Brain Research Bulletin, Vol. 54, No. 4, pp. 399–403, 2001

0361-9230/01/$–see front matter

PII S0361-9230(00)00461-5

Tecto-isthmo-optic transmission in pigeons is mediated by glutamate and nitric oxide

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[Received 16 June 2000, Accepted 13 December 2000]

ABSTRACT: The isthmo-optic nucleus of the centrifugal system in birds receives primarily input from the ipsilateral tectum and projects to the contralateral retina. The present study using brain slices and microiontophoresis shows that synaptic transmission from the tectum to the centrifugal nucleus in pigeons is excitatory. About 75% of tecto-isthmo-optic fibers are glutamatergic, mediated by α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid but not N-methyl-D-aspartate-receptors, and 25% of others may use nitric oxide as a transmitter or modulator. On the other hand, about 60% of isthmo-optic cells receive glutamatergic afferents, 20% receive nitric oxidergic afferents, and 20% of others receive both glutamatergic and nitric oxidergic afferents from the tectum. In the last group, it is more likely that both glutamate and nitric oxide may co-release from the same tecto-isthmo-optic terminals. All the isthmo-optic cells examined in the present study also receive γ-aminobutyric acid (GABA)ergic afferents via $GABA_A$ and $GABA_B$ receptors probably from some extratectal structures. © 2001 Elsevier Science Inc.

KEY WORDS: Glutamate, Isthmo-optic nucleus, Neurotransmission, Nitric oxide, Tectum.

INTRODUCTION

The isthmo-optic nucleus (ION) in ground-pecking birds, such as pigeons, is the most developed and extensively studied retinopetal or centrifugal visual structure in all classes of vertebrates so far examined [7,16,26]. It is located in the caudo-dorsomedial midbrain and projects to the contralateral retina, terminating in either convergent or divergent modes on amacrine and/or displaced ganglion cells [25,28,33]. Electrical stimulation of the isthmo-optic tract or the ION enhances visual responses of retinal ganglion cells [8,23,27]. On the other hand, pharmacological blockade of the ION by lidocaine reduces visual activity of tectal neurons [14]. These findings are supported by immunohistochemical studies showing that ION-retinal fibers may use glutamate/aspartate [29] and nitric oxide [24,35] but not γ-aminobutyric acid (GABA) [21] as transmitters.

This centrifugal nucleus receives afferents primarily from laminae 9/10 of the ipsilateral optic tectum [19–21,30,32] as well as from various brainstem structures [19]. Electrical stimulation applied to the lateral tectum or to the tecto-isthmo-optic tract predominantly excites the centrifugal cells [12,15]. However, immunohistochemical studies have shown GABA-immunoreactivity in interneurons and about half the axons and terminals within the pigeon ION [21,22]. On the other hand, numerous glutamate-immunoreactive terminals exist within the ION [22]. Double labeling experiments have shown the presence of choline acetyltransferase- and nitric oxide synthase-immunoreactivity in the tectal neurons, which may project to the pigeon ION [20], but in disagreement with a recent study on the chicken [35]. Therefore, the present study was carried out to reveal physiological transmitters and their receptors in the tecto-isthmo-optic pathway by using brain slice, electrical stimulation, and microiontophoresis techniques.

MATERIALS AND METHODS

The experiments were performed on brain slices of 29 adult pigeons (*Columba livia*), 270–490 g body weight, following the Policy on the Use of Animals in Neuroscience Research approved by the Society for Neuroscience in 1995. The experimental procedures were as described previously [15]. Briefly, the pigeon was anesthetized with ketamine hydrochloride (30 mg/100 g) and then decapitated. The brain was immediately removed and washed in ice-cold Krebs-Ringer solution containing (in mM) NaCl, 124; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 2; KH_2PO_4, 1.25; $NaHCO_3$, 26; glucose 10 [11], oxygenated with a mixture of 95% O_2 and 5% CO_2. A brain tissue containing tectum and cerebellum was blocked and glued on the stage of Vibroslice (752 M; Campden Instruments Ltd., UK). Slices containing the ION and the tecto-isthmo-optic tract (TTIO) were sectioned horizontally at 400 μm in thickness, and then transferred into the recording chamber (BSC-HT; Medical System Corp., Greenvale, NY, USA) perfused with Krebs-Ringer solution bubbled with a mixture of 95% O_2 and 5% CO_2. The slices were incubated at 30°C for 60 min. Under an operating microscope, the TTIO was seen merging anterolaterally with the ION. A bipolar tungsten electrode with poles 400 μm apart was placed across the tract and 1.0–1.5 mm distant from the ION. Rectangular pulses of 10–30 μs in duration and 90–780 μA in intensity were delivered for electrical stimulation. For extracellular recording and microiontophoresis, a five-barrel micropipette was used. The recording channel was filled with 2 M NaCl, and three other channels contained the following compounds to be ejected

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by appropriate currents: acetylcholine chloride (0.5 M, pH 3.5; Sigma Chemical Co., St. Louis, MO, USA), sodium-L-glutamate (0.5 M, pH 7.3; Fluka, Switzerland), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 mM, pH 8.3; RBI, Natick, MA, USA), 3-rs-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid (CPP; RBI; 10 mM, pH 7.5), γ-aminobutyric acid (GABA, 0.5 M, pH 3.3; Fluka), bicuculline methiodide (10 mM, pH 3.0; Sigma Chemical Co.), 2-hydroxysaclofen (20 mM, pH 3.0; RBI) [34] and N-nitro-L-arginine (10 mM, pH 6.0; Sigma Chemical Co.) [5]. The remaining channel filled with 2 M NaCl was used for minimizing current effects. The micropipette was advanced into the ION in slices under visual control. Action potentials evoked by the tract stimulation and their changes caused by drug application were amplified (Intra 767; WPI, Sarasota, FL, USA), stored on magnetic tapes using a data recorder (TEAC RD-135T; TEAC Corp., Japan), and then analyzed by usually superimposing ten sweeps. All the statistical values reported in the present study represent means ± SD.

RESULTS

Seventy-eight cells were isolated throughout the ION and their responses to the tract stimulation and drug application examined (Fig. 1). Depending on current intensities used in the present study, these ION cells produced 0.1–1 spikes following a single stimulation to the tecto-ION tract, with an average latency of 2.98 ± 1.28 ms ($n = 78$) (Fig. 2). Effects of acetylcholine, glutamate, and GABA on electrically evoked firing activity were examined on 12 isthmo-optic cells (Fig. 2A). Acetylcholine produced no effect on firing activity during its application with current of 5–200 nA in intensity. Glutamate (10–40 nA) increased in 9 of 12 (75%) cells an average firing from 4.0 ± 1.5 to 9.3 ± 1.1 spikes accumulated for 10 sweeps following electrical stimulation (90–320 μA, 10–30 μs). This significantly enhanced responsiveness (*t*-test, $n = 9$, $p < 0.01$) recovered to the control value 0.5–4 min after stopping glutamate application. Three others (25%) showed no responses to glutamate applied with current intensity of up to 200 nA. Spiking activity evoked by the tract stimulation in these cells was completely abolished by GABA (5–40 nA). This suppressive effect was released 0.5–2 min after stopping drug application. Receptor subtypes of GABA were identified in additional 18 cells by iontophoretic co-application of GABA and bicuculline, a $GABA_A$ antagonist, or 2-hydroxysaclofen, a $GABA_B$ antagonist. As shown in Fig. 2B, firing activity evoked by the tract stimulation was completely suppressed by GABA, and the suppression could be released by co-application of GABA and either bicuculline (10–25 nA) or 2-hydroxysaclofen (10–40 nA).

Effects of glutamate and its α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-antagonist CNQX and N-methyl-D-aspartate (NMDA)-antagonist CPP on firing activity evoked by the tract stimulation were examined on 31 cells. In 24 of these (77%) cells, glutamate (5–80 nA) increased an average firing activity from 6.7 ± 2.4 to 9.7 ± 0.6 spikes counted in 10 sweeps following stimulation (130–630 μA, 10–30 μs). This significant enhancement (*t*-test, $n = 24$, $p < 0.01$) was returned to normal 0.5–3 min after ceasing drug application. The electrically evoked spiking of these glutamate-sensitive cells was blocked by CNQX (5–80 nA) but not by CPP applied with current of up to 400 nA (Fig. 2C). The blockade by CNQX was completely released 1–5 min after stopping drug application. Seven others (23%) did not respond to glutamate at all, even though it was applied at much higher dosage of 200 nA.

Effects of CNQX and N-nitro-L-arginine, a competitive inhibitor of nitric oxide synthase, on firing activity evoked by the tract stimulation (170–780 μA, 10–30 μs) were examined on 17 cells. They could be classified into three groups according to their responsiveness to these drugs. Electrically evoked responses in 10 cells (59%) were completely blocked by CNQX (5–80 nA), as shown by reduction of firing activity from 8.8 ± 1.2 spikes to zero in 10 sweeps, but not by N-nitro-L-arginine applied with current of up to 200 nA. Firing activity in 4 cells (23%) was reduced by N-nitro-L-arginine (10–80 nA) from 9.2 ± 0.8 spikes to zero in 10 sweeps but not by CNQX applied even at much higher dosage (400 nA) (Fig. 2D). Evoked responses in 3 cells (18%) were completely blocked by either of CNQX and N-nitro-L-arginine shown by reduction from 9.0 ± 1.4 spikes to zero in 10 sweeps. These suppressive effects were usually started 0.5–5 min and 3–7 min after drug application and recovered to the pre-drug levels 1.5–10

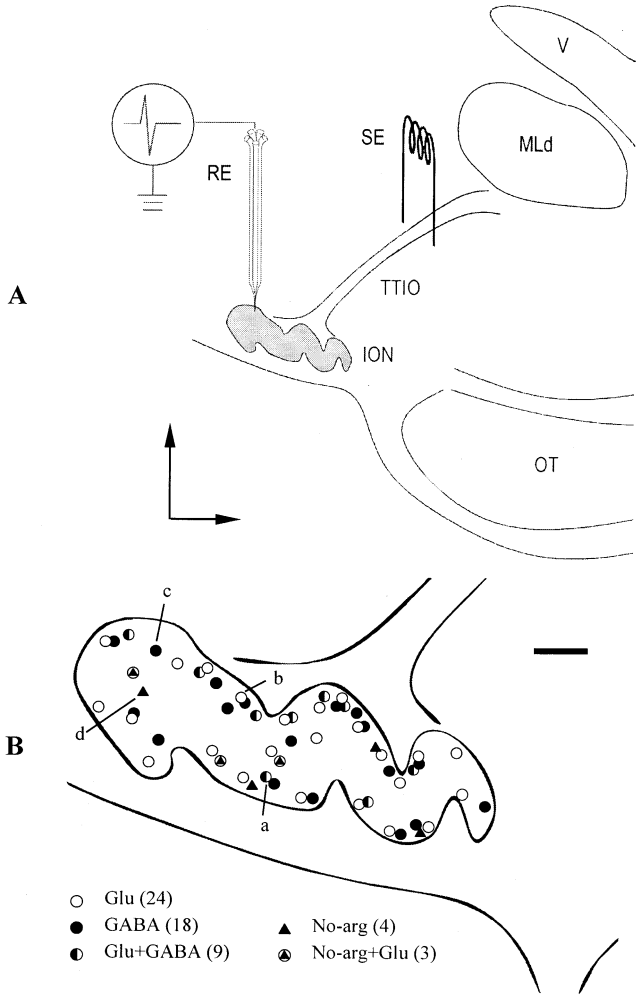

FIG. 1. Experimental set-up (A) showing arrangement of a 5-barrel micropipette (RE), stimulating electrode (SE), the isthmo-optic nucleus (ION), and the tecto-isthmo-optic tract (TTIO) on a horizontal section of the pigeon brain. (B) shows distribution of the recording sites of isthmo-optic cells examined for effects of glutamate (Glu), γ-aminobutyric acid (GABA), both glutamate and GABA (Glu + GABA), N-nitro-L-arginine (No-arg), as well as both N-nitro-L-arginine and glutamate (No-arg + Glu). Numbers in parentheses indicate the number of ION cells responding to drug application in either an excitatory or an inhibitory way. Lower-case letters (a–d) label the cells whose discharge patterns are shown with corresponding letters in Figs. 2A–D. Abbreviations: L, lateral; MLd, nucleus mesencephalicus lateralis, pars dorsalis; OT, optic tectum; R, rostral; V, ventricle. Scale bars: 330 μm in (A); 100 μm in (B).

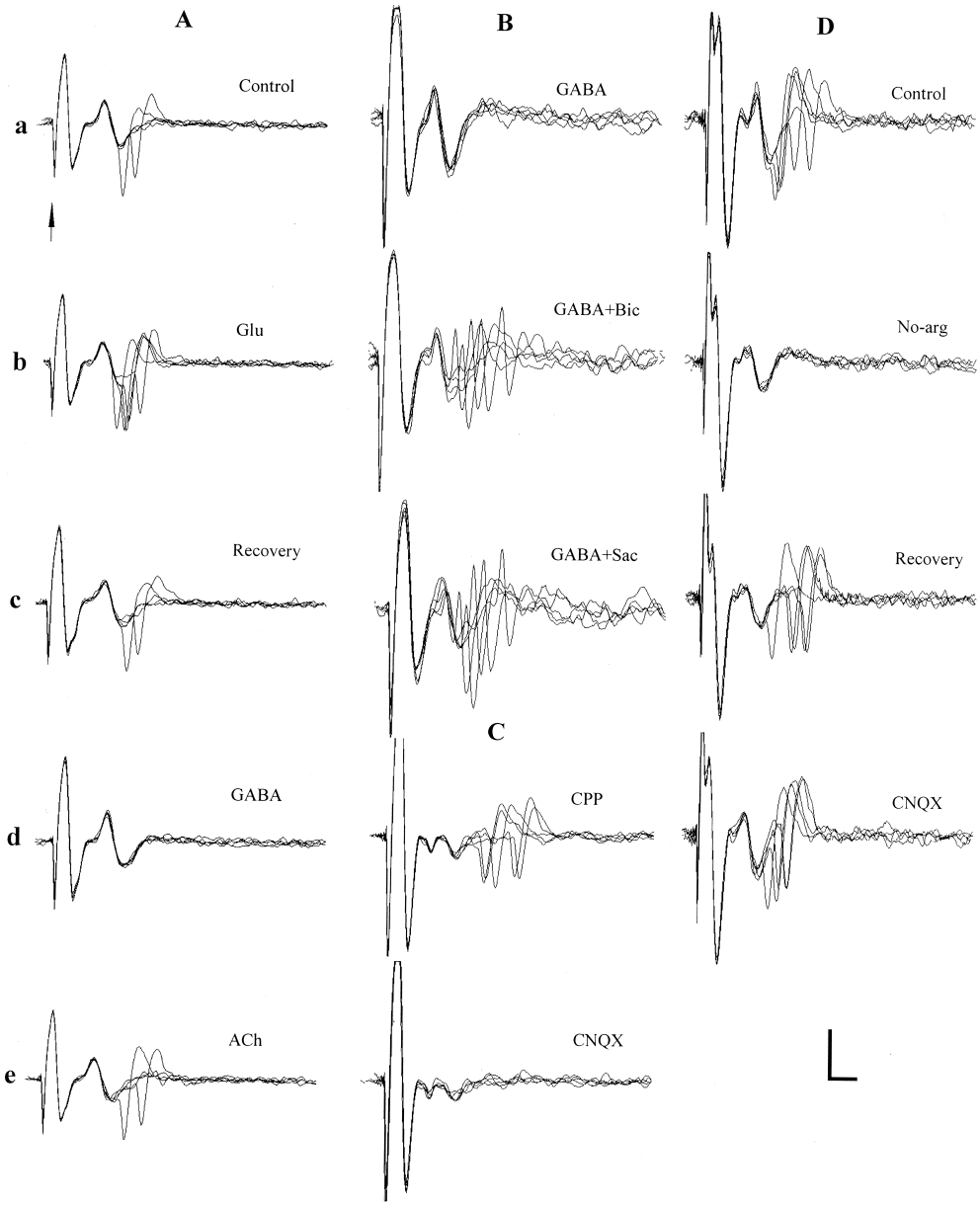

FIG. 2. Effects of drug application on firing activity evoked in four cells (A–D) by electrical stimulation of the tecto-isthmo-optic tract. Cell (A) was excited by glutamate (Glu) but not acetylcholine (ACh) and inhibited by γ-aminobutyric acid (GABA). Electrically evoked spiking in cell B was inhibited by GABA, and this inhibition was released by bicuculline (Bic) and 2-hydroxysaclofen (Sac). Spiking activity in cell (C) was blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) but not 3-rs-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid (CPP). Cell (D) was blocked by N-nitro-L-arginine (No-arg) but not CNQX. The recording sites of cells (A–D) were shown in Fig. 1(Ba–d), respectively. Five sweeps were superimposed. Scale bars: 0.2 mV, 1 ms in (A) and (B); 0.1 mV, 1 ms in (C) and (D). An arrow in (Aa) points to an electrical stimulation artifact.

min and 5–20 min after stopping application of CNQX and N-nitro-L-arginine, respectively.

DISCUSSION

The present study provides further evidence that the tecto-isthmo-optic pathway is excitatory, in agreement with the results obtained by recording field potentials [12] and postsynaptic potentials [15] evoked by electrical stimulation of tectum or the tecto-isthmo-optic tract. Though numerous terminals within the pigeon ION are GABA-immunoreactive [21,22], and GABA is immunostained in cells within tectal layers projecting to the ION [6,10], a recent study has indicated that none of the tectal cells projecting to the ION cells are GABA-immunopositive [20]. Therefore, the present finding that spiking activity of centrifugal cells elicited by the tract stimulation is completely abolished by GABA could be explained by action of GABA on its receptors postsynaptic to

GABAergic afferents from some other brain-stem structures [20] and/or to widely branching axons of interneurons within the ION [21], which can be excited by the tract stimulation.

The main finding of the present study is that the tecto-isthmo-optic fibers may use glutamate and nitric oxide as transmitters. Most (75%) of the fibers are glutamatergic and mediated by AMPA receptors. This is supported by an immunohistochemical study [22] demonstrating the existence of numerous glutamate-immunoreactive terminals within the pigeon ION. It is interesting to note that the retino-tectal [13], tecto-ION (present study), and ION-retinal [29] pathways in pigeons may all use glutamate as their transmitters, and at least the first two pathways are mediated by AMPA receptors. The other (25%) tecto-ION fibers may use nitric oxide as a messenger, because their synaptic transmission can be completely blocked by N-nitro-L-arginine. Abolishment of a response by a nitric oxide synthase (NOS) inhibitor suggests that it is mediated by nitric oxide [9], as shown in the cat visual system [5]. Immunohistochemical studies showing nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d)/NOS activity indicate the presence of NOS within the tectal layers projecting to the ION and/or the nucleus itself in quails [17], pigeons [18] and chicken [1,35]. A double-labeling study [20] indicates that most tectal cells stained for NOS in pigeons project to the ION. However, the tecto-isthmo-optic tract is unstained for the enzyme, and immunostained tectal cells are different from those projecting to the ION in chicken [35]. This discrepancy may be due to a species difference. Though choline acetyltransferase-immunoreactivity has been shown in the ION cells and tectal cells in layers 9/10 in pigeons [20], the centrifugal cells examined in the present study are not responsive to iontophoretic application of acetylcholine. This observation may be an example to imply that choline acetyltransferase is a necessary but not sufficient condition for identifying cholinergic cells. The statistical data shows that about 60% of the ION cells only receive glutamatergic fibers, 20% of the cells receive nitric oxidergic fibers, and 20% others receive both glutamatergic and NOergic afferents from the tectum. In the last case, however, some other possibilities can not be ruled out that glutamate and nitric oxide may co-release from these tecto-ION fiber terminals, or alternatively, nitric oxide acts presynaptically to facilitate glutamate release. These suggestions do not contradict with the facts that one-to-one connection between the tectum and the isthmo-optic nucleus has been postulated [15,30,32], and that nitric oxide in the visual system can act as a neurotransmitter postsynaptically and presynaptically [4].

The physiological significance of the ION in visually guided behaviors in birds is still mysterious. It is interesting to note that the ION in ground-pecking birds is much more developed than in birds of prey [7,31], and it mainly receives input from the dorsal retina via tectum and projects primarily to the ventral retina [3,33]. Furthermore, the two parts of the retina may be connected by intrinsic interneurons [2]. Local enhancement of the ventral retina by, for example, a predator in the sky and long-range action on the dorsal retina would result in visual attention switching [3]. Fast transmission of visual information by glutamatergic pathways via AMPA receptors within the retina-tectum-isthmo-optic nucleus-retina loop would help in rapidly switching visual attention.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China and by the Chinese Academy of Sciences.

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