Neuroscience Letters 612 (2016) 172–177

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Serotonergic projections from the raphe nuclei to the subthalamic nucleus; a retrograde- and anterograde neuronal tracing study

Martin Reznitsky[a,b], Per Plenge[c], Anders Hay-Schmidt[d,*]

[a] Department of Neuroscience and Pharmacology, Panum Institute 24.2, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark
[b] Department of Otorhinolaryngology, Head & Neck Surgery and Audiology, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, 2100 Copenhagen O, Denmark
[c] Department of Neuroscience and Pharmacology, The Panum Institute 18.5, Faculty of Health, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark
[d] Department of Neuroscience and Pharmacology, The Panum Institute 24.2.52, Faculty of Health, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark

HIGHLIGHTS

- 5-HT is important in movement control.
- Serotonergic projections to the STN from the DRN have not been thoroughly described.
- All STN afferents from the raphe system projects from the DRN, mainly DRD and DRL.
- Approximately 34% of the STN afferents from the DRN are serotonergic.

ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form
17 November 2015
Accepted 22 November 2015
Available online 9 December 2015

Keywords:
Dorsal raphe nucleus
STN
5-HT
Parkinson's disease
Immunohistochemistry
In situ hybridization

ABSTRACT

The objective of this study is to establish which subdivision of the dorsal raphe nucleus (DRN) supplies serotonergic projections to the subthalamic nucleus (STN) in the rat brain. Several studies in recent years have shown that serotonin (5-HT) might have a therapeutic role in the most prevalent basal ganglia (BG) movement disorder, Parkinson's disease (PD), but, because of the depletion of dopaminergic input to the BG, L-DOPA has been the main treatment for PD patients. Autoradiography showed that serotonin receptors 5-HT_{1B} and 5-HT_{2C} and the serotonin transporter were present in STN, whereas the 5-HT_{1A} and 5-HT_{2A} not were present. Retrograde tracer FluoroGold or Choleratoxin subunit B were iontophoretically delivered in the STN and combined with immunohistochemistry for 5-HT in order to map the topographic organization in the dorsal raphe system. The study showed that approximately 320+/−137 neurons were retrogradely traced from each STN to the DRN, located mainly in the dorsal- and ventrolateral DRN, and of these 108+/−42 or 34% co-localized 5-HT. Additionally anterograde tracer PHA-L was injected in the DRN to confirm projections to STN and accordingly only a sparse number of axon terminals were observed in the STN.

1. Introduction

Recent studies of the serotonergic system indicate that serotonin (5-HT) plays a more pronounced role in the pathophysiology, and that potential alleviation of L-DOPA-induced dyskinesia for patients with Parkinson's disease (PD) might be possible [1,2]. Clinically, the subthalamic nucleus (STN) is particularly interesting because motor control is compromised when dysfunctioning [3], and it is targeted in surgical treatment of PD with high-frequency deep brain stimulation (DBS) [4].

Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; BG, basal ganglia; CTB, choleratoxin subunit B; CLi, caudal linear nucleus of the raphe; DBS, deep brain stimulation; DRN, dorsal raphe nucleus; DRC, dorsal raphe nucleus, caudal part; DRD, dorsal raphe nucleus, dorsal part; DRL, dorsal raphe nucleus, lateral part; DRV, dorsal raphe nucleus, ventral part; FG, fluoroGold; GP, globuspallidus (GPe, externa, GPi, interna); IR, immunoreactivity; ISH, in situ hybridization; MnRN, median raphe nucleus; PBS, phosphate buffered saline; PD, Parkinson's disease; PHa-L, phaseolus vulgaris-leucoagglutinin; PMnRN, paramedian raphe nucleus; SN, substantia nigra (SNr, pars reticulata, SNc, pars compacta); STN, subthalamic nucleus; ZI, zonaincerta.

* Corresponding author.
E-mail addresses: martin.reznitsky@gmail.com (M. Reznitsky), pplenge@sund.ku.dk (P. Plenge), ahay@sund.ku.dk (A. Hay-Schmidt).

http://dx.doi.org/10.1016/j.neulet.2015.11.035
0304-3940/

1.1. Anatomy of STN projections

In the rat, the STN is an ovoid nucleus in the midbrain bordered by: the zona incerta (ZI) dorsally; the cerebral peduncle ventrally and laterally; by the lateral hypothalamus medially; by the globus pallidus interna (GPi) rostrally and by substantia nigra pars reticulata (SNr) caudally. It differs from the primate STN primarily in relative size. Afferent projections to the STN originate from the dorsal pallidum (globus pallidus externa (GPe)), ventral pallidum, frontal cortex, central gray and dorsal raphe nucleus (DRN) [5,6]. Fine varicose serotonergic fibers within the STN occur throughout the entire nucleus [7]. Some studies report a topographic organization of serotonergic fibers, stating that the concentration is high in the caudal STN compared with the rostral [8], others that the rat STN has a diffuse uniform distribution of serotonergic fibers [7,9], but, the origin of these serotonergic fibers has not been determined. Based on electrophysiological studies the overall serotonergic input probably is very low [10]. The main efferent projections from STN terminate in the output nuclei GPi and SNr [3].

1.2. Physiological effects on the STN

Putative functional 5-HT receptor subtypes in STN include; 5-HT_{1A} [9,11], 5-$HT_{1B/1D}$ [12,13] and 5-HT_{2C} [14]. For review on central 5-HT receptors and their localization see Barnes and Sharp [15]. The 5-HT receptors are mainly situated postsynaptic, and more than one type of 5-HT receptor subtype can be found on the postsynaptic membrane of a particular STN neuron [9,16]. Alteration in the firing pattern of the serotonergic neurons in DRN affects the activity of the STN [16] and 5-HT inhibits glutamate and GABA release in the STN [17]. Administration of 5-HT_{1A} agonist [16] or local 5-HT_{2C} antagonist [11,18] has been suggested to decrease firing in STN and alleviate dyskinesia. Aggression, depression and tendencies towards suicidal thoughts are in many cases seen with low or decreased levels of 5-HT, and represent cognitive symptoms that unfortunately are accompanied with DBS, demonstrating that DBS of the STN potentially inhibits 5-HT input from the DRN in the midbrain [19].

The aim of this neuroanatomical tracing study is to determine the extent and localization of serotonergic projections to the STN.

2. Materials and methods

The animal care and all experimental procedures performed in this study were approved in accordance with the European Community Counciĺs Directive of Nov 24th 1986 (86/609/EEC) by The Danish National Committee for Ethics in Animal Research under the Danish Ministry of Justice (License number 2009/561-1629).

2.1. Animals, injection and fixation

Wistar male rats weighing 250–300 g were kept under standard laboratory conditions with free access to food and water; and a 12 h light and 12 h dark photoperiod. Rats were anesthetized with 1 ml hypnorm/dormicum s.c. and the skull was exposed with a cranio-caudal incision of approximately 1.5 cm. The head was fixated with ear bars and incisor bar in a Kopf stereotaxic instrument and Bregma was localized. An anterograde tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L) (Vector Labs), or retrograde *Choleratoxin* subunit B (CTB) (List Biological Laboratories, CA, USA) or *Fluoro-Gold* (FG, aminostilbamine) (Fluorochrome, LLC, CO, USA) were iontophoretic injected in either DRN or STN using Bregma levels according to the rat brain atlas by Paxinos and Watson [20]. 7 out of 43 rats were suitable for obtaining tracing data and included in the final analysis. In addition 2 rats were used for SERT-immunoreactivity (IR), 6 for in situ hybridization and 6 for autoradiography. After a postoperative period of approximately 14 days the rat was anesthetized and perfused transcardially with phosphate buffered saline (PBS) for 3 min followed by a perfusion with 4% formaldehyde in PBS for 5 min. The rat was decapitated hereafter and the brain was removed. The brain was placed in a 4% formaldehyde phosphate buffer for 24 h followed by 5 days in 30% sucrose in PBS with Na-azide for cryoprotection at +4 °C. Hereafter the brain was frozen on a cryostat and cut into 40 μm sections. Each brain was divided into 6 series containing representative 40 μm sections throughout the brain and stored in PBS containing 0.1% Na-azide at +4 °C. This division of the brain sections has as consequence that each section is separated by 200 μm from the next.

2.2. Immunohistochemistry

Immunolabeling procedures follows those in [21]. In brief: For the tracing experiments the following primary antibodies were used; polyclonal goat anti CTB antibody (cat # 104; List Biological Laboratories, CA, USA) in a concentration of 1:1.000–1:1:6.000; a polyclonal rabbit anti-FG antibody (cat # AB153; Chemicon, CA, USA) in a concentration of 1:6.000-1:10.000; or a polyclonal goat anti-PHA-L antibody (Cat# AS 2224; Vector Labs, CA, USA) in a concentration of 1:3.000. Sections which were to be double-labeled were in case of CTB incubated with polyclonal rabbit anti serotonin antibodies (cat # S-5545; Sigma–Aldrich, LO, USA) in a concentration of 1:2.000–1:8.000; or for FG with polyclonal goat anti-serotonin antibodies (cat # 20079; DiaSorin, MN, USA (now ImmunoStar)), made in a concentration of 1:4.000.

The secondary antibodies were a biotinylated polyclonal donkey anti-goat $(Fab)_2$ antibody (cat # 705-066-147; Jackson Immuno Research Laboratories, PA, USA) or a biotinylated donkey anti-rabbit polyclonal $(Fab)_2$ antibody (cat # 711-066-152; Jackson Immuno Research Laboratories, PA, USA) in a concentration of 1:2.000. The sections were rinsed in 3 × 10 min in PBS-T followed by incubation one hour with ABC complex (VECTASTAIN® Elite ABC Kit (standard) PK-6100; Vector Labs, CA, USA) in a concentration of 1:500. After this the sections were reacted with 3,3′-Diamino-Benzidine (DAB) (Sigma D-5637; Sigma–Aldrich, Denmark) as a chromogen for single labeling sections or the chromogen SG (VECTOR® SG Substrate Kit SK-4700; Vector Labs, CA, USA) for double labeling.

For the double labeling the sections were proceeded to 1% H_2O_2 in PBS-T for quenching residual peroxidase activity from the ABComplex, and rinsed afterwards with PBS-T 3 × 10 min. The sections were incubated for 1 h with a second secondary antibody either a biotinylated donkey anti-rabbit $(Fab)_2$ antibody (cat # 711-066-152; Jackson Immuno Research Laboratories, PA, USA) or a biotinylated donkey anti-goat $(Fab)_2$ antibody (cat # 705-066-147; Jackson Immuno Research Laboratories, PA, USA) in a concentration of 1:2.000 in PBS-T with 1% human serum albumin. The sections were rinsed in PBS-T for 3 × 10 min and incubated one hour in ABC complex in a concentration of 1:500, followed by rinsing 3 × 10 min with PBS-T and then reacted with DAB. The sections were mounted and imbedded in DEPEX. As shown in Hay-Schmidt et al. [21], unspecific cross binding does not take place using this procedure.

For the serotonin transporter a monoclonal anti-SERT antibody (Cat. # MAB1564, Chemicon CA, USA) diluted 1:4.000, was used as primary antibody. Secondary antibody was biotinylated donkey anti-mouse $(Fab)_2$ antibody (cat # 715-066-150; Jackson ImmunoResearch Laboratories, PA, USA).

2.3. In situ hybridization

Free floating in situ hybridization (ISH) was done accordingly to [22].