Effects of Thyrotropin Releasing Hormone on Cerebellar Mutant Mice —A Kinesiological Comparison between Rolling Mouse Nagoya, Weaver and Reeler

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Rolling Mouse Nagoya (RMN), weaver and reeler mice were examined for the clinical effectiveness of drugs (thyrotropin releasing hormone (TRH), reserpine, L-dopa) that are reported to facilitate or alter the metabolic system of noradrenaline (NA). Behavioral changes and ataxic gait were recorded by 16mm cinefilms and EMG of the thigh extensor muscles. In addition, latencies in the reflex discharge of the muscle by tilting load were measured before and after the intraperitoneal injection of each drug and compared to those of normal littermates. TRH injection increased spontaneous behavioral activity in RMN and weaver, and shortened the latency of the reflex discharge by tilting load in RMN. Reserpine injection did not increase behavioral activity, but was accompanied by a slight shortening of latencies in the reflex discharge by tilting load only in RMN. L-dopa did not affect behavioral and EMG activities of these cerebellar mutant mice. No remarkable deficit has yet been in any selective intracortical neurons in RMN and no other characteristic abnormality in central and peripheral nervous, skeleton and muscular systems. It was, therefore, suspected that there was a possibility of metabolic disorders of NA in the cerebellum not only in RMN, but also in patients with certain spinocerebellar degenerations.

Key Words: Rolling Mouse Nagoya, Staggerer, Reeler, Ataxic gait, Spontaneous activity, Latency in response to tilting load, TRH, Reserpine.

Rolling Mouse Nagoya (RMN) is a neurological mutant mouse developed by Oda in 1973¹⁾. The mouse was found in the course of mating experiments between SIII and C57BL/Nga. SIII is characterized by sucking death, and the latter is purely normal. The symptom of RMN is severe gait disturbance. The forward progression is interrupted by creeping and rolling of the hindlimbs toward one side or the other (Fig. 1). The forelimb movements are less disturbed than the hindlimb movements. Oda also suggested that the symptom was caused by hereditary cerebellar disorders.

His genetic studies identified the transmission by a single recessive gene. Therefore, RMN is regarded as a model of human cerebellar degenerative diseases. Pathologically, other neurological murine mutants, such as weaver, staggerer^{2,3)} and reeler^{4,5)} are recognized to have explicit hypoplasia of the cerebellum, but no anatomical and histological abnormalities have been notably found in the cerebellum of RMN except in Nishiura's report⁶⁾.

Adachi et al⁷⁾ reported that the cerebellum of RMN had remarkable increases in number and fluorescent intensity of norad-

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Fig. 1. Gait posture of Rolling Mouse Nagoya before and after administration of TRH, (A): before, Notice that hindlimbs are more ataxic and unsteady than forelimbs. (B): after, (b) right RMN mouse 15 min. after the administration; limbs are steady, (a) left RMN mouse without TRH; limbs are unsteady.

renaline (NA) nerve terminals. These increases were more distinct in the anterior lobe than in the posterior. Similar increases in number and fluorescent intensity of NA nerve terminals were observed through the cerebral cortex of this mouse.

In this study, observation of behavioral changes and kinesiological analysis in these cerebellar mutant mice (RMN, weaver and reeler) were done before and after administration of the drugs, such as thyrotropin releasing hormone (TRH), reserpine and L-dopa, which could influence NA nervous pathways.

MATERIALS AND METHODS

Three type of experimental mice were used. One was RMN, 4-6 weeks after birth with body weights of approximately 20 gr and littermates as normal controls, 4 months old with body weights of 20 to 25 gr. Another type was weaver mutant mouse, 4 to 6 months with body weights of 20 to 23 gr. The third type was reeler mutant mouse, also 4 to 6 months with body weights of 20 and 21 gr. Thyrotropin releasing hormone (TRH, 500 μ g) was administrated to all the mice, but reserpine (1.25 mg) and L-dopa (1.25 mg) only to RMN and normal controls in this experiment. Each drug was intraperitoneally injected. 5 RMN mice, 2 weaver, and 2 reeler mice were examined in this study. Observation of behavioral changes by 16 mm cinefilms was done every five minutes after the injection for 90 minutes. In order to test kinesiological changes by tilting load, the mouse was fixed on a flat board by strong paper tape to the limbs.

Fig. 2. Experimental apparatus.

Fig. 3. Reflex discharge of thigh extensor muscle by tilting load RMN; Rolling Mouse Nagoya, upper row: electromyogram of thigh extensor muscle, lower row: change of tilting angle with potentiometer, calibration; 100 μ V, 20 msec. Latency of control mouse was 10 msec and the one of RMN, 40 msec.

The board was devised to fold downward easily with hinges. EMG activity of thigh extensor muscles, being using bipolar needle electrode (diameter: approximately 0.2 mm), and angle changes of the tilting board using a potentiometer, were simultaneously observed on an oscilloscope and recorded on X-ray film and magnetic tape (Fig. 2, 3). At least five trials of tilting load at the frequency of 0.2 c/sec were recorded every five minutes after the administration. Latency measurements of reflex discharge of thigh extensor muscles by tilting load were statistically analyzed.

RESULTS

Effects of administration

(a) TRH:

TRH was intraperitoneally administered to all the mutant mice. In RMN, behavioral activities were increased 5 to 10 minutes after the injection of TRH. At times elevation of the tail in RMN was observed. The gait was still clumsy, but forward progression appeared easier. The frequency of rolling to each side decreased markedly (Fig. 1, B). This improvement in gait became clearer 10 to 30 minutes after the injection and continued for 40 minutes. Difficulties in gait then reappeared and the frequency of rolling increased gradually. Active behavior of RMN, however, continued for as long as 60 to 90 minutes. On the other hand, behavioral activity in normal littermates was subtly increased or not detectable at all.

Impairment of gait in RMN could be recognized from the EMG activity of the thigh extensor muscles by tilting load, that is, latencies of reflex discharge were shorter than before the injection. The latency of RMN was 40.2 ± 10.3 msec (mean $\pm$ SD) without any drugs, while that of normal mice was 10.1 ± 4.8 msec ($p < 0.01$, Fig. 4). Fifteen minutes to 30 minutes after the injection, the latency of RMN became 13.4 ± 5.9 msec and spontaneous muscle discharge of the thigh appeared from 15 minutes to 30 minutes after the injection. This shortening of

latency could be maintained until 45 minutes (Fig. 4, Table 1). In contrast, the latency of normal mice after the injection was not so remarkably changeable and almost the same as 10.0 ± 5.2 msec before it.

Weaver and reeler, hereditary cerebellar ataxic mice, were given TRH. The former mice seemed to have increases of behavioral activities, and the ataxic gait disturbance

Fig. 4. Changes of latencies in reflex discharge by tilting load after administration of TRH. control; normal mouse, RMN; Rolling Mouse Nagoya, number; time (minute) after administration, Calibration; 100 μ V, 20 msec. upper row; electromyogram of thigh extensor muscle, lower row; change of tilting angle. Notice increase of spontaneous muscle discharge and shortening of latency (40 $\rightarrow$ 13 msec) in RMN.

Table 1. Changes of latency of reflex discharge by tilting load before and after TRH administration to cerebellar mutant mice.

*: $P < 0.01$, RMN: Rolling mouse Nagoya.

	Control (msec)	RMN (msec)	Weaver (msec)	Reeler (msec)
before	10.1 $\pm$ 4.8	40.2 $\pm$ 10.3	33.0 $\pm$ 6.5	60.8 $\pm$ 5.7
5 min.	10.1 $\pm$ 2.7	40.2 $\pm$ 7.3	-----	-----
10	9.8 $\pm$ 3.9	16.8 $\pm$ 5.8*	30.2 $\pm$ 5.7	56.9 $\pm$ 4.6
15	9.6 $\pm$ 5.6	16.8 $\pm$ 5.0*	30.2 $\pm$ 5.3	53.6 $\pm$ 5.1
20	9.8 $\pm$ 3.8	13.8 $\pm$ 5.0*	23.5 $\pm$ 7.3	60.3 $\pm$ 4.7
25	9.6 $\pm$ 5.6	13.4 $\pm$ 5.9*	-----	-----
30	9.8 $\pm$ 5.8	16.4 $\pm$ 5.9*	33.5 $\pm$ 8.6	60.3 $\pm$ 5.8
40	10.2 $\pm$ 4.7	19.5 $\pm$ 8.8	40.2 $\pm$ 6.9	63.6 $\pm$ 11.4
60	15.8 $\pm$ 5.6	23.5 $\pm$ 6.3	40.2 $\pm$ 7.1	70.3 $\pm$ 10.2

Fig. 5. Changes of latencies in reflex discharge by tilting load after administration of TRH in weaver mouse. Calibration; 200 μ V, 20 msec, latencies after administration, were not changable as before. min: minute.

could be improved or changed. The latency of the reflex discharge by tilting load was not so significantly short as before the injection (Table 1, Fig. 5). Spontaneous discharge of the extensor muscles clearly increased without any load, but they gradually decreased from 40 minutes after the injection. In reeler mouse, the spontaneous behavioral activities seemed to be less active than that of the other mice. No marked changes of the EMG activity and the reflex discharge by tilting load could be observed even after the injection in this study (Table 1, Fig. 6).

(b) Reserpine:

No remarkable change of behavior in either RMN or normal mice could be observed after the intraperitoneal injection of reserpine (1.25 mg). Nevertheless, the shortening of latency of the reflex discharge by

tilting load began clearly at approximately 30 minutes after the injection. The latency became 13.2 ± 3.3 msec at approximately 40 minutes after the injection ($p < 0.01$). After 60 minutes the latency returned to the previous value (Table 2, Fig. 7). Administration of more than 1.25 mg of reserpine gave

Fig. 6. Changes of latencies in reflex discharge by tilting load after administration of TRH in reeler mouse; latencies are almost unchangable, calibration; 200 μ V, 20 msec.

Table 2. Changes of latencies in reflex discharge by tilting load before and after reserpine injection to RMN. *: $P < 0.01$.

	Control	RMN
10 ^{min.}	11.5 ± 1.0 (msec)	33.0 ± 3.3 (msec)
20	11.6 ± 2.0	19.2 ± 1.7
30	9.9 ± 1.7	$16.5 \pm 1.7^*$
40	13.2 ± 1.7	$13.2 \pm 3.3^*$
50	9.9 ± 1.0	23.1 ± 3.5
60	9.9 ± 0.7	28.1 ± 5.0

Fig. 7. Changes of latencies in reflex discharge by tilting load after administration of reserpine under the same condition as Fig. 3, calibration; $200\ \mu\text{V}$, 20 msec. Notice shortening of latency (40→13 msec).

the mice diarrhea, decreased activities, caused the more to go into rigid posture and finally to die in most cases.

(c) L-dopa:

No changes of behavioral activities of RMN or normal control were discriminable for 90 minutes, and no change in EMG activity by tilting load was recorded.

DISCUSSION

It has been undoubtedly verified that weaver, staggerer and reeler, well known as hereditary mutant mice with cerebellar ataxia, have each pathological findings. The cerebellum of weaver was found to be 25% heavier than that of the normal mice and devoid of granular cell layer except in the parafolium and the most lateral portion of cerebellar hemispheres⁸⁾. Absence of granule cells in the cerebellar cortex in staggerer mouse was also markedly recognized^{8,9)}. Purkinje cells of the mouse were not ordered, but irregularly scattered in the

granule cell layer¹⁰⁾. In reeler mouse^{4,11,12)} the thinning of granule cell layer was also reported, as was existence of most of Purkinje cells inside of granule cell layer, irregularly located relatively between the granule cell layer and Purkinje cell layer^{12,13)}. On the contrary, it has not yet been reported in detail^{14,15)} that RMN had any microscopic abnormal findings in the cerebellum except in Nishimura's report⁶⁾ which presents the significant decrease in number of granule cells in lingula, lobulus centralis and culmen in cerebellar vermis of RMN.

On the other hand, cerebellar abnormalities in the nerve terminals of NA in weaver, staggerer and reeler was found by Landis et al^{16,17)}, who reported that in weaver mice there were increases of fluorescence intensity and total number of NA nerve terminals. Adachi et al. also proposed abnormalities of the NA fiber system especially in the cerebellar anterior lobe of RMN, and that similar increase in number and fluorescent intensity of NA nerve terminals were observed throughout the cerebral cortex of this mutant, compared with those of littermates. No significant changes in number of fluorescent intensity at locus coeruleus were found.

NA can play a role in direct inhibition of activity of Purkinje cells in the cerebellum^{16,18)}, and be inhibitory to cortical¹⁹⁾ and spinal neurons²⁰⁾ as well. It has been recently reported that NA nerve fibers have concern with some maturation of cerebrocortical neurons²¹⁾. Meanwhile, it has been pointed that there is a kind of accumulation of NA in the cerebellar cortex of ataxic mutant mice. It is suspected that there is accumulation of NA at presynaptic or synaptic sites of NA fiber terminals in weaver and reeler mice with cerebellar hypoplasia or aplasia. In RMN, it is also inferable that the disturbance of turnover in NA fiber may exist at presynaptic or synaptic sites because the turnover disturbance of NA fibers in RMN may result in storage of NA. This abnormal storage of NA may affect the development of the central nervous

system, predominantly the cerebellum of these mice as well as the function of cerebellar nervous pathways. Cerebellar ataxic movements may then result. Therefore, it might be recalled that the drugs which facilitates the turnover of NA metabolism could influence neurological disorders of these mutant mice. Keller et al.²²⁾ proved that TRH enhanced the turnover of NA in the rat brain. It was, furthermore, pointed out that TRH did not affect the metabolism of dopamine and 5-hydroxytryptamine. Yarbrough²³⁾ reported that TRH facilitated excitability of cerebrocortical neuron by acetylcholine because TRH could play a role as antagonist of barbiturate. It was reported by Grim et al.²⁴⁾ that dopamine and NA facilitated the release of TRH and serotonin inhibited it. TRH is localized within neurons and concentrated within, and can be released from, preparations of synaptosomes^{13, 25)}. Exogenous applications of TRH can alter central neuronal excitability^{26, 27)}. On the other hand, reserpine is also known as a releasing and blocking agent of NA. In addition, L-dopa (levodopa) changes into dopamine as a precursor of NA in the plasma through catecholamine system.

On these bases, effects of these drugs were examined in these cerebellar ataxic mice in order to observe behavioral activity and ataxic gait and to record reflex discharge by tilting load. As the results have shown, RMN revealed increases of activities, improvement of ataxic gait and shortening of latency in the reflex discharge. Weaver mice presented only a little increase in behavioral activities while in reeler there was no change of either behavioral activity, ataxic gait, or latency of the reflex discharge. Reserpine injection was followed by slight shortening of latencies in the reflex discharge only in RMN. Neither increase in behavioral activity nor gait improvement was observed. L-dopa was not effective in these mice. Thus, it might be inferred from these kinesiological results that less involvement of intracortical cell architecture of the cerebellum in these mutant mice could

evoke more improvement of cerebellar ataxic motor disturbance. The shortening of latency in the reflex discharge by tilting load began at 5 to 10 minutes after TRH injection, while after reserpine injection, shortening of latency began at 15 to 30 minutes. The difference may be a pharmacological relapse step of NA. Gait improvement of RMN after TRH was verified by the shortening of latency in the reflex discharge which might be considered one of vestibular reflexes probably including not only primitive protection reflex, but also cerebrocerebellar interaction, because the longer latency of the reflexes discharge was shown when eye lids of RMN were closed with black tape although it was not shown in this report.

Conclusively, the gait improvement might be mainly due to facilitation of NA-turnover in the cerebellar mutant mouse, and besides increase of excitability of cerebrocortical motor neuron, mental activity must be also considered. As mentioned above, TRH is reported to be antagonist to barbiturate, so it seems that TRH influenced excitability of cerebral and cerebellar cortical neurons. Reserpine injection was also followed by a slight shortening of latency of the reflex discharge only in RMN but no increase of activity was seen. It is known that reserpine has a role as a tranquilizer²⁸⁾. If so, it is interesting that response to reserpine could result in the slight shortening of latency despite of the sedative reaction. It is also possible that TRH and reserpine play a similar role in NA nerve terminals as a transient metabolic facilitatory agent, while there is some opposite reaction in mental behavior between them.

These experimental results suggest that the possibility of TRH application to the human ataxic patients. In facts, it has been recently reported that TRH administration to patients with spinocerebellar degenerations has made several effects on decrease of the area of stabiligram at the upright position²⁹⁾, improvement of abnormal eye movement³⁰⁾ and shortening of silent period

of gastrosoleus muscle evoked by sudden forward tilting load⁸¹⁾. It was therefore suggested that there was a possibility of metabolic disorders of NA in pateints with spinocerebellar degenerations.

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