Exp Brain Res (1992) 88:313–320

Experimental Brain Research

A role for 5-hydroxytryptamine in descending inhibition of spinal sexual reflexes

L. Marson and K.E. McKenna

Department Physiology, Northwestern University School of Medicine, 303 E. Chicago Ave., Chicago, IL 60611, USA

Received April 3, 1991 / Accepted September 4, 1991

Summary. Neurons in the region of the rostral nucleus paragigantocellularis (nPGi) mediate the inhibition of spinal sexual reflexes. Anatomical and pharmacological evidence is presented supporting a role for 5-hydroxytryptamine (5–HT) in this inhibition. Neurons in the rostral nPGi project to the ventral horn in the vicinity of the pudendal motoneurons. A significant number (78% ipsilateral) of these neurons contain 5–HT. Anterograde tracing with Phaseolus leucoagglutinin (PHA–L) confirmed the nPGi projection to pudendal motoneuron and interneuronal areas of the lumbar cord. 5–HT immunoreactive fibers and presumptive terminals surround the pudendal motoneurons. Urethral stimulation, in the anesthetized male rat, elicited penile erections, ejaculation and rhythmic contractions of the perineal muscles, we have used the term coitus reflex to describe this response. Intrathecal injection of 5–HT (4–50 μg) abolished the coitus reflex. Methysergide (1–10 mg/kg i.v.) prevented the 5–HT induced blockade of the coitus reflex. These data support the hypothesis that 5–HT is involved in the descending inhibition of spinal sexual reflexes.

Key words: Serotonin – Ventral medulla – Sexual reflexes

Introduction

It has long been assumed, on the basis of clinical and experimental evidence, that spinal sexual reflexes are under inhibitory control from the brainstem. However, the source of this inhibition was unknown. A model was developed in our laboratory for evoking sexual reflexes in the anesthetized male and female rat (Chung et al. 1988; McKenna et al. 1991). This reflex consists of a coordinated response involving sympathetic (hypogastric nerve), parasympathetic (pelvic nerve) and somatic (pudendal nerve and perineal muscle EMG) rhythmic activity, penile erections and expulsion of the urethral contents and may model ejaculation and climax in the rat. This reflex is elicited upon urethral distension or probing the urethra with a fine catheter. We have used the term coitus reflex to describe the neural and physiologic events of this reflex (Chung et al. 1988; McKenna et al. 1991). The fact that this reflex is only seen in the spinalized animal suggests that the brainstem exerts a strong inhibition over this reflex. We recently identified an area in the ventral medulla that mediates this inhibition (Marson and McKenna 1990). Brainstem transections rostral to the facial nucleus in the medulla oblongata, failed to release the inhibition. However, bilateral electrolytic or neurotoxic lesions of the ventral medulla in the region of the rostral nucleus reticularis paragigantocellularis (nPGi) consistently evoked the reflex upon urethral distension (Marson and McKenna 1990).

The presence of 5–HT containing neurons in the nPGi, in the region that controls the descending inhibition of spinal sexual reflexes, suggests that serotonergic mechanisms may be involved in the descending inhibition of spinal sexual reflexes. 5-hydroxytryptamine (5–HT) has been implicated in the control of sexual behavior and sexual reflexes (Fernandez-Guasti et al. 1989; Mas et al. 1985; McIntosh and Barfield 1984), however, the location of 5–HT neurons in the brain that exert these effects are largely unknown. Neurons that contain 5–HT are present in the medullary raphe nuclei and ventral medulla reticular formation including the rostral nPGi (Steinbusch 1981; Marson and Loewy 1985). Bulbospinal neurons containing 5–HT project to the lumbar spinal cord in the rat (Bowker and Abbott 1990) and cat (Marson 1989). In addition, 5–HT receptors have been located in the lumbar cord (Monroe and Smith 1983; Fischette et al. 1987).

The present study provides anatomical and pharmacological evidence for the involvement of 5–HT in the descending inhibition of sexual reflexes in the anesthetized male rat.

Offprint requests to: K.E. McKenna

Methods

Anatomical studies

Male Sprague-Dawley rats [Charles River, 275–450 g) were anesthetized with ketamine/xylazine (ketamine 90 mg/kg, (Aveco) xylazine 15 mg/kg, (Rugby)] and tracers were administered into the brain or spinal cord using aseptic procedures. Rats were given the analgesic Buprenex (buprenorphine hydrochloride 0.1–0.3 mg) postoperatively.

Retrograde tracing combined with immunohistochemistry

A laminectomy was performed at L1 vertebrae to expose the sixth lumbar segment of the spinal cord. Rhodamine latex microspheres (RITC beads, 40–400 nl, 4% w/v in distilled water, Lumafluor Inc., NJ) or Flurogold (100–800 nl, 4% w/v in distilled water, Fluorchrome Inc., CO) was pressure injected unilaterally into L6 using a glass micropipette (tip size 10–50 μm). Following 9–20 days survival, rats were reanesthetized with ketamine/xylazine and perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.3. The spinal cord was dissected out and the dorsal roots counted to verify the level of the injection site which was visible when illuminated with an UV lamp. Sections of the spinal cord were cut (50 μm), mounted onto slides and coverslipped with Krystalon (Harleco, EM Diagnostics Systems, Inc.) for microscopic identification of the injection site. The brainstem was cut into sections (30 μm) and every third section was mounted onto slides and examined for retrogradely labelled cell bodies. Every third section of the medulla oblongata was incubated in rabbit antibody to 5–HT conjugated to bovine serum albumin (1:500 dilution, Incstar) for 48 h. Sections were subsequently incubated in fluorescein isothiocyanate (FITC)-conjugated goat anti rabbit IgG (1:50 dilution, Cappel) for 1 h, then mounted onto slides. The sections were examined for identification of RITC retrogradely labelled cell bodies and FITC labelled 5–HT immunofluorescence using a Leitz microscope equipped for epifluorescence. The 5–HT immunoreactive stain was totally blocked with 10 μm 5–HT conjugated to bovine serum albumin as previously reported (Marson and Loewy 1985).

The location of 5–HT immunoreactivity in the lumbosacral spinal cord was examined. Rats were perfused with 4% paraformaldehyde, segments T12–S1 of the spinal cord were dissected out, and horizontal sections (50 μm) cut and incubated in rabbit antibody to 5–HT (1:500 dilution) for 48 h. The sections were then processed using a modified peroxidase anti-peroxidase technique (Sternberger 1977).

Anterograde tracing studies

Rats were placed into a stereotaxic holder and a small dorsal craniotomy was performed. A glass micropipette (tip size 5–15 μm) was lowered into the medulla (coordinates 2–2.5 mm caudal to lambda, 1.3 mm lateral to the midline and 8.5 mm beneath the brain surface). Phaseolus leucoagglutinin (PHA–L) was iontophoresed into the medulla using positive direct current (7 s on 7 s off 5 μA for 15–30 min). Following a survival period of 7–21 days, rats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The brainstem and spinal cord were dissected out and postfixed for 2–4 h, then placed into 25% sucrose solution for 1–3 days. Coronal sections (50 μm) were cut through the medulla and processed for identification of the injection site. Horizontal sections (30 μm) of the spinal cord (T13–S1) were processed for identification of PHA–L immunoreactive fibers and terminals using the avidin-biotin (ABC) procedure. Briefly, sections were incubated in 0.1 M phosphate buffer containing 0.4% Triton X100 and 10% normal rabbit serum for 1–2 h and then incubated in goat anti PHA E+L (1:1000, Vector Labs) for 48 h. Sections were reacted using the Vectastain ABC kit, incubated in diaminobenzidine (50 mg/100 ml 0.1 M phosphate buffer) containing 0.12% hydrogen peroxide. Sections were washed, mounted onto glass slides and coverslipped with DPX mountant (BDH Chemicals, Poole UK).

Pharmacologic studies

Male Sprague-Dawley rats (350–490 g) were anesthetized with urethane (1.2–1.5 g/kg i.p. or s.c.). The carotid artery and jugular vein were cannulated for measurement of arterial blood pressure and administration of drugs, respectively. The trachea was cannulated for artificial respiration using a Harvard rodent respirator. The rectal temperature was maintained between 37–39° C with a thermostatically controlled heating lamp. A catheter (PE 50) connected to an infusion pump and pressure transducer was inserted into the urethra from a bladder incision and tied in place. The rat was then mounted into a stereotaxic frame. Drugs were administered into the spinal cord by a catheter (PE 10, 8–10 μl capacity) passed into the subarachnoid space via an incision in the atlanto-occipital membrane and advanced caudally so that its tip lay at the upper lumbar level of the spinal cord. Drugs were delivered in artificial cerebrospinal fluid (CSF). For drug delivery the intrathecal catheter was prefilled with CSF (10 μl capacity), the drug was injected into the catheter using an Hamilton syringe, the drug was then displaced so as to fill the catheter to its tip by a further infusion of 10 μl of CSF to ensure no drug remained within it. Subsequent administration of the same or a different drug was performed similarly by first displacing the 10 μl CSF and allowing 2–5 min for dispersal. Control injections of 10 μl CSF were performed prior to drug administration and the coitus reflex examined for 2–20 min after CSF injection. At the end of the experiment 10 μl of Chicago Sky Blue was injected into the intrathecal catheter to verify the location and approximate spread of the drugs. The catheter was located around L3–L5 segments and the dye spread over 2–3 segments.

The coitus reflex was elicited by urethral distension accomplished by infusing saline through the urethral catheter and briefly occluding the urethral meatus (Marson and McKenna 1990) and monitored via EMG electrodes implanted into the bulbospongiosus muscle (BC). Prior to spinal cord transections, the urethral distension was ineffective in eliciting the coitus reflex, that is, no penile erections or ejaculatory responses were seen following urethral stimulation. Transections of the spinal cord at C1 removed the descending inhibitory control from the ventral medulla, and unmasked the coitus reflex. The effect of drug administration on the coitus reflex could then be examined. Drug effects were monitored for 1–2.5 h after drug infusion or until the coitus reflex returned. The coitus reflex was evoked at intervals greater of 2–5 min. Control experiments have shown that the coitus reflex can be reproducibly evoked at 2 min intervals for up to 3 h (maximum time tested). Systemic administration of drugs were given 5–20 min prior to intrathecal drug infusions.

Drugs

CSF (NaCl, 4g; Na HCO_3, 2.3g; KCl, 0.18g; KH_2PO_4, 0.9g; $CaCl_2$, 0.25g; $MgCl_2$, 1.7g; Na_2SO_4, 0.07g; glucose, 1.06g in 1 liter H_2O) pH 7.4. 5-hydroxytryptamine creatinine sulfate (5–HT, RBI), methysergide (Sandoz).

Data analysis

The data are presented as mean ± S.D. for the given numbers of rats. Data were analyzed using an unpaired t test.

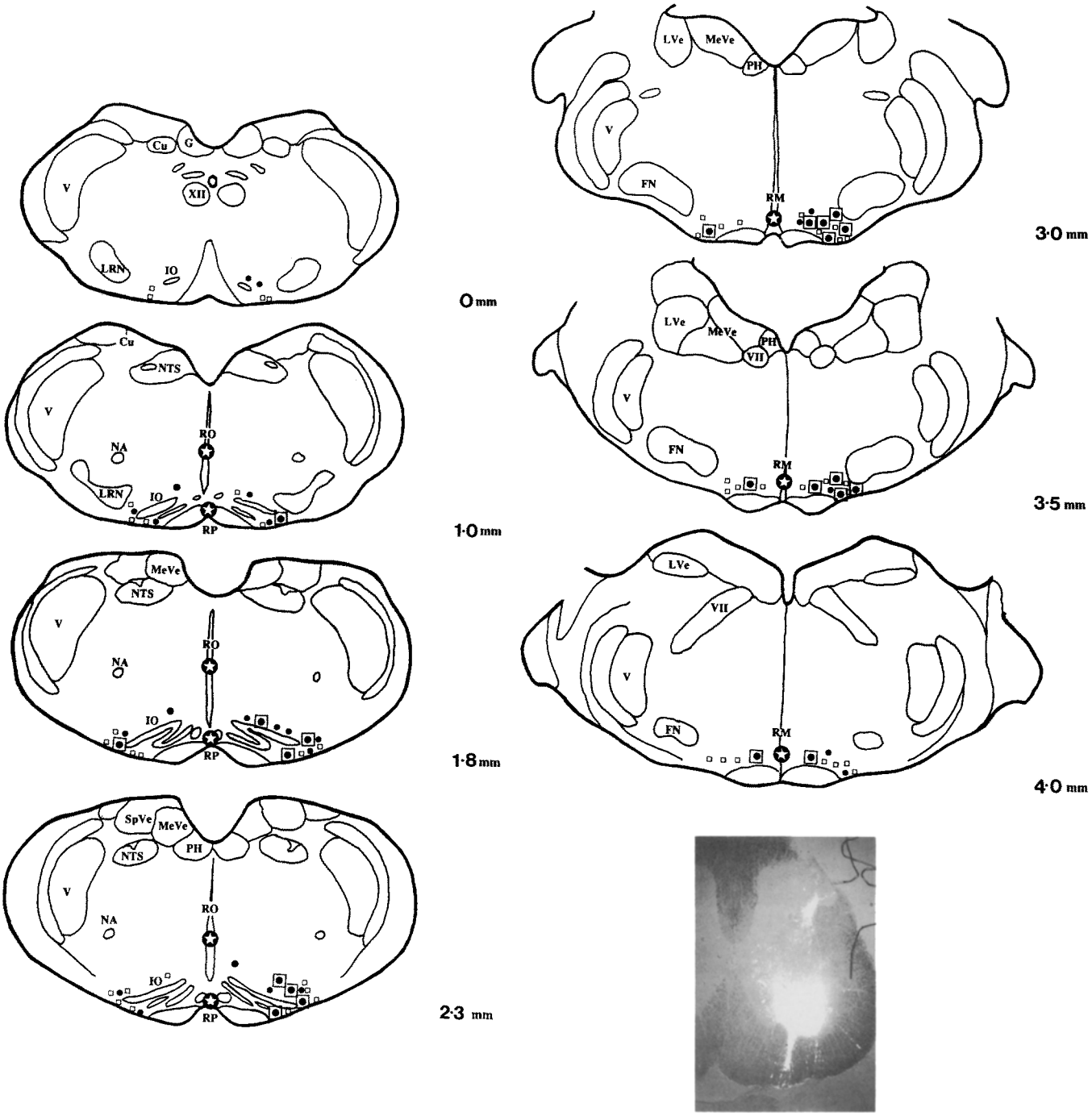

Fig. 1. Drawings of the medulla oblongata illustrating the distribution of spinally projecting neurons after injection of RITC beads into the ventral horn of L6 of the spinal cord (see inset). Filled circles, 1–5 RITC labelled neurons; open squares, 1–5 5–HT labelled cells; filled circles inside squares, 4–10 RITC–5–HT double labelled neurons: stars represent RITC–5–HT double labelled raphe neurons. Abbreviations: G-gracilis nucleus; Cu-cuneate nucleus; XII-hypoglossal nucleas; LRN-lateral reticular nucleus; NTS-nucleus of the tractus solitarius; NA nucleus ambiguus; IO-inferior olivary nuclei; RO-raphe obscurus; RP-raphe pallidus; MeVe-medial vestibular nucleus; SpVe = spinal vestibular nucleus; LVe-lateral vestibular nucleus; V-trigeminal nucleus; FN-facial nucleus; RM-raphe magnus; PH-prepostitus nucleus; VII-facial nerve

Results

Anatomical studies

Microinjection of RITC beads into the 6th lumbar segment of the spinal cord, in the vicinity of the pudendal motoneurons, retrogradely labelled cell bodies in the ventral medulla. These labelled cells were found along the length of the nPGi with the highest number of cells located in the rostral portion of the nPGi, and included cell bodies located on the surface of the medulla. In addition, retrogradely labelled cells were found in the nucleus gigantocellularis, primarily dorsal to the pyra-

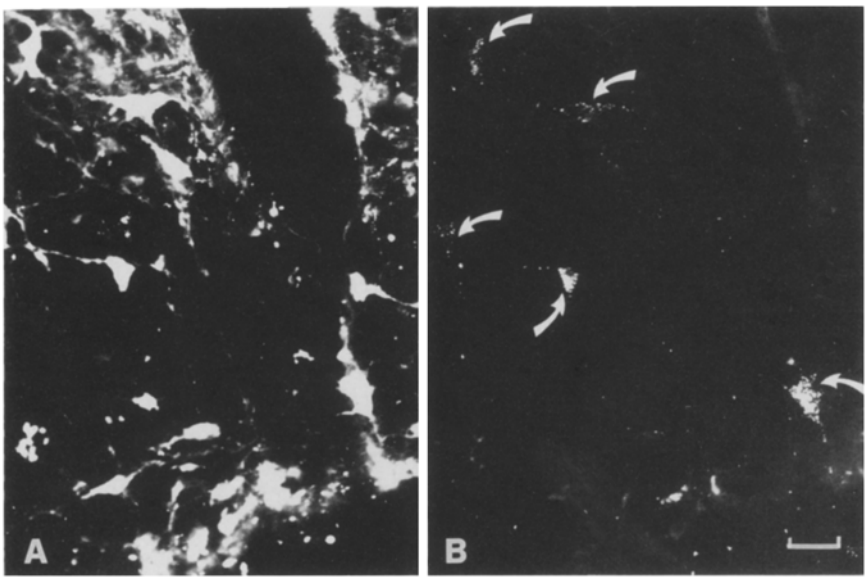

Fig. 2A, B. Photomicrographs illustrating retrogradely labelled neurons in the rostral nPGi after injection of RITC beads into the spinal cord region of the pudendal motoneurons **A.** 5–HT immunoreactivity in the same section **B.** Arrows indicate RITC labelled neurons that contain 5–HT. Scale bar = 50 μm

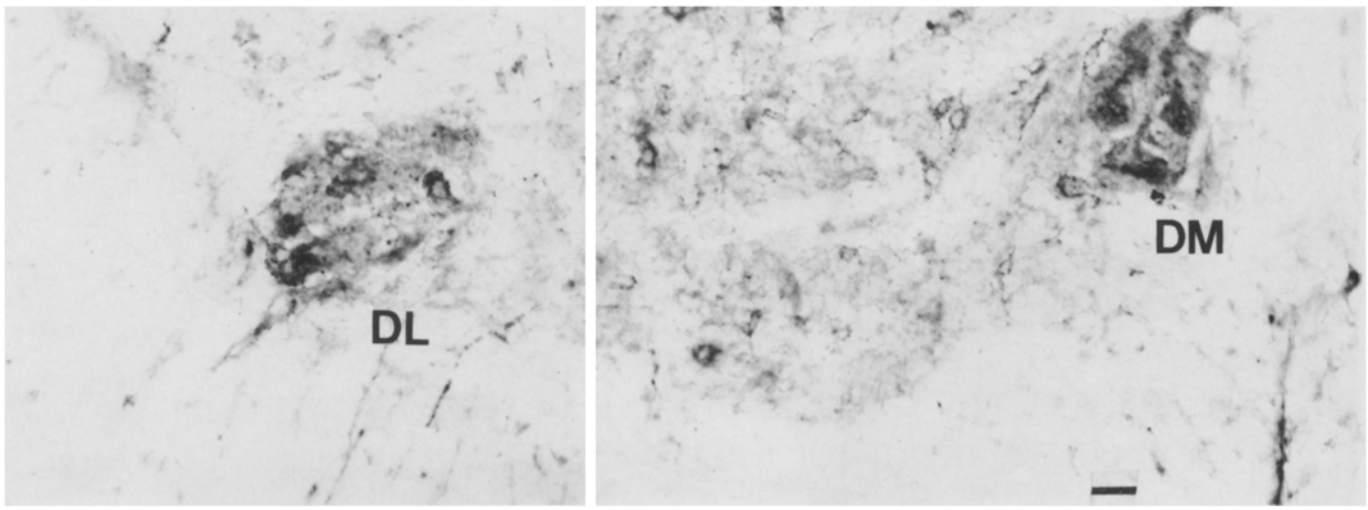

Fig. 3. Photomicrographs illustrating dense PHA–L staining surrounding the dorsolateral (DL) and dorsomedial (DM) pudendal motoneurons in L6 of the spinal cord. Scale bar = 50 μm

midal tract and raphe pallidus; fewer retrogradely labelled neurons were found in the raphe obscurus and raphe magnus (Fig. 1). Retrogradely labelled cells were counted in the rostral nPGi (ie. from the rostral extent of the facial nucleus to the rostral third of the inferior olivary nucleus) within the area containing cell bodies that control the descending inhibition of spinal sexual reflexes (Marson and McKenna 1990). Combined retrograde labelling and immunostaining for 5–HT revealed that 78% of the retrogradely labelled cells ipsilateral to the injection site in the rostral nPGi contained 5–HT (Fig. 2). 15% of the retrogradely labelled cells contralateral to the injection site contain 5–HT, although fewer retrogradely labelled cells were observed. Around half of the 5–HT neurons in the rostral nPGi project to the lumbar cord ipsilateral to the injection site and 5% of the 5–HT neurons contralateral to the injection site contained RITC beads. Virtually all the retrogradely labelled neurons in the raphe pallidus and raphe obscurus contained 5–HT immunoreactivity, whereas fewer retrogradely labelled cell bodies in the raphe magnus contain 5–HT.

Anterograde tracing using PHA–L injected into the rostral nPGi confirmed that the nPGi projects to the vicinity of the pudendal motoneurons. Fibers and presumptive terminals were observed surrounding the pudendal motoneurons in L6 (Fig. 3), the dorsal gray commissure and lamina X surrounding the central canal in spinal segments L5–S1, suggesting that rostral nPGi neurons project to pudendal motoneurons and interneuronal areas in the lumbosacral spinal cord. Anterograde labelling was observed on both sides of the spinal cord. Dense 5–HT immunoreactive fibers and presumptive terminals were observed surrounding the dorsomedial and dorsolateral motoneurons in L6 spinal cord (Fig. 4).

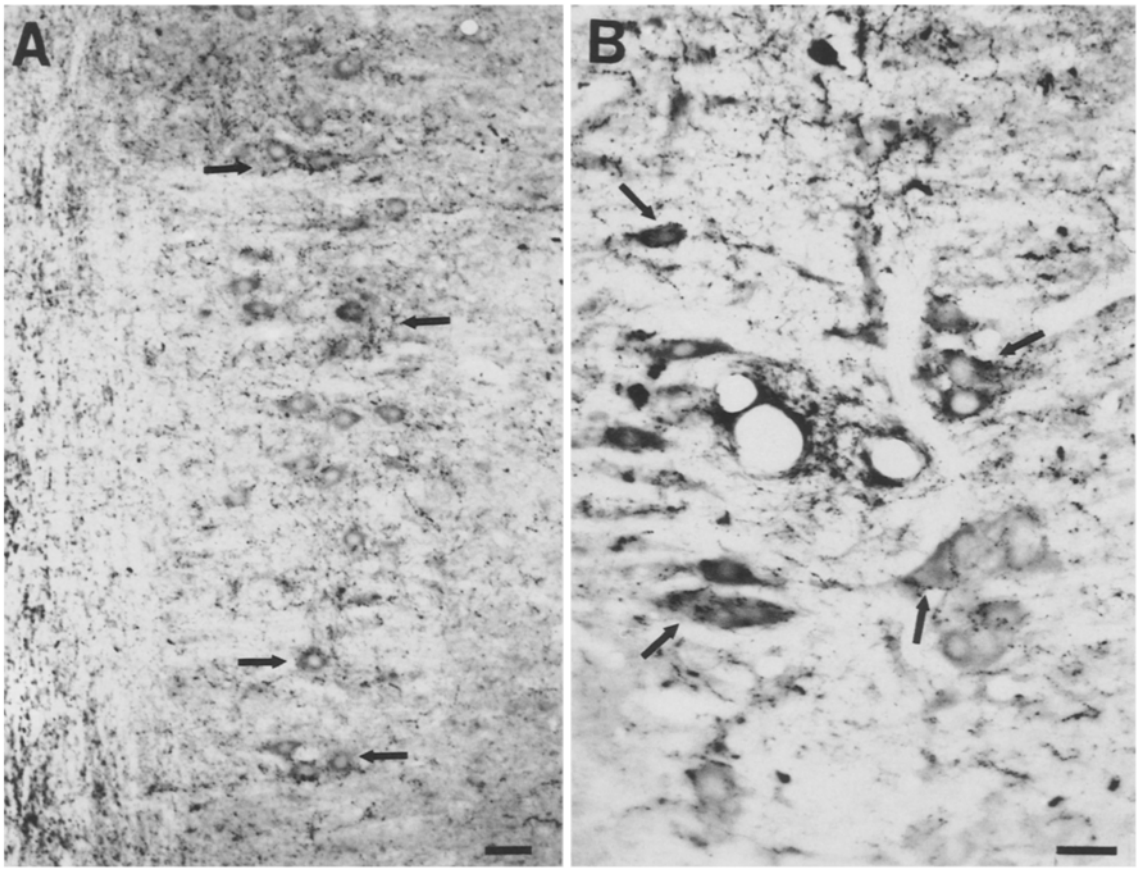

Fig. 4A, B. Photomicrographs illustrating 5–HT immunoreactive fibers and presumptive terminal in the dorsolateral **A** and dorsomedial **B** pudendal motoneuclei at L6, taken from horizontal sections of the spinal cord. Arrows indicate examples of 5–HT immunoreactivity surrounding the motoneurons. Scale bar = 50 μm

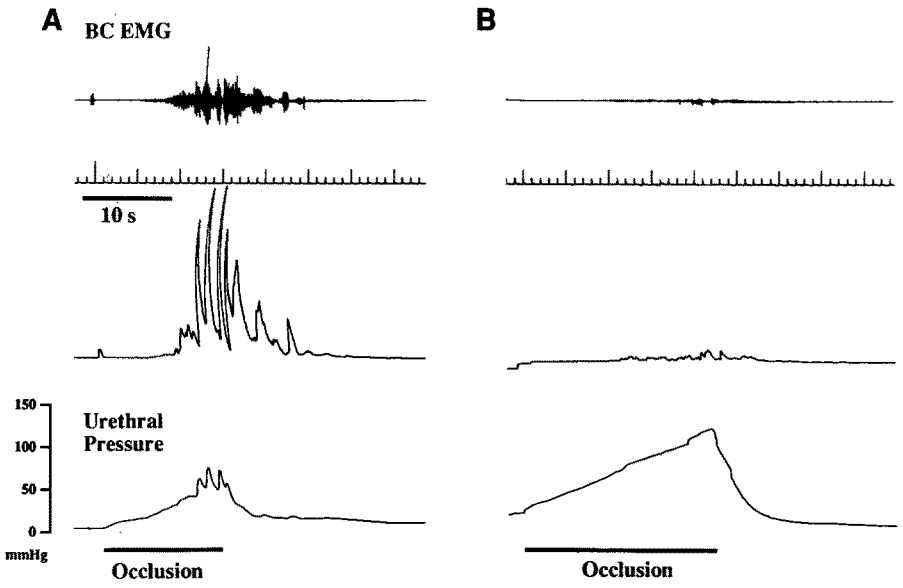

Fig. 5A, B. Polygraph tracings demonstrating the effect of 5–HT (5 μg i.t.) on the coitus reflex in the spinalized rat. The top tracings show bulbospongiosus muscle electromyographic activity (BC EMG). The middle tracings show the rectified and averaged BC EMG activity. The bottom tracings show the pressure within the urethra. Distension of the urethra was accomplished by infusion of saline and occlusion of the urethral meatus. The coitus reflex consisted of rhythmic bursting of the muscle following release of the occlusion. Prior to 5–HT, urethral distension evoked the coitus reflex (panel **A**). Intrathecal 5–HT suppressed the reflex (panel **B**)

Pharmacologic studies

In the anesthetized male, rat bilateral lesions of the rostral nPGi consistently allowed the coitus reflex to be evoked upon distension of the urethra. The coitus reflex consisted of an increase in bulbospongiosus muscle (BC) EMG activity during saline perfusion and occlusion of the urethral meatus. When the threshold perfusion pressure had been reached, release resulted in rhythmic firing of BC EMG activity.

After spinal transection, the coitus reflex could be consistently evoked upon saline infusion and brief occlu-

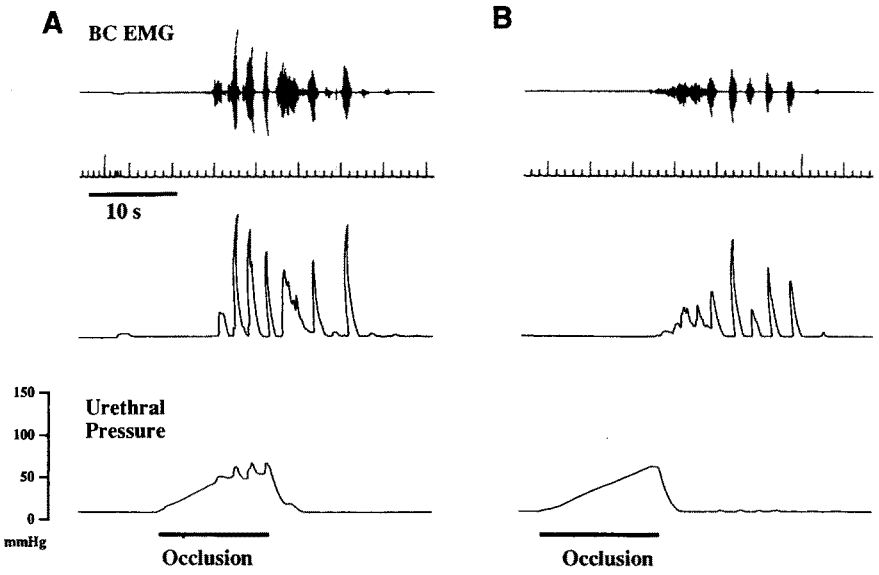

Fig. 6A, B. Polygraph tracings illustrating the antagonism of the 5–HT inhibition by methysergide. Top tracings show bulbospongiosus muscle electromyographic activity (BC EMG). Middle tracings show the rectified and averaged BC EMG. Bottom tracings show pressure within the urethra. The preparation is described in Fig. 5. Prior to methysergide or 5–HT, urethral distension evoked the coitus reflex (panel **A**). After methysergide (5 mg/kg), intrathecal administration of 5–HT (5 μg) failed to abolish the coitus reflex (panel **B**)

sion of the distal penis. Urethral threshold for eliciting the coitus reflex was 65.6 ± 16.4 mmHg (mean ± S.D.), the number of bursts were 7.6 ± 2.1 and the total burst duration was 43.3 ± 26.1 s on average for 18 trials. Intrathecal injection of 5–HT (1–2 μg) increased the threshold for evoking the coitus reflex by 5–10 mmHg and increased the time taken to complete the reflex by 20–60 s, although the number of bursts were similar to the pre-5–HT values ($n = 3$). Intrathecal injection of higher doses of 5–HT (4–50 μg) totally abolished the appearance of the coitus reflex. The coitus reflex was blocked within 8 min after injection of 5 μg 5–HT (Fig. 6). In the majority of experiments, once 5–HT had inhibited the coitus reflex, it could not be re-evoked using physiological threshold pressures (ie 250–300 mmHg). Systemic administration of methysergide, a general 5–HT receptor subtype antagonist, (1–10 mg/kg i.v.) given 5–20 min prior to 5–HT, consistently prevented the 5–HT induced inhibition of the coitus reflex (Fig. 6). The coitus reflex could still be evoked 20 min after intrathecal administration of 5–HT in the presence of methysergide. The number of bursts (10 ± 2.2) and total burst duration (36.3 ± 14.8 s, $n = 4$), were not significantly different from non-treated spinalized trials.

Discussion

These studies provide anatomical and pharmacological evidence for the involvement of 5–HT in modulating the descending inhibition of spinal sexual reflexes. Previous studies demonstrated that 5–HT neurons in the ventral medulla project to the lumbar cord (Bowker and Abbott 1990; Skagerberg and Björklund 1985). The present study demonstrated that a significant number (78%) of retrogradely labelled neurons in the rostral nPGi that project to the ventral horn, in the vicinity of the pudendal motoneurons, contain 5–HT. These data are consistent with the percentage of bulbospinal 5–HT neurons recently reported by Bowker and Abbott (1990). The pudendal motoneurons are located in the dorsolateral and dorsomedial nuclei and project to the bulbospongiosus (BC) and icshiocavernousus (IC) muscles and external anal and urethral sphincters (McKenna and Nadelhaft 1986) and play an important role in sexual and excretory behavior. The BC and IC muscles are essential for the formation of cups and flips, respectively, which are important components of sexual reflexes (Sachs 1982). Many studies have shown that medullary 5–HT neurons project to the cervical, thoracic and lumbar cord. Thus 5–HT containing neurons may send collaterals to more that one spinal cord segment which subserve respiratory, cardiovascular and sexual reflexes.

The neurochemical characteristics of nPGi neurons have been mapped and many 5–HT spinally projecting neurons also contain substance P, met-enkephalin, thyrotropin releasing hormone and GABA (Sasek et al. 1990; Marson 1989; Johansson et al. 1981; Millhorn et al. 1987). While neurons located in the caudal nPGi and ventral medulla play a role in cardiovascular control (Caverson and Ciriello 1987), our data suggest that neurons in the rostral nPGi control the descending inhibition of sexual reflexes (Marson and McKenna 1990). Bilateral electrolytic lesions of the nPGi substantially increase copulatory efficiency in male rats, providing behavioral evidence that this region is involved in sexual function (Maillard and Edwards 1990). Since the execution of sexual behavior involves an integration of cardiovascular, respiratory and pelvic responses, the nPGi appears to be a likely candidate for the integration of these reflexes whose inputs may arise from more rostral sensory and autonomic brainstem inputs.

Anterograde tracing using PHA–L confirmed the projection from the rostral nPGi to the region of the pudendal motoneurons and interneuronal areas of the lumbar cord. Previous anterograde tracing studies have shown that neurons in the ventral medulla, including the region of the nPGi, project to the lumbar cord in the vicinity of the pudendal motoneurons and autonomic areas thought to innervate the genitalia (Holstege and

Kuypers 1987). 5–HT containing fibers and presumptive terminals surround the motoneurons in the lumbar cord (present study, Steinbusch 1981). Moreover, 5–HT immunoreactive staining was significantly reduced after complete spinal transection, suggesting that the 5–HT input arose from supraspinal neurons (Micevych et al. 1986).

The neurophysiological components comprising the coitus reflex in the present model are remarkably similar to those present during ejaculation and climax in the human male (Gerstenberg et al. 1990), supporting the contention that the coitus reflex is an appropriate model for studying sexual reflexes in the anesthetized rat. The coitus reflex appears to be the neural substrates of ejaculatory responses as it is elicited, in the male, by the build-up of seminal fluid in the urethra (McKenna et al. 1991). The coitus reflex may also be viewed as the ability of the spinal cord to coordinate sexual reflexes (Chung et al. 1988; McKenna et al. 1991).

The present study proposes that 5–HT containing neurons in the rostral nPGi that project to the vicinity of the pudendal motoneurons modulate the inhibition of spinal sexual reflexes. Previous work monitoring sexual behavior primarily focused on ascending 5–HT pathways. Electrolytic or neurotoxic lesions of the midbrain raphe or intracerebroventricular injection of neurotoxic agents facilitated sexual behavior (McIntosh and Barfield 1984; Larsson et al. 1978). Microinjection of 5–HT into the midbrain tegmentum decreased the time to ejaculate and the number of intromissions (Hillegaart et al. 1989). These authors postulated that 5–HT was acting on autoreceptors to inhibit their firing and release of 5–HT from projection sites. In contrast, augmentation of 5–HT activity also elicits spontaneous erections and ejaculatory responses (Berendsen and Broekklamp 1987; Renyi 1986). Therefore, 5–HT may have both facilitatory and inhibitory effects on sexual function depending on the receptor subtype, location of receptors and the animal model. These results are further confused by the presence of 5–HT in supraspinal, spinal and peripheral sites involved in sexual behavior.

Several studies have investigated the effect of 5–HT receptor subtypes on sexual behavior. Systemic administration of the 5–HT_2 receptor selective agonist DOI, [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane], or administration of the 5–HT_2 receptor selective antagonists, LY53857 and LY281067, demonstrated an inhibitory role for the 5–HT_2 receptor on male sexual behavior (Foreman et al. 1989). Systemic administration of 5–HT_{1B} receptor agonists, RU24969 ([5-methoxy-3-(1,2,3,6-tetrahydro-4-pyrinyl)-indole), TFMPP ([1-(m-trifluoromethylphenyl)piperazine] and MCPP ([1-(m-chlorophenyl)piperazine]) also inhibit male sexual behavior (Fernandez-Guasti et al. 1989). While, 5–HT_{1A} receptor agonists, 8OHDPAT (8-hydroxy-2-(di-n-propylamino)tetraline), ipsapirone or 5-MeODMT (5-methoxytryptamine) facilitate sexual behavior (Ahlenius et al. 1989; Schnur et al. 1989; Lee et al. 1990; Ahlenius and Larsson 1991). However, 8OHDPAT suppresses ejaculation and erection ex copula (Schnur et al. 1989; Lee et al. 1990), although some evidence for reduced seminal plug weight in copula was found suggesting inhibition of ejaculatory mechanisms (Schnur et al. 1989). In addition, 5–HT_{1A} receptor activation also stimulates arousal, which may explain the differential effects seen ex copula and during mating behavior (Lee et al. 1990). The fact that 5–HT receptor agonists have demonstrated conflicting actions on sexual behavior is obscured by multiple 5–HT pathways which may enroll multiple receptor subtypes in its action.

We have demonstrated that intrathecal injection of 5–HT, in the spinalized anesthetized male rat, blocks the appearance of the coitus reflex and that this blockade is reversed by methysergide. This suggests that endogenous 5–HT may act in the lumbar cord to inhibit sexual reflexes. Systemic administration of MCPP, a 5–HT_{1B} receptor agonist, evoked rhythmic contractions of the pelvic muscles, increased intracavernous pressure and cavernous nerve activity. Spinal cord transection facilitated the neural firing and evoked reflexes suggesting that a supraspinal descending inhibitory input could influence the MCPP response and that MCPP may act either directly or indirectly on autonomic and somatic pathways controlling sexual reflexes (Steers and De Groat 1989). 5–HT_{1B}, 5–HT_{1C} and 5–HT_2 receptor activation facilitates the excitability of spinal cord motoneurons and 5–HT_{1A} receptors may be involved in enhancement of somatomotor outflow at sites presynaptic to the motoneurons (Jackson and White 1990). The effect of MCPP and other 5–HT receptor selective agonists and antagonists on the coitus reflex in the intact and spinalized rat, in addition to recordings of pelvic efferents and afferents would provide further information as to the neural mechanisms controlling ejaculation and climax.

The present study demonstrates a role for 5–HT in the descending inhibition of sexual reflexes by bulbospinal neurons located in the rostral nPGi. This does not preclude the involvement of other 5–HT brainstem pathways in modulating sexual function. Further studies using intrathecal injections of 5–HT receptor agonists and antagonists will further define which receptor subtype/ s are involved in the reflex. This preparation will provide useful information which may be obscured in the behaving animal by conflicting effects of drug actions on sexual reflexes, arousal, or motor activity.

Acknowledgements. The authors would like to thank Marsha List for excellent technical assistance and Cindy Polchow for excellent photographic work. This work was supported by NIH grant number NS 26157.

References

Ahlenius S, Larsson K (1991) Opposite effects of 5-methoxy-N,N-di-methyl-tryptamine and 5-hydroxytryptophan on male rat sexual behavior Pharmacol Biochem Behav 38:201–205

Ahlenius S, Larsson K, Arvidsson L-E (1989) Effects of stereoselective 5–HT_{1A} agonists on male rat sexual behavior. Pharmacol Biochem Behav 33:691–695

Berendsen HHG, Broekklamp CLE (1987) Drug-induced penile erections in rats; indications of serotonin$_{1B}$ receptor mediation. Eur J Pharmacol 135:279–287

Bowker RM, Abbott LC (1990) Quantitative re-evaluation of descending serotonergic and non-serotonergic projections from

the medulla of the rodent: evidence for extensive co-existence of serotonin and peptides in the same spinally projecting neurons, but not from the nucleus raphe magnus. Brain Res 512:15–25

Caverson MM, Ciriello J (1987) Ventrolateral medullospinal neurons involved in the control of circulation. In: Ciriello J, Calaresu FR, Renaud LP, Polosa C (eds) Organization of the autonomic nervous system: central and peripheral mechanisms. Alan R Liss Inc, New York, pp 227–237

Chung SK, McVary KT, McKenna KE (1988) Sexual reflexes in male and female rats. Neurosci Lett 94:343–384

Fernandez-Guasti A, Escalante A, Agmo A (1989) Inhibitory action of various 5–HT_{1B} receptor agonists on rat masculine sexual behavior. Physiol Biochem Behav 34:811–816

Fischette CT, Nock B, Renner K (1987) Effects of 5,7-dihydroxytryptamine on serotonin 1 and 2 receptors throughout the rat central nervous system using quantitative autoradiography. Brain Res 421:263–279

Foreman MM, Hall JL, Love RL (1989) The role of the 5–HT_2 receptor in the regulation of sexual performance of male rats. Life Sci 45:1263–1270

Gerstenberg TC, Levin RJ, Wagner G (1990) Erection and ejaculation in man. Assessment of the electromyography activity of the bulbocavernosus and ischiocavernosus muscles. Br J Urol 65:395–402

Hillegaart V, Ahlenius S, Larsson K (1989) Effects of local application of 5–HT into the medial and dorsal raphe nuclei on male rat sexual and motor behavior. Behav Brain Res 33:279–286

Holstege JC, Kuypers HGJM (1987) Brainstem projection to lumbar motoneurons in rat. I. An ultrastructural study using autoradiography and the combination of autoradiography and horseradish peroxidase histochemistry. Neuroscience 21: 345–367

Jackson DA, White SR (1990) Receptor subtypes mediating facilitation by serotonin of excitability of spinal motoneurons. Neuropharmacology 29:787–797

Johansson O, Hokfelt T, Pernow B, Jeffcoate SL, White N, Steinbusch HWM, Verhofstad AAJ, Emson PC, Spindel E (1981) Immuno-histochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P and thyrotropin releasing hormone -like immunoreactivity in the medullary neurons projecting to the spinal cord. Neuroscience 6:1857–1881

Larsson K, Fuxe K, Everitt BJ, Holmgren BJ, Sodersten P (1978) Sexual behavior in the male rat after intracerebroventricular injection of 5,7-dihydroxytryptamine. Brain Res 141:293–303

Lee RL, Smith DR, Mas M, Davidson JM (1990) Effects of intrathecal administration of 8-OH-DPAT on genital reflexes and mating behavior in male rats. Physiol Behav 47:665–669

Maillard C-A, Edwards DA (1990) The lateral paragigantocellularis nucleus: lesions increase copulatory efficiency in male rats. 22nd Annual Conf. on Reproductive Behavior. Atlanta, GA, p 37

Marson L (1989) Evidence for colocalization of substance P and 5-hydroxytryptamine in spinally projecting neurons of the cat medulla oblongata. Neurosci Lett 96:54–59

Marson L, Loewy AD (1985) Topographic organization of substance P and monoamine cell bodies in the ventral medulla of the cat. J Auton Nerv Syst 14:271–285

Marson L, McKenna KE (1990) The identification of a brainstem site controlling spinal sexual reflexes in male rats. Brain Res 515:303–308

Mas M, Zahradnik MA, Martino V, Davidson JM (1985) Stimulation of spinal serotonergic receptors facilitates seminal emission and suppresses penile erectile reflexes. Brain Res 342:128–134

McIntosh T, Barfield RJ (1984) Brain monoaminergic control of male reproductive behavior. I. Serotonin and the post-ejaculatory refractory period. Behav. Brain Res 12:255–265

McKenna KE, Nadelhaft I (1986) The organization of the pudendal nerve in the male and female rat. J Comp Neurol 248:532–549

McKenna KE, Chung SK, McVary KT (1991) A model for the study of sexual reflexes in anesthetized male and female rats. Am J Physiol 261:R1276–R1285

Micevych PE, Coquelin A, Arnold AP (1986) Immunohistochemical distribution of substance P, serotonin and methionine-enkephalin in sexually dimorphic nuclei of the rat lumbar spinal cord. J Comp Neurol 248:235–244

Millhorn DE, Hokfelt T, Seroogy K, Oertal W, Verhofstad AAJ, Wu J-Y (1987) Immunohistochemical evidence for colocalization of gamma-aminobutyric acid and serotonin in neurons in the ventral medulla projecting to the spinal cord. Brain Res 410:179–185

Monroe PJ, Smith DJ (1983) Characterization of multiple [3H] 5-hydroxytryptamine binding sites in the rat spinal cord tissue. J Neurochem 41:349–355

Renyi L (1986) The effect of selective 5-hydroxytryptamine uptake inhibitors on 5-methoxy-N,N-dimethyltryptamine-induced ejaculation in the rat. Br J Pharmacol 87:639–648

Sachs BD (1982) Role of striated penile muscles in penile reflexes, copulation, and induction of pregnancy in the rat. J Reprod Fert 66:433–443

Sasek CA, Wessendorf MW, Helke CJ (1990) Evidence for co-existence of thyrotropin-releasing hormone, substance P and serotonin in ventral medullary neurons that project to the intermediolateral cell column in the rat. Neuroscience 35:105–119

Schnur SL, Smith ER, Lee RL, Mas M, Davidson JM (1989) A component analysis of the effects of DPAT on male rat sexual behavior Physiol Behav 45:897–901

Skagerberg G, Björklund A (1985) Topographic principles or serotonergic and non-serotonergic brainstem neurons in the rat. Neuroscience 15:445–480

Steers WD, De Groat WC (1989) Effects of *m*-chlorophenylpiperazine on penile and bladder function in rats. Am J Physiol 257:R1441–R1449

Steinbusch HWM (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat- cell bodies and terminals. Neuroscience 6:557–618

Sternberger LA (1979) Immunocytochemistry, 2nd ed. Wiley and Sons, New York