INVESTIGATION OF THE MECHANISM OF CHEMORECEPTION.

REPORT V. THE EFFECT OF TETRAETHYLAMMONIUM ON THE ABILITY OF THE SMALL INTESTINE RECEPTORS TO PERCEIVE VARIOUS CHEMICAL STIMULI

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Sensitivity to acetylcholine and similar substances has been shown for the pressor receptors [10, 16] and chemoreceptors of the carotid body [15], the mechanoreceptors of the skin [9, 14], for pain endings [18], and for endings which react to temperature changes [11]. On the basis of this, several authors [15, 16] regard acetylcholine as the mediator in the transmission of excitation from these sensory formations subsequent to the action of adequate stimuli, while the majority of investigators [4-7, 10, 12, 17] believe that it is perceived by these special structures independent of the basic function of the receptors. The latter concept is based on the fact that by using different gangliolytics (tetraethylammonium (TEA), hexone, etc.), it is possible to block perception of acetylcholine and other gangliostimulators, while leaving intact the basic functioning of the receptors.

The sensitivity of the interoceptors of various organs to the action of acetylcholine and similar substances has been shown extensively in the works of V. N. Chernigovskii [8].

The existence of two basic groups of chemical stimuli, differing in the mechanism of their action [8], has also been established for the receptors of the small intestine and the chemoreceptors of the carotid body [4, 6]. To one of these groups belong acetylcholine, nicotine, lobeline, and other ganglionic poisons, while the other is made up of the so-called anoxic stimuli (anoxia, cyanides, acids).

It is postulated that the action of the latter group is mediated through a change in the metabolism of the cells, which serves as the reason for stimulation of the receptors, while the stimuli of the first group act on the receptors directly. This hypothesis is based on the fact that perception of the anoxic stimuli is markedly easier to disrupt by enzymatic poisons.

In our experiments, investigating the action of monoiodoacetic acid on the intestinal receptors, we confirmed the concept of different points of application for the indicated two groups of chemical stimuli [2, 3]. The question arose of the significance of sensitivity of this region to acetylcholine, and its possible role in the transmission of excitation arising from the action of acids.

We investigated the effect of TEA on the ability of small intestine receptors to perceive various stimuli.

EXPERIMENTAL METHOD

We employed the method of perfusion, using a segment of small intestine with an isolated vascular supply. The action of TEA was realized both by prolonged passage of its solutions ($1 \cdot 10^{-5} - 1 \cdot 10^{-4}$ grams / ml) through the vessels of the intestine, and by simultaneous injection of it into the perfusion fluid. The chemical stimuli (nicotine and acetylcholine in dilutions of from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-3}$, and the acids – lactic 0.5–2% and acetic 0.5–1%) were injected into the current of the perfusion fluid in a quantity of 0.5 ml. The sensitivity of the small intestine receptors was gauged from the magnitude of the reflex changes in arterial pressure, which were recorded in the carotid artery by a mercury manometer. The motor activity of the intestines was recorded with the aid of a Marey capsule connected with a rubber balloon which was inserted into the lumen of the intestinal loop.

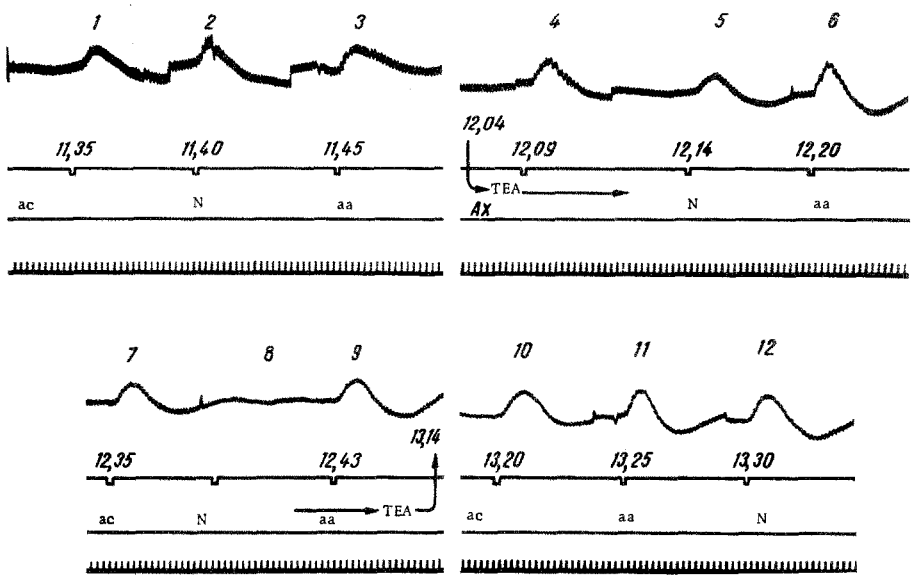

Fig. 1. Reflex changes in the arterial pressure associated with the action of the intestinal receptors of 50 γ of nicotine (N), acetylcholine (ac) and 0.5 ml of 0.5% acetic acid (aa), before passage of TEA solution (1, 2, 3), against the setting of prolonged passage of a $1 \cdot 10^{-5}$ grams/ml solution of TEA (4–9), and with successive washing with Ringer-Lock's solution (10, 11, 12). TEA passage begun at 12:04, ended at 1:14. Meaning of the curves (from above downward): arterial pressure in the carotid artery, mark denoting injection of stimulus; time markings every 5 seconds.

EXPERIMENTAL RESULTS

With prolonged passage of the TEA solution through the vascular system of the intestine, the perception of nicotine by its receptors was completely blocked, which we judged from the absence, under these conditions, of those changes in arterial pressure which normally arise through the action of nicotine. Upon washing out the system with Ringer-Lock's solution, these changes attained the original level. In the pressence of the TEA solution, the reflex reactions which arise from exposing the intestinal receptors to acid initially increased somewhat, and then returned to the original indices, not changing, in the majority of cases, with further passage of TEA through the system. Thus, the experiments of this series indicated that it is possible for TEA to exert a selective blocking action, and also confirmed for the case of the intestinal receptive zone, what had been shown to be true for other sensory formations.

However, in these experiments, investigation of acetylcholine reception yielded an unexpected result. During the course of the perfusion with TEA solution, the reaction in response to exposing the receptors to acetylcholine did not change, or possibly decreased to a varying degree, but never was completely blocked.

Fig. 1 presents the changes in arterial pressure that arose from exposing the intestinal receptors to nicotine, acetylcholine, and acetic acid, under the influence of prolonged passage of TEA solution ($1 \cdot 10^{-5}$ grams/ml). Kymograms 1, 2, and 3 show the starting reactions of the arterial pressure. Kymograms 4, 5, and 6 were obtained 5, 10, and 16 minutes after beginning the passage of TEA, respectively. It is apparent that the stimulatory action of nicotine began to weaken, the reaction to acetylcholine did not change, and the reaction to acetic acid intensified. The potentiating influence of small doses of TEA on the effect caused by the action of acids was observed in the majority of cases.

Thirty minutes after TEA passage was initiated, the reaction to nicotine (8) was completely suppressed, to acetylcholine (7), minimally decreased, and to acid (9) returned to the starting level. On washing with Ringer-Lock's solution all the reactions studied attained their normal levels (10, 11, 12).

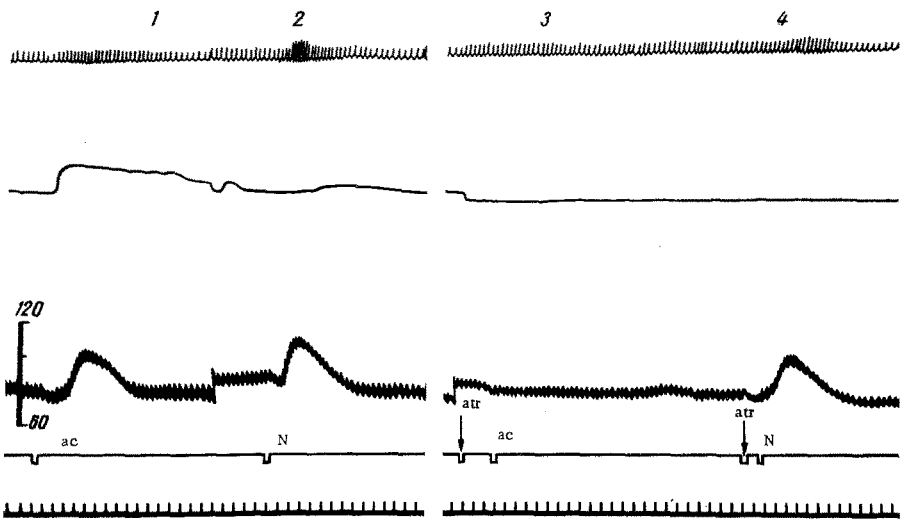

Fig. 2. Reflex changes in the arterial pressure and contraction of the intestinal smooth musculature subsequent to the action on the intestinal receptors of acetylcholine (ac) and nicotine (n), before (1 and 2) and after injection of 1 ml of a solution of atropine in a dilution of $1 \cdot 10^{-5}$ grams/ml (3 and 4). Meaning of the curves (from above downward): respiration, contraction of the intestinal musculature, arterial pressure in the carotid artery, markings indicating injection of the stimuli, time markings (5 seconds).

The same experiments were repeated with a single exposure to the action of TEA. It was established that 1 ml of a 1% solution of TEA completely eliminates the reaction to 50 γ of nicotine, and either slightly decreases or leaves completely unchanged the reaction to the same dose of acetylcholine

These data differ from the results obtained by other authors, in whose works the perception of acetylcholine and nicotine was blocked by a TEA solution in the same amount.

Apparently, the reasons for this contradiction should be sought for in the specifications of the receptive zone which we studied. It is known that, under the influence of acetylcholine, there arises a powerful spasm of the intestinal smooth musculature. It is possible that under these conditions the receptors of the intestinal wall are excited mechanically, and the reflex reaction in response to the action of the preparation, or perhaps a portion of it, is caused specifically by this form of excitation of the receptors, and not by direct action of the acetylcholine.

It is true that nicotine also causes a contraction of the intestinal musculature, but it was shown [1, 9] that the reflex changes in the arterial pressure arise earlier in this case than does the motor discharge. In the case of acetylcholine's action, the reflex shift in arterial pressure and the contraction of the musculature most frequently arise simultaneously.

We undertook an attempt to eliminate this spasm by a preliminary injection of atropine. It was shown that, in the majority of the trials, an injection of 1–2 ml of an atropine solution, ($1 \cdot 10^{-6}$, $1 \cdot 10^{-5}$) significantly diminished the motor activity arising under the influence of 50γ of acetylcholine, and was able to eliminate the spasm completely when smaller doses of acetylcholine were used. It was also demonstrated that, in this case, the reflex reaction of the arterial pressure to acetylcholine administration either disappeared or was decreased substantially. This confirms the hypothesis that was advanced, and indicates the dependence of the latter reaction on contraction of the smooth musculature.

True, the possibility must be considered that atropine exerts a direct blocking action of the cholinoreactive system of the receptors. However, investigation of the reaction to nicotine shows that it was completely unchanged under the influence of the doses of atropine used.

Kymograms 1 and 2 (Fig. 2) present the original reactions of the arterial pressure in response to the action of 5 γ of acetylcholine and 5 γ of nicotine. After injection of 1 ml of atropine ($1 \cdot 10^{-5}$) into the vessels of the

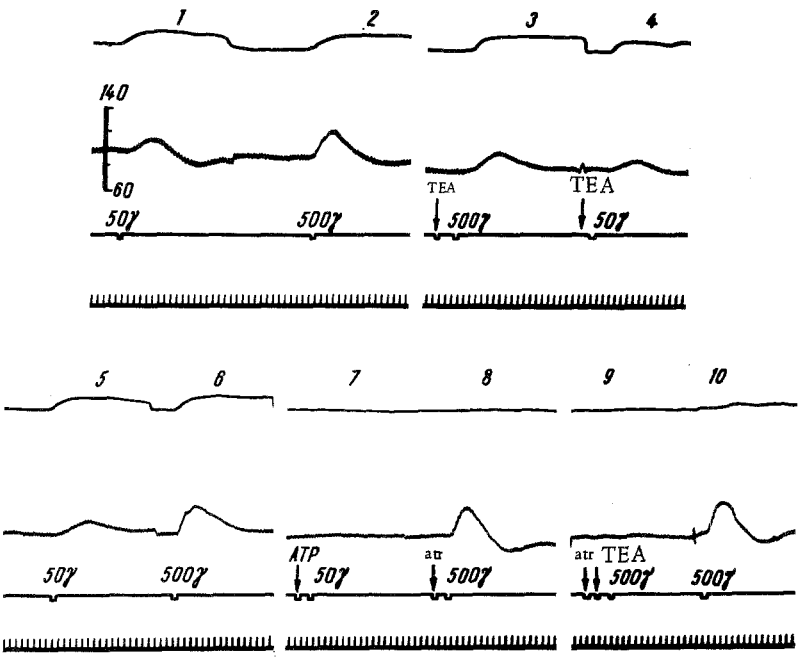

Fig. 3. Changes in the magnitude of the arterial pressure reflex reactions and the intensity of contractions of the intestinal smooth musculature, arising from the action of 50 and 500 γ of acetylcholine, under the influence of simultaneous injections of TEA and atropine. 1 and 2 – injection of acetylcholine; 3 and 4 – the same after injection of 1 ml of a 1% solution; 5 and 6 – repeat injections of acetylcholine; 7 and 8 – the same after injection of 1 ml of atropine ($1 \cdot 10^{-5}$ grams/ml); 9 – the same after the joint action of 1 ml of a 1% solution of TEA and 1 ml of atropine ($1 \cdot 10^{-5}$ grams/ml); 10 – injection of acetylcholine. Meaning of the curves (from above downward): contractions of the intestinal smooth musculature, arterial pressure in the carotid artery, markings indicating injections of stimuli, time markings every 5 seconds.

intestine, contraction of the smooth musculature disappeared, and along with this, the reflex reaction of the arterial pressure to acetylcholine (3), while the effect from nicotine was completely preserved (4).

The obtained results justify regarding the reaction to acetylcholine as consisting of two components, one of which is caused by direct action of the acetylcholine on the cholino-reactive systems of the receptors, while the other is the result of their mechanical stimulation, secondary to contraction of the intestinal smooth musculature; the latter action of acetylcholine, which is not blocked by TEA, has been designated by us as secondary.

The action of small doses of acetylcholine (0.5–5 γ) was completely removed by atropine; apparently, these doses stimulate the receptors primarily through the secondary route. The reaction to 50 γ of acetylcholine, in the majority of trials, was only partially blocked by atropine. This is evidence of increased importance of acetylcholine's direct action on the receptors. With further increase in its concentration, the basic part of the reaction arising in this case was caused by this direct action.

Kymograms 1 and 2 (Fig. 3) show the reactions of the arterial pressure, arising from the action of various doses of acetylcholine on the intestinal receptors. Kymograms 3 and 4 demonstrate the same reactions after exposure to 1 ml of a 1% solution of TEA. It is apparent that the reaction to 500 γ of acetylcholine is depressed to a greater degree than the reaction to 50 γ of the same preparation. Subsequently (5 and 6) there is complete restoration of the reaction levels. With the action of atropine (1 ml $1 \cdot 10^{-5}$), the reaction to 50 γ of acetylcholine (7) is completely blocked, while the reaction to 500 γ of acetylcholine (8) retains its original level, only shortening somewhat. The combination of TEA (1 ml of a 1% solution) and atropine (2 ml $1 \cdot 10^{-5}$) completely blocked this reaction (9). Kymogram 10 shows the restoration of this reaction after TEA and atropine administration. Thus, in this case the reaction also can be clearly divided into two components: TEA lowers it significantly, blocking the direct action of

acetylcholine on the receptors; atropine, taken alone, does not change the magnitude of the reaction, since with the injection of a large dose of acetylcholine the latter preparation's direct stimulatory action on the receptors is sufficient to cause maximal reaction of the arterial pressure. The combination of TEA and atropine, depressing both possible mechanisms of action of acetylcholine, completely eliminates the reaction.

The use of atropine prevents the possibility of secondary action by acetylcholine; after this, the reactions to the latter, as well as to nicotine, are blocked by TEA, but the TEA does not interfere with the reception of acids. This removes the discrepancy between our results and the data of other authors. The obtained results show that acetylcholine does not participate in the conduction of excitation arising from the action of acids on the receptors, and they do not support the presence of acetylcholine transmission in the intestinal receptive zone. The question of the significance of receptor sensitivity in the intestinal region, as well as the sensitivity of other receptor formations toward acetylcholine, remains unresolved. The most widely held opinion is that the action of acetylcholine is a pharmacological phenomenon, reflecting some sort of specialization of the receptor membrane for sensory functioning [13].

SUMMARY

A study was made of the effect produced by TEA on the reception of ACh, nicotine, and acids. As established, TEA blocked the reception of nicotine by the receptors, did not affect the action of acids, and only partially depressed the ACh reception; the part of the reaction in response to ACh which remained after the TEA action was blocked in this case with ATP. A conclusion was drawn that in effecting the reaction of the given area to ACh considerable significance is played both by this direct action upon the receptors and by their mechanical stimulation as a result of musculature spasm; it also followed that the ACh transmission in this area was absent.

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