an important controlling function on the supply of structural material of lipid nature to membrane structures, creating the essential background of a hydrophobic environment for many membrane proteins, including some membrane-bound phospholipid-dependent enzymes. It can accordingly be concluded that a combined administration of EF with α-tocopherol and sodium nucleate in myocardial infarction of coronary occlusive etiology is an effective way of inhibiting LPO and stimulating the course of biosynthesis in the perifocal zones of the necrotic myocardium.

LITERATURE CITED

1. E. B. Burlakova, Kardiologiya, No. 8, 48 (1980).
2. A. A. Engibaryan, K. G. Karagezyan, and S. S. Ovakimyan, Vopr. Med. Khimii, No. 1, 43 (1985).
3. F. Z. Meerson, V. E. Kagan, Yu. P. Kozlov, et al., Kardiologiya, No. 2, 81 (1982).
4. A. Bruni and E. Racker, J. Biol. Chem., 243, 962 (1968).
5. Y. M. Chen, Cancer Res., 34, 3192 (1974).
6. K. R. Chien, R. G. Peak, I. L. Farber, et al., Am. J. Pathol., 97, 505 (1979).
7. I. A. Menon, Canad. J. Biochem., 50, 807 (1972).
8. F. Novello, J. H. Huchrome, B. Bonora, et al., Ital. J. Biochem., 24, 325 (1975).
9. E. Santiago, N. Zoper-Worotalla, and I. L. Segovia, Biochem. Biophys. Res. Commun., 53, 439 (1973).
10. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161, 83 (1945).
11. S. C. Vasdev, K. I. Kako, and I. P. Biro, J. Mol. Cell. Cardiol., 11, 1195 (1979).

BURST DISCHARGES OF A SINGLE NEURON INDUCED BY METRAZOL AND PENICILLIN

G. N. Kryzhanovskii,* N. V. Vorona, Yu. L. Bereznyak, A. A. Chumachenko, V. N. Efimov, and V. A. Safonov

UDC 612.822.3.014.46:[615.334+615.221

KEY WORDS: depolarization shift; metrazol; penicillin

The study of the conditions and mechanisms of formation of generators of pathologically enhanced excitation, which lie at the basis of the development of pathological processes in the nervous system [4], is an urgent task from both theoretical and practical points of view. On its solution depends the solution to the problem of what contribution modifications of relations within the system of neurons and anomalies arising in the neuron itself may make to the development of the pathological process.

The aim of this investigation was to study changes in single unit electrical activity under the influence of convulsants.

EXPERIMENTAL METHOD

Experiments were carried out on the neuron of a slow adapting stretch receptor of a crayfish, isolated from 2-4 abdominal segments. The preparation consisted of a receptor nerve cell with an axon 6-7 mm arising from it and entering the common nerve trunk. Cell dendrites were buried in the receptor muscle, attached to pieces of the shell of two adjacent segments. The receptor cell and muscle of the fast stretch receptor were removed. The preparation was immersed in a bath filled with Harreveld's solution, balanced in its ionic composition with crustacean hemolymph, and maintaining function of the preparation under conditions of complete isolation for a long time. The solution surrounding the cell was changed from a buffer reservoir through a funnel connected to the bath so that this procedure did not give rise to any artefact. Activity was recorded extracellularly by means of a suction

*Academician of the Academy of Medical Sciences of the USSR.

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Research Institute of Neurocybernetics, Rostov-on-Don University.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 11, pp. 550-552, November, 1989. Original article submitted March 28, 1989.

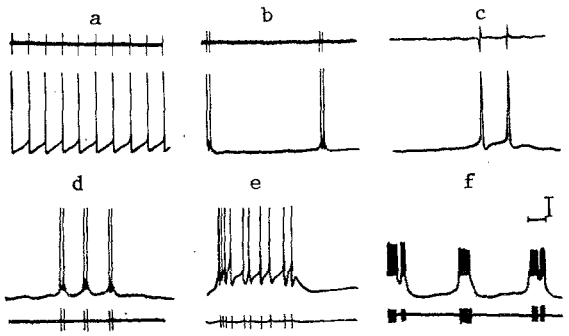

Fig. 1

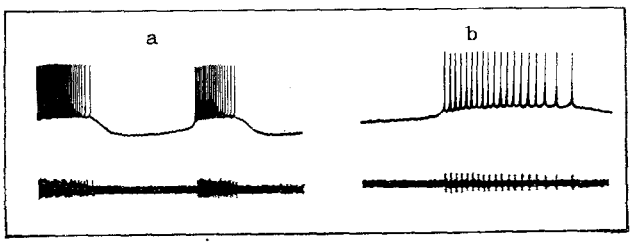

Fig. 2

Fig. 1. Effect of metrazol on firing pattern of a single nerve cell. a, b, c) Top beam – extracellular recording, bottom beam – intracellular; d, e, f) top beam – intracellular recording, bottom beam – extracellular. a) Spontaneous activity; b) 1 h 50 min after exposure to metrazol ("washed" cell, no metrazol in solution), rhythmic flow of single spikes replaced by rhythmic flow of pairs of spikes; c) paired discharge from flow shown in b; d) after 2 h 15 min pairs are grouped into bursts (example shown in one burst consisting of three paired discharges); e) after 2 h 45 min number and frequency of spikes in burst are increased; f) after 4 h 50 min, with increasing depolarization shift there is a further increase in frequency and number of spikes in burst, and with no grouping into pairs. Time marker: a) 100 msec b) 200 msec; c) 20 msec; d) 200 msec; e) 100 msec; f) 1000 msec; calibration: a, b, c) 20 mV; d e, f) 10 mV.

Fig. 2. Burst activity of nerve cell exposed to penicillin. Shift of membrane potential during burst about 4 mV. Time marker: a) 500 msec; 2) 200 msec.

electrode, the tip of which had a diameter corresponding to that of the nerve trunk (30-80 μ). Activity was recorded intracellularly by means of a glass microelectrode filled with 3 M KCl solution (resistance 20-40 MΩ). Activity was recorded on an N-327/5 automatic writer and S8-17 storage oscilloscope. Activity was photographed from the oscilloscope screen. Metrazol in Harreveld's solution in a concentration of 1.5-4 mg/ml and the sodium salt of benzylpenicillin in Harreveld's solution in a concentration of 5000-30,000 IU/ml were used as convulsants. Activity of 18 neurons under the influence of metrazol and 14 under the influence of penicillin was recorded.

EXPERIMENTAL RESULTS

Normally a stretch receptor neuron generates a spike train with a frequency determined by input (Fig.1a). Addition of metrazol to the solution surrounding the cell led to transient inhibition of activity, followed by weak depolarization, accompanied by small increase of frequency. Later the firing rate gradually decreased and regular activity was replaced by a rhythmic flow of pairs of spikes (Fig.1b, c), later being transformed into bursts (Fig. 1d, e, f). The process of conversion of rhythmic into burst activity was quite prolonged, but it was considerably accelerated if the paired discharges were present in spontaneous activity or were obtained by repeated stretching of the receptor muscle or by the use of Harreveld's solution with a low calcium ion concentration. In such cases metrazol induced burst activity more quickly.

With time, grouping into pairs within a burst may either continue or disappear, and the number of spikes in the burst and the following frequency of the burst increased (Fig. 1d, e, f). Intracellular recording clearly demonstrated that burst discharges arise on the wave of the endogenous depolarization shift of the membrane potential.

The effect of metrazol did not disappear after the solution containing it was changed for the ordinary solution, even when the change was made immediately after the appearance of burst activity, and for that reason the solution surrounding the cell was changed several times. Moreover, the effect was enhanced after that time.

The primary response to penicillin consisted of transient inhibition of neuronal activity, followed by a return to the original firing rate. After a certain time regular spike activity became unequal, and the inequality in turn was replaced by burst discharges arising

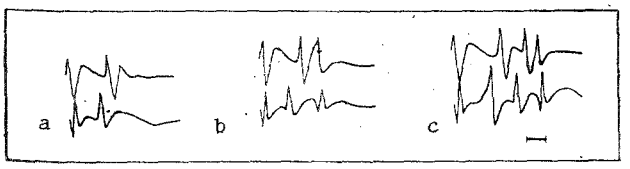

Fig. 3. Appearance of reverberative burst activity in response to extracellular action of potassium ions. Extracellular recording: top beam – electrode in region of initial segment; bottom beam – electrode located more distally in axon; a) first spike in pair arises in region of initial segment and spreads into axon, second spike (extra spike) arises autonomously in axonal locus and spreads antidromically into initial segment; b) second spike in burst is initiated in axonal locus; third (extra) spike of initial segment; c) burst of four spikes arising during reverberative interaction of electrically excitable loci of initial segment and axon. Time marker – 2 msec.

on the wave of the depolarization shift of membrane potential. Examples of such volley activity are shown in Fig. 2.

Under the influence of penicillin, burst discharges were not always preceded by rhythmic paired discharges, but if they were, just as in the case of metrazol, the volleys developed faster and were more clearly defined.

Thus, the appearance of burst activity in a neuron under the influence of the convulsants used is preceded by the appearance of paired discharges in most cases. Whatever the mechanism of their generation, one thing is certain: the presence of paired discharges quickened the appearance of burst activity.

This resembles the effect observed on neurons of Helix pomatia which have spontaneous "burst" activity. Among neurons possessing different type of activity and silent neurons, these nerve cells were found to be the most sensitive to the action of metrazol, which led to the appearance of a paroxysmal depolarization shift in them [5].

A similar model of epileptization of a neuron was obtained by the present writers on the crayfish stretch receptor: epileptiform activity developed in response to application of metrazol and penicillin, typified by a paroxysmal depolarization shift of membrane potential with a high-frequency spike discharge on the plateau.

A characteristic feature of burst discharges obtained in response to the action of metrazol and penicillin was that they appeared on the wave of the endogenous depolarization potential. These burst discharges, consisting of a comparatively small number of spikes, also were observed in response to electrophoretic application of potassium ions to the cell membrane [2]. However, the appearance of these bursts was due not only to a depolarization shift of membrane potential. A spike generated by the stretch receptor neuron is the resultant of interaction between several electrically excited loci [2, 3]. On application of potassium ions to the cell membrane paired discharges are recorded on the axon. The depolarizing action of potassium on the membrane, in the zone of the axonal generation locus, leads to autonomous excitation of that locus and to the spread of the spike in the antidromic direction into the initial segment. A spike may also arise autonomously in the initial segment of the axon and spread into the axon, with the result that bursts containing various numbers of spikes (three, four, or more) of different origin appear (Fig. 3). We observed no such reverberative relations and changes in temporal parameters of interaction between the generative loci of the axon and soma in response to the action of metrazol and penicillin.

Burst activity developing in the crayfish stretch receptor neuron in response to the action of other modifying substances (strychnine and procaine) has been explained by some workers as due to delayed depolarization, persisting in dendrites of the nerve cell, and this disparity in the number of spikes recorded in the soma and axon is regarded as proof of generation of the spikes in different trigger zones of the membrane [6, 7]. However, our experiments with penicillin and metrazol revealed no quantitative disagreement and no disturbance of temporal relations between spikes recorded at different points of the cell membrane, and the irreversible character of the action of these substances is evidence that in this case the appearance of burst discharges is due to activation of an endogenous pace-

maker mechanism, whose function is closely bound with the state of metabolism of the neuron [1, 3].

The problem of whether intraneuronal reverberative relations apply in the genesis of burst activity during paroxysmal depolarization shifts, and whether they are involved in the formation of epileptiform activity of neurons require further study.

LITERATURE CITED

1. G. G. Arakelov, Usp. Fizol. Nauk, 5, No. 4, 52 (1974).
2. Yu. L. Bereznyak, "The role of interaction between loci of spike generation in functioning of the crayfish stretch receptor neuron," Author's abstract of dissertation for the degree of Candidate of Biological Sciences, Rostov-on-Don (1986).
3. N. V. Voronova, "Adaptive modifications of function of a single nerve cell during selective inhibition of its energy supply," Author's abstract of dissertation for the degree of Candidate of Biological Sciences, Rostov-on-Don (1986).
4. G. N. Kryzhanovskii, Determinant Structures in Pathology of the Nervous System [in Russian], Moscow (1980).
5. É. F. Lavretskaya, P. M. Balaban, V. P. Timashov, and I. S. Zakharov, Zh. Nervopatol. Psikhiat., 85, No. 6, 822 (1985).
6. V. S. Rusinov and V. L. Ézrokhi, Mechanisms of Local Response and Spreading Excitation [in Russian], Leningrdad (1970), p. 13.
7. Y. Washizu, Comp. Biochem. Physiol., 15, 535 (1965).

EFFECT OF THE CALCIUM CHANNEL BLOCKER RYODIPINE ON FOCAL AND GENERALIZED EPILEPTIC ACTIVITY

M. N. Karpova, O. Yu. Pankov, R. N. Glebov, S. K. Germane, V. E. Klusha, and G. J. Dubur

UDC 615.31:546.41].015.4:616.853].076.9

KEY WORDS: epileptic activity; calcium channel blockers; ryodipine

An important role in the mechanisms of neuronal epileptic activity is ascribed to a change in Ca^{++} homeostasis [4-6]. A fall in the Ca^{++} concentration in the extracellular space, which always procedes an epileptic discharge, and entry of Ca^{++} into neurons through the cytoplasmic membrane are among the mechanisms of neuronal hyperactivation and they exert a considerable influence on the formation and intensity of discharges arising within an epileptic focus [10, 11]. Since voltage-dependent calcium channels are the main pathway of entry of Ca^{++} inside the cell, their blockade plays an important role in the termination of epileptic activity [7-9].

The aim of this investigation was to study the effect of ryodipine [foridon; 2,6-dimethyl-3,5-dimethoxycarbonyl-4-(o-difluoromethoxyphenyl)-1,4-dihydropyridine], a new calcium channel blocker (the preparation was synthesized at the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR) [3], on focal and generalized epileptic activity (EA). Foridon, as a Ca^{++}-antagonist, has been used in the treatment of hypertension [3]. During the study of its effect on the CNS, both a sedative component of its action and an anticonvulsive effect, when administered perorally [1], have been described.

EXPERIMENTAL METHOD

Experiments were carried out on 102 male Wistar rats weighing 200-220 g. To create a model of focal EA, holes were drilled (2 × 4 mm) 24 h before the experiment, by the method described

---

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga.

(Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 11, pp. 553-555, November, 1989. Original article submitted February 15, 1989.

0007-4888/89/0011-1562$12.50