LITERATURE CITED

1. E. D. Gol'dberg and V. A. Eshchenko, Patol. Fiziol., No. 3, 81 (1989).
2. E. D. Gol'dberg and V. A. Eshchenko, Probl. Éndokrinol., No. 3, 78 (1989).
3. V. A. Eshchenko, Tsitologiya, No. 8, 927 (1978).
4. S. V. Levin, Structural Changes in Cell Membranes [in Russian], Nauka, Leningrad (1976).
5. V. G. Baranov, I. M. Sokoloverova, E. G. Gasparyan, et al., Experimental Diabetes Mellitus [in Russian], Leningrad (1983).
6. K. V. Atchuta, N. Ramajah, and A. Mookerjee, Indian J. Exp. Biol., **16**, 857 (1978).
7. W. J. Bettger and B. L. O'Dell, Life Sci., **28**, 1425 (1981).
8. I. Kadota, J. Lab. Clin. Med., **35**, 563 (1950).
9. A. E. Martell and M. Calvin, Chemistry of the Metal Chelate Compounds, New York (1957).
10. K. Okamoto, Acta Schol. Med. Univ., **27**, 43 (1949).

SEIZURE ACTIVITY OF ALBINO RATS AFTER IMMUNIZATION WITH A CONJUGATE OF SYDNOPHEN AND SERUM ALBUMIN

R. A. Danilova, E. V. Kustova, M. F. Obukhova, and I. P. Ashmarin

UDC 616.853-02:615.214.32]-092.9-07

KEY WORDS: sydnophen, conjugates, immunization, metrazol, seizures.

In the clinical management of epilepsy psychotropic drugs with sedative and tranquilizing properties are widely used as anticonvulsive agents. Antidepressants with powerful stimulating properties, chiefly MAO inhibitors, in most cases have a provocative effect and potentiate the action of convulsive agents. The method of so-called inverse regulation of physiological and biochemical processes, being studied at the present time, involves active immunization against bioregulators, injected in the form of conjugates with carrier antigens. It leads to prolonged and profound effects, the direction of which it is mainly opposite to the effects of the same bioregulator as such. The bioregulator used may be identical with or similar in structure to the endogenous regulator. The regulators bind with antibodies formed during immunization, thus determining their ultimate prolonged physiological effect [1]. We showed previously that immunization of rats with a conjugate of the antidepressant and psychostimulant sydnophen (a partial catecholamine analog) with bovine serum albumin (BSA) can produce a long-term change in the state of rats, as reflected in physiological and biochemical parameters [2, 3, 6]. Changes observed in the behavior of the rats, similar in some respects to the effects of neuroleptics, led us to assess the influence of immunization on seizure activity.

The aim of this investigation was to study the effect of immunizing rats with a conjugate of sydnophen and serum albumin on seizure activity following injections of metrazol.

EXPERIMENTAL METHOD

Experiments were carried out on 115 noninbred male albino rats weighing 180-200 g. Conjugation of sydnophen with BSA was carried out by the glutaraldehyde method, as described previously [2, 3]. Rats of the experimental groups were

Department of Human and Animal Physiology and Department of Higher Nervous Activity, Faculty of Biology, M. V. Lomonosov Moscow State University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 137-139, February, 1991. Original article submitted June 15, 1990.

 0007-4888/91/0002-0154$12.50

TABLE 1. Effect of Single Injection of Metrazol (60 mg/kg) on Seizure Activity of Immunized and Control Rats

Group	Number of rats (in %)	
	with LP of onset of seizure ≤ 10 min	with no seizure for 30 min
Control (n = 23)	60,9 %	8,7 %
Sydnophen (n = 17)	52,9 %	11,8 %
BSA (n = 19)	47,4 %	31,6 %
Conjugate (35-40 days, n = 15)	13,3 %	40,0*
Conjugate (n = 20), (2 months, n = 20)	45,0 %	25,0 %

Legend. *n*) Number of animals in group; $*p < 0.05$ compared with group 1; $**p < 0.01$ compared with group 1 and $p \leq 0.05$ compared with groups 2, 3, and 5.

immunized with the conjugate three times at intervals of 7-8 days; the conjugate was injected subcutaneously at several points in the dorsal region, the first two times mixed with Freund's complete adjuvant (FCA) in the ratio of 1:1. The dose, expressed in protein, was 150-200 μg per rat. Animals of the control groups received: 1) physiological saline, 2) BSA without sydnophen, treated with glutaraldehyde and with FCA, and 3) sydnophen in the dose contained in the conjugate (15-30 μg). The development and severity of the seizure syndrome were studied both after single injections of threshold doses of metrazol and after repeated injections of metrazol in below-threshold doses.

To form a metrazol model of seizures, 35-40 days or two months or more after the beginning of immunization, all the experimental and control groups of rats were given a single injection of metrazol (60 mg/kg, subcutaneously). The state of the animals was assessed according to the latent period of onset of marked clonicotonic convulsions, with the animals falling on to their side. The animals were kept under observation for 30 min after receiving the injection of metrazol. In a separate series of experiments the formation of generalized seizure activity was studied during daily injection of metrazol in subthreshold doses. Three weeks after the first immunization rats of the experimental and control (with physiological saline) groups received 21 daily injections each of 30 mg/kg of metrazol. The animals were kept under observation for 30 min after each injection. Seizures were rated in points according to the criteria described in [5]. After the end of the experiments, antibody titers were determined in all the rats by ELISA, using seeding buffer, pH 7.5). The results were subjected to statistical analysis by the Fisher and Wilcoxon—Mann—Whitney tests.

EXPERIMENTAL RESULTS

In rats of the control group, receiving physiological saline, for 30 min after injection of metrazol marked clonicotonic convulsions, with the animal falling onto its side, were observed in more than 90% of cases, with a latent period ranging from 1 to 30 min. More than half of the rats gave a seizure with a latent period of 10 min. A similar pattern was observed in animals of the control group receiving sydnophen (Fig. 1; Table 1). In the group of immunized rats receiving metrazol on the 35th-40th day after the beginning of immunization (the period of most marked behavioral changes, as indicated previously), a statistically significant increase was observed in the latent period of onset of the seizure compared with other groups (Fig. 1). In addition, the trend of the distribution of the number of animals by latent period of severe seizure manifestations in the control and immunized rats was directly opposite in direction (Fig. 2). In the group of immunized rats (Table 1, group 4) the proportion of rats with high sensitivity to metrazol, i.e., with a latent period of onset of the seizure of 10 min, was statistically significantly reduced (fourfold) compared with all other groups. Meanwhile, the proportion of rats which had no seizures with the animal falling onto its side in the course of 30 min, but in which only weak shaking of the head or twitches of individual trunk muscles were observed, was increased more than fourfold. However, this was statistically significant only by comparison with the control group, which received physiological saline. In the groups of rats immunized only with BSA or with the sydnophen conjugate, but tested with metrazol two months or more after the beginning of immunization (Table 1, groups 3 and 5), an increase in the number of animals without marked seizures also was observed compared with the control. However, these differences were not statistically significant.

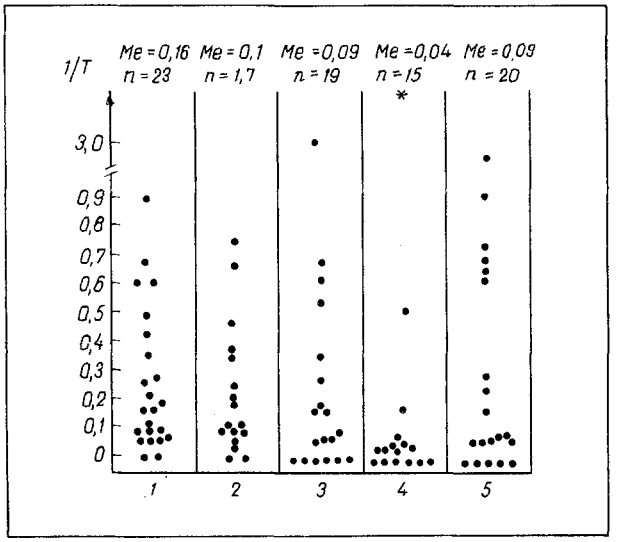

Fig. 1. Temporal parameters of onset of seizure after a single injection of metrazol. Abscissa, serial No. of group: 1) control group; 2) injection of sydnophen; 3) immunization with BSA; 4, 5) immunization with conjugate of sydnophen and BSA; testing 35-40 days after first immunization (40) and 60 or more days thereafter (5). Ordinate, reciprocal of latent period of seizure (in min). Asterisk indicates statistically significant differences compared with groups 1, 2, and 5 ($p < 0.01$) and with group 3 ($p < 0.05$).

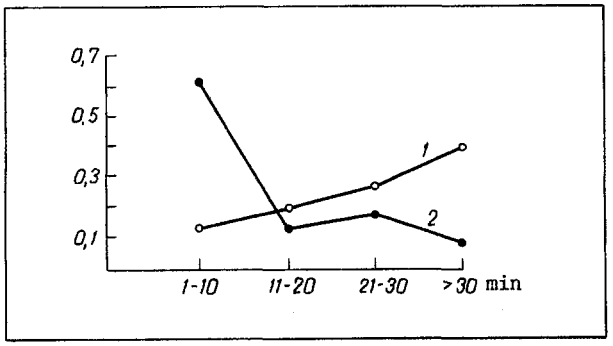

Fig. 2. Distribution of animals by latent period of onset of seizures after a single injection of metrazol. 1) Animals immunized with conjugate of sydnophen and BSA, 2) control group. Abscissa, latent period (in min); ordinate, fraction of animals.

The results led us to compare the control and immunized rats, using a model of increasing predisposition to seizures with repeated injections of subthreshold doses of metrazol, i.e., pharmacological kindling [4]. Just as was shown previously [4, 5], daily injection of 30 mg/kg metrazol causes an increasing degree of predisposition to seizures in the control animals, and the appearance of seizures in response to a subthreshold dose of metrazol. The intensity of the seizure reaction, just as the latent period of its onset and its duration, varied both in individual animals and in the group as a whole. On the 11th day of injection of metrazol seizure reactions with an average intensity of two points were observed in 90% of the rats. The way in which a state of predisposition to seizures was formed in the immunized rats in the first stages differed a little from that in the controls, as follows. First, the appearance of the first seizure responses in the control rats began on the 5th day of metrazol administration, and had reached almost 50% by the end of the first week (Fig. 3). In the experimental group, seizure responses did not appear until the 8th day, and in 20% of the animals. The increase in the level of predisposition to seizures in the experimental group until the 11th day was retarded somewhat, as regards both the number of animals with seizures ($p < 0.05$) and the latent period

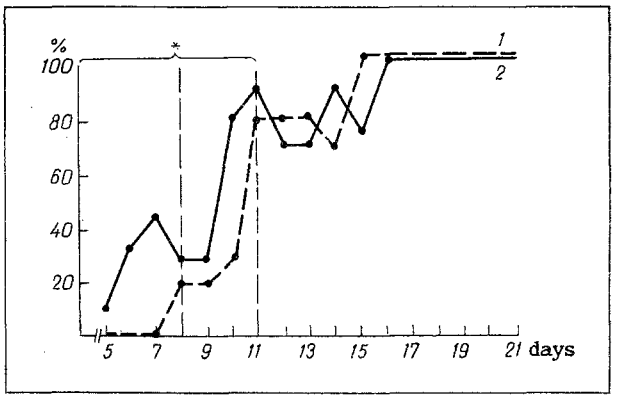

Fig. 3. Dependence of number of animals with manifest seizures on duration of metrazol administration (30 mg/kg). 1) Animals immunized with conjugate of sydnophen and BSA (n = 10), 2) control group (n = 11). Abscissa, duration of observations with daily injection of metrazol (in days); ordinate, number of animals (in %). n) Number of animals in group; *) statistically significant differences ($p < 0.05$) compared with control group in period from 1st through 11th day.

of onset and the mean level of severity of the seizure responses. Starting with the 11th day of metrazol administration, no differences were found. The results of kindling are preliminary in character and require further verification.

The results are thus evidence that immunization of rats with a conjugate of sydnophen and BSA lowers the threshold of predisposition to seizures induced by threshold doses of metrazol and, as the preliminary results show, it may modify the process of formation of pharmacological kindling.

The spectrum and level of antibodies to the various endogenous bioregulators after immunization against sydnophen deserves special examination. The complexity of the pattern thus formed justifies a special communication on the subject. It may be pointed out here only that, besides titers of antibodies to sydnophen, close to 1:600 on average, antibodies to noradrenalin, dopamine, and serotonin of a comparable level (1:320-1:1280) were detected. In control experiments with immunization to FCA and BSA, the corresponding average titers were 2-4 times lower.

Lowering of the plasma noradrenalin and brain dopamine levels in rats immunized against sydnophen was demonstrated previously [6].

The search for correlations between the physiological, immunological, and biochemical parameters brought to light must be continued.

LITERATURE CITED

1. I. P. Ashmarin and O. A. Gomazkov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 1, 11 (1989).
2. R. A. Danilova, M. F. Obukhova, I. P. Ashmarin, and M. V. Mashkovskii, Dokl. Akad. Nauk SSSR, **303**, No. 6, 1512 (1988).
3. R. A. Danilova, M. F. Obukhova, Sh. K. Sagimbaeva, M. D. Mashkovskii, and I. P. Ashmarin, Biol. Nauki, No. 3, 63 (1989).
4. G. N. Kryzhanovskii, A. A. Shandra, L. S. Godlevskii, and R. F. Makul'kin, Usp. Fiziol. Nauk, **19**, No. 4, 12 (1988).
5. A. A. Shandra, L. S. Godlevskii, and N. D. Semenyuk, Byull. Éksp. Biol. Med., No. 4, 20 (1983).
6. I. P. Ashmarin, R. A. Danilova, M. D. Mashkovskii, E. I. Melnik, et al., Biomed. Sci., **1**, 95 (1990).