Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 4, pp. 777–779, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0109-U

# Effects of an Ectodermal Microceptor Preparation on Motor Coordination in Cerebellar Mutant Mice

ROBERT LALONDE,*[1] M. I. BOTEZ,* R. BONTEMPS† AND P. LORON‡

*Hôtel-Dieu Hospital, Neurology Service, Neurobiology Laboratory, Montreal, Quebec, Canada H2W 1T8
†Laboratoires R. Bontemps, Boucherville, Quebec
‡Pitié-Salpêtrière Hospital, Paris, France

Received 1 November 1993

LALONDE, R., M. I. BOTEZ, R. BONTEMPS AND P. LORON. *Effects of an ectodermal microceptor preparation on motor coordination in cerebellar mutant mice.* PHARMACOL BIOCHEM BEHAV **49**(4) 777–779, 1994.—Lurcher mutant mice, characterized by degeneration of the olivocerebellar system, and dystonia musculorum (dt) mutant mice, characterized by degeneration of spinocerebellar fibers, were treated with an ectodermal microceptor preparation (EMP), a compound containing natural substances derived from embryonic bovine ectodermal tissue, or with placebo, and evaluated in motor coordination tests. EMP-treated lurchers, but not dt mutants, were quicker to initiate movement than placebo-treated controls in the inclined beam test. No group differences were found in terms of distance travelled on the beam or in motor coordination assessed in a more challenging coat-hanger test. These results indicate that ectodermal microceptors may improve movement initiation of cerebellar-related disorders in animals, but that these effects are test and disease-specific.

Cerebellum  Lurcher mutants  Movement initiation  Ectodermal microceptor preparation

AN ectodermal microceptor preparation (EMP) is a food supplement in liquid form containing low concentrations of essential vitamins, minerals, amino acids, and lipids derived from embryonic bovine ectodermal tissue (as originally conceived among other microceptor products by one of us, R.B.). In preliminary studies, no toxic properties have been discerned, mainly because of the low concentrations of the natural substances found in this preparation. During the course of preliminary investigations, we wished to determine whether EMP has behavioral effects. Because of our interest in pharmacologic intervention of cerebellar disease in humans (1) and animals (5), we sought to determine whether EMP has an effect on lurcher and dystonia musculorum (dt) mutant mice. The lurcher mutant is characterized by losses in Purkinje, cerebellar granule, and inferior olive neurons (2,3,7,8) and is impaired in tests of motor coordination (4). The dt mutant is characterized by degeneration of spinocerebellar fibers (6). In the present study, two tests were used: the coat-hanger test and the inclined beam test. In the coat-hanger test, the mice must coordinate their movements on a thin (2 mm) horizontal bar until reaching a side-bar (4). In the inclined beam test, the mice must coordinate their movements on a wider (10.7 cm) wooden beam. The former test is more difficult in the sense that lurcher mutants occasionally fall from the bar but rarely from the beam. On the other hand, dt mutants were only tested on the beam because the severity of their motor deficits precluded their being evaluated in the former test.

The rationale for the use of a multivitamin and mineral preparation in such diseases is that, although neural damage has already occurred there may be residual biochemical defects that are reversible with simple manipulations of the nutrional status of the animal.

## METHOD

### *Animals*

Lurcher mutant mice (approximately 6 mo of age) and dt mutant mice (approximately 2 mo of age) were obtained from Jackson Laboratory (Bar Harbor, ME), kept in a tempera-

[1] To whom reprint requests should be addressed.

TABLE 1

MEAN (SD) VALUES PER DAY OF LURCHER MUTANTS INJECTED WITH EMP OR PLACEBO IN COAT-HANGER TEST

| | Latency (s) | | | | Climbs | |
|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | Halfway | To the Top |
| Placebo | 104.6 (24.2) | 116 (11.3) | 116.2 (10.8) | 80 (32.4) | 1.1 (3.2) | 1 (2.8) |
| EMP | 100.4 (24.4) | 116.4 (7.3) | 119.2 (1.4) | 91 (19.7) | 0 | 0 |

ture- and humidity-controlled room with a 12-h light-dark cycle (lights off at 0630 h), and then tested in a separate experimental room. Food and water were available at all times.

*Apparatus*

The steel coat-hanger was triangular in outline, containing a horizontal bar (diameter 2 mm, length 40 cm) and two diagonal side-bars (length 19 cm, inclination 45°) and was placed at a height of 82 cm from a blanket-covered table to cushion the falls of the mice. The wooden inclined beam (length 117 cm, width 10.7 cm, thickness 6 cm) was placed at a 10° inclination from the horizontal over a blanket-covered floor. It contained 13 equally spaced segments marked by means of a felt pen.

*Procedure*

After 1 week of habituation (two trials of 60 s per day) in each apparatus, the lurcher mutants were injected intraperitoneally with either EMP (containing small amounts of water-soluble vitamins, in particular the group B vitamins, minerals such as sodium, potassium, calcium, and magnesium, all essential amino acids and polyunsaturated fatty acids, prepared by R. Bontemps Laboratories, after thawing from frozen samples) at a fixed volume of 0.3 cc ($n = 7$) or placebo at the same volume (0.9% saline) ($n = 8$) twice a week at 2–3-day intervals and tested 24 h after each injection for 5 weeks (total of 10 test days). In the coat-hanger test, four latencies were determined. The mice were placed in the middle of the horizontal bar facing the right side and the time elapsed before they reached either side-bar with two (latency one), three (latency two) or all four (latency three) paws were determined. Latency four was the time elapsed before a fall. The number of successful climbs at the halfway point of the diagonal bar and up to the top of the bar was also tabulated. There were two trials per day with an intertrial interval of 10 min and a cut-off point of 60 s. When a mouse fell before touching either side-bar, a score of 60 s was given for latencies one through three. When a mouse reached the top of the hanger, it was immediately retrieved and a score of 60 s was given for latency four.

In the inclined beam test, conducted on the same day but after the coat-hanger test, the mice were placed in the middle of the beam. The amount of time elapsed before crossing with all four paws the first segment of the beam was measured together with the number of falls. In addition, the number of segments traversed in both ascending and descending orientations was counted. There were two trials per day with an intertrial interval of 10 min and a cut-off point of 120 s. Reported data on this test included the final 3 weeks of testing (6 days of data collection) because, based on preliminary testing, this period gave stable results over time. The first 2 weeks of exposure of the animals to the beam were considered to be an additional habituation period, although the animals were injected during this time. The same procedure was repeated for dt mutants ($n = 7$) in comparison with placebo ($n = 7$). In addition, normal mice of the dt background strain (B6C3/a-a) were evaluated in the same tests as the mutants.

All measures for both tests were evaluated by an observer unaware of group assignments. The Mann–Whitney $U$-test was used for group comparisons on all time-dependent measures, whereas the unpaired $t$-test was used for the other measures with homogeneous variances.

## RESULTS

All animals appeared to be healthy, and there was no group difference in terms of body weight at the end of the treatment period, $p > 0.05$. There was no difference, $p > 0.05$, between EMP-treated as opposed to placebo-treated lurchers for any measure in the coat-hanger test (Table 1). In the inclined beam test, EMP-treated lurchers crossed the initial segment quicker than placebo-treated lurchers, $U(7, 8) = 7.5$, $p < 0.05$ (Table 2). There were negative correlations (Spearman test) between the crossing latency and the number of segments traversed, both in ascending, $\rho = -0.778$, $p < 0.05$ and descending, $\rho = -0.81$, $p < 0.05$, orientations for the placebo-treated lurchers, but not for the EMP-treated lurchers, ascending: $\rho = -0.402$, $p > 0.3$; descending: $\rho = -0.616$, $p > 0.1$. There were no group differences in terms of segments

TABLE 2

MEAN VALUES (SD) PER DAY OF LURCHER MUTANTS INJECTED WITH EMP OR PLACEBO IN INCLINED BEAM TEST

| Group | Crossing Latencies (s) | Ascending Segments | Descending Segments | Number of Falls |
|---|---|---|---|---|
| Placebo | 27.4 (14.7) | 15.7 (7.6) | 15.6 (10.7) | 0.07 (0.1) |
| EMP | 8.9 (4)* | 21.5 (5.6) | 21.5 (4.6) | 0.2 (0.2) |

*$p < 0.05$.

TABLE 3

MEAN VALUES (SD) PER DAY OF DT MUTANTS INJECTED WITH EMP OR PLACEBO IN INCLINED BEAM TEST

| Group | Crossing Latencies (s) | Ascending Segments | Descending Segments | Number of Falls |
|---|---|---|---|---|
| Placebo | 50.8 (32.3) | 2.5 (3.8) | 7.0 (4.3) | 0.6 (1.1) |
| EMP | 53.3 (22.9) | 3.2 (2.3) | 6.5 (8.1) | 0.5 (1.5) |

traversed, $p > 0.05$. Correlations between the number of ascending and descending segments were high for both groups (placebo: $r = 0.99$, 98.9% of variance; EMP: $r = 0.783$, 61.2% of variance). EMP had no effect on dt mutants in the inclined beam test (Table 3), nor were there effects of EMP on normal mice for either test (data not shown).

## DISCUSSION

Lurcher mutants treated with EMP had shorter crossing latencies but did not traverse more segments than did placebo-treated lurchers. Thus, there is evidence of an improvement in movement initiation in EMP-treated animals, but this was not accompanied by an increase in distance traveled. Contrary to placebo-treated lurchers, there were no correlations between movement initiation and distance traveled in EMP-treated lurchers, a further indication that the compound exerted a differential effect on these measures. EMP had no effect on normal mice. This result shows that the compound is not a stimulant, but instead may selectively improve movement initiation in cerebellar disease in animals and possibly other pathologic conditions.

No group differences emerged in the more demanding coat-hanger test. The value of this compound may diminish in more difficult tests. It is interesting to test the effects of EMP in other conditions characterized by a slowness in movement initiation, such as animal models of Parkinson's disease. At the moment, we do not know the mechanism of action of this agent. Future studies must determine whether EMP has an effect on dopamine-mediated mechanisms or on other neurotransmitter functions either presynaptically or postsynaptically.

No group differences emerged in dt mutants. This mutant is more severely affected in motor coordination tests than lurcher mutants (compare Tables 2 and 3). The behavioral effects of this compound are also disease-specific, as improvement was only found in the cerebellar mutant with milder symptoms.

## ACKNOWLEDGEMENT

This research was funded by Laboratoires R. Bontemps, Canada (Mr. Marc Laperle, Laboratory Director, Boucherville, Quebec).

## REFERENCES

1. Botez, M. I.; Young, S.N.; Botez, T. ; Pedraza, O. The treatment of heredodegenerative ataxias with amantadine hydrochloride. Can. J. Neurol. Sci. 18:307–311; 1991.
2. Caddy, K. W. T.; Biscoe, T. J. Structural and quantitative studies on the normal C3H and Lurcher mutant mouse. Phil. Trans. Roy. Soc. London, Series B 287:167–201; 1979.
3. Heckroth, J. A.; Eisenman, L. M. The olivocerebellar projection in "lurcher" mutant mice. Neurosci. Lett. 85:199–204; 1988.
4. Lalonde, R.; Botez, M. I.; Joyal, C. C.; Caumartin, M. Motor abnormalities in lurcher mutant mice. Physiol. Behav. 51:523–525; 1992.
5. Lalonde, R.; Joyal, C. C.; Guastavino, J.-M.; Coté, C.; Botez, M. I. Amantadine and ketamine-induced improvement of motor coordination in lurcher mutant mice. Restor. Neurol. Neurosci. 5: 367–370; 1993.
6. Sotelo, C.; Guenet, J. L. Pathologic changes in the CNS of dystonia musculorum mutant mouse: An animal model for human spinocerebellar disease. Neuroscience 27:403–424; 1988.
7. Wetts, R.; Herrup, K. Interaction of granule, Purkinje and inferior olive neurons in Lurcher chimeric mice. I. Qualitative studies. J. Embryol. Exp. Morphol. 68:87–98; 1982.
8. Wetts, R.; Herrup, K. Interaction of granule, Purkinje and inferior olive neurons in Lurcher chimeric mice. II. Granule cell death. Brain Res. 250:358–362; 1982.