Pharmacology Biochemistry & Behavior, Vol. 9, pp. 401–404. Printed in the U.S.A.

Avoidance Reaction to Painful Stimulation of Another Rat: Effect of Methylglucamine Orotate

W. WETZEL[1] AND P. V. SIMONOV

Institute of Higher Nervous Activity and Neurophysiology
USSR Academy of Sciences, Moscow 117485, USSR

(Received 28 June 1978)

WETZEL, W. AND P. V. SIMONOV. *Avoidance reaction to painful stimulation of another rat: Effect of methylglucamine orotate.* PHARMAC. BIOCHEM. BEHAV. 9(4) 401–404, 1978.—An avoidance reaction to painful stimulation of another animal was developed in rats. Two hundred twenty-five mg/kg methylglucamine orotate was injected intraperitoneally each day, 1 hr before the training session. The orotate treated rats showed an accelerated development of the avoidance reaction during 5 days of training. Depending on the experimental conditions, extinction of the reaction was delayed in orotate treated rats, compared to saline controls. The results from these experiments, using neither footshock punishment nor food reward as reinforcement, give further evidence for the improvement of long term memory by orotic acid.

Methylglucamine orotate Avoidance learning Emotional reinforcement Memory

AN AVOIDANCE reaction to the painful stimulation of another animal can be developed in rats which had never experienced electrical stimulation [13, 14, 16–18]. By this method, unlike other learning experiments using footshock punishment or food reward, emotional reactions act as a reinforcement [15].

In previous investigations, we have found a memory improving effect of the RNA precursor orotic acid in one way avoidance experiments and in brightness discrimination learning [7, 9–12]. Because of the poor water solubility of orotic acid and sodium orotate, we have used in further experiments the good soluble compound methylglucamine orotate (Patent No. 116 036, VEB Fahlberg-List, Magdeburg, GDR). In the present experiments, we investigated the effect of methylglucamine orotate on the development and extinction of the above-mentioned conditioned avoidance reaction to painful stimulation of another rat.

METHOD

We used 80 adult male Wistar rats, 200–300 g body weight. The training box (30×40×30 cm), shown in Fig. 1, consisted of 3 different compartments separated from each other by transparent partitions [13]. A door between the big chamber (1) and the small chamber (2) was permanently open. Chamber 3, provided with an electrifiable grid floor, communicated with Chambers 1 and 2 through small openings for improved sound conduction.

For learning of the avoidance reaction, one rat was put into Chamber 1 and another rat, the victim, was in Chamber 3. For the whole time the first rat was in Chamber 2, the second rat was receiving inescapable electric footshocks (1–2 mA). During each stay in Chamber 1, footshock was off. Each day, 1 training session of 5 min duration was performed. The total of time the rat was in Chamber 2 and the number of entries were recorded for each session. For further details, see [13,14]. Chi square tests [2] and Mann Whitney U tests were used for statistical evaluation of the results. Two experiments (Experiment 1 and Experiment 2) were performed.

Experiment 1

During the first 5 training sessions (5 consecutive days, first week), for each learning rat one other rat was used as victim. From the sixth to tenth training session (5 consecutive days, second week), two victims, together in Chamber 3, were used in order to have a stronger reaction to the footshock stimulation. Two hundred twenty-five mg/kg methylglucamine orotate, corresponding to 100 mg/kg orotic acid, was injected intraperitoneally, 1 hr before each training session. Control rats received an equal volume of NaCl solution (1.0 ml/100 g body weight). During the third and fourth weeks, extinction of the learned avoidance reaction was tested daily using the same conditions as in training but without footshock stimulation. No injection was given.

Experiment 2

In this experiment, 5 training sessions (first week) were performed similar to the second week in Experiment 1, that means, with two victims in Chamber 3. Methylglucamine orotate was given 1 hr before each training session in the same way as in Experiment 1. Extinction was tested from the sixth to tenth day (second week), without injections.

[1]Visiting Scientist from the Institute of Pharmacology and Toxicology, Medical Academy, 301 Magdeburg, German Democratic Republic (to whom correspondence should be sent).

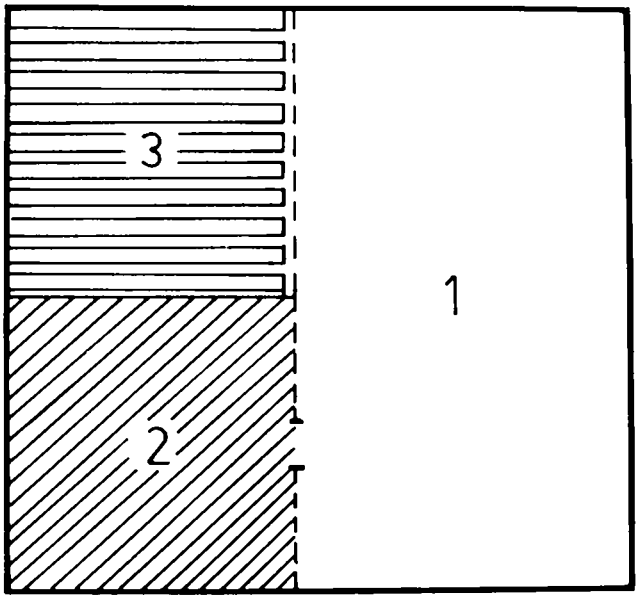

FIG. 1. Training box. For explanation, see text.

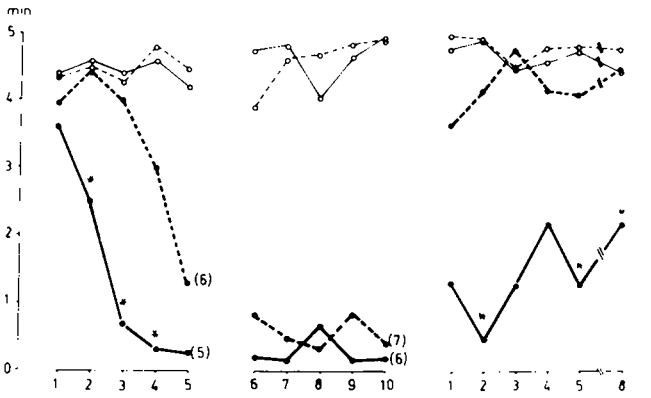

FIG. 2. Experiment 1. Effect of methylglucamine orotate (MGO) on the development (left and middle part of the figure) and extinction (right part of the figure) of an avoidance reaction to painful stimulation of another rat (median values of the groups). Abscissa: number of sessions. Ordinate: time of staying in the small chamber (t-2). Number of animals in parentheses. ●——● MGO learners; ●- - -● control learners; —— MGO non-learners; - - - control non-learners. *p 0.05 (MGO learners versus control learners).

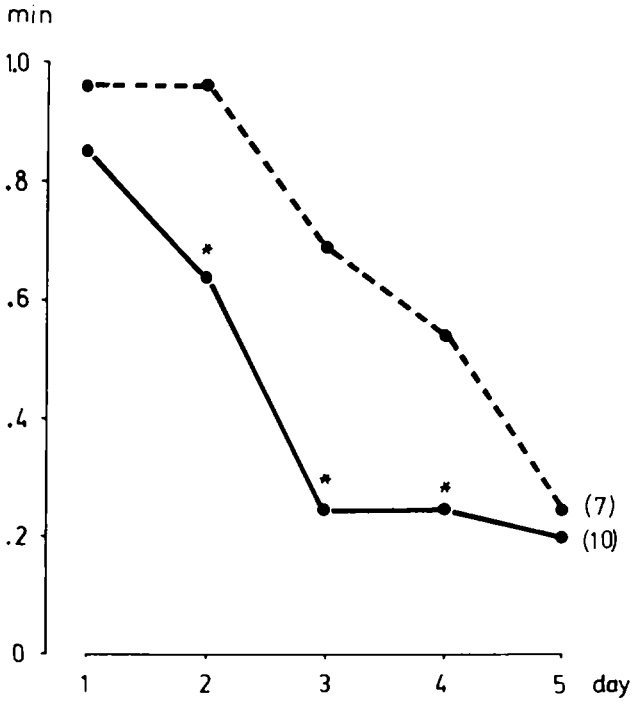

FIG. 3. Experiment 2. Effect of methylglucamine orotate (MGO) on the development of an avoidance reaction to painful stimulation of another rat (median values of the groups). Abscissa and ordinate: same as in Fig. 2. Number of animals in parentheses. ●——● MGO learners; ●- - -● control learners. *p 0.05.

RESULTS

Experiment 1

Results of Experiment 1 are summarized in Fig. 2. The figure shows the time of staying in Chamber 2 (t-2) on each day for the different groups of rats: 6 of the 10 control rats (learners) exhibited the conditioned avoidance, shown by a decrease of t-2 during the first week of training. The remaining 4 control rats (non-learners) persistently entered Chamber 2 for 4–5 min each day during the whole training. Similarly, in the methylglucamine orotate (MGO) treated group, 5 of 10 rats learned the avoidance response, whereas the other 5 showed t-2's of 4–5 min each day. However, the MGO learners developed the avoidance more quickly than the related control rats. This was shown by significant t-2 differences between MGO and controls on second, third, and fourth training sessions, respectively (left part of Fig. 2). During the second week of training, t-2 values of the learners of the control group reached the level of the MGO treated learners between 0 and 1 min. Non-learners of both MGO group and control group showed nearly the same t-2 as in the first week, except for 2 rats which changed to learners, 1 of them in each group (middle part of Fig. 2). During extinction sessions, learners of the control group very soon reached the 4–5 min level of t-2, whereas the extinction of the MGO learners was considerably delayed. This could be demonstrated by significant t-2 differences on second, fifth, and eighth days of extinction, respectively (right part of Fig. 2).

Experiment 2

In Experiment 2, results similar to those of the first week of Experiment 1 were found (Fig. 3). Seven of 10 control rats showed a decrease of t-2 during 5 days of training. However, the MGO treated rats (10 of 10) reached the same low t-2 level after 3 days of training. This resulted in significant t-2 differences between MGO and the control group on the second, third and fourth training sessions, respectively. During the extinction test, no differences between MGO rats and controls were found in this experiment.

DISCUSSION

In both experiments, a clear effect of methylglucamine orotate (MGO) on an avoidance response to footshock stimulation of another rat was shown. Burov and Speranskaya [3] used this behavioral method in psychopharmacological studies and they found different effects of some types of psychotropic drugs. For investigation of memory influencing substances, this special avoidance method had not yet been used. In previous investigations, the memory-improving effect of orotic acid was shown in learning experiments using footshock, heat, water reward, or tape removal as reinforcement [1, 4–12, 19, 20]. In the present experiments, however, learning behavior was reinforced by pain stimulation of another rat. From the results, it can be concluded that the orotic acid compound MGO improve long term memory retention; moreover, it seems to be a relative specific memory-enhancing effect. Such a conclusion is supported by several facts: (1) On the first day of treatment, no t-2 differences between MGO rats and controls were found; but, on the following days, such differences appeared, probably, due to memory differences. (2) The number of entries into Chamber 2 during each session was nearly the same for MGO rats and control rats; that means, the substance was not influencing locomotor activity which could interfere with the avoidance learning. (3) The number of learners related to all animals of a group was not changed by MGO. Thus, the accelerated avoidance learning of the MGO rats is not due to changing of non-learners to learners. Moreover, the behavior of non-learners was not influenced by MGO. (4) Similar MGO effects on avoidance elaboration were observed in Experiment 1 (one victim) and Experiment 2 (two victims). Thus, the substance effect seems to be independent on the strength of reinforcement. (5) In Experiment 1, the MGO effect could be detected both in the learning-relearning situation as in the extinction procedure—a further evidence for a memory-enhancing effect of the substance. In Experiment 2, however, no effect on extinction was observed. Possibly, 5 days of MGO treatment during avoidance elaboration might not be enough for an effect on extinction.

Looking at the extinction curve of the learners of the control group in Experiment 1 (right part of Fig. 2), one may raise a doubt about a real extinction process. However, the figure shows median values of the group, giving not all the information on the behavior of the individuals. The following values, the number of control rats with t-2 smaller than 2.5 min, demonstrate more clearly the development of extinction: Whereas on the last day of training 7/7 animals showed t-2<2.5 min, on the first and second day of extinction test 3/7, on the third and fourth day 2/7, on the fifth day 1/7, and on the eighth day 0/7 animals, respectively, showed t-2<2.5 min. Moreover, on the first and second days of extinction there was a significant difference between median values of control learners and control non-learners, whereas there were no differences on the following days.

If we speak about an "avoidance reaction to pain stimulation of another rat," we must discuss also some other possibilities concerning the motivational aspect of such behavior. Firstly, is the avoidance behavior in our experiments attributed to different levels of fear of the big compartment (Chamber 1)? Then, one may expect also differences in open field behavior. But in the open field test, performed with the same rats after the end of Experiment 1, we found no difference between avoidance learners and non-learners both in the control group and in the MGO group and there were also no differences between control learners and MGO learners. For evaluation, number of squares crossed, number of rearings, and number of boluses were used. Secondly, one may argue that the avoidance reaction described here might be escape rather than avoidance. However, if we assume an escape behavior, the following question is unsolved: Why do not all rats of a group show the development of the reaction? Furthermore, in rats, which learned the reaction, a typical behavior, not consistent with escape, could be observed: Very often, the rat in Chamber 1 is sitting near the door and looks through the door for a long time, without going in. Concluding, we can state that the avoidance response to pain stimulation of another rat is a special behavioral method, suitable for getting further evidence for the memory improving effect of orotic acid.

REFERENCES

1. Becker-Carus, C. Einfluß von Magnesium-Orotat und Orotsäure auf den Lernprozeß bei der Ratte. *Arzneimittelforschung* **22:** 2067–2969, 1972.
2. Berchtold, W. Die Irrtumswahrscheinlichkeit des χ^2-Kriteriums für kleine Versuchszahlen. *Z. angew. Math. Mech.* **49:** 634–636, 1969.
3. Burov, Yu. V. and N. P. Speranskaya. The effect of psychotropic agents on the formation of avoidance reaction in albino rats subjected to electropain stimulation of another rat. *Bull. exp. Biol. Med.* **76** (10): 59–61, 1973.
4. Dési, I., I. Szelényi und J. Sós. Einfluß von Magnesium orotat und Orotsäure auf Lernprozesse gesunder und neurotoxisch geschädigter Ratten. *Arzneimittelforschung* **21:** 780–785, 1971.
5. Ezer, E. and L. Szporny. Tape test as a simple new method for the study of compounds increasing the problem-solving ability of the rat. *Psychopharmacology* **48:** 97–99, 1976.
6. Knoll, B., K. Held and J. Knoll. Rapid screening of drug actions on learning and memory. In: *Symposium on Pharmacology of Learning and Retention*, edited by J. Knoll and B. Knoll. Budapest: Akadémiai Kiadó, 1974, pp. 43–47.
7. Lössner, B. und H. Matthies. Die Wirksamkeit intraventrikulär applizierten Natrium-Orotats auf eine bedingte Fluchtreaktion der Ratte. *Acta Biol. med. germ.* **27:** 221–224, 1971.
8. Lutz, M. P. and E. F. Domino. Some evidence for long term memory retention by orotic acid in rats bar pressing for water. *Arch. int. Pharmacodyn.* **211:** 123–127, 1974.
9. Matthies, H. und W. Lietz. Der Einfluß von Orotsäure auf das Erlöschen einer bedingten Fluchtreaktion der Ratte. *Acta Biol. med. germ.* **19:** 785–787, 1967.
10. Matthies, H. und M. Kirschner. Die Wirkung von Orotsäure auf den Stabsprungtest der Ratte. *Acta Biol. med. germ.* **19:** 789–790, 1967.
11. Matthies, H., C. Fähse und W. Lietz. Die Wirkung von RNS-Präcursoren auf die Erhaltung des Langzeitgedächtnisses. *Psychopharmacologia* **20:** 10–15, 1971.
12. Ott, T. und H. Matthies. Der Einfluß von Orotsäure und Pentetrazol auf Akquisition und Extinktion am Modell der optischen Diskriminierung. *Acta Biol. med. germ.* **26:** 79–85, 1971.

13. Preobrazhenskaya, L. A. and P. V. Simonov. Conditioned avoidance reactions to a pain stimulation of another animal. *Zh. vyssh. nerv. Deyat. I.P. Pavlova* **20:** 379–385, 1970.
14. Preobrazhenskaya, L. A. and P. V. Simonov. Conditioned avoidance response to the pain stimulation of another animal. *Soviet Psychol.* **12**(3): 90–101, 1974.
15. Scott, J. P. The emotional basis of social behavior. *Ann. N.Y. Acad. Sci.* **159:** 777–790, 1969.
16. Simonov, P. V., M. L. Pigareva and F. A. Brazovskaya. Avoidance reaction to pain stimulation of another individual in hippocampectomized rats. *Zh. vyssh. nerv. Deyat. I.P. Pavlova* **21:** 681–685, 1971.
17. Simonov, P. V., M. L. Pigareva and F. A. Brazovskaya. Avoidance reactions in rats to pain stimulation of another individual after lesion of the amygdala. *Zh. vyssh. nerv. Deyat. I.P. Pavlova* **25:** 350–355, 1975.
18. Simonov, P. V. Conditioned avoidance responses to the pain stimulation of another animal. *Activitas nerv. sup.* **19:** 28–29, 1977.
19. Szirmai, E. und J. Klosa. Der Einfluß wasserlöslicher Orotsäure auf den Lernprozess von Ratten. *Arzneimittelforschung* **26:** 1685, 1976.
20. Volokhov, A. A. and L. I. Serebryakova. Influence of orotic acid on the elaboration and retention of an active avoidance conditioned reflex in young rats. *Zh. vyssh. nerv. Deyat. I.P. Pavlova* **26:** 854–856, 1976.