SENSITIVITY OF HYPOTHALAMIC NEURONS TO β-ENDORPHIN, MET-ENKEPHALIN, and THYROLIBERIN*

V. N. Babichev, S. F. Mironov,
V. Ya. Ignatkov, Yu. P. Shvachkin,
A. P. Smirnova, N. I. Zavalishina,
I. L. Kofman, and Yu. A. Pankov

UDC 612.826.4.014.46:547.95:547.943

KEY WORDS: opiods; thyroliberin; microiontophoresis; neurons; hypothalamus.

Opioid peptides participate in the regulation of reproductive function on which they have an inhibitory action [11]. Opioids have been shown not to affect the peripheral component of the reproductive system [6]; the main site of their action is evidently hypothalamus, although sensitive neurons and opiate receptors have also been found in other regions of the CNS [2, 5]. Naloxone, a competitive antagonist of the opiates, if administered to intact animals, has the opposite effect [11]. Thyroliberin, as a neuropeptide and neuromediator, according to data in the literature, not only is the releasing factor of thyrotrophic hormone [7], but also has a blocking agent in the system regulating pituitary gonadotropic function [7, 10]. This action is evidently effected at the hypothalamic level, for specific receptors for the peptide have been found there, and the inhibition is abolished by exogenous luteinizing hormone releasing factor.

The object of this investigation was to study the effect of these neuropeptides when applied microiontophoretically on electrical activity of neurons in the preoptic region (PO), the periventricular nucleus (PN), and arcuate nucleus (AN) of the hypothalamus which play an important role in the regulation of pituitary gonadotropic function [1].

EXPERIMENTAL METHOD

Experiments were carried out on male rats anesthetized with urethane. The animals were immobilized with tubocurarine. Unit activity was recorded extracellularly and the test substances applied microiontophoretically by means of 5-channel glass microelectrodes. The central channel was filled with 2 M NaCl solution and the side channels with solutions of biologically active substances. All the neuropeptides used were obtained in the writers' Institute. Microiontophoresis was carried out with positive currents with a strength of 30-60 nA. Stereotaxic coordinates were taken from the atlas [9]. Unit activity was recorded and subjected to primary analysis on a conventional electrophysiological apparatus. Full details of the technique were given previously [2].

EXPERIMENTAL RESULTS

The results are given in Table 1. In all parts of the hypothalamus investigated both neurons, responding by excitation or inhibition to application of the test substances, and cells not responding to them were found. However, it is an interesting fact that of 78 neurons in PN and AN tested during application of β-endorphin only 15.4 ± 4.1% gave an activation reaction, whereas an inhibitory effect predominated significantly and was observed in 55.1 ± 5.6% of cases. The reactivity of the test structures was very similar, as shown by the chi-square test. PN was found to be more sensitive, but the differences were not statistically significant.

Application of met-enkephalin to PO, PN, and AN showed that the proportion of cells giving an activation response in these hypothalamic formations was 20.3 ± 3.6%, and the percent-

*Thyrotrophin releasing factor.

Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 2, pp. 33-35, February, 1982. Original article submitted June 16, 1981.

 0007-4888/82/9302-0176$07.50 © 1982 Plenum Publishing Corporation

TABLE 1. Effect of Met-Enkephalin, β-Endorphin, Thyroliberin, and Nalorphine on Activity of Hypothalamic Neurons (direction of response, in % ± m)

Hypothalamic structure	Met-enkephalin				β-endorphin			
	A	I	O	total No. of neurons	A	I	O	total No. of neurons
PN	25,5±6,1	45,1±7,0	29,4±6,4	51	18,0±6,3	61,5±7,9	20,5±6,6	39
AN	15,4±5,8	12,8±5,4	71,8±7,2	39	12,8±5,4	48,7±8,0	38,5±7,8	39
PO	18,4±6,3	13,2±5,5	68,4±7,5	38				

TABLE 1 (continued)

Hypothalamic structure	Thyroliberin				Nalorphine			
	A	I	O	total No. of neurons	A	I	O	total No. of neurons
PN	51,3±5,7	26,9±5,0	21,8±4,7	78	7,1±6,9	7,1±6,9	85,8±9,3	14
AN	38,0±5,8	19,7±4,7	42,3±5,9	71				
PO	50,0±9,1	6,0±4,5	43,3±9,0	30	34,4±8,4	6,2±4,3	56,3±8,8	32

Legend. A) Activation of test neuron, I) inhibition, O) no response, m) error of percentage frequency.

age giving an inhibitory response was 25.8 ± 3.8%. Consequently met-enkephalin had an inhibitory action significantly less frequently than β-endorphin ($P < 0.01$), whereas there was no significant difference between the frequencies of their activation response. The reactivity of PN differed significantly from that of the other structures ($\chi^2 = 14.71$ for PO, $\chi^2 = 19.39$ for AN; $\beta f = 2$; $P < 0.01$). This difference was mainly due to the higher sensitivity of PN and the significant predominance of neurons with an inhibitory reaction in it ($P < 0.01$). To assess to what extent the two peptides acted in the same direction, they were applied consecutively to 39 neurons of AN and 39 neurons of PN. The rank correlation coefficients were found to be 0.539 and 0.619 respectively, evidence that their effects on the same cells were substantially similar.

To test the specificity of responses of the neurons (responses mediated by opiate receptors are considered to be specific), in 38 cases the competitive opiate antagonist nalorphine (N-allylnormorphine) was applied similtaneously with the opioid. In PO it blocked the response of four of five neurons with an inhibitory response but only in one of five neurons with an activation response. In PN nalorphine suppressed the response in all four neurons with an activation response. In PN nalorphine suppressed the response in all four neurons with an inhibitory response to met-enkephalin tested by combined application, but it was ineffective against the only cell with an activation response. It is evidently the inhibitory effect of the opioids that is mediated by specific opiate receptors.

Since naloxone, when administered to intact animals, evokes a response opposite to that evoked by the opioid, it can be tentatively suggested that the opioid system also functions under normal conditions [11]. In the investigation cited, the effect of nalorphine was studied on 46 neurons of PN and PO, and the PN neurons were found to be much more sensitive ($P < 0.01$). By contrast to the specific inhibition evoked by opioids, it activated the cells more often. This can evidently be explained by abolition of the inhibitory action of the endogenous peptide. It can be tentatively suggested that the tone of this peptidergic system is higher in PO than in PN.

Besides endogenous opioids, in the present investigation a neuropeptide of a different class, namely thyroliberin, was used. The proportion of neurons with an activation reaction to this oligopeptide in all regions was 45.8 ± 3.7% and the percentage with an inhibitory response was significantly less, namely 29.7 ± 3.0% ($P < 0.01$). Thyroliberin thus evoked activation of the test neurons significantly more often than the opioids ($P < 0.01$). Just as in the previous experiments, PN was most sensitive ($P < 0.01$). This evidently was responsible for the significant difference in reactivity of PN from that of AN ($\chi^2 = 7.12$, $P < 0.05$) and of PO ($\chi^2 = 7.4$, $P < 0.05$). In 109 cases the opioids and thyroliberin were applied consecutively to the same neurons. Calibration of the rank correlation coefficient revealed significant agreement between the direction of the effects of thyroliberin and β-endorphin in PN

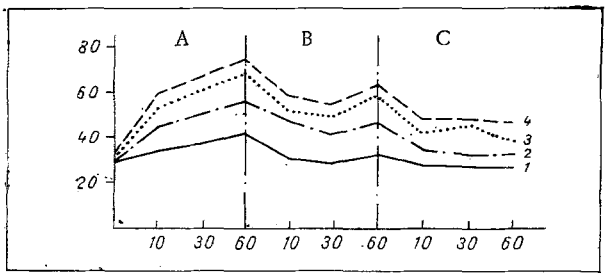

Fig. 1. Quantitative evaluation of state of microcirculation with respect to different vessels during hypotension and after injection of HOB and reinfusion of blood. A) Period of hypotension, B) period of action of HOB, C) recovery period; 1) arteriole, 2) venule 25-30 μ in diameter, 3) capillary, 4) venules 15-20 μ in diameter. Abscissa, time (in min); ordinate, points.

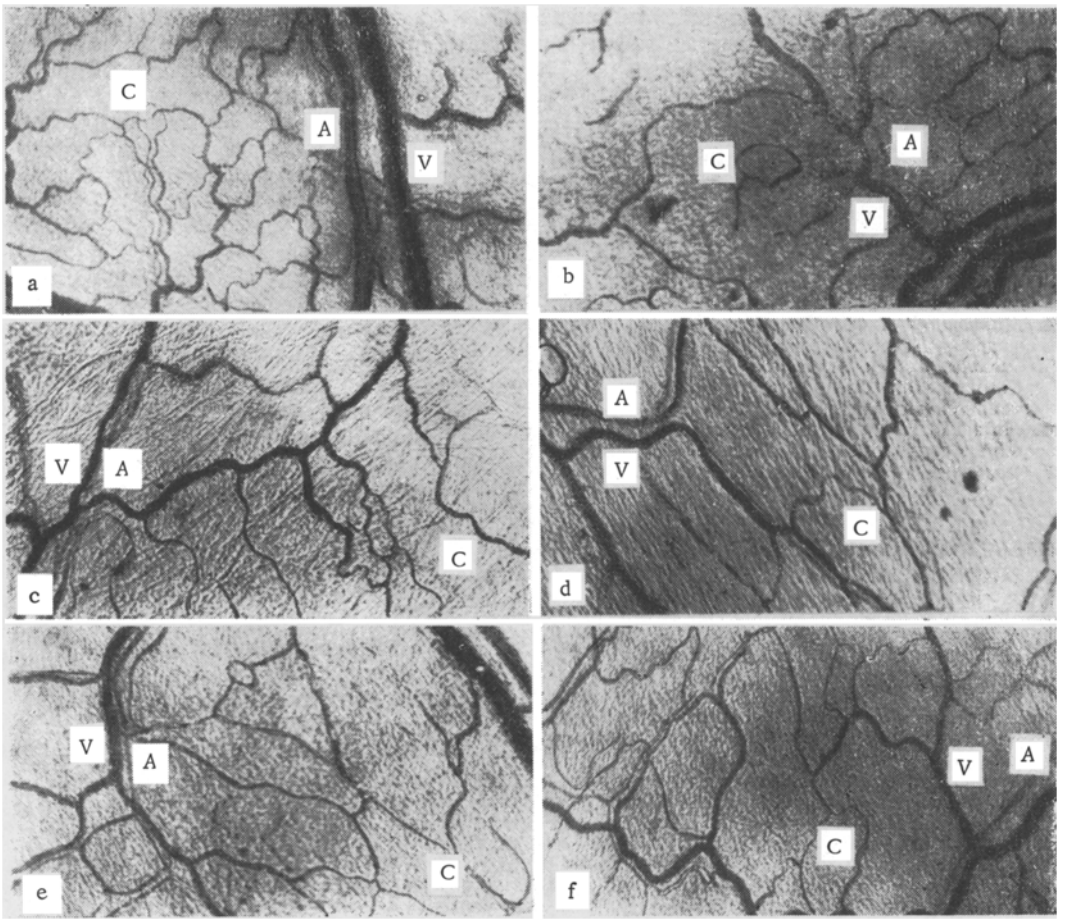

Fig. 2. Biomicroscopy of dog mesentery during hypotension, after injection of HOB, and in recovery period: a) initial state, b) 60 min of hypotension, c, d) 30 and 60 min respectively after injection of HOB, e, f) 10 and 60 min respectively after beginning of recovery period. A) Arteriole, V) venule, C) capillary. Magnification 56 ×.

only the first 10 min, to a more than twofold increase in cardiac output. This was due both to improvement of the pumping function of the heart and to restoration of the venous return. The vascular reaction forming the venous pressure was evidence of a very small rise in CVP and a significant increase in PVP. The increase in PVP could be used to judge the increase

TABLE 1. Parameters of Cardiohemodynamics and Systemic Circulation during Hypotension and after Injection of HOB and Reinfusion of Blood (M ± m)

Parameter	Initial state	60 min of hypotension	Period of action of HOB		Recovery period	
			10—15 min	60 min	10—15 min	60 min
Cardiac output, ml/min	1554±82	589±58[a]	1312±64[b]	1075±68[a, b]	1996±106[a]	1500±86
Stroke volume, ml	10,9±1,2	3,2±0,6[a]	8,6±0,9[b]	7,3±0,8[a,b]	14±1,3	11±1,2
Heart rate, beats/min	143±7	180±8[a]	152±8[b]	154±8[b]	143±10	134±7
Card. index, liter/m^2/min	3,5±0,2	1,3±0,1[a]	2,9±0,1[a, b]	2,4±0,2[a, b]	4,4±0,2[a]	3,3±0,2
Systolic index, ml/m^2	25±1,2	7±0,5[a]	19±0,7[a, b]	16±0,8[a, b]	31±1,2[a]	25±1,1
SBP, mm Hg	140±5	37±1[a]	80±5[a, b]	76±6[a,b]	120±6	124±6
Work of left ven., kg · m/min	2,937±0,5	0,292±0,09[a]	1,416±0,3[a, b]	1,102±0,2[a, b]	3,234±0,6	2,511±0,5
WILV, kg · m/min/m^2	6,5±0,52	0,65±0,20[a]	3,1±0,31[a,b]	2,4±0,28[a, b]	7,2±0,65	5,6±0,51
SWILV, kg · m/m^2	0,045±0,001	0,004±0,0004[a]	0,020±0,0006[a, b]	0,016±0,0005[a,b]	0,050±0,001[a]	0,042±0,0009[a]
N, W	0,469±0,018	0,047±0,009[a]	0,231±0,011[a,b]	0,180±0,010[a,b]	0,528±0,019[a]	0,410±0,021
E, J	0,201±0,020	0,016±0,008[a]	0,091±0,011[a,b]	0,070±0,010[a,b]	0,221±0,018	0,183±0,019
CVP, mm Hg	68±8	42±9[a]	46±7[a]	50±4[a]	72±9	75±7
PVP, mm Hg	89±5	69±6[a]	85±4[b]	88±6[b]	114±7[a]	123±6[a]
TPR, dynes · sec · cm^{-5}	7200±595	5028±518[a]	4888±532[a]	5655±565[a]	4814±621[a]	6606±580
SPR, conventional units	40±2,4	28±4,0[a]	28±2,5[a]	32±2,6[a]	27±1,8[a]	38±2,8

Legend: a) significance of differences of parameters compared with initial state, b) significance of differences of parameters during hypotension and period of action of HOB (differences statistically significant at the $P < 0.05$ level). SPR) Specific peripheral resistance. SWILV) stroke work index of left ventricle; MILV) work index of left ventricle.

in tone of the peripheral capacitive vessels after administration of HOB and a consequent increase in the blood flow to the heart. The increase in the venous gradient and at the same time in SBP led to improvement of the diastolic return blood flow to the heart [5, 6]. Data obtained by the writers previously showed that HOB redistributed K^+ from the extracellular fluid into cardiomyocytes in exchange for Na^+ and H^+, thereby prolonging diastole, and it increased the filling time of the chambers of the heart with blood [3]. Lengthening of the diastolic and systolic periods after administration of HOB was accompanied by an increase in the power and work of the heart muscle. The favorable action of HOB on the cardiohemodynamics, incidentally, did not cause an increase in TPR. Such a vascular response against the background of a marked rise in CO and SBP led to improvement of the peripheral circulation, or with a high cardiac output and low TPR, the tissues were perfused by considerably greater volumes of blood in unit time [7]. This was confirmed by data obtained in a study of the microcirculation. As early as 2-3 min after infusion of HOB the velocity of movement of the blood was increased in the arterioles, and later in the venules and capillaries. By 30 min the number of functioning capillaries and the velocity of the blood flow were close to their values in the initial state. The content of plasma in the lumen of the mesenteric vessels was increased. At the 60th minute after injection of HOB the velocity of the blood flow in the arterioles was rapid, but lower than initially. It remained a little slow in the venous section of the microcirculation.

During the first few minutes after intravenous infusion of heparinized blood and during the next 60 min of observation, the parameters of the cardiohemodynamics and systemic circulation returned to normal and became stabilized. Immediately after correction of the blood loss the velocity of the blood flow increased and accessory capillaries began to function. A fine degree of aggregation was found in the venules and capillaries. No significant changes in the microcirculation were observed 60 min after infusion.

On the basis of these observations, HOB was used for the treatment of shock and correction of blood loss in 108 patients before admission to hospital [8]. The addition of HOB to the treatment program led to an improvement in the principal hemodynamic parameters and enabled them to be maintained steadily at this level while the patients were being transported to hospital.

Experimental investigations and clinical observations thus showed that a single injection of HOB after blood loss improves the pumping function of the heart, increases BP and PVP, without at the same time raising TPR, and it improves the microcirculation in the mesentery (increases the velocity of the blood flow, reduces aggregation of the blood cells, and increases the number of functioning capillaries). The beneficial action of HOB is mani-

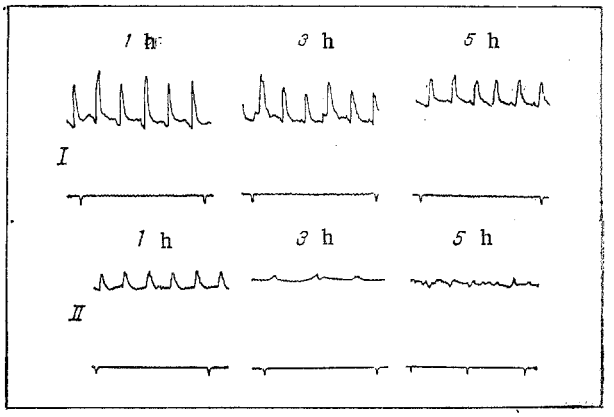

Fig. 1. Recovery of contractile activity of isolated unpreserved and preserved heart after perfusion. I) ECG of preserved heart; II) ECG of unpreserved heart.

liver, and kidney during acute ischemia was investigated at the time of decapitation of the animals and 1, 3, 5, and 24 h later at room temperature (series I).

The time course of the exogenous formaldehyde concentration in the heart during acute ischemia (conservation — series II) was investigated after injection of 5 ml of 0.1% formalin solution (55 μmoles formaldehyde), made up in Ringer's solution, into the inferior vena cava of a rat, which was followed by isolation of the heart and its preservation for 0.5, 1, 2, 3, 4, 5, and 7 h in Ringer's solution containing formaldehyde in a concentration of 0.5 mM at room temperature. The indicator of preservation of viability was recovery of cardiac activity during reperfusion by Langendorf's method.

The rate of removal of the administered formaldehyde from the organ also was studied on a model of the isolated perfused heart (series III). The isolated hearts were perfused with Krebs–Henseleit solution containing 0.6% formalin until they stopped beating. Perfusion was then continued with the same solution without formaldehyde until cardiac activity was restored. The formaldehyde concentration was measured in the outflowing perfusion fluid before cardiac arrest, in the myocardium of the arrested heart, in the outflowing perfusion fluid after recovery of cardiac function, and in the myocardium 1 min after recovery of cardiac function. Formaldehyde was determined in a protein-free extract by a method based on specific interaction between formaldehyde and chromotropic acid in the presence of sulfuric acid, with the formation of a violet color. The sensitivity of the method was 0.1 μmole/g wet weight.

To study the contractile activity of the hearts, the ECG and arterial pressure in the left ventricle were recorded on a Biograph (Harvard Apparatus, USA). The data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Under normal conditions formaldehyde was shown to be present in the liver, heart, and kidneys. Its concentration in the intact organs could be arranged in the following diminishing order: liver 1.04 ± 0.04 μmole/g, kidneys 0.57 ± 0.09 μmole/g, heart 0.27 ± 0.05 μmole/g.

Production of acute ischemia in the organ led to a decrease in the endogenous formaldehyde concentration. By the first hour of ischemia its level had fallen in the liver and kidneys by almost 20% — to 0.91 ± 0.04 and 0.46 ± 0.05 μmole/g respectively ($P = 0.05$). The decrease in the formaldehyde concentration in the heart began later, and by the end of the 3rd hour of ischemia in the organ it was 63% ($P < 0.01$) of its initial value (0.17 ± 0.03 μmole/g). The study of the formaldehyde level in the organs up to the 5th hour of ischemia showed a tendency for its concentration to rise in the heart and kidney; this may possibly be regarded as preservation of short-term ability to process metabolic formaldehyde into the late stages of ischemia. By the 24th hour of ischemia no formaldehyde could be found in the kidney, but in the liver and heart its concentration was down to 25-30% ($P < 0.01$) of that in the intact organ, namely 0.36 ± 0.05 and 0.07 ± 0.01 μmole/g, respectively.

Formaldehyde, as a natural metabolite, thus participates in metabolism when the supply of oxygen and substrates to an organ has ceased completely.

Toward the beginning of perfusion of the isolated heart the formaldehyde concentration in the perfusion fluid was 2 orders of magnitude higher than in the myocardial tissue. At the moment of cardiac arrest the formaldehyde concentration in the myocardial tissue was 84 times higher (up from 0.27 ± 0.05 to 22.9 ± 2.13 μmoles/g), whereas in perfusion fluid flowing from the heart it fell from 66.6 to 42.7 ± 2.7 μmoles/ml. During reperfusion with pure Krebs–Henseleit solution the formaldehyde concentration in the myocardium fell sharply to reach 1.6 ± 0.2 μmole/g after 1 min, whereas in the outflowing perfusion fluid the formaldehyde concentration was 105 ± 10.2 μmoles/ml. This value is 4.5 times greater than the maximal concentration of free formaldehyde in the heart (22.9 ± 2.13 μmoles/g). Since formaldehyde could enter the perfusion fluid only from the heart, it must be assumed that formaldehyde bound with the tissues of the heart was liberated.

The next step was to study the dynamics of the formaldehyde concentration in heart muscle after intravenous injection of that substance, followed by ischemia of the heart for 0.5, 1, 3, 5, and 7 h. Intravenous injection of formaldehyde caused an increase in the concentration of this metabolite, but a much smaller increase than might be expected following injection into the rat in a dose of 55 μmoles. The greater part of the formaldehyde was evidently immediately bound and, as the previous series of experiments showed, it was bound reversibly. After 2-3 min formaldehyde was found in the myocardium in a concentration of 0.52 ± 0.01 μmole/g, i.e., only twice as high as in the intact organ. Its concentration in the myocardium fell after 30 min by 20% ($P < 0.01$). This process was observed throughout the period of investigation. Just as in series I, a brief rise of the formaldehyde level (by 20%, $P < 0.05$) was observed, confirming its participation in metabolism.

The physiological data are evidence of recovery and normalization of the ECG parameters and blood pressure in the left ventricle of the preserved heart after 1, 3, and 5 h, with improvement of myocardial contractility, with lengthening of the perfusion periods. Whereas the values of the parameters in the control experiments indicated profound changes in the myocardium, these changes intensified still more with prolongation of the perfusion period (Fig. 1).

Comparison of the results of the biochemical and physiological investigations showed that once the formaldehyde concentration in the preserved heart exceeded 0.50-0.60 μmole/g, the myocardium was protected against ischemic damage.

It can accordingly be concluded from these results that free formaldehyde is present as a normal metabolite in the heart, liver, and kidneys. Ability of the cell to process formaldehyde is preserved temporarily during acute ischemia of organs, evidence that formaldehyde participates in metabolism. Long-term preservation of an organ is accompanied by a fall in the concentration of this metabolite. The appearance of free formaldehyde in the perfusion fluid during reperfusion of the preserved heart, in an amount more than 4.5 times greater than is present in the heart during preservation, is proof of the reversible binding of formaldehyde by the myocardium.

These experiments show that a very small increase in formaldehyde concentration above its metabolic level is a necessary and sufficient condition for protection of the myocardium against ischemic damage.

LITERATURE CITED

1. É. L. Muzykantskii, "Protection of the myocardium with formaldehyde against anoxic injury," Candidate's Dissertation, Moscow (1980).
2. V. D. Rozvadovskii, in: Technical Meeting of Research Workers Studying the Preparation, Sterilization, Preservation, and Keeping of Homologous and Heterologous Tissues [in Russian], Moscow (1967), pp. 84-86.
3. V. I. Tel'pukhov, in: Current Problems in Transplantation of Organs and Tissues [in Russian], Moscow (1978).
4. V. S. Yagodovskii, The Action of Weak Solutions of Formalin on Biological Tissues [in Russian], Moscow (1975).
5. M. Koivusalo, Acta Physiol. Scand., 39, 131.