Brain Research Bulletin 78 (2009) 132–138

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Functional interrelations between nucleus raphé dorsalis and nucleus raphé medianus: A dual probe microdialysis study of glutamate-stimulated serotonin release

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ARTICLE INFO

Article history:
Received 19 April 2007
Received in revised form 24 July 2008
Accepted 18 September 2008
Available online 26 October 2008

Keywords:
Limbic midbrain
Serotonin
Glutamate
NMDA receptor
AP-5
In vivo microdialysis
5-HT
5-Hydroxytryptamine
Raphe nuclei

ABSTRACT

Dual-probe *in vivo* microdialysis was used to explore the relationships between the two midbrain raphé nuclei, raphé dorsalis (DRN) and raphé medianus (MRN). Infusion of the excitatory neurotransmitter glutamate (10 mM) into the dorsal raphé nucleus produced a large increase in the extracellular 5-HT (5-HT_{ext}) in the dorsal raphé (1400% of control values) that was limited to the time of infusion. This was followed by a significant decrease in extracellular 5-HT below baseline levels that continued for the duration of the experiment (3 h). Extracellular 5-HT (5-HT_{ext}) was also increased to 500% of control values in the median raphé nucleus following infusion of 10 mM glutamate (GLU) into the dorsal raphé nucleus. Infusion of the competitive NMDA receptor antagonist AP5 prior to and during infusion of GLU into the DRN resulted in a decrease in the response to GLU in the DRN and an antagonism of the increase of 5-HT_{ext} in the MRN. Infusion of 10 mM GLU into the lateral midbrain tegmentum, an area of the brain just lateral to the DRN, also increased 5-HT_{ext} in the probe in the lateral midbrain tegmentum (900% of control) but did not alter 5-HT_{ext} in the MRN. When glutamate was infused into the MRN, 5-HT_{ext} was also increased to 1400% of control in a time course similar to that seen with infusion of GLU into the DRN. Infusion of glutamate into the MRN, however, did not alter the 5-HT_{ext} in the DRN. These data suggest a serotonergic innervation of the median raphé nucleus by the dorsal raphé nucleus. A reciprocal innervation from the median raphé to the dorsal raphé is not mediated by glutamate, does not appear to be serotonergic, and does not regulate extracellular serotonin in the dorsal raphé.

1. Introduction

The serotonergic (5-HT) systems of the forebrain are implicated in a diverse number of homeostatic systems of the brain as well as in many neuropsychiatric disorders. Of particular note is the involvement of the serotonergic systems in depression [26], anxiety [25] and schizophrenia [1]. It is clear from a number of anatomical studies that the two midbrain raphé nuclei (dorsalis and medianus) provide the major serotonergic innervation of the forebrain (see Azmitia [4] Azmitia and Segal [5], Parent et al. [23], and Steinbusch and Nieuwenhuys [28] for reviews). Each of these nuclei has a distinctive pattern of forebrain innervation. Further, there are major morphological differences in the serotonergic fiber systems from each nucleus. Molliver [21] reviewed considerable evidence of morphological differences between dorsal raphé and median raphé neurons, strengthening the findings that these two nuclei show many anatomical, and probably, functional differences. The nucleus raphé dorsalis has thin fibers with a diffuse distribution, a high density of serotonin transporters (SERT) and a susceptibility to neurotoxic agents such as methamphetamine, whereas the raphé medianus shows thick beaded axons with a more precise topographic distribution, has a low density of SERT and low susceptibility to neurotoxic agents [9,21]. Furthermore, the dorsal raphé nucleus (DRN) and the median raphé nucleus (MRN) project to different areas of the forebrain [5,6]. The DRN projects to the dorsal striatum, ventral hippocampus, amygdala, nucleus accumbens and the cerebral cortex (Fig. 1). The MRN projects primarily to the septum and dorsal hippocampus. In addition, the DRN and the MRN also project to multiple areas of the lower brainstem (Fig. 1). In the cortex the two nuclei project to distinct areas; the projections of the MRN are distributed more uniformly throughout the cortex while the projections of the DRN are much greater to the frontal cortex [21]. Accordingly, these two midbrain serotonergic systems represent distinct functional pathways. To date, as reviewed below, several studies have used various tract-tracing

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doi:10.1016/j.brainresbull.2008.09.017

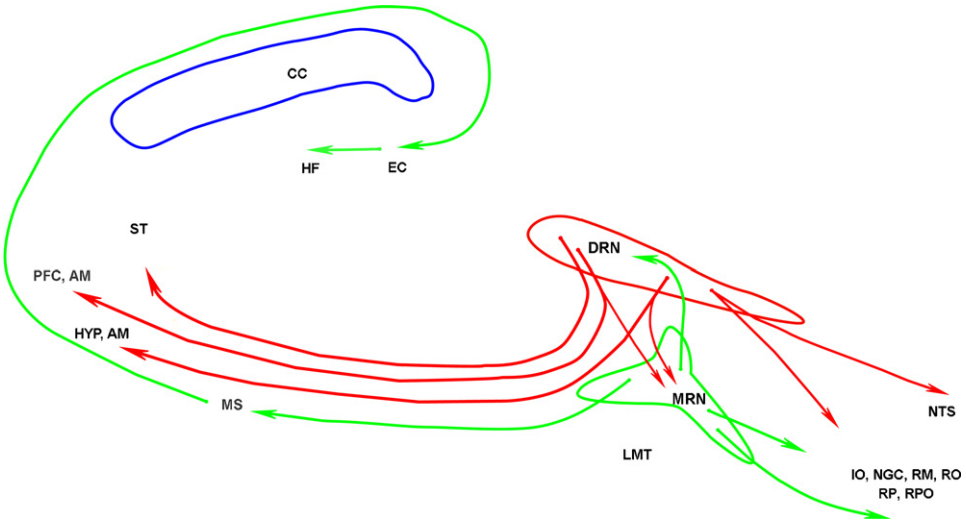

Fig. 1. Schema representing the differential projection systems of the dorsal (DRN) and median raphé nuclei (MRN). This diagram also shows the differential innervation of the forebrain by the rostral and caudal dorsal raphé nucleus (from [10]). Abbreviations (see [28]) - AM: amygdaloid complex, EC: entorhinal cortex, HF: hippocampal formation, HYP: hypothalamus, IO: inferior olive nucleus, LMT: lateral midbrain tegmentum, MS: medial septum, NGC: nucleus gigantocellularis, NTS: nucleus tractus solitarius, PFC: prefrontal cortex, RM: nucleus raphé magnus, RO: nucleus raphé obscurus, RP: nucleus raphé pallidus, RPO: nucleus raphé pontis, ST: striatum.

techniques and autoradiography to examine their anatomical relations.

One of the first studies of anatomical relations between raphé dorsalis and medianus was that of Jacobs et al. [11] demonstrating median raphé projections to the dorsal raphé nucleus. This study also indicated that these serotonergic inputs to the dorsal raphé terminated largely on raphé interneurons (probably GABAergic). These authors postulate that impulse flow in 5HT-containing neurons is subject to regulation via negative neuronal feedback as originally proposed by Aghajanian et al. [3]. Kalen et al. [14] reviewed previous studies showing fibers from median raphé to dorsal raphé. Vertes and Martin [36], using autoradiographic techniques, also demonstrated median raphé projections to the dorsal raphé nucleus. The existence of connections between the two-midbrain raphé raises another possibility, i.e., that manipulations, which increase 5-HT concentrations in the synapse, may depress discharge of raphé neurons directly via inhibitory 5-HT synapses on raphé neurons.

Strong projections have also been demonstrated from the DRN to the MRN. Marcinkiewicz et al. [18], using tract-tracing methods, examined inputs to median raphé and showed strong dorsal raphé inputs to the median raphé. Behzadi et al. [6] using retrograde tract-tracing techniques also demonstrated dorsal raphé inputs to median raphé. Vertes and Kocsis [34], using anterograde tracing methods, showed heavy dorsal raphé projections to median raphé. Thus, anatomical studies have demonstrated that the MRN and DRN are connected reciprocally.

Numerous studies have demonstrated a role of glutamate in the regulation of 5-HT release in the raphé nuclei (see Adell et al. [2] for review). Tao and Auerbach [31] have examined the GABAergic and glutamatergic influence on extracellular 5-HT (5-HT_{ext}) in the median and dorsal raphé nuclei using *in vivo* microdialysis. They showed that there were significant differences in the roles of GABA and glutamate in the regulation of 5-HT_{ext} in these two nuclei. Pallotta et al. have also shown that infusion of 100 μM NMDA into the DRN increases 5-HT_{ext} in the DRN [22]. However, neither group has examined the interactions between these major limbic midbrain nuclei.

To our knowledge, no primarily functional studies of relations between the midbrain raphé nuclei have been carried out. In particular, relations between these two raphé nuclei are needed to examine how their interactions alter serotonin release in key forebrain areas, including the medial prefrontal cortex, hippocampal formation, amygdaloid complex, nucleus accumbens, anterior cingulate cortex, and other limbic forebrain areas (Fig. 1). To date no adequate immunocytochemical studies have revealed the chemical makeup of the interconnecting fibers between the raphé dorsalis and medianus though we have previously proposed [19] they could be serotonergic and/or GABAergic. Many other possibilities exist given the numerous transmitter systems present in both raphé nuclei. From this summary it is clear that functional studies of cross talk between the midbrain raphé nuclei are in order. An understanding of how the midbrain raphé nuclei communicate may be useful in the design of new pharmacologic treatments for neuropsychiatric disorders such as depression, schizophrenia or sleep disorders.

2. Methods

This study was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals in Research, the Society for Neuroscience Handbook for the Use of Animals in Neuroscience Research, and under protocols approved by the Institutional Animal Care and Use Committee of the University of New England.

2.1. Stereotaxic surgery

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing between 300 and 325 g were used in these experiments. Animals were housed in a vivarium with a 12:12 light–dark cycle (lights on at 07:00). Animals had *ad libitum* access to food and water.

For implantation of guide cannulae, animals were anesthetized with 50 mg/kg pentobarbital and 160 mg/kg chloral hydrate i.p. Guide cannulae (CMA 12, CMA/Microdialysis AB, Acton, MA) were implanted above both the median raphé and dorsal raphé nuclei using coordinates from Paxinos and Watson [24]. Coordinates for the midpoint of the active membrane of the microdialysis probe in the MRN were AP + 0.70; L 0.0: DV + 2.0 (Figure 102, Paxinos and Watson [24]) determined from interaural zero; while the coordinates for the midpoint of the active membrane of the DRN probe were AP + 1.70; L 0.0; DV + 4.0 from interaural zero (Figure 94, Paxinos and Watson [24]). Guide cannulae were implanted at an angle of 20° from the vertical to avoid the venous sinus and cerebral aqueduct. Fig. 2 shows histology demonstrating the guide cannula and the probe tracts for the MRN. In a control study with infusion of GLU into the lateral midbrain tegmentum, an area of the midbrain just lateral to the DRN, the midpoint of the active membrane was AP + 1.70, L 2.0 and DV + 5.5 with respect to interaural zero.