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SYNAPTIC TRANSMISSION IN PARASYMPATHETIC GANGLIA IN THE URINARY BLADDER OF THE CAT

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SUMMARY

1. Electrophysiological techniques were used to study the sacral parasympathetic input to pelvic ganglia located on the surface of the urinary bladder of the cat.

2. Synaptic transmission in pelvic ganglia was mediated primarily via nicotinic receptors although muscarinic excitatory receptors were present.

3. The most prominent characteristic of transmission in pelvic ganglia was the marked recruitment elicited by increasing frequencies of preganglionic nerve stimulation. Post-ganglionic action potentials were of low amplitude at low frequencies of stimulation (0·1–0·5 c/s), but commonly increased to five to twenty times control amplitudes during continuous stimulation at frequencies between 5 and 10 c/s. Thus, it is proposed that vesical ganglia may act as 'filters' in the micturition pathway; blocking the excitatory input to the bladder when intravesical pressure and parasympathetic firing is low and facilitating the neural input to the bladder during micturition when preganglionic activity is high.

4. Information was also obtained about the characteristics of the parasympathetic post-ganglionic neurones innervating the bladder. Stimulation of the preganglionic fibres in the pelvic nerve elicited a bimodal contraction consisting of an initial phasic response, which was atropine-resistant and a tonic response which was blocked by atropine. This suggests that two types of neurones, cholinergic and non-cholinergic, may mediate the sacral input to the vesical smooth muscle.

INTRODUCTION

For many years it was believed that peripheral autonomic ganglia functioned simply as relay stations where neural activity arising in the

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central nervous system was distributed to various effector organs. Information conveyed to ganglia by preganglionic nerves was thought to be transmitted with very little modification across the ganglionic synapse. Recently, however, it has been recognized that within ganglia there are complex synaptic mechanisms, both excitatory and inhibitory, which have the potential for exerting important modulating influences on efferent autonomic firing (see reviews by Volle, 1966; Tauc, 1967; Libet, 1970).

Electrophysiological studies in this laboratory have shown that parasympathetic ganglia on the surface of the feline urinary bladder exhibit prominent inhibitory and facilitatory mechanisms which are physiologically and pharmacologically distinct from the processes underlying normal transmission (de Groat & Saum, 1971, 1972; Saum & de Groat, 1972, 1973). The major excitatory input to these ganglia originates in the sacral segments of the spinal cord and is carried peripherally via the pelvic nerves (Langley & Anderson, 1895; de Groat & Ryall, 1969; de Groat, 1975). Transmission in vesical ganglia is cholinergic and mediated via nicotinic receptors. This process in turn can be modified by activation of the vesicosympathetic pathways (hypogastric nerves) which elicit both adrenergic inhibition of transmission, mediated by alpha receptors, and adrenergic facilitation, mediated by beta receptors (de Groat & Saum, 1972).

Cholinergic modulating mechanisms involving muscarinic receptors have also been noted in autonomic ganglia (Eccles & Libet, 1961; Volle, 1966; Libet, 1970) including ganglia on the surface of the bladder (Saum & de Groat, 1973). This has prompted the present investigation in which a more detailed study of the parasympathetic (cholinergic) input to the pelvic ganglia was conducted. It was shown that ganglionic transmission was characterized by a marked facilitation or recruitment at increasing frequencies of preganglionic nerve stimulation. The facilitation was not related, however, to the activation of muscarinic receptors, although activation of these receptors under certain conditions did elicit postganglionic firing. Various indirect evidence suggests that the facilitation was mediated by presynaptic mechanisms.

METHODS

Experiments were performed on forty-five female cats anaesthetized either with a mixture of sodium diallylbarbiturate (70 mg/kg), urethane (280 mg/kg) and monoethylurea (280 mg/kg) administered intraperitoneally or with chloralose (50–70 mg/kg, I.V.) after initial induction with halothane. Following intubation of the trachea, the parasympathetic (pelvic) and sympathetic (hypogastric) innervations to the urinary bladder were exposed through a mid line abdominal incision. After removal

of a portion of the large and small bowel, the hypogastric nerves on both sides and several colonic nerves were dissected free from surrounding connective tissue and sectioned peripheral to the inferior mesenteric ganglia. Several preganglionic nerves (inferior splanchnic nerves) entering the ganglia were exposed and cut at their point of origin from the sympathetic chain. Branches of the pelvic nerve (preganglionic) were freed at a point 2–3 cm from the neck of the bladder. The pelvic nerves either were sectioned central to the point of isolation or were left intact. Ganglia were identified on the surface of the bladder and post-ganglionic fibres were freed of connective tissue and sectioned 0·5–1 cm distal to the ganglia. The urinary bladder was cannulated by inserting a polyethylene tube (i.d. 2 mm) into the urethra through the external orifice and passed into the bladder. The cannula was filled with physiological saline solution and connected to a pressure transducer to record the pressure within the bladder. The spinal cord was transected at the lower lumbar region to block the central parasympathetic outflow to the bladder (de Groat & Ryall, 1969). Close intra-arterial, I.A., administration of drugs to the ganglia was made via a 27-gauge needle inserted in the abdominal aorta proximal to the origin of the inferior mesenteric artery. The external iliac arteries were occluded during drug injections to increase the amount of drug reaching the ganglia. Skin flaps were tied to a metal frame supporting the animal and the area was covered with warmed paraffin oil.

Isolated nerves were mounted on bipolar silver electrodes for stimulation and recording. Stimulation was produced by rectangular pulses of 0·05–0·1 msec duration at varying frequencies and intensities. Action potentials were displayed on an oscilloscope and photographed on 35 mm film. In some experiments potentials were also averaged on a Computer of Average Transients (CAT), the output of which was then plotted on an X–Y paper recorder. The magnitude of an averaged potential was measured with a planimeter as the area under the evoked response. In half of the experiments the animals were paralysed with gallamine triethiodide or decamethonium and artifically respired. End-tidal CO_2 was maintained between 3·5 and 4% by varying the rate and depth of respiration. Systemic blood pressure was measured from the carotid artery with a strain-gauge pressure transducer. The animals' temperature was maintained between 36 and 38° C with the aid of a heating pad. End-tidal CO_2, blood pressure, and bladder pressure were displayed on a rectilinear multichannel paper recorder.

The following drugs were used: acetyl-β-methylcholine chloride, acetylcholine chloride, atropine sulphate (Sigma), bethanechol chloride (Merck), 2-diethoxyphosphenylthioethyldimethylamine acid oxalate (217 AO) (Ayerst), dihydroergotamine methanesulphonate (Sandoz), gallamine triethiodide (Lederle), hexamethonium chloride, tetraethylammonium bromide (Baker). Doses are expressed as the salt and refer to I.A. administration unless otherwise indicated.

RESULTS

Synaptic transmission in pelvic ganglia

Ganglionic response to pelvic nerve stimulation

Electrical stimulation of the pelvic nerve (preganglionic fibres) elicited a bimodal response on nerve filaments arising from ganglia on the surface of the urinary bladder (Fig. 1*C*). The response consisted of a short latency (1–5 msec), transient potential and a longer latency (15–40 msec), more

prolonged discharge (duration 15–30 msec). The threshold stimulus (T) for eliciting the early potential was 0·5–1 V (0·05 msec) and the threshold for the late discharge was 2–5 T. A maximal late discharge was produced by stimulus intensities from 6 to 15 T. The late discharge, in contrast to the early response, was (1) facilitated during repetitive stimulation (2–20 c/s), (2) depressed at high frequencies of stimulation (40–60 c/s) and (3) completely blocked by the I.A. administration of ganglionic blocking agents, e.g. hexamethonium (0·1–1 mg) (Fig. 1 D) and tetraethyl ammonium (1–10 mg). Therefore the late discharge represented post-ganglionic nerve activity, while the early response might be firing in vesical afferents or preganglionic through-fibres. The calculated conduction velocity for the early response was 6–12 m/sec. It is assumed that the late discharge occurred only in parasympathetic fibres since stimulation of the hypogastric nerves or the sympathetic chain did not elicit similar activity, whereas stimulation (1–6 V) of the preganglionic axons in the sacral ventral roots evoked a bimodal response on the vesical post-ganglionic fibres with the same general characteristics as those elicited by pelvic nerve stimulation.

As illustrated in Fig. 1, continuous stimulation (0·5–20 c/s) of the pelvic nerves elicited a post-ganglionic response which gradually increased in amplitude. This augmentation, ranging from 3–20 times increase in amplitude (Fig. 2), will be referred to as ganglionic recruitment. With low frequencies of stimulation (0·5–5 c/s) the post-ganglionic response reached peak amplitude in 5–30 sec after the onset of the stimulus and was maintained for the duration of the stimulus train (1–2 min). With higher frequencies of stimulation (10–20 c/s) maximal recruitment (mean, 12·5 times increase in spike amplitude, five experiments) occurred in 1–4 sec, but the response was not maintained (Fig. 2). After the end of the stimulus train the response declined to control values in 30–180 sec depending upon the frequency and duration of the train. Interestingly, if the stimulus train was terminated before the response had achieved its maximum, the response did not continue to build up, but instead rapidly declined to control. The number of shocks necessary to produce maximal recruitment ranged from 17 to 45 (mean $27{\cdot}7 \pm 6{\cdot}7$ S.D.) in different experiments and seemed to be unrelated to the frequency of stimulation. The averaged results from four experiments showed that maximal responses at frequencies of 1, 2, 5 and 20 c/s occurred after 23, 32, 32 and 20 shocks, respectively.

The increase in the amplitude of the post-ganglionic action potential during repetitive stimulation might be due to two different phenomena: firstly, as noted in many experiments the action potential decreased in duration by 30–50 %, indicating a more synchronous firing of the ganglion cells; secondly, since the area of the evoked response also increased,

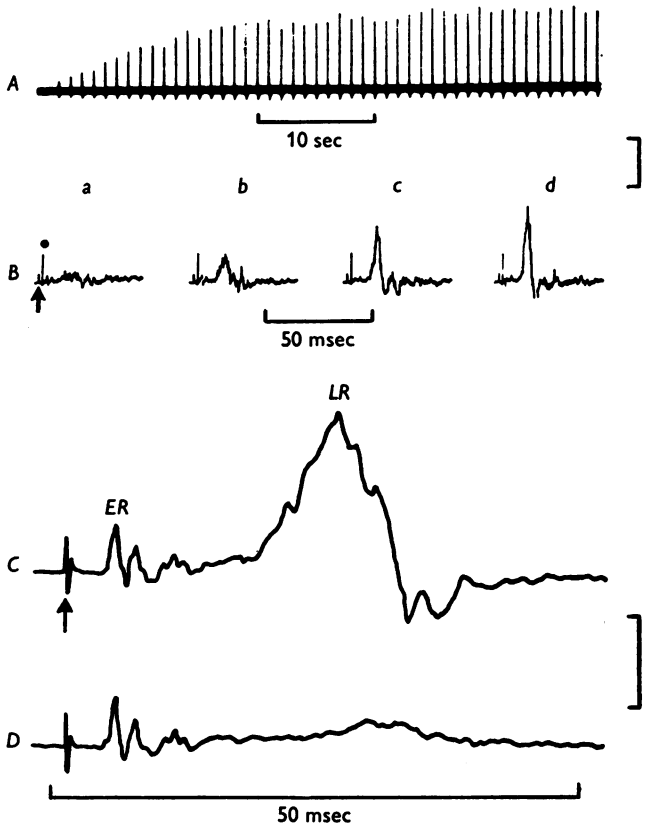

Fig. 1. Recruitment of the post-ganglionic response elicited by repetitive stimulation of the pelvic nerves. *A*, action potentials recorded on a vesical post-ganglionic nerve filament in response to submaximal (5 V) stimulation of the preganglionic fibres at a frequency of 1 c/s. *B*, sample tracings from *A* of the post-ganglionic responses obtained at varying times after the start of the stimulus train. In *B*, *a* was the first response elicited by preganglionic stimulation and *b*, *c* and *d* were obtained, respectively, 5, 10 and 20 sec later. The arrow below *Ba* denotes the stimulus artifact and the dot above denotes the early response. *C*, bimodal response to pelvic nerve stimulation (1·1 V, 1 c/s) recorded on nerve filaments arising from ganglia on the surface of the urinary bladder. *ER* and *LR*, respectively, are the early response and late response. The arrow below *C* denotes the stimulus artifact. *D*, effect of hexamethonium (100 μg, I.A.) on the bimodal response. Responses in *C* and *D* represent the average of twenty individual responses. Time calibration in *D* also applies to *C*; vertical calibration in *A* and *B* is 400 μV and in *C* and *D* is 200 μV, negativity upwards.

greater numbers of ganglion cells must have been activated or multiple firing must have occurred in individual ganglion cells. The maximum increase in area in different experiments ranged from 1·5 to 10 times (Fig. 3). A comparison between the maximal enhancement (increase in area) elicited by submaximal and supramaximal intensities of preganglionic nerve stimulation demonstrated that a greater recruitment was

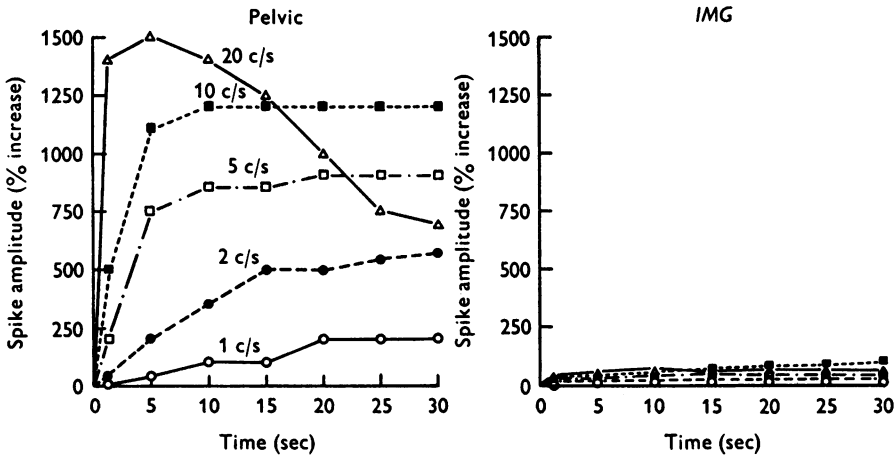

Fig. 2. Time course of the recruitment of the post-ganglionic response in the pelvic ganglion and inferior mesenteric ganglion (*IMG*). The abscissa indicates the time in seconds after the initiation of the stimulus train and the ordinate is the percentage increase in the amplitude of the post-ganglionic response. The post-ganglionic action potentials were elicited by submaximal stimulation of the preganglionic pelvic nerves and inferior splanchnic nerves. Records are included for frequencies of stimulation at 1 c/s (○—○), 2 c/s (●--●), 5 c/s (□-·-·-□), 10 c/s ■----■) and 20 c/s (△—△) for both ganglia.

produced by submaximal stimulation (Fig. 3). This finding is no doubt related to a greater subliminal fringe (i.e. a population of ganglion cells receiving a subthreshold excitatory input) when stimulating at submaximal intensities.

In contrast to the striking recruitment observed in pelvic ganglia, very little recruitment occurred in the inferior mesenteric ganglion (a sympathetic ganglion). Maximal enhancement of the post-ganglionic response elicited by repetitive preganglionic nerve stimulation at 10–20 c/s ranged from 60% to 100% increase in spike amplitude. A comparison of recruitment in the two ganglia is shown in Fig. 2 where enhancement is expressed

in terms of increase in spike amplitude and in Fig. 3 where it is expressed as an increase in the area of the evoked response.

Alterations in ganglionic excitability by homosynaptic and heterosynaptic preganglionic volleys

A series of experiments was conducted using homosynaptic and heterosynaptic testing procedures to determine whether the above-mentioned

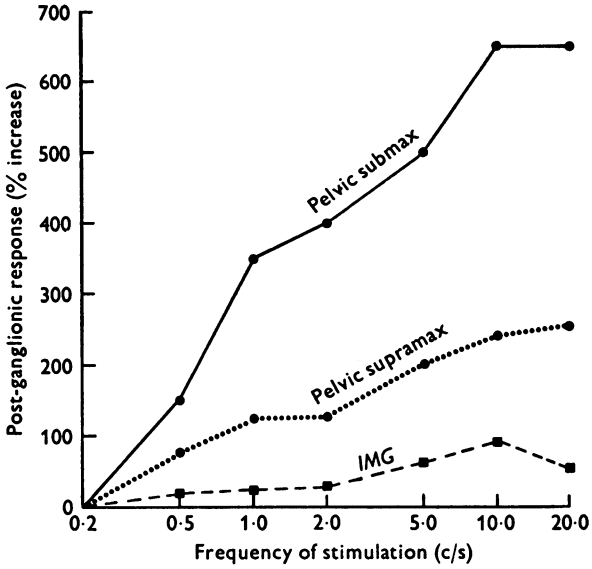

Fig. 3. Plot of the maximum facilitation obtained in parasympathetic (pelvic) and sympathetic (*IMG*) ganglia with varying frequencies of preganglionic nerve stimulation. The abscissa is the frequency of stimulation applied to preganglionic fibres innervating the two ganglia. The ordinate is the percentage increase in the area under the post-ganglionic response. Each point represents twenty individual responses averaged on a computer. Stimulation of the pelvic nerves at submaximal (2 V; ●—●) and supramaximal (20 V; ● · · · ●) intensities. Inferior mesenteric ganglionic transmission was elicited by submaximal intensities of preganglionic stimulation (0·5 V; ■----■).

facilitation in pelvic ganglia was mediated by presynaptic or postsynaptic events. Facilitation of transmission occurred when a test stimulus to the pelvic nerve was preceded by a single conditioning stimulus delivered to the same preganglionic nerve. The test response was elicited by stimulation with submaximal intensities at a frequency (0·2 c/s) which produced no recruitment of the response. Following a single conditioning stimulus of supramaximal intensity there was a prolonged

facilitation of the test response lasting for 1–2 sec (Fig. 4). In six out of twelve experiments, the facilitation seemed to occur in two distinct phases consisting of: (1) an early facilitation persisting for 20–150 msec after the conditioning stimulus and (2) a late facilitation which lasted for 0·5–2 sec (Fig. 4). In the other six experiments the two peaks in the

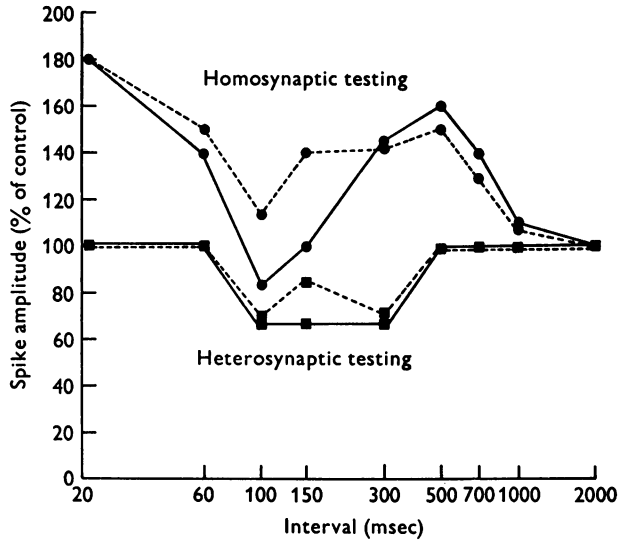

Fig. 4. Homosynaptic and heterosynaptic testing in pelvic ganglia before and after atropine. Test response was elicited by supramaximal stimulation of a preganglionic nerve at a frequency 0·2 c/s. Single conditioning stimuli were applied to the same preganglionic nerve (homosynaptic testing, ●—●) or to a different preganglionic nerve (heterosynaptic testing, ■—■) at supramaximal intensities at a frequency of 0·2 c/s. Homosynaptic (●----●) and heterosynaptic (■----■) testing after the injection of atropine (10 μg, I.A.) are indicated by dashed lines. The abscissa is the interstimulus interval in milliseconds and the ordinate is the amplitude of the pelvic response as a percentage of the control response.

facilitatory curve were not observed. The periods of early and late facilitation will be termed Phase I and Phase II, respectively. The maximum increase in spike amplitude occurring during Phase I ranged from 50 to 300% in six experiments (mean 172%). The enhancement during Phase II ranged from 15 to 75% (mean 49%, six experiments). A depression of transmission which in different experiments amounted to a 20–50% (mean 39%, six experiments) decrease in spike amplitude occurred between Phases I and II (Fig. 4). The depression appeared to be related to the degree of excitation of the ganglion cell population elicited by the conditioning stimulus. For example, when the intensity of the conditioning

shock evoked a submaximal response there was little or no depression of the test response, whereas with supramaximal stimulation the amplitude of the conditioning response increased and there was a concomitant decrease in the test response.

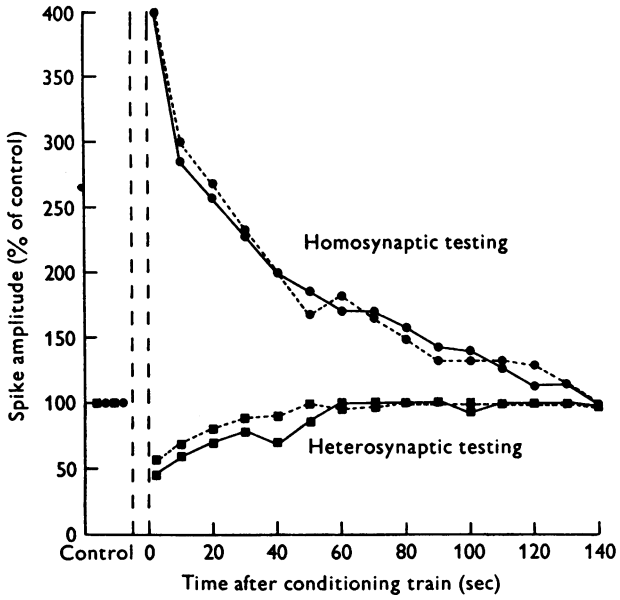

Fig. 5. Effects of homosynaptic and heterosynaptic conditioning trains of stimuli on pelvic ganglionic transmission before and after atropine. The abscissa is the time in seconds, after the conditioning train of stimuli and the ordinate is the amplitude of the pelvic response as a percentage of the control response. The test response was elicited by preganglionic nerve stimulation at a frequency of 0·5 c/s. The conditioning stimuli were applied for 5 sec (indicated by two vertical dashed lines) at a frequency of 20 c/s to the same preganglionic nerve (homosynaptic testing, ●—●) and a different preganglionic nerve (heterosynaptic testing, ■—■). Homosynaptic (●----●) and heterosynaptic testing (■----■) after the injection of atropine (60 μg, I.A.).

Single conditioning shocks of supramaximal intensity applied to a different preganglionic nerve (heterosynaptic testing) produced only depression of the test response at interstimulus intervals of 60–150 msec (five experiments, one of which is illustrated in Fig. 4). Since the heterosynaptic stimulus elicited post-ganglionic firing the period of depression could most reasonably be attributed to refractoriness of the same ganglion cell population.

Consistent with these findings, conditioning trains of stimuli (1–40 c/s for 0·5–30 sec) applied to a pelvic nerve produced a prolonged (0·5–5 min) potentiation (post-tetanic potentiation) of the post-ganglionic response elicited by a test stimulus to the same nerve (homosynaptic testing) (Fig. 5). In these experiments the test response was evoked by submaximal stimulation at a frequency of 0·5 c/s. Maximal facilitation which was produced with frequencies of stimulation from 20 to 40 c/s occurred 1–10 sec after the tetanus and ranged from 230 to 375% increase in the test response (mean 294%, four experiments). As with ganglionic recruitment, the degree of post-tetanic potentiation was directly related to the frequency of the stimulus train and the time for recovery to control levels was dependent on the duration of the tetanus, i.e. the longer the train duration the more prolonged the post-tetanic potentiation.

A depression of transmission was not observed following a homosynaptic conditioning tetanus with frequencies between 1 and 40 c/s. However, a similar tetanus to an adjacent preganglionic nerve produced only depression (Fig. 5, five experiments). With low frequencies of stimulation (1–3 c/s) the depression was detectable within 2–5 sec after the onset of the tetanus and persisted for 30–60 sec after the termination of the stimulus. The maximum decrease in spike amplitude ranged from 25 to 70% in five experiments (mean 49%). In two experiments a conditioning tetanus (20 c/s intratrain frequency) to the post-ganglionic nerve filament produced a similar decrease in the amplitude of the test response.

Muscarinic mechanisms in pelvic ganglia

Since it has been proposed that facilitatory and inhibitory events in various sympathetic ganglia resulted from atropine-sensitive (muscarinic) slow synaptic potentials (Eccles & Libet, 1961; Libet, 1964) it was of interest to determine if similar mechanisms accounted for the facilitation and depression in pelvic ganglia. However, as shown in Fig. 6, the I.A. administration of atropine (2–100 μg, six experiments) to the ganglia did not antagonize the recruitment, or the prolonged facilitation of transmission which occurred after either a single conditioning shock (Fig. 4) or a train of stimuli to the same preganglionic nerve (Fig. 5).

Atropine also did not block the transient depression of transmission produced by homosynaptic (Fig. 4) or heterosynaptic (Figs. 4, 5) conditioning stimuli. Since there is evidence for an adrenergic inhibitory mechanism in pelvic ganglia (de Groat & Saum, 1972; Saum & de Groat, 1972) the possibility was considered that endogenously released catecholamines might be mediating the ganglionic depression evoked by pelvic nerve stimulation. It was of interest, therefore, to study the effects of dihydroergotamine (50–100 μg, four experiments), an alpha adrenergic

blocking agent, on the ganglionic depression elicited by pelvic nerve stimulation. However, it was found that doses (50–100 μg) of dihydroergotamine which blocked the depressant effects of exogenous catecholamines did not alter homosynaptic or heterosynaptic depression.

The I.A. administration of 217 AO (50–200 μg, four experiments), an irreversible anticholinesterase agent (Koelle & Steiner, 1956; McIsaac &

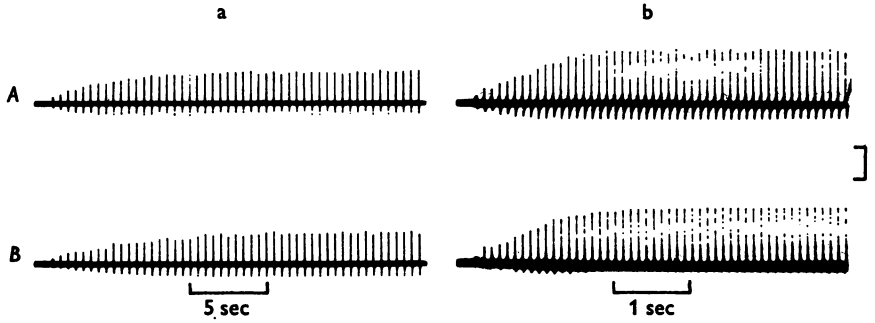

Fig. 6. Effect of atropine on the recruitment of the pelvic post-ganglionic action potential elicited by repetitive preganglionic nerve stimulation. Pelvic ganglionic transmission was recorded as in Fig. 1. Records *a* and *b* correspond, respectively, to the responses elicited by stimulation of the pelvic nerves at frequencies of 2 and 10 c/s. *A* and *B*, correspond, respectively, to before and after the administration of atropine (60 μg, I.A.). Vertical calibration is 1 mV, negativity upwards.

Koelle, 1959; de Groat, 1970), depressed the post-ganglionic action potentials elicited by repetitive (0·5–1 c/s) stimulation of the pelvic nerves. The depressant effect was apparent 20–30 sec after the injection and often persisted for 10–15 min. The amplitude of the post-ganglionic nerve response was reduced by 50–70%. In addition, 217 AO depressed post-tetanic potentiation elicited by homosynaptic conditioning trains of stimuli. The depression was apparent as a decrease in the duration of the post-tetanic potentiation and as a decrease in the amplitude of the post-ganglionic response. These depressant effects were not antagonized by atropine (three of four experiments) or by dihydroergotamine (one experiment).

In ganglia treated with 217 AO (50–200 μg), repetitive preganglionic nerve stimulation (10–50 c/s for 5–30 sec) elicited a prolonged (2–4 min) asynchronous discharge (Fig. 7). The threshold stimulus intensity necessary to elicit the asynchronous discharge ranged from 1 to 5 V. In the majority of experiments (thirteen out of fifteen preparations), 217 AO did not evoke post-ganglionic firing in the unstimulated ganglion. In addition to the late discharge, repetitive stimulation evoked a transient early

discharge immediately following the stimulus (ten experiments, Fig. 7*B*). In one experiment, stimulation of the preganglionic axons in the second sacral ventral root elicited both an early and late discharge.

Atropine (2–10 μg, seven experiments) completely blocked the late discharge and reduced the early discharge in each of five experiments (Fig. 7*C*). The antagonism of the late discharge persisted for the duration of the experiment (4–6 hr). The subsequent administration of the competitive ganglionic blocking agent hexamethonium (1–10 mg) blocked the remaining early discharge as illustrated in Fig. 7*D*. Similar doses of hexamethonium in ganglia not treated with atropine had no effect on the late discharge (five experiments).

Late firing of lower amplitude was also evoked by repetitive preganglionic stimulation in the untreated ganglion (nine experiments). The

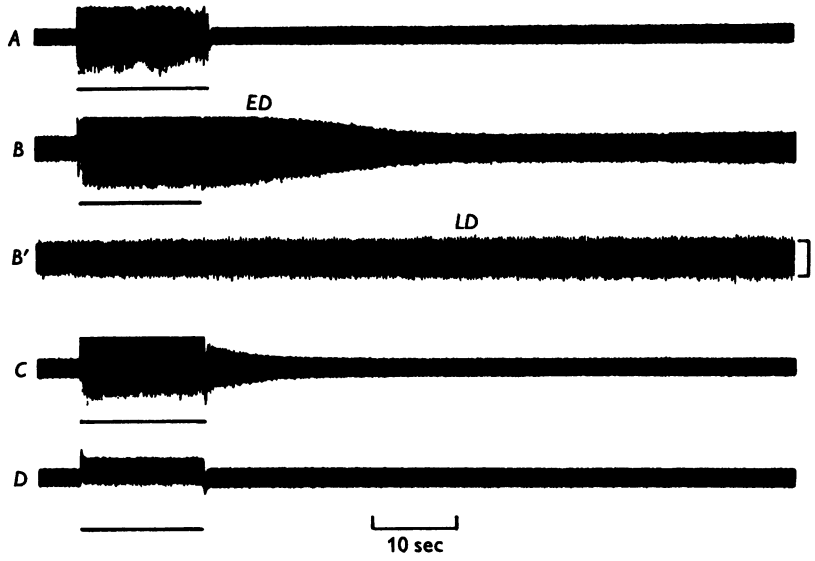

Fig. 7. Muscarinic post-ganglionic firing in a pelvic ganglion treated with an anticholinesterase agent (217 AO). *A*, control response in the untreated ganglion to repetitive (50 c/s for 15 sec) preganglionic nerve stimulation. Note that there is no after-discharge. *B* and *B'*, continuous tracing of the post-ganglionic firing evoked by repetitive stimulation (50 c/s for 15 sec) in the ganglion treated with the anticholinesterase agent 217 AO (100 μg, I.A.). Note the early discharge (*ED*) immediately after the stimulus is terminated, followed by a late discharge (*LD*). *C*, blockade by atropine (10 μg, I.A.) of the late discharge and reduction in the early discharge. *D*, blockade of the remaining early discharge by the subsequent administration of hexamethonium (10 mg, I.A.). The period of repetitive stimulation (50 c/s) is indicated by the horizontal bars below the records. Vertical calibration is 50 μV, negativity upwards.

threshold for producing this response was 2 V (0·05 msec) at a frequency of 15–30 c/s. Like the late discharge evoked in the 217 AO-treated ganglia, atropine (2–100 μg) blocked the response (three out of four experiments).

Cholinergic modulation of bladder activity

Since it has been demonstrated in the present experiments that endogenously released acetylcholine appeared to excite both nicotinic and muscarinic receptors in pelvic ganglia, it was of interest to determine if physiological activation of these sites could modulate bladder motility.

Bladder contractions evoked reflexly by distension of the bladder

Rhythmical bladder contractions were elicited when the bladder was artificially distended and maintained under constant volume conditions (Langworthy, Kolb & Lewis, 1940; Ruch, 1960; de Groat & Ryall, 1969; de Groat, 1975). The contractions which occurred with a frequency between 0·3 and 2/min had durations ranging from 0·5 to 1·5 min and achieved peak intravesical pressures of 60–80 cmH_2O. As illustrated in Fig. 8, the bladder contractions coincided with asynchronous neural firing recorded simultaneously on nerve fibres on the surface of the urinary bladder. The phasing of the efferent discharges and bladder contractions was maintained over long periods of time, and close inspection of the records revealed that the efferent discharges preceded the bladder contractions by approximately 1 sec. Neural firing was not detectable during the interval between the contractions. The spontaneous bladder contractions and the efferent discharges were unaffected by transection of the hypogastric nerves but were completely abolished by bilateral transection of the pelvic nerves or by transection of the spinal cord at the lower thoracic or lumbar levels. These results indicated that the responses were dependent upon a central parasympathetic reflex pathway.

As shown in Fig. 8*A*, the contractions and associated neural firing were completely and reversibly blocked by the administration of the competitive ganglionic blocking agents, hexamethonium (0·5–10 mg, I.A.) and tetraethylammonium (1–10 mg). The blockade occurred 5–10 sec after the injection and, with the larger doses of the agents, persisted for 5–15 min. Increasing the afferent input to the central nervous system by raising bladder pressure to 50–60 cmH_2O, thereby increasing preganglionic firing (de Groat & Ryall, 1969), did not overcome the ganglionic blockade. Thus, it was apparent that the asynchronous firing represented activity in parasympathetic post-ganglionic axons and that transmission in parasympathetic ganglia was mediated primarily via nicotinic receptors.

Atropine (0·002–1 mg, I.A.; Fig. 8*B*), in doses which completely blocked the bladder contractions evoked by acetylcholine (10–40 μg), acetyl-

β-methylcholine (10–50 μg) and bethanechol (10–100 μg), reduced the amplitude (40–60%) and the duration of the spontaneous bladder contractions and decreased base-line vesical pressure ('tone') (Edvardsen, 1968*b*, *c*). The depressant effects of atropine were apparent 0·5–1 min after the injection and persisted for the duration of the experiment (4–6 hr). Maximal reduction in the amplitude of the bladder contractions was produced by 10–20 μg. However, atropine (0·002–1 mg) did not greatly depress the efferent firing although the duration of firing was markedly reduced (Fig. 8*B*). The reduction by atropine of the amplitude and duration of the spontaneous bladder contractions was most reasonably

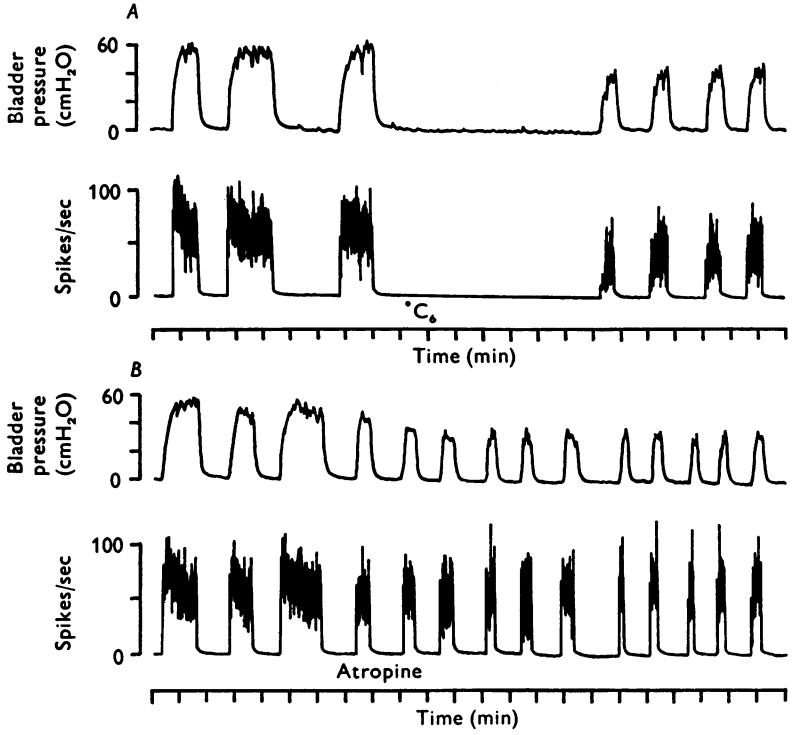

Fig. 8. Effects of cholinergic blocking agents on the isovolumetric contractions of the bladder and the asynchronous post-ganglionic firing evoked reflexly by distension of the bladder. Upper tracing in each record is bladder pressure in cmH_2O and lower tracing is the average rate of efferent firing in spikes/sec (time constant, 1 sec) recorded from the central end of a sectioned pelvic nerve filament on the surface of the urinary bladder. *A* and *B* were obtained in cats with an intact spinal cord. *A*, continuous tracing showing the blockade of both bladder activity and neural firing by the administration of hexamethonium (C_6, 500 μg, I.A.). *B*, continuous tracing before and after atropine (2 μg, I.A.). Drug injections are indicated by a dot below each record. Horizontal calibration is 1 min/division.

attributed to a depressant action at the parasympathetic neuro-effector junction.

Electrical stimulation of the pelvic nerves

Electrical stimulation of the pelvic nerves in cats with an intact spinal cord produced an increase in intravesical pressure (i.e. a bladder contraction; Fig. 9*A*) which was mediated via two mechanisms: activation of vesical afferent fibres in the pelvic nerve which elicited reflex parasympathetic firing ipsilateral and contralateral to the site of stimulation; and activation of the ipsilateral preganglionic fibres to the bladder. To simplify the experimental conditions, in most preparations the spinal cord or the pelvic nerves were transected to block the central reflexes. In these preparations, repetitive electrical stimulation (0·5–30 c/s) of the pelvic nerves commonly elicited bimodal bladder contractions (Fig. 9*A*). The early contraction was of short duration (5–10 sec) and often reached peak intravesical pressures of 60–80 cmH_2O. The late contraction, which was only observed with periods of stimulation exceeding 15 sec, was maintained for the duration of the stimulus (as long as 5 min) and declined to control levels within 20–30 sec after cessation of the stimulus. The late contraction achieved peak intravesical pressures of 30–50 cmH_2O. It was impossible to differentiate the two contractions on the basis of intensity or frequency of preganglionic nerve stimulation. The threshold stimulus intensity necessary to produce a detectable bimodal contraction varied in different animals from 1 to 5 V (0·05 msec duration), while maximal responses were generally produced by stimulus intensities of 5–10 V. With low frequencies of stimulation (0·5–2 c/s) both responses were relatively small and there was not a clear distinction between the two contractions; whereas at higher frequencies (5–30 c/s) of stimulation the two responses were quite prominent. Maximum contractions to pelvic stimulation occurred at frequencies of 20–30 c/s. At frequencies greater than 20 c/s the late contraction was not maintained during prolonged stimulation.

Hexamethonium (0·1–10 mg) and tetraethylammonium (2–10 mg) completely blocked the bimodal contraction elicited by pelvic nerve stimulation (six experiments; Fig. 9*A*). In two of the experiments, however, a transient (10–15 sec) increase in intravesical pressure occurred after cessation of the stimulus (5–30 c/s). A similar response was also noted by Langley (1911). The I.A. administration of atropine (2–500 μg, twelve experiments) to the bladder selectively blocked the late contraction (Fig. 9*C*). The blocking action of atropine occurred with relatively low doses (2–10 μg, I.A.) and larger doses had no further effect.

The I.A. administration of 217 AO (50–200 μg) produced a prolonged increase (10–30 cmH_2O) in resting bladder pressure and also enhanced

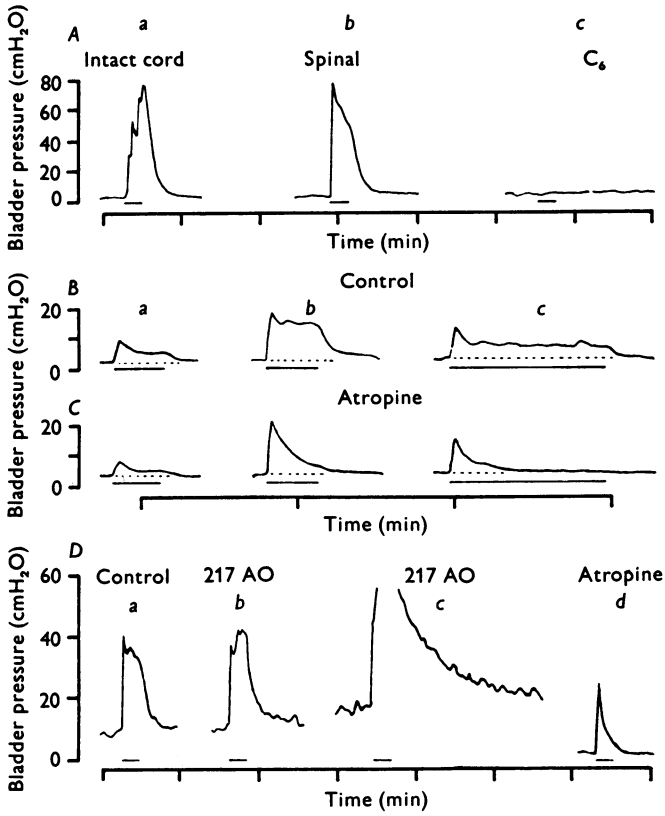

Fig. 9. Effects of pelvic nerve stimulation on bladder activity. *A*: *a*, bladder contraction evoked by pelvic nerve stimulation (15 V, 10 c/s) in a cat with an intact spinal cord; *b*, bladder contraction evoked by pelvic nerve stimulation (20 V, 30 c/s) in a different experiment with the spinal cord transected at the lumbar level (L3); and *c*, pelvic nerve stimulation (same parameters as *b*) after the administration of hexamethonium (C_6, 3 mg, I.A.). *B* and *C*, bladder contractions evoked by pelvic nerve stimulation (15 V) at: *a*, 2 c/s; *b*, 20 c/s; and *c*, 10 c/s; before, *B*, and after, *C*, administration of atropine (5 μg, I.A.). The dashed lines under the tracings indicates the base line. *D*: *a*, control response to pelvic nerve stimulation (20 V, 30 c/s); *b* and *c*, pelvic nerve stimulation after the administration of 50 and 100 μg 217 AO, respectively. Note the increase in the late contraction after 217 AO and the increase in bladder 'tone'; *d*, pelvic nerve stimulation after the administration of atropine (20 μg, I.A.). Note that atropine blocked the late contraction and markedly reduced bladder 'tone'. Pelvic nerve stimulation was applied during the period indicated by the bar under each record; horizontal calibration is 1 min/division.

pelvic nerve-evoked contractions (Fig. 9*D*). Low doses (50–100 μg) of 217 AO selectively potentiated the late contraction, whereas higher doses which produced an increase in bladder 'tone', enhanced both the early and late contractions. The increase in bladder 'tone' and the potentiation of the neurally-evoked contractions were blocked by atropine (2–20 μg). Hexamethonium, in doses (1–10 mg) which completely blocked the bimodal contraction in the untreated preparation, also blocked the pelvic-nerve evoked bladder contractions in a 217 AO-treated preparation. Hexamethonium did not affect the increase in bladder 'tone' elicited by 217 AO.

DISCUSSION

The present results indicate that prolonged facilitation in pelvic ganglia of the cat has an important modulating influence on the parasympathetic input to the urinary bladder. It was shown that single conditioning shocks to pelvic preganglionic fibres facilitated ganglionic transmission for 1–2 sec and that continuous stimulation at interstimulus intervals of less than 2 sec produced a marked recruitment of evoked postganglionic firing which developed gradually during the course of 20–40 stimuli. It can be concluded from these observations that transmission in vesical ganglia is relatively inefficient at low frequencies of preganglionic stimulation (i.e. below 0·5 c/s) and that even with a maximal synchronous preganglionic input more than 70% of the ganglion cells fail to discharge. However, at higher frequencies of stimulation a ganglionic facilitatory mechanism effectively recruits a large population of ganglion cells from the subliminal fringe leading to a considerable amplification of the excitatory input to the bladder smooth muscle.

Mechanisms underlying ganglionic facilitation

It was initially considered that the facilitation might be mediated by slow excitatory post-synaptic potentials (slow e.p.s.p.s) which have been implicated in recruitment and post-tetanic potentiation in sympathetic ganglia (Libet, 1964, 1970). Slow e.p.s.p.s in contrast to normal ganglionic transmission are elicited by activation of muscarinic receptors and are blocked by atropine. Atropine also blocked heterosynaptically induced post-tetanic potentiation as well as a large part of homosynaptically induced post-tetanic potentiation in the rabbit superior cervical ganglion (Libet, 1964). However, the findings in pelvic ganglia were markedly different. Neither post-tetanic potentiation nor facilitation elicited by single conditioning volleys, nor ganglionic recruitment produced by continuous stimulation were affected by the administration of atropine. A similar atropine-resistant late facilitation has been demonstrated

recently in the superior cervical ganglion of the rabbit (Brimble, Wallis & Woodward, 1972).

Although muscarinic mechanisms do not seem to contribute to recruitment in pelvic ganglia, evidence for the existence of excitatory muscarinic receptors in pelvic ganglia was obtained. Repetitive preganglionic stimulation at high frequencies or repetitive stimulation at lower frequencies in ganglia treated with an anticholinesterase agent evoked post-ganglionic firing which was resistant to the effects of competitive ganglionic blocking agents, but was blocked by small doses of atropine. It is well established that under similar conditions a muscarinic form of transmission can occur in various sympathetic ganglia (Takeshige & Volle, 1962; Trendelenburg, 1966; Brown, 1967; de Groat, 1970). However, the physiological role of muscarinic excitatory receptors in pelvic ganglia is uncertain, since the bladder contraction elicited by pelvic nerve stimulation were completely blocked by hexamethonium. Thus, neurally evoked atropine-sensitive contractions of the bladder are not mediated by the activation of ganglionic muscarinic receptors as suggested by Libet (1964) but rather appear to be due to an action of acetylcholine on muscarinic excitatory receptors in the bladder smooth muscle.

The results obtained with homo- and heterosynaptic conditioning procedures suggest, albeit indirectly, that facilitation, post-tetanic potentiation and presumably recruitment in vesical ganglia are mediated by a presynaptic facilitatory mechanism, which leads to an increased release of acetylcholine from the preganglionic nerve terminals. It is noteworthy that similar recruitment has also been observed at certain neuro-effector junctions (see review by Bennett, 1972).

At the frog neuromuscular junction where the mechanism of transmitter release has been extensively studied, Magleby & Zengel (1975) suggested that recruitment of the end-plate potential is due to at least three separate processes which they termed facilitation, potentiation and an intermediate facilitatory process. The importance of calcium in the release of acetylcholine is well recognized and it has been proposed that facilitation (Katz & Miledi, 1968; Rahamimoff, 1968) and post-tetanic potentiation (Rosenthal, 1969; Weinreich, 1971) at the neuromuscular junction might be due to accumulation of calcium in the nerve terminal, although not necessarily the same calcium dependent process (Landau, Smolinsky & Lass, 1973). It has been shown in the present study that: (1) pelvic ganglionic recruitment requires time to attain maximal amplitude, which is dependent on the number of stimuli applied to the preganglionic nerves, (2) the magnitude of the recruitment is dependent on the frequency of stimulation; and (3) the recruitment persists for several minutes after the termination of the stimulus. These findings are consistent with the concept of calcium

accumulation after each stimulus and, therefore, it is tempting to speculate that the mechanisms underlying ganglionic recruitment may be similar to those obtaining at the neuromuscular junction (Magleby, 1973; Magleby & Zengel, 1975).

Functional significance of ganglionic recruitment

Ganglionic transmission in sympathetic and parasympathetic pathways to the urinary bladder exhibited different characteristics, which reflected in part the different functions of the two autonomic systems. In sympathetic ganglia (inferior mesenteric) transmission occurred with a high safety factor and a majority of the ganglion cells could be excited with a single preganglionic stimulus. On the other hand, in parasympathetic ganglia, transmission was relatively inefficient, the subliminal fringe was large and the majority of the ganglion cells only discharged when trains of stimuli were applied to the preganglionic nerves. These characteristics matched the properties of the respective central reflex pathways. For example, vesicosympathetic reflexes were strongly activated by single afferent volleys and exhibited very little recruitment (de Groat & Lalley, 1972), whereas the vesicoparasympathetic reflexes were minimal at low frequencies of stimulation but exhibited marked recruitment during repetitive stimulation at frequencies of 1–5 c/s (de Groat & Ryall, 1969). The properties of the sympathetic reflex seem well suited for a tonically active inhibitory pathway which might be expected to respond in a graded manner to provide a negative feed-back control of bladder activity (Edvardsen, 1968*a*; de Groat & Saum, 1972; de Groat & Lalley, 1972; de Groat & Theobald, 1975). On the other hand, the parasympathetic pathway is only activated periodically and responds in an all-or-none fashion. This parasympathetic pathway seems to function as a gating circuit maintaining the excitatory input to the bladder at or near zero during urine accumulation, when intravesical pressure and afferent firing are low, but responding maximally once a critical level of intravesical pressure is reached. Vesical ganglia must play an important role in this gating mechanism since they can 'filter out' low frequency preganglionic activity but seem to amplify the intense firing which occurs during micturition. The existence of the latter recruiting mechanism as well as adrenergic inhibitory and facilitatory mechanisms in vesical ganglia (de Groat & Saum, 1971, 1972) provides the basis for a potentially complex ganglionic modulation of the parasympathetic outflow to the bladder.

Parasympathetic neuro-effector transmission

It is evident from the present results and those of other investigators (Henderson & Roepke, 1934, 1935; Edvardsen, 1968*b*, *c*; see also reviews by Ambache, 1955; Burnstock, Dumsday & Smythe, 1972; Taira, 1972) that the parasympathetic pathway to the urinary bladder elicits bladder contractions which are only partially sensitive to blockade by atropine. As initially reported by Henderson & Roepke (1934) low doses of atropine blocked the 'tonic' component of the pelvic nerve evoked contractions but did not block the early 'phasic' component. Atropine also reduced the amplitude and decreased the duration of spontaneously occurring bladder contractions, suggesting that 'physiological' activation of the bladder is also mediated in part by stimulation of muscarinic receptors. Since atropine did not influence ganglionic transmission its action must have occurred at the neuro-effector junction. Low doses of an anticholinesterase agent selectivity increased the tonic component of the evoked contractions but higher doses increased bladder tone and had effects on vesical ganglia These data are consistent with the view that the bladder is innervated by both cholinergic and non-cholinergic post-ganglionic neurones. However, the physiological significance of two types of parasympathetic neurotransmission is obscure.

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