

In the present study the effects of microiontophoretically applied acetylcholine and NMDA were investigated on neurones of the avian nucleus isthmi. Whereas acetylcholine affected equally cell firing in the two parts of the nucleus, the magnocellular (Imc) and the parvocellular (Ipc) division, the effect of NMDA was predominantly confined to Ipc. The NMDA induced firing was specifically blocked by the antagonist CPP, while having no effect on acetylcholine. Our results support earlier findings that isthmo-tectal feedback loops can be modulated by distinct mechanisms in separate divisions of nucleus isthmi.

Effect of acetylcholine and NMDA on neurones of avian tectum and nucleus isthmi

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Introduction

The electrophysiology and neuroanatomy of the avian nucleus isthmi, which is a complex of several cellular aggregates¹ have been extensively studied.² Although there is some discrepancy, it is now generally accepted that the two main portions of the nucleus, pars magnocellularis (Imc) and pars parvocellularis (Ipc) are related to visual information processing.^{3,4} In birds, the Ipc receives its input from the ipsilateral tectum and projects back ipsilaterally to superficial tectal layers,⁵ and the Imc projects to deeper layers of the ipsilateral tectum.⁶ Using intracellular recording techniques, Wang and Matsumoto⁷ have shown that stimulation of the nucleus isthmi of the frog exerts inhibition on 70% and excitation on 30% of tectal cells.

The search for the chemical nature of synaptic interconnection within the nucleus isthmi has included autoradiographic, biochemical, histochemical and electrophysiological data (for review see Refs 8 and 9). The excitatory tecto-isthmi pathway possibly uses acetylcholine^{10,11} and/or glutamate¹² as neurotransmitters. There is evidence that isthmo-tectal pathways use glycine and GABA as inhibitory substances^{13,14} and acetylcholine^{15–17} or glutamate¹⁸ as excitatory transmitters. In the central nervous system there is strong evidence that the excitatory amino acid neurotransmitter glutamate is mediated by distinct receptor types, e.g. *N*-methyl-D-aspartate (NMDA), quisqualate and kainate.¹⁹ Furthermore, NMDA receptors might play an important role in information processing.²⁰

Recently, Wang and coworkers²¹ have shown that microinjection of NMDA into Ipc reduces visual responsiveness by activating Ipc-tectal pathway, whereas injection of lidocaine into the same area had no effect. In contrast, injection of lidocaine into Imc produced

significant reduction of tectal firing, while NMDA was not effective. In order to elucidate whether different receptor mechanism are involved in starting this feedback pathway, we tested NMDA, acetylcholine and its specific antagonists in the two parts of the nucleus isthmi.

Materials and Methods

Our experiments were carried out on 13 adult pigeons (*Columba livia*) having body weights of 280–340 g. The animals were anaesthetized with urethane (1 ml 100 g⁻¹ body weight, 20% solution) and placed in a stereotaxic apparatus. The exposure of the surface of the optic tectum was done in a conventional manner. Multibarrelled micropipettes with an overall tip diameter of approximately 4 μm were inserted through the tectal layers and aimed at the nucleus isthmi using coordinates from the atlas of the pigeon brain.²²

Extracellular action potentials were obtained through a 2 M NaCl- and 100 mM cobalt chloride-filled barrel of the five-barrel glass micropipettes and the neuronal discharge was recorded continuously and stored on the magnetic tape of a data recorder (KS-609, Sony) or displayed on an UV-recorder (Bell & Howell, 5-137). The other barrels of the micropipette were used for microiontophoretic application of the compounds being tested. Each substance was ejected from the electrode with the appropriate anionic or cationic current. Substances tested included acetylcholine chloride (ACh, Sigma, 0.5 M, pH 3.5), NMDA (*N*-methyl-D-aspartic acid, Sigma 0.05 M, pH 7.5), sulphate atropine (Sigma, 0.01 M, pH 5.7) and CPP (3-rs-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid, Tocris Neuramin, 0.01 M, pH 7.5).

At the end of each experiment, cobalt ions were

Table 1. Summary of effects of *N*-methyl-D-aspartic acid (NMDA) and acetylcholine (ACh) in different areas of tectum opticum (TO) and subnuclei of nucleus isthmi (lpc, Imc)

| Drugs | Effects | TO | | Imc | lpc |
|-------|------------|--------------------|---------------|-----------|----------|
| | | superficial layers | deeper layers | | |
| NMDA | excitation | 12 (50%) | 20 (91%) | 2 (9%) | 27 (96%) |
| | no effect | 12 (50%) | 2 (9%) | 21 (91%) | 1 (4%) |
| | depression | 0 | 0 | 0 | 0 |
| ACh | excitation | 16 (67%) | 10 (45%) | 23 (100%) | 24 (86%) |
| | no effect | 8 (33%) | 12 (55%) | 0 | 4 (14%) |
| | depression | 0 | 0 | 0 | 0 |

ejected microiontophoretically and subsequently the brain was processed for histological verification of the recording location.

Results

Extracellular recordings were made from a total of 97 cells, 51 lying in the isthmic nucleus area and the remainder in different layers of the optic tectum. Within the nucleus isthmi 23 neurones were located in the magnocellular part and 28 in the parvocellular portion. Visual stimulation was used to identify neurones. The majority of these neurones showed spontaneous activity. Neurones in the magnocellular part of the

nucleus usually showed stronger spontaneous firing than those of the parvocellular part.

The effects of iontophoretically applied ACh and NMDA on tectal and isthmic neurones are illustrated in Table 1. In all areas tested, only excitatory or no effects but no depression of spike activity were observed. In the tectum opticum the two substances tested showed regional distribution patterns in terms of effectiveness. Whereas in superficial layers of the tectum about half of the tested neurones were excited by NMDA, the majority of cells in deeper layers responded with excitatory effects. In contrast, ACh had a stronger excitatory effect on neurones in the superficial layers. This finding is substantiated by the fact, that in superficial areas 10 neurones were found to be excited by ACh only, whereas specifically NMDA-sensitive neurones were located in deeper tectal structures.

In the two parts of the nucleus isthmi a clear distinction between the effects of the substances tested could be seen (Fig. 1A). An increase in the discharge frequency following the ejection of NMDA was predominantly confined to the parvocellular portion of the nucleus. The minimum current used to produce an excitatory effect varied between 5 and 10 nA. Further-

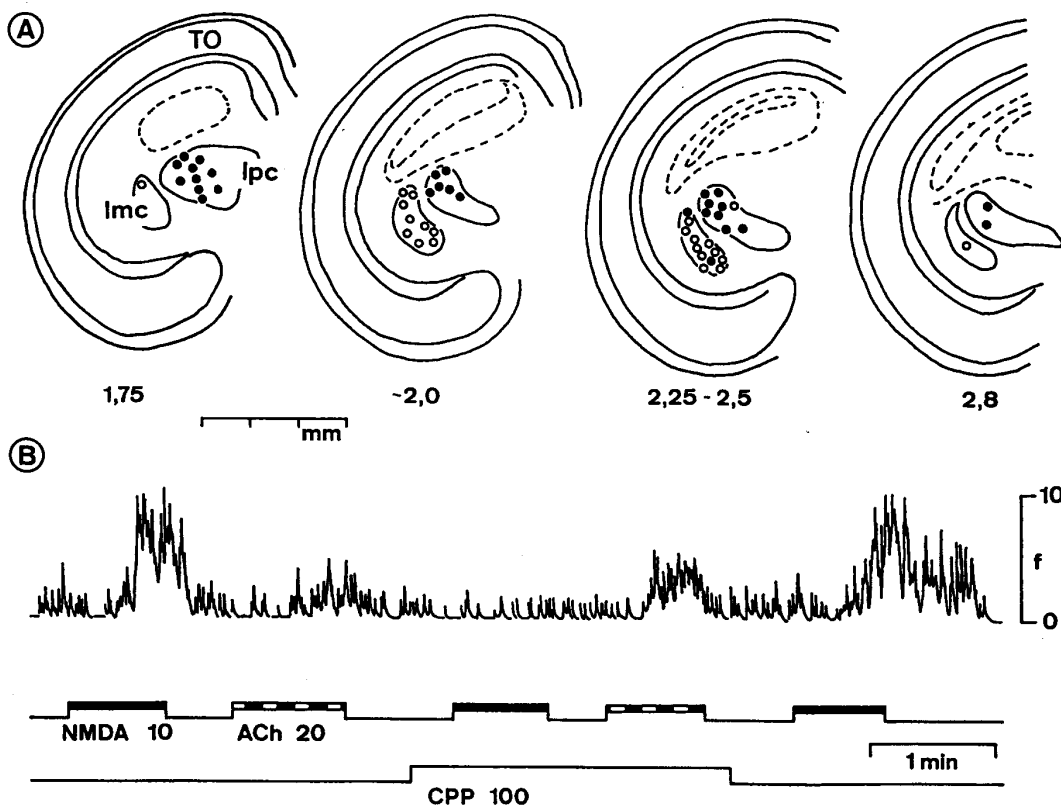


FIG. 1. (A) Effect of NMDA in the two parts of nucleus isthmi. All neurones were identified by histological techniques and mapped on the basis of their response: excitation (closed circles) or no effect (open circles). Anterior-posterior levels according to the atlas of Karten and Hodós.²² (B) Effect of NMDA (20 nA) and ACh (20 nA) before, during and after the ejection of the NMDA antagonist CPP (100 nA). The iontophoretic administration of substances is marked at the bottom lines.

more, the discharge frequency of neurones showed a marked acceleration with an onset of usually less than 2 s. In contrast to the distinct location of NMDA-sensitive cells in Ipc, ACh almost equally affected neurones in both parts of the nucleus. Although the amount of current (10–20 nA) to produce an excitatory effect was relatively low, the average latencies for isthmic acetylcholine-induced excitation were 5.5 s for currents of 20 nA. A comparison of relative potencies of NMDA and ACh on Ipc neurones showed, that out of 28 cells tested, 17 had a stronger excitatory response to NMDA when similar amounts of current were applied, and the remaining 11 cells were equally affected by both agents.

NMDA receptors are one of the best characterized types in the central nervous system and mediate excitatory amino acid synaptic transmission. Having demonstrated that NMDA shows a highly potent excitatory action on Ipc cells, we then proceeded to study the specific competitiveness of the NMDA antagonist CPP. In five experiments CPP was applied iontophoretically in doses from 70–150 nA on Ipc cells showing excitatory action to both NMDA and ACh. The data were analysed with respect to changes in firing frequency, latency of onset and latency for recovery. In all cells tested, the NMDA-induced firing rate was antagonized by CPP in a reversible and specific manner (Fig. 1B). The antagonistic effect started 1–3 min and reached its maximum 3–5 min after the beginning of the application; complete recovery usually occurred 5–8 min after the termination of the ejection. The action of ACh was never blocked by CPP; in comparison the ACh-induced activation was antagonized by atropine sulphate.

Discussion

The electrophysiological data obtained in our investigations show striking differences in terms of neuronal sensitivity towards NMDA in magnocellular and parvocellular divisions of pigeon nucleus isthmi. This is strengthened by the fact that ACh-induced activation did not differ in the two isthmic parts. The findings are in agreement with previously published data from Wang and coworkers,²¹ who showed that the two divisions of nucleus isthmi differentially modulated neuronal firing in avian optic tectum. It has been proposed that the nucleus isthmi play an important role in visual processing, possibly involving delayed inhibitory effects²³ and prolonged excitation.²⁴ A positive feedback loop exists, originating in Imc and projecting into deeper layers of the tectum,^{10,14} as well as an inhibitory feedback loop having its origin in the Ipc and its terminals in superficial tectal layers.^{4,24} Microinjection of NMDA into the Ipc resulted in a reduction of tectal firing, thus activating an inhibitory isthmo-tectal pathway.^{21,25} The present results support the idea that this activation might be mediated by the glutamatergic

NMDA-receptor type. Cholinergic Ipc neurones possibly are involved in the excitatory tecto-isthmic projection.^{5,11} In contrast, the majority of Imc neurones did not respond to NMDA, suggesting that no NMDA receptor population exists in that area. Our data show that Imc contains cholinergic neurones; its afferent input, however, is not known. Imc projection terminates in deeper layers of the tectum,⁶ its chemical nature of transmission is not yet known. The majority of visual neurones in the avian optic tectum receive an excitatory input from the Imc, suggesting that either an excitatory amino acid and/or acetylcholine might be candidates as possible transmitters.

To summarize, the avian optic tectum may receive an excitatory input from the magnocellular portion of the nucleus isthmi and an inhibitory one from the parvocellular division. Both feedback loops can be modulated in the nucleus isthmi: the negative loop in Ipc by NMDA- and acetylcholine receptors; the positive feedback by acetylcholine but not NMDA.

Conclusion

In conclusion, the results of this study indicate that neurones in the optic tectum and the two subnuclei of the nucleus isthmi, Ipc and Imc, are differentially activated by NMDA and ACh. These results extend and support previous observations and suggest that feedback loops between nucleus isthmi and the tectum can be modulated at the isthmic level by NMDA and/or ACh.

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