ROLE OF OPIOID PEPTIDES IN NEUROTROPHIC CONTROL OF SENSITIVITY OF THE RAT SKELETAL MUSCLE FIBER MEMBRANE TO ACETYLCHOLINE

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It is now generally accepted that the differential state of the surface membrane of skeletal muscle fibers, characteristics of the innervated muscle, is maintained through the participation of substances of peptide nature transported to the muscle along axons [2, 5, 8]. It has been shown, in particular, that β-endorphin, secreted from a nerve, controls the spectrum of synaptic acetylcholinesterases in the neonatal period [9-11].

Considering that the sensitivity of the muscle membrane to acetylcholine is determined primarily by the surface properties of the chemosensitive membrane, it can be tentatively suggested that β-endorphin can participate in the neurotrophic control not only of synaptic acetylcholinesterase activity, but also of the properties of the acetylcholine-activated channel-receptor complex. To test this hypothesis the investigation described below was undertaken.

EXPERIMENTAL METHOD

Experiments were carried out on surviving rat diaphragm muscle. A muscle strip 3-4 mm wide, taken from an animal weighing 150-200 g, was cultured in Eagle's medium (USSR) with strengthening carbonate buffer and cysteine (USSR) concentration increased to 0.3 mM, and containing also 5% embryonic calf serum (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), 100 U/ml of penicillin (USSR) with pH 7.2-7.4, in a moist atmosphere of a mixture of 95% O_2 and 5% CO_2 at 36°C, in accordance with the usual method [4, 7]. In some experiments β-endorphin, dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg) or the tripeptide Tyr-D-Ala-Gly was added to the culture medium in a concentration of 10^{-8} M (these preparations were synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR). Series of experiments were carried out with simultaneous addition of dalargin and the tripeptide to the culture medium: in one series they were used in concentrations of $5 \cdot 10^{-8}$ and $5 \cdot 10^{-9}$ M respectively, in the other, conversely, dalargin was used in a concentration of $5 \cdot 10^{-9}$ M and the tripeptide in a concentration of $5 \cdot 10^{-8}$ M.

After culture for 24, 48, 72, 96, and 120 h the muscle strip was transferred into continuously flowing Ringer's solution for warm-blooded animals at 36°C [3]. By means of a standard microelectrode technique, with iontophoretic application of acetylcholine, the extrasynaptic sensitivity of the muscle membrane to the neurotransmitter was recorded [1].

The character of development of postdenervational hypersensitivity of the muscle membrane to acetylcholine in these experiments agreed with data obtained previously in experiments *in vivo*, evidence that the chosen technique was adequate [3].

EXPERIMENTAL RESULTS

Extrasynaptic sensitivity of the muscle membrane to acetylcholine appeared on the 3rd day of culture in 45% of fibers tested, rising to 86% of fibers after 5 days (Table 1). Addition of β-endorphin to the culture medium delayed the development of this phenomenon: it appeared only on the 4th day in 12% and on the 5th day in 30% of muscle fibers (Table 1). These results can be regarded as evidence in support of a role for β-endorphin in the neuro-

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TABLE 1. Appearance of Extrasynaptic Sensitivity to Acetylcholine in Fibers of the Rat Diaphragm Muscle, Kept in Culture Medium

Time of incubation, h	Without preparation	Preparation				
		β-endorphin	dalargin	tripeptide	dalargin (5×10^{-9} M) + tripeptide (5×10^{-8} M)	dalargin (5×10^{-8} M) + tripeptide (5×10^{-9} M)
0—48	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	—	—
72	36/80 (45)	0/53 (0)	0/68 (0)	66/72 (92)	54/71 (76)	0/50 (0)
96	61/79 (77)	9/74 (12)	1/71 (1)	75/79 (96)	68/73 (93)	2/86 (2)
120	81/94 (86)	26/87 (30)	2/89 (2)	68/68 (100)	68/70 (97)	3/85 (4)

Legend. Numerator indicates number of fibers in which extrasynaptic sensitivity to acetylcholine is recorded, denominator gives total number of fibers tested. Each ratio obtained from the results of measurements on four animals. Figure in parentheses is a percentage.

trophic regulation of acetylcholine sensitivity of the muscle membrane. It can be postulated that this activity of β-endorphin is due to its opiate properties.

Experiments with the other opioid peptide, dalargin, gave evidence in support of this hypothesis. The presence of dalargin in the incubation medium virtually completely prevented the appearance of sensitivity to the neutotransmitter in the extrasynaptic region of the muscle membrane (Table 1). The reason why dalargin is more effective than β-endorphin is evidently that it is more resistant to the action of the N-terminal aminopeptidase because its molecule contains D-alanine in place of glycine[2] [6, 10].

It is generally considered that at least the first four amino acids are responsible for the opiate activity of the peptide [6, 10]. It was therefore interesting to test, under the conditions of the model chosen, the effect of a peptide containing one fewer amino acid. Addition of the tripeptide to the culture medium not only did not prevent, but actually facilitated the development of extrasynaptic sensitivity of the muscle membrane to acetylcholine; it did so, moreover, in a larger number of fibers than in the control (Table 1). This confirms our hypothesis that it is the opiate properties which determine activity of the opioid peptides as substances delaying the development of postdenervational hypersensitivity of the muscle membrane to acetylcholine.

Opioid peptides are considered to have two sites for interaction with specific receptors, namely the phenolic rings of N-terminal tyrosine and of the 4th amino acid — phenylalanine [6, 10]. The possibility therefore cannot be ruled out that the tripeptide may interact with opiate receptors, although because binding takes place at only one side, the biological effect typical of opioid peptides is not induced.

Experiments with simultaneous addition of dalargin and tripeptide to the culture medium indicate that the interaction between these substances is competitive in type. For instance, if the tripeptide was present in the medium in a concentration 10 times higher than that of dalargin, it abolished the delaying action of the latter on the appearance of extrasynaptic sensitivity to the neurotransmitter. However, if the ratio between them was reversed, the effect of dalargin was exhibited almost in full measure (Table 1). This is indirect proof that specific binding sites for opioid peptides are present on the muscle membrane. In turn, evidence in support of this conclusion is given by the fact that peptides exert their effect in low concentrations.

On the basis of these results we can also regard the tripeptide Tyr-D-Ala-Gly as candidate for the role of blocker of the specific "input" of the muscle fiber, through which nervous control of acetylcholine receptor metabolism is exerted.

The appearance of extrasynaptic sensitivity to acetylcholine in the presence of the tripeptide in a larger number of fibers than in the control can be attributed to the fact that the tripeptide prevents manifestation of the trophic effect of the corresponding opioid molecules present in the incubation medium. The presence of such molecules in the incubation medium cannot be ruled out. Their source may be either the embryonic calf serum added to the medium or the muscle itself.

It can thus be concluded that neurotrophic control of the properties of the chemosensitive membrane of skeletal muscle fibers may be exerted through the participation of a neurogenic peptide which possesses opiate properties. A possible candidate for this role may be β-endorphin.

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FEATURES OF CERTAIN FORMS OF GOAL-DIRECTED BEHAVIOR AFTER INDUCED CHANGES IN THE ENDOGENOUS β-ENDORPHIN LEVEL IN RATS

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β-endorphin is an endogenous peptide factor which is widely distributed in the CNS and gastrointestinal tract, is present in the blood serum and cerebrospinal fluid of animals and man, and can pass through the blood-brain barrier. It has been shown that β-endorphin is a powerful analgesic, it modifies activity of the cardiovascular and respiratory systems and the body temperature, and induces variously directed changes in hormone secretion and mediator metabolism, and it also exerts a considerable influence on various forms of behavior (feeding, sexual, defensive, etc.) and on the motor activity of animals [5, 6, 9, 10]. However, modern views on the physiological role of β-endorphin are still insufficiently complete. In order to explain the physiological role of the peptide factor, it is necessary to study the effects of selective blocking of its formation or action by binding with appropriate antibodies [2, 11]. It must be pointed out that complex forms of animal behavior after administration of antiserum or immunization against oligopeptides have not been adequately studied [1, 7, 8, 12].

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