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Research Report

Is the periaqueductal gray an essential relay center for the micturition reflex pathway in the cat?

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ABSTRACT

The periaqueductal gray (PAG), especially in a region between the levels the oculomotor nucleus and the trochlear nucleus, was suggested to be the essential relay center that conveys information of bladder fullness to the pontine micturition center (Barrington's nucleus). The present study examined this hypothesis by transecting the brainstem in anesthetized cats. In eight cases of the midbrain transection, all ($n=3$) or most ($n=5$) of the PAG between the levels the oculomotor nucleus and the trochlear nucleus was separated from the intact side of the brain. Furthermore, in the former three cases, the PAG at the level caudal to the trochlear nucleus was separated from the intact brain by more than half ($n=2$) or completely ($n=1$). In all these cases, there were no remarkable differences in the amplitude of the micturition contraction (80–98% of that before transection), irrespective of the levels of the transection. In the cases of the pontine transection, micturition contraction disappeared after transecting through the caudal part of Barrington's nucleus ($n=1$) or through regions caudal to this nucleus ($n=5$). In the one case that received a transection through the rostral part of Barrington's nucleus, the amplitude of the micturition contraction was 43% of that before transection. This study demonstrates that Barrington's nucleus is essential, but the PAG is not essential, for evoking micturition. Our results suggest that the information of bladder fullness in the cat is conveyed to Barrington's nucleus either directly from the lumbosacral neurons or indirectly via relay neurons located below the midbrain.

1. Introduction

In 1921, Barrington showed that the micturition reflex is mediated by a spino-bulbo-spinal pathway that passes through the pontine micturition center, or Barrington's nucleus, in the rostral pontine tegmentum. Barrington's nucleus neurons project their axons directly to the sacral spinal cord (for review, see Holstege, 2005). Excitation of this nucleus causes micturition contraction and urethral relaxation (Griffiths et al., 1990).

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Abbreviations: 3N, oculomotor nucleus; 4N, trochlear nucleus; 5MET, mesencephalic tract of trigeminus; AQ, aqueduct; BC, brachium conjunctivum; CNF, cuneiform nucleus; IC, inferior colliculus; LL, lateral lemniscus; MRF, mesencephalic reticular formation; PAG, periaqueductal gray; Pyr, pyramidal tract; SC, superior colliculus; TR, trochlear decussation; V4, fourth ventricle

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Neuron-tracing studies have revealed a species difference at the ascending limb of the micturition reflex pathway. In rats, the lumbosacral neurons project densely to Barrington's nucleus (Hida and Shimizu, 1982; Ding et al., 1997). Ding et al. (1997) showed that the lumbosacral neurons also project to the midbrain periaqueductal gray (PAG), and the PAG neurons, in turn, project to Barrington's nucleus (also see Valentino et al., 1994). They concluded that there are two micturition pathways from the spinal ascending neurons to Barrington's nucleus: one is a direct pathway and the other is an indirect pathway via the midbrain PAG. However, in cats, Blok et al. (1995) presented ultrastructural evidence for very limited projections from the lumbosacral cord to Barrington's nucleus. Instead, they showed that lumbosacral neurons projected densely to the PAG (Blok et al., 1995; Vanderhorst et al., 1996; Mouton and Holstege, 2000; Floyd et al., 2000). The PAG neurons, particularly those in a specific region between the levels the trochlear nucleus and the center of the oculomotor nucleus, sent fibers to Barrington's nucleus (Blok and Holstege, 1994; Taniguchi et al., 2002; Kuipers et al., 2006). Thus, it was suggested that, in the cat, spinal afferents project to the PAG directly and Barrington's nucleus indirectly, and that the PAG, in turn, activates Barrington's nucleus in order to produce voiding (Blok et al., 1995). Physiological studies have shown that electrical stimulation of bladder afferents in the pelvic nerve evoked negative field potentials in the PAG in rats (Noto et al., 1991). Furthermore, electrical stimulation of various sites in the PAG evoked or facilitated micturition in the cat (Skultety, 1959; Koyama et al., 1962; Gjone, 1966; Taniguchi et al., 2002), in the dog (Fukuda and Koga, 1991), and in the rat (Kruse et al., 1990), but it also inhibited micturition contraction in the cat (Koyama et al., 1962; Gjone, 1966; Liu et al., 2004) and in the dog (Fukuda and Koga, 1991).

On the other hand, Barrington (1921) found that bladder contractions were not abolished when transection was made just in front of the superficial origins of the fifth nerves ventrally and through the posterior parts of the inferior colliculi dorsally. Although this transection appeared to have removed the majority of the midbrain PAG according to an atlas of the cat brain (Barman, 1968), it is unclear whether part of the PAG remained and whether the amplitude of micturition contraction was affected.

The present study aimed to clarify which part of the PAG is essential for the micturition reflex pathway in the cat. To this purpose, the brain was transected at several levels of the midbrain and the pons. We found, however, that transection of the midbrain PAG did not substantially influence the micturition contraction.

2. Results

Fig. 1 illustrates an example of the brain transection made to separate the midbrain PAG from Barrington's nucleus. Micturition contraction was well preserved after this midbrain transection. Transection was made from the dorsal side, toward a ventral and rostral direction (Fig. 1A). Barrington's nucleus remained in the caudal brain (i.e., the intact side of the brain) (Figs. 1B, C). The transection cut through the PAG at the level of the fourth ventricle (Fig. 1C). At the most caudal level where the aqueduct was seen (Fig. 1D) and at the level of the trochlear nucleus (Fig. 1E), the midbrain PAG was completely separated from the intact side of the brain. The transection was wide enough to reach the ventral part of the inferior colliculus or the lateral lemniscus. At a more rostral level, transection lesions were observed in the ventral part of the mesencephalic reticular formation (Fig. 1F) and reached as far as the pyramidal tract (not shown).

The amplitudes of micturition contraction decreased during the transection but increased after the transection was complete and reached a steady level 10 min later (Fig. 1G). In this example, the amplitude recovered to 95% of the initial measurement, and the frequency of micturition contractions was 78% of that observed before transection.

Fig. 2 illustrates an example of a brain lesion made in the rostral part of Barrington's nucleus. The amplitude of micturition contraction decreased after this lesion was made. In this preparation, the boundary between the midbrain and the pons was transected. In addition, a small part of the rostral and dorsomedial portion of the intact side of the brain was aspirated in order to create a lesion in the PAG at the level of the fourth ventricle. Histological examination of brain sections showed that the caudal half of Barrington's nucleus remained intact (Fig. 2A), but the rostral half was partially lesioned (Fig. 2B). Bleeding was also observed around Barrington's nucleus. The PAG at the level of the fourth ventricle was also partially (Fig. 2A) or completely (Fig. 2B) lesioned. Again, in this preparation, the midbrain PAG that surrounded the aqueduct was completely separated from the intact brain.

The amplitudes of micturition contraction decreased during transection (Fig. 2C). After showing large contractions near the end of the transection, the bladder tone remained quiet for a few minutes but was then followed by small contractions with high frequency oscillations (8–10 times/min) (from the point indicated by an arrow in Fig. 2C). The amplitudes of these high-frequency contractions increased gradually up to 30–50 mmH_2O. In addition, lower frequency contractions with somewhat larger amplitudes appeared 20 min after the transection. These were interposed between the smaller, high-frequency contractions (Fig. 2D): the bladder contractility was substantially the same before and after aspirating a rostral and dorsomedial portion of the intact side of the brain. The contraction amplitude of lower frequency contractions was 43% (average 147 mmH_2O) of that before transection (average 340 mmH_2O), and the frequency (0.69 times/min) was comparable to that before transection (0.71 times/min).

We examined in 12 animals whether transecting the brain at various levels of the midbrain (#1–9) or pons (#10–12) affected the micturition contraction (Table 1). In 3 animals (#4, #5, and #10), a second transection was made in the pons (#4[2], #5[2], and #10[2]).

In the midbrain transections, the separation was wide enough to reach the middle or lateral edge of the inferior colliculus and deep enough to transect the PAG as in Fig. 1. In 1 out of 9 cases (#1), the transection passed through the dorsal part of the PAG at the level of the trochlear nucleus; thus, the caudal part of the dorsal PAG remained with the intact brain for that animal. In the remaining 8 cases, the dorsal half of the