PHARMACOLOGICAL SENSITIZATION OF CHOLINERGIC RECEPTORS INDEPENDENT OF CHOLINESTERASE INHIBITION

V. B. Prozorovskii and O. N. Khromova

UDC 615.217.32.015.4:612.814

Experiments on the isolated rat duodenum showed that atropine, in a concentration of $1 \cdot 10^{-13}$ M, does not weaken the response to acetylcholine, while neostigmine, in a concentration of $3.2 \cdot 10^{-8}$ M, increases sensitivity of the duodenum to acetylcholine by 10 times. However, neither atropine nor neostigmine in these concentrations depress cholinesterase activity. Atropine, if added to the bath 10 min before neostigmine, completely prevents neostigmine sensitization. It is postulated that both atropine and neostigmine, in the concentrations used, act on special sensitizing receptors, different from functional receptors. The choline-sensitizing action of neostigmine is, therefore, the result of an independent primary pharmacological reaction unconnected with inhibition of cholinesterase.

* * *

In their experiments on the isolated rat duodenum, Hazard and co-workers [9, 10] showed that atropine, and also other cholinolytics with muscarine-like action, potentiate acetylcholine (AC) effects in concentrations of $10^{-20} - 10^{-15}$ M, do not change these effects in concentrations of $10^{-15} - 10^{-9}$ M, and suppress them in concentrations greater than 10^{-9} M.

Karasik [3], in whose laboratory these experiments were repeated [1], suggested that the potentiating effect of atropine is produced by its action on "allotopic" receptors, causing an increase in sensitivity of the functional receptors to AC. Disappearance of sensitization, in his opinion, can be explained by blocking of the allotopic sensitizing receptor by an increase in the concentration of atropine.

The object of the present investigation was to show that sensitization to AC observed by the application of low concentrations of atropine and neostigmine, actually takes place through their effect on a special receptor, which differs from functional, chlolinesterase receptors. It was necessary to discover whether the potentiating effect of neostigmine is weakened by the action of atropine in a concentration which itself cannot produce sensitization and, at the same time, is too low to inhibit the AC effect.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated duodenum of adult noninbred albino rats. Pieces of intestine were placed in Tyrode solution at 37°, oxygenated with air, and stretched by a 0.5 g weight. After immersion in the bath for 60 min, AC was added in separate portions so that its concentration was increased threefold each time: $1 \cdot 10^{-8}$, $3.3 \cdot 10^{-8}$, $1 \cdot 10^{-7}$, and so on (cumulative curve [13]). Each successive portion of AC was added after the contraction produced by the previous addition had reached a maximum. After a second reproduction of the AC effect, atropine was added to the bath, followed 10 min later by neostigmine. After contact with neostigmine for 30 min, the cumulative curve of AC was again reproduced. The AC concentration at which contraction of the duodenum was equal to the control produced by AC in a concentration of $1 \cdot 10^{-6}$ M (approximately 50% of the maximum) was determined. The ratio between the AC concentrations in the control and experiment ($P = C_c : C_e$) gave the degree of potentiation. If higher concentrations of AC were required in the experiment to obtain an equal effect, P was less than 1, indicating depression of the AC effect. All solutions were made up ex tempore in Tyrode solution. The anticholinesterase activity of neostigmine and atropine was determined in a homogenate of rat small

Central Research Laboratory, Leningrad Pediatric Medical Institute. (Presented by Academician of the Academy of Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 11, pp. 53-55, November, 1969. Original article submitted April 28, 1969.

TABLE 1. Changes in Acetylcholine Effect (M±m) and in Cholinesterase Activity (in %) after Treatment of Isolated Rat Duodenum with Atropine and Neostigmine

Drugs	Concentration (in M)	Change in acetylcholine conc. ($P=C_c:C_e$)	Cholinesterase activity
Atropine	$2{,}4\cdot10^{-3}$	0,01	50
	$1{,}0\cdot10^{-10}$	0,3±0,1	100
	$1{,}0\cdot10^{-13}$	1,4±0,3	—
Neostigmine	$1{,}0\cdot10^{-6}$	—	50
	$3{,}2\cdot10^{-8}$*	10,1±2,8	100
	$1{,}0\cdot10^{-8}$	2,1±0,3	—
Antropine + Neostigmine	$1{,}0\cdot10^{-13}$ + $3{,}2\cdot10^{-8}$	1,1±0,2	95

*Spasm of duodenal musculature develops at this concentration.

intestine (using AC as substrate) by Hestrin's method [11]. Contact with neostigmine and atropine was for 100 min and incubation for 60 min at 37°. Optimum concentrations of atropine and neostigmine were found in control experiments. Either atropine and Tyrode solution or Tyrode solution and neostigmine were poured into the bath for this purpose. By optimum concentration of atropine was meant the greatest concentration in which the AC effect was not suppressed in any of 10 experiments. By optimum concentration of neostigmine was meant the concentration at which the duodenal cholinesterase was not inhibited, but marked sensitization to AC was present.

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show that sensitization of duodenum to AC by neostigmine in a concentration of $3.2\cdot10^{-8}$ M did not occur through cholinesterase inhibition. This possibility was previously demonstrated for the rat's stomach when treated with neostigmine and eserine [8]. Possible subthreshold depolarization likewise was evidently not the cause of the sensitization, for in control experiments in which arecoline was given in a concentration producing an initial contracture of the duodenum, potentiation of the AC effect by only 3.1-3.5 times was observed. The only possible cause of the potentiation was thus a choline-sensitizing action.

According to Table 1, atropine in a concentration of $1\cdot10^{-13}$ M did not reduce the sensitivity of functional cholinergic receptors to acetylcholine (the observed sensitization is not statistically significant). However, atropine in this concentration nevertheless undoubtedly acts on some receptors, because its preliminary (10 min) administration completely prevented the choline-sensitizing effect of neostigmine. The target of its action was evidently the same receptors as which produced potentiation of the AC effect when acted upon by neostigmine. These results indicate that both atropine and neostigmine sensitize the duodenum to AC through their effect on special receptors, which are different from functional receptors. With a change to average concentrations, atropine blocks these receptors, and this is accompanied not only by disappearance of its sensitizing effect proper, but also by depression of the sensitizing effect on neostigmine. Since a concentration at least a million times greater is necessary for blocking the functional cholinergic receptor than for activating the sensitizing receptor, the latter can be considered to have a much greater affinity for atropine and also for neostigmine [1]. This high sensitivity is typical of all allosteric enzymes controlling receptors [4].

The results described above confirm the earlier hypothesis [2, 7] that the cholinergic system contains, as well as a functional receptor responsible for the production of the AC effect, an allotopic sensitizing receptor (or center), action upon which causes an increase in affinity of the functional receptor for AC. The presence of sensitizing receptors is evidently not specific for the rat duodenum alone, for a potentiating effect of low concentrations of cholinolytics has also been observed on the guinea pig intestine [6], the dorsal muscle of the leech [12], and the rat diaphragm [14].

It follows from these results that the choline-sensitizing action is the result of an independent primary pharmacological reaction, unconnected with cholinesterase inhibition. There are grounds for considering that a choline-sensitizing action is possessed not only by neostigmine, but also by other choline-potentiating drugs [5]. Facilitation of mediation by the action of choline-potentiating drugs can thus take place not only through AC stabilization, but also through an increase in the sensitivity of cholinergic receptors to AC. It may be postulated that the increase in amplitude of the effector responses takes place through involvement of some of the sensitized receptors from the receptor pool in the transmission process.

LITERATURE CITED

1. P. E. Dyablova, Byull. Éksperim. Biol. i Med., No. 8, 76 (1964).
2. V. M. Karasik, Uspekhi Sovr. Biol., 21, No. 1, 1 (1946).
3. V. M. Karasik, in: Proceedings of the 10th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], Vol. 1, Moscow – Erevan (1964), p. 63.

4. S. A. Neifakh, in: Problems in General and Age Pharmacology [in Russian], Leningrad (1965), p. 103.
5. V. B. Prozorovskii, in: Problems in General and Age Pharmacology [in Russian], Leningrad (1965), p. 126.
6. A. Ashford, G. B. Penn, and J. W. Ross, Nature, 193, 1082 (1962).
7. J. A. Cohen and C. H. Posthumus, Acta Physiol. Pharmacol. Neerl., 5, 385 (1957).
8. S. Ehrenpreis, Ann. Acad. Sci., 144, 720 (1967).
9. R. Hazard, C. R. Soc. Biol., 155, 1452 (1961).
10. R. Hazard, E. Savini, and A. Renier-Cornec, Arch. Internat. Pharmacodyn., 120, 369 (1959).
11. S. Hestrin, J. Biol. Chem., 180, 249 (1949).
12. H. Lüllmann and W. Förster, Arch. Exp. Path. Pharmakol., 217, 217 (1953).
13. J. M. Van Rossum and F. G. Van der Brink, Arch. Exp. Path. Pharmakol., 143, 240 (1963).
14. H. Walther, Acta Biol. Med. Germ., 18, 733 (1967).