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Sensitivity to indomethacin of tetrodotoxin-resistant contractions of smooth muscle from the base of rabbit bladder

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Tetrodotoxin (TTX) reduced the contractions to field stimulation of strips of rabbit bladder base by 58% of control (at 40 Hz), and increased the spontaneous activity occurring between the evoked responses. The TTX-resistant contractions resembled the spontaneous activity in that they were of comparable size and poorly sustained; in the presence of indomethacin, TTX produced a significantly greater reduction (to 13% of control at 40 Hz), of the evoked contractions. Indomethacin abolished spontaneous activity in the presence and absence of TTX, but did not affect evoked responses in strips that were not exposed to TTX. The results imply that a prostaglandin-like substance may potentiate residual evoked responses in TTX-treated strips, but does not contribute to field stimulation-induced contractions in untreated bladder base smooth muscle.

Introduction Tetrodotoxin (TTX) produces a marked reduction in the response to field stimulation of many types of smooth muscle (Gershon, 1967), including rabbit and guinea-pig bladder detrusor muscle (Downie & Dean, 1977; Krell, McCoy & Ridley, 1981). Such a reduction in response is usually interpreted as revealing the presence of a process dependent upon nerve action potentials. However, in rabbit bladder base, it was observed that TTX treatment alone was relatively ineffective in reducing field stimulation-induced contractions and was associated with an increase in spontaneous activity (Slack, Downie & Elbadawi, 1982). Since prostaglandins have been implicated in the production of spontaneous contractile activity in the detrusor muscle (Abrams & Feneley, 1976), it was of interest to determine whether indomethacin could modify the TTX-resistant contractions in rabbit bladder base.

Methods Male New Zealand White rabbits weighing approximately 1.8 kg were killed by cervical dislocation. Longitudinally oriented strips (5 × 2 mm, unstretched) were cut from the anterior bladder base and prepared for measurement of isometric contractions to field stimulation (Slack *et al.*, 1982) in a modified Krebs solution of the following composition (mM):NaCl 118, KCl 4.69, $MgSo_4$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 2.45, $NaHCO_3$ 25 and glucose 5.55. After an equilibration period of 1 h at a flow rate of 2 ml min^{-1}, tissues were stimulated at 90 s intervals for 30 min with pulses of 0.2 ms width at 5 or 10 Hz and train duration of 5 s. Control frequency-response curves over the range from 2 to 40 Hz were then obtained and tissues were washed at 5 ml min^{-1} for 20 min. The 30 min stimulation period was repeated and a second frequency-response curve was obtained.

Corrections for changes in sensitivity with time of untreated or indomethacin-(10 μM for 50 min) treated strips were determined in each experiment (Downie & Dean, 1977). Responses obtained in the presence of TTX (1 μM for 20 min) were expressed as a percentage of the pretreatment maximum of the same strip. The means of the responses at each frequency obtained before and after TTX treatment were compared using Student's *t* test, and differences were considered to be significant at $P < 0.05$. All data are expressed as means ± s.e.mean.

Results Isolated strips of bladder base smooth muscle exhibited spontaneous contractions but this activity was markedly suppressed when the tissue was being field stimulated (Figure 1a). In the presence of TTX (1 μM) spontaneous activity persisted in spite of field stimulation (Figure 1a). Although TTX alone caused a significant reduction in the electrically-evoked response at all frequencies tested, the mean maximum response (at 40 Hz) was reduced by only 58 ± 6% with respect to the control maximum in the same strips (Figure 1c). Field stimulation of TTX-treated strips caused phasic contractions resembling spontaneous ones rather than the well-sustained contractions obtained under control conditions (Figure 1a). Because of the difficulty in distinguishing be-

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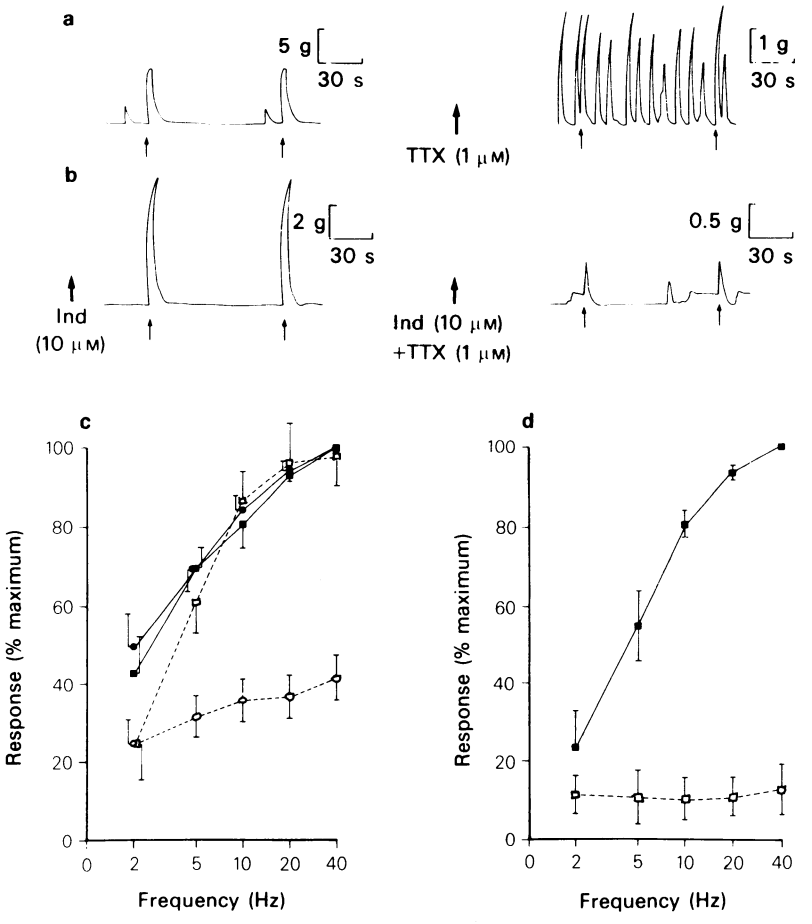

Figure 1 Isometric contractions in strips of muscle from the base of rabbit bladder. (a) Field stimulation-induced contractions (10 Hz at unmarked arrows) before (left) and after (right) tetrodotoxin (TTX, 1 μM). (b) Field stimulation-induced contractions (10 Hz at unmarked arrows) in strips pretreated with indomethacin (Ind, 10 μM) before (left) and after (right) TTX (1 μM). (c) Field stimulation-induced contractions in untreated strips before (●) and after (○) TTX (1 μM, $n = 5$), or before (■) and after (□) indomethacin (10 μM, $n = 4$). Responses are expressed as a percentage of the untreated control maximum. (d) Field stimulation-induced contractions in strips treated with indomethacin (10 μM) before (■) and after (□) addition of TTX (1 μM, $n = 4$). Responses are expressed as a percentage of the maximum achieved after indomethacin treatment. For panels (c) and (d) vertical bars indicate s.e.means. $P < 0.05$; control vs TTX, and TTX (c) vs TTX and indomethacin (d).

tween spontaneous and evoked contractions in TTX-treated strips, an attempt was made to inhibit spontaneous activity in these preparations by pretreatment with indomethacin. Indomethacin alone did not significantly alter the frequency-response curve in preliminary experiments (Figure 1c) but it did suppress the spontaneous activity usually associated with TTX treatment (Figure 1b). Evoked responses in the presence of both TTX and indomethacin were reduced to $13 \pm 6\%$ of control (at 40 Hz) and the magnitude of the contractions was no longer dependent on frequency (Figure 1d). Electrically-induced contractions were significantly larger in strips treated with TTX alone than in strips exposed to both TTX and indomethacin, when expressed as a percentage of their respective control maxima (Figure 1c and d).

Discussion The depression of spontaneous contractile activity during periods of intermittent field stimulation in untreated strips may have been due to release of an inhibitory substance or to refractoriness in the muscle following evoked contractions. After the tissue had been exposed to TTX, the suppression

of spontaneous activity by field stimulation was not observed (see also Slack *et al.*, 1982). Similarly, in the cat stomach, spontaneous contractile activity was found to increase after TTX treatment (Boev, Golenhofen & Lukanow, 1976). These observations imply that TTX may have removed an inhibitory influence normally responsible for suppression of spontaneous activity.

The effectiveness of indomethacin in preventing the increase in spontaneous activity seen with TTX alone implies that prostaglandins or prostaglandin-like substances may be involved in the generation of this activity as has been suggested for detrusor muscle (Abrams & Feneley, 1976, Abrams, Sykes, Rose & Rogers, 1979), among other tissues. If this is indeed the explanation then the usual suppression of spontaneous activity during intermittent stimulation of untreated strips of bladder base muscle may be due to the release of unidentified factors that inhibit prostaglandin synthesis.

TTX substantially reduces evoked contractions in rabbit and guinea-pig detrusor (Downie & Dean, 1977; Krell *et al.*, 1981) but the response to high frequency stimulation in the rabbit bladder base was relatively resistant to TTX treatment. Indomethacin had no effect on field stimulation-induced responses in control strips of the rabbit bladder base in the present study (cf. detrusor, Johns & Paton, 1976), but significantly reduced the magnitude of TTX-resistant evoked contractions. The lack of effect on control strips suggests that the concentration of indomethacin used did not interfere with excitation-contraction coupling. Thus, although we found little evidence for prostaglandin involvement in normally evoked responses in rabbit bladder base, prostaglandins may contribute to contractions evoked in the presence of TTX.

The unmasking of phasic (unsustained) contractions in response to field stimulation of bladder smooth muscle has been observed previously after TTX (Downie & Dean, 1977) or combined atropine and guanethidine treatment (Krell *et al.*, 1981). These TTX-resistant evoked responses resembled ongoing spontaneous activity in the bladder base. Apparently, in the presence of TTX, field stimulation does not elicit a coordinated contraction in the muscle strip but rather 'triggers' a local, perhaps myogenic response. Since these responses were reduced by indomethacin, they may have been due to prostaglandin-mediated sensitization of the muscle (Schulz & Cartwright, 1976) or to enhancement of neurotransmitter release (Schulz & Cartwright, 1976; Daniel, Crankshaw & Sarna, 1979). Direct muscle membrane depolarization by the stimulus seems unlikely since the stimulation parameters were modest (see Downie & Dean, 1977) and the response was not sustained. Further work will be necessary to clarify the mechanism underlying the TTX-resistant indomethacin-sensitive contractions in the rabbit bladder base.

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