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POTENTIATION OF NERVE-INDUCED BLADDER RESPONSES BY TETRAETHYLAMMONIUM IN RELATION TO JUNCTIONAL AND EXTRAJUNCTIONAL MUSCARINIC RECEPTORS

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1 The single stimulus responses elicited in the rat urinary bladder were enhanced up to 3 fold by tetraethylammonium chloride (TEA) in the range 2.5 to 10 mM. Responses elicited by repetitive stimulation at 20 Hz were potentiated much less by 2.5 and 5 mM TEA; 10 mM TEA depressed the responses to less than control levels.

2 The responses elicited in preparations treated with tetrodotoxin or botulinum toxin during the 20 Hz stimulus trains were one third to one half of control, while single stimulus responses were abolished altogether. After 10 mM TEA the response to the 20 Hz stimulus trains were near control but the single stimulus responses were not restored at all.

3 The single stimulus response of control and of TEA-treated bladder preparations were unaffected by atropine (2×10^{-6} M) but responses elicited by a 20 Hz stimulus train were reduced more than 40% by atropine. After 5 mM TEA the responses to the 20 Hz stimulus trains that had been partially blocked by atropine were immediately restored to near control levels.

4 The responses of bladders to carbachol were dose-dependent in the range 10^{-6} to 10^{-5} M and were atropine-sensitive. After 5 mM TEA the means of the responses produced by graded doses of carbachol were less than control; muscarinic receptors that were blocked by TEA are probably also atropine-sensitive.

5 It is suggested that muscarinic receptors in the rat urinary bladder may be divided into: (1) junctional receptors that are resistant to atropine and may be indirectly affected by TEA and (2) extrajunctional receptors that are blocked by atropine and may be directly affected by TEA.

Introduction

The parasympathetic innervation of the urinary bladder seems to be different from most other autonomic effectors which are innervated by cholinergic nerves because large amounts of atropine and other antimuscarinic drugs can only partially block nerve-induced contractile responses of this organ (Ursillo & Clark, 1956; Chesher & Thorp, 1965). The toxin of *Clostridium botulinum* is partially effective as a blocking agent although the mechanism of the blockade is to reduce acetylcholine release from the nerve terminal (Carpenter, 1967).

The action of blocking agents on autonomic effectors is frequently studied on agonist-induced contractions of various muscle preparations in an organ bath. However, there are marked differences between the contractile responses *in vitro* of the rat urinary bladder elicited by a cholinomimetic drug and the responses produced during repetitive stimulation of its motor nerves. The latter may be graded in magnitude by varying the stimulus rate between 1 and 20 Hz. In the range 1 to 5 Hz the responses undergo a visible mechanical summation; all-or-none contractions occur coincident with each stimulus pulse. The single stimulus responses are not abolished by high concentrations of atropine (10^{-5} M) while the responses to a maximal dose of a cholinomimetic drug are abolished by only 5×10^{-7} M atropine. A further difference between the two types of response is related to the magnitude; at the optimal stimulus rate the nerve-induced responses are 50% greater than those resulting from the cholinomimetic drug (Carpenter, 1967).

At low stimulus rates the nerve-induced responses of a smooth muscle innervated by adrenergic nerves have been shown to be greatly potentiated by tetraethylammonium chloride (TEA) (Gillespie & Tilmisany, 1976). In this paper the action of TEA on

nerve-induced responses of the rat bladder is compared with its action on responses elicited by a cholinomimetic drug. These two modes of excitation may depend upon different postjunctional receptors. The nerve terminal may be in such close association with a certain fraction of the receptor population that these receptors are inaccessible to atropine; or the concentration of transmitter to which this fraction of receptors is exposed may be very high in comparison to those receptors that are blocked by atropine.

By its action on transmitter release, TEA should therefore potentiate the responses of the bladder that are elicited by single stimuli. Furthermore, if the presence of a high transmitter concentration is indeed a factor in atropine resistance of the bladder, the responses elicited in the presence of TEA should also be resistant to atropine. Finally, one might expect that TEA should reverse the action of *Clostridium botulinum* toxin on the bladder as has been shown by Lundh, Leander & Thesleff (1977) on the rat neuromuscular junction.

Methods

Urinary bladders from adult male rats (450 to 500 g) were excised at the ureteral-vesical junction and tied to a small glass cannula. A platinum electrode was placed within this cannula and another platinum electrode, in the shape of a coil, surrounded the preparation after it was mounted in an organ bath maintained at 32°C. The bathing solution itself was of the following composition (mM): NaCl 145, KCl 2.7, $CaCl_2$ 2, $MgCl_2$ 0.25, MOPS buffer (morpholino propane sulphonic acid buffer adjusted to pH 7.3) 10, glucose 20. Contractile responses of the bladder were elicited *in vitro* by 'transmural' stimulation; electric pulses (1 ms) of suprathreshold intensity (25 mA) were applied as single pulses or as trains of pulses at a rate of 20 Hz to the electrodes (Carpenter, 1963).

The resting pressure and the pressures that developed during stimulation were measured under isometric conditions since a constant intraluminal volume of 0.10 ml was maintained during a response. A pressure transducer and a carrier wave amplifier were used to record each response on an inkwriting oscillograph. The pressure changes associated with a response were also recorded photographically from a cathode ray oscilloscope.

Since the bladder does not contract after its postganglionic nerves have degenerated, excitation of this organ by electric pulses must be regarded as indirect and mediated by acetylcholine (Carpenter & Rand, 1965). Indeed this transmitter appears in the bathing solution containing physostigmine in proportion to the rate at which stimuli are applied (Carpenter & Rand, 1965).

Type A *Clostridium botulinum* toxin was administered as an acetate buffered solution containing 2 mg of the crystalline protein. Doses 100 to 1000 times larger than the LD were administered intraperitoneally to the rats to reduce the time required for the onset of symptoms to 4 to 6 h; 1000 × LD of type A toxin was found to produce terminal symptoms of botulism within 3 to 5 h (Carpenter, 1967).

After the animals were anaesthetized, the gastrocnemius muscle was indirectly stimulated through the sciatic nerve. A heavy steel needle was inserted in the knee and fixed to a frame which supported both the animal and the nerve electrodes. The muscle tendon was attached to a force transducer; recordings were made of the force developed by the muscle while undergoing stimulation for 1 s at a frequency of 100 Hz.

Overt signs of limb paralysis were rare during the terminal stage of botulism, whether the animals had been treated with large or small doses of toxin. In the terminal state the rats would right themselves at once when placed in the supine position.

After a variable latent period, the motor response diminished progressively to zero. The response of the muscle was equal to that of controls after spontaneous breathing had stopped. Hence, ventilatory arrest did not coincide with a generalized paralysis of skeletal muscle. Total blockade of neuromuscular transmission only developed in those animals that had been sustained by artificial ventilation. Twenty rats receiving 1000 × LD of type A toxin developed total blockade in an average of 5 h.

Drugs

Type A *Clostridium botulinum* toxin was obtained as a solution of the crystalline protein from the U.S. Army Biological Laboratories, Fredrick, Maryland. Diallybarbituric acid and urethane (Ciba), tetraethylammonium chloride (Eastman), carbamylcholine chloride (carbachol, Sigma), atropine sulphate, MOPS buffer (Calbiochem), tetrodotoxin (Sigma), and tetramethylammonium iodide (K & K) were routinely used throughout the experiments.

Results

Potentiation of nerve-induced responses by tetraethylammonium chloride

Single electric pulses applied across the bladder wall produced contractile responses of the rat urinary bladder ranging in magnitude between 14 and 25 cmH_2O pressure. In 37 measurements of untreated preparations the mean response amounted to 18 ± 1 cm. Figure 1 is a recording of an experiment showing

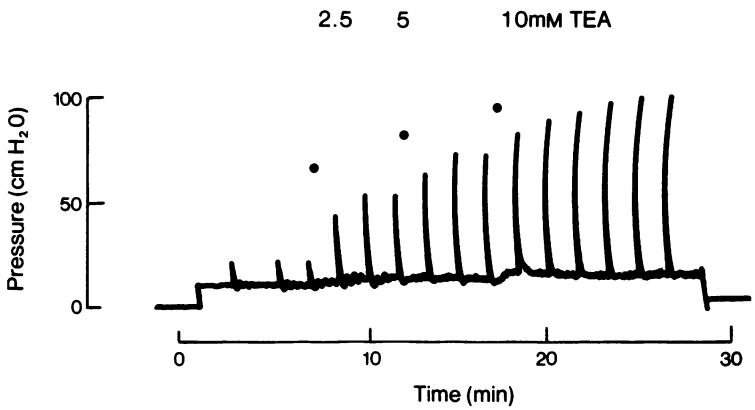

Figure 1 Phasic contractions of the rat urinary bladder *in vitro* elicited by single square wave pulses applied uniformly across the wall of the organ by coaxial electrodes. Potentiation of the initial responses by tetraethylammonium chloride (TEA) can be seen after the concentration was increased to 2.5, 5 and 10 mM as indicated above the recording. Ordinate scale: pressure in cmH_2O. Time (min) is shown below the record.

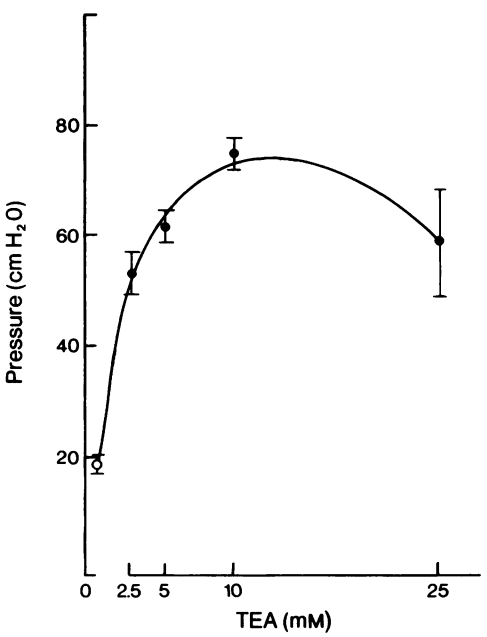

Figure 2. Potentiation of single stimulus phasic contractions of the rat bladder by 2.5, 5, 10 and 25 mM tetraethylammonium chloride (TEA) in the bathing solution (abscissa scale). Ordinate scale: pressure in cmH_2O. The mean values (n = 28) for control (○) and for each concentration of TEA (●) are shown. Vertical lines show s.e. means.

a number of control responses and the responses which were produced in the presence of 2.5, 5.0 and 10 mM TEA. The mean responses of 28 preparations before and after 2.5, 5.0, 10 and 25 mM TEA are plotted in Figure 2. All these values were found to differ significantly and it seems quite clear that 10 mM TEA represents the optimum concentration for potentiation since the mean responses after 25 mM TEA were significantly less than those treated with 10 mM.

The action of TEA on nerve-induced responses appears to be highly specific since mecamylamine, 0.5 mM, a ganglionic blocking agent far more potent than TEA and tetramethylammonium iodide (TMA) a structural analogue, failed to increase the response height of the bladder responses to single stimuli. In fact, TMA in the same concentrations elicited sustained contractions of the bladder similar to a cholinomimetic agent; this action of TMA was effectively prevented by atropine, 5×10^{-7} M.

The contraction of the rat bladder during repetitive stimulation at 20 Hz represents the maximum response of the organ and at this stimulation frequency pressures as high as 200 cm of water were developed (mean 168 ± 8 cmH_2O). These responses were not potentiated as much by TEA as the responses to single stimulus pulses and less TEA was needed in the bathing solution to produce a significant increase of the responses above control. However, when the TEA concentration was increased to 10 mM the mean response was diminished significantly to a value much less than control.

Carbachol-induced contractions of the rat bladder and tetraethylammonium

The addition of carbachol to the bathing solution produced tonic contractile responses of the bladder

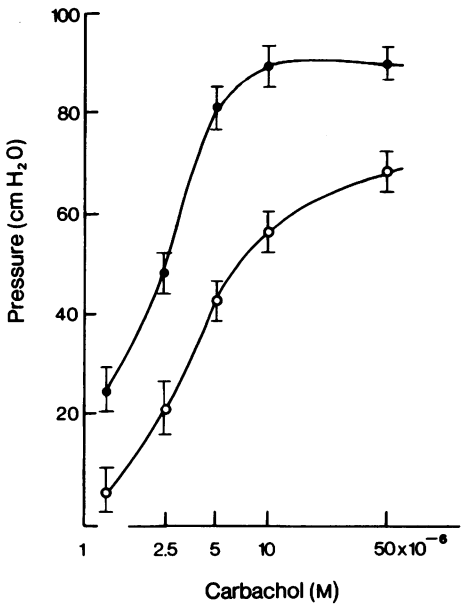

Figure 3 Dose-response curves produced by rat urinary bladder preparations *in vitro* to increasing concentrations of carbachol (abscissa scale); ordinate scale: pressure in cmH_2O. (●): Mean pressure produced by untreated preparations; (○): mean pressure produced by preparations treated with 5 mM tetraethylammonium chloride. The s.e. means (n = 12) are indicated by vertical lines.

in proportion to the agonist concentration. A control dose-response curve for the rat bladder as shown in Figure 3 was obtained from 12 preparations that were progressively exposed to increasing doses of the cholinomimetic; each addition of agonist was made when the previous response had reached maximum height. Contractions which followed the highest concentration of carbachol (5×10^{-5} M) produced much less elevation in pressure than the responses produced during repetitive stimulation at 20 Hz. Thus, the maximum response to 5×10^{-5} M carbachol by any of the preparations was 120 cmH_2O and in the 12 experiments that were performed the mean response amounted to 90 $\pm$ 1 cmH_2O. Bladder contractions elicited by this cholinomimetic were not potentiated by TEA. In fact the presence of TEA (5 mM) in the bathing solution before the addition of the graded doses of carbachol, shown on the abscissa scale, resulted in substantially lower responses than the controls; the dose-response relation was displaced to the right by the TEA and the maximum response was apparently depressed. The action of carbachol is believed to be mediated entirely by muscarinic receptors because intramural ganglia are not present in the rat urinary bladder (Carpenter & Rand, 1965). This finding supports those of others namely, that TEA is an antagonist of muscarinic receptors which normally undergo stimulation by a cholinomimetic drug (Gillespie & Tilmisany, 1976).

Restoration by tetraethylammonium of nerve-induced responses of the bladder blocked by tetradotoxin or by botulinum toxin

A variety of pharmacological agents will abolish or at least diminish substantially the contractile response of the isolated urinary bladder of the rat elicited by electrical stimulation of its motor nerves. Tetrodotoxin (TTX, 1×10^{-8} M) was partially effective as a blocking agent some 20 min after it was added to the bathing solution (Figure 4). The mean response of 5 preparations to repetitive stimulation was reduced from 168 $\pm$ 11 cmH_2O to 50 $\pm$ 6 cmH_2O. However, it should be emphasized that the responses of the bladders to single pulses were completely blocked by TTX but the responses to carbachol were unaffected. Shortly after each of the 5 preparations was treated with TEA (5 mM) their ability to respond to the stimulus trains was dramatically restored (Figure 4); the mean response some 10 min after the drug was added was 143 $\pm$ 6 cmH_2O.

Bladders removed from botulinum intoxicated rats maintained by positive pressure ventilation were less responsive to electrical stimulation than those from control animals. After a single pulse was applied to the electrodes there was no visible sign of a contraction in any of the 11 preparations. After a 5 s train of stimuli was applied at 20 Hz to the same bladders the contractions were only 20% of control, the mean response being 33 $\pm$ 6 cmH_2O. TEA in the range of 2.5 to 10 mM did not affect the responses of the treated preparations to single pulses; there was no suggestion of any reversal or restoration of the contractile properties of the bladders to single pulses. However, in the same range of concentrations TEA was very effective in restoring the response of the bladders to repetitive stimulation. The bladder responses of toxin-treated rats were dramatically enhanced by TEA in proportion to its concentration in the bathing solution. A recording of one of the experiments in Figure 5 illustrates the extent of the potentiation by TEA after the concentration of this agent was increased from 0 to 2.5 and 5.0 mM respectively. In Figure 6 the mean response of the 11 toxin-treated preparations are shown together with the subsequent response at different concentrations of TEA on the lower curve. In the same figure the action of TEA on untreated preparations undergoing repetitive stimulation at 20 Hz is shown on the upper curve. It should be noted that 10 mM TEA clearly diminished the mean response of bladders taken from

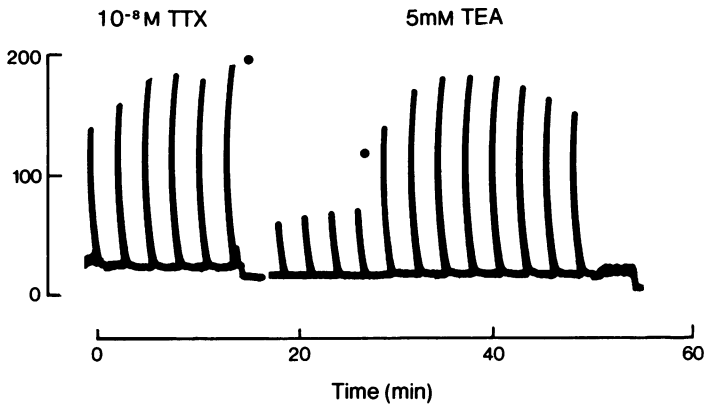

Figure 4 Contractile responses of the rat urinary bladder *in vitro* elicited by a 5 s train of square wave pulses applied at 20 Hz. The addition to the bathing solution of tetrodotoxin (TTX) 1×10^{-8} M, is indicated by the first dot above the recording. Tetraethylammonium chloride (TEA) equal to 5 mM in the bathing solution, was added at the second dot. Ordinate scale: pressure in cmH_2O. Time (min) is shown below the record.

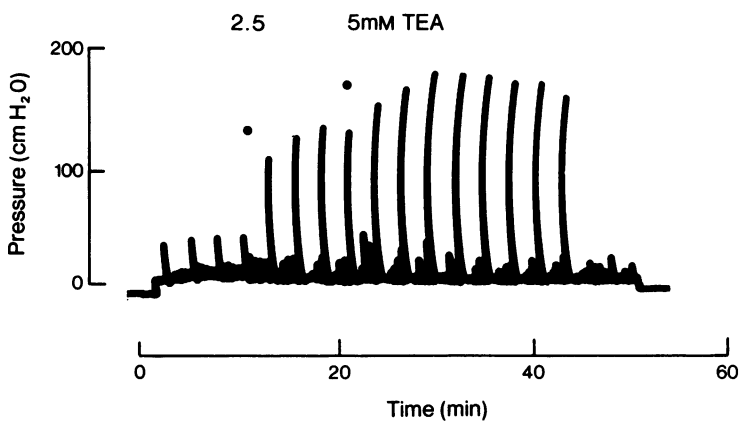

Figure 5 Contractile responses of the rat urinary bladder *in vitro* elicited by a 5 s train of square wave pulses applied at 20 Hz. The preparation was removed from an animal treated with 1000 × LD of crystalline type A *Clostridium botulinum* toxin. Tetraethylammonium chloride (TEA) equal to 2.5 and 5 mM in the bathing solution, was added at the first and second dot respectively.

control animals as compared to the action of 2.5 mM TEA. In contrast the effect of 10 mM TEA on toxin-treated animals was not significantly different from that produced by 5 mM TEA (Figure 6, lower curve).

The action of tetraethylammonium on bladder preparations treated with atropine sulphate

Contractile responses of the rat bladder to single stimulus pulses are unaffected by atropine in amounts sufficient to prevent the contractions produced by an exogenous cholinomimetic agent; 5×10^{-7} M atropine represents the minimum concentration needed to block a maximum dose of carbachol (5×10^{-5} M). Indeed, responses elicited by as many as 5 pulses at 20 Hz were not diminished as significantly by atropine as those elicited by a 5 s train of stimuli applied at the same frequency (Carpenter, 1977). Furthermore, atropine did not interfere with the potentiation by TEA of the responses elicited by single stimuli. In the presence of 10 mM TEA and 10 times the blocking concentration of atropine (5×10^{-6} M) 12 bladder

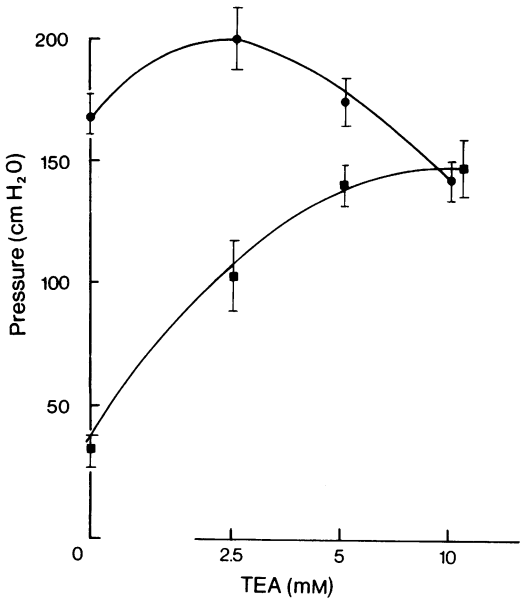

Figure 6 The effect of tetraethylammonium chloride (TEA) on contractile responses of the rat urinary bladder produced *in vitro* by 5 s stimulus trains at 20 Hz. (●): Mean responses of control preparations before (on vertical scale) and after 2.5, 5 and 10 mM TEA (abscissa scale); (■): mean responses of preparations removed from rats treated with type A *Clostridium botulinum* toxin in the absence (on vertical scale) and presence of the same concentrations of TEA. Ordinate scale: pressure in cmH_2O. The s.e. means are indicated by vertical lines.

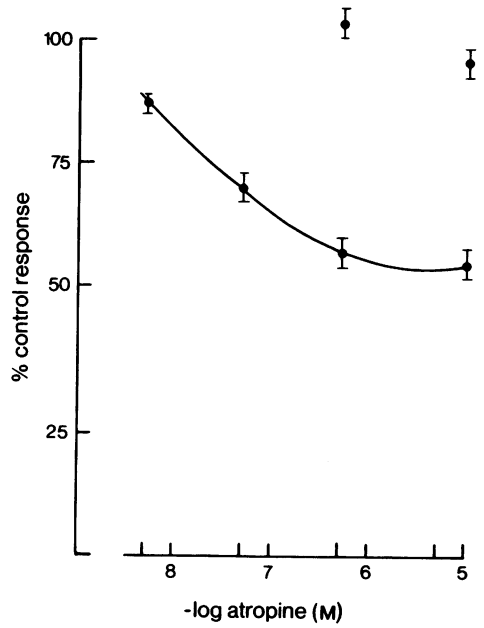

Figure 7 The effect of atropine on contractile responses of the rat urinary bladder produced *in vitro* by 5 s stimulus trains at 20 Hz. The mean responses expressed as % of control, in the presence of 5×10^{-9}, 5×10^{-8}, 5×10^{-7} and 10^{-5} M atropine (abscissa scale) are shown. The two points above the curve are the mean of the responses that were produced after TEA was added in an amount equal to 5 mM in the bathing solution. The s.e. means are shown by vertical lines.

preparations produced a mean pressure of 79 ± 3 cmH_2O after single stimuli were applied. This did not differ significantly from the mean of 27 control preparations (75 ± 3 cmH_2O) treated with TEA alone (Figure 2).

Less than half of the response by a bladder preparation to 5 s stimulus trains is atropine-sensitive. The mean response of 11 preparations was reduced from 180 ± 7 cmH_2O to 105 ± 7 cmH_2O after 5×10^{-7} M atropine. In Figure 7 the effects of 4 different concentrations of atropine on the responses to stimulus trains are expressed as percentages of the control response. With respect to the effective concentration of atropine required for blockade of the 'atropine-sensitive' response, there was no significant difference between 5×10^{-7} and 1×10^{-5} M atropine sulphate (Figure 7). The action of TEA on the atropine-sensitive response is shown by the two points above the concentration-response curve; the partial blockade caused by 5×10^{-7} M and 1×10^{-5} M atropine was relieved by 5 mM TEA. In both cases there was no significant difference between the response after TEA of the atropine-blocked preparations and the control untreated response; the mean response (105 ± 7 cmH_2O) of the atropine-treated preparations was increased to 189 ± 8 cmH_2O after 5 mM TEA.

Figure 8 shows oscilloscope recordings of the pressure that developed during stimulation of the same bladder preparation by 5 s stimulus trains at 20 Hz. A response of the untreated bladders was first recorded (middle trace) followed by another response produced 5 min after the bladder was treated with 10^{-5} M atropine (lower trace). The 3rd response (upper trace) was made after 5 mM TEA had been present in the bathing solution for 5 min. The magnitude of the first contraction was substantially reduced by the atropine, especially during the late phase of the response. However, atropine did not seem to interfere with the response as it developed initially during the first 2 s of the stimulus train. The blocking action of atropine was manifested later during the peak of the contraction; the muscle appeared to be

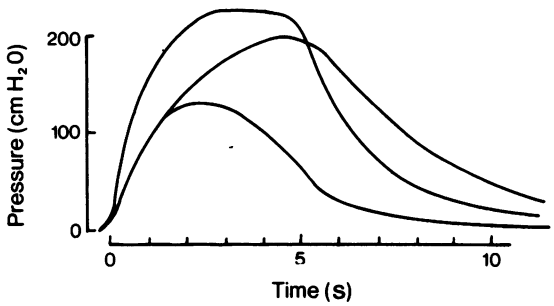

Figure 8 Oscilloscope recording of the contractile responses to 5 s stimulus trains by the rat bladder. The response of the control untreated preparations is shown on the middle trace and the response after 1×10^{-5} M atropine sulphate appears on the lower trace. On the upper trace of the recording is the response produced thereafter following the addition of TEA equal to 5 mM in the bathing solution. Pressure (cmH_2O) is shown on the vertical and time (s) on the horizontal scale.

undergoing relaxation during the final 3 s of stimulation. In the first or the control records the responses were maintained throughout the repetitive stimulation. After TEA was added to the bathing solution the responses of the partially blocked preparations occurred much more rapidly than before and as is also shown in Figure 7, developed greater pressures. Accordingly the apparent effect of TEA was to 'overcome' or to antagonize the atropine blockade; after TEA the atropine-treated bladder did not relax during the stimulus train as was characteristic of the response before it was added. However, it should be pointed out that when the stimulus train ceased the pressure decreased rapidly to a resting level similar to that of the untreated control. That the TEA response occurs more rapidly in the presence of atropine would indeed suggest that a considerable fraction of the muscarinic receptors are totally unaffected by atropine.

Discussion

Single stimulus responses of various skeletal muscles are potentiated by a number of pharmacological agents namely; veratrine (Dun & Feng, 1940), guanidine, 4-aminopyridine (Lundh *et al.*, 1977), and TEA (Koketsu, 1958). Their mechanism of action is generally attributed to a greater than normal release of acetylcholine by the nerve terminals. TEA has been shown to occlude the potassium channels of excitable membranes (Armstrong & Hille, 1972) which prolongs repolarization and the inward migration of calcium ions. Accordingly the release of transmitter is thereby extended (Lundh *et al.*, 1977).

In the rat bladder the most significant consequence of an enhanced transmitter release was manifested in the single stimulus responses. In contrast, responses elicited during the stimulus trains were affected the least. However, when junctional transmission was partially blocked by the toxins, the action of TEA was of no benefit to the single stimulus responses while the responses to the stimulus trains were nearly as large as control. However, there is a limit to the potentiation by TEA not only of the toxin-treated preparations but also of the responses to the stimulus trains. When 10 mM TEA was present in the bathing solution the responses of control preparations were depressed markedly especially as stimulation was continued (Figure 6). The antimuscarinic action of TEA may have been responsible for the diminished response to the stimulus trains. Thus TEA may exert a direct action on the muscle membrane quite apart from its effect upon transmitter release. The weak curariform action of TEA at the neuromuscular junction in the frog (Koketsu, 1958) suggests that it may also exert an antimuscarinic effect, especially at high concentrations. Indeed Feldberg (1951) noted just such an effect on the guinea-pig ileum; acetylcholine and pilocarpine responses were blocked by TEA. However, responses of the ileum and uterus to angiotensin (Prado & Carlini, 1959) and of arterial strips to noradrenaline and adrenaline were potentiated by TEA (Lum & Rashleigh, 1961).

The response to the stimulus trains were slightly greater in magnitude after 2.5 mM TEA than the control responses (Figure 6) and were initiated more rapidly; the peak of the responses was reached sooner. This enhancement also occurred in the presence of atropine (Figure 7) so it is reasonable to believe that the potentiation is restricted to the atropine-resistant receptor population (junctional). Similarly, single stimulus responses when maximally potentiated by 10 mM TEA were completely unaffected by atropine (5×10^{-7} M).

The responses to repetitive stimulation were reduced 43% after 5×10^{-7} M atropine (Figure 7) but the initial rate of rise during the first 2 s of the stimulus trains was unchanged. The recordings in Figure 8 suggest that a certain fraction of the receptors which combined with the transmitter somewhat later during the response was blocked by atropine; the rapid rise in pressure of the untreated preparation (middle tracing) was abruptly terminated by atropine (lower trace). Although the stimuli were applied for 5 s the pressure continued to decline (Figure 8). Since atropine did not diminish the initial velocity of the response it is probably mediated by the atropine-resistant receptors while the later portion of the response is normally mediated by receptors that are blocked

by atropine. Thus, both receptor groups participate in the response of the bladder to the stimulus train but only the atropine-resistant receptors mediate the single stimulus responses. The relief provided by TEA from the partial atropine blockade of the response to the stimulus trains could be the consequence of an increased release of transmitter since atropine and TEA are both antimuscarinic.

The reversal of the partial atropine blockade might also be the result of a 'fast' antagonist effect of TEA; an interaction between two antagonists which dissociated from receptors at different rates has been described by Ginsborg & Stephenson (1972). Accordingly, if muscarinic receptors are occupied by atropine they are, of course, unavailable to an agonist but the antagonist nevertheless undergoes dissociation very slowly. Some antagonists however dissociate very rapidly from the receptors (fast). In the presence of the 'slow' antagonist the 'fast' antagonist would allow more of the agonist to combine as a consequence of the rapid dissociation. Indeed, Ginsborg & Stephenson proposed that the addition of a second agonist would increase rather than decrease an agonist-induced response, providing the second antagonist dissociates more rapidly than the first. The effect of TEA on responses of the bladder treated with atropine could thus be based upon a 'fast' antagonist action of TEA on muscarinic receptors.

It is reasonable to ask if the atropine-resistant responses are mediated by a transmitter other than acetylcholine. It seems most unlikely that such a transmitter is released by single stimulus and/or low frequency stimuli while acetylcholine is released by the 20 Hz stimulus trains. The receptors immediately beneath the nerve terminal are believed to be separated functionally from those receptors that are some distance away from the terminal. The former are either not vulnerable to the atropine, owing to the high concentration of transmitter or they are not accessible to atropine, owing to the close proximity of the terminal to the postjunctional membrane. The atropine-sensitive or extrajunctional receptors may become activated by the transmitter only during the late stage of the response (Carpenter, 1977). The role of extrajunctional receptors in the potentiation of the responses to 20 Hz stimulus trains would undoubtedly be minimal since TEA at least partially blocks those muscarinic receptors that respond to carbachol. Otherwise, more potentiation from the enhancement of mediator release by TEA might have occurred.

TEA restored neuromuscular transmission in skeletal muscle following a complete paralysis caused by botulinum toxin to almost normal twitch and tetanic responses (Lundh *et al.*, 1977). Relief by TEA was greatest when the calcium concentration amounted to 4 mM but there was no reversal when the calcium concentration was maintained at 2 mM, the same concentration as was used in these experiments on the bladder. The acetylcholine release system at the neuromuscular junction may still be intact after botulinum toxin even though its sensitivity to calcium may be diminished (Lundh *et al.*, 1977). The level of calcium ions in the nerve terminal is probably increased by TEA which enhances the release of transmitter sufficiently to overcome the blockade.

The transient sodium channels of the motor nerves in the bladder were incompletely blocked by TTX (Figure 4) since the responses to the stimulus trains were not totally abolished after it was added to the bathing solution. Further, TTX has little direct effect upon the release of acetylcholine (Katz & Miledi, 1969) or upon the postjunctional muscarinic receptors of the smooth muscle fibres (Narahashi, 1974). It is likely therefore that the magnitude of the nerve terminal spike was reduced by TTX and the release of acetylcholine was diminished accordingly. The action of TEA in relieving the block would be to prolong the spike such that the amount of acetylcholine released would return to normal. Thus, in this instance and throughout these experiments it is proposed that the primary action of TEA is to increase transmitter release from the motor nerve terminal. A postjunctional antagonist effect has however been demonstrated upon agonist-induced responses of the rat bladder and may contribute to reversal of atropine blockade.

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