SENSITIZATION OF CHOLINERGIC RECEPTORS OF STRIPED MUSCLE TO ACETYLCHOLINE

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Sensitivity of the frog rectus abdominis muscle to acetylcholine is increased by the action of choline-potentiating drugs not only through inhibition of cholinesterase activity, but also through sensitization of the cholinergic receptors of the muscle.

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Choline-potentiating (anticholinesterase) drugs have been shown to have not only an anticholinesterase action, but also cholinomimetic [5, 12] and facilitatory [7, 13] effects. In Karasik's opinion [3, 4], a possible component of their effect could be an increase in sensitivity of postsynaptic cholinergic receptors to acetylcholine (AC), i.e., a choline-sensitizing action.

In the present investigation an attempt was made to detect this choline-sensitizing action by comparing the potentiation of the AC effect with inhibition of cholinesterase (CE).

EXPERIMENTAL METHOD

Isolated rectus abdominis muscles of male frogs (Rana temporaria) were placed in a bath containing aerated Ringer's solution at 10-12°. The potentiating action was studied by comparing the amplitude of the contracture produced by AC or carbachol in a concentration of 1×10^{-6} M before and after contact for different durations with the choline-potentiating substances. CE activity was determined by Hestrin's method [10] in muscle homogenate diluted 1:10. AC chloride in a final concentration of 1.7×10^{-3} M was used as substrate. Incubation continued for 1.5 h at 25°. Muscles were taken from 4 or 5 frogs for each test. For determining inhibition of CE in time, each muscle was cut into halves. One half acted as control.

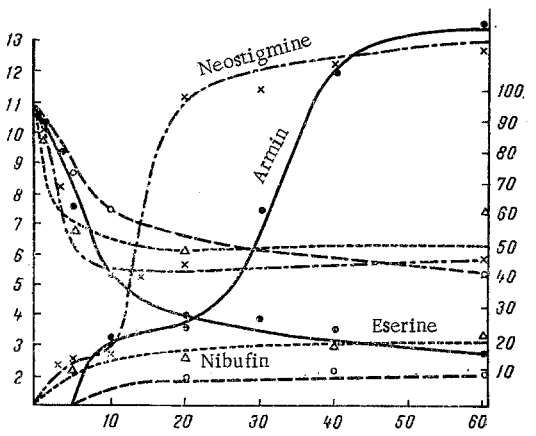

Fig. 1. Increase in amplitude of acetylcholine contracture and change in cholinesterase activity of frog rectus abdominis muscle during contact with choline-potentiating substances for different times. Abscissa: duration of contact (in min). Ordinate: on the left, amplitude of contracture compared with control contracture taken as 1; on the right, percentage of residual cholinesterase activity. Armin used in concentration of 1×10^{-6} M, neostigmine 1×10^{-5} M, eserine and nibufin 1×10^{-4} M.

EXPERIMENTAL RESULTS AND DISCUSSION

At all levels of the effect (Table 1), some degree of correlation was found between potentiating and anticholinesterase activity for contact of muscle and muscle homogenate with choline-potentiating substances for 100 min. However, this correlation was not high enough to indicate that the two were parallel and, consequently, that one effect was thus directly dependent upon the other. The incomplete dependence of the potentiating effect on the degree of inhibition of CE was also confirmed by the actual

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TABLE 1. Comparative Effectiveness of Choline-Potentiating Substances Acting Upon the Frog Rectus Abdominis Muscle

Substance	Negative logarithms of molar concentrations						Increase in acetylcholine effect at $I_{0.5}$
	pP_2	pP_3	pP_{10}	$pM_{0.1}$	$pT_{0.5}$	$pI_{0.5}$	
Nibufin *	5.3	Abs.	Abs.	4.8	Abs.	5.0	2.3
Eserine	5.7	5.4	Abs.	Abs.	Abs.	6.1	1.0
Phosphacol	6.8	6.3	5.1	Abs.	(4.3)	8.1	1.0
Galanthamine	6.8	6.4	5.2	5.3	4.7	5.3	7.5
Neostigmine	6.9	6.6	5.2	4.9	4.8	6.8	2.3
Armin†	7.6	7.2	6.1	5.2	6.1	7.6	2.1
Oxazyl	9.9	9.6	7.1	8.7	6.9	8.1	5.6
r with $pI_{0.5}$	0.71	0.76	0.69	0.04	0.72	—	—0.20
r with $pM_{0.1}$	0.68	0.29	0.52	—	0.55	0.04	0.69
r with $pT_{0.5}$	0.88	0.90	0.96	0.55	—	0.72	0.61

Legend: $pP_{2,3}$ and $_{10}$ denote increase in AC effect by 2, 3, and 10 times; $pM_{0.1}$ denotes mimetic action in 10% of experiments; $pT_{0.5}$ twitches in half the experiments; $pI_{0.5}$ inhibition of CE activity by half.

Note. When calculating the coefficient of correlation r, absence of effect, marked in the table as "absent," was assessed as 0.

* p-Nitrophenyl ester of dibutylphosphinic acid.

† Ethyl-p-nitrophenyl ester of ethylphosphinic acid.

impossibility of achieving a tenfold potentiation with the action of eserine and nibufin, even when they were used in concentrations completely inhibiting the muscle CE. The absence of such a relationship was revealed more clearly still when the magnitude of the potentiation of the AC effect was compared during the action of the compounds in concentrations equally effective relative to CE (inhibition of CE by half—$pI_{0.5}$; Table 1). The results confirm the suggestion that inhibition of CE is not the only reason for the increase in the AC effect.

Changes in potentiation of the AC effect are compared with Fig. 1 with inhibition of CE of the whole rectus abdominis muscle of the frogs for different exposures to the action of armin, neostigmine (in concentrations potentiating the AC effect 10 times in 100 min), nibufin, and eserine (in the maximally effective concentration). The stepwise course of the potentiation curves during the action of neostigmine and armin does not correspond to the smooth course of the curves of CE inhibition, but is associated rather with the nonanticholinesterase action of these compounds. The nonanticholinesterase nature of the second step was confirmed by its disappearance following contact for 100 and 150 min with armin (Fig. 2). Under these circumstances the inhibition of CE did not disappear.

The increase in potentiation in time during the action of armin, oxazyl, galanthamine, phosphacol, and eserine, used in maximally effective concentrations, is illustrated in Fig. 3. These results show that the stepwise character of the effect occurred not only with armin and neostigmine, but also with other choline-potentiating drugs.

The nature of the nonanticholinesterase action cannot be determined from these results. Since the facilitatory action implies an increase in the liberation of AC by the nerve endings, any effect on this liberation must cause a change in the facilitatory action. Addition of magnesium chloride (1.3 mM) to the Ringer's solution, or a decrease in the concentration of calcium chloride (to 0.5 mM), which should lead to inhibition of AC liberation [9, 10], and addition of carbachol (0.03 mM), inhibiting AC synthesis [7], had practically no effect on the potentiation. It can thus be postulated that facilitation of AC liberation has no essential role in the potentiating effect.

The cholinomimetic action ($pM_{0.1}$ in Table 1) did not correlate with the anticholinesterase action but correlated satisfactorily with the degree of potentiation at $pI_{0.5}$ and with potentiation at low concentrations—pP_2. Despite this, no direct relationship between the degree of potentiation and the mimetic action could be deduced because correlation was low, especially with pP_3. It should be added that a mimetic action

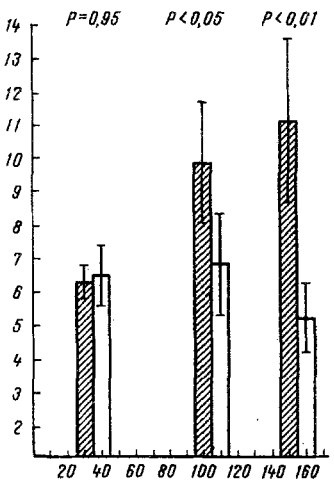

Fig. 2. Changes in potentiation of AC effect by armin after rinsing of the frog rectus abdominis muscle. Abscissa: time (in min) of contact with armin (1×10^{-6} M). Ordinate: amplitude of contracture relative to control. Shaded column—before rinsing; unshaded column—after rising. Mean error is indicated above and below. T calculated by method of paired comparisons.

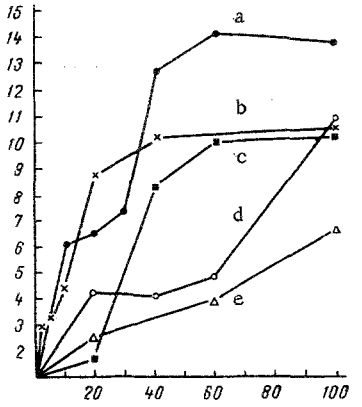

Fig. 3. Increase in acetylcholine effect as a result of treatment of frog rectus abdominis muscle with choline-potentiating substances. Abscissa: duration of contact (in min). Ordinate: increase in acetylcholine effect. Substances used in maximally effective concentrations: a) armin 1×10^{-5} M; b) oxazyl 1×10^{-7} M; c) galanthamine 1×10^{-5} M; d) phosphacol 1×10^{-5} M; e) eserine 1×10^{-4} M.

was observed in 100% of the experiments only during the action of armin and galanthamine, whereas a potentiating effect was always produced.

Hence, the nonanticholinesterase action, concerned along with the anticholinesterase action in potentiation of the AC effect, is neither facilitatory nor mimetic. The only possible solution is to postulate the presence of a sensitizing action. This hypothesis is confirmed by the fact that compounds possessing marked potentiating action (armin, neostigmine, and galanthamine), in the same concentrations and with the same duration of contact, also potentiated the effect of carbachol (a cholinomimetic compound not hydrolyzed by CE). At the same time, compounds not showing any additional potentiating action (eserine and nibufin) did not potentiate the carbachol effect. According to data in the literature, potentiation of the carbachol effect is also observed during the action of phosphacol [1]. These results confirm the original suggestion that the nonanticholinesterase potentiation of the AC effect is based on sensitization of cholinergic systems of the muscles—a choline-sensitizing action.

In the course of the experiments it was noted that compounds possessing high potentiating activity (armin, neostigmine, oxazyl, and galanthamine) caused twitching of the muscle. Twitching was observed in not more than 30% of the experiments in which phosphacol was used. Concentrations necessary to produce twitching ($pT_{0.5}$) were close to the concentrations potentiating the AC effect by 10 times. The mean time of origin of the twitches was close to the time of reaching tenfold potentiation. Since the concentrations causing twitching correlate more closely with the potentiating activity than with the anticholinesterase activity, it may be considered that the twitching phenomenon is based not only on inhibition of CE [15] and facilitation of AC liberation [2, 12], but also on sensitization of the fast muscle fibers to spontaneously liberated mediator.

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