DEPENDENCE OF THE CHOLINE-SENSITIZING ACTION ON THE TEMPERATURE OF THE MEDIUM

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Experiments on the frog rectus abdominis muscle and the rat ileum in vitro showed that potentiation of the acetylcholine effect after preliminary administration of neostigmine depends on the temperature. The increase in sensitivity in the experiments on the frog muscle reached a maximum at 27°C and on the rat ileum at 39°C. Differences in the degree of potentiation are determined by differences in the intensity of the choline-sensitizing action of neostigmine.

Changes in reactivity to acetylcholine (AC) are determined by changes in the properties of the cholinergic receptors, the electrically excitable membrane, and the sarcoplasmic reticulum [3, 6, 8, 13]. The fact that the maxima of reactivity of the organs to AC and of cholinesterase (CE) activity coincide is evidence of the identity of the changes in sensitivity to AC in CE and in the cholinergic receptor [5, 15].

In the present investigation the effect of temperature on the phenomenon of potentiation of the AC effect observed during the administration of anticholinesterase drugs was studied.

EXPERIMENTAL METHOD

The rectus abdominis muscles of the frog were isolated and placed in aerated Ringer's solution containing, in g/liter: NaCl 6.5, KCl 0.14, $CaCl_2$ 0.12, NaH_2PO_4 0.02, and $NaHCO_3$ 0.3, and stretched by a load of 2 g. Segments of the ileum (10 cm from the cecum), 2 cm long, isolated from albino rats deprived of food for 24 h, were placed in aerated Tyrode solution containing, in g/liter: NaCl 8.0, KCl 0.2, $CaCl_2$ 0.1,

TABLE 1. Maximal Contraction P_{max} (in mm) and Sensitivity (pEC_{50}) of Frog Rectus Abdominis Muscle to AC at Different Temperatures of the Medium and After Preliminary Treatment with Neostigmine ($1 \cdot 10^{-5}$ M)

Temperature (in degrees)	Without neostigmine		After neostigmine	
	P_{max}	pEC_{50}	P_{max}	pEC_{50}
7	15,2	5,15±0,05	16,7	5,60±0,10
10	15,5	5,30±0,05	15,5	6,02±0,10
15	17,1	5,48±0,08	17,5	6,50±0,05
20	16,5	5,38±0,08	17,5	6,51±0,06
25	14,9	5,30±0,10	15,4	6,52±0,06
27	14,5	5,25±0,10	14,9	6,53±0,08
30	12,6	4,90±0,03	14,1	5,99±0,09
35	5,6	3,65±0,10	5,4	3,88±0,20
36	0	0	—	—

Note. Here and in Table 2 confidence limits of the means are given at P = 0.05.

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TABLE 2. Maximal Contraction P_{max} (in mm) and Sensitivity (pEC_{50}) to AC and Neostigmine of Isolated Segments of the Rat Ileum at Different Temperatures of the Medium Before and After Treatment with Neostigmine ($1 \cdot 10^{-8}$ M)

Temp., (in degrees)	Without neostigmine				After neostigmine	
	neostigmine		acetylcholine		acetylcholine	
	P_{max}	pEC_{50}	P_{max}	pEC_{50}	P_{max}	pEC_{50}
15	—	—	6,8±0,5	6,12±0,12	5,5±1,0	6,38±0,09
20	7,1±0,6	5,8±0,3	7,6±0,6	6,24±0,03	7,0±0,5	6,58±0,04
25	—	—	9,1±1,0	6,67±0,11	9,7±0,8	7,21±0,06
30	—	—	9,2±0,9	6,81±0,05	10,2±0,6	7,10±0,06
35	8,1±1,0	6,1±0,2	9,6±0,2	6,96±0,10	10,7±0,4	7,72±0,04
37	5,8±0,7	6,3±0,5	9,8±0,6	6,89±0,06	11,4±0,6	7,79±0,06
39	5,2±0,9	5,9±0,1	9,3±0,9	6,74±0,05	12,1±0,5	7,81±0,03
41	2,3±0,4	2,5±0,7	8,9±1,2	6,53±0,05	8,0±1,2	7,54±0,05
43	—	—	3,2±1,3	6,38±0,06	1,9±1,6	6,68±0,08
45	—	—	3,1±0,2	6,27±0,16	0	0
47	—	—	0	0	—	—

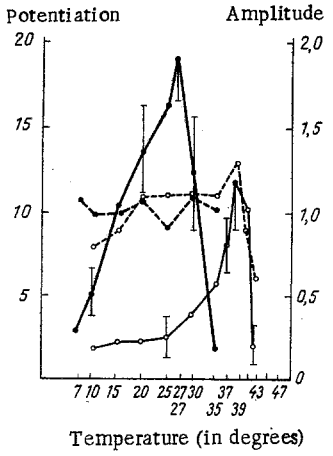

Fig. 1. Intensity of choline-potentiating effect of neostigmine plotted against temperature of medium in experiments on frog rectus abdominis muscle (thick lines) and rat ileum (thin lines). Abscissa: temperature of medium (in deg); ordinate: left) increase in sensitivity to acetylcholine (continuous lines), on right) change in amplitude of contractile reaction when treated with acetylcholine after neostigmine (broken lines). Confidence limits shown by vertical lines.

$MgCl_2$ 0.1, $NaHCO_3$ 0.7, NaH_2PO_4 0.15, and glucose 1, and stretched by a load of 1 g. The isolated organs were bathed at the assigned temperature for 30 min, during which the solution was changed five times. After bathing, neostigmine was added in sufficient quantity to produce a final concentration of $1 \cdot 10^{-5}$ M in the baths containing the rectus abdominis muscles and completely suppressing CE activity. In the experiments on segments of the intestine, neostigmine was used in a concentration at which the hydrolysis of AC was not suppressed: $1 \cdot 10^{-8}$ M [2, 8]. After 30 min without rinsing out the neostigmine, concentration titration curves were plotted [11] and used to find the amplitude of maximal contraction (P_{max}) and negative logarithms of the concentrations required to produce a contraction equivalent to 50% of maximal (pEC_{50}). The degree of potentiation was estimated from the change in EC_{50}.

EXPERIMENTAL RESULTS AND DISCUSSION

The results in Tables 1 and 2 show that treatment with neostigmine for 30 min led in all cases to an increase in sensitivity to AC. However, the degree of potentiation of the AC effect differed at different temperatures (Fig. 1). With an increase in temperature, sensitivity rose and reached a maximum at 27°C in the experiment on the frog rectus abdominis muscle and at 39°C in the experiment on the rat ileum. The potentiation diminished rapidly with a further increase in temperature.

The potentiating effect of neostigmine is determined by its anticholinesterase, facilitatory, depolarizing, and sensitizing actions [1]. The concentrations chosen rule out any effect of the anticholinesterase action on temperature differences in potentiation. The facilitating action is not exhibited on the frog rectus abdominis muscle [10]. Temperature differences in the liberation of AC from nerve endings in the intestine [7, 9, 14] are of no significance in the present experiments, for the decrease in the concentration of Ca^{++} and addition of Mg^{++} to the Tyrode solution did not affect the pattern of change in potentiation. The development of subthreshold depolarization (at least for the rat ileum) can be eliminated as a cause of the increase in potentiation at 39°C because the depolarizing action of neostigmine has a maximum at 37°C. If the increase in potentiation were connected with a change in the depolarizing action, its maximum also would lie at 37°C.

Since all the other possible causes have been ruled out, it must be accepted that the changes in the degree of potentiation of the AC effect with changes of temperature depend on changes in the intensity of the choline-sensitizing action of neostigmine.

The writers showed previously that a change in temperature of the medium leads to significant changes in the properties of both the receptor and the extrareceptor protein systems concerned with the formation of the contractile response of a muscle to AC [3, 4]. Taking these results in conjunction with those of the present investigation, it can be concluded that there is a certain optimum temperature which corresponds to the maximum of complementary interaction between receptor and AC under the conditions used. However, according to Koshland and Neet [12], even at the optimum temperature, complementary interaction with AC is not ideal. Preliminary treatment with neostigmine leads to changes in the cholinergic receptor as a result of which not only is its sensitivity to AC increased, but a different response to temperature is established. As a result the shape of the temperature – effect curve is altered, and the temperature optimum is displaced.

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