ROLE OF SULFHYDRYL GROUPS OF NICOTINE-LIKE CHOLINERGIC RECEPTORS IN THE MECHANISM OF ACTION OF NEOSTIGMINE

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UDC 615.785.4:615-092.254

Against the background of blocking of sulfhydryl groups with cadmium sulfate, the contractile response of the isolated intestine and uterus of rats to neostigmine is inhibited. The response to acetylcholine and to barium chloride remains. Blocking of SH-groups of the intestine and uterus leads to the disappearance of the stimulant effect of the nicotine-like cholinomimetic DMPP. Blocking of nicotine-like cholinergic receptors with nikohexonium has a similar action on the effects of DMPP and neostigmine. The results obtained indicate the role of sulfhydryl groups of ganglionic nicotine-like cholinergic receptors in the mechanism of the stimulant effect of neostigmine on the intestine and uterus.

Recognition of the leading role of the anticholinesterase effect in the mechanism of action of neostigmine and its analogs in no way rules out the possibility of their direct action on cholinergic receptors [3, 5, 8, 15, 27, 29]. There is now weighty evidence to show that sulfhydryl groups are present in the structure of cholinergic receptors [6, 10, 11, 16, 23, 24], and also information concerning the role of these groups in the mechanism of action of cholinomimetics and cholinolytics with cholinergic receptors [4, 9, 12-14, 19-21].

The object of the present investigation was to study the role of the sulfhydryl groups of cholinergic receptors in the mechanism of action of anticholinesterase drugs.

EXPERIMENTAL

The effect of neostigmine was studied in experiments on an isolated segment of ileum and on the isolated uterine cornu of albino rats.

The animals were sacrificed immediately before the experiment. Contractions of the intestine and uterus were recorded on a kymograph.

The isolated oxygen were placed in a 50-ml dish filled with Kravkov's solution, through which oxygen bubbled continuously. The temperature of the solution (38°) was maintained by means of an ultrathermostat.

Sulfhydryl groups in the tissues were blocked with cadmium sulfate [22] in dilutions of 1×10^{-5}-2×10^{-5}. Neostigmine was tested in dilutions of 1×10^{-6}-5×10^{-7}. The substances used for analysis were tested in the following dilutions: acetylcholine 1×10^{-5}-1×10^{-6}, dimethylphenylpiperazinium iodide (DMPP) 1×10^{-5}-2×10^{-5}, nikohexonium 1×10^{-4}, and barium chloride 2×10^{-4}-4×10^{-4}. Altogether 44 experiments were carried out, 21 of them on segments of the isolated ileum and 24 on the isolated uterine cornua of albino rats.

Experimental Department, Kiev Research Institute of Pediatrics, Obstetrics, and Gynecology.
(Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 4, pp. 72-75, April, 1970. Original article submitted August 6, 1969.

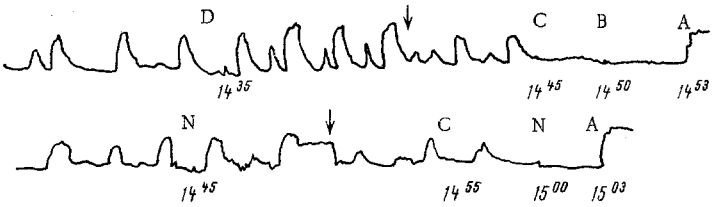

Fig. 1. Comparative effect of neostigmine, DMPP, and acetylcholine on uterine contractions against the background of blocking of sulfhydryl groups (isolated uterine cornu of rat). D) DMPP 1×10^{-5}; C) cadmium sulfate 2×10^{-5}; A) acetylcholine 1×10^{-5}; N) neostigmine 5×10^{-7}; arrow indicates rinsing with Kravkov's solution.

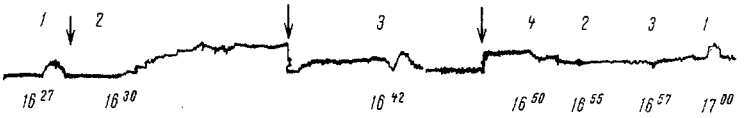

Fig. 2. Inhibition of contractile response of intestine to neostigmine after ganglion blocking (isolated segment of rat ileum). 1) Acetylcholine 1×10^{-5}; 2) neostigmine 1×10^{-6}; 3) DMPP 2×10^{-5}; 4) nikohexonium 1×10^{-4}. Arrow indicates rinsing with Kravkov's solution.

EXPERIMENTAL RESULTS

Administration of neostigmine caused tonic contraction of the muscles of the segments of ileum. When applied to the cornu of the virgin uterus in the same concentrations, neostigmine either had no significant effect or it slightly increased the frequency and amplitude of the contractions.

Administration of cadmium sulfate was usually followed by a decrease in tone of the muscles of the segment of ileum and by complete suppression of contractions of the uterine cornua. Against the background of blocking of sulfhydryl groups by cadmium sulfate, the response of the ileum and uterus to neostigmine either was completely absent or greatly weakened. Meanwhile, the contractile response of the segments of ileum and uterus to acetylcholine and barium chloride either remained unchanged or was only slightly weakened.

Unlike acetylcholine, the nicotine-like cholinomimetic DMPP had no effect whatever against the background of the action of cadmium sulfate, a result very similar to that of the action of neostigmine under similar conditions (Fig. 1).

Repeated additions of neostigmine and DMPP (before administration of cadmium sulfate) for control purposes led to the development of a contractile response similar to that observed initially.

DMPP and neostigmine also produced similar changes against the background of ganglionic blocking of nicotine-like cholinergic receptors due to administration of the gangliolytic drug nikohexonium.

Contractions of segments of the ileum and uterus in response to both drugs were blocked under these conditions, and the response to acetylcholine was substantially unchanged (Fig. 2).

No information on this subject could be found in the literature. Indirect data showing the possible role of SH groups in the mechanism of action of anticholinesterase drugs were obtained by Afonskaya and co-workers [2]. They found that urea, which has the property of loosening the protein molecule and liberating reactive SH groups [1, 7, 26], restored the work of the isolated frog's heart when arrested by armin and nibufin.

Since it is now firmly held that, besides their anticholinesterase action, neostigmine and its analogs also have a direct effect on cholinergic receptors, it can be postulated that the absence of response to

neostigmine in the experiments described above is due to blocking either of the SH group of cholinesterase, or of the SH groups of protein molecules of the cholinergic receptors.

An effect of cholinesterase SH groups seems unlikely because there is no information at the present time to indicate that sulfur-containing amino acids are present in the esteratic area of the active surface of its molecule, whereas the presence of SH groups in cholinergic receptors, as was mentioned above, has been conclusively proved.

The results demonstrate the role of sulfhydryl groups of cholinergic receptors in the mechanism of the response of the ileum and uterus to neostigmine.

In fact, against the background of blocking of SH groups not only was there no response to neostigmine but also to DMPP, which selectively stimulates nicotine-like cholinergic receptors.

Preservation of the response to acetylcholine against the background of blocking of SH groups does not contradict the above hypothesis, but simply suggests that the direct stimulant action of neostigmine evidently extends mainly to the nicotine-cholinergic receptors of the ileum and uterus. After blocking of the nicotine-like cholinergic receptors with nikohexonium, the effect of both DMPP and of neostigmine was absent.

SH groups of noncholinergic components of the ileum and uterus evidently do not play any important role in the mechanism of their contractile response, for the effect of the myotropic agent barium chloride was not significantly modified by previous blocking of SH groups with cadmium sulfate.

These facts now obtained concerning the role of sulfhydryl groups of the protein structures of ganglionic nicotine-like cholinergic receptors in the mechanism of the stimulant action of neostigmine on the ileum and uterus correlate closely with data in the literature concerning the direct nicotine-like cholinomimetic effect of neostigmine and various other anticholinesterase drugs [17, 18, 25, 28, 30, 31].

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