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NADPH–Diaphorase–Reactive Neurons in Rat Basal Ganglia

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Abstract: NADPH–d reaction was performed on nine male Sprague–Dawley rats and morphometry of NADPH–d(+) cells was investigated in caudate–putamen, globus pallidus and ventral pallidum. In caudate–putamen we observed intensely stained NADPH–d (+) cells. Maximum diameter and minimum diameters of the NADPH–d (+) cells in caudate–putamen was measured as 19.19±0.04 µm and 10.42±0.12 µm, consequently. In globus pallidus, moderately stained NADPH–d (+) cells were observed. We measured the maximum and minimum diameters of the NADPH–d (+) cells as 19.93±0.24 µm and 10.42±0.12 µm, consequently. In ventral pallidum, medium–sized, moderately stained cells were present. Maximum and minimum diameters of the NADPH–d (+) cells in ventral pallidum was 19.19±0.04 µm and 12.40±0.2 µm, consequently. NADPH–d (+) cells in caudate–putamen, globus pallidus and ventral pallidum were mostly multipolar in shape. Basal ganglia have recently been suggested to have a role in nociceptive processing. Based on the role of nitric oxide in nociception and NADPH–d which is a form of nitric oxide synthase, the positive reaction in these nuclei is proposed to have a role in nociception in basal ganglia.

Key Words: caudate–putamen, globus pallidus, pallidum, NADPH–diaphorase.

Introduction

The basal ganglia are associated with motor functions of the brain, although it is becoming clear that they may subserve many non–motor functions as well. Recent neurophysiological, clinical and behavioral experiments indicate that nucleus accumbens (Acb) also processes non–noxious somatosensory information. Basal ganglia are rich in many different neuroactive chemicals that may be involved in the modulation of pain processing also (1).

Caudate–putamen and pallidum are structures of basal ganglia localized deep in the cerebral hemispheres. Nitric oxide (NO) is a small gaseous molecule easily able to pass through neuronal membranes with a very short half–life. It is suggested to act as a retrograde transmitter and also as a classical neurotransmitter. It can also act in the neuron where it is produced. NO has been found to have a role in nociceptive transmission (2). It has later been shown that NO plays an important role in mediation of nociceptive processes such as thermal hyperalgesia (3). Aanosen (4, 5), Raigordsky and Urca, Dickenson and Aydar, Woolf and Thompson, Kitto et al. and Meller et al. also reported that NMDA receptor is responsible in nociceptive transmission (2, 4–10). NADPH–d activity has been shown in various regions of the central nervous system (11, 12, 13, 15). Although the function of NO in human brain has not yet clearly been defined, it is accepted to play a role in synaptic transmission in both central and periferic nervous systems (2, 12, 14).

The enzyme that produces NO (nitric oxide synthase, NOS) has been first described by Bredt et al. (16). NOS requires NADPH as a cofactor, so it has been suggested and shown by Hope et al. that neuronal NADPH–diaphorase is a form of NOS (17). Bredt et al. later showed that the localization of nitric oxide synthase is absolutely coincident with NADPH–d staining in the rat and primate brain (18).

In this study, we aimed to present the distribution and morphometry of NADPH–d (+) neurons to reveal the NO

activity in rat basal ganglia, suggesting a possible role of basal ganglia in nociception via the neurotransmitter NO.

Materials and methods

The experiments were performed on nine male Sprague–Dawley rats weighing about 250–300 g. The NADPH–d histochemical reaction was assayed on the forebrain, and spinal cord sections taken from six experimental cases. The staining procedure was modified from the method of Vincent and Kimura (12). Rats were deeply anaesthetised with Nembutal (60 mg/kg, i.p.), intracardially perfused with 10 ml of heparinized saline (25.000 U/l) followed with 500 ml fixative (4–5% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3). After perfusion, the brains were transferred to PB and one day later were blocked and sectioned with vibratome at 50 µm. Coronal sections were collected in PB, rinsed 1–2 hs, and incubated in 0.1 MPB (pH 7.3) containing 0.3% Triton X–100, 0.5 mg/ml nitroblue tetrazolium (Sigma, USA) and 1.0 mg/ml NADPH tetrasodium salt (Sigma, USA) at 37 for 30–60 min. Following the incubation period, sections were rinsed in PB, mounted on gelatine coated slides and left to dry at room temperature. Mounted sections were then immersed in 100% alcohol, cleared in xylene and coverslipped directly with Entellan.

Results

Evaluation of the distribution of NADPH–d (+) neurons and quantitative analysis were performed only in the cases where histochemical analysis was adequate to reveal the largest number of NADPH–d (+) neurons in detail with reduced background precipitation. Our results for the distribution of NADPH (+) cells are in accordance with the studies of Vincent and Kimura, Nisbet and Rodrigo (12, 20, 21).

A large number of medium sized multipolar NADPH–d (+) neurons were scattered through the whole extent of caudate–putamen beginning from Bregma +2.2 mm. to –3.8 mm (Figure 1 A, B). These cells were intensely stained and they gave rise to a dense fiber network within the striatal neuropil (Figure 2A, C). The fiber density was very high throughout caudate–putamen. 250 NADPH–d (+) cells were observed in each 100 µm–thick section. 54% of these cells were multipolar, 16.5% were pyramidal, 16.5% fusiform, 7% flattened and 6% were spheroid in shape. Maximum diameter (D max) of the NADPH–d (+) cells, in caudate–putamen was 19.19±0.04 µm, ranging from 8.34 to 36.11 µm. Minimum diameter (Dmin) was 10.42±0.12 µm, ranging from 4.16 to 16.16 µm.

In globus pallidus, medium–sized, moderately stained, multipolar cells NADPH–d (+) cells were present beginning from Br –0.3 mm. to Br –2.8 mm (Figure 1B). Maximum diameter (D max) of the NADPH–d (+) cells in globus pallidus was 19.93±0.24 µm, ranging from 13.89 to 38.89 µm. Minimum diameter (Dmin) was 10.42±0.12 µm, ranging from 8.33 to 15.37 µm. 50.4% of these cells were multipolar, 6.4% were pyramidal, 23.2% fusiform, 4.8% flattened and 15.2% were spheroid in shape.

In ventral pallidum, medium–sized, moderately stained, multipolar cells were present beginning from Br + 0.7 mm. to Br –1.3 mm (Figure 1 A, B). Maximum diameter (D max) of the NADPH–d (+) cells in ventral pallidum was 19.19±0.04 µm, ranging from 12.5 to 27.78 µm. Minimum diameter (Dmin) was 12.40±0.2 µm, ranging from 4.16 to 15.37 µm. 55.33% of these cells were multipolar, 7.36% were pyramidal, 20.96% fusiform, 6.87% flattened and 9.28% were spheroid in shape (Figure 2 B, D)

Discussion

NADPH–diaphorase activity has been shown in different regions of the central nervous system (12, 14). The reaction was strong in some regions and weaker in some places. Neurons in the striatum, laterodorsal and pedunculopontine tegmental nuclei were intensely stained, while neurons in the dorsal periaqueductal gray matter were moderately stained (12). Both NADPH–d and NOS were found in the similar regions of the spinal cord (10, 12, 14, 22). NADPH–d activity in caudate–putamen and pallidum in human and cat was observed similar to the activity in rat brain also (23).

In this study, we observed intensely stained medium sized NADPH–d (+) neurons in caudate–putamen and moderately stained, medium sized neurons in ventral pallidum and globus pallidus. We found the pattern of distribution and staining properties of NADPH–d (+) cells in these regions was in accordance with the findings of Vincent and Kimura and Valtschanoff et al. (12, 13, 14). We have not observed any literature regarding morphometric data of the NADPH–d (+) cells in caudate–putamen, globus pallidus and ventral pallidum, making it impossible for us to compare the results of our measurements.

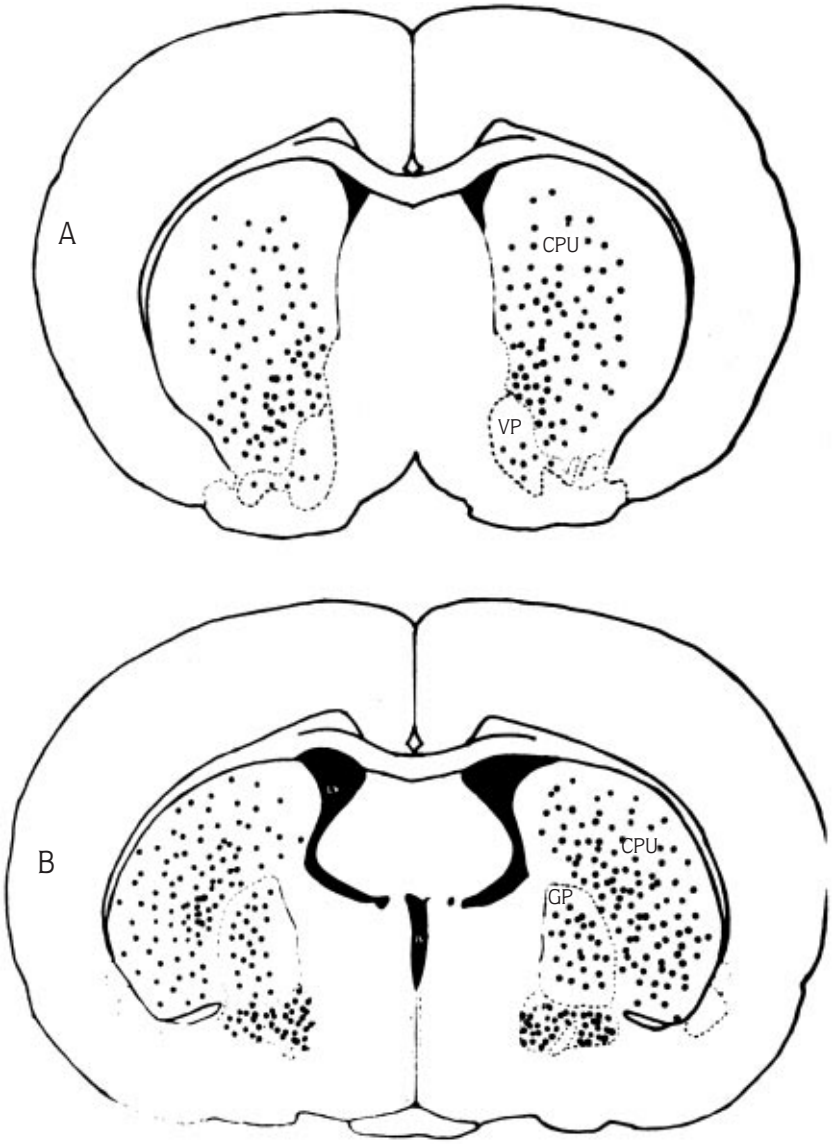

Figure 1. Distribution of NADPH–d (+) cells in a 50–µm–thick section. A. caudate–putamen and ventral pallidum (Br+0.2); B. cauadate–putamen, globus pallidus and ventral pallidum (Br–0.8).

Basal ganglia are rich in many different neuroactive chemicals that may be involved in the modulation of nociceptive transmission. Neuroanatomical experiments suggest several pathways by which nociceptive information may reach the basal ganglia. Also, some patients with basal ganglia disease (Parkinson's disease, Huntington's disease) have alterations in pain sensation in addition to motor abnormalities (1). These lead us to the idea that basal ganglia are now responsible in some levels of pain transmission and modulation.

NO has been found to have a role in nociceptive transmission first in 1992 by Meller et al. (2). It is known that NOS requires NADPH as a cofactor, so it has been suggested that NADPH–diaphorase is a form of NOS. NADPH–diaphorase activity has been shown in different regions of the central nervous system and it has been suggested that this activity reflects the activity of NOS.

Distribution and morphological characteristics of NADPH–d (+) cells in caudate–putamen, globus pallidus and ventral pallidum here are in accordance with the possible role of these cells in nonociceptive processing. Still revealing the exact function of NADPH–d (+) cells in rat basal ganglia and their acting mechanism requires further investigations.

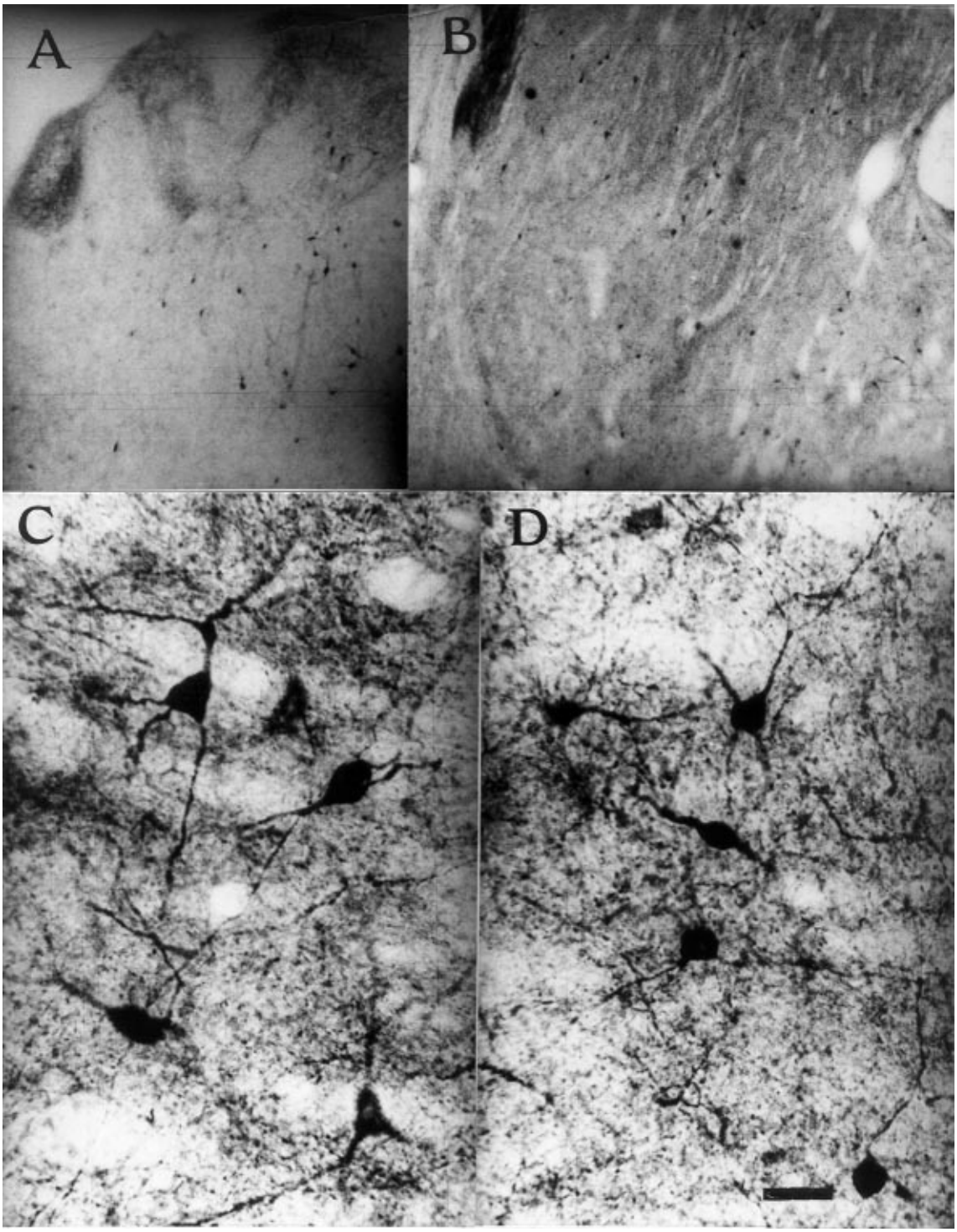

Figure 2. Photomicrographs of NADPH–d (+) neurons in A, C: caudate–putamen (Br –0.8) B, D: ventral pallidum (Br –0.2). Scale bars are 140 µm for A, B and 15 µm for C, D.

References

1. Chudler EH, Dong WK. The role of basal ganglia in nociception and pain. Pain 60: 3–38, 1995.
2. Meller ST, Pechman PJ, Gebhart GF, Maves TJ. Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. Neuroscience 50: 7–10, 1992.
3. Malmberg AB, Yaksh TL. Spinal nitric oxide synthase inhibition blocks NMDA– induced hyperalgesia and produced antinociception in the formalin test in the rats. Pain 54: 291–300, 1993.
4. Aononsen, LM, Wilcox GL. Nociceptive action of excitatory amino acids in the mouse: effects of spinally administered opioids, phencyclidine and sigma agonists. J Pharmacol Exp Ther 243: 9–19, 1987.
5. Aanonsen LM, Lei S, Wilcox GL. Excitatory amino acid receptors and nociceptive neurotransmission in the rat spinal cord. Pain 41: 309–321, 1990.
6. Raigordsky G, Urca G. Intrathecal N–methyl, D–aspartate (NMDA) activates both nociceptive and antinociceptive systems. Brain Res 422: 158–162, 1987.
7. Dickenson AH, Aydar E. Antagonism at the glycine sites on the NMDA receptor reduces spinal nociception in the rat. Neurosci Lett 121: 263–266, 1991.
8. Woolf CF, Thompson SWN. The induction and maintenance of central sensitization is dependent on N–methyl–D–aspartic acid and receptor activation: implications for the treatment of post–injury pain hypersensitivity states. Pain 44: 293–300, 1991.
9. Kitto KF, Haley JE, Wilcox GL. Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neurosci Lett 148: 1–5, 1992.
10. Meller ST, Gebhart GF. Nitric oxide and nociceptive processing in the spinal cord. Pain 52: 127–136, 1993.
11. Dawson T, Bredt D, Fotuhi M, Hwang PH. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc Natn Acad Sci 88: 7797–7801, 1991.
12. Vincent SR, Kimura H. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience 46: 755–784, 1992.
13. Valtschanoff JG, Weinberg RJ, Rustioni A. NADPH–diaphorase in the spinal cord of rats. J Comp Neurol 321: 209–222, 1992a.
14. Valtschanoff JG, Weinberg RJ, Rustioni A. Nitric oxide synthase and GABA colocalize in Lamina II of rat spinal cord. Neurosci Lett 148: 6–10, 1992b.
15. Schottler F, Collins JL, Fergus A, Okonkwo D, Kassell NF, Lee KS Structural interactions between NOS–positive neurons and blood vessels in the hippocampus. Neuroreport 7: 966–968, 1996.
16. Bredt DS, Hwang PM and Snyder SH. Localization of nitric oxide synthase indicating a neuronal role for nitric oxide. Nature 347: 768–770, 1990.
17. Hope BT, Michael GJ, Knigge KM and Vincent SR. Neuronal NADPH diaphorase is a nitric oxide synthase. Proc. Natn. Acad. Sci. 88: 2811–2814, 1991.
18. Bredt DS, Glatt CE, Hwang PM, Fotuhi M, Dawson TM Synder SH. Nitric oxide synthase protein and mRNA are discretely colocalized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron 7: 615–624, 1991.
19. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York, 1986.
20. Nisbet AP, Foster DJ, Kingsbury A, Lees AJ, Marsden CD. Nitric oxide synthase mRNA expression in human subthalamic nucleus, striatum and globus pallidus: a implications for basal ganglia function. Brain Res Mol Brain Res 22: 329–332, 1994.
21. Rodrigo J, Springall DR, Uttenthal O, Bentura ML, Abadia MF, Riveros MV, Martinez MR, Polak JM, Moncada S. Localization of nitric oxide synthase in the adult rat brain. Philos Trans R Soc Lond B Biol Sci 345: 175–221, 1994.
22. Dun NJ, Forstermann U, Tseng LF. Nitric oxide synthase immunoreactivity in the rat spinal cord. Neurosci Lett 147: 217–220, 1992.
23. Mizukawa K, Vincent SR, McGeer PL, McGeer EG Distribution of reduced–nicotinamide–adenine–dinucl eotide–phosphate diaphorase–positive cells and fibers in the cat central nervous system. J Comp Neurol 279: 281–311, 1989.