The principles of information processing in the CNS are based on mechanisms that are universal for all parts of the brain, and based on processes changing the excitability of the neurons. These universal mechanisms regulating intracellular activity include the system of secondary messengers, to which the calcium–calmodulin complex belongs. Calmodulin-dependent phosphorylation and dephosphorylation of membrane and cytosol proteins in the nervous system lead to a change in the characteristics of the neuronal membranes and in relations between individual structural components of the cell. Taken as a whole, these processes enable regulation of the functions of the CNS by calmodulin to take place. It has been shown, for instance, that calmodulin can modify synaptic transmission [7], by its action on mediator biosynthesis (as has been shown for catecholamines and serotonin), mediator secretion, and sensitivity of receptors to neurotransmitters.

Our experiments showed that a low-intensity modulating shf field has a marked influence on calmodulin levels in brain structures, and that the character of this effect is determined by the conditions of modulation. Our results confirm the previous view that the efficacy of low-intensity modulated shf fields is associated with activation of biological amplification mechanisms. Deliberate interference with calmodulin levels in brain structures by means of shf fields may provide a new and nonpharmacologic approach to the correction of nervous and mental disturbances, emotional activity, and memory.

LITERATURE CITED

1. H. F. Harmuth, Sequency Theory: Foundations and Applications, New York (1977).
2. W. R. Adey, TIIER, No. 1, 142 (1980).
3. W. R. Adey, Fiziol. Cheloveka, No. 1, 59 (1975).
4. W. R. Adey, Fiziol. Cheloveka, No. 5, 774 (1977).
5. W. R. Adey, Ann. New York Acad. Sci., **247**, 15 (1975).
6. D. Bingmann, EEG-Lab., **2**, No. 11, 85 (1989),
7. C. O. Broström and D. J. Wolf, Biochem. Pharmacol., **30**, No. 12, 1395 (1981).

ACTIVATION OF ATP-SENSITIVE K^+ CHANNELS OF CARDIOMYOCYTES BY ENDOGENOUS CARDIOPEPTIDES

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KEY WORDS: ATP-sensitive K^+ channels; endogenous cardiopeptides; isolated cardiomyocytes; GTP-γ-S.

Among the various biologically active substances, ever-increasing interest is being displayed in compounds of peptide nature, with cardiotropic effects [9]. Endogenous cardiopeptides obtained by extraction from myocardium constitute a new group of these compounds. Despite the varied manifestations of their action on heart muscle, as experimental and clinical studies have shown [14], the molecular and cellular mechanisms of the physiological activity of endogeneous cardiopeptides still remain unexplained. There is evidence of a direct action of these peptides on activity of K^+ channels in nonmyocardial cells [6, 8, 12], but no attempt has been made to verify the presence of such effects of peptides from the heart on K^+ channels of cardiomyocytes.

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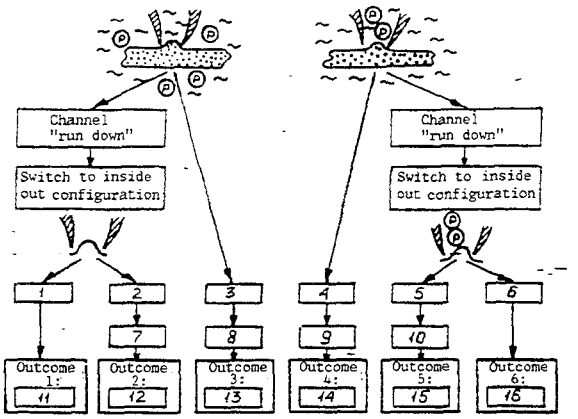

Fig. 1

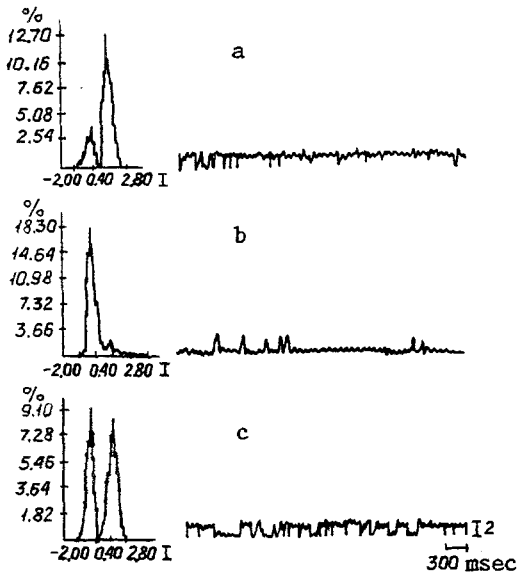

Fig. 2

Fig. 1. Effect of endogenous cardiopeptides on functioning of ATP-sensitive K^+ channels of cardiomyocytes (diagram of experiment). P) Peptides (explanation in text).

Fig. 2. Reactivation of ATP-sensitive K^+ channel by GTP-γ-S Layout of amplitude histograms: horizontally – current (in pA), vertically – length of stay at current level corresponding to each bin (in % of total recording time). Broken line on current tracks denotes zero level of current. Upward deviation on trace corresponds to outward current. Held voltage 0 mV. Signal of trace after passage through low-frequency filter with transmission band up to 1000 Hz. Remainder of explanation in text.

The aim of this investigation was to study the effect of endogenous cardiopeptides on potassium ion transport through single K^+-channels of the cardiomyocyte sareolemma.

EXPERIMENTAL METHOD

Mature isolated Wistar rat cardiomyocytes, tolerant to calcium, isolated by the method described previously [1], were used. Currents through single potassium channels were recorded by the patch-clamp method in configurations with an intact cell ("cell-attached") and on inside-out membrane fragments. Solutions (extracellular, intracellu-

TABLE 1. Reactive ATP-Sensitive K^+ Channels (n = 8) during Action of GTP-γ-S

Conditions	1 min	2 min	GTP-γ-S
Experimental values	0,760	0,151	0,513
	0,802	0,152	0,522
P_o	0,757	0,145	0,507
	0,749	0,146	0,495
	0,778	0,140	0,489
	0,786	0,138	0,531
	0,763	0,136	0,525
	0,754	0,129	0,518
Average	0,769	0,142	0,513
Standard deviation	0,018	0,008	0,015
Error of the mean	0,006	0,003	0,005
RCIM 95 %	0,015	0,007	0,012
At 99 %	0,023	0,010	0,018
P_a		—5,83e+1	
t and P paired		1,15e—10	
For independent		—6,29e+1	
		4,30e—17	

Legend. RCIM) Range of confidence intervals for mean values and t and P given in standard form.

lar, and to identify the channels) used were similar to those described in [1]. A preparation of cardialin, consisting of a combination of bovine heart peptides, isolated by the method in [3] and subjected to ultrafiltration with an exclusion limit of 15 kD, was used as the endogenous cardiopeptides. The working concentration of the polypeptides in the extracellular solutions used was 10^{-8}-10^{-6} mg·ml^{-1}.

EXPERIMENTAL RESULTS

Activity of K^+ channels with abnormal rectification, as studied previously [2], was recorded in the cell-attached configuration by filling the micropipets with extracellular potassium solution. Addition of extracellular sodium solution with 1 mM Ca^{2+} ions (BSC) and with cardialin in concentrations of 10^{-8} to 10^{-6} mg·ml^{-1} in the chamber did not lead in any of the 26 recordings from intact cells to an appreciable change in functioning of channels of this type.

Addition of the peptides to the pipet likewise did not affect the abnormal rectification currents, but in five of 24 cases of established tight junctions with an area of the membrane ("patches") activation of channels of another type was obtained. Their identification showed them to be ATP-sensitive K^+ channels. To abolish currents through the K^+ channels with abnormal rectification, which complicated analysis of activity of the ATP-sensitive K^+ channels, in the subsequent experiments the pipets were filled with BSC solution and the holding potential was higher than the reversal potential. Two methods were used to apply the preparation to the cell membrane (Fig. 1). First, after establishment of a tight junction the micropipets with sarcolemma applied the BSC solution with cardialin to the chamber. In that case the peptides made contact with the whole surface of the cell except that part of the membrane which contained the ionic channel. Second, cardialin was added to the micropipet, and thus only the fragment of sarcolemma immediately surrounding the molecule of the ionic channel was exposed to its action.

Analysis of the results of recording currents through single channels of 50 cells led to outcomes 1, 2, 4, or 6 (Fig. 1). Multiple repeated derivations from the same cardiomyocytes, with no cardialin present in the solution from the pipets, did not reveal operation of the ATP-sensitive K^+ channels. Activation of the ionic channel by the peptides thus evidently took place, not its unblocking as a result of a possible fall of the intracellular ATP concentration.

Since contact between the preparation and the membrane, except in the area containing the channel-forming protein, bounded by the micropipet, did not lead to a change in activity of the ATP-sensitive K^+ channel, but an effect was induced only by the appearance of peptides actually in the region of the channel, it was suggested that there could be a "short" path for transmission of the signal from the outer surface of the membrane to the effector

channel. The hypothesis currently accepted assumes that ion transport through the channel is controlled by an external ligand, in the following way: receptor – coupling G protein – ionic channel [5, 15]. To find out if such a mechanism for controlling the work of ATP-sensitive K^+ channels of the cardiomyocyte sarcolemma is possible, we studied the effect of the G-protein activator on working of channels of this type.

Experiments were carried out with eight channels in the composition of "inside out" fragments with intracellular potassium solution (K_i) on the cytoplasmic side of the membrane. For direct activation of the G-protein we used guanosine-5′-O-(3-triphosphate) (GTP-γ-S). The parameter of activity of the channel was a probability of it existing in the open state (P_o) during every 30 sec of the trace. A detailed study of the dynamics of channel function showed that after detachment of the membrane fragment it was predominantly in the open state. Typical values of P_o for the first minute of work in the "inside out" state were 0.7-0.8. With the course of time there was a gradual decrease in activity of the channel, and by the 20th minute P_o had fallen to about 0.2. Virtually complete disappearance of activity was observed after 1 h of working of the channel in the composition of an isolated fragment. Addition of K_i solution with GTP-γ-S in a concentration of 100 μM to the inner side of the membrane in the presence of Mg^{2+} ions in a concentration of 1 mM at the 40th minute of working of the channel "inside-out" caused reactivation of the ATP-sensitive K^+ channel (Table 1). This amounted to not less than half of the peak value of P_o in the 1st minute of the experiment. One typical example is shown in Fig. 2, which gives amplitude histograms plotted for 30-sec traces of the currents. Short fragments of the traces are shown to the right of the histograms and correspond: a) to activity at the 1st minute after detachment of the membrane fragment; b) after working for 40 min in the "inside-out" state; c) after addition of the reactivating solution to the chamber. It follows from analysis of the amplitude histograms that as a result of inactivation the value of P_o, which was estimated from the area of the right-hand peak of the histogram, corresponding to the open state of the channel, fell from 0.760 to 0.150, but as a result of reactivation it rose to 0.510.

Exclusion of Mg^{2+} ions from the "intracellular" solution led to loss of the activating effect of GTP-γ-S. This indicated an Mg^{2+}-dependent mechanism of action of the GTP analog on the ionic channel, characteristic of G-protein activation.

First, therefore, specific activation of the ATP-sensitive K^+ channel by endogenous cardiopeptides was discovered. Second, regulation of its function by GTP-binding protein was demonstrated. A change in the working of the channel caused by direct activation of G-protein is analogous to its response to the action of the preparation on the outer surface of the membrane. This indicates that the ATP-dependent component of potassium conduction of the cardiomyocyte sarcolemma may be regulated by a mechanism of signal transmission along the short pathway: receptor – G-protein – ATP-sensitive K^+ channel.

In cardiomyocytes, G-protein-dependence of function has been demonstrated for K^+ channels activated by acetylcholine and adenosine [5, 10, 15]. For the ATP-sensitive K^+ channels a reactivating action of GTP-γ-S or of the AlF_4^- complex has been obtained on membranes of transverse T-tubules of rabbit skeletal muscles [11]. A competition of GTP analogs and of the specific blocker of ATP-sensitive K^+ channels glibenclamide for common binding sites has been found on cell membranes from the ventricles of the heart [7]. The possibility cannot be ruled out that this binding took place with G-proteins, coupled with ionic channels of this type.

Cellular reactions due to activation of ATP-sensitive K^+ channels have, as their key mechanism, a hyperpolarization shift of transmembrane potential, which may lead to significant modulation of the cardiomyocyte action potential [13]. Changes in activity of K^+ channels of this type have been shown (for smooth-muscle cells) to correlate with modulation of Ca^{2+} ion exchange between its intracellular depots and the cytosol and with the work of Ca^{2+} channels of L type. It is not an accident that substances influencing the functioning of ATP-sensitive K^+ channels have become objects for intensive pharmacologic research [4].

Thus activation of ATP-sensitive K^+ channels by endogenous cardiopeptides may be responsible for the receptor-mediated effect of the test preparation on the G-protein that regulates its function. The possibility cannot be ruled out that cardialin contains peptides which selectively modify different types of ionic permeability of the cardiomyocyte membrane.

LITERATURE CITED

1. A. P. Babenko and V. O. Samoilov, Fiziol. Zh. SSSR, **77**, No. 11, 55 (1991).

2. N. A. Burnashev and Yu. I. Zil'berter, Dokl. Akad. Nauk SSSR, **281**, No. 5, 1269 (1985).
3. V. G. Morozov and V. Kh. Khavinson, Dokl. Akad. Nauk SSSR, **261**, No. 1, 235 (1981).
4. Nihon Yakurigaku Dzassi (Folia Pharmacol. Jpn.), **96**, No. 3, 10 (1990).
5. L. Birnbaumer, A. Yatani, A. M. J. Vandongen, et al., J. Pharmacol., **30**, Suppl., 13 (1990).
6. N. Esparza and J. Diez, Eur. J. Pharmacol., **166**, No. 2, 349 (1989).
7. J. F. French, L. C. Riera, J. G. Sarmiento, et al., Biochem. Biophys. Res. Commun., **167**, No. 3, 1400 (1990).
8. S. C. Martin, D. I. Yule, M. J. Dunne, et al., EMBO J., **8**, No. **12**, 3595 (1989).
9. P. Needlemar, E. Blaine, J. E. Greenwald, et al., Annu. Rev. Pharmacol., **29**, 23 (1989).
10. A. Noma, Trends Neurosci., **9**, No. 4, 142 (1986).
11. L. Parent and R. Coronado, J. Gen. Physiol., **94**, No. 3, 445 (1989).
12. M. A. Schumann and P. Gardner, J. Membr. Biol., **111**, No. 2, 133 (1989).
13. A. H. Weston, Pflügers Arch., **414**, Suppl. 1, 88 (1989).
14. G. M. Yakovlev (G. M. Yakovlew), V. Kh. Khavinson, V. S. Pavlenko, et al., Cardiovasc. Drug. Ther., **5**, Suppl. 3, 408 (1991).
15. A. Yatani, J. Codina, R. Mattena, et al., Progress in Endocrinology, Vol. 2, Amsterdam (1988), pp. 1009-1016.

PROLONGED ACTION OF TUFTSIN ON PENICILLIN-INDUCED EPILEPSY

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KEY WORDS: peptide; tuftsin; epilepsy; brain structures; behavior; electroencephalogram.

In intact animals the effect of the tetrapeptide tuftsin (Thr-Lys-Pro-Arg) extends to nonspecific immunity, motor activity, and memory [1, 4, 6, 8]. In stress situations it possesses a stress-protective and antidepressant action [2, 3, 6], but in depressions it has an antidepressive effect. Meanwhile, the potential value of tuftsin in other forms of CNS pathology, including the epileptic state, has not yet been elucidated. This is an urgent problem because of the increasing interest of clinicians in this peptide, for if administered intranasally its effect is predominantly on processes in the CNS [5].

In the investigation described below the effect of a single dose of tuftsin on the EEG recorded from brain structures and on behavior of animals with experimental epilepsy, induced by large doses of penicillin, was studied. Particular attention was directed to the delayed effect of administration of a single dose of the peptide: observations on the EEG and behavior of the animals were made during the period of 3-4 days after administration of tuftsin.

EXPERIMENTAL METHOD

The experimental conditions envisaged two versions of tuftsin administration: against the background of frank epileptiform activity, associated with the action of penicillin, and before penicillin was given, i.e., its preventive action. Experiments were carried out on rats (180-200 g) with electrodes implanted into all parts of the cerebral

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