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Thyrotropin-Releasing Hormone: Physiological Concomitants of Behavioral Excitation

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ANDRY, D. K. AND A. HORITA. *Thyrotropin-releasing hormone: physiological concomitants of behavioral excitation.* PHARMAC. BIOCHEM. BEHAV. 6(1) 55–59, 1977. – Two doses (10 μg and 100 μg) of thyrotropin-releasing hormone (TRH) or saline were injected intraventricularly in rabbits pretreated with either saline, pentobarbital or phenobarbital. Behavior, EEG patterns, respiration rate and heart rate were monitored for 100 min posttreatment. TRH significantly altered all physiological indices except heart rate in both barbiturate- and saline-pretreated animals. The results support the contention that TRH modifies central functioning by direct action. Results are discussed in terms of barbiturate antagonism and excitatory effects of TRH.

TRH Pentobarbital Phenobarbital Behavior EEG Barbiturate antagonism

THYROTROPIN-RELEASING hormone (TRH) has been postulated to have direct action on central nervous system (CNS) functioning, in addition to its role in the pituitary-thyroid axis. The hypothesized neurotropic action of TRH has received support from studies demonstrating that TRH potentiates the behavioral effects of DOPA in pargyline-pretreated animals [9,10]. This effect of TRH appears to be independent of its action in the pituitary-thyroid system, since the potentiation occurs in thyroidectomized and hypophysectomized animals [9,10]. The CNS stimulant effect of TRH is further indicated by the increased motor activity and hyperthermia seen after its administration in rabbits [6]. These indices are not antagonized by most adrenergic or serotonergic blockers, amine depletors or depressant drugs.

Although much evidence supports the postulated neurotropic action of TRH, the underlying systems and mechanisms mediating this action are unknown. Consistent findings are not readily apparent regarding biogenic amine or cholinergic substrates [3, 6, 7, 8]. The wide distribution of TRH in brain [15], however, argues for its role in CNS functioning.

Probably one of the most potent effects of TRH is its reversal of alcohol [2] and barbiturate [3, 6, 11] depression in animals. The barbiturate antagonism by TRH extends to behavioral and temperature indices of pentobarbital and phenobarbital narcosis. Hypophysectomy does not decrease the effectiveness of TRH narcosis antagonism and the administration of L-triiodothyronine is ineffective [3,11], indicating a CNS action of TRH. The only depressant drug studied which is resistant to TRH is morphine [6]. Most other sedatives and depressants are behaviorally antagonized by TRH.

Antagonism of barbiturate narcosis by TRH has been observed in mice, rats and rabbits using several different routes of administration. However, behavioral and temperature indices have primarily been stressed in these studies. Other physiological concomitants of TRH narcosis reversal and TRH effects on normal CNS functioning have not been delineated. Thus, electroencephalographic (EEG), respiration rate and heart rate changes associated with the behavioral antagonism of barbiturates by TRH and effects of TRH by itself have not been described systematically.

The present study was designed to characterize the time course of several physiological measures of TRH narcosis reversal and to more adequately delineate the effects of TRH on control animals. The studies were conducted on rabbits using intracerebral-ventricular (ICV) injections of TRH in order to more closely parallel previous and current studies in our laboratory.

METHOD

Animals

Thirty (30) male New Zealand rabbits (Totem) weighing 2.0–2.3 kg at the start of the experiment were used. All animals were housed individually with free access to food and water. The animals were arbitrarily assigned to experimental treatment groups.

Apparatus

Animals were tested in an electrically-shielded chamber. A four-channel Grass (Mdl. 7) polygraph and inkwriter were used to amplify and record electrical potentials. The EEG activity of rabbits was differentially preamplified (Grass, Mdl.7P5B, a.c., x 4000) and actively filtered (1.0–75.0 Hz). Heart rate was measured by transthoracic electrodes led into a low level d.c. preamplifier (Grass, Mdl. 7P1A). Respiration rate was obtained by a water cuff connected to

a pressure transducer which led into a low level d.c. preamplifier (Grass, Mdl. 7P1A). Thus, all recordings were obtained simultaneously on separate polygraph channels.

Procedure

Rabbits were anesthetized with pentobarbital sodium (25 mg/kg) intravenously (IV) and their heads were shaved. All surgical instruments and electrodes were previously soaked in ethanol. A midsagittal incision was made through the scalp and the skull fascia were blunt dissected. A small (0.88 mm) burr hole was drilled through the skull over the lateral ventricle (1.0 mm lateral to midline and 1.0 mm rostral to bregma) and a cannula made of stainless steel tubing (25 ga) embedded in a Plexiglas disc was lowered 12 mm. Placement was confirmed by backflow of cerebrospinal fluid. The cannula was cemented in place with dental acrylic and a cap affixed.

Three more burr holes (1.2 mm) were drilled (one 2 mm lateral to midline and 10 mm rostral to bregma and two 2 mm lateral to midline and 5 mm rostral to bregma on opposite sides of midline). Stainless steel screws (2/56) with male Amphenol pins previously soldered were self-tapped to the underlying meninges. The entire assembly was cemented to the skull with dental acrylic. Wound closures were effected with wound clips and animals were allowed to recover for at least one week before experimentation.

For testing, animals were given IV pretreatments of either saline (N = 10), 25 mg/kg pentobarbital sodium (N = 10) dissolved in saline or 100 mg/kg phenobarbital sodium (N = 10) dissolved in saline. Saline rabbits had been adapted to the chamber for 2–3 days earlier. A ribbon cable, counter-weighted, with female Amphenol pins was secured to the electrodes with the most rostral one serving as animal ground. Stainless steel wound clips soldered to input leads were attached transthoracically. For saline animals, the thorax was locally anesthetized with Efocaine (0.5 cc, intramuscular) prior to wound clip penetration. The water cuff was wrapped around the rabbits and joined to the pressure transducer.

Thirty min after pretreatments, baseline control records of behavior, EEG, respiration and heart rate were taken. Each pretreatment group was then divided into a saline (N = 2), 10 μg TRH (N = 4) dissolved in 10 μl saline and 100 μg TRH (N = 4) dissolved in 10 μl saline treatment groups. Saline or TRH was administered ICV by a syringe microburet (Micro-Metric) in a total volume of 10 μl. Animals were were continuously monitored with one min sample records taken every five min for 100 min posttreatment observation periods. Behavior was concurrently noted.

Behavioral and EEG records were scaled for all pretreatment groups, based on pilot studies demonstrating that in rabbits increasing doses of barbiturates produce characteristic behavioral signs and EEG patterns. Scales for saline-pretreated animals were similarly obtained, using adaptation time in apparatus as the index. Ratings for EEG patterns in saline animals were analagous to those observed for barbiturate rabbits and the same EEG scales were used for both. Figure 1 and Table 1 represent the scales used for EEG and behavioral measures. Respiration and heart rates were recorded as frequency per min. The data from each animal were normalized by calculating percent change from pretreatment control levels. These scores were averaged for each treatment group for illustration purposes. Individual animal scores were subjected to statistical analysis using Kruskal-Wallis and Mann-Whitney tests for scaled data and appropriate analyses of variance and Duncan Multiple Range Test for nonscaled data [14]. Statistical significance was determined at the $p<0.05$ level or less.

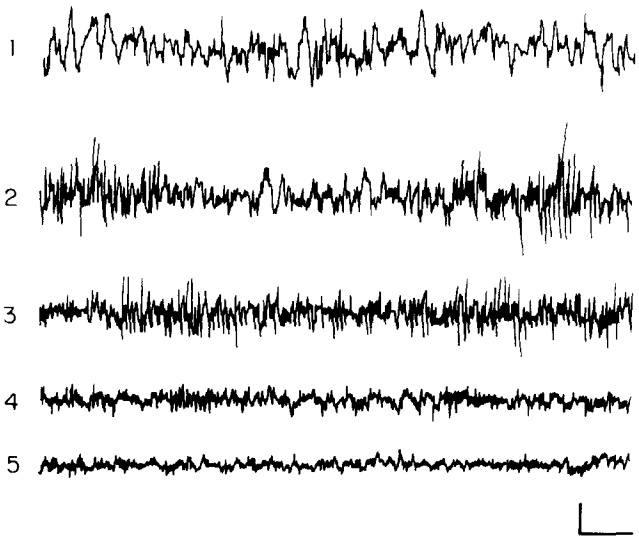

FIG. 1. Sample EEG records illustrating rating of EEG patterns. Traces from top to bottom represent decreasing depths of barbiturate narcosis or increasing EEG activation levels. Trace 5 depicts EEG of untreated, awake rabbits. Records were obtained from surface cortical electrodes differentially amplified. Samples were retraced. Calibration: 100 msec @ 100 μV.

TABLE 1

SCALES FOR ASSESSING TRH PROGRESSIVE EFFECTS ON BEHAVIOR

Drug Pretreatment	Rating	Description
Barbiturate	1	Narcosis, loss of righting
	2	Flexor head movements, nose twitching, sniffing
	3	Limb movements
	4	Attempting to right, scratching
	5	Regain righting, walking
Saline	1	No motor movement, neck flaccid, eyes closed
	2	Head erect, eyes open
	3	Head movements, sniffing
	4	Walking, gnawing, exploring
	5	Running, kicking, biting, chewing

RESULTS

The effects of 10 μg and 100 μg ICV doses of TRH on saline, pentobarbital and phenobarbital pretreated rabbits are presented in terms of behavioral, EEG pattern, respiration rate and heart rate changes from pretreatment control levels for each treatment group. Both the latency and percent change from pretreatment control levels are presented.

Saline pretreatments. Baseline recordings of rabbits receiving IV saline injections displayed resting, drowsy

behavioral and EEG levels (2.0 on the scale). Respiration rate was approximately 100 per min and heart rate averaged 250 per min before TRH or saline administration.

In saline-pretreated rabbits, ICV injections of TRH produced significant changes in behavior, EEG patterns and respiration rates as compared to ICV saline injections. Heart rates were not significantly affected by TRH or saline administration and are not presented in the figures.

Figure 2a depicts the behavioral changes produced by ICV injections of TRH and saline. Both doses of TRH resulted in significant increases in behavioral levels as compared to saline animals. The 100 μg dose of TRH produced significantly greater behavioral increments for a significantly longer period (70–75 min) than the lower TRH dose (20 min). The behavior displayed by 100 μg TRH animals could be described as thrashing, gnawing, greatly increased motor activity, such as running and kicking, and biting. The lower dose produced these same responses but for a shorter length of time and less intensely. The onset latency of behavioral changes was significantly shorter (10 min) for the higher dose than the lower TRH dose (20 min).

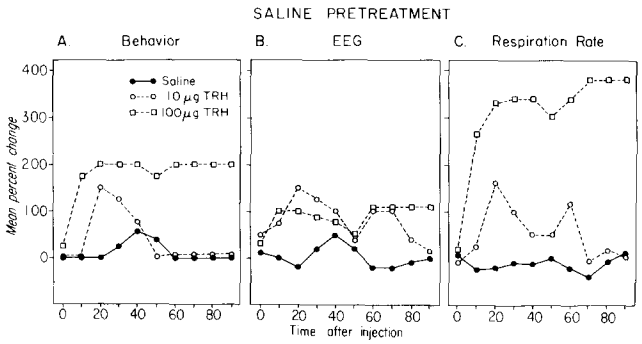

FIG. 2. Effects of TRH and saline on behavior, EEG patterns and respiration rates in saline-pretreated rabbits. Each point represents mean percent change from pretreatment control levels. Behavior and EEG patterns were scaled. Respiration rate was calculated as frequency per min.

As seen in Fig. 2b, the EEG pattern changes of TRH animals closely paralleled the behavioral changes produced by the drug. Both TRH groups showed EEG ratings significantly higher than those of saline animals. Both doses of TRH produced essentially the same EEG changes. However, onset latency for peak effect was significantly shorter for the higher dose (10 min) than the lower dose (20 min).

Figure 2c shows a distinct dose-related increase in respiration rates. Both doses of TRH resulted in significantly increased respiration rates compared to saline treatments, but 100 μg of TRH produced significantly larger respiration rate increases at significantly shorter latencies (10 min) than 10 μg (20 min).

Behavioral, EEG pattern and respiration rate changes produced by each dose of TRH in saline-pretreated animals followed very similar postinjection time courses. Onset latencies of the measured responses to TRH administration were also the same within groups. Thus, TRH affected all three physiological indices more or less simultaneously. The most definitive and obviously immediate effect of TRH, however, was on respiration rate.

Pentobarbital pretreatments. Rabbits pretreated with 25 mg/kg pentobarbital sodium IV showed behavioral and EEG levels of about 1.0 on the scale in Fig. 1. Respiration rate was slowed to about 40 per min and heart rate was maintained at 250 per min. Administration of TRH reversed behavioral, EEG patterns and respiration rates seen in the barbiturate state, but did not alter heart rates.

Figure 3a illustrates the behavioral changes induced by TRH and saline ICV administration in pentobarbital-pretreated rabbits. All animals regained righting reflex within the observation period. However, latencies of the groups were significantly different. Both doses of TRH produced behavioral changes significantly sooner after administration than saline animals. The 100 μg dose, furthermore, produced righting significantly sooner (50 min) than the 10 μg TRH dose (70 min). In fact, behavioral signs of pentobarbital reversal could be detected in the 100 μg TRH group within 10 min after injection and within 40 min for the lower dose group.

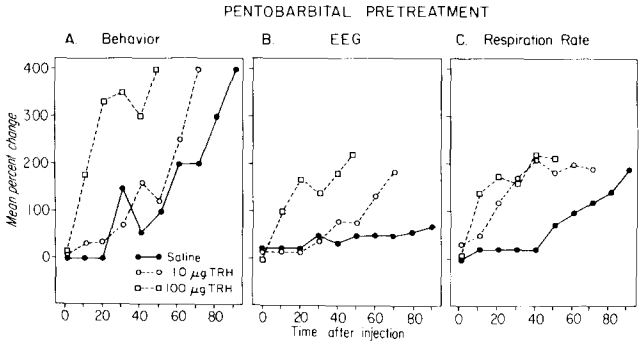

FIG. 3. Effects of TRH and saline treatments on three measures of excitation in rabbits pretreated with pentobarbital sodium (25 mg/kg). Each point represents mean percent change from pretreatment control levels. Behavior and EEG patterns were scaled and respiration rate was calculated as frequency per min.

Significant EEG pattern changes were associated with the TRH-induced behavioral alterations in pentobarbital-pretreated rabbits. Figure 3b depicts the EEG pattern changes of these animals. The modification of EEG patterns recorded from TRH animals occurred significantly sooner for 100 μg animals (10 min) as opposed to 40 min for the 10 μg TRH group, which was significantly sooner than the saline group (90 min). Furthermore, the overall percent change in EEG pattern levels was significantly different for the three groups. Thus, although all animals reached similar behavioral levels, the EEG patterns of TRH animals were significantly elevated over those of saline animals at the time of righting.

As seen in Figure 3c, respiration rate increases produced by the two doses of TRH and saline were of similar magnitude at the time of righting, although the two TRH groups showed significantly shorter latencies of respiration rate increases than the saline group. Both 10 μg and 100 μg TRH doses resulted in the same respiration rate increases over similar time courses. Increases in respiration rates were closely associated in time with behavioral and EEG pattern changes in all pentobarbital groups.

Phenobarbital pretreatments. Rabbits pretreated with phenobarbital (100 mg/kg, IV) showed essentially the same baseline levels as pentobarbital rabbits. Behavioral and EEG

levels were at 1.0, respiration rate was slowed to about 40 per min and heart rate was approximately 200 per min. As with pentobarbital, both doses of TRH significantly altered behavioral, EEG pattern and respiration rate indices, but not heart rate. In contrast to pentobarbital animals, however, changes produced by TRH in phenobarbital-pretreated animals were transient.

Animals administered saline ICV after phenobarbital did not regain righting reflex or show signs of behavioral awakening during the observation period (Fig. 4a). Both doses of TRH, however, did result in significantly increased behavioral levels. Furthermore, the higher TRH dose produced significantly greater increases than the lower dose. However, latency of peak behavioral changes did not differ between the two groups, although the 100 μg dose produced the same amount of behavioral change at significantly shorter latencies than the 10 μg TRH dose.

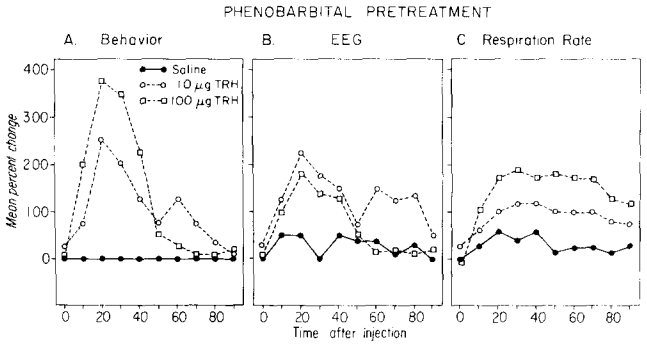

FIG. 4. Effects of TRH and saline on behavior, EEG patterns and respiration rates in phenobarbital-pretreated (100mg/kg) rabbits. Each point represents mean percent change from pretreatment control levels. Behavior and EEG patterns were scaled and respiration rate was measured as frequency per min.

Phenobarbital reversal was transient. Behavioral levels returned to pre-TRH control levels within about 60–70 min after TRH administration. There were no significant differences in total behavioral reversal between the two doses of TRH.

EEG pattern level changes produced by TRH and saline, depicted in Fig. 4b, closely followed behavioral signs. Although the two TRH groups showed significantly higher EEG pattern levels than saline, there were no significant differences between the 10 μg and 100 μg TRH doses in peak effect. However, the duration of TRH effects on EEG patterns was dose-related. The lower dose produced changes in EEG patterns over significantly longer time periods than the higher dose. Similar to behavior, TRH effects on EEG patterns were transient and returned to control levels within 60 min for the higher TRH dose and 90 min for the 10 μg group.

Figure 4c illustrates respiration rate changes produced by ICV injection of TRH and saline in phenobarbital-pretreated rabbits. Both doses of TRH resulted in significantly larger increases than saline. The larger TRH dose, furthermore, led to significantly higher increases than the lower dose at significantly shorter latencies. The most striking effect of TRH on respiration rate in phenobarbital animals was the persistent rate elevations. At times when behavioral and EEG measures had returned to control levels, respiration rates were still significantly elevated. Throughout the observation period, TRH significantly increased respiration rates in a dose-related manner.

DISCUSSION

The results demonstrate that TRH is capable of significantly antagonizing several physiological indices of pentobarbital and phenobarbital narcosis, as well as producing behavioral, EEG and respiration rate excitation in saline control animals. The results not only lend further support for a central action of TRH, but indicate that its actions are not specifically linked with narcotic states. TRH produces profound effects on normal CNS functioning as well.

The TRH reversal of behavior, EEG patterns and respiration rates induced by barbiturates extends previous reports [3, 6, 11] of TRH reversal of behavior and hypothermia following barbiturate administration. Thus, TRH seems to lead to rather comprehensive antagonism of barbiturate narcosis. The lack of TRH effects on heart rates may be due to the fact that barbiturized animals had heart rates similar to control animals.

Changes in behavioral and EEG indices of TRH-induced excitation were closely parallel in time within experimental groups. However, the relationship between EEG pattern modifications and behavior was not correlated between treatment groups. For instance, in pentobarbital-pretreated rabbits at the time of righting, EEG patterns were significantly elevated in a dose-related manner. Thus, TRH produced effects on EEG patterns over and above those seen in saline animals at similar behavioral levels. This was also apparent for phenobarbital- and saline-pretreated animals. Thus, although temporally correlated, behavior and EEG could be dissociated on the basis of between-group comparisons. A particular behavioral level was not strictly associated with specific EEG patterns. This suggests that EEG changes may be produced directly by TRH and are not necessarily a consequence of behavioral changes.

The relationship between behavior and respiration rate was also dissociable, as most clearly illustrated by phenobarbital- pretreated rabbits. The transient behavioral and EEG pattern changes produced by TRH were not reflected in respiration rates within or between treatment groups. Elevated respiration rates persisted even after behavioral and EEG patterns had returned to control levels. It would be difficult, therefore, to consider EEG and behavior as consequences of respiration rate changes. It also seems unlikely that respiration rate changes were the product of compensatory mechanisms related to behavior. Our results suggest the possibility that TRH has direct action leading to respiration rate increases which is separable from its other central actions.

Increased respiration rate was the most dramatic effect of TRH treatments in both saline- and barbiturate-pretreated animals. The mechanisms underlying TRH-induced tachypnea are not known. However, it has recently been demonstrated that tachypnea can be produced from TRH injections into various and diverse brain loci and is not dependent upon diffusion of TRH to medullary respiratory centers [4].

The difference between TRH effects on pentobarbital and phenobarbital animals is distinct. Most likely, the different pharmacological nature of these two barbiturates is at least partly responsible [5]. Thus, the different time courses and effective levels could alter the ability of TRH to antagonize these two barbiturates. More detailed studies, delineating the relationship between effective barbiturate

levels at the time of TRH administration, should provide a more concrete explanation of TRH transient antagonism of phenobarbital narcosis.

Previous investigators [1,7] have not found dramatic changes in spontaneous motor behavior of rats after intraperitoneal TRH. Our results, on the other hand, clearly demonstrate considerable behavioral excitation in rabbits administered TRH by the ICV route. Either species or route of administration differences may explain these disparate findings.

The elucidation of the physiological or pharmacological nature of TRH interactions with other drugs obviously must await further study. However, our results show that TRH by itself is capable of modifying physiological functioning. Consistent with our findings are reports that microiontophoretically applied TRH alters neuronal activity in several brain loci [14,15]. This evidence suggests that TRH reversal of barbiturate narcosis may be the result of TRH alterations of physiological systems antagonizing underlying central substrates mediating barbiturate-induced narcosis.

Our results support the contention that TRH produces CNS alterations. Our descriptive analysis of TRH effects on behavior, EEG patterns and respiration rates in saline and barbiturate animals suggest that TRH is a centrally acting agent with perhaps independent actions on several different central substrates. The hypothesis that TRH interacts with other drugs by modifying central physiological substrates was also supported. The strong potentiation of respiration rates by TRH argues for clinical evaluation of its possible analeptic properties. More mechanistic, as well as descriptive, analyses of TRH should provide a broader framework for deciphering its postulated central actions.

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