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The Effect of Acetylcholine on Neurones of the Amphibian Nucleus Isthmi

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In the bird, histochemical and biochemical data suggest that the tectal input into nucleus isthmi is of a cholinergic nature. In the present study the response of toad (*Bufo bufo gargarizans*) isthmic neurones to acetylcholine was investigated using conventional extracellular recording techniques and ionophoresis. The results show isthmic cells to be strongly excited by acetylcholine (ACh), whereas neurones in the neighbouring areas show minimal sensitivity. Atropine sulphate completely blocked the response to acetylcholine and greatly decreased activity induced by visual stimulus. The present data show that acetylcholine mimics one of the physiological transmitters in the nucleus isthmi.

INTRODUCTION

The nucleus isthmi is a prominent mesencephalic structure found in all classes of vertebrates except cyclostomes and mammals^{1,18}. Reciprocal fibre connections between this nucleus and the optic tectum have been reported in amphibians⁹, reptiles⁷, birds¹³ and teleosts²². In addition, reciprocal projections are topographically arranged in the frog^{9,17,23,26} and the toad^{27,28}. The nucleus isthmi is thought to be homologous to the parabigeminal nucleus in mammals^{8,19,20}.

It has been suggested that the nucleus isthmi in the pigeon is possibly an auditory station⁵. On the basis of an extensive electrophysiological study, Wang and co-workers²⁷ have proposed that the nucleus isthmi in the toad is a restricted part of the visual system. Furthermore it has been shown that this nucleus plays an essential role in visually guided behaviour in frogs¹⁰ and toads²⁸.

A number of possible neurotransmitters in cells of the avian optic tectum and subtectal nuclei have been the subject of extensive investigations (for reviews see refs. 4 and 6). The uptake of choline has been related to the presence of the neurotransmitter acetylcholine within nucleus isthmi pars parvocellularis

(Ipc) neurones. After tectal lesions had been made, acetylcholinesterase (AChE) staining activity stopped in topographically related areas of Ipc neuropil¹⁴, indicating that the tectal-Ipc pathway, whose cell bodies lie in the tectal layer III³, is of a cholinergic nature.

Little is known at present about the chemical nature of synaptic transmission in amphibian nucleus isthmi. However, on the basis of findings in birds one can assume that transmission in the amphibian structure is similar. We have therefore attempted to test the action of acetylcholine on single unit discharges of neurones of the amphibian nucleus isthmi with the aid of microionophoretic techniques. Microionophoretic application of acetylcholine should mimic the effect of the physiological synaptic action of the transmitter on the postsynaptic element.

MATERIALS AND METHODS

All experiments were performed on adult toads, *Bufo bufo gargarizans*. The surgical and stereotaxic procedures used have already been described²⁷. Visual stimuli comprised a 2° spot of light and 3 and 8° black or white discs which were moved automatically

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or manually against a diffuse grey background. For acoustic stimulation the ear was exposed to a whistle or a brief sharp click. Extracellular recordings of action potentials were obtained from isthmic and neighbouring neurons¹⁶ with a barrel of a 4-barrelled micropipette filled with 4 M NaCl (5–15 M Ω resistance, 4.5 μ m diameter). The other channels contained the following compounds to be ejected microionophoretically by appropriate ionic currents: atropine sulphate (10 mM, pH 5.7), acetylcholine (0.5 M, pH 3.5), strychnine hydrochloride (2 mM in 165 mM NaCl); spontaneous and evoked activity were displayed on an oscilloscope and plotted directly onto an UV-oscillograph.

RESULTS

Extracellular recordings were made from a total of 65 cells, 37 lying in the isthmic nucleus area and the remainder in the area medial, lateral or caudal to the nucleus isthmi. The majority (74%) of the neurones discharged spontaneously. Within the nucleus isthmi only units responding exclusively to visual stimulation were found, whereas auditory as well as multi-sensory cells were located outside its boundary (Table IB). The only neurone from the neighbouring region which displayed a visual reaction showed a characteristic ON-OFF response and was presumed to be a tectal unit.

The effects of microionophoretically applied ACh are summarized in Table IA and Fig. 1. An increase in the discharge frequency was predominantly confined to the nucleus isthmi. Depression of spike activity occurred in about 10% of the cells tested. These cells were scattered throughout the whole area ex-

plored. Although only a few cells excited by the ejection of ACh were found outside the nucleus, there was a marked difference in the ionophoretic current required to evoke the excitations. The minimum current used to produce an excitatory effect varied between 15 and 50 nA for isthmic neurons and between 100 and 150 nA for neighbouring cells. Furthermore, the discharge frequency of neurons within the nucleus isthmi showed a marked acceleration when enough ACh was applied to raise firing above the level of spontaneous activity, whereas in non-isthmic cells the excitation evoked by ACh was slow in onset. The average latencies for isthmic acetylcholine-induced excitation were 5.5 s for currents of 20 nA, 2 s for 40 nA and 1.5 s for 100 nA.

It was possible to block or reduce the excitatory effect of ACh by applying atropine sulphate. The doses required to block excitation ranged from 20 to 50 nA. With this amount of current, the antagonist alone produced either no effect or, in the case of two cells, a small decrease in unit firing. Atropine applied in doses exceeding 100 nA, however, blocked all activity. The most sensitive antagonism was observed in neurones within the isthmic nucleus on which ACh caused the most marked acceleration of firing. Thus the specifically ACh-responsive neurones were also the most sensitive to the blocking action of the ACh antagonist. In contrast, ACh-induced firing was not attenuated by strychnine.

In a second series of experiments the influence of the ACh-antagonist atropine was tested on the electrical responses to visual stimulation. Isthmic units

TABLE I
ACh-sensitivity of units and their sensory modality

	<i>Nucleus isthmi</i>	<i>Surrounding area</i>
<i>A Effect of ACh</i>		
Excitation	25	5
No effect	7	22
Depression	5	1
<i>B Sensory modality</i>		
Visual response	30	1
Auditory response	0	7
Visual-auditory response	0	3

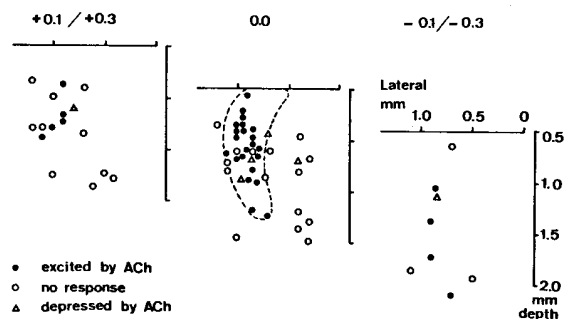


Fig. 1. Effect of acetylcholine (ACh) on the firing of isthmic and non-isthmic neurones. All neurones were mapped on the basis of their response according to the coordinates of the atlas of Kemali and Braitenberg¹⁶. At anterior-posterior level 0.0 the boundary of nucleus isthmi is indicated by a broken line.

usually responded strongly to the movement of the visual targets through their receptive fields and immediately stopped firing when the targets stopped moving. Most units were directionally sensitive and preferred the temporal-nasal direction; an example is given in Fig. 2. Non-spontaneously active cells responded to visual stimulation and were excited by the application of ACh (Fig. 2A). Three minutes after atropine sulphate was ejected ionophoretically the ACh-induced activity was completely blocked, and the response to visual stimulation was reduced but not completely blocked (Fig. 2B). Five minutes after the application of atropine was stopped complete recovery of the response to both natural and chemical stimulation was observed (Fig. 2C). In contrast to the findings concerning visual units within the nucle-

us isthmi, auditory and visuo-auditory responses in the surrounding area of the nucleus were not attenuated by atropine.

DISCUSSION

The electrophysiological data obtained in our investigations on toad isthmic neurones agree with previous findings²⁸ which indicate that isthmic units respond exclusively to visual stimulation. Bimodal units are absent in this region, as has been found in the frog isthmic nucleus²⁵. However, bimodal units have been found in regions equivalent to the antero-dorsal and postero-dorsal tegmental nucleus²¹.

In our study two-thirds of the visual units recorded in the nucleus isthmi showed spontaneous activity. This contrasts, however, with the results of a previous study by Wang and coworkers²⁸ in which three-quarters of the recorded cells were unresponsive. This discrepancy could be explained by the use of multi-barrelled electrodes and the spontaneous efflux of the substances from the tip of the electrode. Although the spontaneous leakage of active material has always been recognized as an undesirable feature of the technique, it has often been disregarded on the basis that leakage is eliminated by applying a retaining current¹⁵.

There is no doubt that cholinergic neurones play an important role in the optic tectum^{2,12}. Biochemical investigations have revealed the distribution of the enzymes choline acetyltransferase (ChAT) and glutamate decarboxylase (GAD), which control the level of acetylcholine (ACh) and γ -aminobutyric acid (GABA)¹¹. The level of activity of both enzymes is high in the nucleus isthmi pars parvocellularis (Ipc) and the distribution of ChAT correlates well with the staining pattern of acetylcholine esterase (AChE). Superficial tectal lesions prevented AChE staining in the topographically corresponding Ipc region¹⁴, a fact which indicates that the tecto-Ipc neurones in pigeon are cholinergic. On the other hand, the somata of Ipc-tectal neurones did not show a positive reaction to AChE. Since lesions in the Ipc do not affect AChE staining in the tectum it seems that Ipc-tectal neurones are unlikely to be cholinergic¹⁴.

The results of our experiments on toad isthmic neurones indicate that this region contains cells which have a strong chemosensitivity to ACh. The

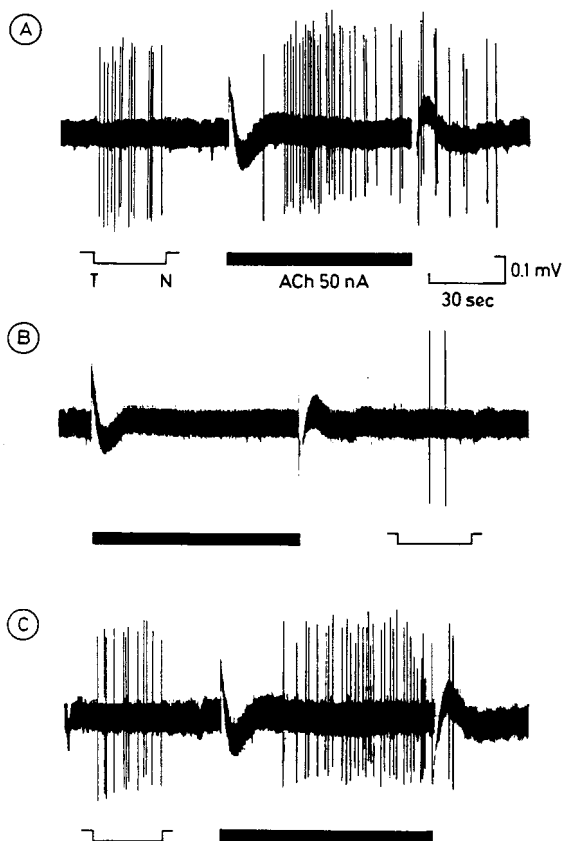


Fig. 2. Effect of visual temporal nasal (T-N) stimulation and of ionophoretically applied acetylcholine (ACh) on a neurone of nucleus isthmi (length of ejection indicated by bars). A: control. B: 3 min after application of atropine sulphate (50 nA). C: full recovery seen 5 min after atropine application was stopped.

results are confirmed by the finding that even low doses lead to an immediate response and that atropine sulphate blocks ACh-sensitive cells as well as stimulus-induced activity by natural visual stimulation. Furthermore, neurons in the neighbouring areas showed no specific sensitivity to ionophoretically applied ACh. In the pigeon the tectal cholinergic input is most probably of the nicotinic type, since the Ipc is completely devoid of muscarinic binding sites². The question of whether nicotinic receptors are also involved in the toad nucleus isthmi needs fur-

ther elucidation.

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