EFFECT OF SEROTONIN AND ITS AGONISTS AND ANTAGONISTS ON BIOCHEMICAL PROCESSES IN THE MYOCARDIUM

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UDC 612.173.014.46:615.362.018.547.756

Serotonin (7-35 μg/kg), when injected into the left ventricle of cats, increases the ATP concentration in the myocardium, as the result of a chemoreflex initiated by serotonin. Under the same experimental conditions serotonin increases the NAD concentration in the wall of the left ventricle but does not change the NAD.H_2 concentration. An increase in the NAD concentration is observed as early as 3 sec after injection of serotonin into the heart, before the onset of reflexes to serotonin. Analysis with α-naphthylbiguanide, tipindole, and morphine suggests that the increase in NAD concentration is associated with excitation of serotonin-sensitive structures of the M-type.

Serotonin is known to evoke a reflex from the receptors of the heart which takes the form of bradycardia and hypotension [11]. Serotonin-reactive structures responsible for this reflex have been called structures of the T-type [4, 5]. Serotonin-reactive structures of the D- and M-types are less well represented in the heart [1].

The object of this investigation was to study the effect of serotonin on the concentration of pyridine and adenine nucleotides in the cat myocardium in the period immediately preceding the onset of hemodynamic changes in response to serotonin and also in the peak period of manifestation of these changes. Adenine and pyridine nucleotides have attracted attention because of their role in key metabolic reactions and also because of the ability of serotonin to interact with NAD and to influence oxidative phosphorylation [9, 17]. The second part of the investigation was devoted to examination of the relationship between the biochemical changes discovered and excitation of serotonin-reactive structures of D-, M-, and T-types.

EXPERIMENTAL METHOD

Experiments were carried out on cats weighing 2.5-3.5 kg, anesthetized with urethane (600 mg/kg) and chloralose (40 mg/kg). The arterial pressure was recorded in the carotid artery by a mercury manometer. The heart rate was determined before the arterial pressure was recorded. To exclude influences from the pulmonary vessels, serotonin was injected directly into the chamber of the left ventricle. To do this, the thorax was opened widely under artificial respiration. A polyethylene catheter was introduced into the left ventricle through the subclavian artery and ascending aorta. Serotonin was injected in doses of 7 and 35 μg/kg in 0.5 ml physiological saline. When injected into the left ventricle in these doses, serotonin evoked reflex bradycardia from receptors of the heart (threshold dose 5-6 μg/kg). The latent period of the reflex is 3.5-4 sec. It reaches a maximum after 8-12 sec [3, 11]. The heart was quickly frozen in situ, 3 or 10 sec after injection of serotonin, by Vollenberger's forceps cooled in liquid nitrogen. The left ventricle was minced in a mortar. The concentration of components of the adenylic system was determined spectrophotometrically after chromatographic fractionation [8]. The concentrations of NAD and NAD.H_2

Laboratory of Pharmacology of the Cardiovascular System, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 10, pp. 35-37, October, 1970. Original article submitted January 4, 1970.

were determined enzymically; the first by Racker's method [15], the second by a modified Stollar's method [16]. The experimental results were subjected to statistical analysis. Arithmetic mean values were calculated with confidence limits at $P=0.05$; differences were regarded as significant when $P \leq 0.05$. At least five animals were used in each series of experiments.

RESULTS AND DISCUSSION

In the control, the ATP concentration in muscle of the left ventricle was 3.35 (4.19-2.51) μmoles/g moist weight of tissue, and the ADP content 0.85 (1.03-0.67) μmole/g. No AMP was found. Serotonin, 3 sec after injection in doses of 7 and 35 μg/kg, had no significant effect on the content of adenine nucleotides. The ATP content was increased after 10 sec to 4.24 (4.57-3.91) μmoles/g. The ADP level was virtually unchanged. The fact that the ATP content was unchanged in the latent period of the reflex, but was increased during the peak period of its manifestation, and also results showing an increase in the ATP concentration in the heart under the influence of acetylcholine or vagus nerve stimulation [2, 7] suggested that the changes observed were reflex in nature. This suggestion is supported by the results of experiments with atropine. Atropine was injected in a dose of 1.5 mg/kg, in which it exhibits marked muscarine-like cholinolytic properties and totally blocks reflex bradycardia. Its antiserotonin properties are ill-defined [10, 13, 18]. Atropine itself in the present experiments did not change the concentration of ATP and ADP in the heart, but completely inhibited the corresponding effect of serotonin. The increase in ATP concentration in the myocardium is thus one of the results of the chemoreflex to serotonin and is not related to the trigger mechanisms of this reflex.

The NAD concentration in muscle of the left ventricle of the control animals was 395.8 (415.86-375.74) μg/g, and the $NAD.H_2$ concentration was 168 (194.7-141.5) μg/g. As early as 3 sec after injection of serotonin in a dose of 7 μg/kg, i.e., before changes had taken place in the heart rate and arterial pressure, the NAD concentration in the myocardium was increased to 451.8 (473.6-430) μg/g. With an increase in the dose of serotonin to 35 μg/kg, no further increase in the NAD concentration took place. The $NAD.H_2$ level under these circumstances showed no significant change. Consequently, the observed increase in NAD content is not the result of a redistribution of its oxidized and reduce forms, but can be attributed either to an increase in its synthesis or a decrease in its breakdown. The fact that the increase in NAD level took place during the first 3 sec after injection of serotonin into the left ventricle, i.e., before the appearance of visible changes in cardiac activity, and that it did not increase during development of reflex bradycardia is evidence that the observed changes are not the result of the reflex. They could be related to its trigger mechanisms, to serotonin-reactive structures of T-type or to processes connected with excitation of structures of M- and D-types. To solve this problem a series of experiments was carried out with α-naphthylbiguanide, which reproduces the effects of serotonin due to stimulation of structure of M- and T-types, but has no effect on serotonin-reactive structures of the D-type [6, 12, 13]. The same increase in NAD concentration as after injection of serotonin in a dose of 445.6 (500.0-391.2) μg/g took place after injection of α-naphthylbiguanide into the left ventricle (7 μg/kg), but the concentration of $NAD.H_2$ was unchanged. This fact suggests that the observed changes in the NAD concentration were due to excitation of serotonin-reactive structures of T- and M-type. Further analysis showed that tipindole, in a dose of 0.8 mg/kg, in which it blocks structures of the T-type like serotonin and α-naphthylbiguanide, increased the myocardial concentration of NAD [to 441.3 (471.6-411.0) μg/g], without changing the $NAD.H_2$ concentration. This result makes any relationship between changes in the concentration of NAD in the heart and excitation of serotonin-reactive structures of the T-type highly improbable.

Morphine, in a dose of 1 mg/kg, in which it blocks serotonin-reactive structures of the M-type, but not of the T- and D-type [4, 13, 14], when injected intravenously does not change the NAD content in the myocardium [399.6 (415.17-384.03) μg/g] and lowers the $NAD.H_2$ concentration to 127.7 (142.26-111.74) μg/g. Serotonin, if injected into the left ventricle 5 min after intravenous injection of morphine, did not raise the NAD level. These results suggest that the increase in NAD concentration taking place in the myocardium under the influence of serotonin was due to its action on structures similar or identical to structures of M-type. Among the effects of serotonin mediated through structures of the M-type are its ganglion-stimulating action and, possibly, its action on chemoreceptors of the aortic and carotid zones [14, 18]. Further investigations will show whether the increase in NAD concentration observed in these experiments is related to any of these effects, and whether it is due to them or is one of the links of the trigger mechanism.

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