SPONTANEOUS SYNCHRONIZATION BY 4-AMINOPYRIDINE OF ACETYLCHOLINE RELEASE IN THE NEUROMUSCULAR JUNCTION

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The effect of 4-aminopyridine (4AP) on the character of acetylcholine release was investigated by intracellular recording of spontaneous synaptic activity in an isolated rate phrenic nerve – diaphragm preparation. No significant changes were found in the mean frequency and amplitude of miniature end-plate potentials (MEPP) in response to 4AP in concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-3}$M. Meanwhile, 4AP caused the appearance of large spontaneous EPP capable of inducing spreading action potentials. 4AP changed the character of distribution of MEPP amplitudes, converting it to polymodal; in some cases the principal mode was shifted into the region of lower values. It is concluded that 4AP changes the character of acetylcholine release and potentiates spontaneous synchronization.

KEY WORDS: 4-aminopyridine; miniature end-plate potentials; synchronization of acetylcholine release; rat diaphragm.

The compound 4-aminopyridine (4AP), whose membrane effect is due to inhibition of the repolarizing flow of potassium ions [12, 13], has a potentiating effect on synaptic conduction [2, 5, 7, 9], which leads to the recovery of conduction if neuromuscular transmission is disturbed by D-tubocurarine or botulinus toxin [2, 3, 7, 14]. Convincing though indirect proof has been obtained that 4AP exerts its action at the presynaptic level: Potentiation of electrogenesis in axon terminals and increased inflow of calcium ions make the incoming nervous impulse more effective as regards acetylcholine release [7, 9]. However, besides the action of 4AP on the presynaptic impulse, its direct effect on the process of mediator secretion cannot be ruled out. In the investigation described below, which was carried out to study this problem, the effect of 4AP on spontaneous acetylcholine secretion was studied in the neuromuscular junction, i.e., in the absence of presynaptic electrogenesis due to the arrival of a nervous impulse.

EXPERIMENTAL METHOD

Male August rats weighing 120-140 g were used. A neuromuscular preparation consisting of the phrenic nerve and a strip of diaphragm muscle was isolated under ether anesthesia and placed in a constant temperature chamber (35-36°) through which flowed oxygenated (95% O_2 + 5% CO_2) Tyrode solution of the following composition (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 2.0, $MgCl_2$ 1.0, $NaHCO_3$ 12.0, NaH_2PO_4 1.0, glucose 11.0; pH 7.2-7.4. In the course of the experiment the original solution was replaced by a solution containing 4AP (Pimadin from Parmachim, Bulgaria)* in concentrations of 10^{-6} to 10^{-3}M. Spontaneous acetylcholine secretion was assessed intracellularly by recording synaptic electrical activity by the usual method with glass microelectrodes filled with 2.5M KCl solution, with a resistance of 10-20 MΩ. The transmembrane potential and, after further amplification, its variable component and, in particular, miniature end-plate potentials (MEPP) were recorded. The effects of 4AP were assessed by two methods: by measuring the frequency and amplitude of of MEPP in a population of neuromuscular junctions before and after addition of the compound, and during continuous recording of these processes in a single synapse during a change of solution.

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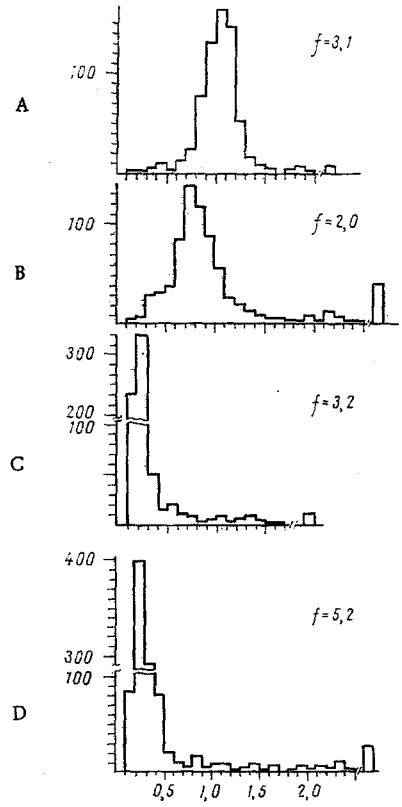

Fig. 1. Effect of 4AP on amplitude of spontaneous synaptic activity in single neuromuscular synapses. Histograms of distribution of MEPP amplitudes in original solution (A) and in solution containing 4AP ($1 \cdot 10^{-4}$M) (B, C, D). Abscissa, amplitude of MEPP (in mV); ordinate, number of impulses; after interruption, number of impulses of higher amplitude than in the last discharge interval. Frequency of MEPP (in Hz) in corresponding fiber shown at top right. Order of histograms (A, B, C, D) corresponds to course of experiment.

EXPERIMENTAL RESULTS AND DISCUSSION

4AP in concentrations of 10^{-6} to 10^{-3}M caused no significant change in the frequency or amplitude of MEPP compared with their mean values in the synapse population before addition of the compound (with a concentration of 10^{-4}M the frequency was 2.5 ± 0.19 spikes/sec before and 2.7 ± 0.5 after addition, and the amplitude 0.56 ± 0.012 mV before and 0.44 ± 0.057 mV after addition; $P > 0.05$). A specific feature of the effects of 4AP was the appearance of "unusual" spontaneous postsynaptic potentials, substantially greater in amplitude than the standard MEPP. These potentials could lead to the development of local or even spreading potentials, causing fibrillation of the muscle fibers. Although the proportion of augmented potentials was low ($8.7 \pm 1.1\%$), it differed significantly ($P < 0.02$) from that in the control ($1.2 \pm 0.4\%$). Analysis of amplitude histograms enables the changes in the amplitude parameters of MEPP under the influence of 4AP to be evaluated more fully (Fig. 1). Under normal conditions [1] the curve of amplitude distribution as a rule is monomodal, more frequently asymmetrical in character, and only in large samples do one or two small additional modes appear (Fig. 1A). Under the influence of 4AP dispersion of amplitudes increased (Fig. 1B, C, D); in most fibers the amplitude histograms had a fairly distinct polymodal appearance, reflecting in particular the formation of spontaneous EPP. Meanwhile, in a considerable number of fibers the principal mode on the amplitude histograms was shifted into the region of lower amplitudes so that the histogram became skew in appearance (Fig. 1C, D). Evidently as a result of this amplitude dissociation a shift in the level of mean values of MEPP under

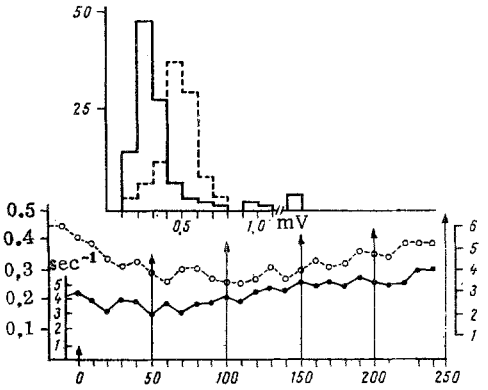

Fig. 2. Effect of 4AP on amplitude-frequency parameters of MEPP in a single fiber. Abscissa, time (in sec) after addition of 4AP in concentration of 10^{-4} M; ordinate: filled circles, frequency of MEPP (in Hz); empty circles, amplitude of MEPP (in mV). Vertical arrows denote fraction of "large" EPSP (in %). Top histogram shows amplitudes before (broken line) and after (continuous line) addition of 4AP.

the influence of 4AP could not be detected. Similar effects of 4AP were observed when the parameters of MEPP were recorded in a single fiber during a change of solution, although in this case, besides the appearance of "giant" MEPP, wavelike changes in the mean value of amplitudes and frequencies of MEPP also were observed (Fig. 2).

Two observations are interesting: a) Grouped MEPP separated from each other by short intervals were as a rule more uniform in shape; b) the spontaneous EPP often had an unusual shape with a more gently sloping peak and relatively long descending limb. This last fact possibly reflects direct postsynaptic effects of 4AP, leading to protraction of repolarization. A special feature of the postsynaptic structures (thin muscle fibers of the diaphragm) distinguished by highly efficient electrogenesis and a low excitation threshold accounts for the ease with which they developed fibrillation. The special sensitivity of the diaphragm to the potentiating effects of 4AP may also be connected with this fact [3]. Meanwhile, dissociation of MEPP amplitudes under the influence of 4AP was evidently due to the presynaptic mechanism of its action. The appearance of unusually large spontaneous potentials could reflect spontaneous synchronization of release of acetylcholine quanta, as had been observed under normal conditions in large samples of MEPP amplitudes [1], but in the present case it was more marked because of the presynaptic membrane effects of 4AP. If the appearance of new peaks on the histograms in the region of lower amplitudes is taken into account, this may indeed be a more complex phenomenon – a change in the actual character of spontaneous release (a "critical point"). The view has recently gained acceptance that spontaneous discharges of mediator, reflected as MEPP, consist of more or less standard sets of subunits, which can also be detected under normal conditions by careful computer analysis because of the polymodal character of distribution of MEPP amplitudes [6, 15]. Under circumstances increasing interaction between calcium ions and special sites on the terminal membrane, this type of situation arises in the posttetanic period [4], and under the influence of 4AP the original character of spontaneous synchronization of mediator release, in the form of a standard set of quanta of mediator, is also evidently disturbed, with the consequent appearance of both subminiature (a "true" quantum response) and unusually large MEPP. Incidentally, this reduction in the calculated value of the quantum could partly resolve one of the contradictions in the quantum-vesicular theory, for the "old" quantum value, estimated from the mean amplitude of MEPP, assumes an unrealistic overfilling of the vesicle with acetylcholine [1].

An alternative to this explanation of the shift in the mode of MEPP amplitude distribution into the region of lower values is to postulate that, just as under the influence of tetraethylammonium [11] or an increase in the external calcium ion concentration [8, 10], besides its presynaptic action 4AP also reduces the sensitivity of the postsynaptic membrane and reduces the original quantum response (the MEPP), in which case the problem is reduced to the further development, or even the appearance de novo, of spontaneous synchroniza-

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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