THE EFFECTS OF URINARY BLADDER AFFERENTS ON RESPIRATION

by

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The electrical activity of phrenic and recurrent laryngeal motoneurons was recorded during activation of urinary bladder afferents in Nembutal anaesthetised, paralysed, artificially ventilated cats. Distension or spontaneous neurogenic contraction of the bladder decreased the amplitude and frequency of the burst activity in these two nerves. The decrease in frequency was due mainly to a prolongation of the interburst interval (duration of expiration). Similar effects were observed during low frequency electrical stimulation of pelvic nerve afferents with conduction velocities in the A gamma-delta range. Contraction or distension of the bladder in spontaneously breathing animals or high frequency stimulation of pelvic nerve afferents increased the amplitude of the burst activity in these two nerves. These results suggest an action of bladder afferents on the brainstem oscillator controlling respiratory frequency and drive. Excitatory and inhibitory effects of bladder afferents on phrenic motoneurons through spinal circuits were demonstrated in acutely spinalised cats.

RESUME

L'activité électrique des motoneurones phréniques et laryngés récurrents a été enregistrée pendant l'activation des afférents vésicaux chez le chat anesthésié, paralysé et ventilé artificiellement. La distension ou la contraction neurogénique spontanée de la vessie a déclenché une diminution de l'amplitude et de la fréquence de l'activité des 2 nerfs. La diminution de la fréquence a été causé le plus souvent par une prolongation de la durée expiratoire. Des effets semblables ont été observés pendant la stimulation électrique en basse fréquence des afférents du nerf pelvien avec une vitesse de conduction dans la groupe des A gamma-delta. La contraction ou la distension de la vessie chez les animaux respirant spontanément ou la stimulation en haute fréquence de nerf pelvien ont augmenté l'amplitude de l'activité des 2 nerfs. Ces résultats suggèrent une action des afférents vésicaux sur l'oscillateur médullaire qui contrôle la fréquence et la commande respiratoire. Des effets excitateurs et inhibiteurs spinaux des afférents vésicaux sur les motoneurones phréniques ont été démontrés chez le chat spinalisé.

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I. INTRODUCTION

This thesis presents an analysis of some of the reflex effects on respiration originating from the urinary bladder. There are several qualitative analogies between the effects on respiration of bladder afferents and of other abdominal visceral afferents, suggesting a common mechanism of action in the CNS. The survey of the literature will therefore include all abdominal visceral afferents. The data concerning the urinary bladder will be reviewed separately from the data dealing with the effects of other abdominal visceral afferents on respiration.

1) Respiratory Effects of Abdominal Visceral Afferents Other Than Those From the Urinary Bladder

By the early part of this century, several reports had appeared in the literature describing inhibition of respiratory activity in experimental animals during distension or manipulation of abdominal viscera. Distension of the bile ducts (Davis et al., 1929) or of the intestine (Crowley, 1941), or traction applied to various abdominal viscera (Brewer et al., 1934; Brewer and Bryant, 1935; Reeve et al., 1951), caused apnea or depression of respiratory movements which was abolished by splanchnic nerve section (Davis et al., 1929; Brewer and Bryant, 1935; Crowley, 1941). Similar depression of respiratory movements could be evoked by electrical stimulation of splanchnic nerve afferents (Graham, 1881; Brewer et al., 1934; Martin et al., 1942). Similar effects have been reported for manipulation of abdominal viscera in humans undergoing abdominal surgery (Rovenstine and

Hershenson, 1938; Reeve, 1951). As in experimental animals, electrical stimulation of the greater splanchnic nerve in humans evokes apnea (Kruta et al., 1950). Inflammation of the viscera seems to enhance the depressant effects of manipulation (Reeve, 1951).

The above observations were paralleled by physiological experiments aimed at explaining the genesis of visceromotor reflexes associated with intestinal trauma (Downman and McSwiney, 1946) or abdominal inflammation. Manipulation of the intestine in cats with acute spinal transection at C1 caused contraction of the abdominal muscles (Downman and McSwiney, 1946). Electrical stimulation of afferents of the greater splanchnic nerve was capable of exciting inspiratory and expiratory muscles both in the CNS-intact cat (Yamamoto, 1961b) and in the acute spinal cat (transected at C1, Miller, 1925; transected at T1, Downman, 1955). Recording of the volley evoked by splanchnic afferent stimulation (Downman, 1955) showed that A gamma and delta fibres were responsible for these reflex effects.

These observations suggest that one possible explanation of the respiratory depression evoked by distension or manipulation of abdominal viscera is that it may result from sustained contraction of inspiratory and/or expiratory muscles. Tonic contraction of the abdominal musculature would oppose the downward excursion of the diaphragm and in conjunction with the tonic contraction of the intercostal muscles would also decrease the compliance of the chest wall (Campbell et al., 1970) and would therefore decrease tidal volume or cause apnea. In this case, the respiratory depression would be due to a spinal reflex since

contraction of the respiratory muscles could be obtained in spinal animals either during manipulation of the intestine or during electrical stimulation of splanchnic nerve afferents.

There is considerable intersegmental spread of the splanchnic afferent input in the acute spinal cat (Fig. 1), which accounts for the widespread activation of respiratory muscles by stimulation of splanchnic nerve afferents. This wide intersegmental distribution is due partly to intraspinal pathways and partly to an extraspinal pathway in the sympathetic chain. The role of these spinal reflexes in the intact animal is not clear. These reflexes are either considerably attenuated or absent in the decerebrate preparation (Alderson and Downman, 1966). This suggests a tonic inhibitory influence of the brainstem onto these spinal circuits. Alderson and Downman were able to show that stimulation of sites within the ventromedial medulla inhibited these reflexes, while lesions of the same sites in the decerebrate preparation led to a facilitation of these reflexes similar to the release seen following spinal transection. In the anaesthetised, CNS intact cat these reflexes are not attenuated.

The respiratory depression evoked by distension or manipulation of abdominal viscera could alternatively be due to an action of splanchnic visceral afferents on the respiratory oscillator in the brainstem. The apnea would then result from the suppression of the excitatory drive that this neural oscillator provides to spinal respiratory motoneurons. Evidence for this action was only recently provided by Garnier and Albano (1978) in the CNS intact cat. Electrical stimulation of low

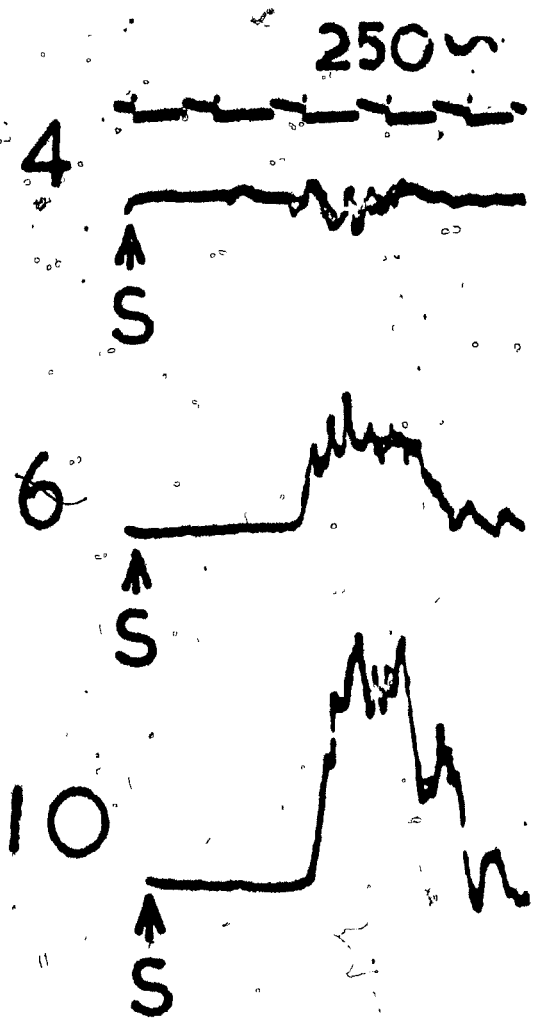

Figure 1. Taken from Downman, 1955. Electrical stimulation of splanchnic afferents evokes reflex discharge of intercostal nerve efferents in cats spinalised at T1. The reflex is attenuated at more rostral spinal segments.

4) reflex recorded from T4 intercostal nerve

6) reflex recorded from T6 intercostal nerve

10) reflex recorded from T10 intercostal nerve

Intensity of stimulation applied to splanchnic nerve is the same in all three cases.

threshold splanchnic visceral afferents inhibited medullary inspiratory neurons and excited medullary expiratory neurons. Some of these neurons have been shown to project to those areas in which spinal respiratory motoneurons are located (Bianchi, 1971). Others, which do not project to the spinal cord, are thought to be involved in the generation of the respiratory rhythm. Repetitive stimulation of splanchnic afferents does depress the inspiration-synchronous activity of phrenic (Garnier and Albano., 1977; Kostreva et al., 1978; Prabhakar et al., 1978) (Fig. 2) and external intercostal motoneurons (Kostreva et al., 1978). The afferents mediating this response have conduction velocities in the A gamma and delta range (Kostreva et al., 1978). The observation that the activity of two pools of inspiratory motoneurons is depressed suggests an action of splanchnic afferents on the common input to both pools, i.e., the descending excitation from the respiratory oscillator.

Repetitive stimulation of splanchnic afferents also decreased respiratory frequency (Prabhakar et al., 1978). Brief trains of stimuli delivered during inspiration shortened inspiration and prolonged the subsequent period of expiration. Similar trains, delivered during expiration, prolonged expiration without affecting the subsequent inspiration. Repetitive stimulation of sympathetic chain afferents (T7-T9) has been shown to selectively prolong expiration (Kostreva et al., 1978). These modifications of respiratory frequency suggest an action on the respiratory oscillator (see DISCUSSION).

To summarise: splanchnic abdominal afferents have two effects on spinal respiratory motoneurons: i) excitation of both inspiratory and

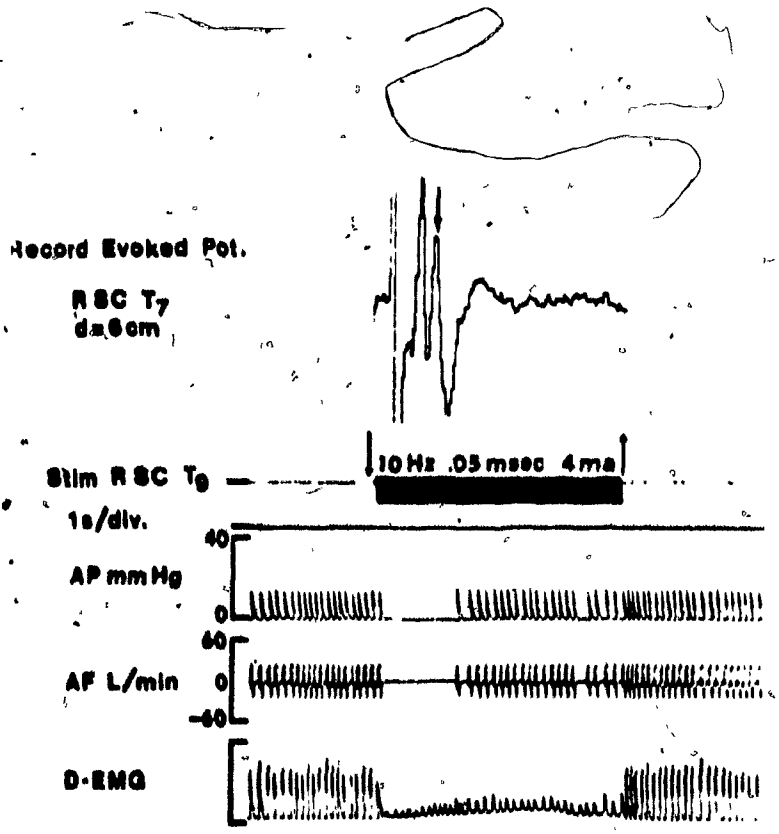

Figure 2. Taken from Kostreva et al., 1978.

expiratory motoneurons through a spinal circuit and ii) depression of inspiratory motoneurons through an inhibition of medullary inspiratory neurons and a prolongation of expiration through an excitation of medullary expiratory neurons.

Investigation of other groups of visceral afferents showed that A gamma-delta afferents in the dorsal and ventral ansa subclavia have effects similar to those of splanchnic nerve afferents on phrenic activity (Kostreva et al., 1978). However, the generalization that all visceral afferents have similar effects on respiratory activity cannot be made since neither renal nor mesenteric afferents (Kostreva et al., 1978) had an effect on phrenic activity.

2) Respiratory Effects of Urinary Bladder Afferents

The respiratory effects evoked by bladder afferents seem more variable than those evoked by splanchnic afferents. Manipulation of the urinary bladder or urethra in decerebrate cats (Barclay and Franklin, 1937) or spontaneous isometric bladder contractions in the decerebrate or anaesthetised cat (Mellanby and Pratt, 1940) caused contractions of abdominal muscles and diaphragm, but from their data it is impossible to infer how respiration was affected. Bladder distension caused an increase in respiratory frequency and chest excursion in anaesthetised cats (Watkins, 1938; Mukherjee, 1957; Evans and McPherson, 1959). In some cases decreases in chest excursion and respiratory rate were observed (Watkins, 1938).

Many afferents from the urinary bladder are found in the pelvic

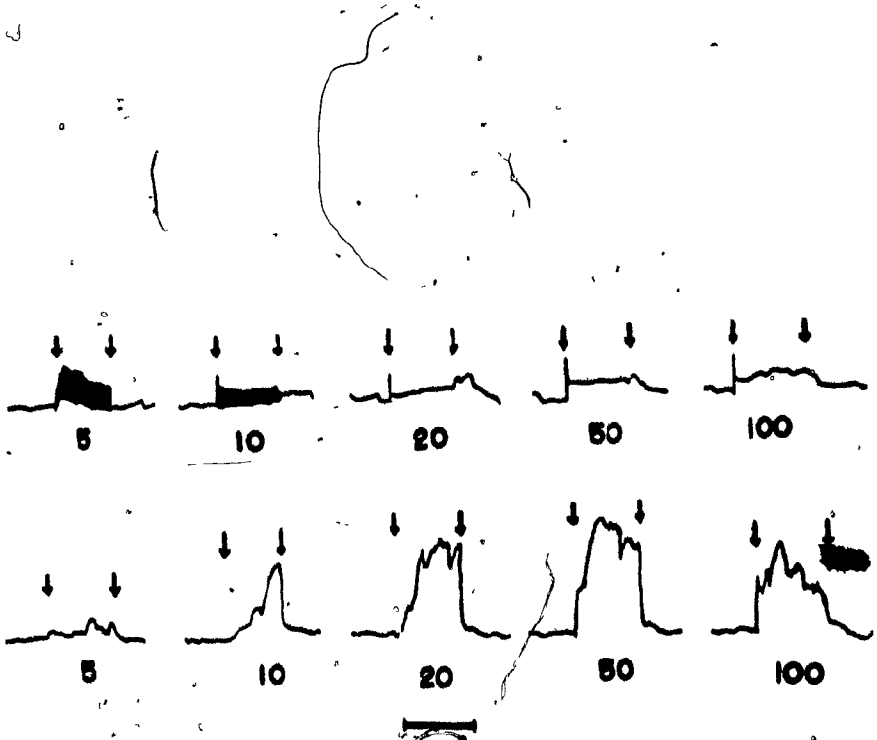

FIG. 1. Kymographic records of abdominal pressure changes following stimulation of splanchnic and pelvic nerves. Upper row, responses to stimulation of splanchnic nerve. Bottom row, responses to stimulation of pelvic nerve. Number below each record indicates frequency of stimulation per second. Square waves of 1-msec duration and 4 volts were used. Stimulation was continued for 10 sec. Beginning and cessation of stimulation are shown by arrows. Time scale, 10 sec.

Figure 3. Taken from Yamamoto et al., 1961a.

nerve (Langley and Anderson, 1896; Langworthy et al., 1940). Electrical stimulation of these afferents increased the EMG activity of the diaphragm, abdominal muscles and intercostal muscles (Yamamoto et al., 1961b). Repetitive stimulation of pelvic nerve afferents (10-50 Hz) evoked a sustained contraction of these muscles with a resultant increase in intra-abdominal pressure (Yamamoto et al., 1961a) (Fig. 3). This reflex was mediated by afferents with conduction velocities between 12 and 25 m/sec, which activated a spinal-bulbospinal circuit.

The ascending path of the reflex was found to lie in the dorsolateral funiculus of the white matter of the cord with extensive crossing in the segments below L4. The reflex centre was found to lie between the ala cinerea and the caudal medullary border (Yamamoto and Araki, 1963). The descending path of the reflex was localised to the ventrolateral funiculus of the white matter of the cord and could be interrupted without abolishing respiration. Transection of the spinal cord at the caudal border of the medulla abolished the increase in intra-abdominal pressure evoked by stimulation of pelvic nerve afferents.

The properties of the afferent limb of this reflex closely resemble the properties of the afferent limb of reflex micturition. Bladder afferents in the pelvic nerve have similar conduction velocities (Winter, 1971) to those pelvic afferents which cause sustained increases in intra-abdominal pressure (Yamamoto, 1961a). Pelvic bladder afferents which convey the sensation of bladder fullness have been localised to the dorsolateral funiculus in cats (Barrington, 1931) and in humans (Nathan and Smith, 1951). These afferents also

undergo extensive crossing in the lower lumbar spinal cord (Barrington, 1931). It therefore seems likely that a large portion of the reflex described by Yamamoto is caused by activation of bladder afferents within the pelvic nerve.

II. AIM OF THE PRESENT STUDY

The present study was designed to evaluate the effects of urinary bladder afferents on respiration. Previous investigators have reported both increases and decreases in the amplitude and frequency of respiratory movements during bladder distension (Watkins, 1938; Evans and McPherson, 1959). Since these studies were performed on spontaneously breathing animals, it is possible that some of the effects observed on respiration were the result of compensatory feedback from chemoreceptors and pulmonary stretch receptors. Moreover, it is known that bladder afferents make reflex connexions with sympathetic preganglionic neurons (Mukherjee, 1957b) as well as with alpha (Evans and McPherson, 1959) and gamma (Evans, 1963) motoneurons. It is therefore possible that some effects of bladder afferents on respiration were secondary to the activation of these neural systems.

In this study an effort was made to rule out those reflexes which may have been responsible for the indirect effects of bladder afferents on respiration. Respiratory activity was studied under open loop conditions by using a preparation that was paralysed, artificially ventilated and in most cases vagotomised. The electrical activity of phrenic and recurrent laryngeal motoneurons was used to evaluate the direct

effects of bladder afferents on respiratory activity. Systemic arterial pressure was recorded and the contribution of fluctuations in arterial pressure during activation of bladder afferents to the effects on respiratory activity was assessed.

The study was directed at answering the following questions:

i) What are the effects of physiological activation of urinary bladder afferents on respiration?

ii) Do bladder afferents have an effect on both the spinal and supra-spinal circuitry involved in respiration?

iii) What are some of the properties of the afferents mediating the effects on respiration?

In comparison with other abdominal visceral afferents, bladder afferents can be activated experimentally with relative ease by physiological stimuli such as contraction or distension as well as by electrical stimulation as has been done with splanchnic afferents.

III. METHODS

The experiments were performed on 27 cats of either sex. The cats were anaesthetised with an intraperitoneal injection of Nembutal (35 mg/kg) which was supplemented intravenously as required. In 2 additional experiments the cats were anaesthetised with ether, and their spinal cords transected at C1. Following anaemic decerebration by occlusion of the carotid and vertebral arteries, the anaesthesia was discontinued. Tracheostomy was performed. The femoral artery was cannulated and systemic arterial pressure (SAP) continuously recorded. Rectal temperature was monitored and maintained between 36 and 38 degrees C by the use of a heating pad and infrared lamp. The urinary bladder was approached through a midline abdominal incision. Two polyethylene cannulas were inserted into the bladder via the urethra and ligated in place. One of these two was used for recording intravesical pressure (IVP) and the other for filling the bladder with physiological saline (Fig. 4).

In most cases, the bladder was slowly filled and IVP recorded under conditions of constant volume, i.e., no evacuation of bladder contents was permitted. Figures 5 and 6 are examples of the behavior of the bladder during the slow filling. As can be seen from Figure 6, slow filling was sometimes associated with small rhythmic fluctuations in IVP which increased in frequency as filling progressed. These small rhythmic fluctuations are most likely due to myogenic contractions of the bladder. These contractions are thought to be myogenic since they are not abolished by section of both the pelvic and hypogastric nerves (Plum, 1960). Once micturition threshold was reached however, large rhythmic spontaneous bladder contractions (SBCs) developed. These SBCs are abolished by spinal

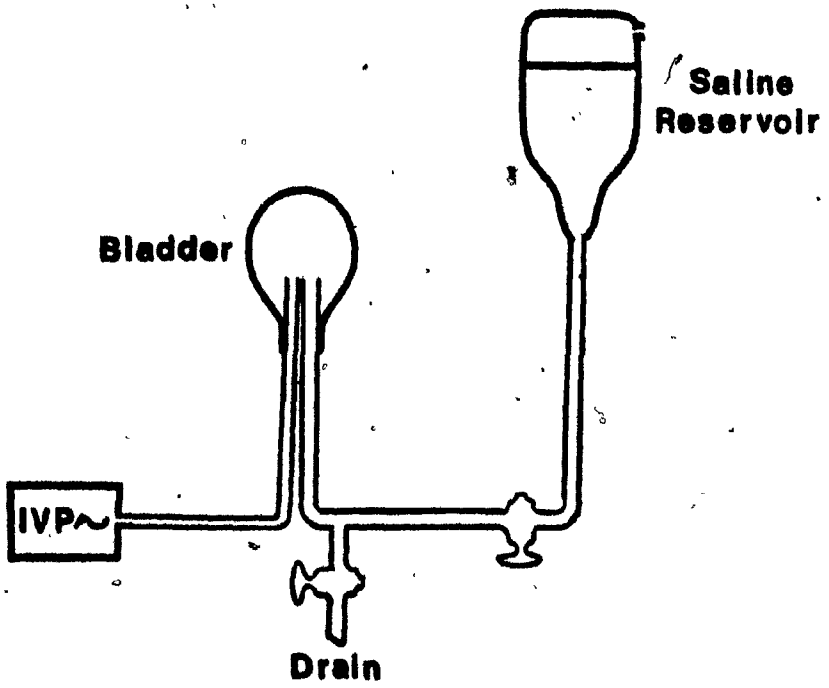

Figure 4. Apparatus used to maintain the bladder under conditions of constant volume or constant pressure. Intravesical pressure (IVP) was monitored in situ. The bladder could be connected to the large saline reservoir and distended to a constant IVP or alternatively, the bladder could be slowly filled in steps and IVP recorded under conditions of constant bladder volume.

Figure 5. Changes in intravesical pressure during slow filling of the bladder. The intravesical pressure is recorded under conditions of constant volume. A large spontaneous contraction develops after the final injection of saline into the bladder. Note that the baseline intravesical pressure changed very little although 29 cc of fluid were injected before a spontaneous contraction was produced.

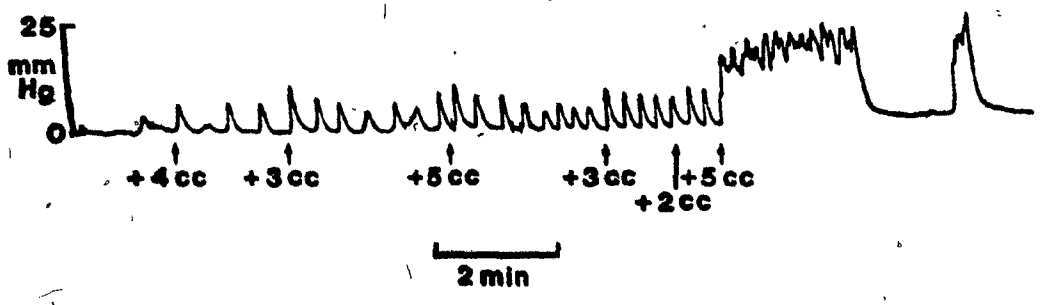

Figure 6. Small rhythmic fluctuations in intravesical pressure (IVP) sometimes develop during slow filling of the bladder. These IVP changes are most likely due to myogenic contractions of the bladder. As filling of the bladder progresses, the frequency of the myogenic contractions increases until micturition threshold is reached and a spontaneous bladder contraction develops.

transection above S1 (De Groat and Ryall, 1969) or by section of the pelvic nerves (Mellanby and Pratt, 1940). Once obtained, the SBCs continue at a constant rhythm without further filling being necessary. In our experiments the SBCs occurred with a mean frequency of 0.53 ± 0.33 contractions/min. The mean peak IVP was 47 ± 9 mmHg and the mean duration of a contraction 62 ± 17 seconds. In some cases the bladder was distended and maintained under conditions of constant pressure by connecting it to a large saline reservoir which could be raised by variable amounts thereby varying IVP (Fig. 4). The term distension will be used in the text to denote the maintenance of the bladder under conditions of constant pressure.

In most cases the animal was paralysed with gallamine (Flaxedil) or pancuronium (Pavulon) and ventilated under positive pressure. End tidal CO_2 was monitored with an infrared analyser and maintained between 3.5 and 4.5%. In many cases bilateral vagotomy was performed to eliminate locking of the phrenic neural discharge to the respiratory pump cycle. Since the axons of recurrent laryngeal motoneurons run in the vagus, vagotomy was not performed in those cases in which RLN activity was to be recorded.

The phrenic and recurrent laryngeal nerves were identified and isolated in the neck. The skin flaps of the open wound were then reflected to form a pool. The nerves were immersed in paraffin oil, desheathed, cut and their central ends placed on bipolar silver hook electrodes for conventional recording (amplification, 20,000 x, low frequency, 30Hz; high frequency, 10KHz).

The low frequency SAP and IVP signals were frequency modulated

and, together with the electrical activity of the phrenic nerves and RLN, recorded on magnetic tape. The recorded signals were subsequently played back off line and filmed. In some later experiments, SAP, IVP, and the integrated electrical activity of the two nerves were also displayed on line on a curvilinear pen recorder.

Both the phrenic nerve and RLN discharge were half-wave rectified and integrated using a "leaky" RC circuit with a time constant of 100 msec. The duration of the inspiratory phase of the respiratory cycle, Ti, was measured from the rapid onset of phrenic nerve activity to the sharp decay in activity. The duration of expiration, Te, was measured from the sharp decay in phrenic activity to the subsequent onset of activity. The peak amplitude of the integrated phrenic discharge and the rate of rise of the phrenic activity (slope = peak amplitude/Ti) were also measured. The Wilcoxon 2 sample test (Rumke and De Jonge, 1964) was used to test the significance of the changes in Ti, Te, amplitude and slope during the various experimental procedures. The Spearman test (Rumke and De Jonge, 1964) was applied to determine whether there was significant correlation between any of the phrenic parameters measured.

In two experiments the hypogastric nerve (Fig. 7) was approached via a midline abdominal incision, identified at the level of the inferior mesenteric ganglion, and prepared for section at some point during the experiment. In cases in which the pelvic nerve was stimulated, the pelvic nerve was approached dorsally and isolated in the lumbosacral plexus. The nerve was placed on silver hook electrodes and stimulated under paraffin oil with square pulses of variable voltage

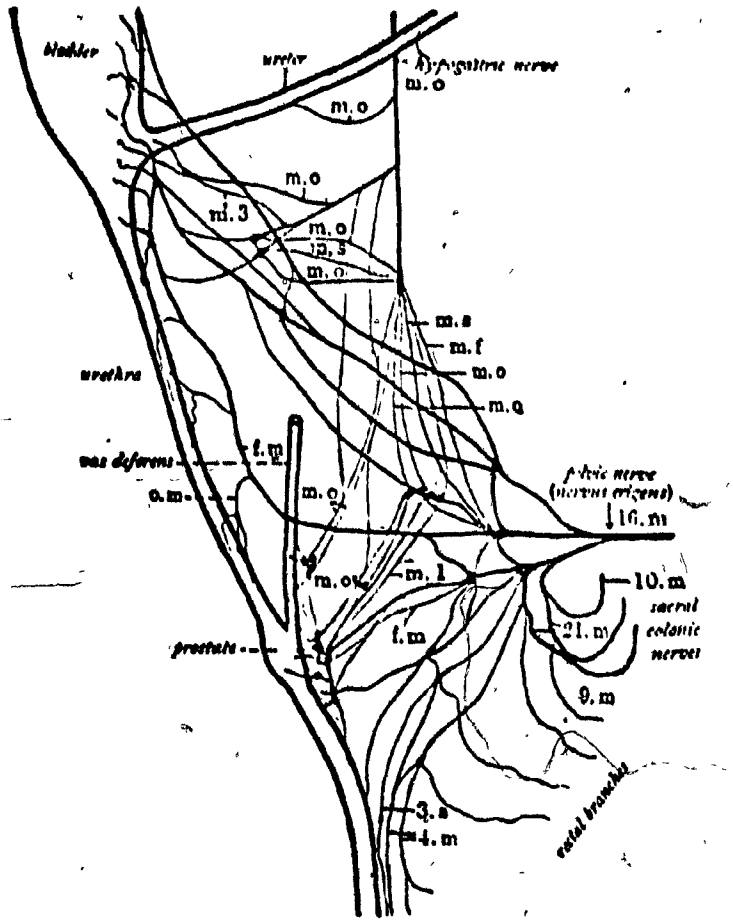

Figure 7. Taken from Langley and Anderson, 1896. Pelvic plexus of a male cat. The pelvic nerve originates in the sacral spinal cord (S_1-S_3), the hypogastric nerve in the lumbar cord (L_2-L_5). Both nerves contain mechanosensitive bladder afferents.

and a duration of 0.2 msec.

In four additional experiments, designed to evaluate the conduction velocities of pelvic nerve afferents, a lumbosacral laminectomy was performed. The animal was rigidly fixed in a stereotaxic frame, and the S2 and S3 dorsal roots were isolated, cut, and placed on silver hook electrodes. Recordings were then made under oil from the dorsal root exhibiting the largest evoked volley to pelvic nerve stimulation and the conduction velocities of the pelvic afferents were then calculated.

IV. RESULTS

1) Activity of Inspiratory Motoneurons in Paralysed Animals During Physiological Activation of Bladder Afferents

The effects of isovolumetric SBCs on phrenic activity were assessed in 11 cats. The peak amplitude of the integrated phrenic activity decreased in 9 of 11 experiments during SBC (Fig. 8), the mean maximum decrease being 18% (range 4-30%, $p < 0.005$). In all of these cases, there was a significant decrease in the slope of the integrated phrenic activity ($p < 0.005$). The decrease in amplitude was positively correlated with the decrease in slope during SBC ($p < 0.005$).

The time course of the depression of phrenic amplitude tended to parallel the time course of the associated SBC but the amplitude usually returned to control before IVP did. The depression began during the increase in IVP, usually before the IVP was half maximal. The peak decrease in phrenic amplitude occurred either during the rise in IVP or during the maximum IVP and returned to control values during the decay in IVP.

Respiratory frequency decreased during SBC in 7 of 11 experiments. The decrease in frequency was due solely to an increase in Te in 5 cases and to an increase in both Ti and Te in the remaining 2 cases. The magnitude of the peak increase in Ti was 13 and 23% respectively ($p < 0.005$). The mean peak increase in Te was 26% (range 4-43%, $p < 0.005$). In the case shown in Figure 9, both Te and Ti increased during SBC. The time course of the Te effect was qualitatively similar to that of the decrease in phrenic amplitude. The decrease in amplitude and frequency

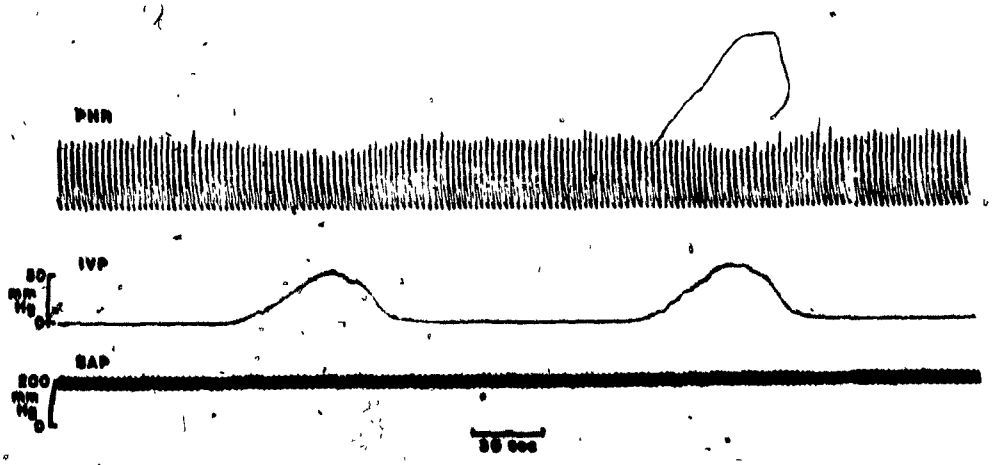

Figure 8. Depression of the peak amplitude of the integrated phrenic activity during a spontaneous bladder contraction. The contour of the amplitude depression parallels the IVP contour. Record was taken from an animal with vagi intact.

PHR - integrated phrenic activity; IVP - intravesical pressure; SAP - systemic arterial pressure.

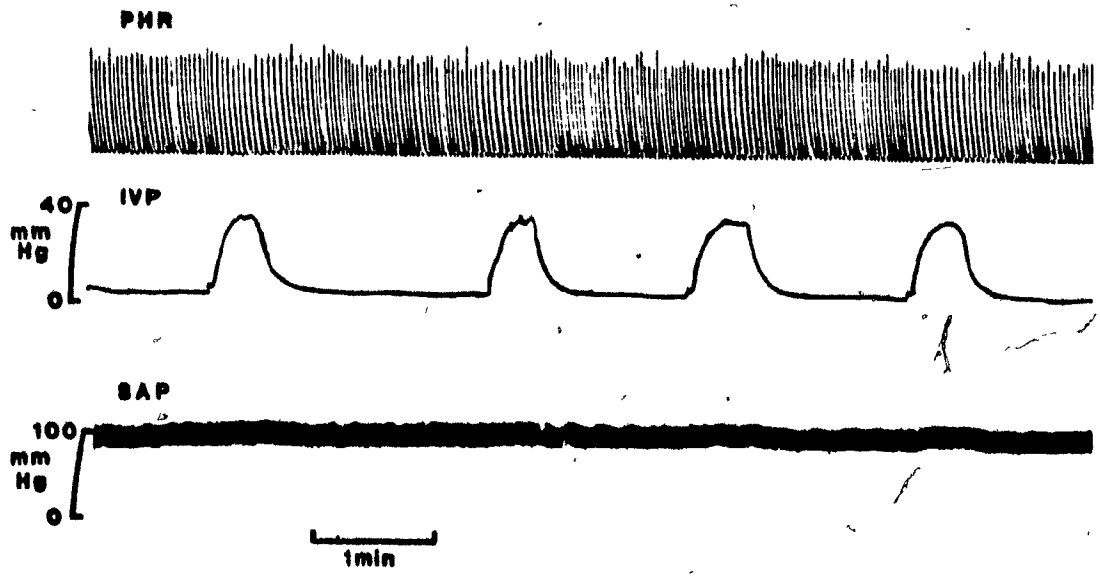

Figure 9. Decrease of respiratory frequency during SBC. The decreased frequency is due in this case to increases in both Ti and Te. Record taken from a vagotomised animal.

PHR - integrated phrenic activity; IVP - intravesical pressure; SAP - systemic arterial pressure.

during SBC could be obtained simultaneously (Fig. 13) or separately (Fig. 8 and 9).

The response of RLN motoneurons to SBC was similar to that described for phrenic motoneurons. SBC was effective in decreasing the inspiration synchronous discharge of the RLN as well as in increasing Te (Fig. 10).

Distension of the bladder was also capable of decreasing phrenic amplitude (Fig. 11) and frequency. In cases in which it was impossible to elicit SBCs by slow filling, distension of the bladder was still effective in altering phrenic discharge.

It seems likely that the depression of phrenic amplitude and frequency results from activation of bladder afferents which occurs during SBC or distension (Iggo, 1955; De Groat and Ryall, 1969; Winter, 1971; Floyd et al., 1976). Some of our observations are consistent with the view that bladder wall tension, rather than IVP, is the stimulus activating the receptors of those bladder afferents which modified phrenic and RLN discharge. Figure 12 is an example of a case in which an abnormally long SBC caused an unusual increase in Te from 3 to 6 seconds. The first noticeable prolongation of Te occurred at an IVP of 14 mmHg. After withdrawal of 15 cc of fluid from the bladder, the subsequent SBC caused an increase in Te only at an IVP of 30 mmHg. If Laplace's law for a sphere can be applied to the bladder, then a decreased intravesical volume and the same bladder wall tension would be expected to produce a greater IVP. This would explain the increase in IVP needed to generate a Te increase after fluid withdrawal. The same peak IVP caused a greater increase in Te during the first SBC. Since the intravesical volume was larger during the first SBC, more tension must have been generated within

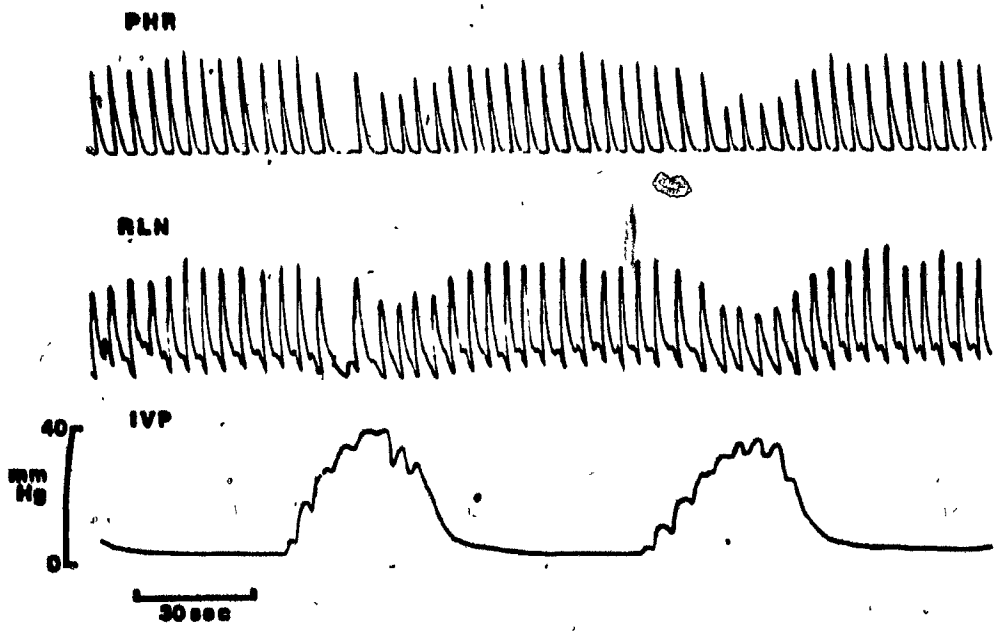

Figure 10. Decrease in the amplitude of the inspiration-synchronous discharge of the recurrent laryngeal nerve (RLN) coinciding with the decrease in phrenic amplitude during SBC.

PHR - integrated phrenic activity; RLN - integrated recurrent laryngeal nerve activity; IVP - intravesical pressure.

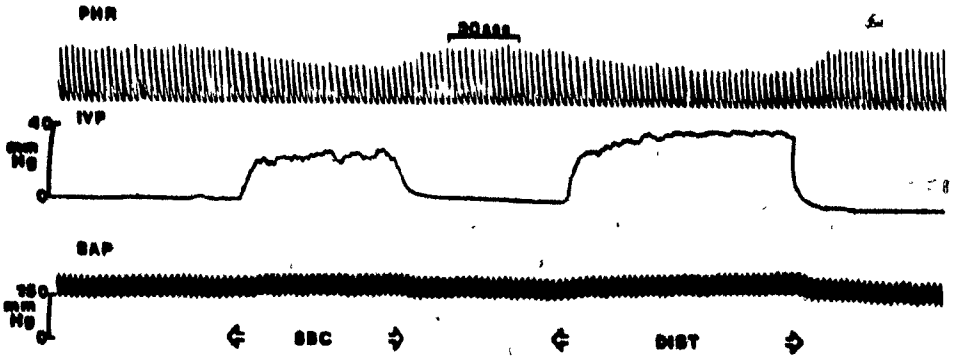

Figure 11. Decrease in the amplitude of integrated phrenic activity during spontaneous contraction or distension (DIST) of the bladder. Record from a vagotomised animal.

PHR - integrated phrenic activity; IVP - intravesical pressure; SAP - systemic arterial pressure.

the bladder wall in order to maintain an IVP identical to that of the second SBC. The fact that distension of the bladder is also effective in altering phrenic activity suggests that bladder tension receptors respond to tension in the passive elastic elements of the bladder, as well as to the activation of the contractile elements which occurs during SBC.

In several cases, contraction or distension of the bladder increased systemic arterial pressure (SAP) between 5 and 10 mmHg. The contour of the SAP increase was similar to the IVP contour (Fig. 13), but usually lagged behind the IVP by 2 - 5 seconds. The increases in SAP are reflexly mediated by bladder afferents in both the pelvic and hypogastric nerves (Watkins, 1938; Taylor, 1968), and can be evoked in both CNS intact (Mukherjee, 1957b) and spinal preparations (Mukherjee, 1957a). It is unlikely that cardiovascular afferents which may have been activated during the rise in SAP were mainly responsible for the effects of SBC or distension on phrenic activity. In many instances similar reductions in phrenic amplitude and frequency were obtained in the absence of an increase in SAP (Fig. 7 and 8). Moreover, intravenous injections of a pressor dose of noradrenaline or a saline bolus did not mimic the effects of SBC or distension on respiratory activity (Fig. 14).

In order to rule out the possibility that the changes in respiratory activity were due to deformation of neighbouring structures by the bladder, the observations during SBC or distension were repeated in 2 animals with an open abdomen. In these experiments, results similar to those obtained in the closed abdomen preparations were observed.

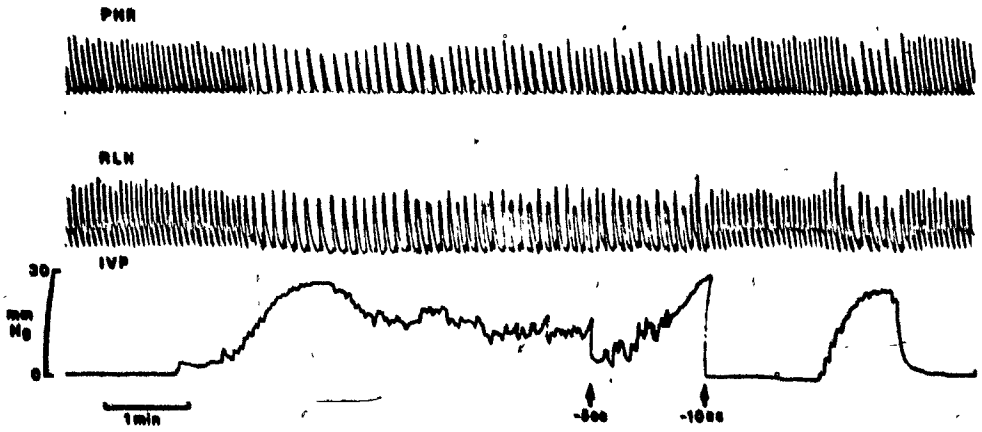

Figure 12. Effects of SBC on respiratory frequency at 2 different bladder volumes. The first SBC caused slowing of respiratory frequency at an IVP of 14 mmHg. Withdrawal of 15 cc of fluid caused the bladder to relax and respiratory frequency to return to control values. A second SBC at the lower intravesical volume caused slowing of respiratory frequency at a higher IVP (30 mmHg).

PHR - integrated phrenic activity; RLN - integrated recurrent laryngeal activity; IVP - intravesical pressure.

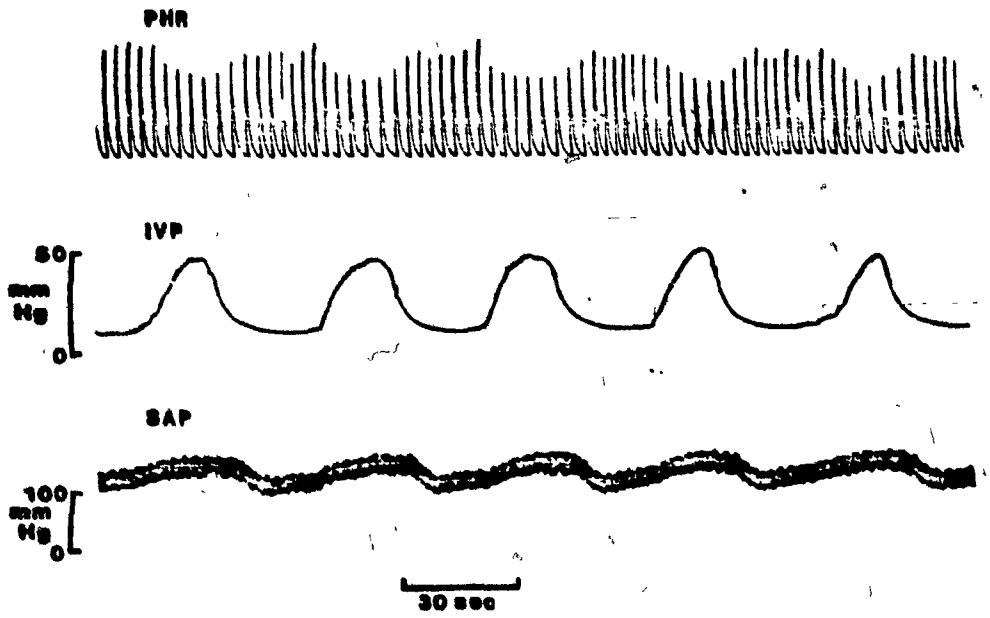

Figure 13. SBC evokes increases in systemic arterial pressure (SAP). The decrease in phrenic frequency and amplitude in this case may be partially due to the rise in SAP. Record from a vagotomized animal.

PHR - integrated phrenic activity; IVP - intravesical pressure.

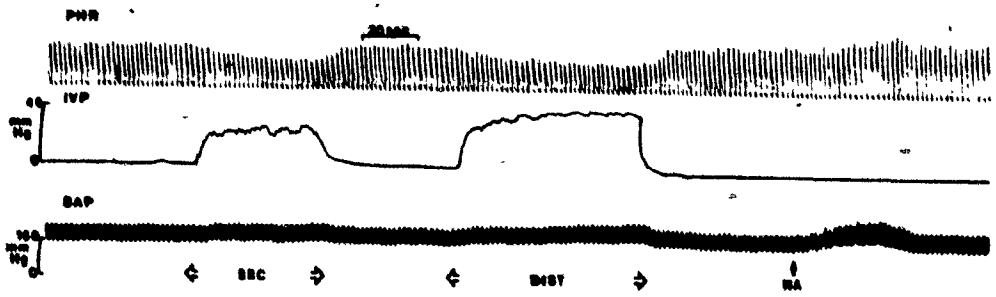

Figure 14. An injection of noradrenaline (NA) to produce an increase in systemic arterial pressure (SAP), similar to the rise seen during SBC or distension (DIST) does not decrease the amplitude of the integrated phrenic activity (Phr). Record from a vagotomised animal.

IVP - intravesical pressure.

As some bladder afferents run in the hypogastric nerve, the effects of SBC on phrenic activity were examined before and after bilateral hypogastric nerve section. Section of the hypogastric nerves in two experiments did not modify the effects of SBCs on phrenic activity.

2) Effects of Electrical Stimulation of the Pelvic Nerve on the Activity of Inspiratory Motoneurons in Paralysed Animals

Since a large number of bladder afferents reach the CNS via the pelvic nerve and since hypogastric nerve section did not modify the decrease in phrenic amplitude and frequency observed during SBC, stimulation of pelvic nerve afferents should mimic the effects seen during SBC. Repetitive stimulation of the pelvic nerve with 20 - 30 second trains at frequencies of 5 - 10 Hz and intensities of 2-4 V decreased the amplitude of the integrated phrenic discharge in 9 of 11 experiments. The mean maximal decrease was 25% (range 11 - 40%). An increase in Te was obtained in two experiments. Thus the incidence of increase in Te was lower with electrical stimulation of the pelvic nerve than with SBC or distension.

In four cats, the effects of graded stimulation of the pelvic nerve at frequencies of 5 and 10 Hz were studied in order to characterize the afferents mediating the effects on phrenic activity (Fig. 15). With repetitive stimulation at these frequencies, depression of phrenic amplitude first appeared at a mean stimulus intensity of 650 mv. Increasing the stimulus intensity increased the magnitude of the depression until a maximum was reached at a mean stimulus intensity of 2.35 V.

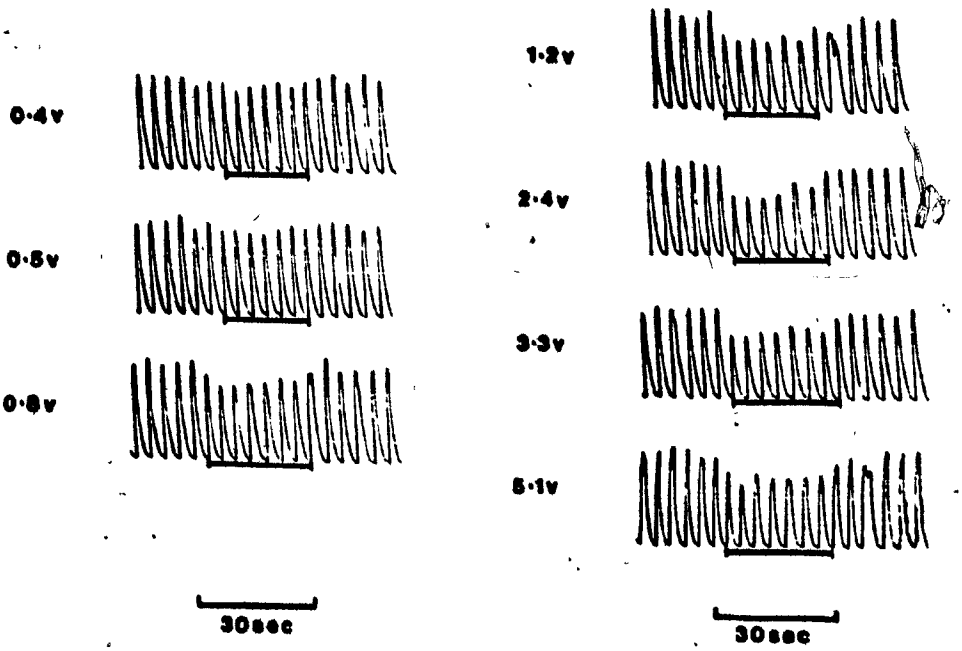

Figure 15. Depression of the amplitude of integrated phrenic activity evoked by repetitive stimulation of pelvic nerve afferents at 10 Hz. Increasing the intensity of stimulation between 0.5 and 2.4 volts increased the magnitude of the depression. Stimulation applied during period marked by the bar.

The intensity of stimulation needed to activate a given group of pelvic afferents was studied in a separate series of experiments. The compound action potential evoked by pelvic nerve stimulation was recorded from either the S2 or S3 dorsal root in four animals. The number of pelvic afferents present in each dorsal root is variable and is largely dependent on the arrangement of the animal's lumbo-sacral plexus (Langley and Anderson, 1896). The average threshold stimulus needed to evoke a compound action potential by pelvic nerve stimulation was 300 mV. Stimulation at this intensity activated fibres conducting at velocities between 30 and 40 m/sec. The compound action potential evoked by pelvic nerve stimulation had three main peaks corresponding to activation of fibres conducting at velocities of 30 - 40, 15 - 20, and 5 - 10 m/sec. At high stimulation intensities (ca 10 V) a C volley was evoked (0.5 - 2 m/sec). Figure 16 is an example of an evoked volley recorded from the S2 dorsal root at various intensities of pelvic nerve stimulation.

As stated above, the depression of phrenic amplitude could be elicited by activating pelvic afferents with stimulation intensities between 0.65 and 2.35 V. Fibres with conduction velocities between 10 and 20 m/sec were activated at these intensities.

The effect of pelvic nerve stimulation on phrenic activity was dependent on the frequency of stimulation used. In four experiments the effects of various frequencies of pelvic nerve stimulation on phrenic amplitude were studied. With supramaximal stimulus intensity (3 - 4 V), the depression of phrenic amplitude, averaged over the period of pelvic nerve stimulation, first appeared at a frequency of

1 - 2 Hz. The maximum depression occurred at 10 Hz and decreased at stimulation frequencies higher than 10 Hz (Fig. 17). When a train of repetitive stimuli of constant frequency and intensity was given, the magnitude of the amplitude depression varied with time. As can be seen from Figure 17, the maximum amplitude depression was usually reached by the first or second phrenic nerve burst following the onset of stimulation. At lower frequencies of stimulation (usually below 10 Hz), the decrease in phrenic amplitude was either maintained (2 experiments), or decayed with time (2 experiments). The tendency to return to control values increased at higher stimulation frequencies in all cases. In all cases with frequencies of stimulation above 30 Hz, the maximum depression of phrenic amplitude was less. In two experiments stimulation at frequencies of 50 - 100 Hz caused, after an initial depression, a reversal, i.e., an increase in phrenic amplitude to levels above control (10 - 15% increase).

In one experiment exhibiting phrenic amplitude reversal and rapid recovery during repetitive pelvic nerve stimulation, the animal was hyperventilated to a CO_2 level which eliminated the inspiration-synchronous phrenic discharge. A stimulus train (4 V, 80 Hz, 70 msec train duration) delivered to the pelvic nerve excited phrenic motoneurons (Fig. 18). This observation shows that pelvic nerve bladder afferents have both excitatory and depressant connexions with phrenic motoneurons, but under most of our experimental conditions, the latter are dominant. In addition, the effects seen during stimulation at different frequencies are the result of the cumulative activation of these two inputs. Higher frequencies either are not followed by the depressant input or

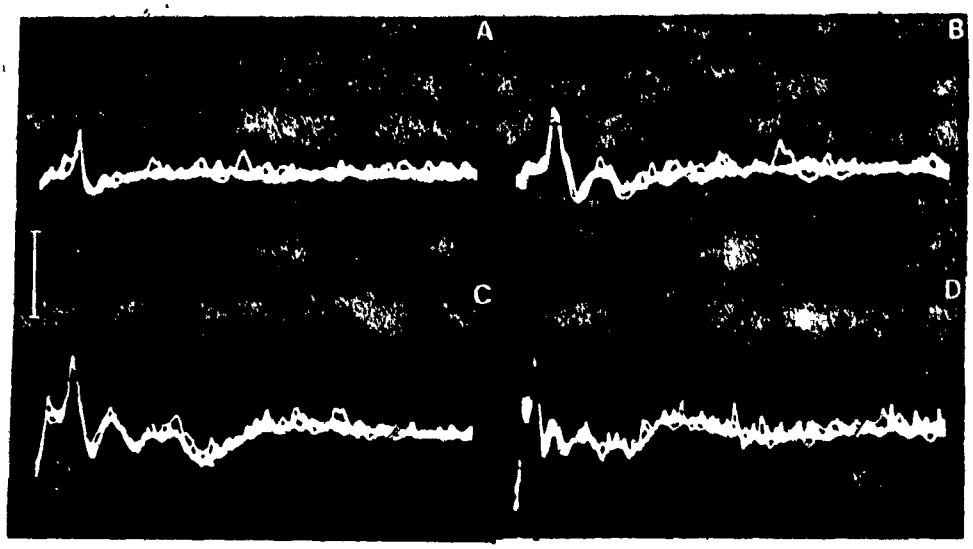

Figure 16. Five superimposed evoked vollies to stimulation of pelvic nerve afferents at different intensities recorded from the S2 dorsal root. Trace A, 300 mV: Trace B, 900 mV: Trace C, 2V; Trace D, 4V. Distance between stimulating and recording electrodes was 3.8 cm. Traces A, B, and C are 10 msec sweeps, trace D is 20 msec. Vertical calibration 250 μv.

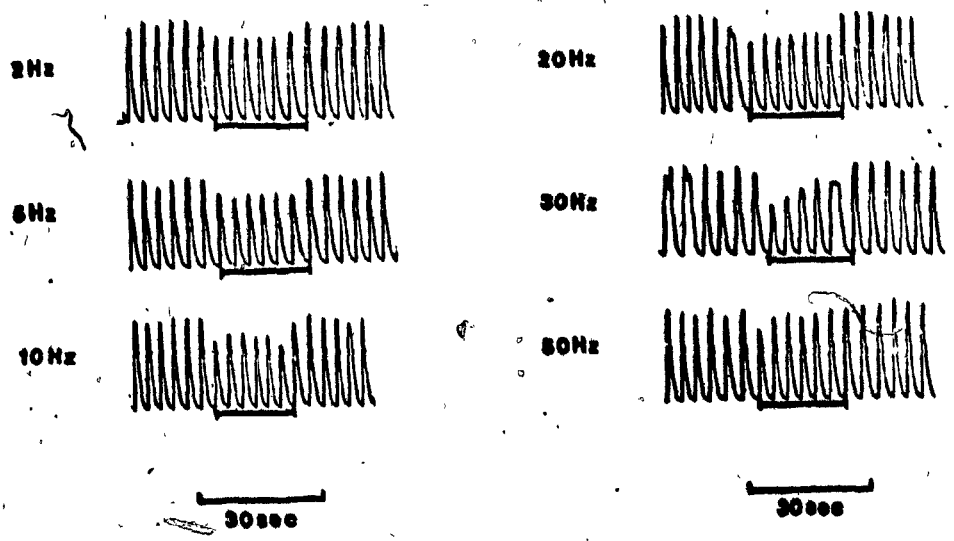

Figure 17. Effects of graded frequency of stimulation of pelvic nerve afferents (2 V) on depression of the amplitude of integrated phrenic activity. Maximal depression is obtained between 10 and 20 Hz. At higher frequencies of stimulation the average amplitude depression is less. Taken from the same experiment as Fig. 15. Stimulation applied during period marked by bar.

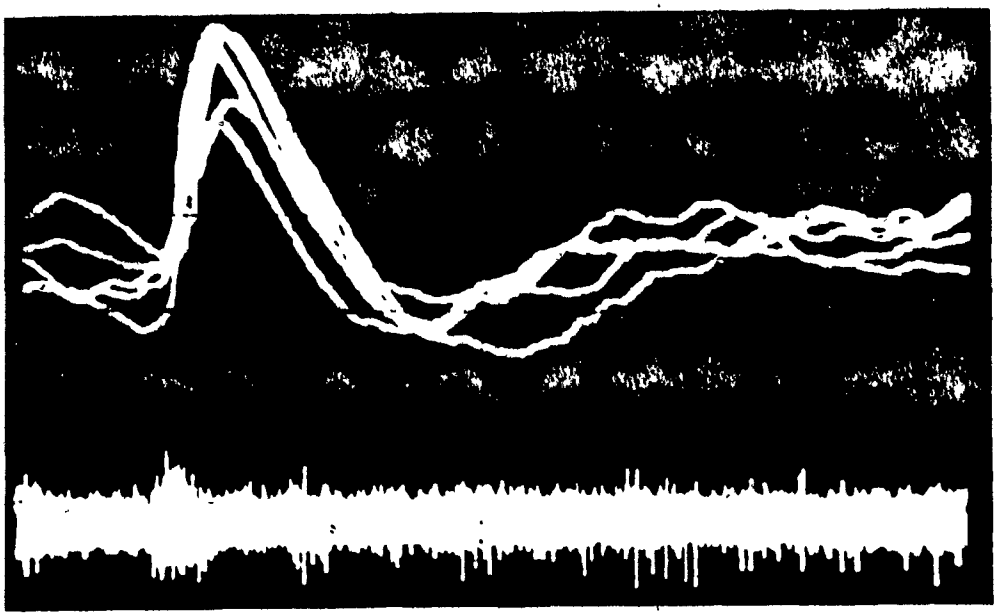

Figure 18. Five superimposed traces of the evoked vollies to pelvic nerve stimulation recorded from the phrenic nerve. The inspiration synchronous activity of phrenic motoneurons was eliminated by hyperventilating the animal (see text). The top trace is the rectified, integrated (5 msec time constant) low and high frequency components of the evoked discharge. The lower trace is the high frequency component of the discharge (30 Hz - 10 kHz). Sweep duration 2 seconds.

alternatively, permit better temporal summation of the excitatory input.

The motoneurons of the RLN behaved in a fashion similar to phrenic motoneurons. The amplitude of the RLN discharge decreased in 4 of 5 experiments during low frequency stimulation of the pelvic nerve. In one experiment, stimulation of the pelvic nerve between 50 and 100 Hz increased the amplitude of both the phrenic and RLN discharge.

3) Effects of Bladder Afferents on Phrenic Motoneurons in Spinal Animals

The observation that two pools of respiratory motoneurons (phrenic and RLN) respond in a similar fashion to activation of bladder afferents suggests that part of the action of these afferents is on an input common to both motoneuron pools. This does not exclude the possibility that pelvic bladder afferents affect phrenic motoneurons at the spinal level as well. The effects of bladder afferents on phrenic motoneurons at the spinal level were assessed in two spinal cats. In one of the two, it was possible to elicit both excitatory and inhibitory responses by activating bladder afferents. In this experiment some phrenic motoneurons were spontaneously active (Fig. 19). The pattern of activity did not resemble in any way the phasic inspiratory activity seen in CNS intact preparations. The motoneurons fired tonically for periods of 10 - 30 minutes and then the activity gradually waned to return at some later time (30 - 60 min). During the period of tonic activity it was possible to demonstrate that passive distension of the bladder (Fig. 19) or repetitive stimulation of pelvic nerve afferents inhibited phrenic motoneurons. Short trains (4 V, 200 Hz, 20 msec) applied to

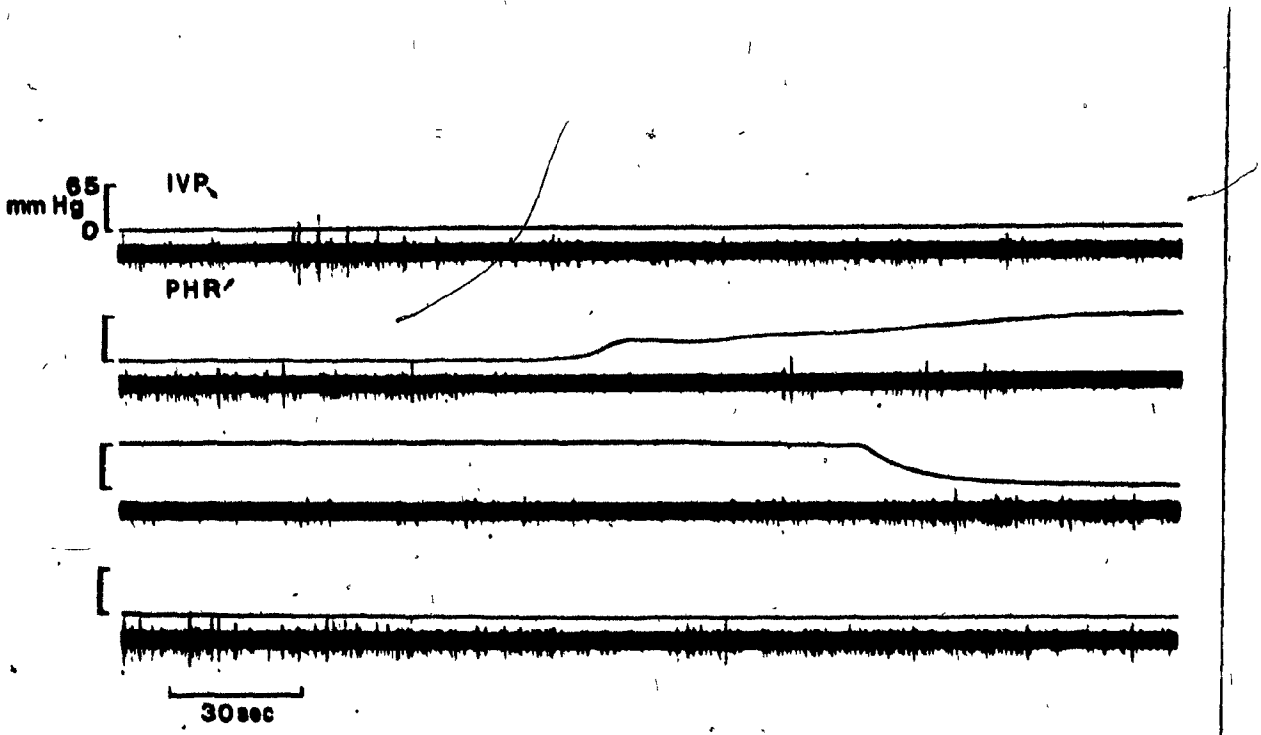

Figure 19. Depression of spontaneous phrenic activity in an acute spinal cat during distension of the bladder. Continuous record. Distension occurs in the second and third panel.

IVP - intravesical pressure: PHR - spontaneous phrenic activity.

the pelvic nerve excited phrenic motoneurons (Fig. 20). The site of action of some of the inhibitory and excitatory effects of bladder afferents on phrenic discharge is therefore at the spinal level.

4) Activity of Inspiratory Motoneurons in the Spontaneously Breathing Animal During Physiological Activation of Bladder Afferents

The decreased phrenic amplitude and frequency observed in the paralysed, artificially ventilated preparation contrasts with the observations of previous investigators who reported enhancement of respiratory movements during manipulation or large distension of the bladder (see INTRODUCTION). Since the animals in their experiments were breathing spontaneously, we evaluated the effects of activation of bladder afferents on inspiratory motoneurons before and after muscle paralysis with Flaxedil.

Distension or active contraction of the urinary bladder increased the amplitude of both the phrenic and RLN discharge in four spontaneously breathing animals (Fig. 21). These effects were either abolished or reversed with an intravenous injection of a paralysing dose of Flaxedil (Fig. 22). Recovery from paraylysis is associated with a return of the amplitude increase seen during SBC or distension.

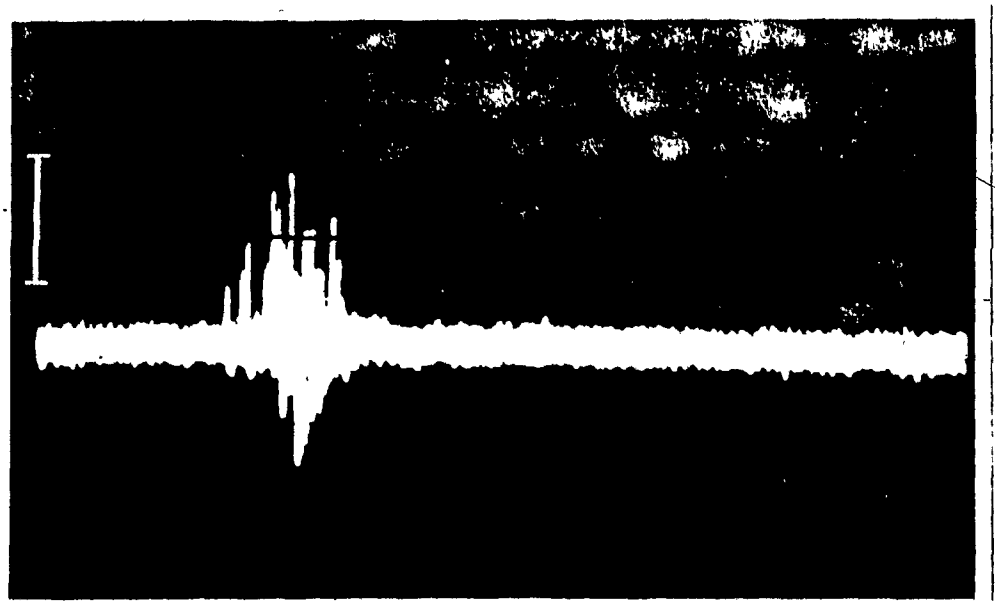

Figure 20. Excitation of phrenic motoneurons evoked by pelvic nerve stimulation in a spinal animal (see text). 20 superimposed sweeps. Sweep duration 50 msec. Vertical calibration 100 μV.

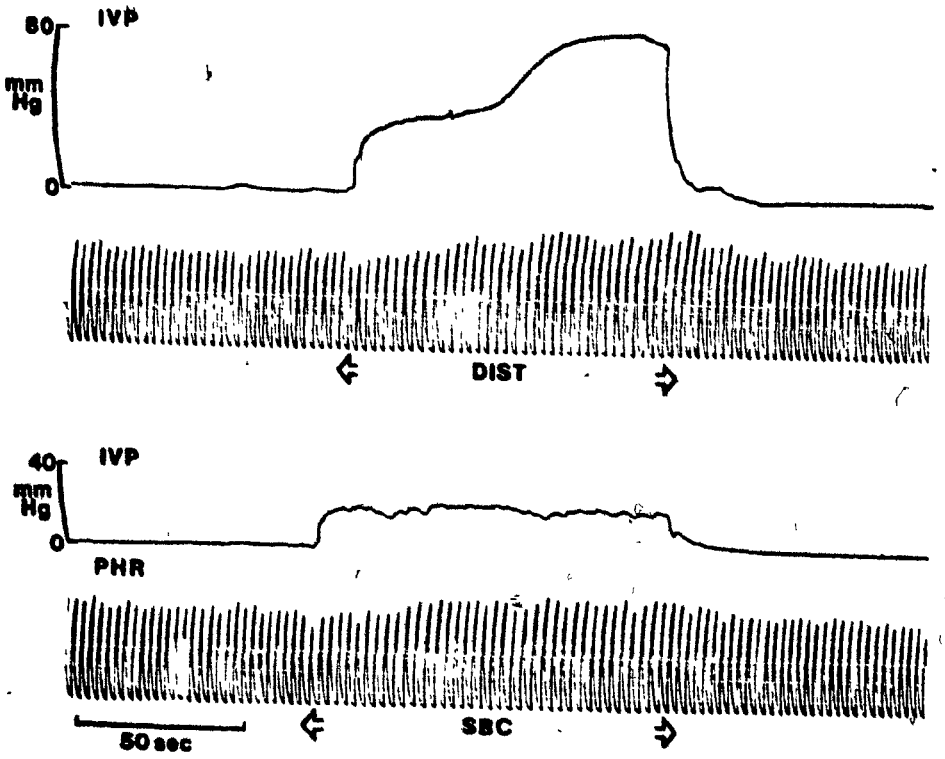

Figure 21. Distension or contraction of the bladder increases the amplitude of phrenic activity in a spontaneously breathing animal. In both cases, note the initial small depression in amplitude which precedes the increase. Record taken from a vagotomised animal.

IVP - intravesical pressure; PHR - integrated phrenic activity.

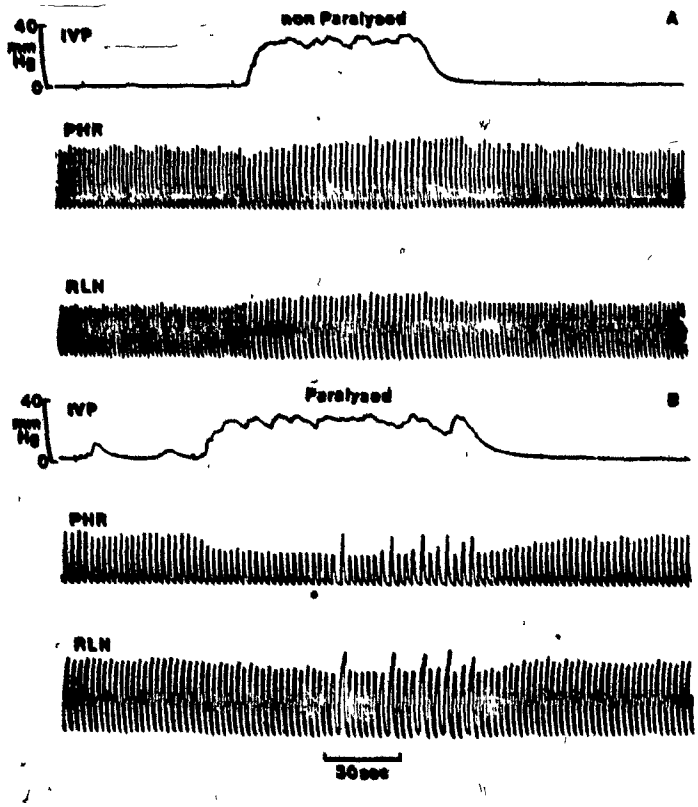

Figure 22. Contraction of the bladder increases the amplitude of integrated phrenic and recurrent laryngeal nerve activity in the spontaneously breathing animal (Panel A). After injection of a paralyzing dose of Flaxedil (Panel B), the amplitude of both the phrenic and RLN discharge decreases during a spontaneous bladder contraction.

IVP - intravesical pressure; PHR - integrated phrenic activity; RLN - integrated recurrent laryngeal nerve activity.

V. DISCUSSION

The results of this study demonstrate that bladder afferents have reflex connexions with the neural circuitry controlling respiration. Spontaneous isovolumetric contraction or distension of the urinary bladder decreased the amplitude and frequency of the phrenic bursts. The reduction in amplitude was associated with a decrease in the slope of the integrated phrenic activity whereas the decrease in frequency was mainly due to an increase in the duration of expiration.

The changes in phrenic activity were not primarily the result of changes in systemic arterial pressure, which at times were associated with SBCs or distension, since similar changes in phrenic activity occurred in some experiments in the absence of any change in SAP. Since the animals were paralyzed, these results could not be due to an increase in somatic motor activity known to occur during SBC (Mellanby and Pratt, 1940). These results were also not due to activation of other abdominal visceral afferents by deformation of viscera adjacent to the bladder since similar results were obtained when the abdominal cavity was widely opened.

It seems likely, therefore, that the decrease in phrenic amplitude and frequency is due to activation of bladder afferents which is known to occur during SBC or passive distension (Iggo, 1955; Floyd et al., 1976). Single unit studies of bladder afferents have demonstrated the existence of both slowly adapting and rapidly adapting mechanoreceptors which are activated during distension or contraction of the bladder.

The majority of bladder afferents in the pelvic nerve behave as slowly adapting mechanoreceptors (Iggo, 1955; Winter, 1971; Arlhac, 1972). These mechanoreceptors are thought to be functionally in series with the passive and active tension developing components of the bladder wall (Iggo, 1966; Leek, 1972), and as shown in Figure 23, their firing frequency approximately parallels the IVP contour during an isovolumetric SBC. These mechanoreceptor afferents exhibit little or no tonic activity at low intravesical volumes (Winter, 1971; Arlhac, 1972). Slow filling of the bladder is associated with an increase in the activity of these afferents with superimposed phasic bursts associated with the small bladder contractions seen during filling (Winter, 1971; Arlhac, 1972) (cf. Fig. 6). Little or no afferent activity is observed between the rhythmic SBCs recorded under isovolumetric conditions.

In addition to the slowly adapting mechanoreceptors just described, the pelvic nerve also contains axons of rapidly adapting mechanoreceptors which have been located in the bladder mucosa and in the peritoneal reflections surrounding the bladder. The units in the mucosa fire with a short burst of action potentials during the rise phase of the SBC. In some cases another short burst accompanies the decline in IVP (Arlhac, 1972).

Both slowly and rapidly adapting mechanoreceptive bladder afferents have been identified in the hypogastric nerve (Fig. 24). Bilateral section of the hypogastric nerves during SBC had no effect on the amplitude or timing of the phrenic discharge; activation of bladder afferents in the pelvic nerve is therefore sufficient to

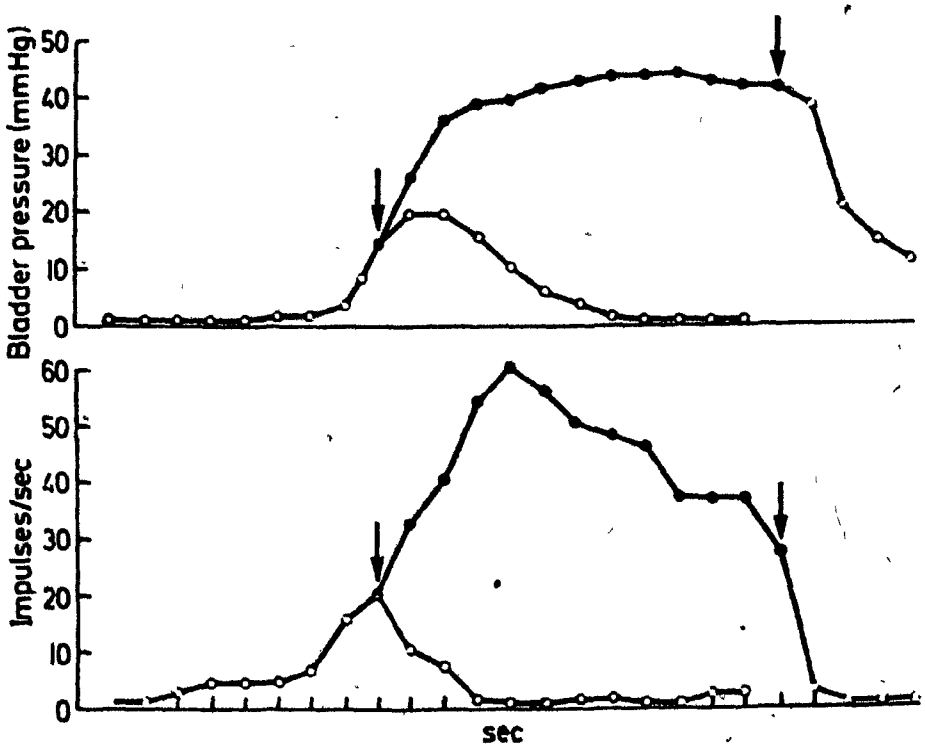

Figure 23. Taken from Leek, 1972. Activity of a single pelvic nerve bladder afferent during a spontaneous bladder contraction (closed circles). The firing of the afferent parallels the bladder pressure contour.

mediate the observed effects. Electrical stimulation of pelvic nerve afferents did decrease the phrenic amplitude and in two cases increased Te. The conduction velocities of the afferents mediating the decrease in phrenic amplitude (10 - 20 m/sec) are in the range of those described for the slowly adapting mechanoreceptors in the pelvic nerve (Winter, 1971). The time course of discharge of these afferents fits the time course of phrenic effects during SBC or bladder distension.

Rapidly adapting mechanoreceptors in the bladder mucosa could not account for the sustained effects of SBCs or bladder distension on phrenic discharge. Similarly, rapidly adapting mechanoreceptors in the peritoneal folds surrounding the bladder could be discounted. In addition, the conduction velocities of these afferents (25 - 40 m/sec; Winter, 1971) are greater than those of the afferents responsible for the decrease in phrenic amplitude.

We have suggested (see RESULTS) that tension within the bladder wall is the stimulus responsible for the activation of afferents mediating the changes in phrenic discharge. Tension changes within the bladder wall have also been suggested to mediate the increase in systemic arterial pressure which occurs during SBC or bladder distension (Watkins, 1938; Mukherjee, 1957b). Localized traction on the trigone, the area in which most of the tension receptors have been localized, also increases SAP (Taylor, 1968). Pelvic nerve afferents conducting in the A gamma-delta range have been shown to activate lumbar (De Groat and Lalley, 1972) and thoracic (our unpublished observations) sympathetic preganglionic neurons. These afferents also cause contraction of expiratory and inspiratory muscles in the

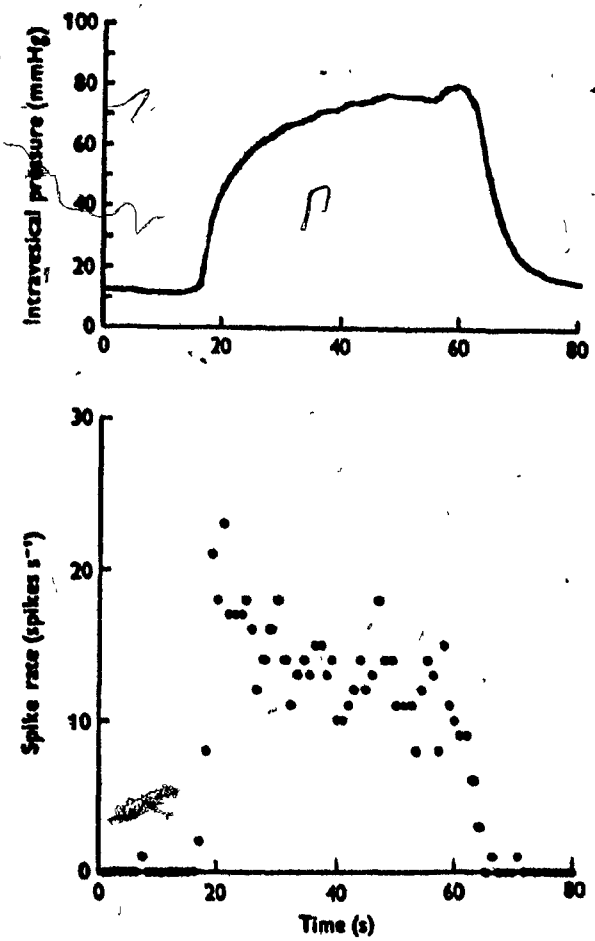

Fig. 5. Intravesical pressure and mean spike rate of one unit in the hypogastric nerve during a prolonged spontaneous contraction.

Figure 24. Taken from Floyd et al., 1976.

spontaneously breathing cat (Yamamoto et al., 1961b).

To summarise, we suggest that the slowly adapting bladder mechanoreceptors with afferents in the pelvic nerve, which respond to changes in bladder wall tension and which have conduction velocities in the A gamma-delta range, are responsible for the effects of SBCs or bladder distension on phrenic discharge.

Although the amplitude of the integrated phrenic discharge decreased during SBC, bladder distension, and low frequency stimulation of myelinated pelvic nerve afferents, observations in particular experimental conditions suggest that bladder afferents actually exert a mixture of facilitatory and inhibitory effects on the neural circuits controlling respiration. At frequencies of pelvic afferent stimulation above 30 Hz, the decrease in phrenic amplitude is less than at lower frequencies of stimulation. In addition, the rate of recovery of the phrenic amplitude towards control values during maintained high frequency stimulation increases. At higher frequencies of stimulation (50 - 100 Hz) phrenic amplitude actually increased. The RLN discharge behaved in a manner similar to that of the phrenic. Evoked excitation of phrenic motoneurons could also be observed in one animal in which rhythmic phrenic nerve activity had been suppressed by hyperventilation in air.

The excitatory effects of bladder afferents on respiration are more easily observed in the spontaneously breathing animal. In this preparation SBC or bladder distension can increase the amplitude of the integrated phrenic discharge. The excitatory effects of bladder afferents are either abolished or replaced by depression after muscle

paralysis. The most likely explanation for these observations is that paralysis abolishes the activity of some somatic afferents which are most probably activated by contractions of respiratory musculature, since this is the only observable somatic motor activity during the experiment. Paralysis would also block the inspiratory synchronous gamma efferent input to the spindles of the respiratory muscles (Critchlow and Von Euler, 1963). The somatic afferents activated during spontaneous respiration may facilitate the excitatory effects of bladder afferents.

Bladder afferents decrease the phrenic frequency, an indication that the action of these afferents is on the brainstem respiratory oscillator responsible for the setting of respiratory cycle duration. The decrease in phrenic amplitude in paralysed preparations during SBC, distension or low frequency pelvic nerve stimulation, or the increase in amplitude during high frequency pelvic nerve stimulation or during SBC or distension in spontaneously breathing preparations is paralleled by similar changes in RLN amplitude. These changes are likely to be due, at least in part, to an action of bladder afferents on the brainstem oscillator since a similarlity of effects on the two pools suggest an action of the afferents on a common driver, i.e., the oscillator. Results obtained in the acute spinal preparation have also shown that activation of bladder afferents can both excite and inhibit phrenic motoneurons through intraspinal circuits. The interaction between spinal and supraspinal circuitry involved in the mediation of the changes in phrenic amplitude cannot be determined from the present data.

An interesting characteristic of the decrease in respiratory frequency caused by bladder afferents was the large number of cases in which Te was prolonged without a concomitant prolongation of Ti. These data contrast with the view that the control of Te is tightly linked to the control of Ti (Clark and Von Euler, 1972). This view is based largely on the results of experiments which demonstrated an approximately linear relationship between Ti and Te. In these experiments, Ti was changed as a result of modifications of pulmonary stretch receptor afferent activity caused by varying the degree of inflation of the lungs. Evidence has been accumulating, however, for an independent control of Te. Te changes independent of similar changes in Ti have been observed in some experimental conditions. A decrease in respiratory frequency due solely to an increase in Te, has been described during static lung inflation (Bartoli et al., 1973), lung inflation in expiration (Knox, 1973; Von Euler and Trippenbach, 1976), distension of the pulmonary vascular bed (Lloyd, 1978), carotid sinus nerve stimulation during expiration (Eldridge, 1976), and repetitive stimulation of sympathetic visceral afferents with conduction velocities in the A gamma-delta range (Kostreva, 1978; Prabhakar, 1978).

Afferents from the urinary bladder in conjunction with other abdominal visceral afferents may contribute to the setting of respiratory drive and frequency. In addition, the excitatory connexions between bladder afferents and inspiratory motoneurons may aid in reflexly increasing intra-abdominal pressure during reflex micturition, thereby ensuring more complete evacuation of the bladder contents. Such reflex mechanisms have previously been suggested (Yamamoto, 1961a; Kuru, 1965).

VI. SUMMARY

The purpose of this work has been to evaluate the effects of urinary bladder afferents on respiratory activity and to examine the circuits through which these effects are mediated.

1) The responses of two pools of inspiratory motoneurons (phrenic and recurrent laryngeal) to activation of urinary bladder afferents has been studied in Nembutal anaesthetized, paralyzed, artificially ventilated cats.
2) Distension or spontaneous neurogenic contraction of the urinary bladder decreased the amplitude and frequency of the inspiratory burst activity of both phrenic and recurrent laryngeal motoneurons.
3) The decreased respiratory frequency was due mainly to a prolongation of the expiratory phase of the respiratory cycle.
4) Section of the hypogastric nerves did not modify the response of phrenic motoneurons to bladder contraction.
5) Low frequency electrical stimulation of pelvic nerve afferents with conduction velocities in the A gamma-delta range decreased the amplitude of both the phrenic and recurrent laryngeal nerve discharge and in some cases prolonged the duration of the expiratory cycle.
6) High frequency stimulation of pelvic nerve afferents with conduction velocities in the A gamma-delta range increased the amplitude of the phrenic nerve and recurrent laryngeal nerve discharge.

7) Contraction or distension of the bladder in spontaneously breathing animals increased the amplitudes of the phrenic nerve and recurrent laryngeal nerve discharge. The increase in amplitude could be either abolished or reversed by paralysis of the animal.

8) Activation of bladder afferents in the acute spinal animal both depressed and excited phrenic motoneurons.

9) These data suggest that bladder afferents make both excitatory and inhibitory connexions with the neural circuitry involved in the control of respiration. Effects of bladder afferents on phrenic motoneurons could be mediated via spinal circuitry. These data suggest, however, that large effects of bladder afferents on respiratory motoneurons are exerted via an action on the brainstem respiratory oscillator.

The work outlined in this summary and presented in detail in the RESULTS section is original.

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