There is indirect evidence to suggest that adjustment of the architectonics of the motoneuron takes place within a fairly short time. For instance, in patients with a disturbance of neuromuscular transmission, when an electromyographic investigation of the muscles must invariably be performed before biopsy, as a rule we did not observe any denervation electrophysiological phenomena — fibrillation potentials (FP) and positive pointed waves in muscles in which considerable degrees of grouping were found. Since we know that FP appear in response to disturbance of contact of the muscle fiber with the corresponding motoneuron in the course of 7-14 days [3, 10], this suggests that the adjustment takes place earlier.

These results demonstate that besides branching of a motoneuron, in one zone of innervation (most probably the central) muscle fibers located at the periphery of MU are excluded from the zone of its innervation, and that the motoneuron actively adjusts its structure in order to create optimal conditions for innervation of a certain population of muscle fibers located in the zone of its innervation.

LITERATURE CITED

1. B. M. Gekht, L. F. Kasatkina, and A. V. Kevish, Zh. Nevropatol. Psikhiat., No. 6, 822 (1980).
2. M. C. Brown and R. Ironton, J. Physiol. (London), 278, 325 (1978).
3. F. Buchtal and P. Rosenfalk, Electroencephalogr. Clin. Neurophysiol., 20, 321 (1966).
4. F. Buchtal and H. Schmalbruch, Physiol. Rev., 60, 90 (1980).
5. V. Dubowitz and M. H. Brooke, Muscle Biopsy: a Modern Approach, London (1973), pp. 7-19.
6. J. Ekstedt and E. Stälberg, in: New Developments in Electromyography and Clinical Electrophysiology, J. Desmedt, ed., Vol. 1, Basel (1973), pp. 89-112.
7. W. K. Engel and J. Warmolz, in: New Developments in Electromyography and Clinical Electrophysiology, J. Desmedt, ed., Vol. 1, Basel (1973), pp. 141-177.
8. A. D. Grinnel and A. A. Herrera, Prog. Neurobiol., 17, 203 (1981).
9. I. Hausmanowa-Petrusewicz (editor), Atlas Chorob Miesni (Atlas of Muscle Diseases), Warsaw (1968), pp. 282-289.
10. I. Luco and C. Eyzaguirre, J. Neurophysiol., 18, 65 (1955).

FORMATION OF GENERALIZED SEIZURE ACTIVITY IN MICE AFTER DAILY INJECTION OF METRAZOL IN SUBTHRESHOLD DOSES

A. A. Shandra, L. S. Godlevskii, and N. D. Semenyuk

UDC 616.8-009.24-092.9-02:615.221

KEY WORDS: metrazol; seizures; "kindling" phenomenon.

A method of inducing seizure activity by repeated subthreshold electrical stimulation of brain structures has been described and is known as the "kindling" phenomenon [3, 5, 6, 13]. Inducing seizure activity by repeated stimulation with chemical agents (convulsants) appeared interesting as a potential model. To study the pathogenesis of the epileptic syndrome and screening of antiepileptic drugs, a model of generalized seizures induced by systematic injection of metrazol is widely used [9, 12].

The object of this investigation was to study whether a seizure syndrome can develop in response to repeated injections of subthreshold doses of metrazol.

EXPERIMENTAL METHOD

Experiments were carried out on inbred (CBA × C57BL/6, BALC/C)F_1 hybrid mice and noninbred albino mice weighing 18-22 g. Each group consisted of at least 15 animals. Metrazol

Department of Pathological Physiology, N. I. Pirogov Odessa Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 20-22, April, 1983. Original article submitted September 30, 1982.

0007-4888/83/9504-0425$07.50

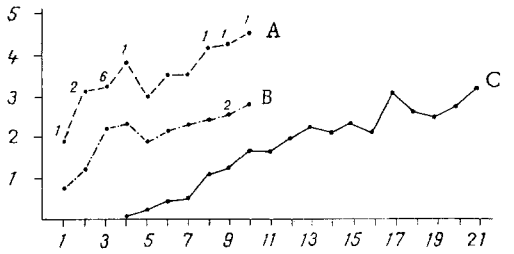

Fig. 1. Severity of seizure response evoked by different doses of metrazol. Dose of metrazol injected: A) 60 mg/kg, B) 40 mg/kg, C) 30 mg/kg. Numbers near broken line indicate number of animals which died. Abscissa, duration of observations with daily injection of metrazol (in days); ordinate, severity of response (in points).

TABLE 1. Effect of Daily Subcutaneous Injection of Metrazol (30 mg/kg) on Intensity of the Seizure Response

Day of experiment	Total number of animals	Number of animals with seizures	Intensity of seizure response, points					Averaged severity of response, points
			1	2	3	4	5	
1	73	—	—	—	—	—	—	0
2	73	—	—	—	—	—	—	0
3	73	—	—	—	—	—	—	0
4	73	5	5	—	—	—	—	0,06
5	73	11	6	4	—	1	—	0,24
6	73	17	10	3	—	4	—	0,44
7	73	23	15	3	1	4	—	0,54
8	73	38	21	4	4	9	—	1,05
9	74	39	19	5	1	14	—	1,20
10	73	43	10	13	—	20	—	1,59
11	73	48	16	14	1	17	—	1,57
12	73	55	14	21	—	19	1	1,87
13	53	49	12	17	—	20	—	2,37
14	53	49	16	16	3	14	—	2,13
15	53	47	10	12	1	21	3	2,56
16	36	33	8	16	—	8	1	2,10
17	36	35	2	10	—	23	—	3,16
18	35	35	6	17	—	12	—	2,51
19	31	31	6	15	—	10	—	2,45
20	31	31	7	12	—	12	—	2,55
21	24	24	2	7	—	15	—	3,

was injected subcutaneously in a dose of 60, 40, or 30 mg/kg in a volume of 0.1 ml daily, under the same conditions (at the same time of day, in a room with the same intensity of illumination and the same background noise level). After injection of metrazol the animals were placed in a glass container and observed for 30-40 min. Behavioral seizures were recorded in points, according to the following system: 0 point) absence of seizure response, 1 point) twitching of the head or individual trunk muscles, 2 points) repeated clonic convulsions of the whole trunk, 3 points) clonic convulsions of the forelimbs, 4 points) clonicotonic convulsions with the animal falling on its side, and with postictal depression, 5 points) repeated marked tonicoclonic convulsions or lethal convulsions. Animals of the control group were given injections of physiological saline under the same conditions. The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS

In the animals of groups 1 and 2, which received 60 and 40 mg/kg respectively of metrazol, seizure responses appeared after the first injection of the drug (Fig. 1). In group 1 convulsions of different degrees of severity were observed in all animals — from twitching of the head and trunk to tonicoclonic convulsions ending in death. The highest mortality among the animals was observed after the 3rd injection of metrazol. In group 2, after the first injection of metrazol, convulsions were observed in four animals but they were very slight and did not cause death. The incresae in severity of the seizure response in the animals of this

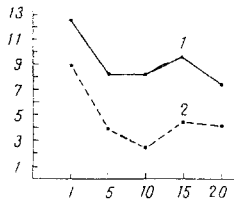

Fig. 2. Dependence of latent period of first seizures and of intensive convulsions on duration of metrazol administration (30 mg/kg daily). 1) Latent period of first seizures; 2) latent period of severe convulsions. Abscissa, duration of metrazol administration (in days); ordinate, latent period of first seizures and period of severe convulsions (in min).

TABLE 2. Effect of Intervals in Metrazol Administration on Intensity of Seizure Responses

Group of animals	Experimental conditions	Ratio of number of animals with seizures to total number of animals	Average severity of convulsions, points	P compared with group 1
1	After 3 weeks of daily injections of metrazol (30 mg/kg)	58/60	2,8	0,05
	Interval			
2	10 days	19/20	3,2	0,05
	15 days	20/20	2,9	0,05
	30 days	7/10	2,2	0,05
	40 days	0/10		0,01

group was slower in character than in the mice of group 1. Severe convulsions (in which one-third of the animals fell on their side) were observed after the 3rd injection of metrazol. After the 7th injection convulsions were observed in all mice, and starting with the next injection the convulsions in some animals ended in death.

In group 3 (receiving 30 mg/kg metrazol) single twitches did not appear until after the 4th injection (Fig. 1). With an increase in the number of injections of metrazol a tendency was observed for the severity of the seizures to rise steadily, and for the number of animals with seizures to increase. A dose of 30 mg/kg of metrazol was chosen for a more detailed study of the pattern of development of the seizure syndrome.

During daily injection of metrazol in the above dose (Table 1) the animals responded in most cases only by weak twitches. On the subsequent days the intensity of the seizure response increased, and by the 15th day marked clonic convulsions were observed in half of the animals with seizures, during which the mice fell on their side, together with postictal depression, production of frothy saliva, and other autonomic responses which accompany a severe convulsive fit. In isolated cases the convulsions terminated in death of the animal. Depending on the times of onset and the severity of the seizure response the animals could be divided conventionally into two subgroups: The first, the most numerous, included animals developing convulsions during the first 10-12 days; the second subgroup consisted of animals with greater resistance to the development of convulsions. They did not arise until after 2 weeks of metrazol injections and they were characterized by very low intensity. As a rule the animals of this subgroup did not develop intensive clonicotonic convulsions.

Aginst the background of a general tendency toward an increase in the number of animals with seizure responses, depending on the times of injection of metrazol these parameters were found to vary on individual days both in the group as a whole and also in single animals (Table 1). Severe convulsions with falling on the side were observed daily in some animals, whereas in others intensive convulsions one day were followed on the next day by very weak twitches, and vice versa. However, starting with the 14th day, seizure responses with an intensity of not less than 2 points were observed in nearly all animals (over 90%), but after 3 weeks convulsions were observed in virtually all animals. Investigation of the change in

the latent period of the first seizures and of the period of development of severe convulsions (falling on the side) in the course of their development showed (Fig. 2) that an increase in the number of metrazol injections was accompanied by shortening of the latent period of the first seizure manifestations from 8.9 ± 0.8 min on the 1st day of injection to 4.1 ± 0.2 min on the 20th day ($P < 0.001$) and of the period of severe convulsions from 12.6 ± 0.6 to 7.4 ± 0.3 min ($P < 0.001$). Hence, like the severity of the convulsions, their latent period also varied on different days both in the group as a whole and in individual animals.

The aim of the next series of experiments was to study the duration of the state of preparedness for seizures, tested by noting if a seizure response developed to a subthreshold dose (30 mg/kg) of metrazol. For 3 weeks mice were given a daily subcutaneous injection of 30 mg/kg metrazol, and this was followed by an interval of varied duration between injections of the drug. As Table 2 shows, a 10-day interval between metrazol injections did not abolish seizure activity. Moreover, the seizure responses observed were rather more severe. Intervals lasting 15 and 30 days also did not cause a statistically significant decrease in the number of animals with seizures or the mean severity of the seizure responses. Only after a 40-day interval did the state of preparedness for seizures disappear completely. Meanwhile an investigation to determine if repeated development of seizure activity was possible in the animals of this group after disappearance of their state of preparedness showed that twitching of individual muscles appeared in three of 20 mice as early as after the 2nd injection of 30 mg/kg metrazol, and after the 3rd injection four mice responded with marked clonicotonic convulsions with falling on their side; after six injections of metrazol convulsions were observed in half of the animals and the mean severity of the seizure response in the group was 1.25 points; this is statistically significantly greater than observed in mice of the same group during the first induction of seizures (0.4 points; $P < 0.05$).

The investigations thus showed that repeated epileptogenic stimulation with the use of subthreshold doses of metrazol causes a progressive increase in the state of preparedness for seizures and leads to the appearance of convulsions in response to a subthreshold dose of metrazol. This phenomenon resembles that of "kindling" [3, 5, 6, 13], in which convulsions are induced by repeated weak subthreshold electrical stimulation of brain structures.

The results are in agreement with those obtained by other workers [2, 4, 7, 10, 11], who showed that repeated administration of cocaine, lidocaine, methionine-sulfoximine, and metrazol potentiates behavioral and electrographic seizures, i.e., causes the onset of a unique kind of pharmacological "kindling".

Convulsions produced experimentally by the method descirbed above differ significantly from acute generalized convulsions evoked by threshold doses of metrazol. The use of clonic injections of subthreshold doses of the convulsant provides a model of increasing preparedness for seizures and enables a broad spectrum of seizure manifestations to be obtained. These features of the model will make it useful not only for screening of antiepileptic agents, but also for studying the pathogenetic mechanisms of development of chronic epilepsy.

LITERATURE CITED

1. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria for Assessing Differences between Two Groups of Observations in Medico-Biological Research [in Russian], Moscow (1969).
2. T. E. Albertson, S. L. Peterson, and L. G. Stark, Neuropharmacology, 20, 597 (1981).
3. J. M. R. Delgado and M. Sevillano, Electroenceph. Clin. Neurophysiol., 13, 722 (1961).
4. A. Downs and N. Eddy, J. Pharmacol. Exp. Ther., 46, 199 (1932).
5. G. V. Goddard, Nature, 214, 1020 (1967).
6. G. V. Goddard, D. C. McIntyre, and C. K. Leech, Exp. Neurol., 25, 295 (1969).
7. B. Harris, Arch. Neurol., 11, 388 (1964).
8. T. Ito, M. Hori, K. Yoshida, et al., Eur. J. Pharmacol., 45, 165 (1977).
9. R. L. Krall, J. K. Penry, H. J. Kupferberg, et al., Epilepsia, 19, 393 (1978).
10. C. R. Mason and R. M. Cooper, Epilepsia, 13, 663 (1972).
11. R. Post, R. Kopanda, and A. Lee, Life Sci., 17, 943 (1975).
12. E. A. Swinyard, in: Antiepileptic Drugs: Mechanisms of Action, G. H. Glaser, J. K. Penry, and D. M. Woodbury, eds., New York (1980), pp. 1-9.
13. J. A. Wada (editor), Kindling, New York (1976).