THE EFFECT OF MORPHINE AND NALORPHINE ON THE LEVEL OF LABILE PHOSPHOROUS COMPOUNDS IN THE BRAIN

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In a previous investigation [1], on the basis of indirectly relevant data, we came to the conclusion that morphine can interfere in the energy metabolism of tissues by inhibiting the resynthesis of high-energy compounds. Analysis of literature data indicating that morphine induces substantial disturbances in the carbohydrate metabolism of nervous tissues [9, 13, 19], plus our own observations, prompted us to study the effect of this analgesic on the content of high-energy phosphorus compounds in the brain.

Experimental Methods

Experiments were performed on white rats weighing 100-200 g each. The brain tissue was fixed by freezing the brain in situ without first opening the cranium. The animal was put under light ether anesthesis, and then gradually lowered by the scruff into liquid air; cardiac activity and respiration were maintained until the brain was completely frozen. The brain was then removed, and chemical analysis of the content of adenosine triphosphoric acid (ATP), phosphocreatine (PhC), and inorganic phosphorus (IP) was carried out in a protein-free filtrate of the ground frozen brain tissue (the cerebellum and medulla oblongata were not used in the test). IP was determined by Fiske and Subbarow's method, the ATP content was determined by precipitation with mercuric acetate, followed by 7-min hydrolysis in normal hydrochloric acid, and the PhC content was determined by 30-min hydrolysis with a 2.5% solution of ammonium molybdate at 37°. The phosphate was precipitated with a mixture of a 0.5% magnesium oxide solution and a 2% calcium chloride solution. The data obtained were processed by variation statistics.

Experimental Results

We performed four series of experiments, using ten animals in each series. The first experimental series was performed on control animals which were injected intraperitoneally with a physiological solution 20-30 min before the investigation. The values obtained for PhC, ATP, and IP (Table 1) approximated the data of other researchers [4,16]. The higher PhC figures cited by some authors [2,6] could be due to the very deep ether anesthesia they employed before freezing the brain tissue. In our experiments, the animal's brain was fixed immediately after the initial signs of anesthetic sleep appeared.

The second series of experiments was performed on rats which were given 40 mg/kg morphine hydrochloride (1% solution) intraperitoneally 30 min before the investigation. After injecting this dose of the analgesic, we noted a decrease in the motor activity of the animals, retardation of the respiration rate, and the appearance of the "caudal phenomenon."

The results of the second experimental series showed (see Table 1) that a statistically authentic decrease in the PhC content in the brain tissue occurred in the rats after the morphine injection. This decrease, which

TABLE 1

Effect of Morphine and Nalorphine on the Level of Labile Phosphorus Compounds in the Brain

Indices	Control			Morphine			Morphine and nalorphine		
	PhC	ATP	IP	PhC	ATP	IP	PhC	ATP	IP
Average content (in mg%) P	7.8	14.9	10.9	5.4	15.2	14.2	7.3	15.1	11.1
Authenticity, T	–	–	–	5.0	0.2	3.3	1.2	0.2	1.3

TABLE 2

Content of High-Energy Compounds in the Brain of Control and Vagotomized Animals

Indices	Control			Vagotomized rats		
	PhC	ATP	IP	PhC	ATP	IP
Average content (in mg%), P	7.8	15.5	11.1	8.4	15.5	10.6
Authenticity, T	–	–	–	1.2	0	0.7

we found in the PhC content can be compared with the observations of Kun and Abood [17], who found that morphine sharply decreased the content of PhC and other intermediate products of the carbohydrate metabolism (hexosephosphates, lactate, and pyruvate) in the liver tissue. We could not, however, detect any substantial changes in the ATP fraction as compared with the control experiments. The average ATP content was 14.9 mg% in the control, and 15.2 mg% after the injection of the analgesic. Statistical processing showed that this difference was not authentic.

The decrease in the PhC content of the brain observed after the morphine injection, and the lack of change in the ATP fraction, agree with the observations of other researchers, the data of whom indicate that various influences which disturb the metabolism of high-energy compounds in the nervous tissue (convulsions, hypoxia, etc.) first affect the PhC content [3,7,11,14]. PhC decomposes sooner than ATP, functioning as a buffer system.

As Table 1 shows, a statistically authentic increase in IP occurred in the second experimental series at the same time as the decrease in the PhC level. The results of this series of experiments, therefore, indicate that morphine can interfere in the metabolism of high-energy phosphorus compounds in the brain, this interference being expressed in a decrease in the content of the labile phosphate of creatine phosphoric acid.

We conducted a third series of experiments studying the nature of the changes in the content of high-energy compounds after the combined administration of morphine and its antagonist, nalorphine, in order to determine the degree to which the effect we observed is specific to morphine. N-allylnormorphine (nalorphine), which was synthesized by Weijlard and Erickson [20] in 1942, is known to be an active antagonist of morphine and other analgesics of similar action. Nalorphine can eliminate almost all the effects of the analgesic agents. After the administration of nalorphine, for example the respiratory depression [5,12] and polysynaptic reflexes [21] induced by morphine disappear, morphine analgesia and hyperglycemia diminish [15,24], etc. The nalorphine we used was synthesized by L. A. Vorotnikova at the AMN SSSR Institute of Pharmacology and Chemotherapy. The preparation (5 mg/kg, 1% solution) was intraperitoneally injected into the rats 15 min after the morphine injection. After another 15 min, the animal was examined. As described in the literature data, the animals regained their normal liveliness and activity three or four minutes after the nalorphine injection, and reaction to painful stimulation was observed.

Analysis of brain tissue from animals which received both morphine and nalorphine showed the latter to have a normalizing effect on the metabolism of labile phosphorus compounds, which morphine had disturbed. As the data cited in Table 1 show, nalorphine promoted restoration of the PhC content to a level approximating the control figures. Statistical processing in this case showed no deviations from the control indices. The changes in the ATP and IP fractions were also negligible.

Consequently, nalorphine's effect on the high-energy compounds of the brain tissue is also clearly antagonistic to that of morphine, which allows one to assume that the changes induced by the analgesic are specific. One objection which could be raised to this conclusion is that morphine, in a majority of cases, induced retardation of the rate of the respiratory movements in the experimental animals (from 140-160 to 80-100 per minute). This effect of morphine could give rise to anoxia of the brain, which could lead to a secondary disturbance in the metabolism of the high-energy compounds of the nervous tissue.

In order to ascertain whether the data obtained were a consequence of a primarily morphine-induced respiratory depression, we conducted a fourth series of experiments: the content of energy-rich phosphorus compounds in the brain of experimental rats after bilateral vagotomy was studied parallel with the content of these compounds in the brain of control animals. The experimental rats were put under ether anesthesia, and both vagus nerves were transected, causing the development of a typical picture of vagus dyspnea in the animals. The respiratory rate was 20-30 times a minute (80-100, after the morphine injection). The brain tissue was analyzed one hour after the operation. The results of this investigation are given in Table 2.

The data cited indicate that retardation of the rate of the respiratory movements does not itself cause any changes in the balance of energy-rich phosphorus compounds in the brain tissue. The results of the second series of experiments, therefore, are adequately authentic.

Reverting to the nature of the antagonism between morphine and nalorphine, one can only say that there is no single opinion held by researchers on this question. Some believe the antagonism to be due to nalorphine's ability to speed up the excretion of morphine [8]; others attribute it to nalorphine's more rapid penetration into the brain tissue, and its more energetic struggle for the receptor formations [22]; a third group believes the reason for the antagonism to be nalorphine's inhibitory effect on the enzyme systems of N-demethylation of the analgesic [10], etc.

Without investigating the substance of these attitudes, one can only point to one curious fact, which could be of value in interpreting the data obtained. Mayer and McCawley [18], studying the effect of morphine and nalorphine on the respiration of brain sections in the presence of different substrates (glucose, pyruvate, etc.), established that brain tissue oxygen absorption is more inhibited by nalorphine than by morphine. If this is true, then it is highly probable that the competition between the two substances is extraneous to the processes of aerobic synthesis of high-energy bonds, possibly occurring at the glycolysis stages. This notion is supported by the observations of Watts [19] and Wolpert et al. [22], which indicate that morphine does not possess the ability to interfere in the processes of oxidizing resynthesis of energy-rich phosphorus compounds. In this connection, one can propose that there is competition between morphine and nalorphine in relation to the key enzymes of the carbohydrate-phosphorus metabolism, specifically in relation to the hexokinase link of glycolysis.

SUMMARY

The author studied the effect of morphine, administered in a dose of 40 mg/kg, and of its specific antagonist N-allylnormorphine (nalorphine) upon the contents of labile phosphorus compounds in the brain of white rats. As established, the administration of this analgesic provokes disturbances in the metabolism of high-energy compounds, manifested, in the statistically authentic reduction of the labile phosphate of creatinephosphoric acid. Nalorphine, administered in a dose of 5 mg/kg against a background of morphine action, exerts a normalizing effect upon the energy exchange in the brain tissue. The above indirect data suggest the existence of antagonism between the two compounds investigated at the level of the hexokinase link of glycolysis.

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