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Effects of Thyrotrophin-releasing Hormone Tartrate and its Sustained Release Formulation on Cerebral Glucose Metabolism in Aged Rats

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Abstract

The effects of a sustained release formulation of thyrotrophin-releasing hormone (TRH) over two weeks (TRH-SR, 10 or 50 mg kg^{-1}, equivalent to 0·56 or 2·80 mg kg^{-1} free TRH, respectively) and repeated treatment with TRH tartrate (TRH-T, 0·3 , 1·0 or 3·0 mg kg^{-1}, equivalent to 0·2, 0·7 or 2·0 mg kg^{-1} free TRH, respectively) on the rate of local cerebral glucose utilization (LCGU) were investigated using the quantitative autoradiographic 2-deoxy-[^{14}C]D-glucose method in various brain regions of aged rats.

In aged rats (28 months old), the LCGU was significantly reduced as compared with young adult rats (3 months old), while treatment with TRH-SR ameliorated the reduction of the LCGU in a dose-dependent manner. The brain regions ameliorated by TRH-SR were the auditory cortex, septal nucleus, substantia nigra, cerebellar cortex and cerebellar nucleus. In contrast, once-daily repeated treatment over one week with TRH-T at a dose of 0·3 mg kg^{-1} (equivalent to 50 mg kg^{-1} of TRH-SR) had no effect on the reduced LCGU in various brain regions in aged rats (27 months old), whereas treatment with a higher dose of TRH-T (0·7 or 2·0 mg kg^{-1} free TRH) significantly ameliorated the reduction.

The comparison of the ameliorating potencies between TRH-T and TRH-SR indicated that TRH-SR had a potency about 7 times greater than TRH-T.

Thyrotrophin-releasing hormone (TRH) is a hypothalamic tripeptide (L-pyroglutamyl-L-hystidyl-L-proline amide) neurohormone which stimulates the release and synthesis of thyrotrophin from the anterior pituitary gland via the hypophyseal portal system. In addition, TRH is known to have profound pharmacological effects on the central nervous system which are independent of the hypothalamic-pituitary-thyroid axis (e.g. modulation of the monoaminergic system, enhancement of learning and memory functions, antagonism of hypnotic, sedative and hypothermic states and amelioration of ataxia due to spinocerebellar degeneration (Burgus et al 1970; Sobue et al 1980, 1983; Sharif 1985; Lechan et al 1986; Giovannini et al 1991; Okada 1991; Toide et al 1993). These actions of TRH on the central nervous system (Griffiths 1985; Horita et al 1986) are marginal because TRH is metabolized easily and has a short plasma half-life (Nagai et al 1980). In a previous clinical study, although TRH significantly improved some symptoms of Alzheimer's disease when the effects were estimated within 90 min of an intravenous injection of TRH, the overall therapeutic effect remained unclear; no effects of TRH intravenous infusions were noted on measures of attention, episodic memory or visual memory, while there was a statistically significant drug-placebo difference in the effect on semantic memory (Peabody et al 1986; Mellow et al 1989). This phenomenon is thought to be closely associated with the short-lived pharmacological action of TRH. For this reason, a sustained release form of TRH (TRH-SR) has been developed (Heya et al 1991a, b), which allows continuous release of TRH over several weeks after subcutaneous injection.

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It has been reported that cerebral energy metabolism in rats decreases with aging or functional deterioration, or both (Nakayama & Nagai 1996; Nakayama et al 1996). This age-related decline has been suggested to be closely related to the degradation of functional activity in the brain, such as occurs in dementia (Sokoloff 1977). Thus, since age is a factor in the pathogenesis of conditions involving dementia, such as Alzheimer's disease, aged animals are considered to form a suitable model for the evaluation of the pathology of progressive neurodegenerative disease with aging. In the present study, using aged Fischer 344 rats, we compared the effect of TRH-SR on the rate of local cerebral glucose utilization with that of TRH tartrate (TRH-T).

Materials and Methods

Animals

Male Fischer 344 rats (3, 25, 27 and 28 months old) obtained from Charles River Laboratories (Japan) were used in these experiments. The animals were maintained under controlled environmental conditions (12 h dark/light cycle, 24 ± 1°C and 55 ± 5% relative humidity) and given free access to a standard diet and water.

Chemicals

TRH-T (Lot No. OB021) was synthesized in our laboratories. TRH-SR (Lot No. Z323H01) and vehicle (Lot No. Z323B03), which was composed of 0·1% Tween 80, 0·9% NaCl, 0·07% Na_2HPO_4 and 2·5% sorbitol in water and adjusted to pH 5·5–7·0 with HCl, were synthesized in the DDS Research Laboratories at Takeda Chemical Ind, Ltd. TRH-SR formula-

tion comprised copoly (DL-lactic/glycolic acid) (copolymer ratio of 75/25) (PLGA) microspheres. PLGA microspheres were prepared in the following method. In brief, 0·5 g of TRH was dissolved in 0·3 mL of water, and 4·5 g PLGA was dissolved in 5·6 mL of dichloromethane. These solutions were vigorously homogenized with a Polytron for a few minutes to make a water in oil emulsion. The emulsion, after being cooled to prevent the evaporation of dichloromethane, was poured into 1000 mL of 0·25% polyvinyl alcohol aqueous solution under stirring and the resulting mixture was stirred for a few minutes to make a water-in-oil-in-water (W/O/W) emulsion. To evaporate the dichloromethane, the W/O/W emulsion was further stirred gently with a propeller mixer for 3 h. The resulting microspheres were collected by centrifuging, rinsed with water three times, and then lyophilized into a powder.

TRH-T and TRH-SR were used after being dissolved in saline and vehicle, respectively. 2-Deoxy-[^{14}C]-D-glucose (specific radioactivity: 1·85–2·22 Gbq mmol^{-1}) was purchased from American Radiolabeled Chemicals. Glucose oxidase (grade II), peroxidase (grade II) and ABTS (2,2′-azino-di-[3-ethyl-benzthiazolinsulphonate]) were purchased from Boehringer–Mannheim. Glucose standard solution (300 mg dL^{-1}) was obtained from WAKO Pure Chemical, Ind. All other chemicals were of analytical reagent grade.

Determination of local cerebral glucose utilization

Animals (male Fischer 344 rats) were given TRH-T (equivalent to 0·2, 0·7 or 2 mg kg^{-1} free TRH) or saline subcutaneously once daily, between 1100 h and 1200 h, for 7 days. The LCGU was determined 30 min after the final dose. TRH-SR (10 or 50 mg kg^{-1}, equivalent to 0·56 or 2·8 mg kg^{-1} free TRH, respectively) or vehicle was injected subcutaneously into other groups of rats and the LCGU was determined 1 week after treatment. Animals were deprived of food for approximately 17 h prior to the experiment to stabilize plasma glucose levels (100 ~ 250 mg (dL plasma)$^{-1}$). Animals with abnormal values in plasma glucose levels and hematocrits were excluded from these experiments.

Indwelling polyethylene catheters (PE50 tubing, Cray Adams, Parsippany, NJ) were implanted into the femoral vein and artery under 2% halothane anaesthesia. The lines were kept patent with heparinized saline (10%). The animals were allowed at least 3 h to recover, then [^{14}C]deoxy-D-glucose (100 μCi kg^{-1}) was injected over 10 s via the venous catheter. Collection of 12 timed arterial samples began simultaneously and proceeded at designated intervals for 45 min. Blood samples were immediately centrifuged, and plasma samples were analysed for ^{14}C by liquid scintillation spectroscopy and for plasma glucose by the glucose oxidase method (Werner et al 1970). Forty-five minutes after the radiotracer had been injected, the animals were decapitated. The brain was dissected, coated with chilled embedding medium (Lipshaw Manufacturing Co., Detroit, MI) and frozen in isopentane with dry ice. Once fully frozen, the brain was stored at −70°C prior to sectioning. Frozen coronal sections (20 μm) were obtained using a cryostat (−22°C), mounted onto glass coverslips and rapidly dried on a hotplate (60°C). Sections were apposed to Kodak SB X-ray film for 10 days, together with a set of [^{14}C] plastic standards. Images were processed using a Fuji photo film developer and fixer. Autoradiograms were analysed for LCGU using a quantitative imaging system (Micro Computer Imaging Device, Imaging Research, Canada) which utilized the operational equation of Sokoloff et al (1977) to calculate the LCGU from the plasma glucose and [^{14}C]deoxy-D-glucose levels, regional [^{14}C] density and the density of the calibrated standards co-exposed with the tissue on the film. The rate of LCGU was calculated using reported values for the kinetic constants and lumped constant in the equations derived by Sokoloff et al (1977). A stereotaxic atlas of the rat brain compiled by Pellegrino & Cushman (1967) was used to define the different brain areas.

Determination of plasma TRH concentration

Male Fischer 344 rats (25 months old) were injected subcutaneously with TRH-T (3 or 10 mg kg^{-1}, equivalent to 2 or 7 mg kg^{-1} free TRH, respectively) as a single dose or once daily between 1100 h and 1200 h for 7 days (repetitive dosing). After the final dose, TRH plasma levels were determined at designated times (0, 0·25, 0·5, 1, 2, 4 and 24 h) using radioimmunoassay. Indwelling polyethylene catheters (PE50 tubing, Cray Adams, Parsippany, NJ) were implanted into the femoral artery under 2% halothane anaesthesia. The lines were kept patent with heparinized saline (10%). Disodium edetate (1·5 mg mL^{-1}), Tween 20 (0·5 mg mL^{-1}) and 8-hydroxyquinoline (0·1 mg mL^{-1}) were immediately added to blood samples to prevent inactivation of TRH by serum and blood samples obtained at the designated times were immediately centrifuged, and plasma samples were analysed for the determination of plasma TRH concentrations .

Likewise, TRH plasma levels after a subcutaneous injection of TRH-SR (50 or 100 mg kg^{-1} (2 weeks)$^{-1}$, equivalent to 2·8 or 5·6 mg kg^{-1} (2 weeks)$^{-1}$ free TRH, respectively) as a single dose were determined in 60 Male Fischer 344 rats (25 months old), five rats per group, by radioimmunoassay. Blood samples were collected from the abdominal aorta 1, 3, 7, 10, 14 and 21 days later.

Statistical analysis

Differences of the LCGU between the young group and the aged controls, and between the TRH-T (repetitive treatment for 7 days with 0·3 mg kg^{-1} day^{-1}) group and the aged controls, were evaluated using Student's *t*-test. Differences of the LCGU between the TRH-T (1 and 3 mg kg^{-1}) or TRH-SR (10 and 50 mg kg^{-1}) groups and the aged controls were evaluated using Dunnett's test. The differences between repetitive and single treatment with TRH-T (3 and 10 mg kg^{-1}) on the time-TRH plasma concentration parameters were compared using Student's *t*-test following analysis of variance. P values of $< 0·05$ were considered statistically significant.

Results

Effect of TRH-SR on the LCGU in aged Fischer 344 rats

As shown in Table 1, the overall mean rates of LCGU for the 35 brain regions investigated in the vehicle-treated young adult rats and aged control rats were 73·2 ± 4·7 μmol min^{-1}/100 g and 40·6 ± 3·0 μmol min^{-1}/100 g, respectively (data not shown), indicating that the LCGU in aged control rats (28 months old) was decreased to 55·5% of that in young rats (3 months old). The LCGU was decreased in almost all brain regions in aged rats, and this decrease was significant in 27 out of the 35 regions. When the LCGU in aged rats treated with

Table 1. Effect of TRH-SR on the local cerebral glucose utilization rate (μmol min^{-1}/100 g) in aged rats.

Structure	Young	Aged		
		Vehicle	TRH-SR	
			10 mg kg^{-1}	50 mg kg^{-1}
Visual cortex	64·7 ± 6·0	34·4 ± 6·4**	38·7 ± 3·8	45·8 ± 4·3
Auditory cortex	100·9 ± 5·0	43·7 ± 9·0**	65·8 ± 5·5#	71·1 ± 4·7#
Parietal cortex	76·3 ± 6·1	47·5 ± 9·8**	53·2 ± 6·5	53·4 ± 4·2
Sensory-motor cortex	92·0 ± 8·4	50·1 ± 11·2**	62·5 ± 8·8	70·2 ± 6·1
Olfactory cortex	93·2 ± 8·2	61·7 ± 15·1*	68·8 ± 4·3	84·5 ± 9·7
Frontal cortex	70·2 ± 9·5	35·9 ± 6·8**	42·4 ± 5·2	53·5 ± 4·8
Thalamus:				
Lateral nucleus	78·4 ± 9·5	54·4 ± 9·8	50·1 ± 6·4	56·8 ± 3·2
Ventral nucleus	68·2 ± 6·7	45·1 ± 5·1*	46·0 ± 7·8	48·5 ± 1·3
Dorsomedial nucleus	85·1 ± 6·6	57·6 ± 9·2**	58·3 ± 5·3	69·0 ± 2·3
Habenula	97·9 ± 6·8	59·3 ± 9·0**	58·4 ± 4·0	72·3 ± 2·3
Subthalamic nucleus	60·8 ± 6·1	37·8 ± 7·6	38·1 ± 6·7	49·7 ± 5·1
Medial geniculate body	72·4 ± 8·2	36·2 ± 4·9**	39·8 ± 4·7	50·4 ± 4·7
Lateral geniculate body	67·1 ± 6·4	35·5 ± 8·8**	40·5 ± 5·9	48·1 ± 3·6
Hypothalamus	45·7 ± 5·7	25·0 ± 4·0**	28·4 ± 4·6	36·0 ± 5·4
Mammillary body	103·3 ± 7·9	67·0 ± 9·3**	72·8 ± 8·7	84·1 ± 8·6
Hippocampus:				
Ammon's horn	61·9 ± 8·7	36·5 ± 3·7**	37·8 ± 6·7	46·5 ± 4·7
Dentate gyrus	49·2 ± 6·8	22·0 ± 3·8**	27·5 ± 4·7	33·5 ± 3·7
Amygdala	30·7 ± 5·4	15·4 ± 5·6	12·5 ± 4·6	25·3 ± 4·8
Septal nucleus	40·7 ± 3·6	16·3 ± 6·7**	19·0 ± 4·8	32·3 ± 4·7#
Caudate-putamen	67·7 ± 10·8	36·7 ± 8·1	42·6 ± 7·6	54·2 ± 4·9
Nucleus accumbens	66·2 ± 7·7	32·2 ± 10·7**	39·9 ± 7·2	50·3 ± 6·5
Globus pallidus	30·8 ± 5·2	10·7 ± 5·2*	8·1 ± 4·9	18·6 ± 4·4
Substantia nigra	45·4 ± 4·5	19·3 ± 5·4**	18·5 ± 4·9	36·7 ± 4·2#
Raphe nucleus	71·0 ± 8·2	33·0 ± 7·4**	36·0 ± 5·7	52·1 ± 4·1
Locus coeruleus	72·6 ± 10·7	42·4 ± 8·1*	39·5 ± 6·0	54·7 ± 2·4
Vestibular nucleus	102·2 ± 7·9	63·1 ± 6·5**	60·0 ± 4·8	78·8 ± 3·6
Cochlear nucleus	121·9 ± 8·6	50·5 ± 7·9**	56·0 ± 8·4	70·6 ± 8·4
Superior olivary nucleus	121·7 ± 10·8	60·9 ± 8·7**	55·3 ± 6·6	75·9 ± 3·6
Lateral lemniscus	92·8 ± 10·0	46·0 ± 8·3	39·2 ± 8·0	59·9 ± 3·2
Inferior colliculus	150·9 ± 12·4	95·2 ± 12·3**	96·1 ± 5·4	115·8 ± 7·5
Superior colliculus	60·4 ± 7·0	38·0 ± 6·5*	34·2 ± 6·9	50·7 ± 5·4
Pontine gray matter	42·7 ± 6·1	25·4 ± 6·5	21·2 ± 5·9	38·8 ± 5·6
Cerebellar cortex	46·4 ± 4·6	18·9 ± 5·3**	16·9 ± 3·3	33·0 ± 5·7##
Cerebellar nucleus	79·1 ± 5·4	48·0 ± 9·0	47·4 ± 6·3	70·9 ± 4·8#
Internal capsule	32·1 ± 6·8	18·4 ± 3·8	18·2 ± 3·3	26·0 ± 4·0

Male Crj:Fischer 344 rats aged 3 and 28 months were used as the young and aged group, respectively, in this experiment. TRH-SR (10 or 50 mg kg^{-1}/2 weeks, equivalent to 0·56 or 2·8 mg kg^{-1}/2 weeks^{-1} free TRH, respectively) or vehicle was injected subcutaneously 1 week before determination of the local cerebral glucose utilization (LCGU) rate. All values represent the means ± s.e.m. for 5 animals. *$P < 0·05$, **$P < 0·01$ compared with the young group (Student's t-test), #$P < 0·05$, ##$P < 0·01$ compared with the vehicle group (Dunnett's test).

TRH-SR (10 or 50 mg kg^{-1}) were determined 1 week after treatment, their mean values were 42·6 ± 3·2 μmol min^{-1}/100 g and 54·8 ± 3·4 μmol min^{-1}/100 g, respectively (data not shown). These rates were 58·2% and 74·9% of that observed in the young rats, respectively. Thus, dose-related amelioration appeared to be induced by TRH-SR treatment, especially in the auditory cortex, septal nucleus, substantia nigra, cerebellar cortex and cerebellar nucleus.

Treatment with TRH-SR did not significantly alter plasma glucose levels (data not shown), and the levels in the TRH-SR and vehicle groups were similar throughout the experimental period (during the measurement of the LCGU rate) (data not shown). The values of other peripheral physiological valuables such as arterial blood gas partial pressures and hematocrits in all rats used in the experiment were in a normal physiological state throughout the experiment period (data not shown). Animals in an abnormal physiological state during this experiment were excluded.

Effect of repetitive treatment with TRH-T on the rate of LCGU in aged Fischer 344 rats

As shown in Table 2, the overall mean rates of LCGU for the 35 brain regions investigated in the saline-treated young adult rats and aged control rats were 97·8 ± 5·6 μmol min^{-1}/100 g and 58·6 ± 3·1 μmol min^{-1}/100 g, respectively (data not shown), indicating that the LCGU in aged control rats (27 months old) was decreased to 59·9% of that in young rats (3 months old). When aged rats were treated with TRH-T (0·3 mg kg^{-1}) once daily for 7 days, the mean LCGU was 56·2 ± 2·9 μmol min^{-1}/100 g (equivalent to 57·5% of that in young rats) (data not shown) and remained unchanged in comparison with the aged controls, indicating that repetitive treatment with a daily dose of 0·3 mg kg^{-1} TRH-T was ineffective.

As shown in Table 3, however, when aged rats (25 months old) were treated with TRH-T (1 or 3 mg kg^{-1}) once daily for 7 days, mean rates of LCGU were dose-dependently increased as compared with the rate in the aged controls (TRH-T

Table 2. Effect of repeated subcutaneous treatment with TRH-T on the local cerebral glucose utilization rate (μmol min^{-1}/100 g) in aged rats.

Structure	Young	Aged	
		Saline	TRH-T (0·3 mg kg^{-1})
Visual cortex	88·2 ± 5·4	50·9 ± 4·5***	54·5 ± 2·0
Auditory cortex	125·6 ± 7·0	75·9 ± 2·3**	74·5 ± 1·5
Parietal cortex	118·8 ± 7·8	71·0 ± 4·5***	65·4 ± 1·8
Sensory-motor cortex	113·4 ± 4·4	83·4 ± 4·3**	71·7 ± 2·6
Olfactory cortex	119·6 ± 1·9	70·3 ± 6·1***	77·6 ± 2·6
Frontal cortex	101·4 ± 2·3	61·8 ± 3·3***	61·0 ± 1·5
Thalamus:			
Lateral nucleus	96·2 ± 5·4	60·9 ± 4·6**	60·1 ± 1·5
Ventral nucleus	87·9 ± 3·2	65·8 ± 3·1**	61·4 ± 3·2
Dorsomedial nucleus	115·9 ± 4·4	75·4 ± 3·0***	70·2 ± 2·8
Habenula	136·3 ± 8·2	89·4 ± 7·1**	78·3 ± 2·0
Subthalamic nucleus	86·9 ± 3·3	58·7 ± 4·3***	54·5 ± 1·5
Medial geniculate body	100·1 ± 4·7	65·7 ± 2·6***	63·3 ± 1·7
Lateral geniculate body	99·2 ± 2·4	57·1 ± 2·8***	55·5 ± 1·7
Hypothalamus	57·6 ± 4·0	35·8 ± 2·3**	35·1 ± 1·5
Mammillary body	130·4 ± 10·0	77·2 ± 4·7**	76·2 ± 2·0
Hippocampus:			
Ammon's horn	68·7 ± 3·8	42·3 ± 3·1***	43·2 ± 2·1
Dentate gyrus	64·1 ± 3·4	41·8 ± 2·4***	40·4 ± 1·6
Amygdala	49·1 ± 2·8	31·0 ± 1·4***	30·5 ± 0·8
Septal nucleus	70·3 ± 2·9	41·2 ± 4·8***	38·1 ± 1·2
Caudate-putamen	103·7 ± 4·3	56·1 ± 3·2***	53·0 ± 1·4
Nucleus accumbens	88·1 ± 4·6	52·7 ± 2·4***	49·2 ± 1·2
Globus pallidus	55·2 ± 2·5	31·2 ± 1·2***	30·1 ± 1·1
Substantia nigra	67·0 ± 2·7	39·9 ± 2·6***	39·8 ± 1·6
Raphe nucleus	91·1 ± 1·2	54·3 ± 2·4***	53·6 ± 1·9
Locus coeruleus	110·1 ± 7·0	68·3 ± 2·5**	61·6 ± 3·0
Vestibular nucleus	122·4 ± 5·9	74·1 ± 2·7***	74·6 ± 3·9
Cochlear nucleus	147·4 ± 9·4	72·6 ± 3·8***	73·6 ± 4·9
Superior olivary nucleus	156·3 ± 10·4	68·5 ± 4·3***	66·7 ± 4·1
Lateral lemniscus	110·6 ± 6·7	53·2 ± 2·8***	49·8 ± 1·8
Inferior colliculus	190·5 ± 11·4	100·1 ± 6·5***	90·5 ± 2·9
Superior colliculus	90·2 ± 3·5	62·9 ± 2·2***	62·1 ± 1·8
Pontine gray matter	57·9 ± 2·0	35·8 ± 2·7***	34·2 ± 1·8
Cerebellar cortex	63·1 ± 3·2	38·0 ± 2·1***	35·1 ± 1·6
Cerebellar nucleus	105·9 ± 5·5	69·1 ± 3·0***	67·2 ± 2·4
Internal capsule	33·5 ± 2·4	17·1 ± 1·8***	16·0 ± 0·7

Male Crj:Fischer 344 rats aged 3 and 27 months were used as the young and aged group, respectively, in this experiment. TRH-T (0·3 mg kg^{-1}, equivalent to 0·2 mg kg^{-1} free TRH) or saline was injected subcutaneously once daily for 7 days. The local cerebral glucose utilization (LCGU) rate was determined 30 min after the final dose. All values represent the means ± s.e.m. for 5 animals. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the young group (Student's t-test).

1 mg kg^{-1}: 66·6 ± 3·4 μmol min^{-1}/100 g, equivalent to 84·5% of LCGU in young rats 78·8 ± 4·0 μmol min^{-1}/100 g (data not shown), TRH-T 3 mg kg^{-1}: 68·4 ± 3·6 μmol min^{-1}/100 g, equivalent to 86·8% of LCGU in young rats, aged rats: 58·5 ± 3·2 μmol min^{-1}/100 g, equivalent to 74·2% of LCGU in young rats (data not shown)), especially in the sensory-motor cortex, olfactory cortex, thalamic dorsomedial nucleus, lateral geniculate body, amygdala, septal nucleus, globus pallidus, substantia nigra, superior olivary nucleus and internal capsule.

Once-daily repeated treatment over one week with TRH-T did not significantly alter plasma glucose levels (data not shown), and the levels in the TRH-T and saline groups were similar throughout the experimental period (during the measurement of the LCGU rate) (data not shown). The values of other peripheral physiological variables such as arterial blood gas partial pressures and haematocrits in all rats used in the experiment were in a normal physiological state throughout the experimental period (data not shown).

Comparative efficacy of TRH-T and TRH-SR on local cerebral glucose utilization in aged rats

The ratio of the net increase in the average LCGU after treatment with TRH-T or TRH-SR against the value obtained by subtracting the average LCGU in each aged control from that in the young rats was semi-logarithmically plotted against the dose of TRH-T or TRH-SR received per day (Fig. 1). For example, in the case of TRH-SR (10 mg kg^{-1}, s.c.) in Table 1, net LCGU increase ratio was calculated by the following equation:

Net LCGU increase ratio = 42·6 (overall mean value of LCGU in aged rats treated with TRH-SR (10 mg kg^{-1})) − 40·6 (overall mean value of LCGU in aged rats treated with vehicle)]/[73·2 (overall mean value of LCGU in young rats) − 40·6 (overall mean value of LCGU in aged rats treated with vehicle)] = 0·06135.

The resulting ED25 values, which showed the 25% net increase in the average LCGU, revealed that TRH-SR had a potency about 7 times greater than TRH-T.

Table 3. Effect of repetitive subcutaneous treatment with TRH-T on the local cerebral glucose utilization rate (μmol min^{-1}/100 g) in aged rats.

Structure	Young	Saline	Aged	
			TRH-T	
			1 mg kg^{-1}	3 mg kg^{-1}
Visual cortex	74·3 ± 5·5	56·4 ± 5·7	63·8 ± 3·6	70·2 ± 3·3
Auditory cortex	90·5 ± 3·1	77·2 ± 4·8	87·4 ± 2·5	92·3 ± 6·0
Parietal cortex	88·5 ± 3·6	73·4 ± 5·4	81·4 ± 3·3	82·7 ± 6·7
Sensory-motor cortex	86·9 ± 1·8	75·6 ± 4·0*	89·0 ± 4·2	95·2 ± 6·2#
Olfactory cortex	88·0 ± 3·3	78·3 ± 3·4	87·1 ± 4·9	96·1 ± 6·6#
Frontal cortex	83·6 ± 3·0	71·5 ± 3·9*	79·1 ± 3·5	78·9 ± 5·4
Thalamus:				
Lateral nucleus	81·5 ± 4·3	60·2 ± 3·4**	69·5 ± 2·6	70·2 ± 5·5
Ventral nucleus	76·4 ± 3·3	68·3 ± 3·7	74·7 ± 2·9	77·0 ± 6·0
Dorsomedial nucleus	93·8 ± 3·4	72·9 ± 2·8***	83·8 ± 2·8	87·5 ± 6·1#
Habenula	109·9 ± 2·4	80·7 ± 4·0***	91·7 ± 4·7	93·1 ± 7·5
Subthalamic nucleus	65·7 ± 5·2	54·1 ± 4·1	62·2 ± 3·2	67·0 ± 4·7
Medial geniculate body	86·3 ± 5·5	61·8 ± 2·5**	67·9 ± 4·2	73·0 ± 5·0
Lateral geniculate body	81·6 ± 3·4	57·1 ± 2·6***	68·0 ± 3·1	70·0 ± 4·3#
Hypothalamus	49·3 ± 2·8	34·9 ± 2·2**	39·4 ± 2·6	39·9 ± 2·5
Mammillary body	92·5 ± 5·5	82·1 ± 3·3	85·2 ± 3·8	92·8 ± 6·7
Hippocampus:				
Ammon's horn	71·7 ± 5·3	55·6 ± 3·7*	65·2 ± 3·9	64·5 ± 4·0
Dentate gyrus	59·0 ± 4·6	39·7 ± 2·5**	47·3 ± 3·1	47·5 ± 2·9
Amygdala	39·1 ± 2·2	24·5 ± 1·6***	31·7 ± 2·0#	33·4 ± 2·4#
Septal nucleus	56·1 ± 3·7	32·9 ± 2·7***	41·9 ± 3·2	43·4 ± 2·5#
Caudate-putamen	84·8 ± 3·4	54·6 ± 4·4***	71·2 ± 3·1#	67·8 ± 4·4
Nucleus accumbens	57·9 ± 5·0	42·7 ± 2·7*	51·9 ± 2·5	50·6 ± 4·2
Globus pallidus	47·4 ± 3·0	28·6 ± 1·4***	36·3 ± 1·9#	38·6 ± 2·2##
Substantia nigra	61·7 ± 3·1	38·1 ± 1·5***	45·5 ± 2·2#	45·8 ± 2·7#
Raphe nucleus	80·9 ± 1·9	56·9 ± 2·6***	65·6 ± 2·6	63·1 ± 3·7
Locus coeruleus	95·8 ± 6·0	72·9 ± 2·9**	78·3 ± 3·3	76·6 ± 5·1
Vestibular nucleus	104·5 ± 6·4	81·4 ± 2·2*	88·5 ± 4·5	87·3 ± 4·8
Cochlear nucleus	111·4 ± 6·1	74·7 ± 2·0**	84·4 ± 4·3	80·4 ± 4·9
Superior olivary nucleus	105·4 ± 4·9	66·4 ± 2·5***	78·2 ± 4·5	82·7 ± 6·1#
Lateral lemniscus	87·3 ± 3·0	52·1 ± 1·2***	55·7 ± 2·9	56·3 ± 3·6
Inferior colliculus	146·1 ± 6·4	93·1 ± 2·6***	101·6 ± 4·6	108·8 ± 9·3
Superior colliculus	73·2 ± 5·8	58·7 ± 2·4*	63·3 ± 3·1	66·7 ± 2·9
Pontine gray matter	49·9 ± 4·9	37·2 ± 1·2	42·6 ± 2·3	42·9 ± 2·0
Cerebellar cortex	57·9 ± 3·2	42·0 ± 2·0**	47·8 ± 1·8	46·8 ± 1·9
Cerebellar nucleus	93·5 ± 4·0	75·4 ± 3·5**	84·3 ± 3·5	82·7 ± 5·0
Internal capsule	27·0 ± 1·4	15·4 ± 1·0***	19·9 ± 1·8	21·1 ± 1·5#

Male Crj:Fischer 344 rats aged 3 and 25 months were used as the young and aged group, respectively, in this experiment. TRH-T (1 or 3 mg kg^{-1}, equivalent to 0·7 or 2 mg kg^{-1} free TRH, respectively) or saline was injected subcutaneously once daily for 7 days. The local cerebral glucose utilization (LCGU) rate was determined 30 min after the final dose. All values represent the means ± s.e.m. for 5–6 animals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the young group (Student's t-test), #$P < 0.05$, ##$P < 0.01$ compared with the saline-treated aged group (Dunnett's test).

Effect of repetitive treatment with TRH-T on various parameters of the time-TRH plasma concentration curve

Fig. 2 shows the time course of TRH plasma concentrations after single and once daily repeated injections of TRH-T (3 or 10 mg kg^{-1}) over one week to aged rats (25 months old) and Table 4 show the effect of repetitive treatment with TRH-T (3 or 10 mg kg^{-1}) on the time-TRH plasma concentration parameters. As shown in Fig. 2 and Table 4, there were no apparent differences in several pharmacokinetic parameters, such as time to reach a maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half-life (t1/2) and area under the curve (AUC).

TRH plasma levels after a subcutaneous injection of TRH-SR

Fig. 3 shows changes in immunoreactive TRH plasma levels after a subcutaneous injection of TRH-SR to aged rats (25 months old). A moderate increase in immunoreactive TRH plasma levels (0·1–1·0 ng mL^{-1} plasma at both doses of TRH-SR) was noted 1–10 days after treatment with TRH-SR. These levels were corresponding to 10–100 times higher than a basal level (0·01 ng mL^{-1}). A single subcutaneous injection of TRH-SR maintained steady TRH plasma concentrations over two weeks.

Discussion

The sustained release form of TRH (TRH-SR) used in this experiment was designed to maintain steady TRH plasma concentrations over two weeks after a single subcutaneous injection (Heya et al 1991a, b), thus compensating for TRH's short duration of action. The present study was performed to compare the effects of TRH-SR and TRH-T on the rate of LCGU in aged Fischer 344 rats.

We revealed that significant decreases in the LCGU in aged rats were observed as compared with young adult rats and that

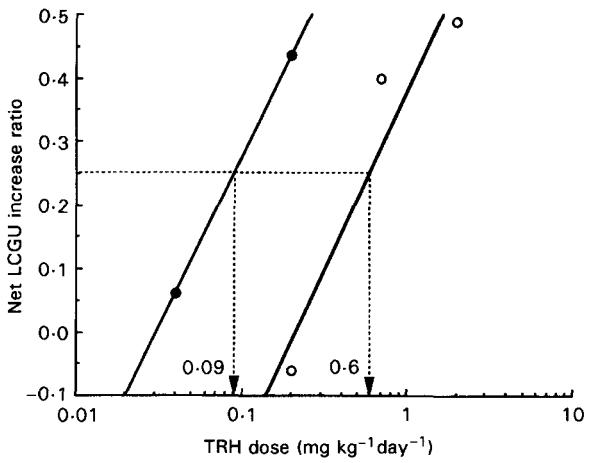

FIG. 1. Dose-related effects of TRH-T (○) and TRH-SR (●) on local cerebral glucose utilization (LCGU) in aged rats. The figure represents the ratio of the average increase in the rate of LCGU in 35 brain regions after subcutaneous treatment with TRH-T or TRH-SR. From this graph, the ED25 values of TRH-T and TRH-SR are 0·6 and 0·09 mg kg^{-1} day^{-1}, calculated as free TRH, respectively.

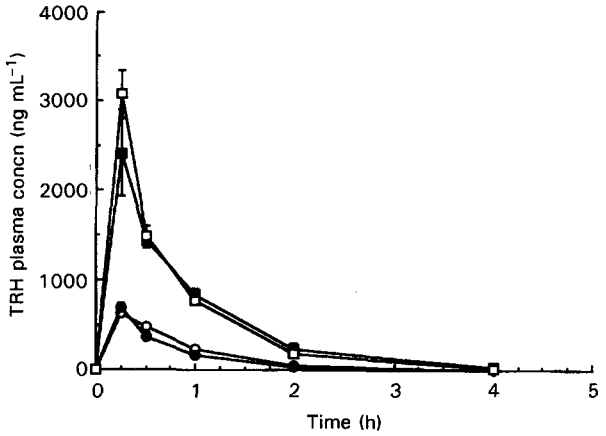

FIG. 2. TRH plasma concentrations after single and repetitive treatment over one week with TRH-T. Male Crj:Fischer 344 rats (25 months old) were injected subcutaneously with TRH-T (3 or 10 mg kg^{-1}, equivalent to 2 or 7 mg kg^{-1} free TRH, respectively) as a single dose or once daily for 7 days (repetitive dosing). After the final dose, TRH plasma levels were determined by radioimmunoassay at designated times. ○, single dosing of TRH-T (3 mg kg^{-1}, n = 6); ●, repetitive dosing of TRH-T (3 mg kg^{-1}, n = 6); □, single dosing of TRH-T (10 mg kg^{-1}, n = 6); ■, repetitive dosing of TRH-T (10 mg kg^{-1}, n = 5).

treatment with TRH-SR ameliorated the reduction of the LCGU observed in aged rats in a dose-dependent manner. It was suggested from the brain regions affected that TRH-SR might act via the activation of cholinergic (the auditory cortex, septal nucleus) or dopaminergic (the substantia nigra) systems, or both. These actions of TRH on these nervous pathways are coincident with some previous reports (Breese et al 1975; Marek & Haubrich 1977; Schmidt 1977; Heal et al 1983; Giovannini et al 1991; Okada 1991; Toide et al 1993). However, repetitive treatment over one week with TRH-T at a dose equivalent to the highest dose (50 mg kg^{-1}) of TRH-SR used had no effect on the reduction of LCGU in various brain regions in aged rats. To estimate the ability of TRH-T to increase the LCGU in aged rats, we tried using higher doses of TRH-T. As a result, TRH-T (equivalent to 0·7 or 2 mg kg^{-1} free TRH) significantly ameliorated the reduction of LCGU predominantly via the activation of cholinergic or dopaminergic systems, or both, as with TRH-SR. It was also proved from the dose-response effects of TRH-T and TRH-SR on the net increase in average LCGU, that TRH-SR is about 7 times more potent than TRH-T. Although we have used a well defined standard method to measure LCGU, in some brain regions there are variations between the LCGU values in 25- or 27-month-old control groups (i.e. saline injected) and those in 28 months old control group (i.e. vehicle injected). It is considered from these results that a drastic decrease in the rate of LCGU in some brain regions in aged rats comes into existence in rats ranging from 27 to 28 months old.

Miyamoto et al (1993) previously reported that TRH-SR (0·56 and 2·8 mg kg^{-1} free TRH) given subcutaneously 7 days before the acquisition trial markedly ameliorated scopolamine-induced amnesia in mice, as evaluated with a passive avoidance task, as did repeated subcutaneous administration of TRH for 7 days at a dose of 5 mg kg^{-1} but not at doses of 0·2 and 1 mg kg^{-1}. Furthermore, they showed, using rats with bilateral intracerebroventricular injection of a cholinergic neurotoxin, ethylcholine aziridinium ion (AF64A), that TRH-SR (0·56 and 2.8 mg kg^{-1} free TRH) exhibited a dose-dependent ameliorating action on a significant impairment in the water maze task 2 weeks after surgery (Miyamoto et al 1993). Also they showed that TRH-SR (0·7 and 2·8 mg kg^{-1}) improved learning and memory impairments in aged rats, which were assessed by the step-down type passive avoidance and water maze tasks, suggesting continuous infusion of TRH may be useful for therapy of dementia including Alzheimer's disease (Miyamoto et al 1992). Our present results appear to be mutually related to their reports on the behavioural changes of aged rats treated with TRH-SR.

A decrease in cerebral glucose metabolism is a well-known characteristic of aging (Smith et al 1980; Kuhl et al 1982), and Smith (1984) also suggested that a decrease in the activity of

Table 4. *Effect of repetitive subcutaneous treatment with TRH-T on* T_{max}, C_{max}, $t_{\frac{1}{2}}$ and AUC of the time-TRH plasma concentration curve.

	TRH-T dose 3 mg kg^{-1}		10 mg kg^{-1}	
	Single	Repeated	Single	Repeated
T_{max} (min)	15	15	15	15
C_{max} (ng mL^{-1})	624	695	3075	2417
$t_{\frac{1}{2}}$ (min)	29·2	29·8	31·7	36·5
$AUC_{(0-4\ h)}$ (ng h mL^{-1})	570	493	2193	2161

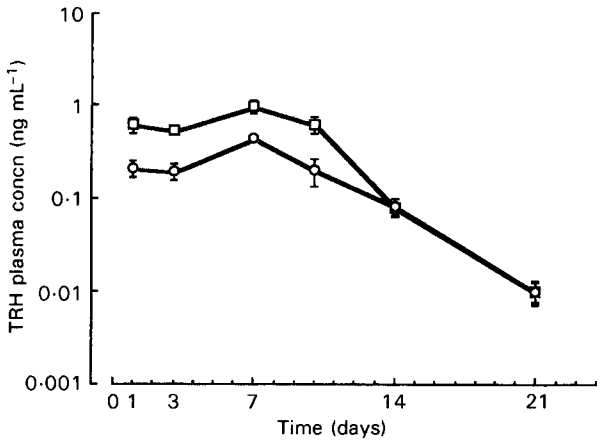

FIG. 3. Time course of TRH plasma concentrations after treatment with TRH-SR. Male Crj:Fischer 344 rats (25 months old) were injected subcutaneously with TRH-SR (50 or 100 mg kg^{-1} (2 weeks)$^{-1}$, equivalent to 2·8 or 5·6 mg kg^{-1} (2 weeks)$^{-1}$ free TRH, respectively) as a single dose. After a single subcutaneous injection of TRH-SR, TRH plasma levels were determined by radioimmunoassay at designated days. ○, 2·8 mg kg^{-1} as free TRH (n = 5); □, 5·6 mg kg^{-1} as free TRH (n = 5).

certain enzymes in the glycolytic pathway, such as hexokinase and phosphofructokinase, occurs by aging. This glucose hypometabolism with aging appears to result from a decline in rate constant K_3 but not K_1 in Sokoloff's equation (Ogawa et al 1996). Thus, a greater potency of TRH-SR may at least in part result from intensive induction of certain enzymes, which are involved in the glycolytic pathway, by maintenance of steady TRH plasma level through continuous infusion in comparison with TRH-T. As the rates of local cerebral glucose utilization in the brain have been shown to correlate closely with local functional activity (Sokoloff 1977), some of the changes in glucose utilization observed in the aged rats were thought to be due to reduced functional activity, pathological damage, or both. In dementia of the Alzheimer type, the rate of LCGU decreases especially in the parietal and temporal cortex (Benson et al 1983; Foster et al 1984; Friedland et al 1985; Duara et al 1986). Since TRH-SR but not TRH-T, at the relatively low doses examined, ameliorated the reduced rate of LCGU in the auditory cortex in aged rats, it is expected to be useful for the treatment of Alzheimer's disease. It was apparent from the result of our present study that both TRH-T and TRH-SR act at the same sites (cholinergic or dopaminergic systems, or both) to enhance glucose metabolism, despite the difference in their potencies. At present, though, it is difficult to consider that the mechanism of action of TRH-T and TRH-SR is likely to be same, since they have different effects at comparable doses. Further intensive investigation is necessary to strictly evaluate the difference between the mechanism of action of TRH-T and TRH-SR.

Furthermore, we examined the effect of repetitive treatment with TRH-T on the TRH plasma concentration profile. No difference was observed between single and repetitive treatment. Therefore, it is reasonable to propose that the beneficial effects of repetitive treatment with higher doses of TRH-T (0·7 or 2 mg kg^{-1} free TRH) on the reduced cerebral glucose metabolism in aged rats could be reproduced by a single injection of TRH-T. It can be speculated that the increase in LCGU seen after subcutaneous injection of TRH-SR is not due to a compensatory change over the one-week evaluation period but that it is due to a direct effect.

In conclusion, treatment with TRH-SR ameliorated the reduction of the LCGU in aged rats at a dose which was ineffective by repetitive treatment with TRH-T over one week. The brain regions in which this amelioration occurred suggest that TRH-SR might act via activation of the cholinergic or dopaminergic systems, or both. From a comparison of the efficacy of the formulations, we found that TRH-SR had a potency about 7 times greater than TRH-T. These results suggest that a sustained-release form of TRH may be clinically beneficial for the treatment of deteriorating brain function accompanied by reduced energy metabolism, including memory deficit associated with aging.

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