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Effects of some drugs on the responses of the rat isolated, innervated urinary bladder to indirect electrical stimulation

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Summary

1. The effects of some drugs known to inhibit transmission in the superior cervical ganglion and at the neuromuscular junction were investigated on the cholinergic nerve-smooth muscle junction, using the rat isolated innervated urinary bladder preparation.

2. HC-3 and Win 4981 inhibited the indirectly evoked contractions; the block was typically slow in onset, depended on the rate of stimulation and was partially reversed by choline. The moderate coincident inhibition of acetylcholine-responses disappeared after washing the tissue, while the block of the neuronally evoked contractions persisted.

3. Morphine, methylpentynol carbamate, chloral hydrate and strychnine inhibited indirectly evoked contractions without inhibiting the responses to acetylcholine. Paraldehyde inhibited both types of response.

4. Hexamethonium, mecamylamine and tubocurarine had no effect on either type of response. Tetraethylammonium augmented both types of response; the augmentation due to lower concentration was followed by a moderate block of the neuronally evoked contractions.

5. Small concentrations of procaine markedly inhibited responses to acetylcholine and produced a partial block of the neuronally evoked contractions.

6. None of the drugs affected conduction in the isolated phrenic nerve.

7. All the drugs other than paraldehyde and procaine appeared to act at the nerve terminals. The results are generally consistent with the view that HC-3 and Win 4981 act by limiting transport of choline across nerve membrane and that the other drugs act by inhibiting the release of acetylcholine. A reduction in sensitivity of the effector cell membrane may account, wholly or in part, for the action of paraldehyde and procaine.

Introduction

Many drugs of different types impair transmission in the superior cervical ganglion and at the neuromuscular junction. Among such drugs are tetraethylammonium (TEA), procaine and many central nervous depressants (Matthews & Quilliam, 1964) including morphine (Trendelenburg, 1957; Agarwal & Bhargava, 1969), strychnine (McKinstry & Koelle, 1967; Alving, 1961), inhibitors of the synthesis of acetylcholine such as hemicholinum-3 (see Bowman, 1962) and the

more specific blocking drugs such as hexamethonium and tubocurarine. The high degree of congruence in action of these drugs at two cholinergic junctions suggests the interesting possibility of a similar action also at the cholinergic nerve-smooth muscle junction, though this exhibits a remarkably different histology and electrophysiology. This hypothesis appears to be supported by the reports that morphine (Paton, 1957), procaine (Cox & Weinstock, 1966), methylpentynol (Marley, 1959) and strychnine (Takagi & Takayanagi, 1966) reduce the contraction and the acetylcholine output of the coaxially stimulated guinea-pig ileum.

We have now studied the effects of some of these drugs and of *bis* (3-diethyl-aminopropoxy) pyridazine bismethiodide (Win 4981), which exerts hemicholinium-3 like action at the neuromuscular junction (Gesler, Lasher, Hoppe & Steck, 1959), using a different and relatively unexplored cholinergic nerve-smooth muscle preparation, the rat isolated, innervated urinary bladder (Huković, Rand & Vanov, 1965).

Methods

Rat isolated, innervated urinary bladder preparation

The rat bladder preparations (obtained from male or female Norwegian rats weighing from 150 to 300 g) were set up as described by Huković *et al.* (1965) in a 30 ml organ bath and stimulated indirectly as described in **Results**. The bathing medium was Tyrode solution (composition, g/l., NaCl 8·0, KCl 0·2, $CaCl_2$ 0·2, $MgCl_2$ 0·1, NaH_2PO_4 0·1, $NaHCO_3$ 1·0 and glucose 1·0) maintained at 32°–34° C and gassed with 5% carbon dioxide in oxygen. Contractions were recorded with a light isotonic frontal writing lever (magnification, × 12) on a smoked kymograph paper; tension on the tissue was about 0·5 g. The preparations were stabilized for 45 min and were washed 2–3 times during this period.

Responses to added acetylcholine. Anti-acetylcholine activity of a drug at the effector cell membrane was estimated from the ratio of concentrations of acetylcholine required to produce 50% of the maximal contraction in the presence and in the absence of the drug. The ratio was computed from log concentration/contractile effect plots worked out during the control period and then at a time when the drug had maximally inhibited the indirect twitches.

Rat phrenic nerve-diaphragm preparation

This preparation was used to indicate any blocking effect of the drug on nerve conduction as described by Matthews and Quilliam (1964). Both the "nerve" and "muscle" chambers were bathed by Tyrode solution at 32° C and gassed separately by 5% carbon dioxide in oxygen.

Drugs. Acetylcholine chloride, strychnine hydrochloride, morphine sulphate, methylpentynol carbamate, chloral hydrate, procaine hydrochloride, tetraethyl-ammonium bromide, (+)-tubocurarine chloride, hexamethonium chloride, mecamylamine hydrochloride, choline chloride and *bis* (3-diethyl-aminopropoxy) pyridazine bismethiodide (Win 4981) were used throughout the experiments; doses refer to the salts. Hemicholinium-3 (HC-3) was made up fresh in 0·9% sodium chloride solution. Paraldehyde was diluted in Tyrode solution; only fresh dilutions were used.

Results

Rat isolated urinary bladder preparation

Indirect stimulation. Indirect stimulation by rectangular pulses (10–20 V, width 1·0 ms) at 3, 5, 10, 20 or 60 Hz elicited reproducible contractions of the bladder, provided the tissue was rested sufficiently between stimulation periods (Fig. 1). In individual experiments, the height of neuronally evoked contractions gradually declined in the first few stimulation periods, but thereafter it remained fairly constant for 3 to 4 h; during this time individual contractions varied within a range of 6% of the control.

Responses to added acetylcholine. The preparations responded to acetylcholine regularly for 2·5 to 3 hours. The regression of response on log concentration of

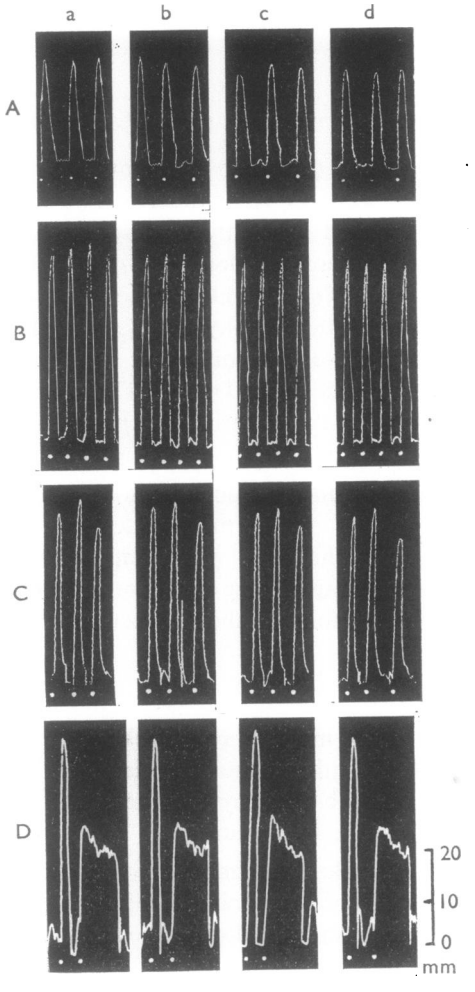

FIG. 1. Rat isolated urinary bladder. At white dots, responses to indirect stimulation (rectangular pulses, 15 V, 1·0 ms) at 10 Hz (50 shocks) in A; at 60 Hz (300 shocks) in B; at 5, 10 and 3 Hz (30 shocks, consecutive in each panel) in C and at 60 and 20 Hz (600 shocks, consecutive in each panel) in D. a, Control responses; b, c and d, responses 50, 100 and 150 min respectively after a.

acetylcholine was linear in the range of 25 to 70% of the maximal contraction (response to 400 μg/ml of acetylcholine). The mean ratio of ED50 of acetylcholine 150 min after setting up the preparation to that during the initial 30 min was 0·931 (S.E., $\pm$0·102, n=16).

Effect of drugs on neuronally evoked and acetylcholine-induced contractions

HC-3 and Win 4981. HC-3 (300 μg/ml) and Win 4981 (100 μg/ml) produced a gradually developing block of neuronally evoked contractions (Table 1a and e). The effect of Win 4981 was preceded by augmentation of contractions. At the time of maximal block responses to added acetylcholine were partially inhibited (Table 1) by both drugs. This finding accords with the reports that HC-3 and Win 4981 exert inhibitory effects on the postjunctional membrane (Gesler & Hoppe, 1956; see also Bowman, 1962). However, the anti-acetylcholine effect of Win 4981 disappeared totally after a single wash while that of HC-3 persisted for 15–20 min; on the other hand, the block of neuronally evoked contractions persisted unchanged for at least 1 h despite repeated washes.

Choline chloride (300 μg/ml) totally antagonized the HC-3-induced block of neuronally evoked contractions in one experiment, caused a recovery by 30–40% in four experiments (Fig. 2A) and was ineffective in one experiment. Choline antagonized the blocking effect of Win 4981 by 25–40% in five out of six experiments (Fig. 2B).

Lower concentrations of HC-3 or Win 4981 were without much effect (Table 1b and f), and the block was also slight if the number of shocks delivered per stimulation period was smaller (Table 1c and g); this accords with the findings of Huković *et al.* (1965) with HC-3. Increasing the number of shocks per stimulation period beyond 300 (Table 1d and h) did not further influence the intensity of the blockade due to HC-3 or due to Win 4981 (Fig. 3).

Strychnine, morphine, methylpentynol and paraldehyde. Neuronally evoked contractions were blocked by all drugs to a variable extent (Table 2). A similar

TABLE 1. *Influence of hemicholinium-3 and Win 4981 on responses of the rat isolated urinary bladder to indirect electrical stimulation and to acetylcholine*

Concentration in bath (μg/ml) (n=number of experiments)	Neuronally evoked responses				ACh responses
	A	B	C	D	
Hemicholinium-3					
(a) 300 (n=7)	300	60 Hz	70 (40–100)	90–120	1·21 $\pm$0·06*
(b) 100 (n=3)	300	60 Hz	18 (12–22)	90–160	—
(c) 300 (n=3)	50	10 Hz	20 (12–30)	60–80	—
(d) 300 (n=3)	600	5 Hz	50 (40–60)	90–120	—
	600	20 Hz	52 (40–60)	90–120	—
Win 4981					
(e) 100 (n=7)	300	60 Hz	65 (50–80)	60–90	1·49 $\pm$0·09*
(f) 30 (n=3)	300	60 Hz	19 (15–26)	60–90	—
(g) 100 (n=3)	60	10 Hz	25 (18–34)	60–90	—
(h) 100 (n=3)	1,200	20 Hz	54 (45–60)	40–60	—
	1,200	60 Hz	49 (40–65)	40–60	—

* Value significantly (P<0·05) different from control. The effect, however, disappeared within a short time after the preparations were washed.
A, Number of shocks per stimulation period. B, Frequency of stimulation (rectangular pulses, 10–15 V, 1·0 ms). C, Mean maximal block (% of control); range in parentheses. D, Time for blockade (min). ACh responses, mean ratio ($\pm$S.E., n=5) ED50 of acetylcholine after: ED50 of acetylcholine before the drug. The ratio in control experiments, 0·931 $\pm$0·102 (n=16).

depth of blockade was noticed in other experiments where effects of strychnine (90 μg/ml, $n=4$), morphine (10 μg/ml, $n=3$, Fig. 4), methylpentynol (200 μg/ml, $n=4$) and paraldehyde (0·004 ml/ml, $n=4$) were studied using slow and fast frequencies of stimulation (3, 5, 20 or 60 Hz) as shown in Fig. 1c.

Paraldehyde differed from the other three drugs in the following respects. (1) Paraldehyde inhibited responses to added acetylcholine (Fig. 5A). Strychnine and methylpentynol did not alter, while morphine enhanced responses to acetylcholine

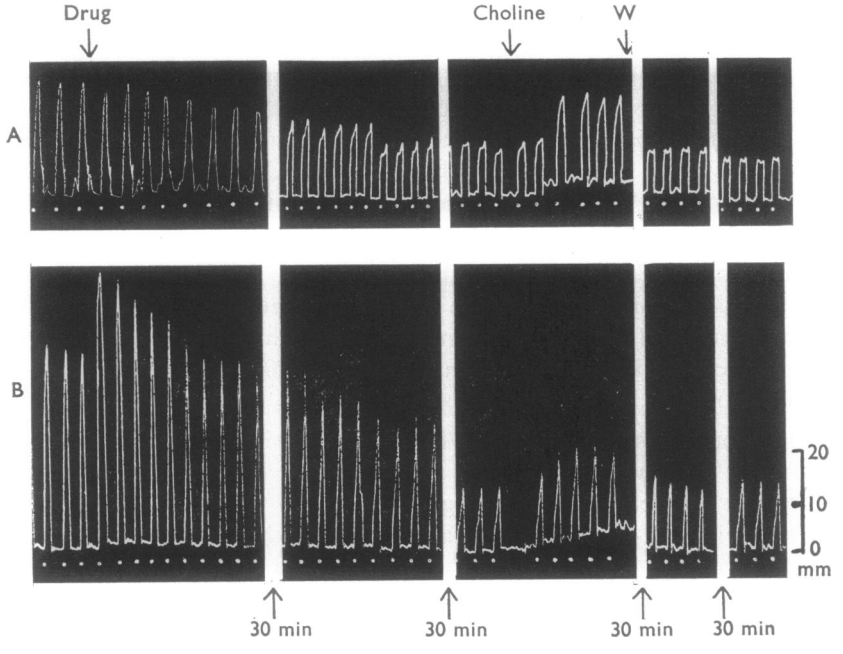

FIG. 2. Rat isolated urinary bladder. Effect of hemicholinium-3 (300 μg/ml in A) and Win 4981 (100 μg/ml in B), added at "drug", on responses (at white dots) to indirect stimulation (rectangular pulses, 10 V, 1·0 ms, 60 Hz for 5 s every 2 min). Choline chloride (300 μg/ml) added at "choline"; W, Wash.

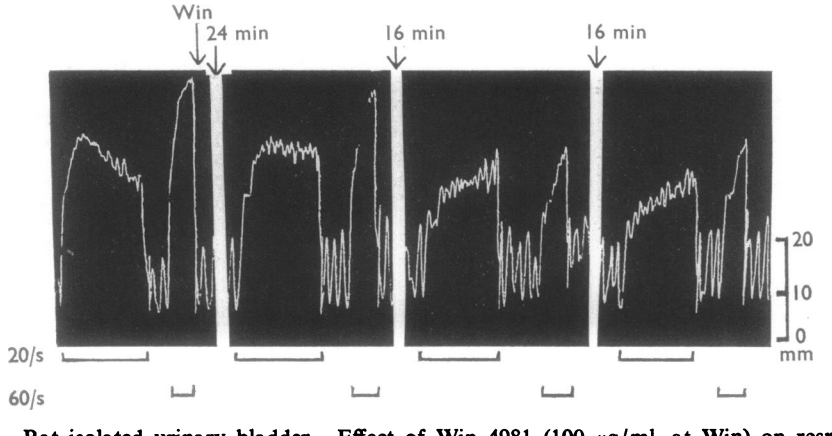

FIG. 3. Rat isolated urinary bladder. Effect of Win 4981 (100 μg/ml, at Win) on responses to indirect stimulation (rectangular pulses, 10 V, 1·0 ms, 1,200 shocks at frequencies indicated below and during the periods shown by bars).

(Fig. 4). (2) In four out of six experiments where paraldehyde had blocked the neuronally evoked contractions by more than 50% of the control, choline chloride (300 μg/ml) restored the contractions to 75–80% of the control (Fig. 5B); choline chloride had no effect on blockade due to the other three drugs. (3) Within 4 to

TABLE 2. *Influence of drugs on the responses of the rat isolated, innervated urinary bladder to indirect electrical stimulation and to acetylcholine*

Concentration in bath	Number of experiments	Neuronally evoked responses a	b	c	ACh responses
Strychnine					
60 μg/ml	5	30–40	45 (43–50)	60–70	—
90 μg/ml	5	10–20	73 (50–80)	20–30	0·943±0·16
Morphine					
10 μg/ml	6	5–10	78 (63–84)	20–30	0·595±0·08 ($P<0·01$)
Methylpentynol carbamate					
100 μg/ml	3	4–10	35 (30–40)	30–40	—
200 μg/ml	8	4–10	68 (62–80)	20–40	0·961–0·07
Paraldehyde					
0·002 ml/ml	4	15–20	28 (20–44)	30–40	—
0·004 ml/ml	12	4–12	76 (60–85)	15–25	1·299–0·07 ($P<0·05$)
Tetraethylammonium					
50 μg/ml	9	15–25	28 (20–32)	25–40	0·747±0·05
Procaine					
10 μg/ml	5	6–12	15 (12–19)	15–25	—
20 μg/ml	5	6–12	21 (16–31)	15–25	2·174±0·17 ($P<0·01$)

Stimulation, rectangular pulses, 10–15 V, 1·0 msec, at 10 Hz for 5 s every 2 min. a, Latency (min); b, mean maximal block (% of control); range in parentheses; c, time for blockade (min) (inclusive of latency period). ACh responses, mean ratio (±S.E., $n=4$) ED50 of acetylcholine after: ED50 of acetylcholine before the drug. P=Probability for the difference between the value and the control value, 0·931±0·102 ($n=16$).

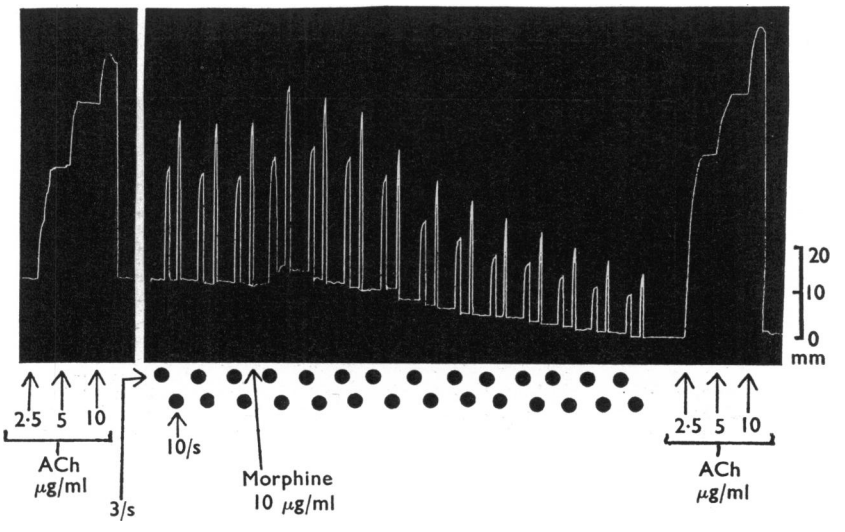

FIG. 4. Rat isolated urinary bladder. Effect of morphine on responses to cumulative administration of acetylcholine (ACh) and to indirect stimulation (rectangular pulses, 10 V, 1·0 ms, 30 shocks at frequencies indicated below) at black dots.

10 min of a single wash, the effects of paraldehyde disappeared nearly completely (Fig. 5A); blockade due to other drugs was much more persistent.

Chloral hydrate. Chloral hydrate (10 μg/ml, $n=8$) reduced the height of neuronally evoked contractions by 30–50% of the control. The effect was seen after 30 to 45 min and was maximal in 60 to 90 min after addition of the drug; the blockade was comparable for both the slow (10 Hz) or fast (60 Hz) frequencies used to deliver either 120 or 300 shocks per stimulation period. At this time, responses to acetylcholine were enhanced, the ratio ED50 after: ED50 before the drug being $0{\cdot}699 \pm 0{\cdot}098$ (the ratio in control experiments, $0{\cdot}931 \pm 0{\cdot}102$). Choline chloride (300 μg/ml) had no effect on the blockade of neuronally evoked contractions ($n=3$). The blockade disappeared nearly completely in 20–30 min after wash.

Ganglion blocking drugs and tubocurarine. Hexamethonium (200 μg/ml, $n=5$), mecamylamine (50 μg/ml, $n=5$) or tubocurarine (20 μg/ml, $n=4$) did not affect the neuronally evoked contractions induced either by a slow (10 Hz) or fast (60 Hz) stimulation delivering either 120 or 300 shocks per stimulation period for approximately one hour. Responses to acetylcholine were also unaffected.

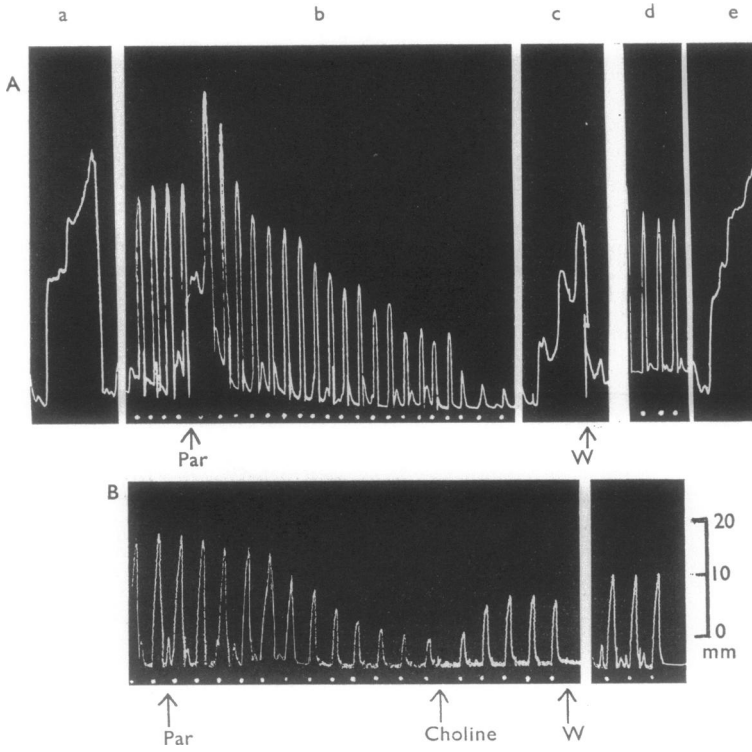

FIG. 5. Rat isolated urinary bladder. A, At a time when the responses to indirect stimulation (rectangular pulses, 15 V, 1·0 ms, at 10 Hz for 5 s every 2 min; at white dots) were severely reduced (panel b) by paraldehyde (0·004 ml/ml, added at Par) the responses to acetylcholine (cumulative administration of 2, 4 and 8 μg/ml) were partially inhibited (panel c; compare with control panel a). Six min after washing out the drug (at W) responses to indirect stimulation and to acetylcholine were considerably restored (panels d and e respectively). B, Blockade of neuronally evoked contractions (stimulation as in A) by paraldehyde (0·004 ml/min, added at Par) was partly antagonized by choline chloride (300 μg/ml, added at Choline; panel a). There was a further recovery of contractions 6 min after washing out (W) the preparation (panel b).

Tetraethylammonium. TEA (50 μg/ml) immediately enhanced the height of neuronally evoked contractions by 15 to 20% of the control. The enhancement was gradually followed by a moderate blockade (Table 2). The blockade was stable in presence of the drug in five experiments, while in four it tended to subside spontaneously. At the time of maximal blockade, responses to acetylcholine were somewhat enhanced. Choline chloride (300 μg/ml) had no effect on the blockade ; however, recovery was complete within 10 to 20 min after the wash.

TEA (500 μg/ml, $n=5$) markedly raised the tonus of the preparations ; the height of neuronally evoked contractions was immediately augmented by 20 to 50% of the control. The changes in the tone and height of contractions subsided to near control levels within a short time if the drug was washed out or within 20–30 min of continued stimulation in the presence of the drug. In the latter case, however, further stimulation for periods up to one hour did not reveal any blocking activity of the drug.

Procaine. Blockade of neuronally evoked contractions due to procaine (10 or 20 μg/ml) was only moderate (Table 2) and was unaffected by choline (up to 400 μg/ml). Procaine considerably inhibited the responses to acetylcholine. Procaine (20 μg/ml, $n=3$) blocked the effect of slow (5 Hz) and fast (20 Hz) stimulation to the same extent as it blocked the effect of stimulation at 10 Hz (Table 2). The effects of procaine disappeared in 10–20 min after washing the preparations repeatedly.

Rat phrenic nerve-diaphragm preparation

When added to the " nerve bath ", none of the drugs (in the highest concentrations used in bladder experiments) had any effect on the indirectly induced contractions of the diaphragm for 30 to 40 min as revealed in three experiments with each drug.

Discussion

All the drugs other than paraldehyde and procaine, which blocked the indirectly evoked contractions of the bladder, appear to act exclusively at a prejunctional site as they did not reduce the sensitivity of the effector cell to added acetylcholine. The drugs did not affect conduction in the nerve trunk, but an action at the nerve terminals is a possibility, since the trunk and the terminal differ in their physiological and pharmacological properties. Consequently, it should be considered whether the drugs affect the neurosecretory mechanism or the traffic of nerve impulses at the nerve terminals.

Our results are consistent with the concept that HC-3 and Win 4981—which had a qualitatively similar action on the bladder—act by limiting the carrier-mediated transport of choline to the intraneuronal sites of acetylcholine synthesis. The brisk initial augmentation of neuronally evoked responses by Win 4981 could be due to a faster initial release of preformed transmitter from the nerves. Such an action has been shown in the superior cervical ganglion for the quaternary ammonium compounds, HC-3 and hexamethonium (Matthews, 1966).

The inference that morphine, methylpentynol carbamate, chloral hydrate and strychnine act prejunctionally to block the neuronally evoked responses of the rat bladder, accords with the reports that these drugs inhibit the output of acetylcholine in the superior cervical ganglion and from the nerve endings in the ileum (see

Introduction for references). In view of the high lipoidal solubility of methylpentynol, chloral hydrate and paraldehyde, some membrane stabilizing effect of these drugs on nerve terminals in the bladder is a possibility. However, paraldehyde appears to have a dual mode of action, namely, an anti-depolarizing activity at the postjunctional site and an inhibitory action on the output of acetylcholine; these actions have been demonstrated at the superior cervical ganglion and at the neuromuscular junction (Matthews & Quilliam, 1964; Payton, 1966). Chloral hydrate (Brown, 1962) and morphine (see Sollmann, 1957) inhibit cholinesterase; this could have enhanced the responses of the bladder to added acetylcholine, and possibly obscured, in part, the manifestation of prejunctional blocking activity of these drugs.

That both hexamethonium and mecamylamine in high doses had no inhibiting effect substantiates the observations of Huković *et al.* (1965) that the rat bladder innervation is purely postganglionic. The results with hexamethonium further indicate that cholinoceptors in the rat bladder are different from those in the frog motor end plate, where hexamethonium is a competitive antagonist of acetylcholine (Payton, 1966) and prevents depolarization.

Initial augmentation of the height of indirectly evoked contractions following TEA could be due to increased release of acetylcholine or due to inhibition of cholinesterase; the characters of the block following TEA (50 μg/ml) seen in our experiments exclude any HC-3-like action of TEA at the nerve terminals (see Freeman, 1968, for references). The blockade could be due to reduced output of acetylcholine as in the superior cervical ganglion (Matthews & Quilliam, 1964).

Though procaine had an inhibitory effect on the responses of the bladder to added acetylcholine, it may also have some prejunctional action. The nerve terminals and the adjacent effector cell membrane are particularly sensitive to procaine (Paton, 1954) and the agent is reported to impair transmission at the neuromuscular junction (Straughan, 1960), at the ganglia (Matthews & Quilliam, 1964) and at the nerve-smooth muscle junction of the ileum (Cox & Weinstock, 1966) both by a pre- and a postjunctional action.

The responses of the rat bladder preparation to indirect stimulation and to added acetylcholine are reproducible and stable for sufficiently long periods. The drugs can react with and are washed out from the preparation with ease. The preparation, hence, appears to be a suitable test model at least for preliminary studies on drug action at the cholinergic nerve-smooth muscle junction.

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