5. M. N. Kondrashova and A. A. Ananenko, Textbook of the Study of Biological Oxidation by the Polarographic Method [in Russian], Moscow (1973), pp. 106-129.
6. M. N. Kondrashova, Yu. V. Evtodienko, G. D. Mironova, et al., Biophysics of Complex Systems and Radiation Disturbances [in Russian], Moscow (1977), pp. 249-271.
7. M. N. Kondrashova, Yu. V. Evtodienko, A. M. Babskii, and V. A. Khazanov, Molecular Mechanisms of Oxygen Homeostasis [in Russian], Novosibirsk (1987), pp. 44-48.
8. G. F. Lakin, Biometrics [in Russian], Moscow (1973).
9. L. D. Luk'yanova, B. S. Balmukhanov, and A. T. Ugolev, Oxygen-Dependent Processes in the Cell and Its Functional State [in Russian], Moscow (1982).
10. S. Nemecek, Introduction to Neurobiology [Russian translation], Prague (1978), pp. 57-67.
11. E. B. Okon, Reactions of Living Systems and the State of Energy Metabolism [in Russian], Pushchino (1979), pp. 126-139.
12. B. Chance and B. Hagihara, J. Biol. Chem., **237**, No. 11, 3540 (1962).
13. E. Grigorenko and N. Kondrashova, Third European Bioenergetics Conference: Reports, Vol. 3B, Hannover (1984), pp. 533-534.

ANTIEPILEPTIC EFFECTS OF NIFEDIPINE

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The antiepileptic effects of blockers of voltage-dependent Ca-channels have been discovered on various different models of epileptic activity (EPA) [1-4, 6-10] and also in man [11]. However, the particular features of the action of these preparations and of the abolition of EPA remain unclear and require further study. Their elucidation is of great importance for a solution to the problem of the use of these preparations in the treatment of epilepsy.

In the investigation described below the effect of nifedipine (1,4-dihydropyridine) on penicillin-induced focal EPA, an acute generalized seizure reaction induced by systemic administration of metrazol, and also the syndrome of increased sensitivity to an epileptogen during its chronic administration (metrazol kindling) and the seizure reaction of animals after the end of metrazol kindling.

EXPERIMENTAL METHOD

Experiments were carried out on 157 male Wistar rats. The animals were kept under ordinary animal house conditions and on a standard diet. A focus of EPA was created by application of filter paper soaked in a solution of the sodium salt of benzylpenicillin in a concentration of 20,000 IU/ml to the sensomotor cortex. By a method described previously [2] burr holes 2 × 4 mm in diameter were drilled 24 h before penicillin application in the animal's skull above symmetrical regions of the sensomotor cortex, the dura was removed, and monopolar cortical silver electrodes were applied

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TABLE 1. Effect of Nifedipine on Penicillin-Induced Focal EPA (M ± m)

Series	Group of animals	No. of animals	Before injection			30 min after injection			Duration of existence of focus, min
			number of IID/min	amplitude of IID, mV	number of ID/min	number of IID/min	amplitude of IID, mV	number of ID/min	
I	Control, DMSO	10	13,14±0,75	1,18±0,16	0,51±0,05	9,81±1,32	1,02±0,16	0,43±0,04	115,63±16,34
	Nifedipine, 1 mg/kg	13	13,69±1,40	1,26±0,18	0,52±0,08	11,13±1,21	1,17±0,39	0,48±0,04	112,80±13,73
II	Control, DMSO	7	12,76±1,20	0,99±0,23	0,23±0,07	8,86±3,13	0,85±0,23	0,16±0,06	127,53±12,52
	Nifedipine, 2 mg/kg	8	14,12±4,14	1,40±0,30	0,27±0,03	8,37±3,64	1,21±0,21	0,37±0,13	131,34±21,34
III	Control, DMSO	8	10,18±2,85	0,98±0,08	0,51±0,06	8,14±2,12	0,68±0,23	0,28±0,09	118,56±14,83
	Nifedipine, 5 mg/kg	8	9,87±2,15	0,95±0,06	0,48±0,06	7,97±2,15	0,66±0,22	0,27±0,10	126,83±11,69
IV	Control, DMSO	11	10,84±1,53	0,70±0,08	0,37±0,08	11,01±1,25	0,61±0,06	0,19±0,06	128,51±16,31
	Nifedipine, 10 mg/kg	10/13	10,04±0,51	0,82±0,06	0,35±0,05	6,95±1,09*	0,42±0,12**	0,10±0,03***	82,79±10,84*
V	Control, DMSO	8	18,74±1,98	1,23±0,06	0,42±0,08	15,27±3,16	1,19±0,13	0,31±0,11	139,65±15,53
	Nifedipine, 30 mg/kg	7/8	20,34±2,50	1,19±0,13	0,40±0,11	10,96±1,36*	0,72±0,12*	0,03±0,01**	91,46±10,18*

Legend. Numerator gives number of animals in which Nifedipine caused suppression of EPA; denominator gives total number of animals in group. *$p < 0.05$, **$p < 0.02$, ***$p < 0.001$.

to derive electrical activity in the cortical regions specified (ECoG). The ECoG was recorded on an EEG-8S electroencephalograph (Hungary) in unrestrained animals. Nifedipine ("Serva") was dissolved in dimethylsulfoxide (DMSO) and injected intraperitoneally in doses of 1, 2, 5, 10, and 30 mg/kg against a background of persistent generation of ictal discharges in the focus. The control animals received an injection of the same volume (0.1 ml) of the solvent. The experimental data were analyzed on a M-44 computer system ("Olivetti"). Amplitude versus frequency characteristic curves were drawn and the duration of existence of the EPA foci determined.

Acute generalized EPA was induced by intraperitoneal injection of metrazol in a dose of 75 mg/kg. The latent period of the first seizure manifestations, the time of onset of the clonic and tonicoclonic phases of the generalized seizure reaction, and their duration and mortality were determined. Nifedipine was injected intraperitoneally 30 min before injection of metrazol, in a dose of 10 mg/kg (10 rats). This dose caused suppression of EPA in the penicillin-induced focus in the cerebral cortex of the rats. The solvent was injected under similar experimental conditions into the control animals (13 rats).

Before the experiments with **pharmacological kindling** began, in order to select animals with similar sensitivity to metrazol, we used a method of our own design [4, 5], in which their sensitivity was tested beforehand in accordance with the character of the response to a single application of the epileptogen in the minimal active dose (40 mg/kg). Rats in which a seizure response to this minimal effective dose was observed could be considered to be relatively more sensitive to the action of the epileptogen, and they were subsequently used in the experiment (40 rats). Kindling was carried out 1 week after testing, by daily intraperitoneal injection of metrazol in a subconvulsive dose of 30 mg/kg for 28 days. Nifedipine was dissolved in DMSO and injected intraperitoneally in a dose of 10 mg/kg, 30 min before each injection of metrazol (10 rats). Animals which, under similar experimental conditions, were given an injection of physiological saline (20 rats) or DMSO (10 rats) before metrazol served as the control. The severity of the seizure reaction in all the animals was tested daily on a 6-point scale [4, 5].

After the end of kindling, the effect of preliminary (30 min before injection of metrazol in a dose of 30 mg/kg) intraperitoneal injection of nifedipine in a dose of 10 mg/kg (10 rats) and 30 mg/kg (10 rats) on the severity of the seizure reaction was determined in control animals which received injections of metrazol and physiological saline during the kindling process.

The significance of differences was estimated in all the experiments by Student's test.

EXPERIMENTAL RESULTS

Penicillin-Induced Focal EPA. Application of penicillin led to the appearance of EPA after 3-6 min: single interictal discharges (IID) appeared, and after 8-16 min paroxysmal ictal discharges (ID) appeared; after 25-35 min the stage of stable generation of ID began, and continued for 30-40 min; this was followed by a gradual decrease in the frequency of ID generation and also of the frequency and amplitude of IID. The duration of existence of the foci from the time of application of penicillin to complete disappearance of EPA was 110-130 min.

Injection of nifedipine against the background of stable ID generation in the focus (25-30 min after penicillin application) in doses of 1, 2, and 5 mg/kg proved ineffective (Table 1). The antiepileptic effect of the drug in a dose of 10 mg/kg was discovered in 70% of animals and expressed as a decrease in the frequency of IID and ID generation, and in the amplitude of IID and shortening of the time of existence of the EPA focus. The effect of nifedipine in a dose of 30 mg/kg was characterized only by a greater decrease in the frequency of generation of ID compared with the effect in a dose of 10 mg/kg. Injection of DMSO into animals of the control groups during this period did not change the character of EPA in the focus (Table 1).

Acute Generalized EPA. Preliminary injection of nifedipine in a dose of 10 mg/kg reduced the duration of the clonic phase of the seizure reaction from 29.9 ± 3.5 sec in the control to 21.8 ± 1.4 sec in the experiment ($p < 0.05$) and increased the time of onset of the tonicoclonic phase (from 115.6 ± 5.7 sec in the control to 213.8 ± 42.9 sec in the experiment $p < 0.05$), and also shortened the duration of the tonic phase of the generalized seizure reaction (from 20.9 ± 1.1 sec in the control to 15.9 ± 1.7 sec in the experiment $p < 0.05$). The mortality in the group of control animals was 54.8% (seven of 13 rats), compared with 20% in the group of experimental animals (two of 10 rats).

Metrazol Kindling. During development of EPA as a result of kindling, injection of nifedipine in a dose of 10 mg/kg before each injection of metrazol had no effect on the development of increased seizure susceptibility or on the severity of the seizure reaction in response to each injection of metrazol during kindling. In the control animals (physiological saline + metrazol), which responded after the end of kindling to the testing dose of metrazol (30 mg/kg) by a seizure reaction estimated at 3-5 points, injection of nifedipine (30 min before metrazol) in doses of 10 and 30 mg/kg had no significant effect on the severity of the seizures: their intensity was the same as in response to injection of metrazol alone.

Thus nifedipine, in a dose of 10 mg/kg; suppressess EPA in a penicillin-induced focus in the rats cerebral cortex. Nifedipine has a similar inhibitory effect on acute generalized clonicotonic seizures induced by metrazol. Meanwhile, in the same dose, the drug is ineffective during the development of EPA in the brain in response to onic administration of an epileptogen, in the form of metrazol kindling; it did not delay the development of enhanced seizure susceptibility and did not reduce the severity of the seizure reaction. After the end of kindling nifedipine, in doses of 10 and 30 mg/kg, had no effect on the severity of the seizure reaction in response to the testing dose of metrazol.

It has been shown [13] that nifedipine, while suppressing certain forms of EPA, does not affect seizures induced by injection of quinolinic and kainic acids into the hippocampus in a dose of 40 mg/kg. However, it reduces the severity of seizures after hippocampal kindling induced by electrical stimulation, in a dose of 20 mg/kg in 57% of animals. Taking these data into consideration, we can postulate that the reason why nifedipine has no action on the development of increased seizure susceptibility during metrazol kindling in the present experiments may be connected both with the use of the drug in a relatively small dose and also with the type of kindling (pharmacological, by contrast with that due to electrical stimulation). At the same time, it must be pointed out that Ca^{2+} antagonists such as verapamil, riodipine (a blocker of dihydropyridine-sensitive Ca-channels) and MK-801, while delaying the development of enhanced seizure susceptibility, did not affect the seizure reaction of rats after the end of kindling induced by metrazol [4] or by electrical stimulation of the amygdala and hippocampus [12], even if the dose of the drug was increased by 2-3 times [4]. The question of possible differences in the mechanisms of the generalized seizure reaction in postkindling and intact animals deserves special attention and investigation.

LITERATURE CITED

1. R. N. Glebov and M. N. Karpova, Patol. Fiziol., No. 3, 57 (1990).
2. M. N. Karpova, R. N. Glebov, G. N. Kryzhanovskii, et al., Byull. Éksp. Biol. Med., No. 7, 54 (1987).
3. M. N. Karpova, O. Yu. Pankov, R. N. Glebov, et al., Byull. Éksp. Biol. Med., No. 11, 553 (1989).
4. G. N. Kryzhanovskii, M. N. Karpova, and O. Yu. Pankov, Byull. Éksp. Biol. Med., No. 10, 348 (1990).

5. G. N. Kryzhanovskii, M. N. Karpova, and O. Yu. Pankov, Byull. Éksp. Biol. Med., No. 4, 351 (1991).
6. D. Ashton and A. Wauguier, Psychopharmacology, **65**, 7 (1979).
7. G. B. De Sarro, B. S. Meldrum, and G. Nistico, Brit. J. Pharmacol., **93**, 247 (1988).
8. L. K. C. Desmedt, C. J. E. Niemegeers, and P. A. J. Janssen, Arzneimittel-Forsch., **25**, 1408 (1975).
9. S. I. Dolin, A. B. Hunter, M. J. Halsey, et al., Eur. J. Pharmacol., **152**, 19 (1988).
10. F. B. Meyer, R. E. Anderson, T. M. Sundt, et al., Mayo Clin. Proc., **61**, 239 (1986).
11. J. Overweg, C. D. Binnie, J. W. Meijer, et al., Epilepsia, **25**, 217 (1984).
12. K. Sato, K. Morimoto, and M. Okamoto, Brain Res., **463**, 12 (1988).
13. A. Vezzani, H. Q. Wu, M. A. Stasi, et al., Neuropharmacology, **27**, 451 (1988).