ROLE OF TRICARBOXYLIC ACID CYCLE IN MECHANISMS OF CHEMORECEPTION

Têng Hsi-hsien

Laboratory of General Physiology (Head – Academician V. N. Chernigovskii) of the Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. V. Parin), AMN SSSR, Moscow
(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 1, pp. 3-8, January, 1961
Original article submitted March 2, 1960

A large number of works [2,9,10,11] devoted to a study of the mechanism of chemoreception of the carotid sinus zone and the intestine have been carried out so far. Many investigators have tried to solve this question by studying the effect of enzyme poisons on the reception of a number of chemical stimuli [5,7,13].

In a study of the chemoreception of the intestine, V. N. Chernigovskii suggested that different chemical stimuli could be divided into two groups according to their mode of action. Stimuli of the first group (carbon dioxide, hypoxia, cyanide, etc.) bring about a change in tissue metabolism, and this results in excitation of the chemoreceptors. Stimuli of the second group (acetylcholine, nicotine, etc.) act directly on the receptors. The experimental basis of this hypothesis is the fact that derangement of tissue metabolism by monoiodoacetic acid or sodium fluoride causes a weakening or the disappearance of the response of intestinal receptors to carbon dioxide, whereas the response to acetylcholine and nicotine remains unchanged [6,7,8].

However, in the works of C. V. Anichkov and M. L. Belen'kii [3,4], it was shown that disturbance of the metabolism in the tissue of the carotid gland by various poisons, particularly sodium fluoride and potassium cyanide, led to a partial or complete suppression of the response of receptors to chemical stimuli of both groups. As a result of these investigations, the authors concluded that the mode of action of these stimuli was bound up with carbohydrate metabolism.

In a study of the chemoreception of the intestine, N. A. Anikina [1] showed that the disagreement between the results of the investigators cited above might have been due to the different doses of enzyme poisons which they used.

In research on chemoreception, various substances have been employed so far for causing derangement of metabolic processes, but the most widely used have been sodium fluoride, monoidoacetic acid, and potassium cyanide. Sodium fluoride and monoidoacetic acid inhibit glycolysis, while potassium cyanide inhibits the cytochrome – cytochrome oxidase system.

In the present work, we investigated the role of the main reactions of the tricarboxylic acid cycle in chemoreceptive reflex processes.

Experimental Method

Thirty-six short-term experiments were carried out on cats under urethane narcosis. Isolated intestines, retaining only nerve connections with the organism, were perfused. We determined the excitability of the receptors from the response of the blood circulation and respiration of the animal to the introduction of a 1 ml solution

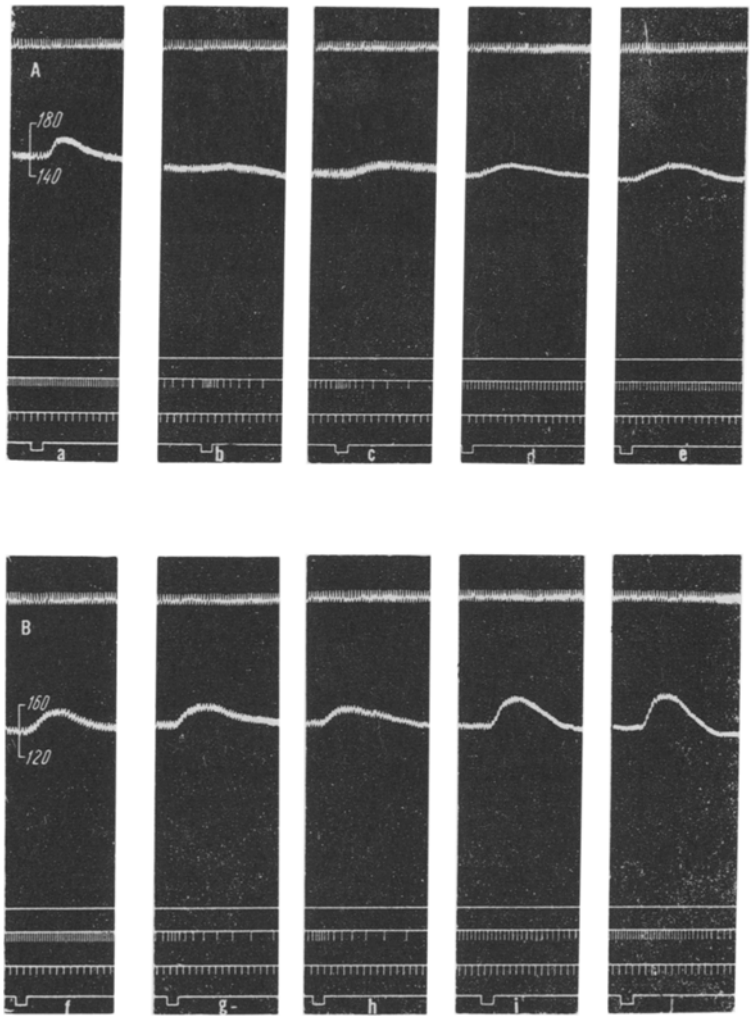

Fig. 1. Effect of sodium fluoride on excitability of receptors of small intestine with sodium pyruvate present. A) Perfusion of intestine with Ringer-Locke solution: a) reflex to acetylcholine 5 min before introduction of sodium fluoride (NaF) into perfusate; b, c, d, e) 10, 25, 60, and 70 min after introduction of NaF (5 ml, 10^{-2} g/ml); B) perfusion of intestine with Ringer-Locke solution containing sodium pyruvate in concentration 10^{-4}g/ml; f) reflex to acetylcholine 5 min before introduction of NaF; g, h, i, j) 10, 25, 60, and 70 min after introduction of NaF (5 ml, 10^{-2} g/ml). Meaning of curves (from top to bottom): respiration, arterial pressure, zero line, rate of perfusion, time marks (5 sec), stimulation marks.

containing 10^{-4} g of acetycholine into the isolated portion of the intestine. Sodium fluoride (3-5 ml of solution in concentration 10^{-2} g/ml) and hydroxylamine (5 ml of solution in concentration 10^{-2} g/ml) were used as inhibitors of tissue metabolism.

The latter substance, as we know, blocks the tricarboxylic acid cycle [12]. Changes in total blood pressure and respiration were recorded by the usual methods.

Experimental Results

In the first series of investigations, nine experiments were carried out. The results of one of the experiments are shown in Fig. 1. In this experiment, as in other experiments of this series, a record was made of the initial

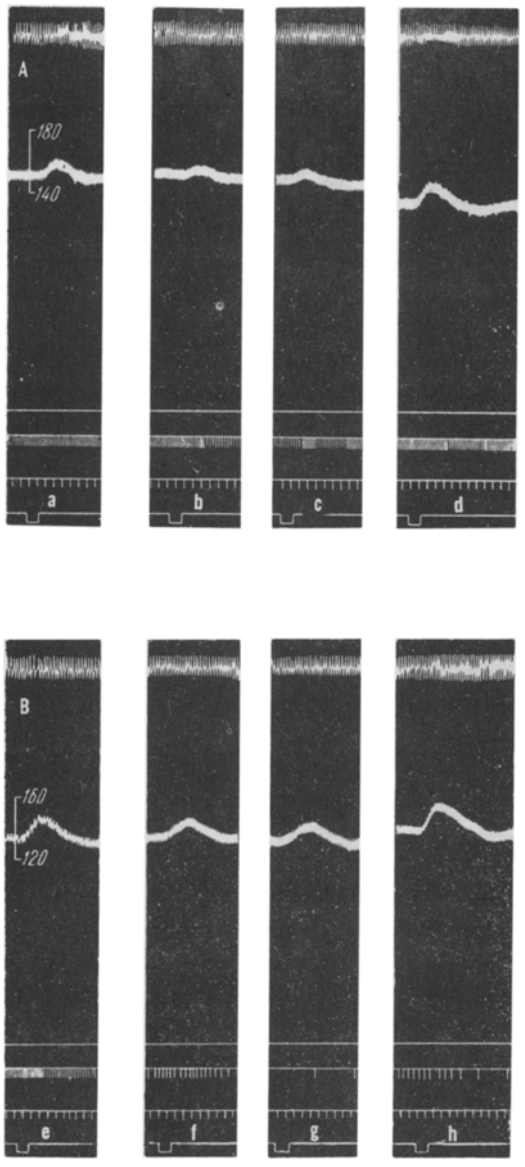

Fig. 2. Effect of sodium fluoride on excitability of receptors of small intestine with neutralized lactic acid present. A) Perfusion of gut with Ringer-Locke solution: a) reflex to acetylcholine 5 min before addition of sodium fluoride (NaF) to perfusate; b, c, d) 5, 15, and 55 min after introduction of NaF (5 ml, 10^{-2} g/ml). B) Perfusion with Ringer-Locke solution containing neutralized lactic acid in concentration 10^{-4} g/ml: e) reflex to acetylcholine 5 min before introduction of NaF; f,g,h) 5, 15, and 55 min after introduction of NaF (5 ml, 10^{-2} g/ml). Meaning of curves as in Fig. 1.

reflex to the introduction of 1 ml acetylcholine solution in concentration 10^{-4} g/ml into the vessels of the perfused intestine (Fig. 1a). This was followed by the introduction of 5 ml of sodium fluoride solution in concentration 10^{-2} g/ml. Ten to seventy minutes after the introduction of sodium fluoride we tested the response of the intestinal receptors to acetylcholine. We found that 10 min after the introduction of sodium fluoride the reflex to acetylcholine was considerably reduced (Fig. 1b).*

After 25, 60, and 70 min, the reflex to acetylcholine was gradually restored (Figs. 1c,d,e). When the reflex to acetylcholine was almost fully restored, we began to perfuse the gut with a Ringer-Locke solution containing sodium pyruvate in concentration 10^{-4} g/ml.

The reflex produced by acetylcholine was distinctly expressed in these conditions (Fig. 1f). After this, sodium fluoride was again added to the perfusate, and over a long period (10, 25, 60, and 70 min after introduction of sodium fluoride), the reflex to acetylcholine not only failed to disappear, but even exceeded the level of the original reflex (Figs. 1g,h,i,j).

The obtained facts can be explained in the following way. As we know, pyruvic acid, being a product of glycolysis, is incorporated into the tricarboxylic acid cycle and is oxidized to carbon dioxide and water. Sodium fluoride, by disturbing glycolysis, particularly the function of enolase, prevents the formation of pyruvic acid. Thus, this inhibitor, by preventing glycolysis, at the same time inhibits the tricarboxylic acid cycle. This is probably why the chemoreception of the intestine is depressed. The addition to the perfusate of sodium pyruvate against a background of glycolysis inhibited by sodium fluoride makes the tricarboxylic acid cycle possible, and inhibition of chemoreception of the intestine is not manifested in this case.

To test this hypothesis, we conducted another series of experiments on seven cats. In this series, we replaced the pyruvic acid by neutralized lactic acid. When oxygen is present, lactic acid is transformed under the action of a specific dehydrogenase into pyruvic acid and is thus incorporated in the tricarboxylic acid cycle. If, in the case of inhibition of glycolysis, the restoring action of sodium pyruvate on chemoreception does, in fact, depend on the pyruvic acid promoting the tricarboxylic acid cycle, then, in these conditions, the lactic acid, by undergoing transformation into pyruvic, ought to have a similar effect.

* The disagreement between our results and those of V. N. Chernigovskii in regard to the effect of NaF on the response to acetylcholine was probably due to the use of different doses of NaF. V. N. Chernigovskii used 3 ml of NaF solution in concentration 10^{-3} g/ml in his experiments [8], while we used 5 ml in concentration 10^{-2} g/ml.

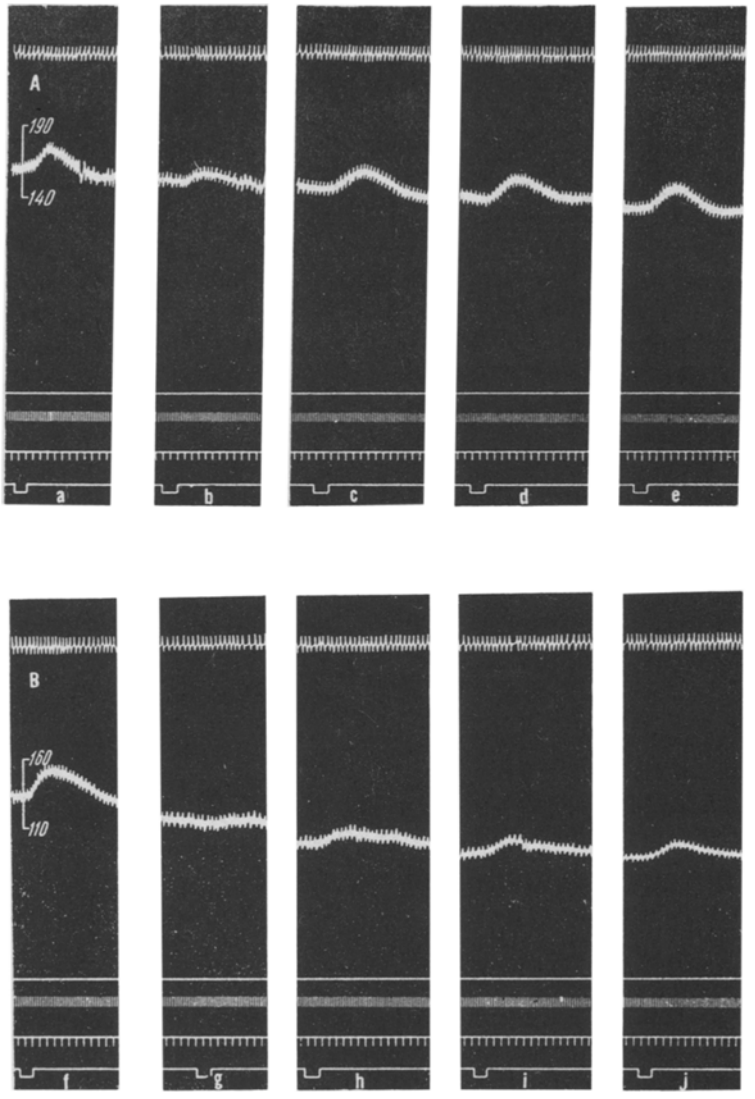

Fig. 3. Reactions of circulation and respiration to stimulation of receptors of small intestine by acetylcholine (1 ml, 10^{-4} g/ml). A) Perfusion of cat intestine with Ringer-Locke solution: a) reflex to acetylcholine 5 min before introduction of hydroxylamine (NH_2OH) to perfusate; b,c,d,e) 10,30,45, and 60 min after introduction of NH_2OH (5 ml, 10^{-2} g/ml). B) Perfusion of intestine with Ringer-Locke solution containing sodium pyruvate in concentration 10^{-4} g/ml; f) reflex to acetylcholine 5 min before introduction of NH_2OH; g,h,i,j) 10,30,45, and 60 min after introduction of NH_2OH (5 ml, 10^{-2} g/ml). Meanings of curves as in Fig. 1.

The obtained results confirmed this hypothesis. Figure 2a shows the initial reflex to acetylcholine before poisoning of the intestine with sodium fluoride. Five and 15 minutes after the introduction of sodium fluoride, the reflex to acetylcholine was greatly reduced (Figs. 2b,c), and after 55 min it was completely restored (Fig. 2d). After this, the intestine was perfused with Ringer-Locke solution containing neutralized lactic acid in concentration 10^{-4} g/ml. Against this background we tested the reflex to acetylcholine before (Fig. 2e) and after introduction of sodium fluoride (Figs. 2f,g,h). We found that the addition of neutralized lactic acid to the perfusate considerably accelerated the restoration of the acetylcholine reflex, which had been inhibited by sodium fluoride.

It was of considerable interest to test how the chemoreception of the intestine was affected by hydroxylamine, an enzyme poison which inhibits several reactions of the tricarboxylic acid cycle (condensation of acetic and oxaloacetic acids, dehydrogenation of ketoglutaric acid, and so on), thus disturbing the tricarboxylic acid cycle as a whole. For this purpose, we added hydroxylamine (5 ml in concentration 10^{-2} g/ml) to the perfusate in eight experiments conducted by the same method. Figure 3a shows the initial reflex produced by acetylcholine before the addition of hydroxylamine. Ten minutes after the introduction of hydroxylamine, the reflex to acetylcholine was reduced (Fig. 3b), and after 30, 45, and 60 min it was gradually restored (Figs. 3c,d,e). Switching to perfusion with Ringer-Locke solution containing sodium pyruvate in concentration 10^{-4} g/ml did not remove the inhibiting effect of hydroxylamine on the reflex produced by acetylcholine (Figs. 3g,h,i,j).

These data indicate that the effect of sodium pyruvate on restoration of the reception of acetylcholine after the action of sodium fluoride (see Fig. 1, B) is due to the fact that this acid makes the tricarboxylic acid cycle possible when glycolysis is inhibited. In the case of blocking of the tricarboxylic acid cycle by hydroxylamine, the inhibition of the reflexes to the acetylcholine cannot be removed by pyruvic acid.

From the results outlined above, we can conclude that substances inhibiting chemoreception of the intestine include not only glycolysis inhibitors, but inhibitors of the tricarboxylic acid cycle. Hence, all the links of tissue energy metabolism are connected with the chemoreception of the intestine. However, the roles of different links of metabolism in the mechanism of receptor excitation are apparently not the same. Glycolysis creates the necessary conditions for realization of the tricarboxylic acid cycle. It can be replaced, to some extent, by the end product – pyruvic acid, which promotes the tricarboxylic acid cycle. Thus, it appears that the process of chemoreceptive reflexes is more closely associated with oxidative metabolism than with glycolysis.

SUMMARY

Short-term experiments were performed on cats. The isolated intestine, retaining connections with the organism, was perfused with various substances. The glycolysis inhibitor (sodium fluoride) and inhibitor of the tricarboxylic acid cycle (hydroxylamine) depress the intestinal sensitivity to acetylcholine. Addition of the final product of glycolysis to the perfusate (pyruvic or lactic acid) accelerates the restoration of intestinal receptor sensitivity disturbed by sodium fluoride; however, it does not aid in restoration of the intestinal receptor sensitivity disturbed by hydroxylamine.

The data presented above lead to the conclusion that not only inhibitors of glycolysis, but also of the tricarboxylic acid cycle belong to the group of substances inhibiting chemoreception. Consequently, all the links of tissue-energy metabolism are connected with the intestinal chemoreception.

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