

Reciprocal connections between the periaqueductal gray matter and other somatosensory regions of the cat midbrain: a possible mechanism of pain inhibition

Mikael Wiberg

Department of Human Anatomy, University of Uppsala and Department of Plastic Surgery, Uppsala University Hospital, Uppsala, Sweden

ABSTRACT

Lectin-conjugated horseradish peroxidase was injected or implanted in crystalline form into various parts of the periaqueductal gray matter (PAG) in the cat. After varying survival periods, the animals were fixed and the mesencephalon was sectioned and incubated for HRP histochemistry.

Outside PAG, labelled cells and terminal labelling were observed in the cuneiform, parabrachial and intercollicular nuclei, in the deep and intermediate gray layers of the superior colliculus, in the anterior and posterior pretectal nuclei and in the nucleus of Darkschewitsch.

This study has shown that the region of PAG that is known to receive heavy ascending somatosensory input from the spinal cord and to be part of descending pain-inhibiting systems, also has reciprocal connections with other somatosensory areas of the midbrain.

The results are discussed in relation to nociception and nociceptive inhibiting mechanisms.

INTRODUCTION

It is well known that the periaqueductal gray matter (PAG) receives a heavy projection from the spinal cord in several species including man (see e.g. 39), and recent studies have revealed somatotopy with the lumbar spinal cord projecting to the caudal region of the PAG and with the cervical spinal cord projecting to the middle region of the PAG (8, 36, 39). The origin of these spinal projections mainly

consists of lamina I neurones of the superficial dorsal horn (36, 39), which are known to be excited chiefly by noxious stimuli (see e.g. 41). Of special interest in this context is the finding that the same regions of PAG that receive this ascending spinal input are also able to produce suppression of pain; both opiate injections and electrical stimulation of this area inhibit the response of the superficial dorsal horn to noxious stimuli (see e.g. 11, 17).

As it has been demonstrated that other areas of the midbrain, especially the intercollicular region, receive pronounced input from the dorsal column and lateral cervical nuclei (7, 9, 18, 37), the present study was undertaken to determine whether there are any connections between these latter sites of projection and the site of the spinal projection to PAG, thus providing a possible means of activating descending pain-inhibiting systems.

MATERIAL AND METHODS

The study was carried out on 10 adult cats. In 3 cats 20-40 nl of 2% wheat-germ agglutinin conjugated horseradish peroxidase (WGA-HRP) was injected into the lateral part of PAG, and in the other 7 the WGA-HRP was implanted in crystalline form into the same part of PAG (see e.g. 14)). The WGA-HRP was aimed stereotactically, in 8 cats at the anterior level 0-0.5 and in the other 2 at the anterior level 1.5-2.0. After a survival period of 24-48 hours the animals were fixed transcardially with an aldehyde mixture and after removal the brains were stored overnight in cold (4^o C) 10% sucrose buffer. Serial frozen sections, 40 µm, were cut and 2 out of every 5 were processed for the TMB peroxidase reaction (28). One set of sections was stained with neutral red or cresyl violet and the other set was left unstained; in the latter the peroxidase activity was examined in a light microscope.

RESULTS

The injections/implantations were made within the boundaries of PAG, and occupied its ventral and lateral regions (Fig. 1A), but they were centered at different

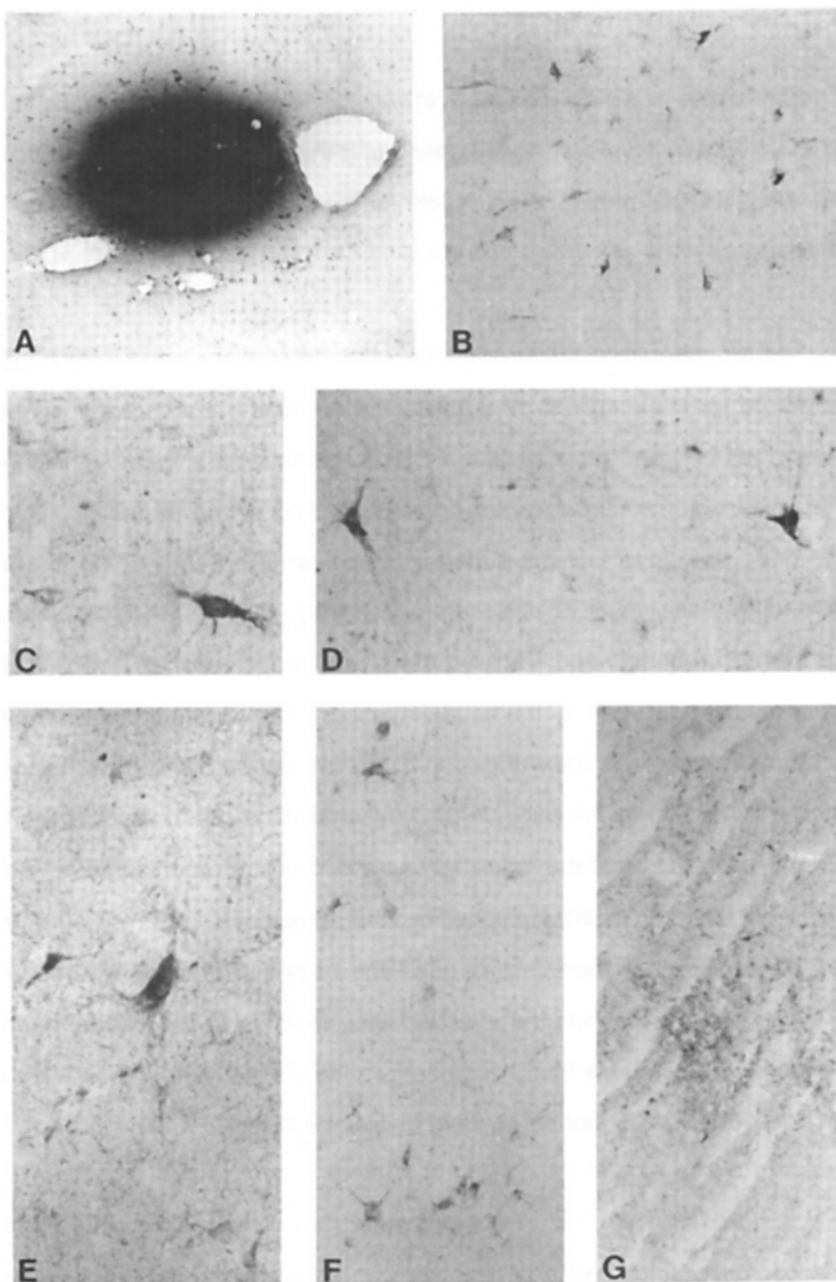


Fig. 1. A. The reaction product after implantation of crystalline WGA-HRP into the lateral PAG. x 120

Labelled cells and terminals in:

B. the parabrachial nucleus (PBN). BC = brachium conjunctivum. x 200

C. the intercollicular nucleus (Inc). x 330

D. the deep gray layer of the superior colliculus (SGP). x 380

E. the intermediate gray layer of the superior colliculus (SGI). x 350

F. the posterior pretectal nucleus (PTP). x 240

Anterograde terminal labelling:

G. in the anterior pretectal nucleus (PTA). x 180

rostrocaudal levels of PAG (see above). In those cases where WGA-HRP was injected there was a minor spread of tracer along the needle-track into the dorsal part of PAG, while in the implantation technique the tracer was confined to the implantation site.

Labelling was observed in several of the somatosensory areas of the midbrain. Labelled fibres were traced from the injection site either (1) ventrolaterally between the cuneiform (CNF) and parabrachial (PBN) nuclei into the mesencephalic reticular formation, where some fibres turned dorsally, ending in the intercollicular nucleus (Inc) and deep grey layers of the superior colliculus (SGI,SGP), or (2) laterally and dorsally, ending directly in SGP and SGI, respectively.

Both terminal labelling and retrogradely labelled neurones were observed ipsilaterally in CNF and PBN in the caudal midbrain (Fig. 1B), in Inc, SGI and SGP in the caudal and middle midbrain (Figs 1C-E), in the anterior and posterior pretectal nuclei (PTA, PTP) and in the nucleus of Darkschewitsch (D) in the rostral midbrain (Fig. 1F-G). In all structures the labelling was evenly distributed without any clearcut pattern and only scattered terminal labelling and peroxidase- positive neurones were observed contralateral to the injection site. The heaviest labelling was seen in the intercollicular region and when the injection was confined to the caudal half of the ventrolateral PAG. Injections into the dorsal or rostral PAG yielded only very sparse labelling. The possible reasons for this are discussed below.

DISCUSSION

Technical considerations: WGA-HRP was used in this study for two reasons: (1) It has a higher sensitivity for both anterograde and retrograde neuronal tracing compared with native HRP, which means that very small amounts can be used (22) and (2) it is not taken up by undamaged fibres (10). The tracer was introduced into PAG either by injection or by implantation, and the latter gave a more restricted injection site. It is important to bear in mind that the area injected - as defined by the histochemical method - is not necessarily the area from which effective uptake of

tracer has taken place (see e.g. 39), and it need not be the most sensitive method that best defines this latter area. In the present study a sensitive method, with TMB, was used to identify the effective area, since it has been demonstrated that the uptake zone is more widespread with retrograde than with anterograde tracing (1, 39).

Another methodological problem to be discussed is the possibility of misinterpreting the results on account of transneuronal transport (see e.g. 31). In the present study such transport seems unlikely, since (1) the injected amounts were small, (2) the survival periods were kept short and (3) labelled fibres were traced from the injection site to the terminal areas.

Anatomical projections: In this study PAG was found to have reciprocal connections with CNF, PBN, Inc, SGI, SGP, PTA, PTP and D. The heaviest labelling was observed in the intercollicular nucleus.

In two earlier studies in the cat (20, 21) with use of a degeneration technique, efferent projections were observed from PAG to CNF, SGI, SGP and the pretectum, projections that have since been shown to exist in the rat and monkey (6, 19, 26). A projection from PAG to the pretectum was not found by Berman (1977) in her autoradiographic study in the cat (5), while in the present study terminal labelling and retrogradely labelled cells were observed in both PTA, PTA and D, and at least in the rat (15, 19) a projection from PAG to PTA and D has been found. In earlier reports on studies in the cat nothing has been said about reciprocal connections between PAG and other midbrain structures, except for CNF; Edwards (1975) found in an autoradiographic study that efferent fibres projected from CNF to PAG (16), and this observation together with the findings of Hamilton and Skultety (20) indicates a reciprocal connection between these two structures.

Reciprocal connections have also been demonstrated indirectly in some other studies in the rat and monkey, by showing either afferent or efferent connections between PAG on the one hand and SGI, SGP, CNF and PBN on the other (24, 25, 26, 27), but there is only one report of a finding of a reciprocal connection between PAG

and some other somatosensory part of the midbrain in the same study, namely the connection between PAG and CNF demonstrated in the rat by Bernard et al (6). Connections such as those demonstrated in the present study are of special interest, since they might imply that PAG has a more integrative function in relation to other somatosensory structures of the midbrain. Also of interest in the present study was the finding that it was virtually only the caudal half of the ventrolateral region of the PAG that projected reciprocally to the rest of the somatosensory midbrain. This is contradictory to the report by Hamilton and Skultety (20), who found that more rostral regions of PAG also had efferent projections to other midbrain structures, but these authors used the degeneration technique, in which there is a risk of damaging fibres of passage through PAG. It seems reasonable to assume that the caudal half of the ventrolateral region of the PAG is the part that has reciprocal connections with the rest of the somatosensory midbrain, since it is precisely that part of PAG that has an ascending somatosensory input, originating from the spinal cord (36, 39).

Functional considerations: Heavy projection to the caudal half of the ventrolateral region of PAG has been demonstrated in several studies in several species (39). This projection originates mainly in lamina I neurones of the superficial dorsal horn, neurones known to be related to nociceptive transmission (see e.g. 41). Besides the knowledge that PAG is involved in nociceptive transmission, it has been known for several years that electrical stimulation of or opiate microinjections into PAG of animals or humans also produce analgesia (see e.g. 11, 17). It has therefore been proposed that in PAG there is some sort of interaction between afferent nociceptive and efferent nociceptive inhibiting transmission (39).

Of special interest in this context are the findings, in recent studies undertaken to localize these pain-inhibiting regions of PAG, that they are situated in that part of PAG that receives the ascending spinal input, and that they contain different neurotransmitters, acting either directly or indirectly through one or several interneurons to inhibit the nociceptive transmission at the spinal level (2, 29, 40).

Since PAG is not the only region in the midbrain that receives an ascending somatosensory input, the question was considered whether the descending anti-

nociceptive system originating in PAG could be activated by other somatosensory regions of the midbrain. Inc receives a considerable ascending input, originating in the dorsal column nuclei, the lateral cervical nucleus and the trigeminal system (7, 9, 18, 37, 38, 39). In accordance with its cells of origin (36, 39) and its neurophysiological properties (see e.g. 12, 13), Inc seems to receive a different kind of input than PAG, being not specifically nociceptive but preferentially receiving light and moderate tactile information. As extensive reciprocal connections were found between Inc and the ventrolateral region of PAG in the present study, it seems reasonable that transmission through this nucleus might also inhibit a nociceptive transmission through PAG. In SGI and SGP, close to Inc and part of the intercollicular region, moderate labelling was observed. The superior colliculus has a complex function, but at least the deep part, i.e. SGI and SGP, seems to have multimodal integrative properties (see e.g. 35), and the relationship between the deep part of the superior colliculus and PAG opens a way for nociceptive inhibition through PAG after multimodal integration in SGI and SGP. The possibility of involvement of SGI and SGP in pain response is supported by earlier findings concerning the topographical connections between this part of the midbrain and cortical areas involved in the escape response threshold to nociceptive stimuli (4, 34).

The present study shows that not only the different parts of the intercollicular region but also other parts of the midbrain have reciprocal connections with PAG. The function of these projections is not clear, but it seems reasonable to assume that at least the connections with PBN, CNF and PTA have nociceptive or nociceptive-inhibiting properties, as suggested by the results of several recent studies (6, 19, 23). Undoubtedly PAG plays an important role both in nociceptive transmission and in nociceptive inhibition, and this role becomes no less complex with the demonstration that PAG not only is connected with the spinal cord and brainstem, but also has extensive connections with the hypothalamus, thalamus and cortex (see e.g. 3, 25, 32, 33), thus providing a possibility not only for spinal pain inhibition but also for such inhibition at the supraspinal level (30).

ACKNOWLEDGEMENTS

This work was supported by grants from the Norwegian and Swedish Medical Research Council.

REFERENCES

1. Ahlsen, G ., Retrograde labelling of retinogeniculate neurones in the cat by HRP uptake from the diffuse injection zone. *Brain Res* 223: 374-380, 1981.
2. Barbaresi , P. & Manfrini, E., Glutamate decarboxylase-immunoreactive neurons and terminals in the periaqueductal gray of the rat. *Neuroscience* 27: 183-191, 1988.
3. Behbehani, M.M., Park, M.R. & Clement, M.E., Interactions between the lateral hypothalamus and the periaqueductal gray. *J Neurosci* 8: 2780-4787, 1988.
4. Berkley, K.J. & Parmer, R., Somatosensory cortical involvement on responses to noxious stimulation in the cat. *Exp Brain Res* 20: 363-374, 1974.
5. Berman, N ., Connections of the pretectum in the cat. *J Comp Neurol* 174: 227-254, 1977.
6. Bernard, J.F., Peschanski, M. & Besson, J.M., Afferents and efferents of the rat cuneiformis nucleus: an anatomical study with reference to pain transmission. *Brain Res* 490: 181-185, 1989.
7. Björkeland, M. & Boivie, J ., An anatomical study of the projections from the dorsal column nuclei to the midbrain in cat. *Anat Embryol* 170: 29-43, 1984a.
8. Björkeland, M. & Boivie, J., The termination of spinomesencephalic fibers in cat. An experimental anatomical study. *Anat Embryol* 170: 265-277, 1984b.
9. Björkeland, M. & Boivie, J., Anatomy of the midbrain projections from the lateral cervical nucleus in cat. In M Rowe and WD Willis Jr (eds): *Development, Organization, and Processing in Somatosensory Pathways.* : Alan R Liss, Inc., New York ,1985, pp. 203-214.
10. Brodal ,P., Dietrichs, E., Bjaalie, J.,G, Nordby, T. & Walberg, F., Is lectin-coupled horseradish peroxidase taken up and transported by undamaged as well as damaged fibers in the central nervous system? *Brain Res* 278: 1-9, 1983.
11. Carstens, E., Hartung , M., Stelzer, B. & Zimmermann, M ., Suppression of hind limb flexion withdrawal reflex by microinjection of glutamate or morphine into the periaqueductal gray in the rat. *PAIN* 43: 105-112, 1990.

12. Danielsson , I. & Norrsell , U., Somatosensory, short latency activation in the intercollicular region of the cat's mesencephalon. *Acta Physiol Scand* 124: 479-482, 1985.
13. Danielsson , I. & Norrsell, U., Somatosensory units in the cat's intercollicular nucleus. *Acta Physiol Scand* 128: 579-586, 1986.
14. Dietrichs, E., Walberg, F. & Nordby T ,The cerebellar nucleo-olivary and olivary-cerebellar nuclear projections in the cat as studied with anterograde and retrograde transport in the same animal after implantation of crystalline WGA-HRP.I. The dentate nucleus. *Brain Res* 3: 52-70, 1985.
15. Eberhart, J.A., Morrell, J.I., Kriegers, M.S. & Pfaff, D.W., An autoradiographic study of projections ascending from the midbrain central gray, and from the region lateral to it, in the rat. *J Comp Neurol* 241: 285-310, 1985.
16. Edwards, S.B., Autoradiographic studies of the projections of the midbrain reticular formation: Descending projections of nucleus cuneiformis. *J Comp Neur* 161: 341-358, 1975.
17. Fields, H.L., Brainstem mechanisms of pain modulation. In: *advances in Pain Research and Therapy*, 6 (1984) 241-253, Kruger L, Liebeskind JC (eds) Raven Press, New York.
18. Flink, R., Wiberg, M. & Blomqvist., AThe termination in the mesencephalon of fibres from the lateral cervical nucleus. An anatomical study in the cat *Brain Res* 259: 11-20, 1983.
19. Foster, G.A., Sizer, AR., Rees,H. & Roberts, M.H.T., Afferent projections to the rostral anterior pretectal nucleus of the rat: a possible role in the processing of noxious stimuli. *Neuroscience* 29: 685-694, 1989.
20. Hamilton, B.L. & Skultety, F.M., Efferent connections of the periaqueductal gray matter in the cat. *J Comp Neurol* 139: 105-114, 1970.
21. Hamilton, B.L.,Projections of the nuclei of the periaqueductal gray metter in the cat. *J Comp Neurol* 152: 45-58, 1973.
22. Heimer, L. & Zaborszky, L., *Neuroanatomical tract-tracing methods 2. Recent progress.* Plenum Press, New York and London, 1989.
23. Hylden, J.L.K., Anton ,F. & Nahin ,R.L., Spinal lamina I projection neurons in the rat: collateral innervation of parabrachial area and thalamus. *Neuroscience* 28: 27-37, 1989.
24. Mantyh, P.W., The ascending input to the midbrain periaqueductal gray of the primate. *J Comp Neurol* 211: 50-64, 1982a.
25. Mantyh, P.W., Forebrain projections to the periaqueductal gray in the monkey, with observations in the cat and rat. *J Comp Neurol* 206: 146-158, 1982b.
26. Manthy, P.W., Connections of midbrain periaqueductal gray in the monkey. II. Descending efferent projections. *J Neurophysiol* 49: 582-594, 1983.

27. Marchard, J.E. & Hagino, N., Afferents to the periaqueductal gray in the rat. A horseradish peroxidase study. *Neuroscience* 9: 95-106, 1983.
28. Mesulam, M.-M., Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *J Histochem Cytochem* 26: 106-117, 1978.
29. Millar, J. & Williams, G.V., Effects of iontophoresis of noradrenaline and stimulation of the periaqueductal gray on single-unit activity in the rat superficial dorsal horn. *J Comp Neurol* 287: 119-133, 1989.
30. Morgan, M.M., Sohn, J-H. & Liebeskind, J.C., Stimulation of the periaqueductal gray matter inhibits nociception at the supraspinal as well as spinal level. *Brain Res* 502: 61-66, 1989.
31. Peschanski, M. & Ralston, III H.J., Light and electron microscopic evidence of transneuronal labeling with WGA-HRP to trace somatosensory pathways to the thalamus. *J Comp Neurol* 236: 29-41, 1985.
32. Rinvik, E. & Wiberg, M., Demonstration of a reciprocal connection between the periaqueductal gray matter and the reticular nucleus of the thalamus. *Anat Embryol* 181: 577-584, 1990.
33. Sesack, S.R., Deutch, A. Y., Roth, R.H. & Bunney, B.S., Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with phaseolus vulgaris leucoagglutinin. *J Comp Neurol* 290: 213-242, 1989.
34. Stein, B.E., Spencer, R.F. & Edwards, S.B., Corticotectal and corticothalamic efferent projections of SIV somatosensory cortex in cat. *J Neurophysiol* 50: 896-909, 1983.
35. Stein, B.E. Development of the superior colliculus. *Ann Rev Neurosci* 7: 95-125, 1984.
36. Wiberg, M. & Blomqvist, A., The spinomesencephalic tract in the cat: Its cells of origin and termination pattern as demonstrated by the intraaxonal transport method. *Brain Res* 291: 1-18, 1984a.
37. Wiberg, M. & Blomqvist, A., The projection to the mesencephalon from the dorsal column nuclei. An anatomical study in the cat. *Brain Res* 311: 225-244, 1984b.
38. Wiberg, M., Westman, J. & Blomqvist, A., The projection to the mesencephalon from the sensory trigeminal nuclei. An anatomical study in the cat. *Brain Res* 399: 51-68, 1986.
39. Wiberg, M., Westman, J. & Blomqvist, A., Somatosensory projection to the mesencephalon: An anatomical study in the monkey *J Comp Neurol* 264: 92-117, 1987.
40. Williams, F.G. & Beitz, A.J., A quantitative ultrastructural analysis of neurotensin-like immunoreactive terminals in the midbrain periaqueductal gray: Analysis of their

possible relationship to periaqueductal gray - raphe magnus projection neurons. *Neuroscience* 29: 121-134, 1989.

41. Willis, W.D. Jr., Projections of the superficial dorsal horn to the midbrain and thalamus. In: *Processing of sensory information in the superficial dorsal horn of the spinal cord*: 217-237, Cervero F, Bennet GJ, Headlay PM (eds), Plenum Publishing Corporation, 1989, pp 217-237.

Adress for reprints:

Mikael Wiberg
Department of Anatomy, Box 571
S-75123 Uppsala, SWEDEN