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MECHANISMS UNDERLYING RECURRENT INHIBITION IN THE SACRAL PARASYMPATHETIC OUTFLOW TO THE URINARY BLADDER

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SUMMARY

1. In cats with the sacral dorsal roots cut on one side electrical stimulation (15–40 c/s) of the central end of the transected ipsilateral pelvic nerve depressed spontaneous bladder contractions. The depression was abolished by transecting the ipsilateral sacral ventral roots.

2. Electrical stimulation of acutely or chronically transected ('deafferented') sacral ventral roots depressed spontaneous bladder contractions and the firing of sacral parasympathetic preganglionic neurones innervating the bladder. The depression of neuronal firing occurred ipsilateral and contralateral to the point of stimulation, but only occurred with stimulation of sacral roots containing preganglionic axons and only at intensities of stimulation (0·7–4 V) above the threshold for activation of these axons.

3. The inhibitory responses were not abolished by strychnine administered by micro-electrophoresis to preganglionic neurones, but were blocked by the intravenous administration of strychnine.

4. The firing of preganglionic neurones elicited by micro-electrophoretic administration of an excitant amino acid (DL-homocysteic acid) was not depressed by stimulation of the ventral roots.

5. It is concluded that the inhibition of the sacral outflow to the bladder by stimulation of sacral ventral roots is related to antidromic activation of vesical preganglionic axons. Collaterals of these axons must excite inhibitory interneurones which in turn depress transmission at a site on the micturition reflex pathway prior to the preganglionic neurones.

INTRODUCTION

In a previous investigation (de Groat & Ryall, 1968*b*) evidence was obtained for a recurrent inhibitory pathway in the sacral autonomic outflow to the urinary bladder of the cat. It was shown that electrical

stimulation of the central end of a transected sacral ventral root depressed the firing of contralateral preganglionic neurones innervating the bladder and depressed spontaneous and evoked bladder contractions mediated by the contralateral sacral outflow. The inhibition only occurred with stimulation of ventral roots containing parasympathetic preganglionic axons and only at intensities of stimulation above the threshold for activating these axons. It was proposed that the depression of bladder activity by ventral root stimulation might be mediated by an inhibitory pathway similar to the one involved in recurrent inhibition of somatic motoneurones. It was not established, however, if (1) the site of inhibition was directly on the preganglionic neurones, (2) activation of bladder preganglionic axons specifically produced the inhibition or whether other efferents might be involved, (3) ventral root afferents (Mikeladze, 1965; Dimsdale & Kemp, 1966; Ryall & Piercey, 1970; Coggeshall, Coulter & Willis, 1973, 1974; Clifton, Vance, Applebaum, Coggeshall & Willis, 1974) might produce the inhibition. These questions have been examined in the present experiments.

METHODS

Experiments were performed on twenty-five cats of either sex anaesthetized with chloralose (50–70 mg/kg, I.V.). Anaesthesia was induced in all animals with halothane. In three animals the left sacral ventral roots had been transected intradurally 11–15 days before the experiment to produce degeneration of the ventral root afferent fibres (Coggeshall *et al.* 1974). The hypogastric nerves were sectioned bilaterally to block the sympathetic inhibitory input to the bladder. In some experiments, branches of the pelvic nerves were isolated 1–3 cm from the neck of the bladder and prepared for stimulation or recording. In these experiments the ipsilateral sacral dorsal roots were also transected.

The urinary bladder was cannulated by one of two different methods. In some cats (mostly females) a polyethylene tube (inside diameter, 2 mm) was introduced into the urethra either through the external orifice or through an incision in the urethra and passed into the bladder (de Groat & Lalley, 1972). 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 was inserted into the lumen (de Groat & Ryall, 1969). The cannula was filled with physiological saline solution and connected to a pressure transducer to record the pressure within the bladder. The cannula 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 spinal cord was exposed by a laminectomy extending from the 3rd sacral, S 3, to the 3rd lumbar, L 3, vertebrae. The sacral ventral roots on one side or on both sides were sectioned and mounted on bipolar electrodes from stimulation with rectangular pulses of 0·05–0·1 msec duration. Monopolar recordings from the surface of the spinal cord were obtained with platinum or silver ball electrodes. Surface potentials were displayed on an oscilloscope and photographed on 35 mm film. Potentials were also averaged on a Computer of Average Transients (CAT) or a PDP 8 computer and were then plotted on an X–Y paper recorder or a Cal Comp Plotter.

The techniques for locating and recording from parasympathetic neurones and for the micro-electrophoretic application of drugs were described in detail in previous papers (de Groat & Ryall, 1968*a*, *b*, 1969; de Groat, 1970, 1971). Sacral parasympathetic neurones were identified by antidromic invasion in response to stimulation of the ventral roots. Extracellular action potentials were recorded with single micropipettes or by means of the centre barrel (4 M-NaCl) of five-barrel micropipettes of 4–8 μm tip diameter. Action potentials were monitored on an oscilloscope and rate of firing was displayed on a rectilinear paper recorder.

Glycine (0·5 M), noradrenaline (0·5 M) and strychnine (either 2 or 10 mM in 165 mM-NaCl solution) were applied electrophoretically to parasympathetic neurones firing in response to distension of the urinary bladder or to the local administration of an excitant amino acid, DL-homocysteic acid (0·2 M, pH 8).

The majority of animals were paralysed with gallamine triethiodide and artificially respired. Experience with unparalysed preparations indicated that the dose of chloralose administered was sufficient to produce surgical anaesthesia for the duration of the experiments. In addition during the experiments depth of anaesthesia was often checked by discontinuing the administration of gallamine and allowing the animal to recover from the paralysis. End-tidal CO_2 was maintained at approximately 4 % by varying the rate and depth of respiration. Systemic blood pressure was measured from the carotid or femoral arteries with a strain-gauge pressure transducer. The animal's temperature was maintained at 36–38° C with the aid of a heating pad.

RESULTS

Recurrent inhibition elicited by stimulation of branches of the pelvic nerve to the bladder

Experiments were conducted in four cats to determine whether antidromic activation of parasympathetic preganglionic axons to the urinary bladder would produce depression of bladder activity. In these animals the sacral dorsal roots were sectioned on the left side; and the left pelvic nerve was transected 1–2 cm below the neck of the bladder. The central end of the sectioned nerve was stimulated at varying frequencies and intensities. In every experiment stimulation at intensities above the threshold for activation of preganglionic axons (1–3 V, 0·05 msec) at frequencies between 15 and 40 c/s depressed spontaneous bladder contractions mediated by the contralateral sacral outflow. In one experiment the inhibition was abolished by transecting the left S 2 and S 3 ventral roots (Fig. 1). The inhibitory effects persisted for 2–5 min after the termination of the stimulus.

These results are consistent with the view that antidromic activation of the preganglionic outflow to the bladder depresses bladder activity. The experiments do not exclude however that the inhibitory effects might be mediated by vesical afferent fibres passing to the sacral cord by the ventral roots.

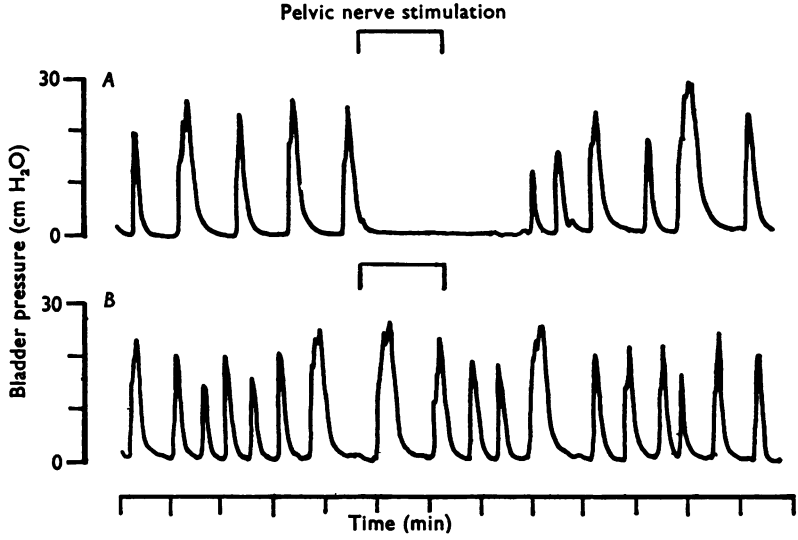

Fig. 1. Recurrent inhibition of spontaneous bladder contractions by stimulation of the central end of a transected pelvic nerve. Stimulation applied at a frequency of 20 c/s during period indicated by bar. *A*, obtained with ipsilateral sacral dorsal roots transected, but the ventral roots intact; *B*, after transection of the ipsilateral sacral ventral roots.

Recurrent inhibition elicited by stimulation of de-afferented sacral ventral roots

Experiments were conducted on three cats in which the sacral ventral roots had been transected intradurally 11–15 days before the experiment. It is reasonable to expect that this is a sufficient period for degeneration of the 'aberrant' afferent axons arising from the dorsal root ganglion cells (Clifton *et al.* 1974).

In these experiments electrical stimulation of the sacral ventral roots produced parasympathetic antidromic surface potentials on the cord dorsum (Fig. 2) which were similar to the potentials recorded in cats with intact or acutely transected roots (de Groat & Ryall 1968*a*). Threshold intensities (0·7–1·5 V, 0·05 msec), latencies for antidromic field potentials and distribution of preganglionic axons in different sacral roots were indistinguishable from data obtained in normal animals. Stimulation of ventral roots giving an antidromic parasympathetic field depressed spontaneous bladder contractions and depressed the firing of preganglionic bladder cells, whereas stimulation of roots lacking parasympathetic efferents did not affect bladder activity or neuronal activity (Fig. 2*B*). The threshold for producing inhibition correlated with the thresholds for eliciting the antidromic field, and for eliciting a *B*-fibre volley on the same

ventral root. Inhibition was elicited at frequencies of stimulation as low as 5 c/s and was usually maximal at 20–30 c/s.

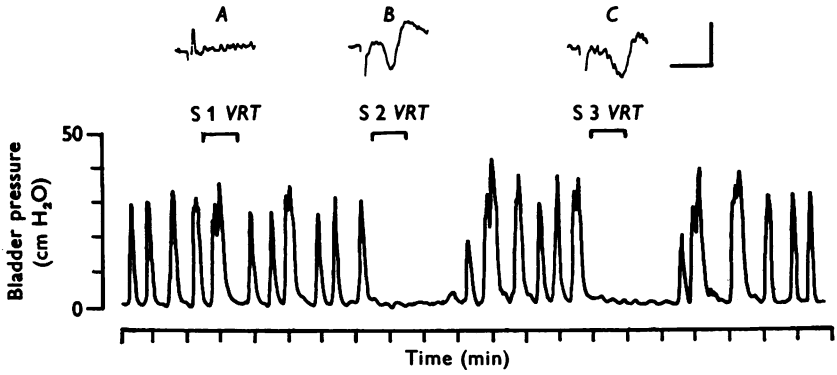

Fig. 2. Recurrent inhibition of spontaneous bladder contractions by stimulation of the central end of 'de-afferented' sacral ventral roots. The roots were transected 15 days before the experiment. *A*, *B*, and *C*, records of the antidromic potentials recorded from the cord dorsum in response to stimulation of central ends of the transected S 1, S 2 and S 3 ventral roots, *VRT*, respectively. Stimulus intensities 5 V, 0·05 msec. Vertical calibration 200 μV, negativity upward; horizontal calibration 5 msec. *D*, spontaneous bladder contractions during stimulation of the ventral roots at a frequency of 20 c/s. Stimulus applied during period indicated by bar.

Site of recurrent inhibition

In previous studies recurrent inhibition was only demonstrated contralateral to the point of stimulation. In the present experiments inhibition of neuronal firing was also obtained by stimulating an ipsilateral root from an adjacent segment or a root from the same segment. As shown in Fig. 3 stimulation of the S 2 ventral root, the same root which was used to antidromically activate the preganglionic neurone produced a prolonged inhibition of the neuronal firing that was elicited by distending the bladder. The inhibition was not due to refractoriness following antidromic activation since it could be elicited by intensities of stimulation (1·6 V) below the threshold (3 V) for activating the cell. At this intensity of stimulation the inhibition was equivalent to the inhibition elicited by higher-intensity stimulation of the same or contralateral ventral roots. Similar results were obtained with six other parasympathetic neurones that were synaptically activated by distension of the bladder.

Refractoriness following antidromic stimulation can contribute to depression of preganglionic firing as shown in Fig. 4. In this cell recurrent inhibition was minimal at intensities of stimulation below the threshold for antidromically activating the cell. However, a slight increase in the

intensity of stimulation (to 2 V) sufficient to activate the cell antidromically produced a depression of neuronal firing lasting for 30 sec. Although recurrent inhibition cannot be entirely excluded in this instance, it seems likely that since depression occurred in an all-or-none fashion at the

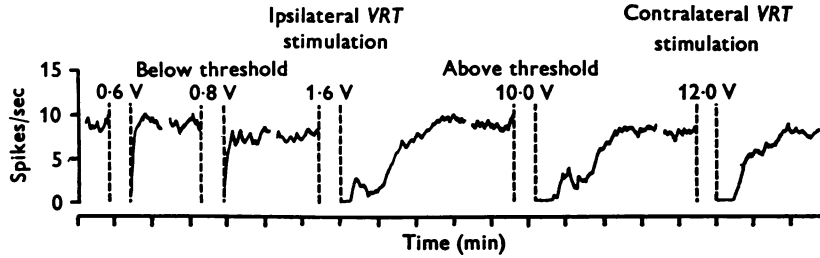

Fig. 3. Recurrent inhibition of the firing of a preganglionic parasympathetic neurone. Firing induced by increasing intravesical pressure to 10 cm H_2O. Ipsilateral ventral root, *VRT*, stimulation applied to the root (S 2) used to antidromically activate the cell (threshold, 3 V). Ventral roots stimulated at 20 c/s between dashed lines at the indicated intensities. The counter was turned off during stimulation. Stimulation also applied to the contralateral S 2 ventral root.

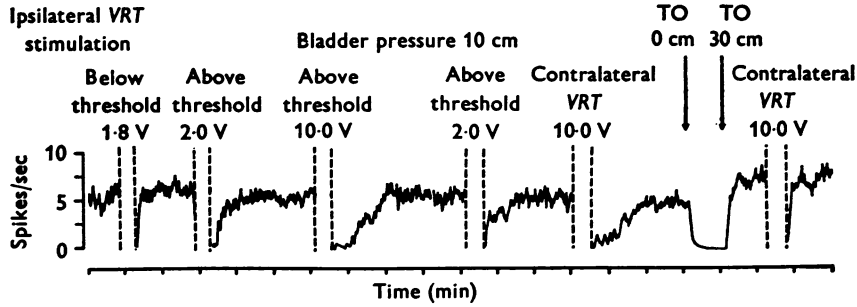

Fig. 4. Recurrent inhibition of the firing of a preganglionic parasympathetic neurone. Firing induced by increasing bladder pressure to 10 cmH_2O. Ipsilateral ventral root, *VRT*, stimulation applied to the root (S 2) used to antidromically activate the cell (threshold, 2 V). In this experiment the sacral ventral roots were transected bilaterally. Ventral roots stimulated at 20 c/s between dashed lines at the indicated intensities, during which the counter was turned off. At arrows intravesical pressure was lowered and raised, respectively, to 0 and 30 cmH_2O.

threshold for antidromic activation that it was primarily due to post-tetanic refractoriness. At higher intensities of stimulation the inhibition was considerably prolonged. This depression must be attributed to recurrent inhibition. As noted in previous experiments recurrent inhibition was reduced at increased bladder pressures (Fig. 4).

This prolonged period of depression of bladder activity or preganglionic firing following repetitive ventral-root stimulation might be attributed to post-tetanic potentiation of the inhibitory pathway or possibly to the withdrawal of the excitatory input to the bladder which then causes a reduction in vesical pressure and in turn vesical afferent firing. Recovery therefore of preganglionic firing might be a function of the peripheral neuromuscular and afferent mechanisms which maintain the firing as well as central mechanisms. The role of the peripheral components in the recovery from inhibition could be minimized by maintaining the bladder at constant pressure as shown in Figs. 3 and 7. However, to exclude this complication completely it was necessary to interrupt the micturition reflex loop by blocking the efferent input to the bladder. In this manner the afferent drive on the preganglionic neurones could be held constant during the experiment. This was done in four cats by transecting the sacral ventral roots bilaterally and then activating the preganglionic neurones by applying a constant pressure stimulus to the bladder. As shown in Fig. 4, under open loop conditions, ventral root stimulation still produced a prolonged depression of neuronal firing, indicating the duration of the inhibition is dependent entirely on a central mechanism.

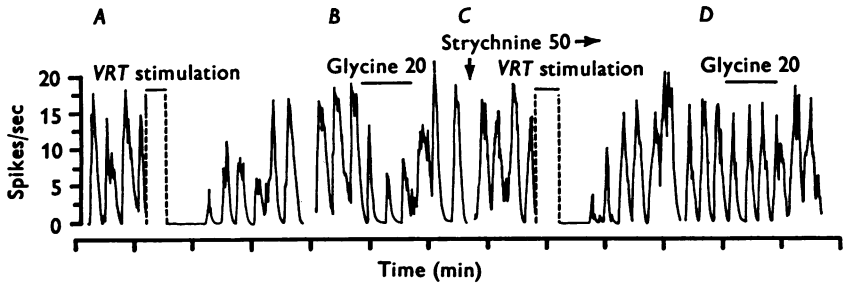

Fig. 5. Effect of strychnine (50 nA) on recurrent inhibition and glycine depression of the firing of a parasympathetic preganglionic neurone. Firing induced by increasing bladder pressure to 10 cmH_2O. S 2 and S 3 ventral roots transected bilaterally. Stimulation at 20 c/s applied to the contralateral S 2 ventral root, *VRT*, between dashed lines; counter turned off during stimulation. *A*, *B*, and *C*, *D*, obtained, respectively, before and after the administration of strychnine.

Strychnine administered intravenously blocks recurrent inhibition (de Groat & Ryall, 1968*b*), suggesting that glycine or a related amino acid may be the inhibitory transmitter released on to preganglionic neurones (de Groat, 1970). However, in the present experiments it was shown that strychnine applied locally to bladder preganglionic neurones blocked the depressant effects of glycine but did not block recurrent inhibition (ten cells, Fig. 5). Strychnine was applied electrophoretically with currents

10–20 times (20–100 nA) those necessary to block the effects of glycine. These quantities of strychnine did not block the depressant effects of noradrenaline (Fig. 6) (Ryall & de Groat, 1972), but often had direct depressant effects on cell firing and spike amplitude. The effects of strychnine were studied under 'open' as well as 'closed' loop conditions. While there are several interpretations of these negative results, an obvious one

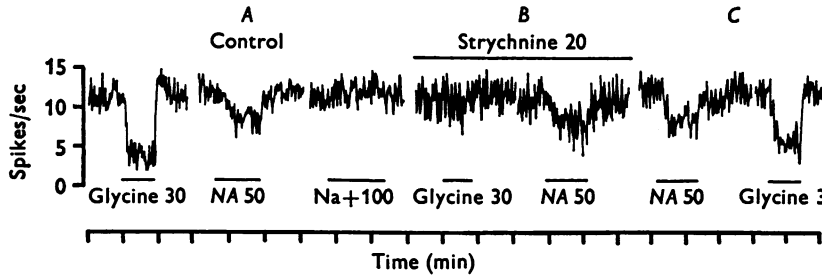

Fig. 6. Effect of strychnine (20 nA) on glycine and noradrenaline, *NA*, depression of the firing of a parasympathetic preganglionic neurone. Firing induced by increasing bladder pressure to 15 cmH_2O. Electrophoretic currents indicated in nA. Strychnine applied during *B*. *C*, taken 20 min after the cessation of strychnine administration. Na + 100, 100 nA of positive current applied through a barrel containing sodium chloride solution.

is that recurrent inhibition may be mediated by glycine, but not at synapses on the parasympathetic preganglionic neurones, but at an interneuronal site earlier on the micturition pathway. One approach to answer this question would be to determine whether recurrent inhibition is accompanied by a direct depression of the preganglionic neurones either using intracellular recording or by estimating the level of excitability of the cell by measuring the discharge elicited by an excitant amino acid. The latter technique was used in the present experiments. As shown in Fig. 7 a preganglionic cell was fired synaptically by maintaining the bladder at elevated constant pressure. Stimulation of the appropriate sacral ventral root inhibited this firing. Then bladder pressure was reduced to zero, which reduced the cell firing to zero and the cell was then activated by the electrophoretic administration of DL-homocysteic acid. The current through the DL-homocysteic acid barrel was adjusted to give approximately the same frequency of firing. It was a consistent finding in seven experiments (ten cells) that firing induced by DL-homocysteic acid was completely resistant to recurrent inhibition. These findings further indicate that recurrent inhibition is not mediated by a direct post-synaptic inhibition of the preganglionic neurones. In this regard it is interesting that a few

interneurones were encountered in the sacral cord which were inhibited by tetanic stimulation of the sacral ventral roots. Two of the interneurones were only inhibited by intensities of stimulation above the threshold for activating preganglionic axons. One of the interneurones was fired by bladder inflation.

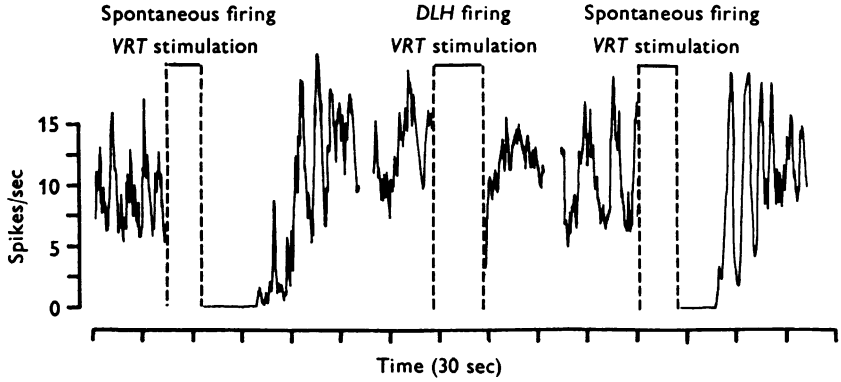

Fig. 7. Effect of recurrent inhibition on spontaneous firing and DL-homo cysteic-acid-induced, *DLH*, firing of a parasympathetic preganglionic neurone. Spontaneous firing induced by increasing bladder pressure to 10 cmH_2O. Contralateral S2 ventral root, *VRT*, stimulation applied at 30 c/s between dashed lines during which time counter was turned off.

DISCUSSION

The present results indicate that repetitive activation of preganglionic axons in the sacral outflow to the urinary bladder produces an inhibition at some point in the micturition reflex pathway. The inhibition is apparently mediated by axon collaterals which excite inhibitory interneurones in the region of the autonomic nucleus (de Groat & Ryall, 1968*b*). It is not known, however, if the inhibitory mechanism is activated exclusively by bladder efferents or whether it also receives an input from preganglionic fibres to other pelvic organs (e.g. colon and sex organs). Studies on 'de-afferented' ventral roots indicated that afferent fibres were not involved in the inhibition.

Recurrent inhibition was distributed contralaterally as well as ipsilaterally to the same and adjacent segments and had a long duration often persisting for several minutes following repetitive stimulation. Initially it was considered that the prolonged inhibition observed under closed loop conditions was related to a peripheral mechanism (e.g. slow recovery of tone in vesical smooth) which would reduce vesical afferent firing and in turn the synaptic input to the preganglionic neurones. However, in the

present experiments recurrent inhibition of long duration was also noted when the bladder was decentralized (open loop). Under these conditions input from the bladder would be expected to remain constant during and after the inhibitory response. Thus, the prolonged nature of recurrent inhibition must be central in origin, possibly related to post-tetanic potentiation in the inhibitory pathway.

The action of strychnine, administered I.V., to block recurrent inhibition suggests that glycine maybe the inhibitory transmitter. However, the failure of electrophoretically applied strychnine to block the inhibition while at the same time blocking the effects of glycine suggests that the inhibitory action must occur by disfacilitation by depressing transmission at a site earlier on the micturition reflex pathway. The failure of ventral root stimulation to alter the DL-homocysteic-acid-induced firing of preganglionic neurones or to elicit inhibitory post-synaptic potentials (de Groat & Ryall, 1968*a*) is consistent with this view. The site of inhibition must be at the spinal level since it has been observed in chronic spinal animals (de Groat & Ryall, 1968*b*). It is noteworthy that somatovesical inhibitory reflexes may also be mediated by inhibition at an interneuronal site (de Groat, 1971).

The role of acetylcholine in the recurrent inhibitory pathway is uncertain. As reported in a previous study, dihydro-β-erythroidine administered I.V. did not block recurrent inhibition, although it is well known to block recurrent inhibition in the somatic system. The effects of atropine upon recurrent inhibition were inconclusive due to its peripheral actions on the bladder (de Groat & Ryall, 1968*b*). In some experiments the I.V. administration of the anticholinesterase agent, physostigmine, prolonged or accentuated recurrent inhibition and depressed spontaneous and evoked bladder contractions (W. C. de Groat & P. M. Lalley, unpublished). However, in many experiments the drug increased bladder activity, presumably via its action on cholinergic synapses in the peripheral pathway to the bladder.

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