CHOLINERGIC NATURE OF HYPOTHALAMO-CORTICAL EXCITATORY INFLUENCES

S. N. Kozhechkin

UDC 612.825.5-06:612.826.4.014.424

KEY WORDS: visual area; lateral hypothalamus; microiontophoresis; acetylcholine; atropine.

The writer obtained evidence previously of excitation of visual cortical neurons in rabbits during electrical stimulation of some hypothalamic structures: the ventrocaudal zone of the lateral hypothalamic region, the perifornical region, and the supramammilary region [2]. The question of identification of the mediator of these hypothalamo-cortical excitatory connections has arisen.

The most likely mediator transmitting nonspecific information to the neocortex from these phylogenetically old parts of the brain such as the reticular formation, hypothalamus, and so on, is acetylcholine (ACh). It is known that ACh, if applied microiontophoretically, excites single cortical neurons [7, 11] and participates in the functioning of the cortical arousal system [6]. ACh and enzymes of its synthesis and inactivation are present in the cerebral cortex [4, 8], and their content is reduced by undercutting of the cortex and its isolation from the subcortex [3, 5].

In the investigation described below ACh and atropine were applied by microiontophoresis to single visual cortical neurons in order to obtain direct evidence that ACh is the mediator of hypothalamo-cortical excitatory influences.

EXPERIMENTAL METHOD

Experiments were carried out on 19 adult rabbits weighing 3.0-3.5 kg. General anesthesia was not used. Areas of possible painful sensations were infiltrated with the long-acting local anesthetic proloncain. The animals were curarized (5 mg/kg diplacin) and artificially ventilated. The central barrel of a five-barreled micropipet, filled with 3 M NaCl, was used to derive action potentials extracellularly from visual cortical neurons in the focus of maximal activity. The remaining barrels were used for microiontophoretic application of the following substances: acetylcholine chloride (0.3 M, pH 4.0), atropine sulfate (0.3 M, pH 4.5), and sodium glutamate (0.5 M, pH 7.1). One of the barrels, filled with 3M NaCl, was used to compensate for current artifacts.

Bipolar nichrome electrodes (diameter 0.15 mm, interelectrode distance 0.2 mm) were used to stimulate the hypothalamus. The parameters of the stimulating pulses were: 12-200 μA, 0.1 msec, 75 sec^{-1}; 1 min. Electrodes were inserted into the lateral hypothalamic region (AP −2.5 to AP +2.5; S 1.5-2.5; V −2 to V −4) in accordance with stereotaxic coordinates from Fifkova and Marsala's atlas [1]. The location of the tip of the stimulating electrode was verified histologically (staining by Nissl's method) after electrolytic destruction.

The ongoing mean frequency of neuronal discharges was recorded in analog form by means of a frequency meter actually during the experiment (Fig. 1). Unit activity was recorded simultaneously on magnetic tape and later subjected to statistical analysis by means of a Neiron-1 analyzer and Biokod-1 converter (Figs. 2 and 3). Integral (cumulative) histograms based on 50 realizations (Δt = 5.0 msec) were obtained by the synchronous summation method.

EXPERIMENTAL RESULTS

On microiontophoretic application ACh increased the spontaneous discharge frequency of more than half (56 of 95) of the visual cortical neurons (Table 1). Some neurons (30 of 95) were insensitive to ACh and the activity of other cells was reduced by ACh (9 of 95).

Laboratory of Pharmacology of the Nervous System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 10-12, May, 1982. Original article submitted January 4, 1981.

 0007-4888/82/9305-0546$07.50

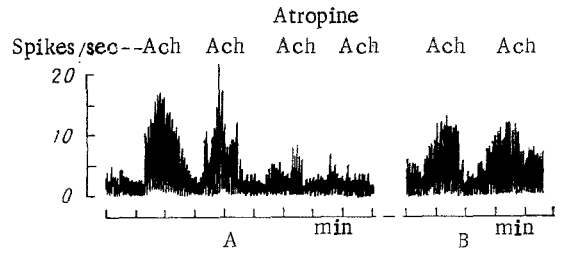

Fig. 1. Reduction of excitatory effect of ACh by atropine. A) Responses of neuron to ACh after microiontophoretic application of atropine; B) responses of neuron to ACh 5 min after end of application of atropine. Abscissa, time (in msec); ordinate, discharge frequency of neuron (spikes/sec). Horizontal lines mark duration of microiontophoretic application of substances; numbers beneath them show strength of currents (in nA). Broken line represents control application of Na^+ by a current of 50 nA. Spontaneous activity of neuron maintained by constant application of sodium glutamate (5 nA).

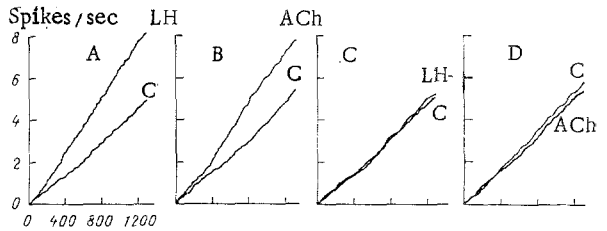

Fig. 2. Blocking by atropine of excitation of a neuron evoked by stimulation of ventrocaudal zone of lateral hypothalamic region (AP +2.5, S 1.5, V −3.75) and microiontophoretic application of ACh. A, B) Before microiontophoretic application of atropine; C, D) after atropine. LH) Lateral hypothalamus; C) control, 1 min before effects of LH and ACh. ACh applied by current of 16 nA, atropine by a current of 7 nA. Duration of application of both agents and of hypothalamic stimulation 1 min. Abscissa, time (in msec); ordinate, frequency of spikes.

Atropine reduced or completely blocked the excitatory effect of ACh (53 and 56 neurons). It will be clear from Fig. 1A that ACh, when applied microiontophoretically by a current of 40 nA, caused a well reproducible increase in the spontaneous discharge frequency of the neuron from 2.5-5 spikes/sec to over 15 spikes/sec. If ACh was applied by a current of the same strength, applied 0.5 min after microiontophoretic application of atropine (14 nA), it gave a much weaker excitatory effect, and if the test was repeated 2.5 min after the beginning of application of atropine, it had no effect at all on unit activity.

The blocking of the excitatory effect of ACh usually lasted several minutes (from 2 to 15 min) after the end of application of atropine. Recovery of the excitatory effect of ACh 5 min after the end of application of atropine can be seen in Fig. 1B.

Evidence of the specificity of the cholinolytic effect of atropine is given by the fact that the excitatory action of sodium glutamate was not reduced after application of

TABLE 1. Correlation between Different Types of Visual Cortical Unit Responses to Electrical Stimulation of Lateral Hypothalamus and Microiontophoretic Application of ACh

ACh	Lateral hypothalamus			Total
	+	−	No effect	
+	21	18	17	56
−	0	0	9	9
No response	3	6	21	30
Total . . .	24	24	47	95

Legend. Numbers denote number of neurons; plus sign indicates increase, minus sign a decrease in discharge frequency.

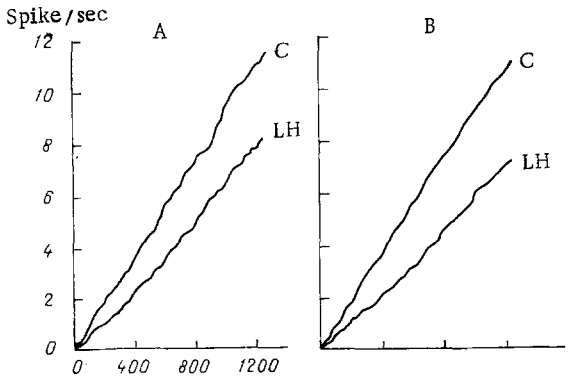

Fig. 3. Resistance of neuronal depression evoked by stimulation of rostral zone of lateral hypothalamus (AP 0; S 2.0; V −2.5) to atropine. A) Before, B) after application of atropine (48 nA). Remainder of legend as to Fig. 2.

atropine in a dose sufficient to exhibit distinct antagonism with ACh. The spontaneous discharge frequency of the neuron illustrated in Fig. 1 was kept artifically at the assigned (increased) level (3-5 spikes/sec) by constantly applying sodium glutamate. Atropine thus does not affect spontaneous activity, i.e., it does not reduce the excitatory effect of sodium glutamate.

The cholinolytic effect of atropine could be observed when comparatively weak microiontoelectrophoretic currents were used (5-30 nA). The membrane-stabilizing (local anesthetic) action of atropine, expressed as a reduction in amplitude of action potentials of the neurons, was observed only when currents of over 50 nA were used.

During electrical stimulation of different zones of the lateral hypothalamic region, both an excitatory (24 of 95 neurons) and an inhibitory (24 of 95 neurons) effect was observed on visual cortical unit activity. The discharge frequency of half of the neurons tested (47 of 95) was unchanged by hypothalamic stimulation. An excitatory effect was observed more often during stimulation of the ventrocaudal zones of the lateral hypothalamic region (16 of 24). Characteristically, mainly those neurons whose activity increased under the influence of microiontophoretically applied ACh were excited (Table 1).

In 17 of 24 neurons excitation evoked by hypothalamic stimulation decreased or was completely suppressed by microiontophoretic application of atropine. It will be clear from Fig. 2A that in response to stimulation of the ventrocaudal zone of the lateral hypothalamic

region unit activity increased from 5 to 8 spikes/sec (an upward shift of the curve). A similar effect was observed during microiontophoretic application of ACh to this same neuron by a current of 16 nA (Fig. 2B). Microiontophoretic application of atropine (7 nA) completely abolished both the facilitatory effect of hypothalamic stimulation (Fig. 2C) and excitation of the neuron evoked by ACh (Fig. 2D).

The more rostrally the tip of the stimulating electrode was located in the lateral hypothalamic region the more often visual cortical unit activity was observed to be inhibited. This inhibition, by contrast with hypothalamic excitation, was not reduced in a single case (0 of 24) by atropine. It will be clear from Fig. 3 that depression of unit activity (a downward shift of the curve, from 11 to 8 spikes/sec) under the influence of stimulation of the rostral zone of the lateral hypothalamic region not only was not reduced, but was actually increased a little by microiontophoretic application of atropine (48 nA). It must be emphasized that the activity of neurons inhibited by hypothalamic stimulation was not reduced by ACh (Table 1).

It follows from the data described above that excitation of visual cortical neurons evoked by stimulation of the ventrocaudal zone of the lateral hypothalamic region is due, unlike hypothalamic inhibition, to activation of acetylcholine receptors, evidently of the muscarinic type. This is shown both by the correlation between the ability of the neurons to increase their activity under the influence of hypothalamic stimulation and their ability to be excited by ACh, and the possibility of blocking of both excitatory effects by the muscarinic cholinolytic drug atropine.

The results of these experiments agree with those of histochemical investigations, showing that cholinergic fibers in the cerebral cortex arise in various subcortical structures, including hypothalamic [9, 10]. The cholinergic nature of nonspecific connections of the mesencephalic reticular formation with the visual and sensomotor cortex was convincingly proved in [12, 13].

It is not yet clear whether hypothalamo-cortical influences are due to activation of mono- or (and) polysynaptic pathways. The method of microiontophoretic application, by contrast with systemic administration of drugs, enables the mediators to be identified with a sufficient degree of reliability, at least in the last, cortical stage of these connections.

LITERATURE CITED

1. J. Bures, M. Petran, and I. Zachar, Electrophysiological Methods of Investigation [Russian translation], Moscow (1962), p. 384.
2. S. N. Kozhechkin, S. D. Zhadina, G. G. Bondar', et al., Zh. Vyssh. Nerv. Deyat., No. 6, 1274 (1975).
3. B. Collier and J. R. Mitchell, J. Physiol. (London), 188, 83 (1967).
4. C. O. Hebb, Physiol. Rev., 37, 196 (1957).
5. C. O. Hebb, K. Krnjević, and A. Silver, Nature, 198, 692 (1963).
6. K. Krnjević, Anesthesiology, 28, 100 (1967).
7. K. Krnjević and J. W. Phillis, J. Physiol. (London), 166, 296 (1963).
8. K. Krnjević and A. Silver J. Anat., 99, 711 (1965).
9. P. R. Lewis and C. C. D. Shute, Brain, 90, 521 (1967).
10. C. C. D. Shute and P. R. Lewis, Brain, 90, 497 (1967).
11. R. Spehlmann, J. Neurophysiol., 26, 127 (1963).
12. R. Spehlmann, Brain, 94, 139 (1971).
13. R. Spehlmann and K. Downes, Brain Res., 74, 229 (1974).