EFFECT OF STROPHANTHIN AND DIGOXIN ON ACTIVITY OF AN EXPERIMENTAL EPILEPTOGENIC FOCUS IN THE FROG HIPPOCAMPUS

I. B. Mikhailov

UDC 616.831.314-009.24-02:615.22:547.918:[582.937+582.951.64]-092.9

KEY WORDS: frog hippocampus; cardiac glycosides; epileptogenic focus

Cardiac glycosides are known to penetrate into the CNS [11] where they interact with receptors (Na^+, K^+-ATPase) on membranes of neurons and their axons [10]. This leads to changes in neurotransmitter activity [4, 6, 12]. The number of these receptors and their affinity for cardiac glycosides is known to be much greater in the CNS than in the heart [3, 10].

The aim of this investigation was to study the effect of the glycosides strophanthin and digoxin on activity of an epileptogenic focus in the frog hippocampus, i.e., to study their action on neurons with pathologically enhanced excitability. We know that neurotropic effects of drugs are less marked on the pathologically changed function than on the normal function of the CNS [1]. When starting this study we expected that the neurotropic effect of cardiac glycosides would be directed more especially toward activity of an epileptogenic focus.

EXPERIMENTAL METHOD

Experiments were carried out in the fall on 54 male grass frogs (*Rana temporaria*) weighing 20-30 g and living in the Leningrad area. The frogs were anesthetized with a 25% solution of urethane (5 mg/g) and the bones of the vault of the skull were removed to expose the hemispheres of the forebrain. Monopolar and bipolar recordings of electrical activity from the left and right primordial hippocampi were obtained by means of a chemical electrode and a bipolar electrode glued together. This combination was so designed that after fixation in the electrode holder of an SEZH-2 universal stereotaxic apparatus the chemical electrode was buried in the substance of the left hippocampus and the bipolar electrode was located at the same time in the symmetrically opposite point of the right hippocampus. The chemical electrode, inserted into the left hippocampus, consisted of a cannula-needle (0.4 mm in diameter), insulated with transparent plastic, for injecting penicillin into the brain (it also served as one of the two electrodes constituting the bipolar chemical electrode). The second electrode consisted of nichrome wire, insulated with transparent plastic, 0.10 mm in diameter and glued to the cannula. The bipolar electrode inserted into the right hippocampus of the frogs differed from the chemical electrode only in the fact that, instead of a needle-cannula, nichrome wire 0.18 mm in diameter was used to make it. The reference electrode consisted of a silver plate, placed in the frog's mouth. The electrode and the chemical electrode were inserted into the brain substance for a distance of 0.5-0.6 mm, taking coordinates from an atlas of the frog's brain [7], to reach the highest concentration of hippocampal pyramidal cells. Hippocampal potentials were recorded on a ÉÉGU-16-02 16-channel electroencephalograph, with simultaneous visual monitering on the screen of a VEKS-4M oscilloscope. An epileptogenic focus was created in the frog hippocampus by injection of 0.4 µl of a solution of the sodium salt of penicillin, containing 1000 U of the antibiotic, by means of a micromanipulator. As the results of preliminary experiments showed, this dose of penicillin is a minimal which led to the appearance of an epileptogenic focus in the hippocampus in all experiments. In the course of 120 min after injection of the penicillin the EEG was recorded to count single interictal discharges (2-3 phasic spikes or 2-3 pointed waves) during 1 min of recording of the EEG in each 10-min interval of the experiment and the mean number of electrographic correlates of fits (a continuous long epileptic discharge on the EEG), also in every 10-min interval of the experiment. The cardiac glycosides were injected into the dorsal lymph sac either 5 min before injection of penicillin into the hippocampus or 50-60 min after its injection, when activity of the focus had stabilized. The following doses of glycosides were used (in µg/g): strophanthin (L'vov Pharmaceutical Chemical Factory) 1.8 (subtoxic dose) and 0.18; digoxin

Department of Pharmacology, Leningrad Pediatric Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, G. N. Kryzhanovskii.) Translated by Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 586-587, November, 1987. Original article submitted December 8, 1986.

 0007-4888/87/0011-1572$12.50

TABLE 1. Changes in Activity of Epileptogenic Focus in Frog Hippocampus under the Influence of Cardiac Glycosides Injected into Dorsal Lymph Sac 50-60 min after Beginning of Experiment and Stabilization of Pathological Activity

Substance	Dose, mg/kg	Number of experiments	Interictal discharges (mean number per minute of EEG recording from 60th to 120th minutes of experiment)	Electrographic correlates of fits (mean number during 10 min of observation from 60th to 120th minutes of experiment)
Control	—	18	7,1	2,9
Strophanthin	1,8	6	8,5	3,2
Digoxin	1,2	6	11,8*	4,9*

Legend. *p < 0.05.

TABLE 2. Changes in Activity of Epileptogenic Focus of Frog Hippocampus after Preliminary Injection of Cardiac Glycosides into Dorsal Lymph Sac (5 min before injection of penicillin into hippocampus)

Substance	Dose, mg/kg	Number of experiments	Interictal discharges (mean number per minute of recording EEG from 0 to 120th minute of experiment)	Electrographic correlates of fits (mean number per 10 min of observation from 0 to 120th minute of experiment)
Control		18	12,8	2,6
Strophanthin	1,8	6	14,5	3,4*
	0,18	6	14,0	2,9
Digoxin	1,2	6	18,8*	4,2*
	0,12	6	9,3	1,1**

Legend. *p < 0.05, **p < 0.01.

(Gedeon Richter, Hungary) 1.2 (subtoxic dose) and 0.12. Injection of the glycosides into frogs with no epileptogenic focus did not change the character of their EEG. Distilled water was injected into the dorsal lymph sac of the control frogs before the focus was produced and 50-60 min after its formation. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney U test [2].

EXPERIMENTAL RESULTS

The data in Tables 1 and 2 are evidence that preliminary injection of subtoxic doses of cardiac glycosides (the subtoxic dose is the largest dose of strophanthin or digoxin which, in 100% of cases, caused no toxic effect — cardiac arrest — in the frogs in the course of 2 h) or their injection after 50-60 min (against the background of an actively functioning, stabilized epileptogenic focus) caused an increase in the frequency of interictal epileptiform discharges and in the number of electrographic correlates of fits on the frogs' EEG.

The injection of preparations in a dose lower than subtoxic by a factor of one, up to epileptogenic focus, brought about a different effect (Table 2). Strophanthin, in this dose, induced pathological activity, although to a lesser degree, while digoxin suppressed it. In this case the number of electrographic correlates of the fits decreased quite significantly.

Under experimental conditions proof was thus obtained for the effect of cardiac glycosides, injected parenterally, on excitability of CNS neurons. It must be emphasized that different glycosides differed in this respect: digoxin, in a near-toxic dose, was 1.5 times more active than strophanthin, evidently because digoxin passes more easily through the blood-brain barrier (BBB) or because of the characteristics of its interaction with Na^+, K^+-ATPase [9, 13]. With a tenfold decrease in the dose of the preparations the effect of stophanthin was weakened whereas the effect of digoxin was diametrically opposite, possibly due to its effect on brain structures, a change in the excitability of which led to suppression of activity of the epileptogenic focus created and, in particular, the circulation of excitation from it.

The provocative effect of large doses of cardiac glycosides on the epileptogenic focus was evidently linked with inhibition of Na^+, K^+-ATPase [8], which is accompanied by disturb-

ance of mediator activity in the CNS [4, 6, 12], and with retention of Na^+ in the neurons and their swelling. The inhibitory action of digoxin in a dose of 0.12 μg/g, on the other hand, may perhaps be explained by the activating effect of small doses of glycosides on the Na^+, K^+-pump [5].

The results of this investigation show the danger of use of cardiac glycosides (especially lipophilic) in patients with epilepsy, for they may provoke fits. Moreover, in this pathology the permeability of the BBB is increased, which facilitates possible central effects of glycosides.

LITERATURE CITED

1. R. G. Biniaurishvili, A. M. Vein, B. G. Gafurov, and A. R. Rakhimdzhanov, Epilepsy and the Functional State of the Brain [in Russian], Tashkent (1985).
2. E. V. Gubler, Computerized Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978), pp. 72-75.
3. T. Godfraind and L. D. Tona, Eur. J. Pharmacol., 60, No. 4, 329 (1979).
4. K. J. Collard and S. R. Williams, Biochem. Soc. Trans., 9, No. 1, 109 (1981).
5. T. J. Hougen, N. Spicer, and T. W. Smith, J. Clin. Invest., 68, No. 5, 1207 (1981).
6. T. Katsuragi and T. Furukawa, Arch. Int. Pharmocodyn., 238, No. 1, 4 (1979).
7. M. Kemali and V. Braitenberg, Atlas of the Frog's Brain, Berlin (1969), pp. 57-65.
8. R. H. Kennedy, T. Akera, and T. M. Brody, Eur. J. Pharmacol., 89, No. 3-4 (1983).
9. J. Kuhlmann, E. Erdmann, and N. Rietbrock, Naynyn-Schmiederbergs Arch. Pharmakol., 307, No. 1, 65 (1979).
10. F. S. La Bella, Trends Pharmacol. Sci., 3, No. 9, 354 (1982).
11. L. Matos, M. Istfanffy, R. M. Halmagyi, et al., Ergebn. Exp. Med., 37, 51 (1980).
12. Y. Nakazato, S. Ito, and A. Ohga, Eur. J. Pharmacol., 89, No. 1-2, 77 (1983).
13. A. C. Swann, Eur. J. Pharmacol., 119, No. 1-2, 67 (1985).

ROLE OF THE GABA-ERGIC SYSTEM IN THE MECHANISM OF THE STRESS-REGULATING ACTION OF FENIBUT

G. V. Kovalev, A. A. Spasov, N. A. Bogachev, V. D. Petryanik, and O. V. Ostrovskii

UDC 615.214.22:547.466.3].015.4

KEY WORDS: GABA-ergic system; stress; fenibut*

An important stage in the mechanisms of emergency adaptation to extremal influence is "activation" of the adaptive function of the GABA-ergic inhibitory system of the brain [1, 2, 7, 12]. In the modern view, fenibut is a GABA-positive agent [10]. Meanwhile, it has been shown that fenibut, in relatively small doses (1-25 mg/kg) increases the resistance of animals to the action of various external factors, exerts a stress-regulating influence [3, 4], and protects the myocardium against stress-induced injury [5].

The aim of this investigation was to study the effect of fenibut on activity of GABA-system under normal and stress conditions. For this purpose concentrations of GABA and glutamic acid (GA) and activity of enzymes of GABA metabolism, namely glutamate decarboxylase (GDC) and GABA-transaminase (GABA-T) were studied in the rat thalamus and hypothalamus. The intensity of the stress reaction was determined by measuring the peripheral blood plasma levels of 17-hydroxycorticosteroids (17-HCS) and glucose — parameters closely linked with the intensity and duration of action of the stress factor [14].

EXPERIMENTAL METHOD

Experiments were carried out on 75 noninbred male albino rats weighing 120-170 g. The

*β-Phenyl-GABA.

Department of Pharmacology and Central Research Laboratory, Volgograd Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR, A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 588-590, November, k987. Original article submitted June 17, 1986.

0007-4888/87/0011-1574$12.50