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The Effect of Metal Complex of Thyrotropin-Releasing Hormone on Locomotor Activity of Neonatal Chicken

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TONOUE, T., S. MINAGAWA, N. KATO, M. KAN, T. TERAO, K. NONOYAMA AND K. OHKI. *The effect of metal complex of thyrotropin-releasing hormone on locomotor activity of neonatal chicken.* PHARMAC. BIOCHEM. BEHAV. **10**(2) 201–204, 1979.—Neonatal chickens were injected intraventricularly with Ni(II), Pd(II), Cu(II) or Zn(II) complex of TRH and the potencies of stimulating locomotor activity were compared with that of TRH. Ni(II)-TRH was more potent than the ligand while Pd(II)-TRH was inert. Cu(II)- and Zn(II)-TRH induced the response to the same extent of the ligand. These results indicate that the action of TRH in the CNS resulting in the locomotor hyperactivity is dependent on the tertiary conformation of molecule which is modified by chelate formation.

TRH Metal complex Locomotion Chicken

A CONSIDERABLE body of evidence indicating the involvement of Thyrotropin-Releasing Hormone (TRH), pyroglutamyl-histidyl-proline amide, in the regulation of the central nervous system (CNS) activity has been accumulated [1, 3, 6, 7, 8, 9, 11, 12, 13, 14, 15]. Intracranial injection of TRH produced many behavioral responses such as grooming, preening, sniffing, head shaking and self-biting of forelegs as well as simple locomotor hyperactivity [1, 3, 7, 9, 11, 13]. We have noticed the simplicity of the behavior and the naivety of the neonatal chicken and used it as the experimental animal for the quantitative analysis of the effect of TRH on the locomotor activity. We report here the stimulation of locomotor activity of the one-day old chicken by the intraventricular injection of TRH and the modification of its potency by forming the complex with some metal ions. The present study of the difference in potencies of metal complex of TRH may provide an approach for elucidating the required orientation of the moieties of this tripeptide in its action in the CNS.

METHOD

Ni(II) complex of TRH (Ni(II)-TRH) was obtained by combining ethanol solution of TRH (Takeda Chem. Ind.) and NH_4OH (25–28%) solution of $Ni(OH)_2$. NH_4OH was evaporated and excess $Ni(OH)_2$ was removed by precipitation. The solution containing complex was evaporated to give the powder of Ni(II)-TRH. Anal. Calcd. for $Ni(C_{16}H_{21}O_4N_6)_2 \cdot 3H_2O$; C, 46.0; H, 5.8; N, 20.1; Ni, 7.0. Found: C, 46.0; H, 6.1; N, 20.4; Ni, 7.8. Pd(II)-TRH was obtained by mixing the aqueous solutions of TRH and $PdCl_2$ and raising pH over 11.5 by an addition of NaOH. The complex was extracted into ethanol and the evaporation of solvent left the purple residue. Anal. Calcd. for $Pd(C_{16}H_{21}O_4N_6) \cdot 3H_2O$; C, 36.8; H, 5.2; N, 16.1; Pd, 20.4. Found: C, 37.0; H, 5.3; N, 15.7; Pd, 21.5. Cu(II)-TRH was obtained from TRH and lauric acid-Cu(II) in ethanol solution. The solution was washed with $CHCl_3$ and the complex was extracted into water. The powder of complex was left after evaporation. Anal. Calcd. for $Cu(C_{16}H_{21}O_4N_6)_2 \cdot 2H_2O$; C, 46.7; H, 5.6; N, 20.4; Cu, 7.7. Found: C, 46.9; H, 6.0; N, 20.3; Cu, 8.0. Zn(II)-TRH was obtained from TRH and $Zn(OH)_2$ in aqueous NH_4OH(25–28%). After an addition of ethanol the solvent was evaporated. Anal. Calcd. for $Zn(C_{16}H_{21}O_4N_6)_2 \cdot 2H_2O$; C, 46.6, H, 5.6; N, 20.4; Zn, 7.9. Found: C, 46.2; H, 6.2; N, 19.8; Zn, 7.2.

White Leghorn male chickens (34–42 g), within 24 hr after hatching, were used throughout the experiments. The chickens were kept at the ambient temperature of 30–32°C

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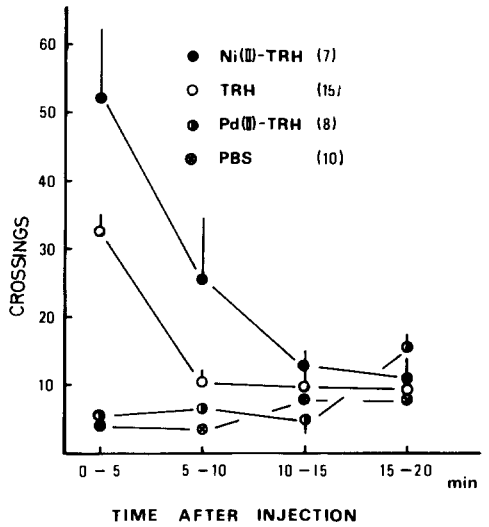

FIG. 1. Spontaneous locomotor activity of one-day old chicken following an injection of vehicle (PBS) or 3 nmoles (amount of ligand) of TRH, Ni(II)-TRH or Pd(II)-TRH dissolved in 1.5 μl of phosphate buffered saline (pH 7.4) into the third ventricle. Each value is the mean count of crossings and the vertical bar represents the standard error. Number of chickens is shown in parenthesis.

without supply of food and water. Intraventricular injection of test substance in 1.5 μl of phosphate buffered saline, pH 7.4, was made under the light ether anesthesia by a microsyringe. The needle was introduced into the third ventricle through a guide of a stereotaxic apparatus which was made so that the head of chicken was fixed by positioning the beak and eyes on the templet. Intracardial injection of test substance in 0.1 ml phosphate buffered saline, pH 7.4, was made on the unanesthetized chicken. Immediately after injection the individual chick was placed in an arena box (27×45×30 cm) and the crossings by the chick of light beams set at 15 cm interval were counted by means of an event recorder. The experiment was carried out under the condition of 25–28°C of temperature and 100 lux of lightness without sound-proofing.

The circular dichroic (CD) curves of 3 mM solution in phosphate buffered saline, pH 7.4, were recorded between 220–700 nm using a JASCO J-40A spectropolarimeter. Infrared (IR) spectra were obtained with an IR spectrophotometer (Hitachi-Model 215) with sodium chloride optics. The concentration of ligand was 20% in 99.8% D_2O as the solvent.

Student's *t*-test was used for statistical analysis.

RESULTS AND DISCUSSION

Within 1 min after an intraventricular injection of 3 nmoles of TRH the chicken commenced to move vigorously from an end of the floor to the other end. The hyperactivity of locomotion lasted for about 5 min and declined sharply thereafter (Fig. 1). The control chicken which received the saline injection moved only occasionally. The effect of

TABLE 1

EFFECTS OF INTRAVENTRICULAR INJECTION OF TRH OR METAL COMPLEXES OF TRH ON THE LOCOMOTOR ACTIVITY OF CHICKENS

Exp. No.	Treatment	Dose (nmoles)*	No. of Chickens	Crossings During First 5 Min (Mean ± SE)	Statistics vs PBS	
1	TRH	3.0	15	32.7 ± 3.0	0.01	
2	TRH	1.5	9	22.9 ± 2.9	0.01	
3	TRH	0.75	7	16.9 ± 1.9	0.01	
4	TRH	0.375	8	13.1 ± 3.5	0.05	
5	TRH	0.1875	7	7.6 ± 2.2	N.S.	
6	Ni (II)-TRH	3.0	7	52.0 ± 11.2	0.01	vs 1, 0.05
7	Ni (II)-TRH	1.5	4	43.5 ± 7.6	0.01	vs 2, 0.01
8	Ni (II)-TRH	0.75	4	24.3 ± 2.4	0.01	vs 3, 0.05
9	Ni (II)-TRH	0.375	4	20.3 ± 5.3	0.01	
10	Ni (II)-TRH	0.1875	4	11.0 ± 4.4	0.05	
11	Pd (II)-TRH	3.0	8	5.5 ± 0.9	N.S.	
12	TRH	1.5	12	21.8 ± 1.8	0.01	
13	Ni (II)-TRH	1.5	14	36.3 ± 2.5	0.01	vs 12, 0.05
14	Cu (II)-TRH	1.5	10	20.7 ± 2.6	0.01	
15	Zn (II)-TRH	1.5	16	18.1 ± 1.8	0.01	
16	$NiCl_2$	1.5	4	4.5 ± 1.9	N.S.	
17†	TRH $PdCl_2$	1.5 1.5	6	19.5 ± 2.8	0.01	
18	PBS		10	4.0 ± 1.0		

*Amount of ligand for metal complex of TRH.
†Mixture of saline solution (pH 7.4) of TRH and $PdCl_2$

TABLE 2

EFFECTS OF INTRACARDIAL INJECTION OF TRH OR METAL COMPLEXES OF TRH ON THE LOCOMOTOR ACTIVITY OF CHICKENS

Treatment	No. of Chickens	Crossings During First 5 Min (Mean ± SE)
Saline	8	2.6 ± 1.9
TRH	8	14.4 ± 4.2*
Ni (II)-TRH	8	13.8 ± 3.2*
Cu (II)-TRH	8	12.2 ± 1.3*
Pd (II)-TRH	8	5.9 ± 1.1

*Difference from saline control, significant at 1% level. 150 μg of ligand was injected.

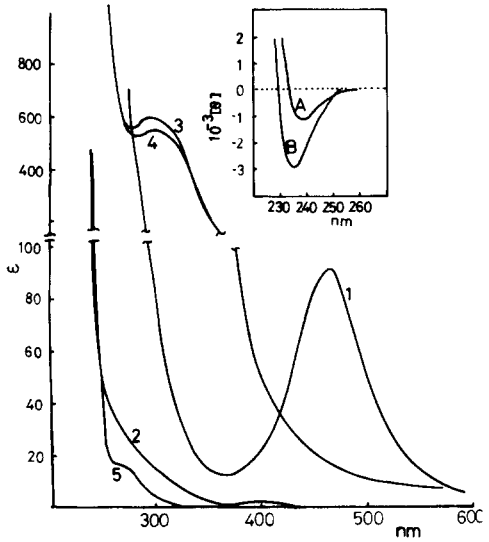

FIG. 2. Absorption and CD spectra of TRH, Ni(II)-TRH and Pd(II)-TRH in aqueous solution. Absorption spectra: 1, Ni(II)-TRH pH 11.5; 2, Ni(II)-TRH pH 7.4; 3, Pd(II)-TRH pH 11.5; 4, Pd(II)-TRH pH 7.4; 5, TRH pH 7.4. CD spectra: A, TRH, pH 7.4; B, Ni(II)-TRH, pH 7.4.

Ni(II)-TRH (3 nmoles of ligand) was more prominent compared with the free TRH (Fig. 1). Any other unusual behaviors such as circling or rotational movement were not produced but defecations and headshakings were sometimes observed. The locomotion hyperactivity induced by TRH or Ni(II)-TRH was dose-dependent and Ni(II)-TRH was always more effective than TRH at the same dose of the ligand (Table 1). On the other hand, Pd(II)-TRH (3 nmoles of ligand) was found to be inert in producing the locomotor hyperactivity (Fig. 1). Cu(II)-TRH or Zn(II)-TRH induced the response to the same extent of free TRH (Table 1). Ni(II) ion itself was shown to be inactive in eliciting the response. Mixing of saline solution (pH 7.4) of $PdCl_2$ with TRH does not form the complex and this mixture produced the hyperactivity to the extent expected from the effect of free TRH (Table 1). This indicated that Pd ion did not inhibit the TRH effect.

Intracardial injection of 150 μg of TRH was also effective

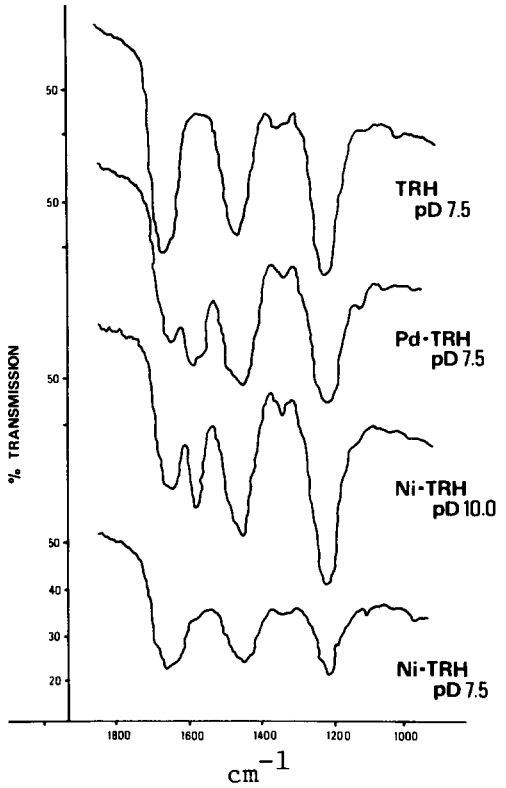

FIG. 3. Infrared spectra of TRH, Pd(II)-TRH and Ni(II)-TRH in aqueous (D_2O) solution.

in producing locomotion hyperactivity (Table 2). But the high potency of Ni(II)-TRH found in the intraventricular treatment was not reproduced by the intracardial treatment. This was presumably due to that Ni(II)-TRH was decomposed in the circulation before reaching the CNS receptors. This finding may imply that the high potency of intraventricular Ni(II)-TRH is not due to the high resistibility of this complex to the enzymatic degradation of ligand molecule.

Pd(II)-TRH was again inert in the intracardial treatment (Table 2). This seemed to indicate that the Pd(II)-TRH was stable and did not release the free TRH. The possibility of chemical change of TRH molecule during chelate formation was ruled out since the decomposed Pd(II)-TRH which was made by lowering pH once to 4.0 and resuming pH to 7.4, showed the same potency as TRH.

These results indicate that the action of TRH in the CNS resulting in the locomotor hyperactivity is dependent on the conformation of molecule which is modified by the chelate formation.

Pd(II) ion has been known to form the stable square planar complex. This situation was shown in the stability of visible absorption spectrum of Pd(II)-TRH irrespective of the change in pH of aqueous solution (Fig. 2). IR spectrum of Pd(II)-TRH showed the remarkable difference from that of ligand in the bands of 1640, 1580 and 1560 cm^{-1} (Fig. 3). Comparison of the 1660 cm^{-1} band of the ligand with the 1640 cm^{-1} band of Pd(II)-TRH indicates association of the protonated peptide linkage with the Pd(II) ion [5]. We can assume that Pd(II) ion produces a stable square planar complex con-

taining bondings with amide of TRH. The assignment of coordination sites of TRH with Pd(II) ion may add an information of the required configuration of TRH in its action.

Ni(II)-TRH showed a characteristic single absorption peak in the visible range at pH 11.5, indicating the formation of a square planar complex [4,5]. But this peak disappeared when the pH was lowered to 7.4 (Fig. 2). IR spectral change with lowering pD from 10.0 to 7.5 was in accordance with the above finding. At high pD (pH) Ni(II)-TRH showed essentially the same spectrum as Pd(II)-TRH whereas at pD 7.5 no distinct difference was apparent in IR spectrum of Ni(II)-TRH compared with that of ligand (Fig. 3). These findings with the data of elementary analysis indicating 1:2 metal ligand ratio suggest that at pH 7.4 Ni(II)-TRH is in the conformation such that two molecules of TRH are associated by Ni(II) ion linking at the site other than amide nitrogen or carbonyl group. The nitrogen of imidazole ring of histidine moiety seems to be the possible coordination site.

CD spectrum of aqueous solution of Ni(II)-TRH at pH 7.4 showed a 3 nm blue shift of the negative band peaking around 238 nm compared with that of ligand. This band has been suggested to contain the contribution from the histidyl-prolin peptide in cis-conformation [2,10]. A close analysis of structure of Ni(II)-TRH in aqueous solution seems promising for elucidating the active configuration of TRH in the CNS and the study including ^{13}C NMR analysis is in progress.

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