EFFECT OF HYALURONIDASE ON QUANTUM COMPOSITION AND BINOMIAL PARAMETERS p AND n OF NEUROMUSCULAR TRANSMISSION IN FROGS

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The juxtamembranous layer, which can retain cations, creates the microenvironment of the cell [10] and influences transport of materials through cell membranes [7]. One of the components of the ground substance found in intercellular spaces and in the composition of the basement membrane of the synaptic cleft, is hyaluronic acid [14].

This paper describes an attempt to estimate the possible consequences of enzymic degradation of hyaluronic acid for synaptic transmission in the neuromuscular junction.

EXPERIMENTAL METHOD

Miniature end-plate potentials (MEPP) and evoked end-plate potentials (EPP) were recorded in the dermosternalis muscle of the frog during stimulation of the nerve with a frequency of 1 or 50 Hz. The normal microelectrode technique was used and the microelectrodes were filled with 3 M KCl solution (resistance 10-15 MΩ, tip potential not more than 5 mV). The amplitudes of MEPP and EPP were corrected for lowering of the membrane potential during the experiment; no correction was introduced for nonlinear addition of quanta. The quantum composition (QC) of transmission was calculated as the ratio of the mean amplitudes of EPP and MEPP (QC_1) and as the reciprocal of the coefficient of variation of the amplitudes of EPP (QC_2). The probability of release of a quantum (p) was estimated by the equation $p = 1 - QC_1/QC_2$, and the transmitter reserve n by the equation $n = QC_1/p$ [6]. The solution used had the following composition (in mM): NaCl – 110.0; KCl – 2.5; $CaCl_2$ – 1.0; $MgCl_2$ – 4.0 (pH 7.2-7.4). In some experiments a solution containing 8 mM $CaCl_2$ and $2.6 \cdot 10^{-6}$ M D-tubocurarine, and not containing Mg^{++} ions, was used. Testicular hyaluronidase (from Reanal, Hungary) was added to the corresponding solution immediately before the experiment up to a concentration of 0.01 or 0.1%. In most experiments acetylcholinesterase (AChE) was blocked by armin* (10^{-5} M for 10-15 min, followed by rinsing out with Ringer's solution for 1 h).

EXPERIMENTAL RESULTS

Hyaluronidase in a concentration of 0.01-0.1%, acting in a solution containing 1 mM Ca^{++} and 4 mM Mg^{++}, caused a decrease in amplitude of EPP, and of QC_1 and QC_2 of transmission in response to stimulation with a frequency of 1 Hz (Fig. 1) and an increase in the half-decay time of EPP (to 135% of its initial value in one of the fibers tested in the presence of 0.1% hyaluronidase solution). Lengthening of EPP was connected with the anticholinesterase action of hyaluronidase, for in preparations with inhibited AChE shortening of the half-decay time of EPP was observed with the same reduction of QC. Under the influence of 0.1% hyaluronidase the frequency of MEPP increased by 8% of its initial value, namely 3.15 ± 2.92 MEPP/sec in fibers with intact AChE, and by 1% of the original value, namely 4.71 ± 0.95 MEPP/sec in fibers with inhibited AChE (N = 5 and N = 3 respectively; here and subsequently mean values and errors of the means are given). It is therefore unlikely that the effect of hyaluronidase treatment was connected with a change in osmotic pressure of the solution as a result of formation of products of hydrolysis of substrates by the enzyme. Rinsing out the hyaluronidase led to partial recovery of the amplitude of EPP and of QC of transmission (Fig. 1).

*Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

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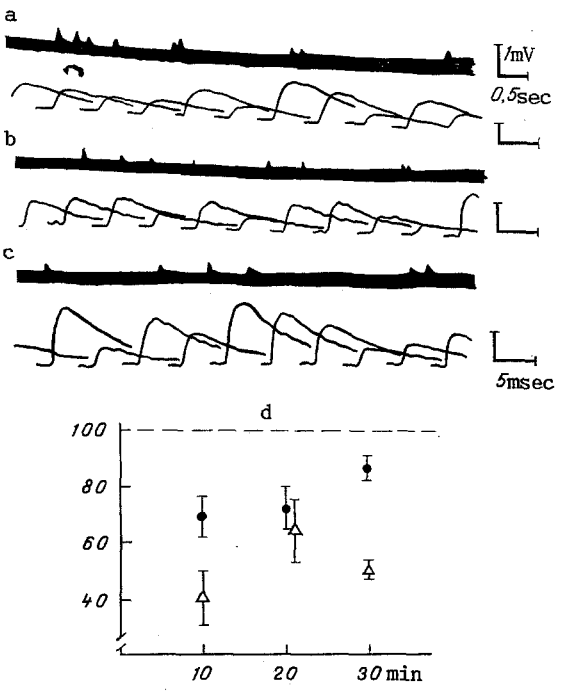

Fig. 1. Effect of hyaluronidase on synaptic potentials, QC, and reserves of accessible transmitter under the influence of a solution containing 1 mM Ca^{++}, during inhibition of AChE. a) Miniature EPP and EPP in background; b) the same, at 10th minute of action of 0.1% hyaluronidase; c) the same after 30 min of rinsing out of the enzyme; d) changes (in % of background value) in QC_1 (circles) and reserves of accessible transmitter (triangles) during the action of hyaluronidase. Mean data for eleven fibers for QC_1 and seven fibers for n ($\bar{X} \pm m$).

Calculation of the parameters p and n, taking into consideration only positive values of probability, showed that in five fibers with intact AChE the value of n at the 10th minute of action of a 0.1% solution of the enzyme was reduced to 36 ± 7% of its initial value 24 ± 11. In two fibers with inhibited AChE, the value of n at the 10th minute of action of the enzyme was 21 ± 2% of its original value of 16 ± 4. During the action of a 0.01% solution of the enzyme, after 20 min n had fallen to 68 ± 12% of its original value of 44 ± 27 in five fibers with intact AChE and to 66 ± 21% of its original value of 13 ± 5 in four fibers with inhibited AChE. The decrease might be explained on the grounds that marginal regions of the synapse were first to be subjected to the action of hyaluronidase, i.e., regions in which recycling of vesicular membranes and endocytosis of choline and other substances conjecturally take place [9]. In that case the appearance of depression of EPP during high-frequency stimulation of the nerve in hyaluronidase solution would be expected. However, in none of the four fibers stimulated for 1-2 sec with a frequency of 50 Hz were any signs of depression of EPP observed under the influence of a 0.1% solution of hyaluronidase (Fig. 2). Under these circumstances, however, a tendency for reduction in the amplitude of the second EPP in the frequency series must be noted (Fig. 2d).

In four of ten fibers tested under the influence of a 0.1% solution of hyaluronidase in preparations with inhibited AChE in a solution with a low Ca^{++} concentration, negative values of p occurred (Table 1). This effect was manifested after the action of the enzyme for more than 15 min, and at the beginning of hyaluronidase treatment an increase in p was observed (Table 1).

In five fibers on which 0.1% hyaluoronidase acted in a solution with a high (8 mM) Ca^{++} concentration, an increase in the amplitude of EPP was observed, and in this case it served as an indicator of QC. Rinsing out of the enzyme caused a further increase in amplitude of the EPP (Fig. 3). Under these circumstances, unlike the action of hyaluoronidase in a solution with 1 mM Ca^{++}, an increase and not a decrease in QC_2 was observed (Fig. 3).

TABLE 1. Effect of Hyaluronidase on Probability of Transmitter Release p in Preparations with Inhibited AChE

No. of fiber	Background value of p	Time of action of hyaluronidase, min			Duration of rinsing out of enzyme, min		
		10	20	30	10	20	30
1	0,197	0,242 (123)	0,527 (268)				
2	0,378	—0,140			0,250 (660)		
3	0,602		0,590 (98)	0,596 (99)	0,317 (53)	0,558 (93)	0,698 (116)
4	0,545		0,322 (59)			0,136 (25)	
5	0,175	0,262 (150)	0,240 (137)			0,264 (151)	0,382 (218)
6	0,067	0,412 (616)	0,515 (769)	0,090 (134)	0,189 (282)	0,407 (607)	0,409 (610)
7	0,087	0,199 (229)	0,423 (486)	—0,335	—1,617	—0,122	0,267 (306)
8	0,354	0,375 (106)	0,405 (114)	0,125 (35)		0,474 (134)	
9	0,187	0,187 (100)	0,247 (132)	—0,748		—0,406	
10	0,266	—0,313	0,146 (550)	—0,142	—0,506	0,176 (660)	—0,007
$\bar{X}\pm m$	0,323±0,064	0,186±0,100	0,361±0,053	—0,069±0,186	—0,273±0,367	0,186±0,114	0,350±0,114

Legend. Changes in p expressed as a percentage of initial value shown in parentheses.

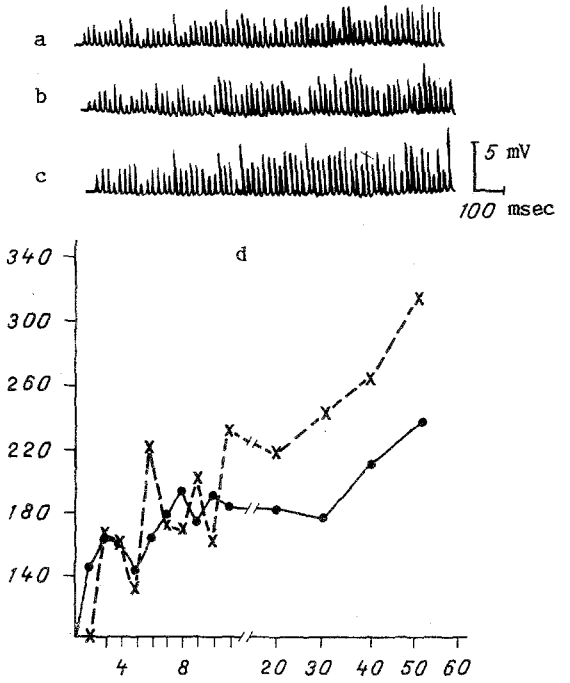

Fig. 2. Effect of hyaluronidase on facilitation during repetitive stimulation in preparations with inhibited AChE. a) Facilitation in background; b) the same after 30 min of action of 0.1% hyaluronidase solution; c) the same after 30 min of rinsing out of the enzyme; d) course of facilitation in background (circles, continuous line), and after 30 min of action of hyaluronidase (crosses, broken line). Mean data for four fibers. Abscissa, serial No. of EPP; ordinate, amplitude of EPP (in % of amplitude of first EPP in series).

These results can be regarded as evidence that the effect of hyaluronidase on QC of neuromuscular transmission is connected with destruction of glycosaminoglycans, which delay diffusion of ions and are incorporated into the matrix of the synaptic cleft and glycocalyx of the nerve terminal [1, 13]. The list of ions which accumulate in the synaptic cleft during activity of the synapse includes acetylcholine and K^+. The more rapid removal of these ions from the cleft on destruction of part of the diffusion barrier could affect the process of synaptic transmission, but at higher rhythms of activity [5]. Changes in QC of transmission observed during the action of hyaluronidase can therefore be more easily connected with changes in the conditions for Ca^{++} exchange between the juxtamembranous layer

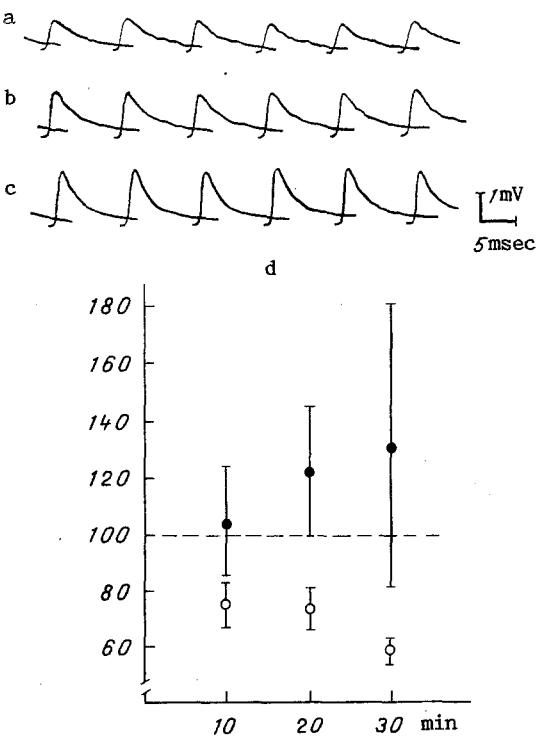

Fig. 3. Effect of hyaluronidase on synaptic potentials in solution containing 8 mM Ca^{++}. a) EPP in background; b) the same after action of 0.1% hyaluronidase solution for 30 min; c) the same after rinsing out of the enzyme for 30 min; d) change (in % of background) in QC_2 of EPP under the influence of hyaluronidase, in solution containing 8 mM Ca^{++} (filled circles), and in solution containing 1 mM Ca^{++} (empty circles). Mean data for 5 fibers (8 mM Ca^{++}) and 11 fibers (1 mM Ca^{++}).

of the nerve terminal and the surrounding solution. We know that hyaluronic acid, like other glycosaminoglycans, possess cation-exchange properties [14]. This suggests that the concentration of ions and, in particular, of Ca^{++} in the layer formed by hyaluronic acid may exceed their concentration in the free solution, if the latter is low enough, and it may be below the Ca^{++} concentration in the solution if the Ca^{++} concentrations in it are high enough. Thus the juxtamembranous layer containing hyaluronic acid can evidently play the role of Ca buffer. The action of hyaluronidase leads to destruction of the normal structure of the juxtamembranous layer and to improvement of exchange with the surrounding solution. It can be tentatively suggested that in a solution with a low Ca^{++} concentration this leads to Ca^{++} deprivation of the juxtamembranous layer and to a fall of QC, whereas in solutions with a high Ca^{++} concentration it leads to an increase in its juxtamembranous concentration and to an increase in QC.

The action of hyaluronidase in a solution with a low Ca^{++} concentration leads primarily to the exclusion of zones with a low probability of transmitter release, located in distal parts of the synapse [3], i.e., to a decrease in n while relatively high values of p are preserved. Meanwhile removal of part of the bound Ca^{++} of the glycocalyx can evidently facilitate binding of Ca^{++} by sialic acids, transmitting Ca^{++} into the terminal during its depolarization [11]. This is confirmed by an increase in p during the first minutes of action of the enzyme in a solution with a low Ca^{++} concentration and by an increase in the amplitude of EPP in a solution with a high Ca^{++} concentration. The continued action of the enzyme in a solution with a low Ca^{++} concentration leads to an even greater fall in the Ca^{++} concentration in more proximal regions of the synapse, and this is accompanied by the appearance of nonhomogeneity and of negative values of p [4, 8]. Insufficiency of Ca^{++} transport into the terminal may be indicated by the decrease in facilitation of the second EPP in the series, observed during high-frequency stimulation. The small increase in the intensity of facilitation subsequently observed in the course of continued stimulation may be linked both with

"switching" of linear facilitation into stepwise, which takes place when the Ca^{++} concentration is lowered [2], and also with the accumulation of K^{+} in the juxtamembranous layer, displacing Ca^{++} from nonspecific binding sites and facilitating the entry of Ca^{++} into the terminal [12]. On the whole, the absence of synaptic depression under the influence of hyaluronidase is evidence against a possible disturbance of recycling under these conditions.

The partial reversibility of the effect of hyaluronidase when acting in a solution with a lowered Ca^{++} concentration might be connected with the high rate of metabolism of glycosaminoglycans [15]. However, the increase in the amplitude of EPP after rinsing out of the enzyme with solution containing 8 mM Ca^{++} indicates that the effects of hyaluronidase are partially connected also with the formation of disaccharide hydrolysis products of the substrates of hyaluronidase. These disaccharides may perhaps also prevent diffusion of Ca^{++}, and rinsing them out improves the access of Ca^{++} to the terminals, thereby increasing QC of transmission in a solution with low, and increasing QC in a solution with high calcium concentration.

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