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# SYMPATHETIC INHIBITION OF THE URINARY BLADDER AND OF PELVIC GANGLIONIC TRANSMISSION IN THE CAT

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## SUMMARY

1. Electrophysiological techniques were utilized to study the mechanisms underlying adrenergic inhibition in the urinary bladder of the cat.

2. It has been shown that catecholamines administered by close intra-arterial injection or released endogenously by electrical stimulation of the hypogastric nerves elicit two distinct inhibitory responses in the bladder: (1) a direct depression of the vesical smooth muscle and (2) a depression of transmission in vesical parasympathetic ganglia.

3. Pharmacological studies revealed that the inhibitory mechanisms were mediated via different adrenergic receptors: $\beta$-receptors on the smooth muscle and $\alpha$-receptors in the parasympathetic ganglia.

4. We have been unable, however, to demonstrate that either of these mechanisms is activated by naturally occurring sympathetic firing.

## INTRODUCTION

The urinary bladder receives an innervation from both the parasympathetic and sympathetic divisions of the autonomic nervous system (Langley & Anderson, 1895, 1896). The parasympathetic pathway represents the principal excitatory input to the bladder; and its integrity is essential for the normal performance of micturition (see reviews by Langworthy, Kolb & Lewis, 1940, and Kuru, 1965). The function of the sympathetic innervation, on the other hand, is still uncertain. It is well known that electrical stimulation of the sympathetic fibres (hypogastrics) inhibits bladder contractions; however, evidence for a similar action by naturally occurring sympathetic activity has been obtained in only a few investigations. Gjone (1965) and Edvardsen (1968*a*) noted that in acute experiments on cats transection of the vesical sympathetic nerves resulted in an enhancement of spontaneous and reflexly evoked contractions of the bladder. They proposed therefore that the sympathetic fibres exerted

a tonic inhibitory influence on the bladder. Their findings and conclusions, however, are in contrast to a considerable body of earlier data which indicated that the sympathetic pathways were relatively unimportant in the regulation of bladder function (see Langworthy *et al.* 1940, for references).

The mechanisms underlying sympathetic inhibitory action in the bladder are also poorly understood. According to traditional concepts, sympathetic influences are mediated by a direct action of the adrenergic transmitter on the vesical smooth muscle cells. Recently, however, it was suggested (Hamberger & Norberg, 1965*a*) that sympathetic inhibition might also result from a depression of ganglionic transmission in the parasympathetic excitatory pathway to the bladder.

This proposal was based on the finding that adrenergic terminals in the bladder were located primarily in the intramural nerve plexus, often in apparent synaptic contact with the parasympathetic (cholinergic) ganglion cells, and only sparsely in the layers of the detrusor muscle. Since exogenous catecholamines depress ganglionic transmission (see reviews by Volle, 1966; de Groat, 1967; Libet, 1970), the possibility exists that endogenously released catecholamines might have a similar action.

The present study was undertaken to examine by more direct means the site of sympathetic-induced inhibition in the urinary bladder of the cat. We utilized electrophysiological techniques (de Groat & Ryall, 1969; de Groat, 1970) to monitor transmission in pelvic ganglia, while simultaneously recording intravesical pressure. In the majority of animals we were able to demonstrate a depression of pelvic ganglionic transmission during stimulation of the hypogastric nerves. However, we conclude, on the basis of pharmacological data, that under the conditions of our experiments, this action must have contributed only slightly to sympathetic depression of bladder activity. Preliminary accounts of some of these observations have been published (de Groat & Saum, 1971*a*, *b*).

## METHODS

Experiments were performed on cats of both sexes anaesthetized either with chloralose (50–70 mg/kg, I.V.) or with a mixture of sodium diallylbarbiturate (70 mg/kg), urethane (280 mg/kg) and monoethylurea (280 mg/kg) administered intraperitoneally. Chloralose anaesthesia was induced with halothane. Following intubabation of the trachea, the urinary bladder and its neural innervation were exposed through a mid line abdominal incision. Hypogastric nerves on both sides and one or two preganglionic nerves to the inferior mesenteric ganglia were dissected free from surrounding connective tissue. Branches of the pelvic nerve (preganglionic) were freed at a point 2–3 cm from the neck of the bladder. In some experiments the pelvic nerves were crushed or sectioned central to the point of isolation and in other experiments the nerves 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 one of two different methods. In some cats (primarily females) a polyethylene tube (i.d. 2 mm) was introduced into the urethra either through the external orifice or through an incision in the urethra and passed into the bladder. The cannula was secured in place by a ligature around the urethra. In other cats an incision was made in the fundus of the bladder and a thin-walled rubber condom mounted on the end of a flexible tube (i.d. 6·4 mm) was inserted into the lumen (de Groat & Ryall, 1968). The cannulae were filled with physiological saline solution and connected to a pressure transducer to record the pressure within the bladder. The cannulae could also be connected via a three-way stopcock to a reservoir of large surface area, the height of which could be adjusted to maintain a constant pressure in the bladder over a range of 0–60 cm $H_2O$. The ureters were ligated; and in many experiments the spinal cord was transected in the lower thoracic or lower lumbar region. The inferior mesenteric artery was cannulated for close intra-arterial administration of drugs to the bladder and the external iliac arteries were ligated to increase the amount of drug reaching the bladder. 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 platinum or silver electrodes for stimulation and recording. Stimulation was produced by rectangular pulses of 0·01–0·2 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. Most animals were paralysed with gallamine triethiodide and artificially respired. End-tidal $CO_2$, which was monitored continuously with a Beckman Medical Gas Analyzer, was maintained at approximately 4 % by varying the rate and depth of respiration. Systemic blood pressure was measured from the carotid artery or femoral artery with a strain gauge pressure transducer. The animals' temperature was maintained at 36–38° 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: (−)-adrenaline hydrochloride, (±)-isoprenaline hydrochloride, (−)-noradrenaline bitartrate (NADR), dopamine hydrochloride, $\gamma$-aminobutyric acid (GABA), propranolol hydrochloride, 1-(*p*-nitrophenyl)-2-isopropyl aminoethanol hydrochloride (INPEA), dihydroergotamine methanesulphonate (DHE), phentolamine methanesulphonate, phenoxybenzamine hydrochloride, picrotoxin, tetraethylammonium bromide, guanethidine monosulphate, bretylium tosylate, atropine sulphate. Doses are expressed as the salt when compounds were administered in this form and refer to I.A. administration unless otherwise indicated.

## RESULTS

### *Adrenergic inhibition in vesical ganglia*

As illustrated in Fig. 1, electrical stimulation of the pelvic nerve elicited a bimodal response on post-ganglionic fibres arising from vesical parasympathetic ganglia. The response consisted of a short latency, transient potential (marked by an arrow in record *A*) and a longer latency, more prolonged discharge. 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 administration of ganglionic blocking agents, e.g. tetraethylammonium or $\gamma$-aminobutyric acid (GABA; de Groat, 1970). We assume, therefore, that the late discharge was activity in post-ganglionic fibres, while the early response represented firing in vesical afferents or preganglionic through-fibres. The amplitude of the post-ganglionic discharge was used as a monitor of ganglionic transmission.

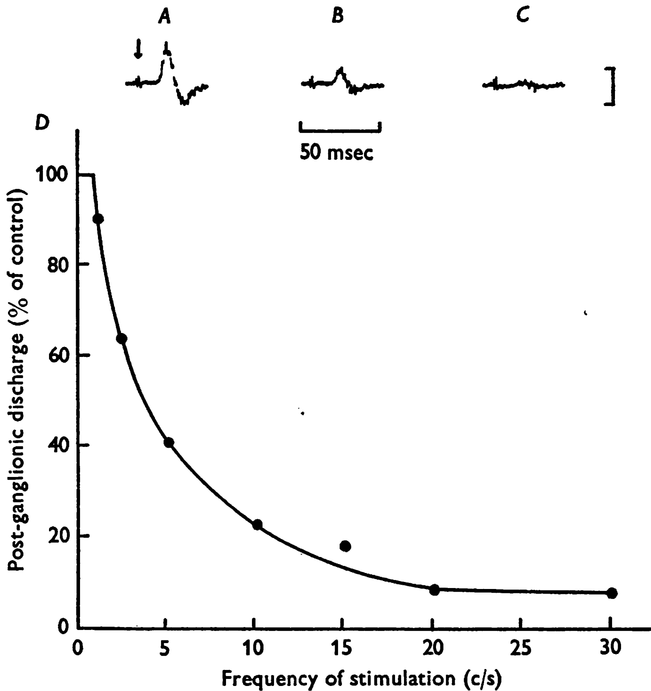


Fig. 1. Depression of transmission in pelvic ganglia by stimulation of the ipsilateral hypogastric nerve (HGN) at various frequencies. *A* is the control discharge recorded on a vesical post-ganglionic nerve filament in response to stimulation of the pelvic nerve (0·5 c/s) at submaximal intensity (2 V). Arrow denotes through-fibre response. In *B* and *C* the post-ganglionic discharge was depressed by stimulation of the HGN (10 V) at 5 c/s and 20 c/s, respectively. *D* is a plot of the depression of the amplitude of the post-ganglionic discharge (ordinate) against frequency of HGN-stimulation (abscissa). Vertical calibration in *A*–*C* is 200 $\mu$V, negativity upwards.

*Stimulation of sympathetic nerves.* In a majority of experiments (twenty-five of thirty-seven) repetitive stimulation of the hypogastric nerve (HGN) ipsilateral to the recording site or stimulation of preganglionic nerves to the ipsilateral inferior mesenteric ganglion (IMG) depressed the evoked post-ganglionic firing on vesical nerve filaments, but did not block the through-fibre responses (Fig. 1). Stimulation of the contralateral HGN

produced inhibition in three of eight experiments. However, the inhibition was abolished by crushing or sectioning the HGN central to the site of stimulation; a finding which suggests that the inhibition was evoked by an axon or Sokownin reflex through the opposite IMG and HGN (see Langworthy *et al.* 1940), rather than by fibres crossing at the level of the bladder. Stimulation of the HGN at intensities greater than 10–12 V (0·05 msec) also elicited firing on vesical nerve filaments at latencies of 60–70 msec. Since the firing was resistant to ganglionic blocking agents,

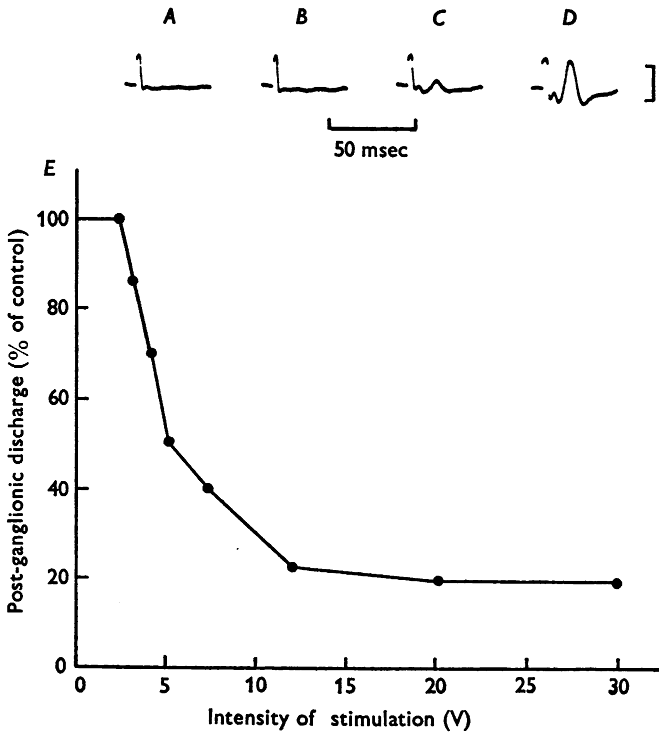


Fig. 2. Depression of transmission in pelvic ganglia by stimulation of the ipsilateral HGN at various intensities. *A*, *B*, *C* and *D* are the post-ganglionic *C*-fibre responses recorded on the HGN to antidromic stimulation of the HGN at 5, 9, 15 and 30 V, respectively. The threshold for the C-fibre response was 10 V. *E* is a plot of the depression of the amplitude of the pelvic post-ganglionic discharge (ordinate) against intensity of HGN stimulation (abscissa) at 20 c/s. Vertical calibration is 250 $\mu$V, negativity upwards.

it is assumed that it occurred as a result of stimulation of afferent fibres or sympathetic post-ganglionic axons. The calculated conduction velocity in this pathway was 1–1·5 m/sec.

The inhibitory responses to HGN-stimulation were apparent at intensities of stimulation below those which produced a detectable discharge on vesical nerves. Thresholds ranged from 2 to 5 V (0·05 msec duration), while maximal inhibition was generally produced by stimuli between 8 and 12 V (Fig. 2). Similar threshold and maximal values were obtained when stimulating preganglionic nerves to the IMG. Based on direct electrical recordings from the HGN it would appear that this range of voltages activates preganglionic B-fibres but excites relatively few post-ganglionic C-fibres (Fig. 2). It seems, therefore, that the sympathetic inhibitory pathway is comprised in part of preganglionic axons which pass directly through the IMG to make synapses with neurones in the pelvic plexus.

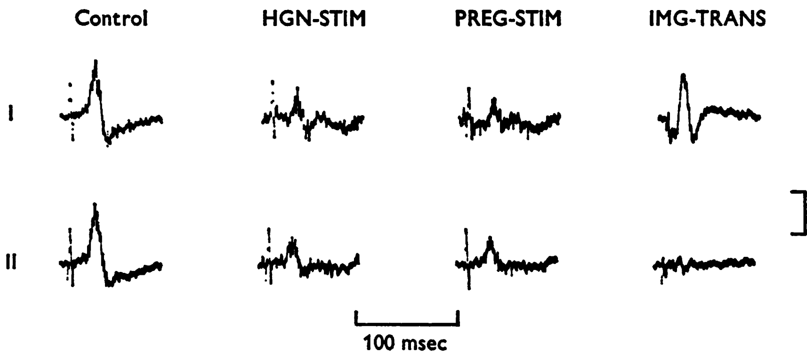


Fig. 3. Depression of transmission in pelvic ganglia during stimulation of either the ipsilateral hypogastric nerve (HGN-STIM, 10 V, 20 c/s) or preganglionic nerves (PREG-STIM, 10 V, 20 c/s) to the ipsilateral inferior mesenteric ganglion (IMG). Records I and II were taken, respectively, before and after the topical application of nicotine hydrochloride (0·1 % solution) to the IMG. This concentration of nicotine blocked synaptic transmission in the IMG (record II, IMG-TRANS). Pelvic ganglionic transmission was recorded as in Fig. 1. Note that the initial through-fibre response was not depressed during the inhibition. IMG-TRANS: the post-ganglionic response recorded on the ipsilateral HGN to stimulation (10 V, 0·5 c/s) of two preganglionic nerves to the ipsilateral IMG. Vertical calibration is 100 $\mu$V, negativity upwards.

The existence of a non-synaptic pathway through the IMG is also indicated by results shown in Fig. 3. In this experiment, stimulation of either the HGN or a preganglionic nerve to the IMG depressed pelvic ganglionic transmission (records I). After the topical application of nicotine to the IMG, in a concentration which completely blocked IMG-transmission (records II), stimulation at both sites produced the same degree of inhibition. Clearly, in this animal, the preganglionic axons made the majority of their synaptic connexions peripheral to the IMG. Similar results were

obtained in another experiment; however, in two additional experiments the local application of nicotine reduced the inhibition to preganglionic stimulation without altering the response to HGN stimulation. These findings suggest that neurones in the IMG are also involved in the inhibitory pathway.

The ganglionic inhibition elicited by activation of the sympathetic

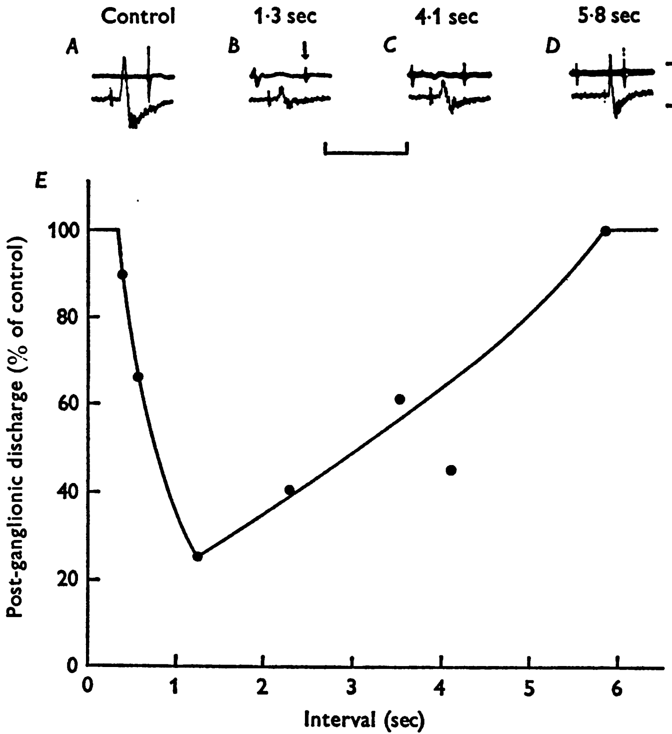


Fig. 4. Time course of the inhibitory effect in pelvic ganglia to activation of the HGN with trains of stimuli (100 msec train duration, 20 V, 200 c/s intra-train frequency). *A* is a control response evoked by stimulation of the pelvic nerve (0·16 c/s) at submaximal stimulation (2 V). Top trace in this and remaining records is taken at slow sweep to show the time relation between the HGN-stimulus train (at the beginning of the sweep) and the pelvic-evoked response (at the arrow). Bottom trace is the pelvic-evoked response on an expanded sweep. Interval (in sec) between the start of the HGN-train and the pelvic response is above each record. *E* is a plot of the depression of the amplitude of the pelvic discharge (ordinate) against the interval between the start of the train and the occurrence of the pelvic discharge. For the upper traces the horizontal calibration represents 2 sec in *A* and *B*, 5 sec in *C* and 10 sec in *D*. For the lower traces the calibration represents 100 msec in *A*–*C* and 200 msec in *D*. Vertical calibration represents 240 $\mu$V for top tracings and 200 $\mu$V for bottom tracings.

fibres was only observed at frequencies of stimulation above 2 c/s and commonly reached a maximum at frequencies of 20–30 c/s (Fig. 1). The inhibition was detectable within 1–5 sec after the onset of stimulation, persisted for the period of stimulation (15–40 sec) and for 5–40 sec after the termination of the stimulus. A facilitation of transmission lasting 1–2 min occurred during the recovery period in approximately 50% of the experiments. In two experiments, hypogastric nerve stimulation (HGS) elicited only a facilitation of transmission without a preceding inhibition.

Ganglionic inhibition was also produced in a few experiments by stimulating the HGN with short trains of pulses (50–300 msec train duration, 40–200 c/s intra-train frequency). The inhibition occurred at a considerable latency (100–600 msec after the beginning of the train) and persisted for 1–5 sec following the stimulus (Fig. 4).

The degree of HGN-induced inhibition was inversely related to the level of excitation in the pelvic ganglionic pathway. For example, inhibition was marked when the pelvic nerves were stimulated at submaximal intensities and at low frequencies (0·5–1 c/s), whereas little if any inhibition occurred when the pelvic excitatory stimulus was applied at supramaximal intensities and at higher frequencies (5–10 c/s).

In addition not all ganglia exhibited inhibition. The degree of inhibition not only varied in different animals, but also varied between different post-ganglionic fibres in the same animal. Occasionally it was observed that inhibition was prominent on one post-ganglionic fibre and not detectable on another. There was no obvious relationship between the presence of inhibition and the anatomical location of the ganglia or the post-ganglionic fibres.

Sympathetic inhibition of pelvic ganglionic transmission was not altered by the administration of $\beta$-adrenergic blocking agents ($\beta$-ABA, propranolol, 0·1–1 mg; INPEA, 0·1–2 mg) (Fig. 5*A*), atropine (10–100 $\mu$g) or picrotoxin (100–600 $\mu$g), but, as illustrated in Fig. 5*B*, was completely antagonized by $\alpha$-adrenergic blocking agents ($\alpha$-ABA, dihydroergotamine, 50–200 $\mu$g; phentolamine, 0·4–1 mg). The antagonism persisted for the duration of the experiment (4–6 hr). $\alpha$-ABAs also reduced or abolished the post-inhibitory facilitation in two of seven experiments. Propranolol reduced the facilitation in two of three experiments.

*Response to injected catecholamines.* The I.A. administration of adrenaline (ADR, 0·1–10 $\mu$g), noradrenaline (NADR, 0·1–10 $\mu$g) and dopamine (DA, 1–20 $\mu$g) mimicked the depressant actions of HGS (Fig. 5). Threshold doses to produce a detectable inhibition (10–15% reduction in the amplitude of the discharge) varied in different experiments; however, the order of potency was invariably ADR > NADR > DA. NADR was generally 5–10 times more potent than DA. The depressant effects of the catechol-

amines appeared 2–10 sec after injection and persisted for 30 sec to 4 min depending upon the dose and the state of the animal's circulation. Isoprenaline (1–10 $\mu$g), an amine with potent *beta* adrenergic actions, exhibited no ganglionic depressant actions, but occasionally produced a slight enhancement of ganglionic transmission (Fig. 5*A*).

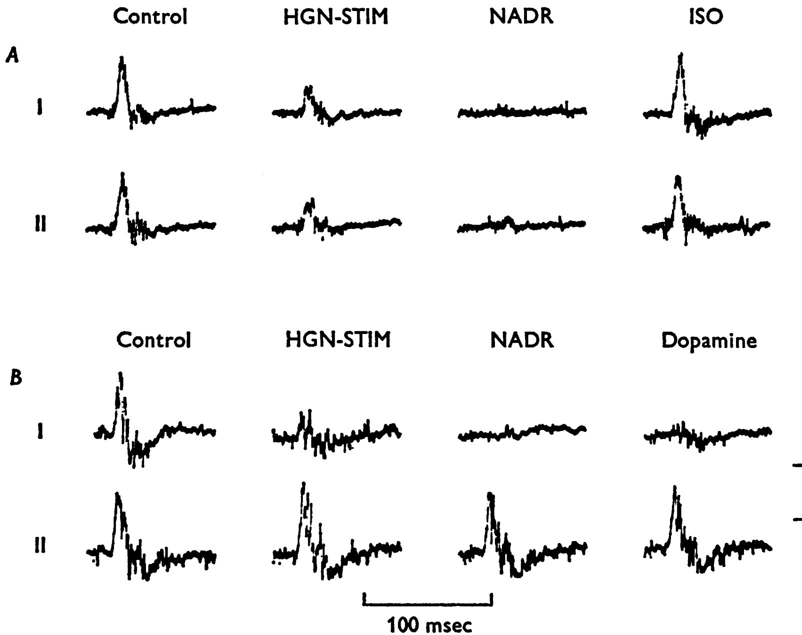


Fig. 5. Effects of electrical stimulation of the hypogastric nerve (HGN-STIM 20 V, 20 c/s) and injected catecholamines on transmission in pelvic ganglia. Recordings were made on a post-ganglionic nerve filament on the surface of the urinary bladder. Discharges were elicited by electrical stimulation of the ipsilateral pelvic nerve (1·5 V, 0·5 c/s). *A*: records I and II are, respectively, before and after the administration of propranolol (0·5 mg). Isoprenaline (ISO) and noradrenaline (NADR) were administered in doses of 1 and 5 $\mu$g, respectively. *B*: records I and II were obtained, respectively, before and after the administration of dihydroergotamine (150 $\mu$g). Noradrenaline (NADR) and dopamine (DA) were administered in doses of 5 $\mu$g. Vertical calibration is the same as in Fig. 1 and applies to records *A* and *B*.

Like the depression produced by HGS, the ganglionic depressant actions of the catecholamines were antagonized by $\alpha$-ABAs, dihydroergotamine (50–200 $\mu$g) and phentolamine (100–300 $\mu$g), but were unaffected by $\beta$-ABAs or picrotoxin (Fig. 5). On the other hand, the effects of another ganglionic depressant, GABA (1–100 $\mu$g; de Groat, 1970), were reduced by picrotoxin (50–300 $\mu$g) but were not altered by either $\alpha$- or $\beta$-ABAs. The

depressant actions of ADR and NADR unlike those of DA and GABA were often followed by a facilitation of transmission. The latter action was not consistently altered by adrenergic blocking agents. In some experiments, it was reduced or completely abolished by dihydroergotamine, whereas in others it was antagonized by propranolol. In other experiments it was not affected by either type of blocking agent.

### *Adrenergic modulation of vesical activity*

*Stimulation of the sympathetic nerves.* In most experiments (eighteen of twenty) repetitive stimulation of the HGNs or preganglionic nerves to the IMG elicited an initial transient rise in intravesical pressure (i.e. contraction of the bladder) followed by a fall in pressure to below control levels (see Langworthy *et al.* 1940; or Edvardsen, 1968*c*, for references to earlier studies). The contractile response was of short duration (20–30 sec) even during continued stimulation, whereas the secondary inhibitory effect persisted during prolonged stimulation (2–3 min) and was detectable for a period of 30–90 sec following the termination of the stimulus. The threshold stimulus to produce a detectable vesical excitatory or inhibitory response varied in different animals from 2 to 5 V (0·05 msec duration), at frequencies of 2–5 c/s. Maximal responses were generally produced by stimulation at 10–30 V, 20–30 c/s.

In comparison to the vesical excitatory response elicited by pelvic nerve stimulation (peak intravesical pressures reaching 80–90 cm $H_2O$), the excitatory response to HGS was relatively small, commonly attaining peak intravesical pressures of 20–30 cm $H_2O$. It was present following bilateral transection of the pelvic nerve or after complete transection of the spinal cord at T10 to T12, a procedure which blocks parasympathetic excitatory reflexes to the bladder (de Groat & Ryall, 1969). Thus, the response to HGS was not dependent upon a central parasympathetic outflow to the vesical smooth muscle. Interestingly, however, stimulation of the HGN in intact animals often induced reflex parasympathetic firing, presumably as a result of activation of vesical afferents during the initial contractile response. The reflex parasympathetic firing complicated the analysis of adrenergic responses; and, thus, most experiments were conducted in animals with pelvic nerves or spinal cord transected.

The vesical contractions to HGS were reduced in amplitude immediately following a spontaneously occurring bladder contraction (in intact animals) or following a contraction elicited by electrical stimulation of the pelvic nerves. This inhibitory interaction would suggest either that the pelvic and hypogastric fibres activate the same population of smooth muscle cells (refractoriness, thereby accounting for the depression) or

that an inhibitory mechanism is activated following a bladder contraction. This particular phenomenon was not studied in further detail.

The vesical contractions elicited by HGS were increased in duration following the administration of propranolol (0·1–1 mg) and INPEA, (1–2 mg) (Edvardsen, 1968*b*). The contractions were blocked by guanethidine (0·7–2 mg), an agent which blocks the release of the adrenergic transmitter (Boura & Green, 1965), but they were not altered by the

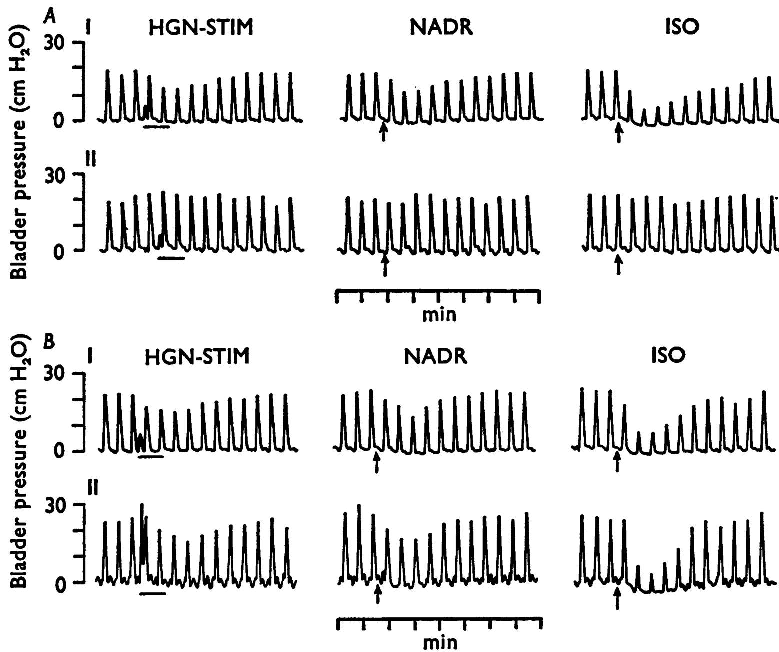


Fig. 6. Effects of electrical stimulation of the hypogastric nerve (HGN-STIM, 20 V, 20 c/s) and injected catecholamines on bladder contractions evoked by intermittent stimulation of the pelvic nerve (15 V, 10 c/s every 30 sec for a period of 5 sec). *A*: records I and II were obtained, respectively, before and after the administration of propranolol (0·5 mg). HGN-STIM was applied during the period indicated by the bar under each record. Note the small bladder contraction which appears at the beginning of each period of HGN-STIM. Noradrenaline (NADR) and isoprenaline (ISO) were injected at the arrows in doses of 5 $\mu$g. *B*: records I and II were obtained, respectively, before and after the administration of dihydroergotamine (100 $\mu$g). Doses of NADR and ISO are the same as in *A*. Horizontal calibration is 1 min per division for both *A* and *B*.

administration of dihydroergotamine (0·1–0·7 mg) or phenoxybenzamine (0·5–2 mg). The latter finding confirms an earlier report by Sigg & Sigg (1964), but conflicts with the results of Edvardsen (1968*b*) which indicated that an $\alpha$-ABA blocked the vesical contraction to HGS. The discrepancy

between our results and those of Edvardsen (1968*b*) is difficult to explain since we have never observed an antagonism even with very large doses of the blocking agents, i.e. 10–15 times the dose required to block adrenergic inhibition in pelvic ganglia. If the bladder contractions to HGS are indeed mediated via $\alpha$-adrenergic receptors, then these receptors must have different pharmacological characteristics than those receptors which are involved in ganglionic inhibition.

The vesical inhibitory effects to HGS were evident as a decrease in the frequency and amplitude of spontaneously occurring bladder contractions or as a decrease in the amplitude of contractions evoked by intermittent electrical stimulation of a pelvic nerve (see Fig. 6). The intrinsic activity of the vesical smooth muscle which was present following bilateral transection of the pelvic nerves or transection of the spinal cord at the lower thoracic levels was also inhibited by HGS. Depending on the frequency and intensity of stimulation, the inhibition ranged up to 100% depression and persisted for as long as 7 min following the cessation of stimulation. The inhibitory effects of HGS were antagonized by the administration of propranolol (0·5–2 mg) (Fig. 6*A*), INPEA (0·5–2 mg) and guanethidine (1–2 mg). The antagonism by propranolol was rapid in onset and persisted for 2–3 hr. On the other hand, $\alpha$-ABAs (dihydroergotamine, phentolamine, phenoxybenzamine) did not modify the inhibition (Fig. 6*B*).

*Response to injected catecholamines*. The I.A. administration of NADR (0·5–10 $\mu$g), ADR (0·5–10 $\mu$g) and isoprenaline (0·1–10 $\mu$g) to the urinary bladder depressed spontaneously occurring and pelvic nerve-evoked contractions of the bladder, decreased the resting bladder 'tone' and depressed the low amplitude contractions of the decentralized bladder. The depression occurred within 20 sec of the injection and persisted for 1–5 min depending upon the dose administered. Dopamine (0·5–50 $\mu$g) did not alter the activity of the urinary bladder. The depressant effects were reduced or completely blocked by the $\beta$-ABAs but were unaffected by the various $\alpha$-ABAs mentioned in preceding sections of this paper (Fig. 6).

The monoamines never produced a detectable contraction of the bladder under any of the conditions encountered in these experiments. GABA 5–100 $\mu$g) produced a transient (20–40 sec) depression (30–50% reduction in peak amplitude) of spontaneous and evoked bladder contractions without producing a reduction in bladder 'tone'. Picrotoxin (100–400 $\mu$g) blocked the depressant actions of GABA in three of six experiments. In some experiments, GABA also evoked a transient contraction of the bladder; this response was not studied in detail.

*Physiological role of sympathetic inhibitory mechanisms in the regulation of bladder activity*

If sympathetic fibres produce a tonic inhibition of the vesical smooth muscle or the vesical parasympathetic ganglion cells, then it might be expected that a surgical or pharmacological interruption of the sympathetic pathways would enhance the activity of these cells. Experiments were conducted to examine this possibility. It was found that neither spontaneous or pelvic nerve-evoked contractions of the bladder nor transmission in pelvic ganglia were consistently altered by transections of the HGN or by the administration of $\alpha$- or $\beta$-adrenergic blocking agents. It was observed as reported previously by Sigg & Sigg (1964), that dihydroergotamine increased base line pressure ('tone') in the bladder; however, this effect occurred in decentralized, as well as normally innervated preparations and would therefore appear to be related to a direct excitatory effect on the vesical smooth muscle rather than to a block of adrenergic inhibition. Other $\alpha$-ABAs did not produce this effect.

The administration of propranolol (0·5–2 mg) enhanced bladder activity and 'tone' in only a small percentage of experiments in which it was tested. Large doses of propranolol (2–3 mg) exerted direct depressant actions on pelvic ganglionic transmission. This action has been observed in sympathetic ganglia following the administration of other $\beta$-ABAs (de Groat & Volle, 1966*a*, *b*) and probably represents a local anaesthetic or membrane stabilizing action, which is unrelated to adrenergic blocking properties of the drugs.

## DISCUSSION

It has been shown in the present investigation that catecholamines administered by close I.A. injection or released endogenously during stimulation of the hypogastric nerves (HGN) elicit two distinct inhibitory responses in the urinary bladder of the cat (see summary diagram in Fig. 7). One type of inhibition, which was apparent as a depression of spontaneous or evoked bladder contractions, was antagonized by $\beta$-ABAs. Since this inhibition could be demonstrated in normally innervated as well as decentralized unstimulated bladder preparations, it must have been mediated by a direct action on the non-neural elements in the bladder, i.e. the vesical smooth muscle cells. This will be termed 'post-junctional inhibition'. The second type of inhibition occurred in the parasympathetic ganglia on the surface of the urinary bladder. This inhibition was unaffected by $\beta$-ABAs, but was completely antagonized by $\alpha$-ABAs. Thus, adrenergic modulation of bladder activity seems to involve depressant actions at different sites in the vesical neuromuscular apparatus and also

actions on different adrenergic receptors: β-receptors in vesical smooth muscle and α-receptors in parasympathetic ganglia.

*Adrenergic inhibition in ganglia.* The inhibition observed in parasympathetic ganglia is of particular interest, since it represents the first demonstration of ganglionic blockade by an adrenergic synaptic mechanism. The existence of such an inhibitory mechanism has been a subject of speculation for many years (Marrazzi, 1939; Bülbring, 1944) and is now supported by a considerable body of experimental evidence. Catecholamines are present in sympathetic and parasympathetic ganglia in nerve

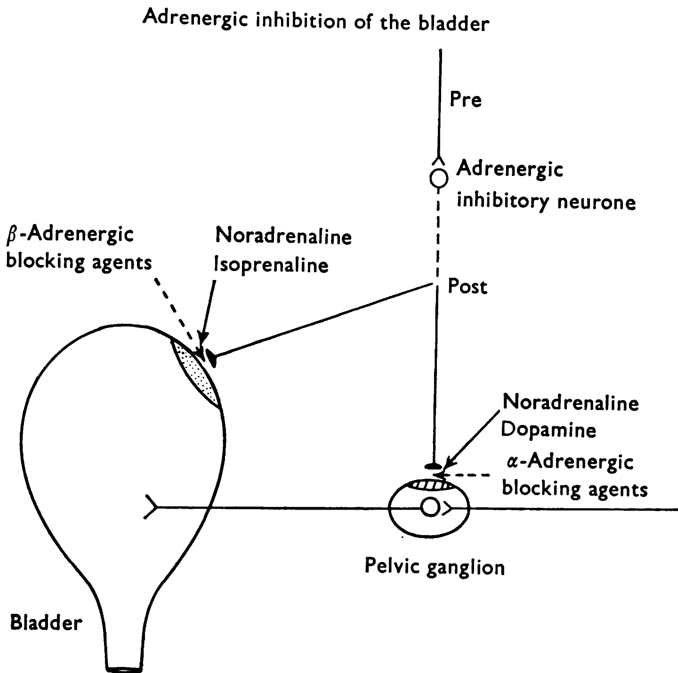


Fig. 7. Diagrammatic representation of receptors mediating adrenergic inhibition in the urinary bladder of the cat. The dashed line from the adrenergic inhibitory neurone indicates that the post-ganglionic axons to the bladder and the pelvic ganglia do not necessarily originate from the same adrenergic inhibitory neurone. See Discussion for further details.

terminals which form basket-like synaptic structures around the ganglion cells (Hamberger & Norberg, 1965*a*, *b*; Jacobowitz, 1970); and catecholamine-like substances are released into the venous effluent of isolated perfused ganglia during preganglionic stimulation (Bülbring, 1944). Exogenous catecholamines are potent ganglionic depressants, which hyperpolarize sympathetic ganglion cells (Lundberg, 1952; de Groat &

Volle, 1966*a*, *b*; Libet & Kobayashi, 1969); and there is evidence from the studies of Eccles & Libet (1961) and more recently by Libet and co-workers (see review by Libet, 1970, for references) that endogenously released catecholamines have a similar hyperpolarizing action. It is tempting to speculate, therefore, that inhibition observed in vesical ganglia is also mediated by a hyperpolarization of the ganglion cells. However, a presynaptic inhibitory mechanism cannot be eliminated (Christ & Nishi, 1971).

An anatomical substrate for adrenergic inhibition in vesical ganglia has been described by Hamberger & Norberg (1965*a*, *b*), El-Badawi (1967) and El-Badawi & Schenk (1971). Using histochemical techniques these investigators showed that adrenergic terminals occur in close apposition to the vesical cholinergic ganglion cells. Since the terminals did not degenerate following section of the HGNs or the lumbar sympathetic chain, it was concluded that they were derived from adrenergic neurones located in the pelvic plexus. We now suggest that these neurones receive synaptic connexions from preganglionic fibres in the HGN. This proposal is based on two observations. First, that the threshold stimulus (2–5 V) for producing inhibition in pelvic ganglia was below the threshold for activating unmyelinated post-ganglionic fibres and was similar on both the HGNs and on preganglionic fibres to the IMG. Secondly, that in one half of the experiments, complete block of transmission in the IMG by the topical application of nicotine did not reduce adrenergic inhibition elicited by stimulation of preganglionic nerves. This proposed pathway is also supported by the histological findings of Kuntz & Moseley (1936) and Moseley (1936) that a large percentage of pelvic ganglia receive a preganglionic input via the HGNs.

By analogy with synaptic transmission on to other peripheral adrenergic neurones, it is reasonable to assume that the inhibitory neurones in the pelvic plexus were activated by a cholinergic mechanism. Furthermore, since atropine did not block ganglionic inhibition to HGS it would appear that these neurones are excited via nicotinic receptors. This is in contrast to the Eccles & Libet model for sympathetic ganglia (Libet, 1970) in which adrenergic inhibitory neurones are supposedly excited via muscarinic receptors.

*Relative importance of ganglionic and 'post-junctional inhibition' in the bladder.* Of the two adrenergic inhibitory mechanisms 'post-junctional inhibition' ($\beta$-type) obviously had the greatest influence on bladder activity under the conditions existing in the present experiments. Depression by HGS of pelvic nerve-evoked bladder contractions was invariably completely blocked by $\beta$-ABAs and unaffected by $\alpha$-ABAs. The failure of ganglionic inhibition ($\alpha$-type) to alter bladder activity is probably related

both to the nature of the inhibitory mechanism and to the parameters of stimulation which we employed. Ganglionic inhibition was a relatively weak mechanism, which could be demonstrated in only 70% of the preparations and only when the excitatory input to the parasympathetic ganglia was at low level, i.e. submaximal stimulation at low frequencies (0·5–1 c/s). This mode of stimulation was unsuitable for evoking reproducible activity of the entire bladder; and in most experiments it was necessary to stimulate the pelvic nerve at higher intensities and frequencies (5–15 c/s). Under these conditions, ganglionic inhibition evoked by HGS, or by injected catecholamines, was undetectable; and depression of bladder activity was mediated exclusively by a direct inhibition of the vesical smooth muscle. It should be noted, however, that these findings do not necessarily indicate that ganglionic inhibition is unimportant in the regulation of bladder function. The synchronous type of activity which was elicited in these experiments on both inhibitory and excitatory pathways is far removed from the asynchronous discharge which occurs physiologically. It is therefore difficult to estimate the relative importance of the two types of inhibition under more physiological conditions.

*Physiological role of ganglionic and 'post-junctional inhibition' in the regulation of bladder activity.* Adrenergic inhibition in vesical parasympathetic ganglia and smooth muscle occurred at frequencies of HGS within the physiological range of sympathetic firing; it is therefore conceivable that these inhibitory responses might also be elicited by physiological sympathetic activity. Indeed, Edvardsen (1968*a*) and Gjone (1965) noted that bladder contractions were enhanced following section of the HGNs and on this basis suggested that the sympathetics produced a tonic inhibitory action on the bladder. Since Edvardsen (1968*a*) obtained this result only when bladder pressure was high he proposed that afferent activity from the bladder maintained a reflex discharge in vesical sympathetic inhibitory fibres. Recently, a vesico-sympathetic reflex has been demonstrated electrophysiologically (Lalley, de Groat & McLain, 1971); however, in the present experiments we have been unable to show that this pathway produces inhibition in the bladder. For example, neither ganglionic transmission nor evoked or spontaneous bladder activity was consistently changed by transection of the HGNs. Following the administration of dihydroergotamine, we have observed an increase in bladder contractions and tone; however, this was noted in normally innervated as well as decentralized unstimulated bladders and was not duplicated by other $\alpha$-ABAs. Therefore, the action seems attributable to a direct effect of the agent on vesical smooth muscle rather than an antagonism of a ganglionic inhibitory mechanism (see also Sigg & Sigg, 1964). The administration of propranolol enhanced bladder activity and tone in only a small

percentage of the experiments. This action was observed more consistently by Edvardsen (1968*c*) and was ascribed to an antagonism of adrenergic inhibition in the vesical smooth muscle.

In summary, the present experiments demonstrate the potentiality for two types of adrenergic inhibition in the urinary bladder of the cat; however, they provided little evidence that these mechanisms are functionally important in the anaesthetized animal. The discrepancies in this regard between our results and those of Edvardsen (1968*a*, *c*) and Gjone (1965) are difficult to explain.

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