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AN ELECTROPHYSIOLOGICAL STUDY OF SOMATIC AND VISCERAL CONVERGENCE IN THE REFLEX CONTROL OF THE EXTERNAL SPHINCTERS

BY S. B. McMAHON, J. F. B. MORRISON AND KATHY SPILLANE
From the Department of Physiology, University of Leeds, Leeds LS2 9JT

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SUMMARY

1. Mass wave and single unit discharges have been recorded from pudendal efferents innervating the external anal and urethral sphincters in chloralose anaesthetized or decerebrate cats.

2. Reflex discharges in these neurones were elicited by electrical stimulation of the contralateral pudendal nerve, the posterior cutaneous nerve of thigh, or the vesical or colonic branches of the pelvic nerve. The latencies of the evoked responses were 5·5–20 msec. The vesical branches of the pelvic nerve produced discharges less consistently than the other nerves.

3. Irrespective of whether afferent stimulation produced an early evoked response there was always a prolonged period of depression of pudendal nerve excitability following the stimulus. Condition–test interactions showed that this depression began within 50 msec of the stimulus and that its duration varied between 150 and 2500 msec in single units, with a modal value of 500 msec.

4. No evoked response or depression of excitability was seen when afferents in the hypogastric or lumbar colonic nerves were stimulated.

5. Increasing intravesical or intracolonic pressure, within physiological limits, produced a graded reduction in the size of evoked discharges.

6. Short trains of stimuli (four shocks in 20 msec) applied to the raphé nucleus, were capable of inhibiting test responses in pudendal efferents for periods of up to 800 msec.

7. The possible functional roles of two groups of sphincteric reflex interneurones, with either excitatory or inhibitory receptive fields, are discussed.

INTRODUCTION

In a previous paper, McMahon & Morrison (1982*b*) studied the properties of a group of sacral interneurones with visceral inputs, and suggested a classification based on their receptive fields and stimulus–response curves. These receptive fields were found to be present either in a single viscus or in more than one viscus. If the receptive field was in a single viscus it was said to be *simple*, whereas multivisceral receptive fields were described as *compound*. In compound type I receptive fields, natural stimulation of each visceral receptive field had the same effect (either

excitation or inhibition); in compound type II receptive fields, natural stimuli, applied separately to different viscera had opposing effects (inhibition from one viscus, and excitation from another). McMahon & Morrison (1982*a*, *b*, *c*) suggested that interneurones with compound type I receptive fields might be involved in the viscero-sphincteric reflexes, because of reports that discharges in the external sphincters were reduced by an increase in either bladder or colonic pressure (Bishop, Garry, Roberts & Todd, 1956; Garry, Roberts & Todd, 1959).

In this paper we have examined the properties of viscero-sphincteric reflexes and correlated the activities of pudendal motoneurones and sacral interneurones with compound type I or with simple visceral receptive fields; an attempt has been made to lay down some criteria to define sphincteric interneurones. We have previously shown that the raphé nucleus can exert a profound inhibitory effect on the micturition reflex (Morrison & Spillane, 1981) and it was therefore of interest also to determine the effects of raphé stimulation on both pudendal reflexes and sacral interneurones.

METHODS

Experiments were performed on seventy-four cats, of either sex, in the weight range 1·7–5·1 kg. The animals were initially anaesthetized with a fluothane in oxygen mixture and then either decerebrated or maintained under chloralose anaesthesia (60 mg/kg I.V.; BDH Chemicals, Poole). Blood pressure, end tidal CO_2 concentration, and deep body temperature were monitored and maintained as described in McMahon & Morrison (1982*a*).

Preparation of nerves for recording and stimulating. A diagrammatic summary of the innervation of the pelvic viscera is given in Floyd, McMahon & Morrison (1982). The pudendal nerves were approached from the front after division of the symphysis pubis. This allowed the different branches of the pudendal nerve to be dissected and made ready for either mass recording or single unit recording. The vesical and colonic branches of the pelvic nerve, the contralateral pudendal nerve, a posterior cutaneous nerve of thigh and the hypogastric or lumbar colonic nerves were prepared for electrical stimulation.

Distension of the bladder or colon. The bladder was cannulated via the anterior urethra, and bladder pressure could be servo-controlled as described previously by McMahon & Morrison (1982*a*). Distension of the colon was achieved by filling either an intracolonic balloon or a closed, innervated sac of the viscus with air. In some preparations the cannulae were inserted through the anus, and tied to the perianal skin, but in others an incision was made in the descending colon and cannulae were tied into the colon. In four experiments two colonic pouches were produced, one innervated by the pelvic and the other by the lumbar colonic afferents, by cannulating the colon in both directions from an incision placed about 3 cm below the inferior mesenteric vessels. The pressure in each of these sacs of the bowel could be servo-controlled independently.

Stimulation of the raphé nucleus. Electrical stimuli were delivered to the raphé nucleus through a stainless steel concentric needle stereotaxically placed in the brain stem. The position of the electrode was confirmed following fixation of the brain in formal saline, using histological methods. Currents of 40–100 μA were used, and these stimuli were used either to condition a test stimulus or as a repetitive train of pulses at rates of up to 100/sec.

Recordings from sacral neurones. Full details of the methods used to record from and identify local sacral interneurones are given in McMahon & Morrison (1982*b*), which includes a less detailed account of the properties of some of the interneurones used in this study. Briefly, a long lumbosacral laminectomy was performed to give access to the cord. Single units were recorded with tungsten or glass micro-electrodes and were designated as sacral interneurones if they did not have axonal projections extending rostrally into the lumbar cord (tested by stimulation of the cord at L5) and were not antidromically driven from pudendal or visceral nerves.

RESULTS

Resting discharge in pudendal motoneurones

Resting discharge was present in some pudendal nerve efferent units; it was more common in decerebrate preparations than in animals anaesthetized with chloralose, and tended to be less common after several hours of experimentation. There was a tendency for resting activity to be less common in preparations in which manipulations of the colon, anal canal or bladder had been extensive, and it was always greatly reduced or abolished by small or moderate increases in intracolonic or intravesical pressure (5–30 mmHg), accompanying distensions or spontaneous contractions of these viscera. Some units discharged at rest for a few minutes and then stopped without any intervention having taken place; it was often possible to provoke these units into activity for a further few minutes by manipulation of hind limb structures, such as touching the skin on the back of the thigh, or movement of the hip or knee joint. Light mechanical stimulation of the perineum could also trigger tonic activity in quiescent motoneurones. The variability of the resting discharges of these neurones made it difficult to assess the effects of visceral pressures on discharge in these units; the effects of changes in visceral pressures could, however, be determined with good reproducibility if their effects on either unitary or mass wave evoked responses in the pudendal nerve were studied.

Evoked activity in pudendal nerve efferents: the influence of visceral pressures

It was possible to evoke reflex discharges in pudendal nerve efferents by electrical stimulation of the contralateral pudendal nerve, the posterior cutaneous nerve of thigh or the vesical or colonic branches of the pelvic nerves. No evoked potentials could be recorded in response to stimulation of the hypogastric or lumbar colonic nerves. The latencies of the responses evoked from the somatic pudendal or posterior cutaneous nerve of thigh were 5·5–10 msec, and from the pelvic nerve branches were 5·5–20 msec. The responses evoked from the visceral nerves were often of smaller magnitude than those from somatic afferents. In all twenty-two single pudendal units studied, excitatory responses were obtained from the colonic branches of the pelvic nerve, and consisted of either a single spike or a burst of up to ten spikes with peak frequencies around 500/sec; in twenty-one of the units these excitatory effects were followed immediately by a prolonged period of depression of reflex excitability. Similar mixed effects were observed in four of the twenty-two units following stimulation of the vesical branches of the pelvic nerve, and in seventeen of the remaining units the only effect of a vesical branch stimulus was a depression of resting or reflexly evoked discharge. The depression was maximal within 50 msec of the stimulus; its precise time of onset was not obtainable in the absence of high resting rates of discharge, but inhibitory effects were observed as early as 20 msec in some of the condition–test interaction studies. The responses to electrical stimulation were tested only once every 4 sec, because repetition of the stimuli at higher rates resulted in a reduction in the amplitude of the evoked potentials. The latency of evoked responses was increased by distension of the bladder or distal colon, but not by distension of a colonic pouch innervated only by lumbar colonic afferents. The probability of obtaining a unitary evoked potential and the amplitude of a mass

evoked response were both reduced during distensions of the bladder and distal colon. Fig. 1 is a comparison of the effects of intracolonic and intravesical pressure on pudendal evoked responses, and shows that the intracolonic pressure that produces 50% inhibition of the evoked response is about 15 mmHg greater than the corresponding value of intravesical pressure. This was true whether the responses were recorded from the anal or urethral branches of the pudendal nerve.

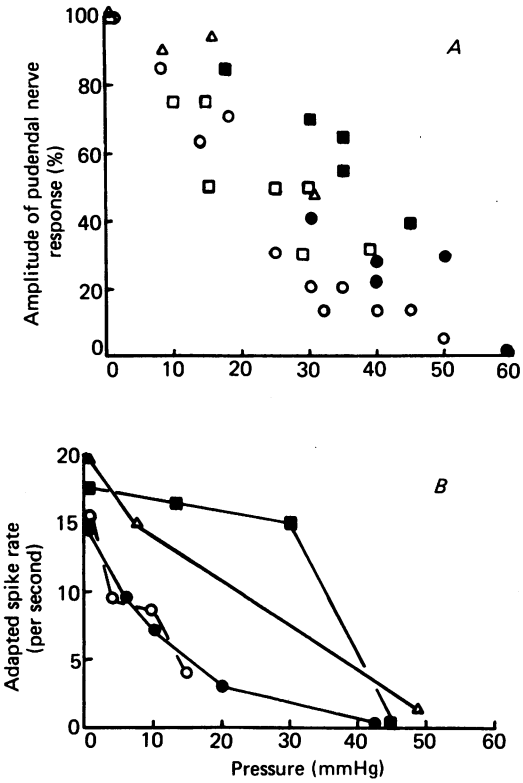

Fig. 1. *A*, the effects of intravesical (open symbols) and intracolonic pressure (filled symbols) on the amplitude of the mass response evoked in the anal branches of the pudendal nerve by electrical stimulation of the afferents in the contralateral pudendal nerve (squares; 4 mA, 0·2 msec), the posterior cutaneous nerve of thigh (circles; 0·5 V, 0·2 msec) and the colonic branches of the pelvic nerve (triangles; 10 V, 1 msec). *B*, the effects of intravesical (open symbols) and intracolonic pressure (filled symbols) on the rate of discharge in three sacral interneurones with compound type I receptive fields in the bladder and colon.

Condition–test interactions in pudendal reflexes

Units which exhibited resting discharges always showed a prolonged period of depression of the resting discharge following the primary evoked response. The time course of this depression was monitored using conditioning stimuli to the colonic or vesical branches of the pelvic nerve. In a few experiments conditioning stimuli were also given to the hypogastric or lumbar colonic nerves, but these stimuli gave neither evoked responses nor inhibitory interactions. The time course of the inhibitory

interaction between the vesical or colonic branches of the pelvic nerve had a duration of 150–2500 msec in different units, with a modal value of about 500 msec (Fig. 2). In only one out of twenty units studied was an inhibitory interaction absent.

Effect of the raphé nucleus on sphincteric reflexes

Stimulation of the raphé nucleus at a mid line site approximately 6·5 mm behind and 9 mm below the interaural line with currents of 40–100 μA (four pulses of 400 μsec duration in 20 msec) was effective in inhibiting pudendal nerve responses evoked from

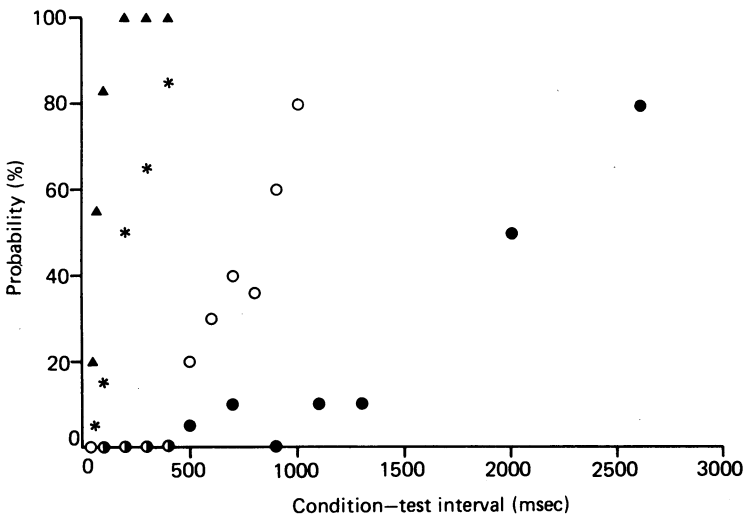

Fig. 2. The graph shows the range of time courses of recovery of pudendal nerve efferents following stimulation of the colonic branches of the pelvic nerve. Ordinate: probability of firing in four single pudendal nerve efferents (denoted by different symbols). The control (100 %) response was the number of spikes evoked in response to electrical stimulation of the colonic branches of the pelvic nerve (10 V, 1 msec, 0·25/sec). The effects of conditioning electrical stimuli to the vesical branches of the pelvic nerve (10 V, 1 msec pulses) at different intervals before the colonic stimulus (the condition–test interval) are indicated.

the pelvic nerve, the posterior cutaneous nerve of thigh or the contralateral pudendal nerve. The inhibitory effect was increased by raising intravesical or intracolonic pressure, and could be seen as an increase in latency (of up to 6 msec), a decrease in the probability of firing of efferent pudendal units (by up to 100 %) or a reduction of amplitude of the mass evoked response. In some experiments it was found that the inhibitory effect of the raphé stimulus was greater in pudendal reflexes evoked from somatic nerves than from visceral nerves, as can be seen in Fig. 3, in which the time course of the inhibition was approximately 800 msec.

Studies on sacral interneurones

Out of sixty-five sacral interneurones that did not have long projections outside the sacral cord, seventeen had simple, five had compound type I and seven had compound type II receptive fields in the bladder and colon. The remainder had no visceral mechanosensitive receptive fields. None of the interneurones had inputs from

the hypogastric or lumbar colonic nerves. Thirteen cells in this sample had somatic as well as visceral receptive fields and all could be evoked by pudendal (somatic) nerve stimuli. Table 1 summarizes the different types of visceral and somatic receptive fields seen in the sacral interneurones. As light mechanical stimuli to the perineum or anus cause the external sphincter to increase its tone and the bladder to relax, the effects of somatic and colonic stimuli might be expected to be synergistic in neurones

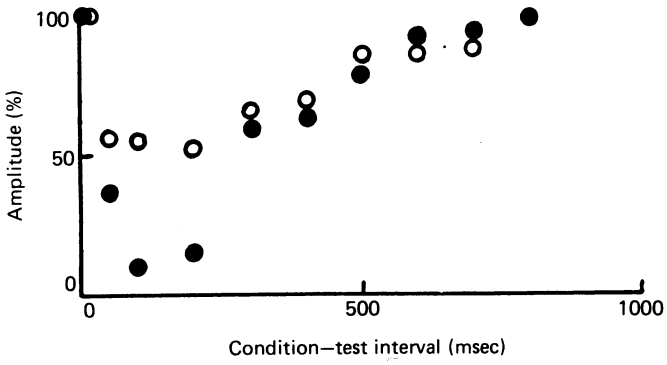

Fig. 3. The time course of excitability in the pudendal nerve motoneurone pool following conditioning electrical stimulation of the raphé nucleus (four × 60 μA, 0·2 msec pulses in 20 msec). The test stimulus which gave the maximal amplitude mass wave in the pudendal nerve was applied to the pelvic nerve (○) or to the contralateral pudendal nerve (●). Both of these nerves received 10 V, 1 msec pulses at 0·25/sec.

TABLE 1.

Function	Sign and position of receptive field: Bladder	Colon	Perineum	Number
S.g. interneurones	− or 0	− or 0	+ or 0	12
Interneurones with simple RFs	+ or 0	+ or 0	+ or 0	10
M.g. interneurones	−	+	+ or 0	7
	+	−	− or 0	

S.g. interneurones: sphincteric gating interneurones. M.g. interneurones: micturition gating interneurones.

involved in micturition, and antagonistic in interneurones involved in sphincteric reflexes. It is of interest therefore that interneurones with compound type I receptive fields that were inhibited by distensions of the bladder and the colon, were never seen to be inhibited by perineal stimulation. Perineal receptive fields were present in two out of six cells in this group and both were excitatory.

Cells in the second row of Table 1 never had compound receptive fields; the possibility that these participate in producing i.p.s.p.s in pudendal and pelvic efferent neurones will be discussed later. The remainder of the cells illustrated in this Table have characteristics consistent with a role in the inhibition of micturition by colonic or perineal stimulation. The cells with simple or compound type I receptive fields had latencies from the pelvic and pudendal nerves in the range 5–30 msec. Table 2

summarizes the differences between interneurones with direct and inverse stimulus response functions; the vesical gating interneurones of Table 1 are excluded from this analysis. Cells which showed a direct relationship between spike rate and intravisceral pressure had lower resting rates of discharge, longer latencies for primary evoked responses (in cells with simple receptive fields) and shorter secondary inhibitory responses than cells with an inverse stimulus–response curve (see Table 2). Cells with compound type I receptive fields all had inhibitory visceral mechanosensitive receptive fields. Some cells with inverse stimulus–response functions did not have any

TABLE 2.

	Direct stimulus–response curves	Inverse stimulus–response curves
Resting rate of discharge	1·2/sec	10/sec
Latencies of primary response (simple cells)	13 msec	5·7 msec
Latencies of primary response (compound cells)	—	15·2 msec
Duration of inhibitory response	37–400 msec	100–800 msec

excitatory evoked response and a pelvic nerve afferent stimulus caused inhibition of resting discharge for up to 500 msec in these cells; responses of this nature were never seen in neurones with direct relationships between firing and pressure.

The effects of stimulation of the nucleus raphé magnus using currents of 100 μA or less were examined in eighteen of the interneurones and in ten cells which responded, a reduction in the resting activity was present in seven. In two of these cells and in three others there were early evoked responses with latencies of 16–52 msec. The inhibition of resting activity was complete for about 60 msec, and condition–test interaction studies showed that the time course of the descending inhibitory effect was 300–400 msec.

DISCUSSION

Sphincteric motoneurones are known to differ from other sacral motoneurones in that they lack Renshaw inhibition (Mackel, 1979) and crossed disynaptic inhibition (Jankowska, Padel & Zarzecki, 1978) and have rather weak monosynaptic inputs from afferents originating in the muscle they innervate. They are excited by light cutaneous stimuli to the perineum, anus and the back of the thigh and decrease their discharge in response to distension of either the bladder or the colon (Bishop *et al.* 1956). The present results indicate that all the motoneurones studied in the anal or urethral branches showed a reduction in discharge rate when vesical or colonic pressure was increased, and the great majority gave evidence of prolonged inhibitory influences from the pelvic nerves innervating these viscera. Most of these cells were evoked by pudendal stimulation with latencies similar to those obtained for the pelvic nerve, and none were evoked from the hypogastric or lumbar colonic nerves. Interneurones with pelvic and pudendal, but without hypogastric or lumbar colonic

nerve inputs have been described previously by McMahon & Morrison (1982*b*) and these units often had long inhibitory time courses and receptive fields in bladder, colon, perineum and adjacent structures. It was pointed out in that paper that units with compound type I receptive fields could be sphincteric reflex interneurones, and the present work examines that hypothesis and extends it to include a set of criteria that define the properties of a group of 'gating' interneurones that control the excitability of the viscero-sphincteric reflexes. The criteria are: (*a*) they should be evoked from the pelvic nerve, and often from the pudendal nerve with latencies consistent with the viscero-sphincteric reflexes; (*b*) they should not be evoked from the hypogastric or lumbar colonic nerves; (*c*) they should not be antidromically driven from the pudendal nerve; (*d*) they should have either simple inhibitory fields in the bladder or colon and an excitatory perineal receptive field, or a compound type I inhibitory receptive field in the bladder and colon, and perineal influences, if present, should be excitatory.

Relatively few of the interneurones studied exhibited all these characteristics but none of the cells that satisfied these criteria had properties compatible with a role in the gating of the micturition reflex, such as antagonism between bladder and colonic inputs, and synergistic activity between colonic and perineal stimuli (McMahon & Morrison, 1982*c*). Table 1 shows the varieties of mechanosensitive receptive fields which satisfy the criteria of the sphincteric interneurones. Half of this group had compound type I receptive fields and these were always inhibitory: these units have properties compatible with a rôle in the reflex. The cells with such compound receptive fields usually had longer latencies (mean = 15·2 msec) than cells with simple receptive fields (mean = 5·7 msec) from which their complex properties are presumably derived. The latencies of the most complex cells were too long to account for the earliest parts of the pudendal evoked responses, and it is assumed that cells with simpler characteristics are responsible for the early components of these potentials. The cells with simple visceral receptive fields fall into two main categories, depending on whether they are excited or inhibited by natural stimulation of the viscera. Cells with simple inhibitory receptive fields have been included in the group of sphincteric interneurones in Table 1. Their excitatory responses to perineal stimulation and the long periods during which their discharge is depressed following pelvic nerve stimulation (mean = 450 msec) is a feature which distinguishes them from the cells with simple excitatory receptive fields (mean = 284 msec) and is one they share with the interneurones which have compound receptive fields (mean = 425 msec) and with the pudendal motoneurones (mean = approx 500 msec).

It is proposed that the cells which satisfy the above criteria are excitatory interneurones that provide tonic excitation to the pudendal motoneurones and disfacilitate them when colonic or intravesical pressure is raised, or following a pelvic nerve stimulus. Their resting discharge may contribute to the tonic activity of the pudendal efferents, and appears to have a major influence on the excitability of reflexes induced in pudendal motoneurones from visceral and from somatic inputs. They are termed sphincteric gating interneurones in Table 1 to distinguish them from interneurones that are believed to be involved in modulating the excitability of the micturition reflex (see Table 1, and McMahon & Morrison, 1982*c*).

Cells with simple excitatory receptive fields in the viscera have properties that differ

in a number of respects from those described above in addition to the sign of their response function. Their resting rates of discharge are considerably slower, and their latencies are longer than simple cells with inhibitory receptive fields. They discharge during the period of the composite i.p.s.p. recorded from pudendal motoneurones by Bradley & Teague (1977) following stimuli to the pelvic or pudendal nerves, and one possibility is that they are inhibitory interneurones that mediate that i.p.s.p.

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