全身麻酔における意識消失メカニズムの電気生理学的研究

（研究課題番号　１４３７０４８５）

平成14年度～平成16年度科学研究費補助金

（基盤研究(B)(2)）

研究成果報告書

平成17年3月

研究代表者　竹之下　眞

（滋賀医科大学医学部助教授）

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はしがき

麻酔薬と抑制性シナプス電位 (IPSP/IPSC)

全身麻酔の作用機序は未だ確定していないが、シナプス伝達、特に GABA 系抑制性シナプス伝達に及ぼす作用が注目されている。静脈麻酔薬、ガス麻酔薬、揮発性麻酔薬にわたる広範な麻酔薬により IPSP/IPSC 振幅の変化や持続 ・減衰(decay)の延長が報告されている。

意識と thalamo-cortical oscillation (40Hz リズム)

近年、30-100Hz の視床 - 大脳皮質間 や海馬 - 大脳皮質間に GABA 作動性相互抑制性の共鳴振動 (thalamo-cortical oscillation、別名 40Hz リズム)が発見され、認知や意識との関連が示唆されている (Llinas 1993)。この thalamo-cortical oscillation は覚醒時や REM 睡眠時に自発的に発生し、また麻酔によりその共鳴振動周波数が減少する (Munglani 1993)。イソフルレンによる IPSC 減衰 (decay) の延長と thalamo-cortical oscillation 周波数の減少が報告されている (Antkowiak 1997)。すなわち、麻酔薬による IPSP/IPSC の持続 (duration)・減衰(decay)延長が thalamo-cortical oscillation 周波数の減少を介して意識消失を来す可能性がある。

本研究の目的は、全身麻酔薬の抑制性シナプス伝達 (IPSP/IPSC) に及ぼす作用、特に IPSP/IPSC の持続や減衰(decay)に及ぼす作用に焦点を当てて、全身麻酔における意識消失のメカニズムを究明しようと試みた。

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平成16年度	1,100,000	0	1,100,000
総計	14,000,000	0	14,000,000

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研究 I

吸入麻酔薬による視床部 LD 部位神経細胞の GABA 性抑制電流の変化に及ぼす麻酔拮抗薬の効果

実験方法

ラット (17-20 days) をハロセン麻酔下に断頭後直ちに、全脳をシャーベット状の Cutting Solution (Sucrose (204), KCl (2.5), D-Glucose (10), NaH_2PO_4 (1.25), Na Pyruvate (2), myo-Inositol (3), Na Ascolvate (0.5), $MgCl_2$ (4), $CaCl_2$ (1) (単位 mM) (Osm. = 310 m mol kg^{-1})) 中でブロック状に切り出した。続いてマイクロスライサー (DKT-1500 (DOSAKA)) を用い、シャーベット状の Cutting Solution 中で厚さ 250 ・m のスライスを作成した。得られた脳スライスは、Ian Solution (NaCl (125), KCl (2.5), D-Glucose (10), NaH_2PO_4 (1.25), Na Pyruvate (2), myo-Inositol (3), Na Ascolvate (0.5), $MgCl_2$ (1), $CaCl_2$ (2) (単位 mM) (Osm. = 310 m mol kg^{-1})) 中で、温度 36℃ で 1 時間溶液を環流しながらインキベートした。この脳スライスを環流式のチャンバーに移した後、混合ガス (O_2 (95 %), CO_2 (5 %)) バブリングした Ian Solution を環流しながら、顕微鏡下にガラス電極 (ピペット内液 (CsCl (135), NaCl (4), EGTA (10), HEPES (10), Mg ATP (2), Na_3 ATP (0.3), Na_2 Creatine Phosphate (8.8)) (単位 mM)で視床の LD GABA 神経細胞をパッチした。ホールセルの状態にした後、吸入麻酔薬 (Halothan (0.5 %)) を含む混合ガスでバブリングした細胞外液 (CNQX (5), AP-5 (20), Strychnine (0.5), DMSO (1.4 ~ 4.2) (単位 μ M)を含む Ian Solution) を環流しながら、スチュムレーター (MASTER-8- Vp (A.M.P.I)) を用いて刺激した(15 ~ 25 V, 0.2msec, every 5 sec)。得られた信号は、増幅器 (AXOPATCH 200B (Axon Instruments)) で増幅した後、デジタル変換器 (DEGIDATA 1322A (Axon Instruments)) を経由してコンピューターに取り込んだ。吸入麻酔薬を含む細胞外液を 5-15 min 環流して抑制系電流のシグナルが安定したのを確認した後、吸入麻酔薬を含む混合ガスでバブリングした拮抗薬 (Myrystate in DMSO) を含む細胞外液細胞外液 (CNQX (5), AP-5 (20), Strychnine (0.5), Myrystate (10 , 30) DMSO (1.4 , 4.2) in Ian Solution) (単位 μ M) を、刺激に対する応答が安定するまで 10 min 環流した。その後、吸入麻酔薬含有細胞外液で 15 分間 Wash out した。

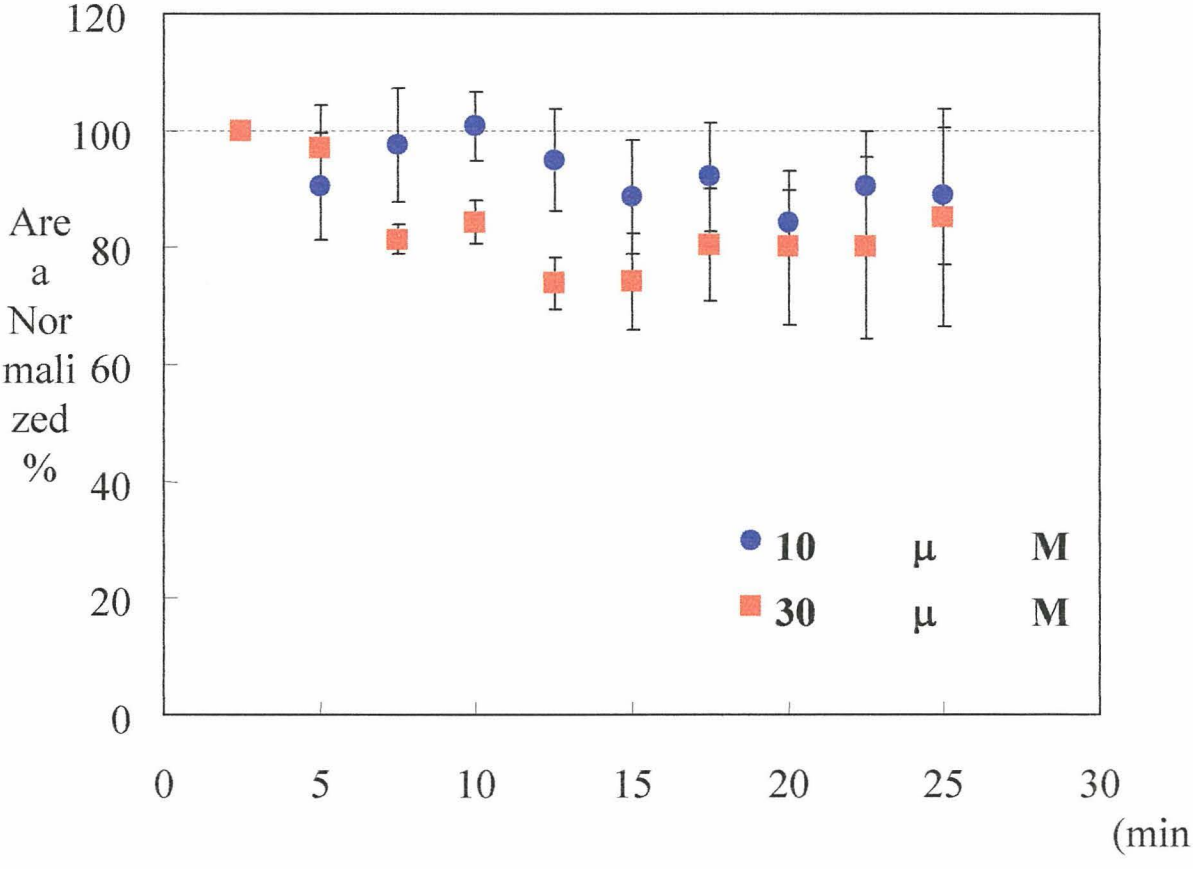
吸入麻酔薬 (Halothan) の存在下における拮抗薬 (Doxaprm in Water) の効果を調べる

ため、Myrystate と同様の実験を行った。

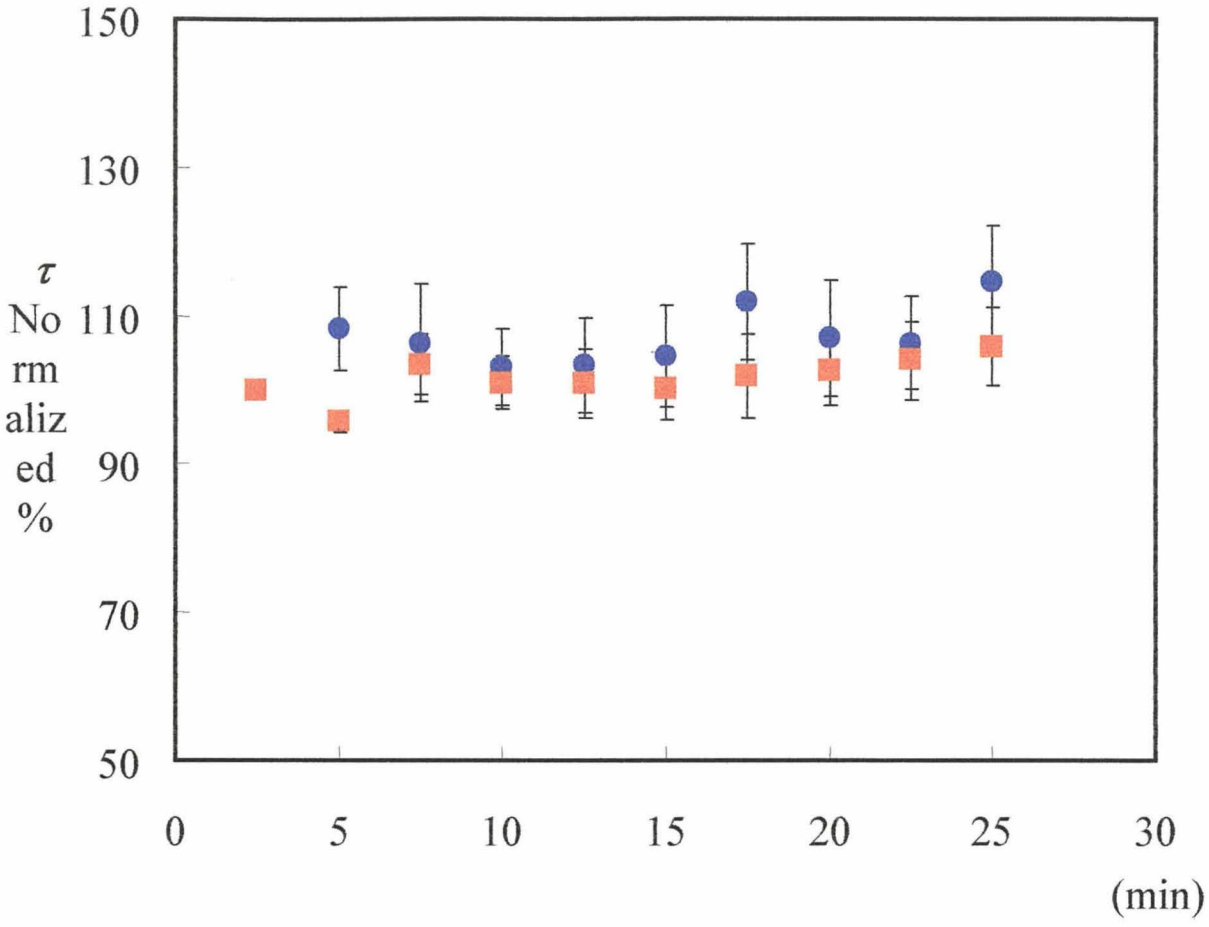
さらに、吸入麻酔剤（Sevoflurane（0.5 %））存在下における拮抗薬（Doxaprm in Water）の効果を調べるため同様の実験を行った。

結果

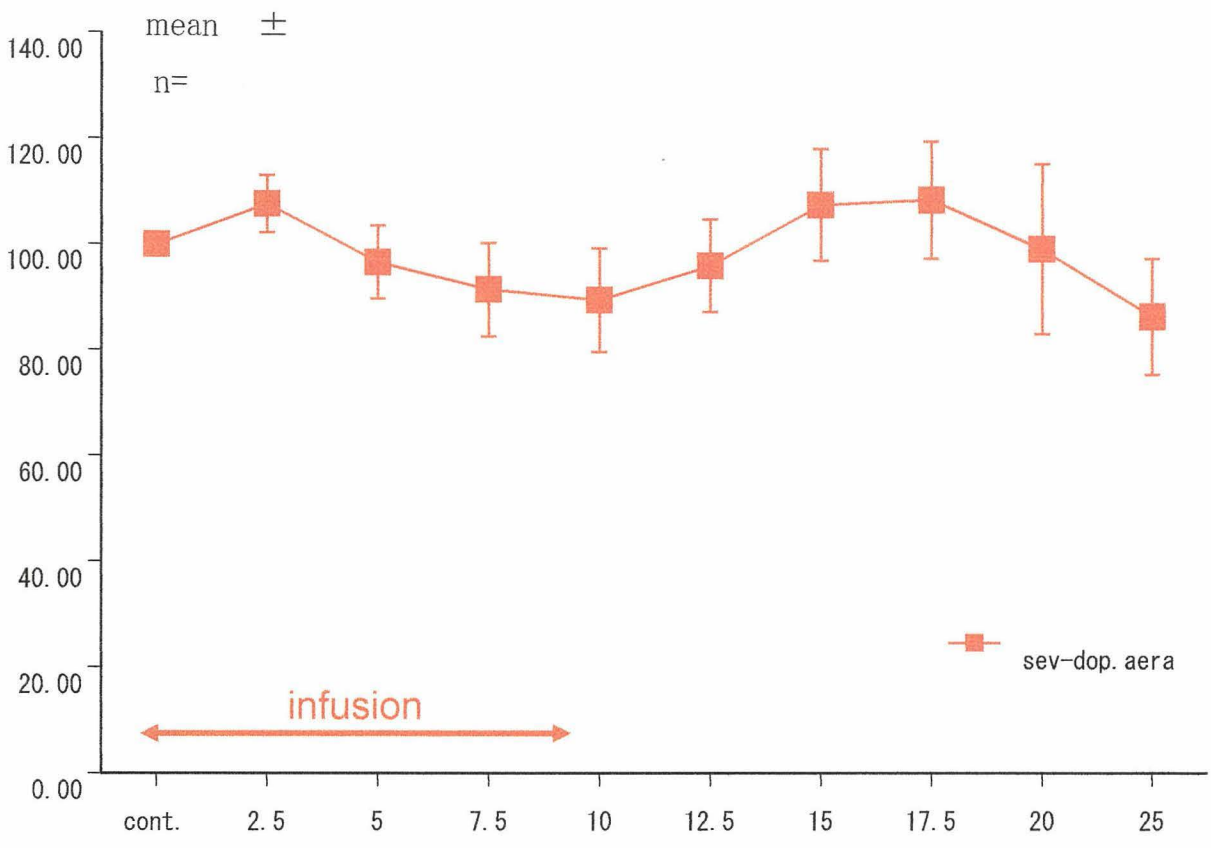
(1) 0.5 % ハロセンにより増強した GABA 電流を麻酔拮抗薬ミリスチン酸は減少させる傾向を示した。以下の図は誘発性 GABA 電流と基線との間の面積の相対変化を示したものである。0-15分間のミリスチン酸投与により面積は減少する傾向を示し、15-30 分間の wash-out により元に戻る傾向を示した。



(2) 0.5 % ハロセンにより延長した GABA 電流の時定数 τ はミリスチン酸による影響を受けなかった。下図は 0-15分間のミリスチン酸投与と、15-30 分間の wash-out を示す。

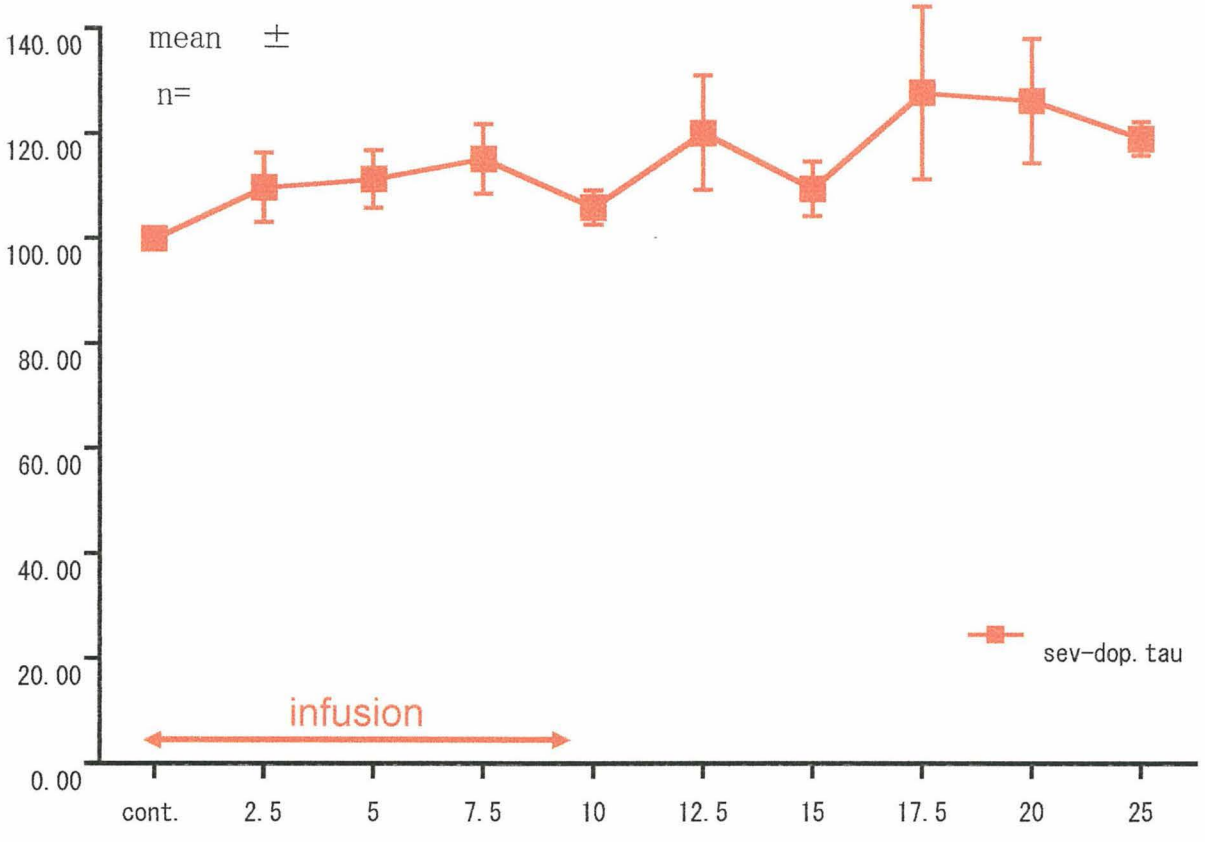


(3) 0.5 % セボフルレンにより増強した GABA 電流を麻酔拮抗薬ドキサプラムは減少させる傾向を示した。以下の図は誘発性 GABA 電流と基線との間の面積の相対変化を示したものである。0-15分間のドキサプラム投与により面積は減少する傾向を示し、15-30 分間の wash-out により元に戻る傾向を示した。



(4) 0.5 % セボフルレンにより延長した GABA 電流の時定数 τ はドキサプラムによる影響を受けなかった。下図は 0-15分間のドキサプラム投与と、15-30 分間の wash-out を示す。

sev-dop タウ


mean ±
n=
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
infusion
sev-dop.tau
cont.
2.5
5
7.5
10
12.5
15
17.5
20
25

研究 II

麻酔拮抗薬の研究の一環として重水とTRHによる影響を調べた。

いかに別刷りを添付する

Title

TRH REDUCES HALOTHANE-INDUCED SLEEPING TIME IN RATS

Running Title

TRH REDUCES HALOTHANE SLEEPING TIME

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Keywords

TRH, halothane, sleeping time

Abstract

Background and objective: The analeptic effect of TRH (protirelin) on narcosis induced by pentobarbital has long been recognized. But only a few works have been reported on halothane anesthesia, and the results are conflicting. The aim of the present study is to confirm the effect of TRH on the sleeping time of halothane anesthesia.

Method: Twenty 4-week-old rats were equally divided into 4 groups; two of them were saline control groups and the rest were TRH groups (5, 10 mg/kg). After 15 min of 2.5 % halothane exposure, saline or TRH were intra-peritoneally injected. Then another 15 min was allowed for halothane anesthesia. Anesthesia sleeping time was measured from the point of halothane removal to the point when the rat started to escape with the upper limbs.

Results: Sleeping times were reduced by TRH. The reductions were by 27 % (5 mg/kg group, from 670 to 488 sec) and 53 % (10 mg/kg group, from 662 to 308 sec), and only the latter was significant.

Conclusions: TRH reduced the sleeping time of halothane anesthesia in rats.

Introduction

The analeptic effect of TRH (Thyrotropin Releasing Hormone) on narcosis induced by pentobarbital has long been recognized. The sleeping time was shortened by TRH in a dose-dependent manner [1-8]. But another study demonstrated no shortening effect. Fewer works have been done about inhalation anesthetics. On halothane anesthesia, conflicting results were reported. Smith et al [9] observed that TRH had no effect on the sleeping time of halothane-induced anesthesia in rabbits, whereas Kruse et al [10] demonstrated that TRH shortened halothane's sleeping time in mice. In the present study, we examined the effect of TRH on halothane's sleeping time in rats.

Methods

Four-week-old male SD rats were used. Rats were housed in cages at room temperature about 20-25 °C with water and food ad libitum. All experiments were done during 16:00-19:00 period, and were in compliance with international guidelines for humane care of experimental animals. Adequate measures were taken to minimize pain or discomfort of rats according to the experimental regulations of Osaka University Medical School, Osaka, Japan. Twenty rats were equally divided into 4 groups; two of which were saline control groups and the rest were TRH groups (5, 10 mg/kg). Control saline groups were not pooled and each drug group had its own saline control group, because experiment dates were different. At a time, five rats were put in an anesthetizing box with warming pad (37 °C) and fan in it. About 10 L/min of oxygen was fed to the box through a halothane vaporizer, which was set to 2.5 %. After 15 min of halothane exposure, intra-peritoneal (ip) injections of saline or drugs were made with the volume of 1 ml per 100 g of body weight. Then another 15 min was allowed for halothane anesthesia after the injection. Rats were transferred form the anesthetizing box onto a warming pad (37 °C) in the room air. The body of the rat was touched by hand every

~10 seconds. Anesthesia sleeping time was measured from the point of transfer to the point when the rat started to escape with the upper limbs. In many cases, they started moving spontaneously in between the hand touches.

Drugs: TRH (Protirelin tartrate, Takeda Pharmaceutical Co. Ltd., Osaka, Japan) was diluted in saline.

Statistical analysis; Data were expressed as mean ± SD, and were analyzed using unpaired t-test. $P < 0.05$ was considered significant.

Results

Sleeping times were reduced by TRH. The reduction was from 670 ± 348 sec to 488 ± 282 sec in 5 mg/kg group, and from 662 ± 196 sec to 308 ± 85 sec in 10 mg/kg group, and only the latter was significant.

Discussion

In the present study, we confirmed the analeptic effect of TRH against halothane anesthesia. Smith et al [9] reported that pre-treated TRH (25 μg, intra cerebro-ventricular injection) had no effect on the sleeping time of 1.5 % halothane in rabbits, whereas Kruse et al [10] reported that TRH injected subcutaneously just after the termination of 1.2 % halothane inhaled for 60-90 min shortened sleeping time in mice. They observed significant reduction by 33%, 55% and 63% with 1, 5 and 10 mg/kg TRH, respectively. In our experiment, 5 mg/kg TRH insignificantly reduced the sleeping time by 27%, and 10 mg/kg TRH significantly reduced it by 53%. These discrepancies may be due to species difference, or may be due to the timing of TRH injection, in which rather short duration of action of TRH might have played some role. Smith et al did not describe the exact injection time. Kruse et al injected TRH just before the termination of anesthesia, while we injected TRH 15 min before the termination of anesthesia.

Miyamoto et al [5] reported a short duration of action of intravenously administered TRH. They showed that pre-treatment of TRH significantly reduced pentobarbital-induced sleeping time only when TRH was administered within 15 min before pentobarbital, and 30 min earlier injection of TRH had no significant effect. So in our experiment, the effect of 5 mg/kg TRH might have been reduced at the time when anesthesia was terminated.

The mechanism of the antagonistic effect of TRH on halothane was not investigated in the present study. For the sleeping time of pentobarbital, cholinergic mechanisms were postulated to be involved for the antagonistic effect of TRH, because atropine pretreatment significantly reduced the analeptic action of the drug[8,11]. The stimulatory effect of TRH on respiration and circulation has long been recognized[12]. Although analeptic effect of TRH on intravenous anesthesia was reported to be independent from its effect on respiration[13], analeptic effect on inhalation anesthesia should be profoundly influenced by TRH's stimulatory effect on respitration and circulation. In the present study, we confirmed the overall arousing effect of TRH on halothane anesthesia.

In conclusion, TRH (ip) reduced the sleeping time of halothane in rats.

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