Raphe Magnus Neurons Respond to Noxious Colorectal Distension

Thaddeus S. Brink and Peggy Mason

Committee on Neurobiology and Department of Neurobiology, Pharmacology and Physiology, University of Chicago, MC 0926, Chicago, Illinois 60637

Submitted 18 September 2002; accepted in final form 28 October 2002

INTRODUCTION

Neurons in the medullary raphe magnus (RM) and the adjacent nucleus reticularis magnocellularis (NRMC) project to the dorsal horn of the spinal cord (Basbaum and Fields 1978, 1979; Holstege and Kuypers 1982) where they modulate cutaneous nociceptive input (Fields et al. 1991; Mason 2001; Sandkuhler 1996). Activation of RM and NRMC neurons can both suppress and facilitate noxious heat-evoked motor and cellular responses (Zhuo and Gebhart 1990, 1992a, 1997), while inactivation or lesioning of RM and NRMC eliminates both facilitation and suppression (Behbehani and Fields 1979; Chung et al. 1987; Gebhart et al. 1983; Kaplan and Fields 1991; Sandkuhler and Gebhart 1984). This evidence indicates that at least two cell classes exist within RM—one that facilitates and one that inhibits cutaneous nociception. Electrophysiological studies have characterized two classes of neurons that are hypothesized to underlie the nociceptive inhibitory and facilitatory effects of RM activation (reviewed in Fields et al. 1991; Mason 2001). On cells are excited by noxious cutaneous stimuli and inhibited by opiates and are thought to facilitate nociceptive transmission. In contrast, off cells are inhibited by noxious cutaneous stimuli, excited by opiates, and are thought to inhibit nociceptive transmission. Both on and off cells are nonserotonergic (Gao and Mason 2000; Mason 1997; Potrebic et al. 1994).

These results suggest that one physiological response—that evoked by noxious tail heat—can predict the function of on and off cells. This is surprising because on and off cell responses to noxious tail heat are not perfectly correlated with responses to noxious stimuli of other modalities (mechanical, chemical) or applied to other cutaneous regions (Ellrich et al. 2001). Because there is limited information available regarding the responses of RM and NRMC neurons to visceral stimulation (Chandler et al. 1994; Guilbaud et al. 1980; Lumb 1986; Snowball et al. 1997), the present experiments were designed to determine how on and off cells respond to a physiological visceral stimulus, distension of the colon and rectum (CRD).

A third type of nonserotonergic RM/NRMC neuron, the neutral cell, does not respond to either noxious tail heat or opioids. However, neutral cells typically respond to other cutaneous somatosensory stimuli (Ellrich et al. 2000; Leung and Mason 1998), leading to the idea that they modulate responses to noxious stimulation of a different intensity or modality than the tail heat stimulus used in most studies. Finally, serotonergic neurons comprise a distinct cell class distinguished by their unique neurochemistry and physiological discharge rate and pattern (Gao and Mason 2000; Mason 1997). Although most serotonin-containing RM and NRMC neurons do not respond to either noxious cutaneous heat or to opioids (Gao and Mason 2000; Gao et al. 1998), a majority respond to mechanical stimulation within the retroperitoneum (Gao and Mason 2001b). Together with the finding that serotonin receptor antagonists attenuate the heterotopic antinociception evoked by CRD (Zhuo and Gebhart 1992b), the sensitivity of serotonergic cells to retroperitoneal stimulation suggests that serotonergic cells may respond to CRD.

The studies reviewed in the preceding text provide indirect evidence that cells in each of the physiological cell classes contained in RM and NRMC may play a role in the modulation of visceral nociception. The present investigation was designed to address the physiological plausibility of such roles by examining whether cells in each cell class respond to CRD. Therefore the responses of on, off, neutral, and serotonergic RM and NRMC cells to CRD were tested in lightly anesthetized rats.
METHODS

Surgery

Male Sprague-Dawley rats (n = 68, 250–500 g; Charles River, Portage, MI) were treated with atropine sulfate (40 μg in 0.1 ml sc) and anesthetized with halothane. In early experiments, a Y tube was inserted into the trachea, whereas in later experiments, halothane was administered through a nose cone adapted for the stereotaxic. Halothane was maintained at 1.8–2.0% in oxygen for the surgery. A catheter was inserted into the femoral artery to record blood pressure.

Needle electrodes were placed bilaterally into the thorax to record the electrocardiogram and into the paraspinalis, quadriceps, and biceps femoris muscles to record the electromyographic activity of the tail and hindlimb muscles. A craniotomy was made over the cerebellum, and the exposed dura was cut. Core temperature was maintained at 36–38°C via a water-perfused heating pad. After the surgery, the halothane concentration was lowered to 1% and the animal was allowed to equilibrate for 30–60 min.

Electrophysiological methods

Both metal and glass microelectrodes were used. The glass micropetites were filled with 2% Neurobiotin (Vector Labs, Burlingame, CA) in a 0.1 M Tris (hydroxymethyl)aminomethane buffer (pH 7.4) and 0.15 M KCl and were used with an impedance of 5–10 MΩ. Metal microelectrodes were made of tungsten (A-M Systems, Pullman, WA). Microelectrodes were lowered into the region of the RM and NRMC (P 10.5–11.8 mm from bregma, L 0.0–1.0 mm, V 8.5–10.5 mm from cerebellar surface). Neurons were isolated by their spontaneous activity, and each extracellular unit that was successfully isolated was studied. The unit waveform was acquired at 40 kHz by a CED Micro1401 interface (CED, Cambridge, UK). Spike2 acquisition software (CED) stored the time of the spike and 30 digitized points from the waveform. Individual waveforms were discriminated off-line using template-matching software.

Stimulation methods

Cells were tested for their responses to noxious tail or paw heat and colorectal distension (CRD). Heat stimuli were applied using a peltier device (Yale Instrumentation, New Haven, CT). For tail heat, the peltier was placed on the ventrum of the tail at a point 6–7 cm from the distal tip, and for paw heat, the peltier was placed on the footpad and toes of the hindpaw. Both the paw and the tail were affixed to the peltier platform (2 cm square) so that they were exposed to the full duration of the stimulus. Each heat stimulus consisted of a 2- to 3-s ramp from 32 to 49–56°C and a 4-s plateau at the peak temperature. The peltier platform then ramped back down to 32°C over the course of 3–7 s. Between thermal stimuli, the peltier platform was maintained at 32°C.

To inflate the colon, the finger portion of a glove was secured onto flexible tubing (Tygon R3603, 2 mm OD). This tubing was connected to a 60-mm syringe with a side port connected to a manometer. The glove finger was coated with petroleum jelly (Vaseline) and inserted 7–9 cm into the anus to a point just past the external sphincter muscle. Unless otherwise stated, all distensions were applied for 20 s.

Experimental protocol

On isolation of a unit, repeated (2–5) trials of noxious cutaneous heat (tail and/or paw heat) were interleaved with CRD trials. All stimuli were separated by intervals of 3–5 min. In early experiments, all cells were tested with heat intensities to 56°C and colorectal distensions of 80 mmHg. In later experiments, the peak temperature of the cutaneous stimulus was chosen to be the minimum that reliably elicited a robust motor withdrawal and was typically 49 or 52°C and initial CRD stimuli were applied at an intensity of 60 mmHg. If cells did not respond to the 60 mmHg CRD, the maximal intensity of 80 mmHg was applied. For each stimulus, three to five trials were recorded.

The recording site for at least one cell per animal was labeled by injection of current (5 min, 10-nA depolarizing for glass electrodes and 4 min, 20-μA hyperpolarizing for metal electrodes).

Histology

Animals were overdosed with 5% halothane and perfused with a fixative containing 4% paraformaldehyde and 7% sucrose in 0.1 M phosphate-buffered saline. The brain stem was removed, postfixed for 2–12 h, and then immersed in 30% sucrose in 0.1 M PBS. Coronal sections (50 μm) were cut on a freezing microtome. Sections from external points with glass electrodes were processed for a DAB reaction as previously described, and the site of Neurobiotin injection was identified (Gao and Mason 1997). Lesion sites from experiments using metal electrodes were recovered. Sections were mounted on gelatin-coated slides and then stained with cresyl violet. Under ×50 magnification, the mediolateral and dorsoventral distances from midline and the ventral midline edge of the section, respectively, were measured. Sites were assigned an anterior-posterior location by comparison of sections with a standard atlas (Paxinos and Watson 1986). Unlabeled sites were located by their stereotaxic distance from marked recording sites.

The nuclear boundaries used are modified from Newman (1985). According to Newman, raphe pallidus (RP) extends rostrally to the rostral pole of the inferior olive but does not continue into levels that include the facial nucleus. It should be noted that this is different from the nuclear boundaries illustrated in Paxinos’ atlas (Paxinos and Watson 1986). Thus we considered RM to include a region 300 μm wide centered on the midline and extending from the base of the brain to a point 1,500 μm dorsal, at levels from −11.6 to −10.4 relative to bregma. A box representing this area is illustrated in Fig. 1. inset. NRMC was considered to include a region that stretched laterally from RM to the lateral edge of the pyramids and had a dorsal extent of 1,000 μm. Cells dorsal to RM or NRMC were considered to be located in nucleus reticularis gigantocellularis (NRGC).

Cellular analysis

Neurons were classified by their discharge pattern and their response to noxious heat as ON, OFF, NEUTRAL, or serotonergic cells (Leung and Mason 1998). The mean (x) and SD (SDSD) of the interspike intervals (ISI) were calculated from 15 min of discharge. In addition, the coefficient of variation of the ISIs (CVISI) was calculated. Cells were classified as physiologically defined serotonergic (p5HT) or nonserotonergic (non-p5HT) using a previously described algorithm based on the rate and variability of discharge (Mason 1997). For each cell, the value of the function, y(x, SDISI) = 146 −x + 0.98 SDISI was calculated, where x and SDISI are in ms. Cells were classified as p5HT if the function value was less than zero and as non-p5HT if greater than zero (Mason 1997). This algorithm has been tested on nearly 100 cells and the probability of observed misclassification rate is <10% (Gao et al. 1998; Gao and Mason 1997a,b, 2000, 2001a,b; Mason 1997, 2001).

As described previously, a quantitative method was used to determine whether responses to stimulation were different from spontaneously occurring changes in discharge (Leung and Mason 1998). Briefly, the SD of the change across 10-s bins was calculated and normalized to spikes/s (SDSIS) as a measure of spontaneous variability in discharge. Unit responses to stimulation were then calculated as the difference in discharge before and after stimulus application (in spikes/s). For both cutaneous heat and CRD stimulation, four sequential 10-s response periods were analyzed. Stimulus-evoked increases in discharge that were >2 SDISI were considered excitatory responses and evoked decreases that were >2 SDISI were considered inhibitory
Responses. This method allows us to have confidence, at a $P < 0.05$ level, that a change evoked by a single stimulation trial represents a response and is unlikely to have occurred spontaneously.

Any cell that responded to a majority of heat trials in the first response period was considered responsive. Non-p5HT neurons that were excited by tail or paw heat were considered ON cells, whereas non-p5HT neurons that were inhibited were OFF cells. Non-p5HT neurons that failed to respond to CRD in most trials, the chance of misidentifying a stimulation responsive cell is vanishingly small.

Statistics

Each variable is expressed as a mean ± SE. Statistical tests were performed using Microsoft Excel (Redmond, WA) or SigmaStat (SPSS Science, Chicago, IL).

Results

Characterization of recorded cells: resting discharge and response to noxious heat

A total of 115 cells were recorded from 68 rats. The recording sites were located within RM ($n = 67$), RP ($n = 3$), NRMC ($n = 25$), or the ventral portion of NRGC ($n = 7$; Fig. 1). Recording sites were concentrated at the level of the facial nucleus but extended rostrally to the level of the trapezoid body and caudally to the level of the inferior olive. The locations of 13 cells from eight animals could not be determined.

Using a quantitative analysis of the resting discharge (see Methods), 24 cells that fired slowly and steadily were classified as p5HT. It should be noted that p5HT cells were located either in RM ($n = 14$) or the ventral portion of NRMC ($n = 8$), regions that contain many serotonergic cells (Fig. 1). The mean discharge rate for p5HT cells was $2.0 ± 0.2$ spikes/s and the mean CV$_{ISI}$ was $0.41 ± 0.03$. A minority of the p5HT cells (5/24) responded to noxious tail or paw heat with small changes in discharge rate (<14 spikes in 10 s), consistent with our previous report (Gao and Mason 2000). Of those that responded, three were excited and two inhibited.

The remaining cells ($n = 91$) were non-p5HT and were characterized as ON ($n = 31$; 34%), OFF ($n = 15$; 16.5%), or NEUTRAL ($n = 45$; 49.5%) by their response to noxious tail ($n = 38$) or paw ($n = 53$) heat (see Figs. 3 and 7). The background discharge rates of ON, OFF, and NEUTRAL cells did not differ ($P = 0.9$) and averaged $10.5 ± 1.3$ spikes/s. Although the majority of these cells (50/91) discharged in a bursting pattern (CV >1), a minority ($n = 28$; 31%) had a regular discharge pattern (CV < 0.5). Regularly discharging neurons were distributed across each of the non-p5HT cell classes (13 NEUTRAL cells, 11 on cells, 4 off cells) at the same proportion as nonregularly discharging neurons ($x^2$, $P = 0.99$). All regularly discharging non-p5HT cells had higher discharge rates (range: 6.4–80.4 spikes/s) than did p5HT neurons (range: 0.5–3.7 spikes/s).

Cellular response to CRD

Most of the p5HT cells (17/24; 71%) were unaffected by the 20-s CRD stimulus (Fig. 2A). Of the seven p5HT cells that responded to CRD, three were excited and four inhibited (Fig. 2, B and C). Both the excitatory and inhibitory responses of p5HT cells were small when present, averaging 13.6 ± 6.6 and $–11.1 ± 4.5$ spikes/20 s, respectively. The ranges of excitatory (3.8–26.3 spikes/20 s) and inhibitory (–4.7 to $–24.3$ spikes/20 s) responses were limited to less than one order of magnitude. p5HT cell responses were also brief, being limited to the duration of the stimulus presentation or less for five of the seven p5HT cells responding (see Fig. 5).

Nearly two-thirds of non-p5HT cells (59/91; 65%) responded to CRD (Figs. 3 and 4). More than half of these CRD-responsive non-p5HT cells that responded to CRD were excited ($n = 32$) with a mean increase in cell discharge during the 20-s CRD stimulus of $215.3 ± 45.9$ spikes (Fig. 4, A and B). Excitatory responses ranged over more than two orders of magnitude from 7.3 to 1,223.7 spikes (Fig. 5). When the magnitudes of all non-p5HT cell responses were graphed in ascending order, there was a discontinuity between responses of ≤100 spikes and those that were 100 spikes/s (Fig. 4C). Cells with the former responses were termed “small respond-
ers” (n = 18), and cells with the latter responses were termed “large responders” (n = 14); the mean responses for each of these groups are shown in Fig. 4, A and B. The majority (21/32) of excitatory neuronal responses to CRD did not persist after termination of the stimulus (Figs. 4, A and B, and 5). The remaining cells, constituting a third of the total excited population, responded to CRD for 10s (Fig. 5).

Just under half of the non-p5HT cells that responded to CRD were inhibited (n = 27; Fig. 4, D–F). The mean CRD-evoked decrease in cell discharge during the 20-s stimulus was 176.4 ± 44.7 spikes and ranged over more than two orders of magnitude from 7.7 to 1,147.8 spikes (Fig. 5). Most inhibitory responses (19/27) did not persist after stimulus offset, either returning to baseline values (n = 6; Fig. 4F) or rebounding to a discharge rate above the baseline level (n = 10; Fig. 4E). Because 40% of the inhibitory responses (n = 11) lasted ≥10 s longer than the stimulus, the mean total inhibitory response to CRD (215.9 ± 48.1 spikes) was much greater than the mean response during the stimulus presentation alone (Figs. 4D and 5).

A small proportion of the non-p5HT cells responded to CRD in a complex pattern. For example in the cell illustrated in Fig. 6A, the cell’s discharge increased during the stimulus and then went below baseline values after the stimulus offset. Such an
For non-p5HT cells, the response to CRD was randomly associated with the response to noxious heating of the paw or tail ($\chi^2, P = 0.7$). Nearly equal numbers of ON cells were excited (9/31), inhibited (n = 11), or unaffected (n = 11) by CRD (Fig. 7A, top). Similarly, nearly equal numbers of neutral cells responded to CRD with an increase (16/45), decrease (n = 13), or no change (n = 16) in discharge (Fig. 7A, middle). Finally, almost half of the OFF cells (7/15) were excited by CRD and minorities were either inhibited (n = 3) or unaffected (n = 5) (Fig. 7A, bottom). It is interesting to note that the largest responses to CRD were observed in OFF cells that were excited by CRD and ON cells inhibited by CRD (Fig. 7A).

As illustrated in Fig. 7B, there was no obvious difference between the response to noxious heat among cells with different responses to CRD. For example, the inhibitory responses of OFF cells to noxious heat were similar among subpopulations with dramatically different responses to CRD (compare Fig. 7, A and B, bottom).

Excitatory responses to CRD were relatively nonadapting in comparison with excitatory responses to heat that peaked immediately and then declined (Fig. 7). However, the inhibitory responses to CRD and to heat were both nonadapting as they were maintained throughout the stimulus. The inhibitory responses to CRD and to heat differed in their poststimulus form. At stimulus offset, the discharge of most CRD-inhibited cells returned quickly toward or beyond baseline values. In contrast, among OFF cells, the return to baseline discharge rate after heat offset was slow and gradual.

It is possible that responses to CRD and to heat are related in some way that is obscured by the cell classification system used. Therefore we compared the total change in spikes evoked during the CRD and heat stimuli, regardless of whether that change met our criteria for a response (Fig. 8). Figure 8 is a double logarithmic plot that illustrates the mean change in

excitatory-inhibitory response was seen in three cells. Of the 10 cells that were inhibited by CRD and then rebounded to a greater than baseline discharge rate, 6 increased their discharge rate by $>2^*$ SDI5 in the 20- to 30-s response period after stimulus offset (Fig. 6B). It is interesting to note that such biphasic patterns, where the changes during and after the stimulus are in opposite directions, were never observed in response to noxious cutaneous heat (n = 46 in the present study). Nine cells had no response to CRD during the stimulus but consistently increased (n = 6; Fig. 6C) or decreased (n = 3; Fig. 6D) their discharge after the stimulus offset. Similarly, poststimulus excitatory (n = 9) and inhibitory (n = 1) responses to noxious cutaneous heat in neutral cells were observed (n = 10).

Relationship between responses to CRD and cutaneous heat

The majority of p5HT (13/24; 54%) cells did not respond to either cutaneous heat or CRD. Most of the p5HT cells that responded to CRD did not respond to heat stimulation (6/7), and most of the cells that responded to heat stimulation did not respond to CRD (4/5). One p5HT cell was inhibited by both cutaneous heat and CRD.
spikes evoked by CRD and heat for all p5HT and non-p5HT cells. There are numerous non-p5HT cells in each quadrant of the graph, supporting the idea that the response of non-p5HT cells to heat cannot predict that cell’s response to CRD. It is interesting to note that there are no p5HT cells that increase their discharge in response to CRD but decrease their discharge in response to heat.

Although no attempt was made to match the intensity of the CRD and heat stimuli, the increases and decreases in non-p5HT cell discharge evoked by the two stimuli were similar in magnitude (Fig. 8). Most cells, therefore fall close to the unity lines illustrated in Fig. 8. Further, non-p5HT cells were equally distributed on either side of the unity lines, demonstrating that the mean population responses to CRD and to heat are approximately equal.

The changes in p5HT cell discharge evoked by CRD and heat were consistently smaller than those observed in almost all non-p5HT cells, even non-p5HT cells that were considered unresponsive (Fig. 8). This reflects the finding that neutral cells and cells unresponsive to CRD may change their discharge significantly in response to a minority of stimulation trials (Leung and Mason 1998). However, these cells are not considered responsive to the stimulation because they do not consistently change their discharge to a group of stimulation trials.

**Relationship between responses to CRD and cell location**

There were no differences between the responses to CRD among cells with different nuclear locations or cells located at different mediolateral, dorsoventral or anterior-posterior sites.

**DISCUSSION**

The present study demonstrates that most nonserotonergic RM and NRMC cells respond to noxious intensities of CRD. In contrast, few serotonergic neurons respond, and those that do have weak responses. Although the response of nonserotonergic cells to noxious cutaneous heat predicts important pharmacological characteristics, it is clearly not predictive of the response to CRD. The heterogeneity of responses to CRD among ON, OFF, and neutral cells suggests that either important functional subclasses of these cells exist or that the functional importance of this classification system is limited.

**CRD is a noxious visceral stimulus**

In awake rats, CRD evokes a relaxation of the external anal sphincter, a motor component of defecation, at intensities of 13–20 mmHg (Ness and Gebhart 1988b). At higher pressures,
Serotonergic neurons discharge in a slow and steady fashion (Aghajanian and Vandermaelen 1982; Bayliss et al. 1997; Li and Bayliss 1999a,b; Mason 1997; Wang et al. 2001). In the present study, RM and NRMC neurons were classified as p5HT or non-p5HT by an algorithm that utilizes their slow discharge rate and steady pattern of firing. This algorithm has been tested repeatedly by our laboratory (reviewed in Mason 2001). Notably, of 60 p5HT cells tested, 56 contained serotonin immunoreactivity, whereas none of the 56 cells classified as non-p5HT contained serotonin immunoreactivity. This would suggest that all of the non-p5HT cells are nonserotonergic, whereas about two of the p5HT cells recorded in the present study may in fact be nonserotonergic. Such an error would not change the basic findings of the present study—namely, that most nonserotonergic RM and NRMC cells respond to CRD and that most serotonergic cells do not.

Serotonergic cells fail to respond to CRD

The paucity of serotonergic neurons that respond to CRD is unexpected. Serotonin release from bulbo spinal terminals has been implicated in both the reaction to CRD and in the modulation of cutaneous nociception by CRD. Regarding the former, an intracerebroventricularly administered 5HT3 receptor antagonist attenuates the blood pressure reaction elicited by CRD in dogs (Miura et al. 1999). Together with the finding that the pressor reaction evoked by CRD is greatly attenuated in the spinalized rat (Ness and Gebhart 1988b), input from brain stem serotonergic neurons may be critical to the production of the behavioral reaction to CRD. Serotonin release has also been implicated in the suppression of cutaneous heat-evoked withdrawals by CRD because serotonin receptor antagonists attenuate this suppression (Zhuo and Gebhart 1992b). However, only 7 of 24 serotonergic neurons recorded in the present study responded to CRD, and the observed responses were weak. The most likely explanation for these findings is that serotonergic neurons responsive to CRD are located outside of RM and NRMC or tonic serotonergic discharge is sufficient for the production of the full reaction to CRD and for CRD-evoked antinoceception.

Nonserotonergic cell responses to CRD

CRD-responsive RM and NRMC cells respond at short latency and may therefore receive direct input from spinal neurons. However, anatomical studies have revealed relatively few spinal projections to RM and NRMC. Instead, ventral medullary neurons are likely to receive somatosensory input indirectly via projections from the PAG, hypothalamus, and amygdala (Abols and Basbaum 1981; Hermann et al. 1997; Holstege 1987; Murphy et al. 1999; Van Bockstaele et al. 1991). The same projections may contribute to both cutaneous and visceral responses in RM neurons.

Regardless of the route by which CRD information reaches the ventral medulla, RM and NRMC responses to CRD are ultimately dependent on input from dorsal horn cells. Dorsal horn cells respond to CRD in at least six different ways, most of which are relevant to the present study (Ness and Gebhart 1987, 1988a; Qin et al. 1999). Neurons with “abrupt” excitatory responses are excited by CRD at short latency and maintain discharge during the stimulus presentation but not afterward. Neurons with “sustained” excitatory responses differ from abruptly responsive cells because they maintain an elevated level of discharge after termination of the CRD stimulus. A third group of dorsal horn cells, the excitatory-inhibitory cells, are excited by CRD during the stimulus and then show an inhibition after stimulus termination. Among cells that are inhibited by CRD, the inhibition is either confined to the stimulus presentation, sustained beyond the stimulus presentation, or followed by an excitatory response after stimulus termination (Qin et al. 1999). Nonserotonergic RM and NRMC cells with excitatory responses to CRD stimulation that are restricted to the stimulus presentation likely receive input primarily from the abrupt excitatory class of dorsal horn cells. RM and NRMC cells with excitatory responses to CRD stimulation that persist beyond the stimulus presentation may receive input from both abrupt and sustained excitatory types of dorsal horn cells. RM and NRMC cells with inhibitory responses to CRD may receive sign-inverting input from short-latency abrupt and sustained cells as well as sign-conserving input from one or more types of CRD-inhibited dorsal horn cells. Finally, cells with biphasic response, either excitatory-inhibitory or inhibitory-excitatory, are found in both the dorsal horn and in the medulla. Because some of the different classes of dorsal horn cells can be distinguished by different thresholds (Ness and Gebhart 1987, 1988a), an examination of the threshold for RM and NRMC cell responses to CRD would aid in distinguishing between these possibilities. Such an examination is in progress.

Dorsal horn cells that respond to CRD receive convergent cutaneous input from scrotal, perineal, and lower abdominal regions (Ness and Gebhart 1987, 1988a). The present experiments demonstrate that many putative pain modulatory neurons in the brain stem also receive convergent cutaneous and visceral input (30 of 91 non-p5HT cells; 1 of 24 p5HT cells). This is certainly an underestimate of the true proportion of convergent neurons as cells may respond to stimulus modalities or locations other than those used in this study.

Somatic versus visceral responses of nonserotonergic RM cells

Most on (22/31), off (12/15), and neutral (29/45) cells respond differently to noxious CRD than to noxious heat. For instance, of 45 neutral cells, defined by their lack of a response to noxious heat, 16 were excited and 13 inhibited by CRD. Our finding that cells do not respond in the same direction to visceral and cutaneous stimulation is consistent with observations from a previous study of RM cell responses to bladder stimulation in the cat. Chandler et al. (1994) showed that nearly equal numbers of RM cells inhibited by noxious pinch were excited, inhibited, or unaffected by bladder disten-
sion. Interestingly, in this study, RM cells that were excited by noxious pinch were never excited by bladder distension but rather were either inhibited or unaffected by the visceral stimulus. Such an absence of excitatory responses to visceral stimulation in cells that were excited by noxious cutaneous stimulation was not observed in the present study using colorectal stimulation in the rat. These conflicting results may reflect the different species used because there is evidence of a RM cell that is excited by noxious cutaneous stimulation and urinary bladder distension in the rat (see Fig. 3 in Lumb 1986). Another study to test RM or NRMC responses to both visceral and cutaneous stimulation reported that the responses to intraperitoneal injection of bradykinin were always in the same direction as the responses to noxious thermal and mechanical stimulation of the skin (Guilbaud et al. 1980). While these results are interesting, intraperitoneal bradykinin is a nonphysiological stimulus that may affect a variety of primary afferent types. Our finding that the response to noxious CRD is not predicted by the response to noxious cutaneous heat also supports previous reports that an individual cell’s response to noxious somatic stimulation varies with stimulus location and modality. Specifically, the response of a RM or NRMC cell to noxious tail heat is not always predictive of that cell’s response to stimuli applied to other regions of the body surface or to noxious mechanical stimulation of the tail (see, for examples: Ellrich et al. 2000, 2001; Leung and Mason 1998).

Nonserotonergic cell responses to CRD and to noxious cutaneous heat were similar in absolute magnitude, although not always in direction. This is interesting particularly since cells were tested with only one or two intensities of each stimulus. One interpretation of this data is that nonserotonergic cells have a nearly binary discharge pattern, and are either on (near the maximal discharge rate) or off (near the minimal discharge rate). A second possibility is that nonserotonergic cells code stimulus intensity poorly, responding similarly to any stimuli of threshold. In support of this idea, the responses of ON and OFF cells to brush and laser heat are indistinguishable in awake rats (Leung and Mason 1999).

Functional implications

The response of spinal neurons to CRD increases after spinalization and is unaffected by collicular decerebration, providing evidence for a tonic inhibitory input from the brain stem and possibly upper cervical cord (Ness and Gebhart 1987, 1989; Qin et al. 1999). In contrast, the cardiovascular reaction to CRD is greatly attenuated by spinalization at either C1 or T6 while being unaffected by decerebration, suggesting that nociceptive modulation descends from the brain stem (Ness and Gebhart 1988b). In support of this lesion data, Zhuo and Gebhart recently demonstrated that the behavioral and cellular responses evoked by 80 mmHg CRD stimulation are either attenuated or facilitated by RM stimulation depending on the site and intensity of stimulation (Zhuo and Gebhart 2002; Zhuo et al. 2002). Thus RM is at least one of the brain stem sites, perhaps the primary site, that provides descending inhibitory and facilitatory modulation to CRD-responsive circuits in the spinal cord.

Nonserotonergic RM and NRMC cells that are classified by their response to cutaneous noxious heat share additional class-specific physiological features. For instance, the response to noxious cutaneous heat also predicts a cell’s pattern of discharge across the sleep/wake cycle (Leung and Mason 1999). Cells within a class also have consistent responses to other manipulations such as nonnoxious thermoregulatory challenges (Young and Dawson 1987) and volume expansion (Morgan and Fields 1993). Most importantly, the response to noxious tail heat predicts the response to analgesic doses of opioids in the anesthetized rat (Barbaro et al. 1986; Cheng et al. 1986; Heinricher and Drasner 1991; Heinricher et al. 1992). Because the descending antinociceptive influence from RM/NRMC is active (i.e., depends on activation of cells), RM and NRMC cells that exert antinociceptive effects on spinal neurons must be activated by opioids (reviewed in Fields et al. 1991). Thus a cell’s response to opioids is critical to predicting that cell’s function. On a cautionary note, opioids do not alter the background discharge rate of ON cells in unanesthetized rats (Martin et al. 1992). Further, the effects of opioids on ON and neutral cell discharge have never been tested in the unanesthetized rat. Thus the opioid response of RM and NRMC cells in the natural, unanesthetized state may not be predicted accurately by the response to noxious cutaneous stimulation tested in anesthetized animals.

Short of testing each cell’s response to opioid administration, the most reliable predictor, albeit not perfect, of a cell’s response to opioids in the anesthetized rat is its response to noxious thermal stimulation of the tail. The observation that many ON, OFF, and neutral cells respond differently to tail heat and to other cutaneous stimuli (Ellrich et al. 2000, 2001; Leung and Mason 1998) is then puzzling as it suggests that the response to tail heat, in particular, holds a special significance. Our results and those of Chandler et al. (1994) further increase the known physiological heterogeneity of cells in the ON, OFF, and neutral cell categories. If the important functional classes of RM and NRMC cells were uniform in their responses to peripheral stimulation, the number of functional classes would be quite large. Another possibility is that a smaller number of moderately heterogeneous cell classes exist. For instance, a cell inhibited by noxious thermal stimulation of most sites and excited by CRD, but not one inhibited by CRD, may mediate CRD-evoked suppression of cutaneous withdrawal reflexes. Overall, the data suggest the existence of subgroups of ON, OFF, and neutral cells, with varying functional roles in nociceptive processing and modulation.

The authors thank Drs. Hayley Foo, Jonathan Genzen, Malcolm Nason, and Madelyn Baez for comments on the manuscript.

T. Brink was supported by a National Institute of General Medicine Training Grant (T32 GM-07839). This research was supported by the Brain Research Foundation.

REFERENCES


Gao K and Mason P. Serotonergic raphe magna cells that respond to noxious tail heat are not on or off cells. J Neurophysiol 84: 1719–1725, 2000.


