The organization of the central control of micturition in cats and humans
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Chapter 7

Direct Projections from the Periaqueductal Gray to the Pontine Micturition Center (M-region).
An Anterograde and Retrograde Tracing Study in the Cat

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ABSTRACT

Micturition is a spino-bulbo-spinal reflex. The bulbospinal part of this reflex is formed by the projections from the M-region, also called the pontine micturition center or Barrington’s nucleus, to the preganglionic parasympathetic motoneurons in the sacral cord innervating the bladder. In respect to the spino-bulbar part of the micturition reflex, our group recently showed that the sacral cord projections to the brainstem terminate mainly in the periaqueductal gray (PAG). In this study it was investigated whether the PAG might serve as a link between the sacral cord and the M-region, by examining the possible connections using the tracers wheat germ-agglutin horseradish peroxidase and tritiated leucine. The results demonstrate that a specific circumscribed rostrocaudally oriented cell group within the ventrolateral PAG and parts of the dorsomedial PAG project specifically to the M-region. A concept is put forward in which specific parts of the PAG are involved in the control of micturition and that information concerning bladder filling is conveyed via the PAG to the M-region.

INTRODUCTION

Normal micturition is a coordinated action between the detrusor muscle of the bladder and the external striated urethral sphincter. In the cat the parasympathetic motoneurons innervating the detrusor muscle are located in the intermediolateral cell group (IML) in the S2-S3 segments of the spinal cord (Nadelhaft et al., 1980). The motoneurons innervating the striated external urethral sphincter are located in the nucleus of Onuf in the S1 and S2 ventral horn (Sato et al., 1978). In adult mammals the area responsible for the synergistic action of both muscles (detrusor-sphincter synergy) is not located in the spinal cord but in the brainstem. The region involved is located in the dorsolateral pons, and is known as Barrington’s area (1925), M-region (Holstege et al., 1986) or pontine micturition center (Loewy et al., 1979). In this paper the term M(medial)-region will be used, because there exists a L(lateral)-region also. Neurons in the M-region project to the IML in the sacral cord (Loewy et al., 1979; Holstege et al., 1986), while cells in the L-region maintain connections with the nucleus of Onuf (Holstege et al., 1986). In the cat electrical stimulation in the M-region results in relaxation of the pelvic floor and urethral sphincter, followed in about two seconds by contraction of the bladder, thus mimicking normal micturition (Holstege et al., 1986). Bilateral lesions of the M-region cause an inability to empty the bladder resulting in a retention of urine (Barrington, 1925; Holstege et al., 1986). The question arises which areas in turn control the M-region. Several physiological studies have demonstrated that most of these areas belong to the limbic system, e.g. cingulate gyrus, preoptic area of the hypothalamus, amygdala, and bed nucleus of the stria terminalis (Torrens and Morrison, 1987). Electrical stimulation in these areas and in
Retrogradely labeled cells after a WGA-HRP injection in the dorsolateral pontine tegmentum, including the M-region

WGA-HRP injection sites in the PAG and adjacent areas

Tritiated leucine injection sites in the PAG and adjacent areas

Fig. 1. Schematic drawing of retrogradely labeled neurons in the PAG after a WGA-HRP injection in the M-region in case 2178 (on top). The arrowheads indicate an accumulation of retrogradely labeled cells in the ventrolateral PAG (see text). The WGA-HRP and the $^3$H-leucine injection sites are shown in the middle and at the bottom, respectively. The injection sites of the cases with projections to the M-region are indicated on the left, those without such projections the right.
the periaqueductal gray (PAG), can evoke micturition or micturition-like contractions of the bladder. In the cat specific fiber projections to the M-region have been shown to originate from the preoptic area (Holstege, 1987), but the connections from the PAG to the M-region have not been studied. Therefore, in a retrograde and anterograde tracing study it was attempted to find out whether PAG - M-region projections exist in the cat.

MATERIALS AND METHODS
A total of 20 adult male cats was used and the surgery procedures, pre- and postoperative care, handling and housing of the animals followed protocols approved by the Faculty of Medicine of the University of Groningen. The animals were anesthetized with intravenous pentobarbital sodium 20 mg/kg diluted with 1:1 sodium hydrochloride. In order to identify the M-region neurons, the caudal lumbar vertebrae were laminectomized and a total of 5 µl 20% HRP was injected bilaterally in the S2 and S3 segments of the sacral cord in two cases (2129 and 2226). To determine whether this pathway was ipsi- or bilateral, a hemisection was made on the right side at upper lumbar levels prior to the injection.

All the injections in the brainstem were placed stereotaxically. In order to localize the PAG neurons projecting to the M-region, in 3 cases (2153, 2178 and 2185; Figure 1) injections of 50-100 nl 5% wheat germ-agglutinin HRP (WGA-HRP) were made in the M-region, and in two control cases (2189 and 2224) in adjacent areas, but not in the M-region itself. In anterograde transport studies, 20-30 nl 5% WGA-HRP was injected in the PAG in two cases (2239 and 2241). The injections were centered on those parts of the PAG that had been shown to contain retrogradely labeled neurons after WGA-HRP injections in the M-region.

In one further case (2250) the injection was made in adjacent areas of the PAG and mesencephalic tegmentum (Figure 1). After 3 days survival time, the (WGA)-HRP cats were re-anesthetized and perfused through the left ventricle with 1.5 L saline, followed by 1.5 L fixative solution containing 2% glutaraldehyde and 1% paraformaldehyde. Brainstems and sacral cords were cut in four series of 40 µm sections. One series was processed using the tetramethyl-benzidine (TMB) method, and one series using the ammonium paratungstate (PT) method (Weinberg and VanEyck, 1991). In order to determine the extent of the injections, of the third series the sections with injection sites were processed with the
diaminobenzidine (DAB) method. In additional anterograde studies of projections from the PAG, in 10 cases (1337, 1409; 1410, 1434, 1435, 1481, 1486, 1487, 1495, 1497; Figure 1) 0.5 µl L-[4,5-3H]-leucine was injected in the PAG and the surrounding tissue. These animals were allowed to survive for 6 weeks after which they were perfused with 1.5 L saline and 1.5 L 10% paraformaldehyde. The brains were processed for autoradiography, as described by Holstege (1987).

RESULTS
After the HRP injections in the S2 and S3 segments (cases 2129 and 2226) a specific group of labeled neurons was found ipsilaterally in the dorsolateral pons just medial and ventromedial to the mesencephalic trigeminal tract (Figure 2, on the left). The labeled cell group forms a rostro-caudally oriented column, extending from the level of the inferior colliculus, rostrally, to the level just rostral to the motor trigeminal nucleus, caudally. It corresponds with the M-region as described previously (Holstege et al., 1986). In 3 cases with WGA-HRP injections in the M-region and adjacent areas, a large number of anterogradely labeled fibers was observed in the sacral intermediomedial and intermediolateral cell group, containing the preganglionic parasympathetic motoneurons in the sacral cord. In the same 3 cases retrogradely labeled cells were found in the ventral, lateral and dorsomedial parts of the ipsilateral PAG, but they were especially numerous in a specific region of the ventrolateral PAG between the levels the trochlear nucleus and the center of the oculomotor nucleus (Figure 1, indicated by arrow heads in the upper row). In control case 2189, the WGA-HRP injection site involved the superior and lateral vestibular nuclei, deep nuclei of the cerebellum, and the caudal part of locus coeruleus, but not the M-region. In this case only very few retrogradely labeled cells were present in the PAG. In the other control case (2224), with an injection in the pontine tegmentum just ventral to the M-region, retrogradely labeled cells were found scattered throughout the ventrolateral PAG, mainly at caudal levels. A specific accumulation of labeled neurons, however, was not found.

In cases 1434, 1435, 2239 and 2241, with injections in the lateral PAG, and in cases 1486 and 1495, with bilateral injections in the dorsal PAG (Figure 1), thin labeled fi-

Fig. 3. Schematic representation of the spinal and supraspinal structures involved in micturition control. Excitatory pathways are indicated by “(+).”
bers descended from the injection site into the area of the cuneiform nucleus dorsal to brachium coniunctivum. From this fiber bundle labeled fibers passed medially and dorsally to the brachium coniunctivum to terminate in the M-region (Figure 2, on the right). Only a limited number of labeled fibers terminated in the areas surrounding the M-region. In the cases with injections in the dorsomedial PAG the projection to the M-region was less pronounced than in the cases with injections in the ventrolateral PAG. However, in the former cases the projections were bilateral, due to bilaterality of the injection sites. In the other six 3H-leucine cases and one WGA-HRP case the injections were placed more ventrally, laterally or dorsolaterally in the PAG, but did not involve the area of the PAG revealing an accumulation of labeled neurons after WGA-HRP injections in the M-region. In these control cases weak diffuse anterograde labeling was observed throughout the dorsolateral pontine tegmentum, but specific anterograde labeling in the M-region was not found.

DISCUSSION

The present results provide evidence for a major projection from the ventrolateral and a minor projection from the dorsomedial PAG to the M-region in the cat. In a study on the PAG projections to the rostromedial pericoerulear region in the rat (Ennis et al., 1991), PAG fibers were found in Barroton’s area as defined by Paxinos and Watson (1986). The present results not only show that a similar projection exists in the cat, but also that it originates mainly from a rostrocaudally oriented cell group in the ventrolateral PAG and from a more loosely arranged rostrocaudally oriented cell group in the dorsal PAG. The results suggest that the PAG plays a substantial role in micturition and they might explain why stimulation in the PAG results in voiding (Torrens and Morrison, 1987; Blok, unpublished results). However, the exact role of the PAG in the framework of micturition is not so clear. Micturition is considered to be a spino-bulbo-spinal reflex (Torrens and Morrison, 1987). The spino-bulbar part of this reflex is formed by the projection from the sacral cord to the brainstem, conveying information on bladder filling or bladder extension. Recent findings in the cat (VanderHorst et al., 1996) have demonstrated that particularly the sacral segments of the spinal cord project strongly to the PAG. In view of the finding that the sacral cord does not, or only to a limited extent, projects directly to the M-region (Blok et al., 1995), it is possible that the PAG serves as a major “receiving” station for ascending sacral projections to the brainstem. The present results show that the PAG, in turn, might activate the M-region in order to produce voiding. Thus, the PAG may take part in the spino-bulbo-spinal reflex of micturition. In summary, a concept is presented in which the ascending projections from the sacral cord, conveying information on bladder filling, terminate in the PAG. In case the bladder is enough extended that voiding is necessary, the PAG, in turn, “stimulates” the M-region, which results in micturition. Rostrally located limbic structures, as the preoptic area, might control this spino-bulbo-spinal reflex and possibly determine, in respect to the safety of the individual, the beginning of the act of micturition (Figure 3). The last projection, thus, might serve as a “safe signal”, i.e. allows micturition only when the individual finds itself in a safe situation.