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

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Chapter 5

Location of External Anal Sphincter Motoneurons in the Sacral Cord of the Female Domestic Pig

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ABSTRACT

The location of the striated external anal sphincter motoneurons in the spinal cord was investigated in 12, between 3 and 4 months old, female domestic pigs using the retrograde tracer horseradish peroxidase (HRP). Their motoneuronal cell bodies were found in the spinal segments S1-S3, and were not located in the ventral horn, but dorsolateral to the central canal. This location within the spinal gray matter strongly differs from the location of the external anal sphincter motoneurons in rat, cat, dog, monkey and humans, but is similar to that in the Mongolian gerbil. The possible relevance of this “aberrant” location is discussed.

INTRODUCTION

Anal atresia or imperforate anus is a congenital disease in humans caused by agenesis of the dorsal part of the cloacal plate. In order to study how to reestablish normal continence for faeces in humans, the pig is often used as an animal model (VanderPutte, 1986; Lambrecht and Lierse, 1987), because anal atresia also exists in this species, and pigs with this disease can be obtained relatively easy. Moreover, the anal anatomy in the pig is similar to that in humans (VanderPutte, 1986). Usually, in anal atresia the striated external anal sphincter (EAS) is rudimentary and dysfunctional (Stephens and Smith, 1971). The surgical methods used at present to reestablish normal continence for faeces in anal atresia are not effective in the long term (Langemeijer and Molenaar, 1991), possibly because of neuronal dysfunction. However, neither in sick, nor in healthy humans and pigs it is known how the neuronal control of the EAS is organized. In order to elucidate this organization, in the present study the motoneurons of the EAS were localized in the spinal cord of the healthy pig.

The EAS forms the main closure muscle of the anus. Together with the levator ani, the coccygeus muscle and the external urethral sphincter (EUS), the EAS takes part in the pelvic floor, and all pelvic floor muscles are innervated by the pudendal nerve. In cat, dog, rhesus monkey, and probably also in humans, the motoneurons innervating the pelvic floor are located in a cell group (Onufrowicz, 1899; Sato et al., 1978; Kuzuhara et al., 1980; Roppolo et al., 1985), which was first described in 1899 by Onufrowicz, who called himself Onuf. This nucleus of Onuf extended from the caudal S1 to the rostral S3 segments of the human spinal cord. Later retrograde tracing studies in cat, dog and rhesus monkey using horseradish peroxidase (HRP) demonstrated that the EAS and EUS motoneurons are located, respectively, in the ventrolateral and the dorsomedial part of Onuf’s nucleus (Sato et al., 1978; Kuzuhara et al., 1980; Roppolo et al., 1985). However, some other animals show a different somatotopic organization. For example, in the rat the EAS motoneurons are located in a cell group at the medial border of the ventral horn, and the EUS motoneurons in another cell group.
at the lateral border (Schrøder, 1980). Finally, in the Mongolian gerbil the EAS motoneurons are not located in the ventral horn, but dorsolateral to the central canal (Ulibarri et al., 1995). On the other hand, the EUS in this animal is located laterally in the ventral horn in a position similar to Onuf’s nucleus in cat, dog, monkey and man.

MATERIALS AND METHODS
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 Maastricht. The animals were preanaesthetized with intravenous pentobarbital sodium 20 mg/kg diluted with 1:1 sodium hydrochloride and with Stressnil (Janssen Pharmaceutica, Belgium). The animals were ventilated with 1-2% Halothane in a mixture of 66% nitric oxide and 34% oxygen. Twelve adolescent female pigs were injected (weighing 30-50 kg; between 3 and 4 months old). In 7 animals (B1, B2, B6, B9, B11, B13, B14) a total of 20 µl of the retrograde tracer HRP (type VI Sigma; 20% solution dissolved in saline) was injected in the left EAS using a Hamilton syringe. In 5 other pigs (B20, B21, B27, B29 and B30) the EAS was injected bilaterally with a total of 40 µl HRP in order to label a maximum number of motoneurons. After a survival time of 48 hours the pigs were deeply anaesthetized and perfused with 8 liters of saline, followed by 4 liters of a fixative containing 2% glutaraldehyde (GA) and 1% paraformaldehyde (PF) in 0.1 M phosphate buffer, pH 7.4. The lumbosacral cord was removed and postfixed for 2 hours using the same fixation solution, and dehydrated overnight in a 25% sucrose solution. The segments of the lumbosacral cord were identified on the basis of their dorsal roots. In all cases the L5-S4 segments were cut transversally, with the exception of cases B29 and B30 which were cut longitudinally, in 60 µm thick sections on a cryostat and processed using the tetramethylbenzidine (TMB) impregnation method. For each case, except the cases with longitudinal sections, the total number of labeled neurons was counted in every other section. The labeled neurons were plotted. The diameter of 41 labeled neurons was determined in the S2 segment of cases B9, B14 and B27. The largest diameter of a cell body was measured, and only labeled cells with two or more labeled dendrites were considered labeled motoneurons.

RESULTS
In 7 animals (B1, B2, B6, B9, B11, B13, B14), which were injected unilaterally in the left EAS, many retrogradely labeled motoneurons were found ipsilaterally to the injected muscle. Sporadically, a contralat-
eral located cell was observed, probably due to spread of the tracer to the contralateral side.

The labeled motoneurons were not located ventrally in the ventral horn as in most other mammalian species, but dorsolateral to the central canal. In the cases with bilateral injections in the EAS (B20, B21, B27, B29 and B30) labeled motoneurons were found bilaterally in the same location as in the ipsilateral cases (Fig. 1). In none of the cases labeled cells were found in the ventral horn. Occasionally, retrogradely labeled axons could be followed from their exit from the ventral white matter through the ventral horn and intermediate zone to their soma of origin. Some retrogradely labeled dendrites of EAS motoneurons were found to cross the midline towards the contralaterally located motoneurons. No clear rostrocaudal periodicity was found in the labeled motoneuron pool.

The precise rostrocaudal distribution of the labeled motoneurons varied among the cases. Consistently, motoneurons were found in the caudal S1 to rostral S3 seg-

Fig. 2. Schematic drawings with small dots indicating the location of the retrogradely labeled motoneurons in cases B11, B13 and B14. Each drawing represents 12 alternate sections.
ments, but in three cases (B1, B11 and B20) labeled motoneurons were already found in the middle of S1. Caudally, in two other cases (B13 and B14) the motoneuronal cell group extended until the level of middle S3 instead of upper S3 (Fig. 2). Most of the cells were round to polygonal, and some cells had a fusiform shape. The mean diameter was 47 µm, varying between 31 and 65 µm. The number of EAS motoneurons counted in the ipsilaterally injected cases (B1, B2, B6, B9, B11, B13, B14) varied between 54 and 90. In the cases B20, B21 and B22, which were injected bilaterally in the EAS, the number of EAS motoneurons on the left varied between 42 and 78 and on the right between 43 and 80. In all cases the bulk of the labeled motoneurons was present in the second sacral segment.

**DISCUSSION**

The results demonstrate that the EAS motoneurons of the female domestic pig are located dorsolateral to the central canal in the sacral cord. In the cat, according to Rexed (Rexed, 1954), this area corresponds with the transition area of the medial part of lamina VII and the lateral part of lamina X. Lamina X contains many neurons of yet unknown nature, but motoneurons have never been described in this area. In the cat the medial part of the intermediate zone (lamina V to VII) is known to contain many interneurons, as well as a few preganglionic parasympathetic motoneurons forming the most medial extent of the dorsal band of the sacral parasympathetic nucleus of Nadelhaft et al. (1980).

Since somatic motoneurons in species as rat, cat, dog, monkey and man are exclusively located in the ventral horn, one might wonder why in the pig and the Mongolian gerbil (Ulibarri et al., 1995) the EAS motoneurons are located in such an “aberrant” location. First, EAS motoneurons cannot be considered as normal somatic motoneurons. Although they partly function in the framework of abdominal pressure control, they also play a role in the more “autonomic” function of defaecation. Although EAS motoneurons innervate a striated muscle, which is clearly under voluntary control, they also behave as autonomic motoneurons in respect to defaecation and receive, unlike somatic motoneuronal cell groups, direct hypothalamic afferents (Holstege and Tan, 1987). Moreover, they also behave as autonomic motoneurons in respect to the disease of amyotrophic lateral sclerosis (ALS), in which all somatic but no autonomic preganglionic motoneurons are affected (Mannen et al., 1977). It has, therefore, been proposed to consider these motoneurons to belong to a separate class of motoneurons between somatic and autonomic (Holstege and Tan, 1987). The fact that autonomic motoneurons are located much more dorsal in the spinal gray than somatic ones, might explain the dorsal location of the EAS motoneurons in the pig.

Second, in a recent study, in which pseudorabies virus was injected in the EUS of the rat [8], it was found that the strongest input to the EUS motoneurons originates from premotor interneurons dorsal and dorsolateral to the central canal, the same location as that of the EAS motoneurons in the pig. In the cat dendrites of Onuf’s nucleus motoneurons (EAS and EUS) also extend into this area (Sasaki, 1994).

These findings indicate that the area dorsolateral to the central canal, in which in the pig the EAS motoneurons are located, contains premotor interneurons for pelvic floor motoneurons in other species. Since in rat and cat this same area receives strong input from Barrington’s nucleus in the pons (Holstege et al., 1979; Loewy et al., 1979), one might speculate that in the pig Barrington’s nucleus might have direct access to the EAS motoneurons.