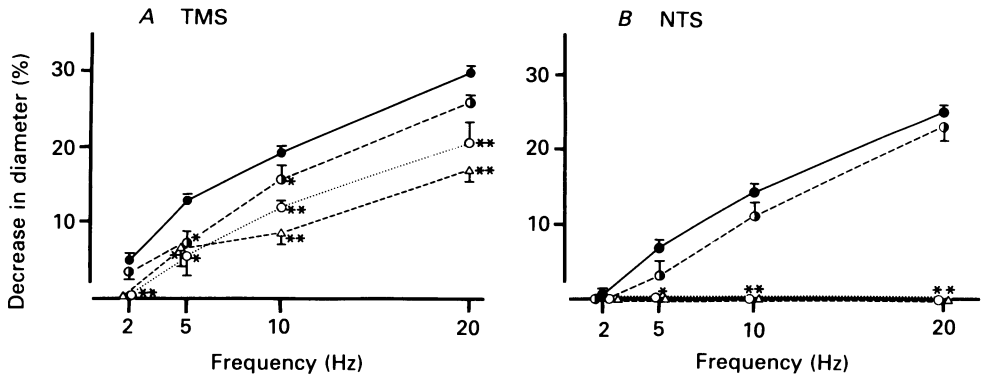

Fig. 8. Comparison of transmural and nerve trunk stimulation-induced contractions under control conditions (●) and in the presence of tetrodotoxin (0·3 μM, △), prazosin (0·5 μM, ○) and α,β-methylene ATP (0·5 μM, ◑). Nerves were stimulated until maximum steady contraction was reached at frequencies between 2 and 20 Hz (0·3 ms duration, 6 V). *A*, percentage decrease in diameter with TMS. Significantly less contraction is obtained with 5 and 10 Hz stimulation in the presence of α,β-methylene ATP and for all frequencies with TTX or prazosin. *B*, percentage decrease in diameter with NTS. Contraction is not significantly decreased by α,β-methylene ATP but is abolished with prazosin or TTX.

with TMS and NTS. TTX completely abolished NTS responses at all frequencies. Contractions with TMS were reduced in magnitude but not abolished (Fig. 8, $n = 9$). Contractions initiated with TMS using longer pulse durations (i.e. 1–2 ms, 10 V, 5 Hz) were not significantly reduced with TTX whereas those with NTS were abolished.

Effect of prazosin

In six vessels (five preparations) exposed to prazosin alone (0·5 μM) 8 min prior to beginning nerve stimulation, contraction with NTS (5–20 Hz) was abolished, whereas with TMS (5–20 Hz) contraction was reduced to 47–55% of control, but not abolished. In contrast, contraction with TMS at 2 Hz was abolished with prazosin. The difference in the action of prazosin on contractions with NTS and TMS was significant (ANOVA) (Fig. 8).

Effect of α,β-methylene ATP

In five preparations exposed to α,β-methylene ATP alone (0·5 μM) 20 min prior to beginning nerve stimulation, contraction was significantly reduced at 5 and 10 Hz with TMS but not with NTS (Fig. 8).

DISCUSSION

The present study has compared the intracellular and contractile responses obtained with two commonly used methods of nerve stimulation, namely transmural stimulation (TMS) and nerve trunk stimulation (NTS). Our results indicate that responses to NTS in the mesenteric artery can be reliably attributed to the initiation of action potentials in perivascular nerves and the subsequent release of transmitter. In contrast, responses to TMS appear to be more complex.

Differences between NTS and TMS are most likely due to the direct association between the stimulating electrodes and the blood vessel with TMS. As a result of this association, stimulation may lead to more than one of the following events, namely: (1) transmitter release by initiation of action potentials in perivascular nerves, (2) transmitter release by directly depolarizing nerve terminals, (3) direct stimulation of the arterial smooth muscle, (4) activation of a non-neural, non-muscle component. The results which we obtained with TMS and NTS will be discussed in the context of these four possible mechanism.

EJPs initiated with NTS at all voltages and durations were completely abolished in the presence of the fast Na^+ channel antagonist TTX, confirming their dependence upon action potentials in nerve fibres. In contrast, EJPs with TMS were not completely blocked by TTX. These TTX-insensitive responses were most apparent at larger stimulus voltages and/or durations. In many studies by others, it has been assumed that if a response is not blocked by TTX then it must not be neural. However, with transmural stimulation there are nerve terminals present in the direct field of stimulation. It is known that transmitter can be released from nerve terminals by directly depolarizing them in the presence of TTX (Katz & Miledi, 1967). Furthermore, in more recent studies TTX-insensitive EJPs which are abolished by guanethidine have been reported (Keef & Neild, 1982; Neild & Kotecha, 1985). From the effects of TTX on the EJP we must conclude that responses with TMS depend to a certain extent upon propagated action potentials but that additional mechanisms, which may involve direct release of transmitter, are also present.

Small amplitude EJPs (i.e. up to 7 mV in amplitude) were identical in time course for TMS and NTS. However, with larger stimulus voltages and/or durations, significant differences appeared between the two modes of stimulation, i.e. in the case of NTS, a plateau in EJP amplitude was observed, whereas with TMS larger amplitude EJPs and action potentials could be obtained. It is apparent from the relation between responses and distance from the point of stimulation that these larger amplitude responses were not a conducted phenomenon but rather were directly related to the close proximity of the electric field to the point of recording. This has also been shown in the guinea-pig ear artery (Keef & Neild, 1982). Since action potentials were never generated in these experiments with NTS, required longer stimulus durations to initiate with TMS and were not conducted away from the point of stimulation, it may be that these events are an artifact of TMS which do not occur with the natural asynchronous release of transmitter *in vivo* (Neild, 1983).

The actions of phentolamine and the selective α_1-adrenoceptor antagonist prazosin

were tested on EJPs, slow depolarization and contractions generated with TMS and NTS, to determine the extent to which α-adrenergic mechanisms contribute to these events with the two types of stimulation. Prazosin and phentolamine did not abolish EJPs elicited with either NTS or TMS which is in agreement with other studies (e.g. Holman & Surprenant, 1980*a*; Hirst & Neild, 1980). The potentiation of EJPs by phentolamine was probably related to antagonism of prejunctional α_2-adrenoceptors involved in autoinhibition of transmitter release (Duckles, 1979; Surprenant, 1980; Kügelgen & Starke, 1985).

Unlike the EJP, slow depolarization and contraction with TMS and NTS were effectively antagonized by prazosin, as shown previously (e.g. Hottenstein & Kreulen, 1987; Suzuki *et al.* 1987). The actions of prazosin differed with NTS and TMS in that: (1) slow depolarization with TMS in the presence of prazosin could be restored by increasing stimulus voltage and/or duration (slow depolarization with NTS was not), (2) contraction with TMS in the presence of prazosin was reduced but not abolished (contraction with NTS was abolished). These studies suggest that both slow depolarization and contraction in the mesenteric artery with NTS are due to activation of α_1-adrenoceptors. On the other hand, with TMS α_1-adrenoceptor activation contributes to slow depolarization and contraction, but as the stimulus voltage is increased other mechanisms become more important.

Since the EJP in this and other vessels is not blocked by α-adrenoceptor antagonists it has been proposed that another transmitter may be released simultaneously with noradrenaline. The 'second transmitter' proposed is ATP and evidence in support of this hypothesis includes both electrophysiological and pharmacological studies (Fedan, Hogaboom, O'Donnell, Colby & Westfall, 1981; Muramatsu, Kigoshi & Oshita, 1984; Sneddon & Burnstock, 1985; Kügelgen & Starke, 1985; Ishikawa, 1985). Since these previous studies implicate ATP as the transmitter responsible for the EJP we investigated the effects of ATP receptor desensitization with α,β-methylene ATP on the contractile and electrical responses to NTS and TMS. α,β-Methylene ATP abolished equal amplitude EJPs obtained with either NTS or TMS. This is in general agreement with the work of Ishikawa (1985) with TMS in the same vessel. Although α,β-methylene ATP had a pronounced effect on the EJP, it had only minimal effects on contractions induced with either TMS or NTS suggesting that purinergic mechanisms do not play a major role in the generation of contraction in this tissue. In contrast, the marked effect of prazosin in reducing contractions with TMS and abolishing contraction with NTS suggest that adrenergic mechanisms are very important to the generation of contraction in this vessel. This conclusion was also reached by Itoh, Kitamura & Kuriyama (1983) with the use of α-receptor antagonists.

When vessels were stimulated transmurally, combined TTX, prazosin and α,β-methylene ATP gave rise to greater inhibition of EJPs than TTX alone. In addition prazosin produced greater inhibition of contraction than TTX. These observations suggest that there is an additional neural component elicited with TMS which is TTX insensitive. A likely mechanism for this component would be the direct release of transmitter from nerve terminals present in the field of stimulation, as discussed previously. Because the TTX-insensitive EJPs were blocked by low-calcium/high-magnesium it is likely that transmural stimulation releases substances via a calcium-

dependent mechanism. At least part of this may be from nerve terminals in the field of stimulation; it has not been ruled out that the remainder of the response may be from direct activation of smooth muscle cells.

The EJPs which persist in the presence of combined TTX, α,β-methylene ATP and prazosin occur with larger stimulus voltages and/or longer pulse durations. This is the general direction which favours direct muscle stimulation. Another possibility which can not be excluded, however, is the stimulation of a third non-neural, non-muscle component. In the rabbit basilar artery and the guinea-pig coronary artery transient depolarizations in response to TMS which are insensitive to TTX, prazosin and α,β-methylene ATP have been reported (Nagao & Suzuki, 1987; Surprenant, Neild & Holman, 1987; Keef & Kreulen, 1988). Nagao & Suzuki (1987) have suggested that these events involve stimulation of the endothelium since they are eliminated following removal of the endothelium. However, in contrast to the findings of Nagao & Suzuki (1987), in the present study endothelium removal had no effect on TTX-resistant EJPs. Our results therefore provide no direct evidence in favour of either direct muscle stimulation or activation of a third cellular component. None the less, both possibilities should be considered for the drug-insensitive component of the response to TMS.

The amplitude of EJPs recorded at various sites along the artery was uniform with NTS. On the other hand, with TMS the EJP amplitude was dependent on the distance from the stimulating electrode and the direction from the point of transmural stimulation. The results show that when perivascular nerves are stimulated 'locally' with TMS, activation travels further in the efferent or orthodromic direction than it does in the antidromic direction. Furthermore, the amplitudes of EJPs in the efferent direction are uniform and of the same amplitude as the EJPs evoked with NTS.

In conclusion, we have shown that NTS produces electrical and mechanical activity which can readily be explained in terms of the propagation of action potentials along paravascular nerve trunks and release of transmitter from perivascular nerve terminals. TMS on the other hand produces responses whose mechanism is to a large extent dependent upon the stimulus parameters selected, with low stimulus intensities producing responses dependent upon action potentials in perivascular nerves and high stimulus intensities involving multiple forms of activation.

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