tion of acetylcholine release under the influence of 4AP, i.e., a unique method of activating the process of mediator secretion. Only by direct evaluation of the state of nicotinic cholinergic receptor structures in the subsynaptic membrane could this problem be solved, and for the moment only the discrete character of the shift in amplitude parameters during observation of the effects of 4AP over a period of time makes the first alternative preferable. However, in either case the phenomenon of spontaneous synchronization of mediator release leading to the appearance of spontaneous EPP, can be regarded as a unique model of the process of acetylcholine liberation in response to the arrival of a nervous impulse and to the entry of calcium ions into the nerve ending. The action of 4AP on synaptic transmission is evidently not confined to a change in the character of pulsed electrogenesis in the presynaptic membrane, but also involves the specific mechanism of acetylcholine secretion.

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MICROIONTOPHORETIC STUDY OF INTERACTION BETWEEN CYCLIC PURINE NUCLEOTIDES AND MEDIATORS ON CORTICAL NEURONS

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Statistically significant evidence of selective interaction between noradrenalin and cyclic AMP and also between acetylcholine and cyclic GMP was found by microiontophoretic application of the cyclic purine nucleotides and mediators to neurons of the rabbit cerebral cortex. These investigations of interrelations of cyclic AMP and cyclic GMP at the single unit level yielded facts suggesting their functional interaction with other systems of intracellular regulators at several different levels.

KEY WORDS: cyclic purine nucleotides; mediators; neurons of the cerebral cortex.

The intracellular mechanism responsible for effects of external signals and stimuli of different types is one of the most important of general biological problems at the present time. There is no question that the "secondary messenger" hypothesis put forward by Sutherland [14] has led to a more intensive and purposive

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TABLE 1. Responses of Cortical Neurons to Mediators and Cyclic Nucleotides when Applied Separately and Together

Type of response	NA	ACh	Cyclic AMP	Cyclic GMP	NA + cyclic AMP	ACh + cyclic GMP	NA + cyclic GMP	ACh + cyclic AMP
Excitation	2	63	4	37	0	41	13	11
Inhibition	70	2	68	6	59	3	17	20
No response	24	26	22	31	6	14	12	16
Total number of neurons	96	91	94	74	65	58	42	47

study of this problem. This hypothesis has also proved constructive for the study of the molecular basis of the activity of brain systems and particularly in connection with the experimental study of some aspects of integrative single unit activity. In the writers' view, investigations of the first stage in the "integrative activity" of the neuron, when interaction between neurotransmitters and specific receptors of the postsynaptic membrane "triggers" complex biosynthetic processes in the cytoplasm, on the basis of which incoming information is processed by the nerve cell [1], is most interesting. At the present level of our knowledge we are still a long way from understanding the concrete mechanisms of interaction between neurotransmitters and their secondary messengers – the cyclic purine nucleotides: cyclic adenosine-3',5'-monophosphate (AMP) and cyclic guanosine-3',5'-monophosphate (GMP), or indeed of their functional relations with each other. In particular, there is very little information in the literature on investigation of these interactions at the single neuron level.

The object of the present investigation was accordingly to make an experimental study of interaction between cyclic purine nucleotides and various neurotransmitters and to examine the character of interaction between cyclic AMP and cyclic GMP on the same neuron.

EXPERIMENTAL METHOD

Acute experiments were carried out on uncurarized and unanesthetized adult cats, fixed in a stereotaxic apparatus. Spike activity of sensomotor cortical neurons was recorded extracellularly and biologically active substances were applied to these cells by microiontophoresis. Five-barreled coaxial microelectrodes, designed in the laboratory, were used in the experiments. The recording micropipets and the side barrels containing glass wool were filled immediately before the experiment with aqueous solutions of the following substances: the recording electrode with 3M NaCl, the side barrels with 0.5M noradrenalin (NA) pH 7.0, 0.5M acetylcholine (ACh) pH 5.5, 0.1M cyclic AMP pH 7.5, and 0.01M cyclic GMP pH 7.5. These biologically active substances were conducted by currents of between 45 and 230 nA, the mediators by positive and the cyclic nucleotides by negative currents. The application time varied from 10 sec to 1 min. In most experiments the biologically active substances were applied by currents of 60 nA for 30 sec. Potentials recorded after preamplification were standardized into square pulses, which were then recorded on an automatic pen writer. At the same time, by means of a simple integrator, the pulse repetition rate was transformed into voltage and recorded as an integral curve. The response of the neuron to application of biologically active substances was considered to be significant if spontaneous activity was changed by not less than 50% toward either an increase or a decrease in frequency. The significance of the results was assessed by nonparametric methods, using the criterion of signs.

EXPERIMENTAL RESULTS

In 28 experiments, 106 spontaneously discharging cortical neurons were recorded and studied. The results are summarized in Table 1.

Of the 96 neurons tested, 72 (75%) were sensitive to NA, and 73% ($P < 0.05$) gave an inhibitory type of response. Application of ACh evoked a response in 71% of neurons, most of which (69%, $P < 0.05$) responded by excitation of spike activity. The response to the mediators reached a maximum on average 2-5 sec after the beginning of application. The action of the substances lasted 5-30 sec after their application had ended (Fig. 1: 1). Sensitivity to cyclic AMP was found in 77% of cortical neurons and the predominant type of response was a decrease in the frequency of spike activity (in 70% of cases, $P < 0.05$). Of 74 neurons tested by application of cyclic GMP, 43 (58%) responded, and the predominant response (50% of cases, $P < 0.05$) was an increase in the frequency of spike activity. Responses of the nerve cells to cyclic nucleotides differed from their responses to neurotransmitters. For instance, the response to cyclic AMP reached maximal intensity 5-25 sec after the beginning of application, and their effects were still well marked for 1-5 min after the end of application (Fig. 1: 1).

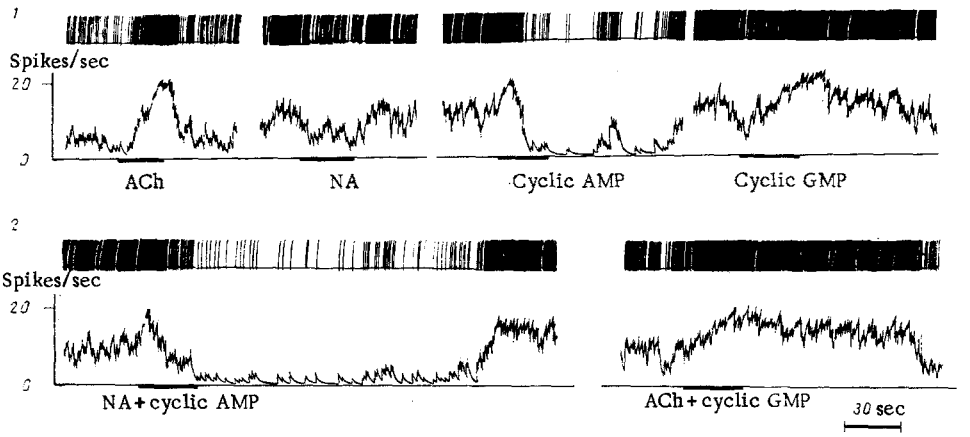

Fig. 1. Responses of cortical neuron to microiontophoretic application of cyclic purine nucleotides and mediators. 1) Responses to NA, ACh, cyclic AMP, and cyclic GMP; 2) responses to simultaneous application of NA and cyclic AMP, and ACh and cyclic GMP. Time of application of all biologically active substances 30 sec, strength of current 60 nA. Top part of each fragment shows unit activity, bottom part synchronous trace of frequency integrator.

Sensitivity to both cyclic nucleotides was shown by 73% of cortical neurons. Most neurons responded in different directions to application of the two cyclic nucleotides: by inhibition of spike activity to cyclic AMP and by excitation to cyclic GMP. Responses in the same direction were found in only five cases, two neurons increasing and three reducing the frequency of their spike activity. During repeated applications of the nucleotides mutual lowering of the threshold of sensitivity of the cells was observed, the period from the beginning of application to appearance of a marked response was shortened, and the time of action after the end of application was lengthened. Cyclic AMP evoked responses to cyclic GMP in some neurons that did not so respond before its application.

Analysis of types of responses of the neurons to application of cyclic nucleotides and neurotransmitters revealed statistically significant correlation ($P < 0.05\%$) between the character of responses to NA and cyclic AMP and also to ACh and cyclic GMP. Selectivity of interaction between the corresponding biologically active substances was exhibited as lowering of the threshold of sensitivity of the neuron to the mediator after application of its synergic nucleotides, or even the appearance of a response, hitherto absent, to the mediator and prolongation and potentiation of the responses to mediators by the nucleotides.

During simultaneous application of NA and cyclic AMP the absolute majority of neurons (91%, $P < 0.05$) responded by inhibition, and often complete inhibition of spike activity was observed (Fig. 1: 1, 2). It is interesting to note that all the neurons tested responded in this way, even if their responses to separate application of these substances had been in opposite directions.

Meanwhile, combined application of ACh and cyclic GMP usually evoked an excitatory response in 71% ($P < 0.05$) of neurons studied. The combined application of these biologically active substances characteristically evoked a stronger and more prolonged response than their application separately (Fig. 1: 1, 2). As a result of simultaneous application of the opposite pairs – NA and cyclic GMP, ACh and cyclic AMP – no statistically significant differences ($P > 0.1$) were found in the responses of the neurons (Table 1).

The correlations between responses of cortical neurons to neurotransmitters and cyclic purine nucleotides described above were confirmed by experiments in which all the biologically active substances were applied to the same neuron. Of 80 neurons tested 67 ($P < 0.05$) responded in the same direction to NA and cyclic AMP, whereas 44 of 70 neurons ($P < 0.05$) responded to ACh and cyclic GMP.

The results of these experiments, showing the selectivity of interrelations between NA and cyclic AMP and also between ACh and cyclic GMP, agree with data in the literature on the specificity of action of cyclic purine nucleotides on cholinergic and adrenergic mediator systems obtained by investigation of cerebellar neurons and Betz cells [5-7, 15]. This supports the hypothesis that cyclic AMP and cyclic GMP are secondary messengers of these mediator systems at the single neuron level in the mammalian CNS [8, 9]. Although certain difficulties arise in the interpretation of microiontophoretic data [2], the considerable difference observed in the principal temporal parameters of responses of the cortical neurons to mediators and cyclic

nucleotides, and also the effects of interaction between these substances, indicate that cyclic nucleotides, when applied extracellularly, as a rule exert their effects through cytoplasmic rather than membrane receptors [4, 12]. The present experiments with application of cyclic AMP and cyclic GMP also showed that most cells of the mammalian cerebral cortex are sensitive to both nucleotides. It can evidently be concluded that both systems of secondary messengers are present in most central neurons and are in an active functional state [13]. Meanwhile the character of their interaction observed on the same neuron suggests that the physiological effects of cyclic purine nucleotides cannot be satisfactorily explained purely on the basis of antagonistic relations between them. In the writers' view, cyclic purine nucleotides can perform the functions of intracellular regulators in the nervous system sufficiently effectively only through specific interaction, at many different levels, with other "universal" intracellular and extracellular regulators such as calcium and magnesium ions, prostaglandins, neuropeptides, and vitamins. Experimental data obtained mainly by biochemical methods using relatively simple biological models have recently been obtained which confirm this hypothesis [3, 10, 11].

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