ACTION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITORS ON THE ACETYLCHOLINE POTENTIAL

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Interaction between acetylcholine (ACh) and its receptor leads to a change in ionic conductance of the postsynaptic membrane of the muscle fiber [8, 9]. The action of ACh can be inhibited in various ways: through a reduction in the number of functioning ACh receptors [10], through disturbance of the kinetics of the ACh receptor channel [1, 11]. The attention of investigators working in this field has recently been drawn to a class of compounds which specifically block Ca channels. It has been shown that verapamil, D-600, and papaverine inhibit or completely suppress the response of the muscle membrane to ACh, blocking the ionic channel of its receptor [1, 3]. However, it is well known that papaverine is a powerful phosphodiesterase (PDE) inhibitor [6]. The question accordingly arises of whether papaverine (and other calcium blockers) can act indirectly on activity of ACh receptors by disturbing the normal cyclic nucleotide level in the cell.

The aim of this investigation was to study the action of PDE inhibitors (theophylline, isobutyrylmethylxanthine, and caffeine) on the sensitivity of muscle fibers to ACh.

EXPERIMENTAL METHOD

Experiments were carried out on the mouse diaphragm muscle. An isolated preparation of the muscle was placed in a constant temperature chamber (28-30°C) through which Liley's solution, of the following composition (in mM): Na^+ 152, K^+ 4, Cl^- 145, Mg^{++} 1, Ca^{++} 2, $H_2PO_4^{-2}$ 0.9, HCO_3^- 16.2, glucose 11; pH 7.2-7.4 maintained by bubbling in carbogen (96% O_2 + 4% CO_2), flowed continuously. PDE inhibitors were used in concentrations of 10^{-5}-$5 \cdot 10^{-3}$ M. In one series of experiments dibutyryl-cAMP ($2.5 \cdot 10^{-4}$, $5 \cdot 10^{-4}$ M) was used after preliminary PDE blockade. ACh was applied to the membrane ionophoretically, only once or with a frequency of 3 Hz. A standard microelectrode technique was used to record the ACh-potential intracellularly.

EXPERIMENTAL RESULTS

The action of Ca blockers and PDE inhibitors was compared with respect to the following parameters: duration and amplitude of the ACh potential during single application of ACh, the rate of development of desensitization, during application of ACh at a frequency of 3 Hz. Previous experiments showed that D-600 and verapamil in a concentration of 10^{-5} M, and papaverine in a concentration of $5 \cdot 10^{-5}$ M, reduce the amplitude of the ACh potential by 50% of the control level (Fig. 1a) and induce strong desensitization of ACh receptors during repetitive application of ACh (Fig. 2c).

PDE inhibitors theophylline and isobutyrylmethylxanthine, in concentration of 10^{-5} and 10^{-4} M, did not affect sensitivity of the muscle fibers to ACh. In higher concentrations ($5 \cdot 10^{-4}$ and 10^{-3} M), theophylline reduced the amplitude and increased the duration of the ACh potential (Fig. 1b). The resting potential and input resistance of the membrane were unchanged. The increase in duration of the ACh potential began immediately after addition of theophylline to the surrounding solution, and reached a maximum after 5 min. Removal of theophylline from the solutions led to rapid and complete recovery of the duration of the ACh potential. For instance, after incubation of the preparation in theophylline (10^{-3} M) for 1 h the recovery time was 30 min. Conversely, the decrease in amplitude of the ACh potential took place uniformly throughout the period of incubation in theophylline. During incubation of the preparation in theophylline (10^{-3} M) for 1 h the amplitude of the ACh potential fell by 40% of its initial level. Removal of theophylline from the chamber and prolonged perfusion

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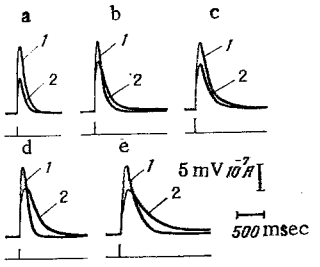

Fig. 1

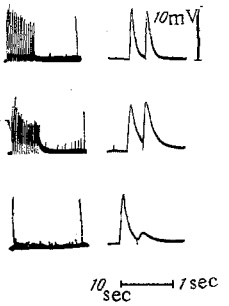

Fig. 2

Fig. 1. Changes in parameters of ACh potential of innervated (a-d) and denervated (e) muscle fibers under the influence of papaverine, theophylline, and dibutyryl-cAMP. Top beam – superposition of ACh potentials recorded in response to application of single doses of ACh before (1) and 30 min after (2) addition of test substances to incubation solution. Bottom beam – strength of pulse of current applied. a) Papaverine ($5 \cdot 10^{-5}$ M), original solution – Liley's solution; b) theophylline (10^{-3} M), original solution – Liley's solution; c) dibutyryl-cAMP, original solution contains 10^{-3} M theophylline; d) caffeine ($5 \cdot 10^{-3}$ M), original solution – Liley's solution.

Fig. 2. Desensitization of ACh receptor of muscle fiber after ACh application with a frequency of 3 Hz in control (a) and in the presence of 10^{-3} M theophylline (b) and $5 \cdot 10^{-5}$ M papaverine (c). On left – series of responses; on right – first two responses. Strength of pulses of current used for application: a) $4 \cdot 10^{-8}$ A, b) $5 \cdot 10^{-8}$ A, c) $1 \cdot 10^{-7}$ A. Duration 5 msec, frequency 3 Hz.

with normal solution led to only very slight recovery of the response to ACh observed during the first few minutes of perfusion. Isobutyrylmethylxanthine behaved similarly.

The other PDE inhibitor, caffeine, in concentrations of $2.5 \cdot 10^{-3}$ and $5 \cdot 10^{-3}$ M, also caused a fall of amplitude and increase of duration of the ACh potential (Fig. 1d). The change in duration of the potential took place similarly to that described for theophylline. The fall of amplitude was not protracted, as occurred after addition of theophylline, but developed parallel to the increase in duration. As a result maximal development of the two effects coincided and was observed 5 min after addition of caffeine to the solution. After removal of the substance from the solution the duration and amplitude of the ACh potential quickly returned to their original values.

PDE inhibitors can thus affect the working of the ACh receptor of the muscle fiber, causing reduction of the ACh potential and an increase in its duration. This last effect was completely absent in the cation of Ca blockers (Fig. 1a).

PDE inhibitors are known to raise the intracellular cAMP level [6]. If the effects of theophylline and isobutyrylmethylxanthine described above are in fact the result of inhibiton of PDE activity, it might be expected that the increase in the cAMP content in the muscle cell caused by addition of dibutyryl-cAMP to the surrounding solution ought to facilitate the development of similar effects. It was found that dibutyryl-cAMP ($5 \cdot 10^{-4}$ M), against the background of theophylline (10^{-3} M) potentiates the action of the latter on the amplitude of the ACh potential (Fig. 1c). This action could not be completely abolished even after rising for several hours. No effect of dibutyryl-cAMP on the duration of the ACh potential could be found. The increase in duration of the ACh potential after preliminary treatment with the test compounds might be explained by their effect on acetylcholinesterase (AChE) activity. However, in experiments on the denervated muscle, when no

AChE is present in the extrasynaptic region, the action of caffeine, theophylline, and isobutyrylmethylxanthine on the kinetics of the ACh potential was preserved (Fig. 1e). Further investigations are needed in order to explain the nature of the observed increase in duration of the ACh potential. Two phases can be distinguished in the action of PDE inhibitors on the amplitude of the ACh potential: 1) the rapid decline of the ACh potential, immediately after additon of the test compounds to the solution, and disappearing quickly after their removal from the solution; 2) a gradual decline of the ACh potential, developing throughout the period of incubation with the test compounds, and not disappearing after their removal from the solution. It is evidently the gradual decline of the ACh potential that is associated with elevation of the cAMP level in the muscle fiber. The biphasic course of the inhibitory effect of the test compounds distinguishes their action from that of Ca blockers. As the writers showed previously, D-600, verapamil, and papaverine cause a marked decrease in value of ACh potential as early as 5 min after their addition to the solution, and the action of these substances is reversible [3]. A difference also exists between effective concentrations of PDE inhibitors and Ca blockers. The latter reduce or completely suppress the ACh potential in concentrations of $8 \cdot 10^{-7}-2 \cdot 10^{-4}$ M. This is two or three orders of magnitude lower than effective concentrations of PDE inhibitors.

Depression of the ACh potential in the presence of Ca-blockers increased substantially during repeated application of ACh at a frequency of 3 Hz (Fig. 2c). The action of PDE inhibitors under similar conditions was therefore studied in a series of experiments. The character of the change in amplitude of the ACh potentials was compared during application of ACh with a frequency of 3 Hz in normal solution (Fig. 2a) and after addition of PDE inhibitors (Fig. 2b). It was found that theophylline, isobutyrylmethylxanthine, and caffeine, in concentrations of $5 \cdot 10^{-4}-5 \cdot 10^{-3}$ M, can accelerate desensitization of ACh receptors during repeated application of ACh. However, the degree of potentiation of desensitization under the influence of these substances differed significantly from the degree of potentiation of desensitization under the influence of Ca blockers. PDE inhibitors never caused such a marked decrease in amplitude of the second response to ACh as papaverine, D-600, and verapamil. The increase in the rate of desensitization under the influence of PDE inhibitors developed immediately after their addition to the solution, and reached a maximum after 5 min; it was not potentiated by the addition of dibutyryl-cAMP and was quickly washed out by normal solution. The desensitizing action of PDE inhibitors is evidently unconnected with any rise of the intramuscular cAMP level. The rate of desensitization of ACh receptors is known to increase with an increase in the Ca^{++} concentration near the inner surface of the muscle membrane [2]. Potentiation of desensitization after preliminary administration of PDE inhibitors may arise as a result of their ability to increase the free Ca^{++} concentration in muscle fibers. This may also be the cause of the rapid decline in amplitude of the ACh potential described above, which was most marked in experiments with caffeine, a substance with a strong Ca^{++}-releasing action on the sarcoplasmic reticulum and surface membrane of the muscle fiber [7].

Cyclic nucleotide PDE inhibitors can thus affect the working of ACh receptors, but their action is not necessarily due to a change in the intracellular cyclic nucleotide level. This conclusion is in agreement with data obtained on other objects [4, 5]. The mechanism of the blocking action of D-600, verapamil, and papaverine on the ionic channel of the ACh receptor is connected neither with inhibition of PDE activity nor with elevation of the cAMP level in the muscle fiber.

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