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Involvement of nicotinergic mechanisms in thyrotropin-releasing hormone-induced neurologic recovery after concussive head injury in the mouse

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Abstract

A behavioral study was performed in an attempt to understand the neuronal mechanisms involved in the thyrotropin-releasing hormone (TRH)-induced improvement of consciousness after concussive head injury in the mouse. Intravenous administration of TRH dose dependently shortened the duration of unconsciousness after concussion in the mouse (ED_{50} = 3.2 mg/kg). The improvement of recovery evoked by TRH (3 mg/kg i.v.) after concussion was not affected by i.p. pretreatment with *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine, α-methyl-*para*-tyrosine, *p*-chlorophenylalanine, scopolamine or methylscopolamine. However, mecamylamine or hexamethonium i.p. pretreatment completely inhibited the TRH-induced improvement of outcome in traumatic brain injury. The results imply that TRH-induced improvement of recovery after concussion is not associated with increased activity of monoaminergic neurons in the brain. These results suggest that the inhibitory effect of TRH upon unconsciousness after concussion in mice is mainly produced by activation of central cholinergic systems via nicotinic receptors whereas muscarinic receptors seem to be not implicated.

Keywords: TRH (thyrotropin-releasing hormone); Trauma; Nicotine; (Mouse)

1. Introduction

Thyrotropin-releasing hormone (TRH) was initially isolated from the hypothalamus as a releasing factor for pituitary thyroid stimulating hormone (Burgus et al., 1970). In addition to its hypophysiotropic function, arousal properties of TRH on central nervous system (CNS) have been reported for both experimental animals (Yarbrough, 1979) and humans (Prange et al., 1972; Manaka et al., 1977). TRH and analogs promote behavioral recovery from the loss of consciousness induced by concussive head injury in mice (Manaka and Sano, 1978; Yamamoto and Shimizu, 1987; Matsushita et al., 1993) and neurological recovery in spinal cord or traumatic brain injury in various species (Faden, 1989; Faden and Salzman, 1992). Furthermore, the disturbance of consciousness of patients with head trauma and cerebrovascular diseases is ameliorated by administration of TRH (Manaka et al., 1977) and TRH is currently used to treat disturbance of consciousness in patients with cerebral vascular diseases, head or spinal cord injury (Sano, 1979; Aii et al., 1986; Faden, 1987). Attempts to define the mechanisms by which TRH produces its beneficial effects in the treatment of traumatic CNS injury have not been detailed and the mechanisms of action of TRH remain at best speculative. The level of consciousness is considered to be controlled by the balance between the excitatory and inhibitory systems of the CNS, and the cholinergic, dopaminergic, noradrenergic and serotoninergic systems appear to play important roles in their regulation (Osward, 1968; Jouvet, 1972; Jones et al., 1973; Yamamoto, 1988). In animal studies, TRH has been shown to interact with several neurotransmitters. Of these interactions, the positive neuromodulatory effects of TRH on cholinergic and monoaminergic systems are those best delineated (Breese et al., 1975; Ushijima et al., 1984; Heal et al., 1987). Based on these findings, the present study was carried out to explore the possibility of the involvement of these neuronal systems in the analeptic properties of TRH in concussive head injury in mice.

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To this end, the effects of monoamine-depleting agents and acetylcholine receptor antagonists on TRH-induced shortening of the duration of unconsciousness after concussion were studied.

2. Materials and methods

2.1. Animals

Five-week old male CD1 mice (Charles River, France), weighing 22–24 g on the day of experiment, were used. All animals were kept at an ambient temperature of 21 ± 1°C and relative humidity (60 ± 5%) under a regular 12/12 h light/dark cycle (0700/1900) before the experiments. They were allowed free access to a pellet diet and water. Procedures involving animals and their care were performed according to NIH guidelines for the care and use of laboratory animals (NIH publication No. 85–23, 1985).

2.2. Concussive head injury

The procedure used was similar to that designed by Manaka and Sano (1978) as an animal model which mimics the most prevalent form of clinical head injury. The dorsal skin of the neck was held and the head of the mouse was immobilized on the Teflon baseplate of the injury apparatus as described by Hall (1985). A Teflon impounder was lowered onto the center of the head. The striking surface of the impounder was flat and sound and had a 1-cm diameter. A 48-g stainless steel weight was released by a pin. The weight fell a distance of 12 cm along a stainless steel shaft and struck the impounder producing an approximated force of 576 g cm (48 g × 12 cm). Anaesthesia was not required since this injury consistently caused immediate unconsciousness as judged from the loss of righting reflex and the loss of any pain response (tail pinch). Immediately after the mechanical shock, clonic convulsions occurred for 1–10 s, followed by loss of consciousness, and then the mice remained motionless in a crouching or prone position for a variable period. The time from concussion to the start of spontaneous movement was recorded as the disturbance time of consciousness (DT time). Animals not recovering the righting reflex and spontaneous movement within 600 s were removed and assigned a score of 600. TRH (Bachem, Budendorf, Switzerland) or saline was administered intravenously 10 min before the concussive test. The ED_{50} value, the dose required for 50% reduction of the DT time (s), was obtained from the dose–response curve and was calculated by the method of Lichtfield and Wilcoxon (1949).

2.3. Noradrenergic lesions

Noradrenergic neurons were lesioned using the method of Dooley et al. (1983). Mice were injected i.p. with *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP4) (50 mg/kg). The effect of DSP4 lesions on monoaminergic neurotransmission was determined in naive mice 8 days after the administration of DSP4. Briefly, the animals were killed using focused microwave radiation (Guidotti et al., 1974), the brains were rapidly removed and stored at −80°C until assayed for monoamine content. The level of monoamines and their metabolites was measured by high performance liquid chromatography (HPLC) with electrochemical detection using the method of Mayer and Shoup (1983).

2.4. Monoaminergic depletion

Serotoninergic depletion was induced by administration of *p*-chlorophenylalanine (Jequier et al., 1967). *p*-Chlorophenylalanine was suspended in 0.9% saline containing 1% Tween 80. The mice received two i.p. injections of *p*-chlorophenylalanine (300 mg/kg) at 24-h intervals and were killed 24 h after the second injection. Control mice received an injection of vehicle alone. The effect of *p*-chlorophenylalanine treatment on monoaminergic neurotransmission was assayed as described above.

Catecholaminergic depletion was obtained by administration of α-methyl-*para*-tyrosine at a dose of 125 mg/kg i.p. The effect of α-methyl-*para*-tyrosine on levels of monoamines and their metabolites was determined 4 h after α-methyl-*para*-tyrosine administration by HPLC with electrochemical detection (Mayer and Shoup, 1983).

2.5. Drugs

The following drugs were used (source within parentheses): (−)-scopolamine hydrobromide and (−)-scopolamine methylbromide (Merck, Darmstadt, Germany), mecamylamine hydrochloride, DSP4, DL-α-methyl-*para*-tyrosine hydrochloride, *p*-chlorophenylalanine (Sigma, St. Louis, MO, USA), hexamethonium dichloride (RBI, Illkirch, France). All drugs were dissolved in 0.9% NaCl (5 ml/kg for i.v. injection or 20 ml/kg for i.p. administration) except for *p*-chlorophenylalanine (1% Tween 80). The dosages are shown per kilogram body weight as the free base. TRH was injected i.v. 10 min before the concussion whereas other drugs were administered i.p. 40 min before head trauma except for *p*-chlorophenylalanine (24 h before), DSP4 (8 days before) and α-methyl-*para*-tyrosine (4 h before).

2.6. Histopathological examination

A total of 10 mice were used to define the gross brain tissue changes induced by the impact. A saline solution of 1% Evan's blue in 5% albumin was injected i.v. (5 ml/kg) before the production of injury to reveal any changes in the permeability of the blood–brain barrier. After concussion, the mice were killed, the brains were removed and cut in the frontal plane to evaluate any modification of the blood–brain barrier.

2.7. Statistical analysis

For concussive head injury, the values represent means ± S.E.M. or medians. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the non-parametric log-rank test (Lee and Desu, 1972). ED_{50} values were calculated by log probit analysis (Lichtfield and Wilcoxon, 1949).

Data (means ± S.D.) from experiments measuring monoamine brain concentrations were routinely analysed with Student's unpaired *t*-test.

3. Results

3.1. Histopathological examination

A few of the animals (30%) had a small amount of subarachnoid hemorrhage near the cortical surface at the point of impact. However, no skull fracture or gross damage of the brain tissue was noticed (i.e., brain contusions). Significant Evan's blue staining was observed in regions both near and remote from the primary site of impact in 20% of the animals.

3.2. Effect of TRH in concussive head injury in mice

Fig. 1 shows the values for DT time of consciousness after concussion with different doses of TRH. TRH, i.v. (10 min before concussion), induced a dose-dependent shortening of the duration of unconsciousness after concussion. TRH started to decrease significantly the duration of unconsciousness after concussion at a dose of 1 mg/kg i.v. The ED_{50} value (mg/kg i.v.), the dose required for 50% reduction of the duration of unconsciousness, was 3.2 (confidential interval: 1.7–5.9).

3.3. Monoamine brain concentrations

The effects of α-methyl-*para*-tyrosine, DSP4 and *p*-chlorophenylalanine on tissue concentrations of noradrenaline, dopamine, 3,4-dihydroxyphenylacetic acid, homovanilic acid, serotonin (5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA) are presented in Table 1. α-Methyl-*para*-tyrosine (125 mg/kg i.p.) provoked a significant reduction of catecholamine brain concentrations 4 h post-injection. Dopamine, 3,4-dihydroxyphenylacetic acid and homovanilic acid concentrations were decreased by −55.3% ($P < 0.001$), −74% ($P < 0.001$) and −61.8% ($P < 0.001$), respectively. Noradrenaline concentrations were decreased by −41.9% ($P < 0.001$). Only a small decrease in 5-HIAA, −18.1% ($P < 0.05$), was observed after α-methyl-*para*-tyrosine administration. DSP4 (50 mg/kg i.p.) induced a significant reduction (−44.7%; $P < 0.01$) of noradrenaline brain concentrations 8 days post-injection. This treatment also moderately reduced the levels of 5-HIAA (−15.5%; $P < 0.05$). *p*-Chlorophenylalanine (2 × 300 mg/kg i.p. at 24 h intervals) provoked a massive and selective depletion of indolamine concentrations (5-HT: −61.6%; $P < 0.001$ and 5-HIAA = −79.7%; $P < 0.001$) 24 h after the last injection.

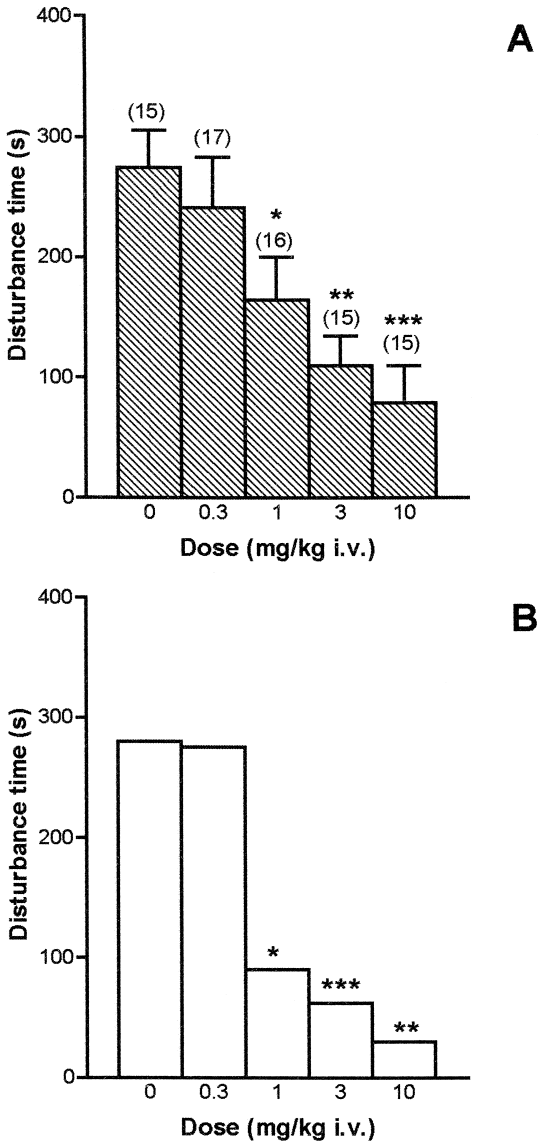

Fig. 1. Effect of TRH on the disturbance of consciousness induced by concussive head injury in mice. Each value represents the mean ± S.E.M. (A) or median (B) for the number of animals given in parentheses. DT time = time from head injury to the start of spontaneous movement. TRH was administered i.v. to mice 10 min prior to the head injury. Significantly different from the value for saline group: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (log rank test).

3.4. Effects of monoaminergic depletion on TRH-induced improvement of outcome in concussive head injury in mice

α-Methyl-*para*-tyrosine, DSP4 or *p*-chlorophenylalanine treatment, as described in the previous section, had no significant effects on the DT time of consciousness after concussion in mice (226 ± 55 vs. 219 ± 58 s; 263 ± 65 vs. 245 ± 70 s; 249 ± 62 vs. 289 ± 69 s, respectively, $n = 9–10$ animals/group). Moreover, pretreatment with α-methyl-*para*-tyrosine, DSP4 or *p*-chlorophenylalanine

Table 1
Changes in levels of monoamines and their metabolites in mouse brain after α-methyl-*para*-tyrosine (α-MPT), *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzamine (DSP4) or *p*-chlorophenylalamine (PCPA) treatment

Treatment	Monoamine brain concentrations (nmol/g net wt)					
	NA	DA	DOPAC	HVA	5-HT	5-HIAA
Vehicle ($n = 5$)	2.41 ± 0.25	9.57 ± 0.70	0.73 ± 0.07	1.31 ± 0.16	3.63 ± 0.22	1.21 ± 0.08
α-MPT ($n = 5$)	1.40 ± 0.12[c]	4.28 ± 0.22[c]	0.19 ± 0.03[c]	0.50 ± 0.03[c]	3.42 ± 0.30	0.99 ± 0.16[a]
Vehicle ($n = 10$)	2.46 ± 0.22	7.16 ± 0.43	0.59 ± 0.10	1.10 ± 0.14	3.61 ± 0.21	1.42 ± 0.20
DSP4 ($n = 11$)	1.36 ± 0.40[b]	7.37 ± 0.88	0.56 ± 0.08	0.99 ± 0.18	3.52 ± 0.26	1.20 ± 0.15[a]
Vehicle ($n = 9$)	2.35 ± 0.26	11.05 ± 0.83	0.80 ± 0.05	0.74 ± 0.10	4.27 ± 0.36	1.18 ± 0.10
PCPA ($n = 9$)	2.16 ± 0.21	10.85 ± 0.62	0.74 ± 0.10	0.74 ± 0.10	1.64 ± 0.21[c]	0.24 ± 0.05[c]

Values are means ± S.D. for the number of animals given in parentheses. Mice were killed 4 h after administration of α-MPT (125 mg/kg i.p.), 8 days after DSP4 administration (50 mg/kg i.p.), or 24 h after the last administration of PCPA (300 mg/kg i.p., once daily for 2 days).
NA = Noradrenaline; DA = Dopamine; DOPAC = 3,4-Dihydroxyphenylacetic acid; HVA = Homovanilic acid; 5-HT = Serotonin; 5-HIAA = 5-Hydroxyindolacetic acid.
Student's *t*-test was used for statistical evaluation: [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$.

failed to modify significantly the effects of TRH, 3 mg/kg i.v., on the DT time of consciousness in mice (approximately −50% in DT time) (105 ± 24 vs. 125 ± 26 s; 135 ± 33 vs. 138 ± 29 s; 113 ± 51 vs. 119 ± 31 s, respectively, $n = 9–10$ animals/group).

3.5. Effects of acetylcholine receptor antagonists on DT time induced by concussion in mice

The muscarinic receptor antagonists studied (i.e., scopolamine and methylscopolamine) had no significant effects on DT time induced by concussion (Table 2). In contrast, nicotinic receptor antagonists at high doses (30 mg/kg i.p. for mecamylamine and hexamethonium) induced a significant increase in DT time after concussion (mecamylamine: +113%; $P < 0.01$, and hexamethonium: +72%; $P < 0.05$).

Table 2
Effects of acetylcholinergic receptor antagonists on the disturbance of consciousness induced by concussive head injury in mice

Treatment	Dose (mg/kg i.p.)	DT time (s)	Effect (%)
Vehicle	–	256 ± 39 (10)	–
Scopolamine	0.3	252 ± 52 (10)	+2
	1	252 ± 60 (10)	+2
	3	182 ± 53 (10)	−29
Vehicle	–	235 ± 12 (9)	–
Methylscopolamine	1	226 ± 51 (8)	−4
	3	212 ± 27 (10)	−10
Vehicle	–	219 ± 46 (10)	–
Mecamylamine	1	236 ± 61 (8)	+8
	3	217 ± 52 (10)	−1
	10	260 ± 66 (10)	+19
	30	467 ± 76 (8)	+113[b]
Vehicle	–	259 ± 53 (10)	–
Hexamethonium	3	223 ± 53 (10)	−14
	10	298 ± 67 (10)	+15
	30	446 ± 76 (9)	+72[a]

Each value is the mean ± S.E.M. for the number of animals given in parentheses.
DT time = time from head injury to the start of spontaneous movement. All the drugs studied were administered i.p. 40 min before the start of concussion.
Significantly different compared with appropriate saline-treated controls: [a]$P < 0.05$, [b]$P < 0.01$.

3.6. Effects of acetylcholine receptor antagonists on TRH-induced improvement of DT time after concussion in mice

TRH at a dose of 3 mg/kg i.v. provoked a decrease in DT time of approximately 50% (Fig. 2). Pretreatment with

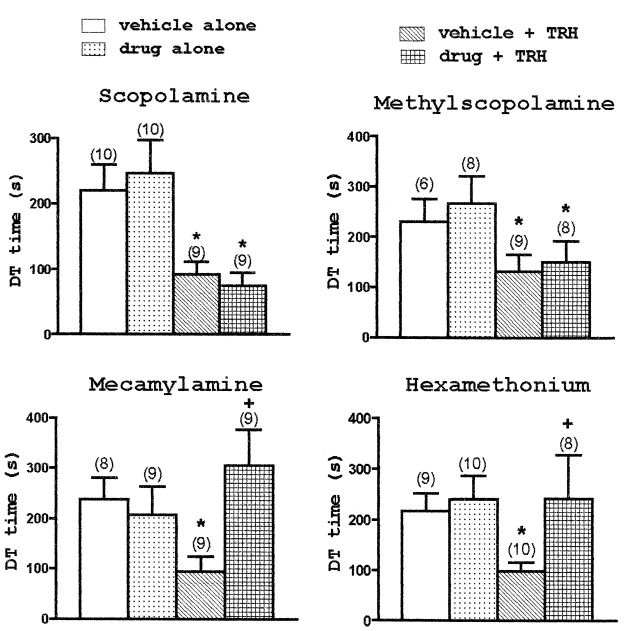

Fig. 2. Influence of acetylcholine receptor antagonists on TRH-induced improvement of outcome in concussive head injury in mice. Values are means ± S.E.M. for the number of animals given in parentheses. DT time = time from head injury to the start of spontaneous movement. Scopolamine, methylscopolamine, mecamylamine, hexamethonium or saline was administered i.p. 40 min before concussion. TRH (3 mg/kg) or saline was injected i.v. 10 min before concussion. Significantly different compared with appropriate saline-treated controls: * $P < 0.05$. Significantly different compared with appropriate TRH-treated controls: + $P < 0.05$.

scopolamine (1 mg/kg i.p.) or methylscopolamine (1 mg/kg i.p.) did not modify the effect of TRH on DT time after concussion. In contrast, pretreatment with low doses of mecamylamine (1 mg/kg i.p.) and hexamethonium (3 mg/kg i.p.) abolished the effect of TRH on DT time after concussion. Pretreatment with a high dose of hexamethonium (10 mg/kg i.p.) which had no significant effect on DT time after concussion, induced a large increase in DT time after concussion in TRH-treated mice (+265% vs. TRH-treated mice: $P < 0.01$; +112% vs. saline control group: $P < 0.05$).

4. Discussion

This study showed that TRH is remarkably potent and effective to promote recovery from unconsciousness in mice after concussive head injury, presumably due to a direct action of TRH on the central nervous system. A single intravenous dose of 3 mg/kg significantly improved recovery from post-injury unconsciousness. Our results with TRH are consistent with those of previous studies with rodents (Yamamoto and Shimizu, 1987; Manaka and Sano, 1978; Matsushita et al., 1993).

Many authors have reported that concussive head trauma disturbs the central dopaminergic and noradrenergic systems in discrete brain areas (Osterholm and Matthews, 1972; Marshall et al., 1978; Huger and Patrick, 1979; Kmieciak-Kolada et al., 1987). Some authors have, therefore, suggested that TRH-induced recovery from the loss of consciousness in concussion could be mediated by catecholaminergic systems (Miyamoto et al., 1982; Yamamoto and Shimizu, 1987). In the present study, a large decrease of catecholamine brain concentrations induced by DSP4 or α-methyl-*para*-tyrosine treatment had no significant effect on the outcome after concussion and failed to antagonize the effects of TRH on recovery from unconsciousness after head trauma in mice. This result does not suggest an implication of the dopaminergic and/or noradrenergic systems in the action of TRH, although the possibility cannot be excluded that a 50–60% depletion may have been insufficient to overcome inherent compensatory mechanisms. However, in our hands, a single i.p. injection of DSP4 produced 44.7% depletion of the noradrenaline concentration in whole brain, leaving dopaminergic and serotoninergic neurons apparently unaffected as previously described for rodents (Jaim-Etcheverry and Zieher, 1980; Jonsson et al., 1981). It should be pointed out that, for a similar range of whole brain noradrenaline depletion, it has been shown that, regarding discrete brain areas, DSP4 produces almost total depletion of noradrenaline in cortex and hippocampus with lesser noradrenaline depletion in hypothalamus and midbrain (Dooley et al., 1983; Introini et al., 1984).

In the case of α-methyl-*para*-tyrosine-induced depletion, the large depletion of dopaminergic neuronal pathways indicates that functional integrity of these systems is not necessary for the loss of concussion and is not involved in the action of TRH in concussion. Similarly, a massive and selective depletion of indolamine brain concentrations by *p*-chlorophenylalanine had no significant effect on the DT time of consciousness after concussion in mice and failed to modify significantly the action of TRH. This result showed that central serotoninergic transmission is not involved in the loss of consciousness after head trauma in mice. The present results thus imply that TRH-induced shortening of unconsciousness after concussion in mice is not associated with the well-known TRH-induced increase in activity of noradrenergic, dopaminergic and serotoninergic neurons in the brain (Yarbrough, 1976, 1979, 1983; Horita et al., 1986).

The analeptic action of TRH is considered to be mediated mainly by activation of cholinergic neurons (Horita et al., 1976; Miyamoto et al., 1982). Involvement of central cholinergic systems in the antagonistic effect of TRH on pentobarbital- and ethanol-induced sleeping time is widely accepted (Breese et al., 1975; Cott et al., 1976). It has been shown that TRH reduces pentobarbital-sleeping time in rodents in a dose-dependent manner (Miyamoto et al., 1982; Yamamoto and Shimizu, 1989) and attenuates the pentobarbital-induced reduction of high-affinity choline uptake (Schmidt, 1977). Moreover, pretreatment with atropine antagonizes the ability of TRH to reduce sleep, induced by pentobarbital in mice, whereas pretreatment with haloperidol or phentolamine is ineffective (Yamamoto and Shimizu, 1989). Pentobarbital-induced sleep and concussive head trauma disturb the central cholinergic systems (Santori et al., 1981; Miyamoto et al., 1982). In the present study, muscarinic receptor antagonists were without effect on DT time of consciousness after concussion in mice and failed to modify the action of TRH. Since the TRH-induced improvement of recovery after concussion was not eliminated by moderate doses of scopolamine and methylscopolamine, the possibility of a relationship between enhancement of cholinergic mechanisms via muscarinic receptors and the analeptic properties of TRH in concussion can be excluded. In contrast, nicotinic receptor antagonists increase the DT time after concussion in mice and reverse the action of TRH. The increase in DT time of consciousness with high doses (30 mg/kg i.p.) of mecamylamine could be linked to its antagonistic properties at the NMDA receptor complex (McDonough and Shih, 1995). However, it should be pointed out that the antagonism of the TRH-induced shortening of DT time after concussion in mice, by a low dose (1 mg/kg i.p.) of mecamylamine, could not be attributed to NMDA activity (McDonough and Shih, 1995). Our results seem to suggest potentiation of the behavioral effect of mecamylamine and hexamethonium by TRH. Hexamethonium was active in our experiments probably due to blood–brain barrier disruption as indicated by significant Evans' blue staining of various cerebral areas after concussion in some of the

mice. This finding is consistent with the definition of the cerebral concussion which is a transient disturbance of brain function followed by permeability disorders of brain capillaries without permanent anatomical changes (Denny-Brown, 1961; Bakay et al., 1977). Moreover, it should be pointed out that, in the rat, even after a relatively limited impact, which was accompanied by a short period of unconsciousness (4–10 min), there was a clear increase in regional cerebral permeability 2 h after concussion, particularly in septal area, frontal cortex, olfactory bulb and tubercule (Goldman et al., 1991). To date, no direct action of TRH at the acetylcholinergic receptors has been demonstrated. Our results suggest that the mechanism of action of TRH in concussion includes presynaptic and/or postsynaptic interactions with cholinergic neurons via nicotinic receptors.

Presynaptically, TRH has been shown to facilitate cholinergic transmission by stimulating the turnover rate and release of acetylcholine from various brain areas (Schmidt, 1977; Giovannini et al., 1991). Postsynaptically, TRH increases the excitatory effects of acetylcholine on cerebral cortical neurons (though this action has not been observed by all investigators) (Winokur and Beckman, 1978), and directly excites cholinergic septohippocampal neurons (Lamour et al., 1985). A number of studies have shown that activation of the cholinergic pathways by TRH is mainly mediated via muscarinic acetylcholine receptors (Yarbrough, 1976, 1983; Horita et al., 1986). However, Miyamoto et al. (1982) have reported an antipentobarbital action of TRH mediated mainly via nicotinic mechanisms in the hypothalamus and partly via non-cholinergic neural mechanisms in other regions. At present, our results are the first evidence of specific mediation of cholinergic activation by TRH via nicotinic mechanisms. The lack of action of muscarinic receptors in our experiments is consistent with previously published reports that activation of nicotinic and muscarinic mechanisms can exert opposite or different functional roles within the same cholinergic system (Dilsaver et al., 1991; Abdulla et al., 1994). Oxotremorine may mobilize or activate a nicotinic mechanism which counteracts its muscarinic effects (Dilsaver et al., 1991). Westfall (1973) reported that nicotinic and muscarinic receptor agonists trigger and inhibit the release of catecholamines in the hypothalamus, respectively. This opposite effect in the hypothalamus is of great interest since the antagonistic action of TRH on pentobarbital anaesthesia has been shown to be mainly produced by activation of the posterior hypothalamic area (Miyamoto et al., 1982).

In conclusion, our results indicate that the analeptic properties (accelerated recovery from disturbed consciousness) of TRH after head trauma in mice do not involve the monoaminergic systems. In this regard, Mushiroi et al. (1996) have demonstrated that in pentobarbital-anaesthetized rats, the analeptic effect of a TRH analog was blocked by neither atropine nor prazosin, but was blocked when both were given together, suggesting a multitransmitter mechanism. However, it is possible that the TRH effects in drug-induced vs. concussion-induced loss of consciousness have very different mechanisms. Current evidence supports the hypothesis that the analeptic properties of TRH after concussive head trauma in mice are mediated by central cholinergic mechanisms via nicotinic receptors. However, the possibility that other neurotransmitters, i.e., γ-aminobutyric acid (GABA), excitatory amino acids or opioids within the brain could be involved in this response, cannot be excluded as a cerebrovascular action of TRH (Shrewsbury-Gee et al., 1998).

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