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Thyrotropin Releasing Hormone: Antagonism of Pentobarbital Narcosis in the Monkey

GARY W KRAEMER

*Primate Laboratory and Wisconsin Psychiatric Research Institute
University of Wisconsin, Madison WI 53706*

ROBERT MUELLER

*Departments of Psychiatry, Pharmacology and Anesthesiology
University of North Carolina School of Medicine and the
Biological Sciences Research Center, Chapel Hill NC 27514*

GEORGE R BREESE

*Departments of Psychiatry and Pharmacology, University of North Carolina
School of Medicine and the Biological Sciences Research Center
Chapel Hill NC 27514*

ARTHUR J PRANGE

*Department of Psychiatry, University of North Carolina School of Medicine
and the Biological Sciences Research Center, Chapel Hill NC 27514*

JONATHAN K LEWIS

*Departments of Psychiatry and Psychology and Primate Laboratory
University of Wisconsin, Madison WI 53706*

HELEN MORRISON

*Department of Psychiatry, University of Wisconsin Medical School
Madison WI 53706*

WILLIAM T MCKINNEY, JR.

*Department of Psychiatry and Wisconsin Psychiatric Research Institute
University of Wisconsin Medical School, Madison WI 53706*

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KRAEMER, G. W., R. MUELLER, G R. BREESE, A. J PRANGE, J K. LEWIS, H MORRISON AND W. T. MCKINNEY, JR. *Thyrotropin releasing hormone antagonism of pentobarbital narcosis in the monkey* PHARMAC BIOCHEM. BEHAV 4(6) 709–712, 1976. – The effect of TRH on pentobarbital narcosis in 12 rhesus monkeys was examined. Vital signs monitored included respiration rate, heart rate, temperature, sleeping time, and time of reappearance of certain reflexes Blood samples were obtained for pentobarbital assay. Two dose schedules for TRH administration were used. One group of 6 animals received a single dose of 20 mg/kg 30 min after barbiturate administration, while the other group received 3 injections of 20 mg/kg spaced at 30, 40 and 50 min after injection of pentobarbital Both groups were sex balanced TRH administration resulted in dramatically increased respiration and heart rates and arrested the progress of barbiturate induced hypothermia The extended dose schedule prolonged increased respiration rate and a differential effect of TRH on pentobarbital induced hypothermia across sexes was observed. All animals regained reflexes sooner and sleeping time was reduced by 22%. No differences in pentobarbital blood levels with TRH were observed These results extend earlier work in rodents to primates and suggest a possible use of TRH in cases of acute barbiturate intoxication

Thyrotropin releasing hormone Barbiturate antagonist Pentobarbital

[1] Address reprint requests to Dr McKinney

PREVIOUS studies have shown that thyrotropin releasing hormone (TRH) has effects on the central nervous system which are distinct from its function as a releaser of thyrotropin and prolactin from the anterior pituitary [4, 6, 7] Plotnikoff and associates [9] reported that TRH potentiated L-DOPA induced excitation in mice following pargyline pretreatment. Recently, TRH was found to decrease the sedation and hypothermia produced by pentobarbital [1, 3, 10] and by ethanol [1, 2, 5] in rodents. Such results have suggested a possible therapeutic role for TRH in cases of acute intoxication by these depressants The purpose of the present study was to determine whether barbiturate depression of physiological mechanisms in primates including those concerned with respiration, heart rate, and reflexes might be altered by TRH.

METHOD

Twelve rhesus monkeys comprising 2 groups of 3 males and 3 females, ranging between 2–4 years of age with a group average of 3 25 years, were used in this study Animals in the first group had received barbiturates previously but not within a month of the present study The second group had never received barbiturates Each animal was food deprived for 18 hr and then treated with an intravenous dose of 26.5 mg/kg of pentobarbital Vital signs including respiration rate, heart rate, body temperature, and reflexes were monitored. Body temperature was recorded every 5 min using a thermister rectal probe (Yellow Springs Inst.). Respiration rate, the electrocardiogram (ECG) and the electroencephalogram (EEG) from the frontal, temporal, and occipital lobes were recorded on a Grass polygraph. Needle skin electrodes were used for the ECG and the EEG. A Beckman pressure transducer was used for respiration All drug and placebo solutions were given through an intravenous catheter with a 20 millimicron millipore filter set in the inferior saphenous vein Three 5 ml blood samples were drawn for serum pentobarbital samples at 20, 40, and 60 min following barbiturate injection. Room temperature was maintained at 22° C The times at which a pain reflex to ear pinch, blink reflex to corneal stimulation, spontaneous body movement, and head lift occurred after pentobarbital treatment were noted.

The first group received a single dose of 20 mg/kg of TRH (Pyro GLU-HIS-PRO-NH_2, Abbott Laboratories, North Chicago, Ill.) in saline vehicle 30 min following pentobarbital injection. The second group received 3 injections of 20 mg/kg of TRH spaced at 30, 40, and 50 min following pentobarbital injection Subsequently, each group received identical treatment with injections of the saline vehicle alone an average of 2 weeks after the first condition

Analyses of variance were used to determine the significance of effects due to TRH vs control, dose level, sex and time blocks for vital signs. Time block effects were evaluated with and without the initial 30 min control period. Similar analyses were performed on the data for reflexes and pentobarbital levels.

RESULTS

Analyses of the data for respirations and heart rate showed a significant main effect of TRH ($F(1,18) = 23\ 74$, $p<0.005$, $F(1,8) = 41.69$, $p<0\ 001$) with increases in both measures. A drug × time blocks interaction was significant ($F(13,104) = 18.11$, $p<0.001$, resp., $F(13,104) = 6.08$, $p<0.001$ heart) with both signs increasing after TRH but showing a downward trend in the control condition (Figs 1, 2) The data for body temperature showed significant first order interactions of TRH × time blocks ($F(13,104) = 34.93$, $p<0\ 001$) (Fig 3) and sex × time blocks ($F(13,104) = 2\ 81$, $p<0.005$) with a second order interaction of TRH × sex × time blocks ($F(13,104) = 5.48$, $p<0.001$). Males showed a sharper decline in temperature than females. TRH arrested the decline in temperature within 10 min of administration in all females and in the males receiving a single dose of TRH but did not have this effect in the males receiving 3 doses of TRH (Fig. 4). Thus, out of 12 animals 3 males did not show an arrest of pentobarbital induced hypothermia following TRH.

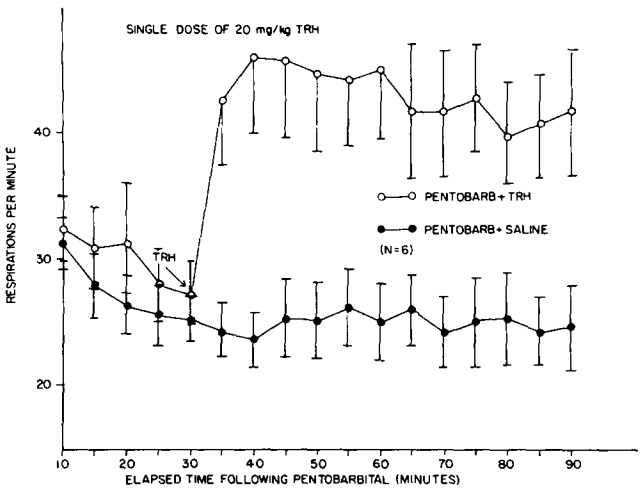

FIG. 1 Effect of 20 mg/kg of thyrotropin releasing hormone (TRH) on respiration rate when given 30 min after 26 5 mg/kg pentobarbital (respirations per min ± SEM).

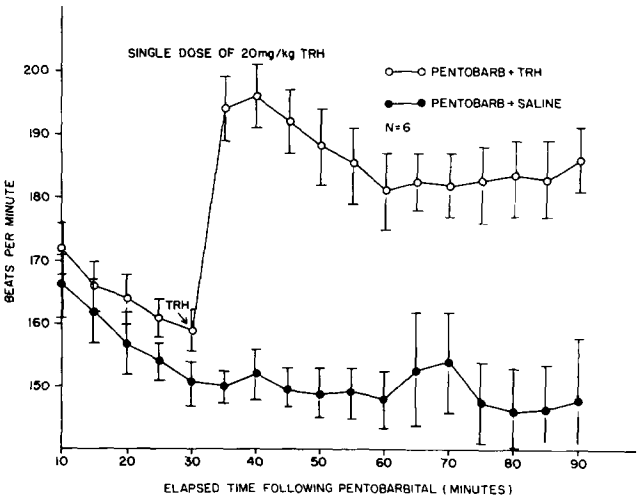

FIG 2 Effect of 20 mg/kg of thyrotropin releasing hormone (TRH) on heart rate when given 30 min after 26.5 mg/kg pentobarbital (beats per min ± SEM)

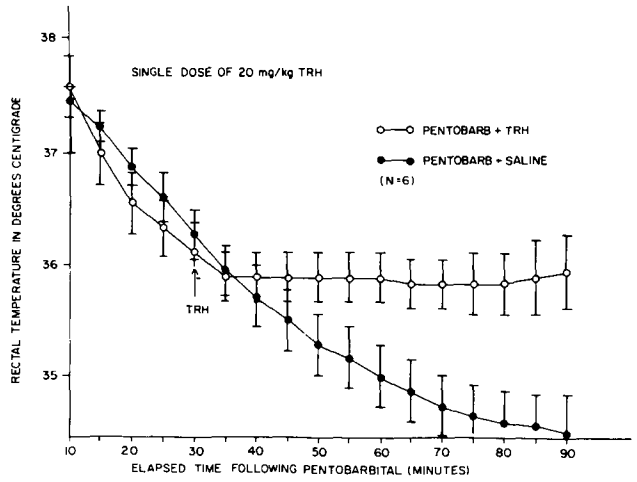

FIG 3 Effect of 20 mg/kg of thyrotropin releasing hormone (TRH) on rectal temperature when given 30 min after 26 5 mg/kg pentobarbital (degrees centigrade ± SEM)

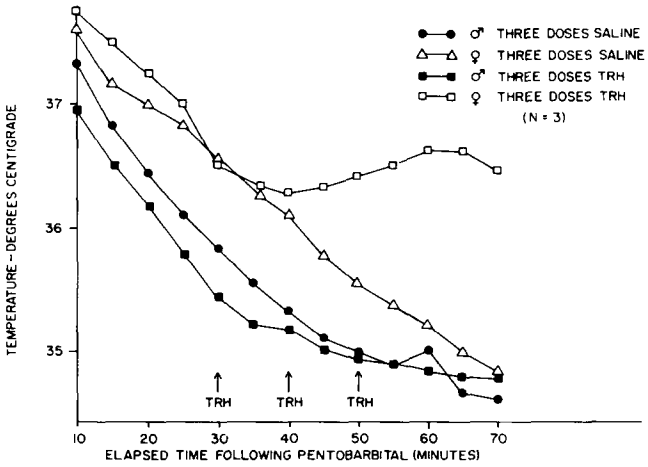

FIG 4 Differential effect of thyrotropin releasing hormone (TRH) on pentobarbital induced hypothermia in male (N = 3) and female (N = 3) rhesus monkeys receiving three doses of 20 mg/kg TRH 30 min after 26 5 mg/kg pentobarbital

Analysis of the data without the initial control block yielded the same results for main effects. However, a second order interaction between TRH dosage level and time blocks was significant in that repeated doses of TRH maintained or slightly increased high respiration rate while one dose caused an increase in respirations which then declined over time ($F(8,64) = 3\ 22$, $p<0.005$ (Fig 5). No other effects of repeated vs. single dose schedules were found.

Head lift, pinch and corneal reflexes occurred significantly sooner in TRH treated animals ($F(1,18) = 14.02$, $p<0\ 01$, head lift, 8.44, $p<0.025$, pinch; 12 70, $p<0\ 01$, corneal reflex) (Fig 6). However, time of spontaneous movement was not reduced significantly and no interactions of these measures with sex or drug dosage were significant. A whole body tremor resulting from fine muscle fasciculations which occurred within 10 sec of injection of TRH made evaluation of the EEG from skin electrodes

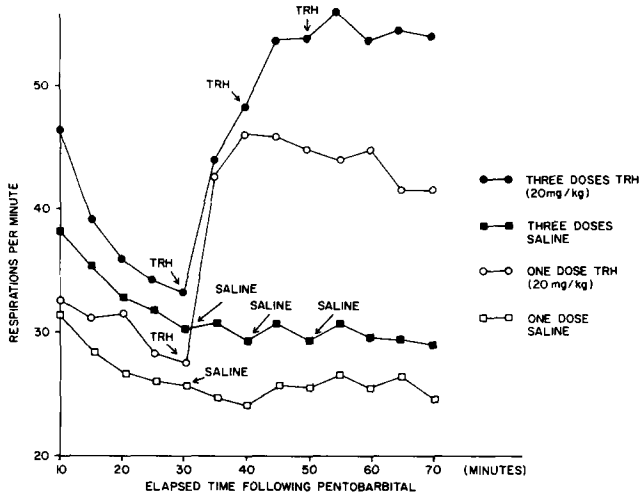

FIG. 5 Effect of repeated vs single dose of 20 mg/kg of thyrotropin releasing hormone (TRH) on respiration rate when given 30 min after 26 5 mg/kg pentobarbital (N = 6 for each condition)

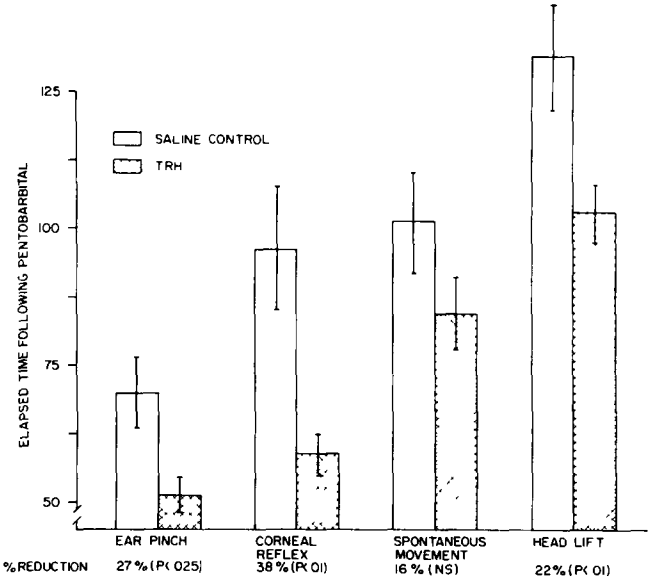

FIG 6. Effect of thyrotropin releasing hormone (TRH) on time of reappearance of reflexes (single and multiple dose schedules combined, N = 12)

impossible No adverse effects of the TRH treatments were observed TRH did not affect serum levels of pentobarbital (Fig. 7).

DISCUSSION

These results confirm previous work in rodents showing that TRH administration reduces pentobarbital sleeping time and hypothermia [1, 3, 10] and extend these findings to show that TRH has pronounced effects on respiration rate, heart rate and basic reflexes in pentobarbital intoxicated monkeys. The antagonistic effects of TRH on pentobarbital could not be attributed to changes in pentobarbital metabolism as serum levels of pentobarbital were unaffected by TRH.

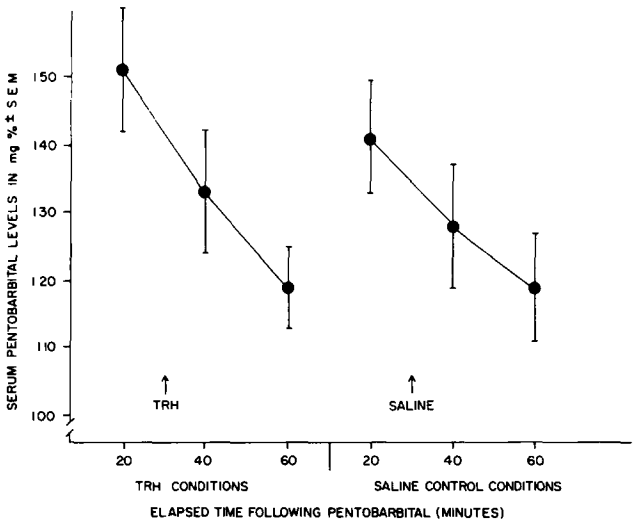

FIG 7. Serum pentobarbital levels in rhesus monkeys receiving thyrotropin releasing hormone (TRH 20–60 mg/kg) or saline beginning 30 min after injection of 26.5 mg/kg pentobarbital (N = 12)

The onset of action of TRH marked by whole body muscle fasciculations occurred within 10 sec of intravenous administration. Respirations and heart rate increased attaining maximum values within 5–10 min following a single dose. The duration of effect is interesting in view of the short half life of TRH in plasma [8].

Repeated doses of 20 mg/kg did not improve upon results obtained with one dose with the exception that respiration rate increased and maintained maximum levels over a longer time period. Absence of a dose response relationship as previously reported for sleep time and hypothermia in mice [3] with these and other measures in monkeys may be due to the maximal response obtained with the initial dose of 20 mg/kg Further studies with a lower dose schedule may establish a dose response relationship

Arrest of pentobarbital induced hypothermia was not observed in 3 male animals Differences in rate of temperature decline and barbiturate experience may account for persisting hypothermia in these males. However, these animals displayed all of the other features of the TRH response suggesting that the effects of TRH on other measures including respirations, heart rate, and sleeping time are independent of the effect on temperature regulation

The results of the present study suggest a possible therapeutic use of TRH in the treatment of barbiturate overdose

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