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Effects of TRH on the Central Nervous System of the Rabbit

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HORITA, A., MONSERRAT A. CARINO AND JOHN R. SMITH. *Effects of TRH on the central nervous system of the rabbit.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 111–116, 1976. – Thyrotropin releasing hormone (TRH) administered intraventricularly to rabbits produces tachypnea, hyperthermia, behavioral excitation and, with larger doses, compulsive scratching. These effects of TRH were unaffected by various catecholamine and serotonin antagonists or depleting agents. When TRH was administered to pentobarbital-narcotized animals, tachypnea and rapid recovery of the righting reflex occurred. The antagonism of narcosis or sedation was evident against other barbiturates, diazepam, chlorpromazine, and reserpine, but not against morphine. Morphine actually appeared to antagonize the excitatory actions of TRH. Scopolamine pretreatment prevented the arousal from pentobarbital narcosis, but not the tachypnea or hyperthermia. TRH represents a new class of psychoactive chemical which may play a role in brain function.

Tachypnea Hyperthermia Behavior Narcosis Arousal TRH

AMONG the many neuropeptides now known, TRH represents one of the most fascinating because of its ability to exert profound effects on the central nervous system. Interest in its neuropsychopharmacologic properties was triggered by the earlier reports of a possible antidepressant effect in man [16,19], although conflicting evidence [1,10] has subsequently appeared in the literature. Plotnikoff *et al.* [18] first demonstrated the potentiation by TRH of the pargyline + DOPA-induced excitation in mice. Other reports also indicated a possible relationship between TRH and norepinephrine turnover in brain [8, 14, 17], although Reigle *et al.* [20] found little or no change in norepinephrine metabolism in rats treated with TRH. Still other researchers found a possible relationship between the serotonergic system and TRH activity in the central nervous system [9].

A most interesting property of TRH is in its ability to shorten the sleeping time of rats and mice given alcohol, barbiturates and other depressant drugs [2, 3, 4]. It is clear that this effect is unrelated to the TSH-stimulating function of TRH and is central in origin. The mechanism of the arousal action of TRH has not been elucidated, but the possibility of a cholinergic component has been suggested [3].

Our work with TRH has been done almost exclusively in the rabbit. The effects of this hormone in the rabbit are in many ways similar to those produced by amphetamine [11]. Subsequently, we found that the pentobarbital-narcotized rabbit could be aroused with extremely small doses (0.1 μg) of TRH [12]. Thus, the rabbit represents a suitable animal for TRH studies in both the conscious and narcotized states. The present article reviews and extends some of our earlier findings on the central actions of TRH in the rabbit.

METHOD

Animals and Procedure

Male-New Zealand rabbits weighing between 2.3 - 2.7 kg were employed. All injections of TRH were made via the intracerebroventricular (ICV) route. The direct-puncture technique as described by Jacob *et al.* [15] was employed, but slightly different coordinates were utilized. One or two days prior to the experiment a midline incision was made in the head and a small hole (0.8 mm dia.) was drilled in the skull of animals anesthetized with pentobarbital (25 mg/kg IV). The hole was located 1 mm lateral to the midline and 1 mm rostral to the bregma. The incision was swabbed with antiseptic and closed. On the day of the experiment, just prior to the injections, local anesthetic was sprayed on the skin wound and the incision reopened. A No. 26 needle was inserted vertically to the depth of 12 mm from the surface of the skull. Proper positioning was indicated by the appearance of cerebrospinal fluid. The needle was withdrawn, filled with drug solution, and reinserted to the same depth. All injections were in a volume of 10 μl over a 30–60 sec period and were made with a syringe microburet. Controls consisted of animals given the same volume of sterile saline. All drug doses are expressed as their bases.

Behavior was assessed by gross observation of various parameters, including changes in motor activity, behavioral excitation, pupillary dilatation, etc. In addition, certain compulsive responses, such as the scratching behavior, were observed. In order to designate the intensity of such responses we employed an arbitrary point-scoring system as follows: 0 = normal, control animal; +1 = excitation, some scratching, increased movement, increased respiratory rate, increase in muscle tone; +3 = hyperactivity, intensification of all +2 symptoms, compulsive and intense scratching; +3

= marked depression or anesthesia, loss of righting reflex.

Colonic temperatures were measured by rectal thermistor probes connected to a YSI Scanning Telethermometer wired to a Leeds-Northrup Speedomax recorder. The experiments were carried out in a constant temperature room of 22.0 ± 1.0°C. Respiratory rates were measured with an impedance pneumograph and recorded on a Physiograph recorder (Narco Bio-systems, Houston, Texas). Arousal from narcosis was based on the time of recovery of the righting reflex from the depressant drugs.

RESULTS

Effects of TRH in the Conscious Rabbit

Given a dose range of 0.5–100 μg TRH produced tachypnea, hyperthermia, behavioral excitation and a compulsive scratching. The hyperthermia and respiratory effects were first seen with doses of 0.5–1.0 μg. Behavioral excitation, characterized by increased locomotor activity, piloerection, pupillary dilatation and further respiratory stimulation, was evident with 10–20 μg. Above the 20 μg dose all these effects were intensified, and, in addition, accompanied by frequent and vigorous scratching of the head and neck region by the hind feet. The most well defined dose-related response to TRH was the hyperthermia. A dose of 0.5 μg produced increases in rectal temperature of 0.2–0.5°C, increasing to about 1.5°C with 100 μg of TRH.

Interactions between TRH and Various Drugs in Conscious Rabbits

Because earlier reports indicated a possible role of the biogenic amines in some of the TRH effects we investigated the influence of catecholamine and serotonin antagonists and depleting agents on the actions of TRH in rabbits. Several depressant drugs were also tested as possible antagonists of the excitatory actions of TRH. As indicated in Table 1, the TRH-induced behavioral excitation and hyperthermia were only minimally affected by pretreatment of the animals with various blocking agents and amine depletors. Some of the antagonists or depletors, such as phenoxybenzamine, chlorpromazine, reserpine and α-methyl-p-tyrosine produced marked sedation, but were ineffective in blocking the TRH-induced excitation. In fact, TRH rapidly reversed sedation and produced tachypnea and hyperthermia.

Of those agents listed in Table 1 only two noticeably affected the normal responses of TRH. One was the α-adrenergic-blocking agent, phentolamine, which, when given ICV, delayed the onset of all effects of TRH. However, the antagonism was only transient, and after 10 min the usual TRH effects reappeared. The other agent which was effective against the TRH-induced behavioral excitation was morphine.

Other depressants, such as the barbiturates and diazepam, were ineffective as TRH antagonists. In fact, TRH acted as an antagonist of these depressants. Animals pretreated with most of these agents recovered from depression or regained their righting reflexes within 10–15 min after TRH administration. With phenobarbital the TRH-induced arousal from narcosis was temporary. The animals righted themselves within 10–15 min after TRH, but within 60 min thereafter they again fell into deep sleep for the long duration typical of this agent. Because ketamine and the gaseous anesthetics have short durations of action, it was difficult to determine shortening of their narcosis time. However, if TRH was given prior to these agents, it was possible to determine whether onset of

TABLE 1

DRUG INTERACTIONS WITH TRH IN CONSCIOUS RABBITS

	Dose	Pretreatment time (min)	N	Behavior	Temperature °C
TRH	100 μg	—	10	+3	1.6 ± 0.2
Phenoxybenzamine	5 mg/kg	90	5	+2	1.8 ± 0.2
Phentolamine	100 μg ICV	10	9	delayed, then +2	1.7 ± 0.3
Cyproheptadine	1 mg/kg IV	30	3	+3	1.6
Cinanserin	10 mg/kg IV	20	3	+3	1.4
p-Chlorophenylalanine	2 × 300 mg/kg IP	48, 24 hr	5	+3	1.5
6-OH Dopamine	2 × 150 μg ICV	48, 20 hr	3	+3	2.1 ± 0.3
α-Methyl-p-tyrosine	200 mg/kg IP	180	3	+3	1.7 ± 0.2
Pimozide	4 mg/kg IP	90	3	+3	1.7 ± 0.3
Haloperidol	1 mg/kg IV	30	5	+3	1.7 ± 0.2
Atropine	2.5 mg/kg IV	30	3	> +3	1.5 ± 0.2
Reserpine	2.5 mg/kg IV	3 hr	5	+2	3.0 ± 0.5
Propranolol	10 mg/kg IP	30	3	+3	1.5
Morphine	4 mg/kg IV	45	5	3	1.0 ± 0.2
Pentobarbital	25 mg/kg IV	30	9	delayed, then +2	1.2 ± 0.2
Phenobarbital	100 mg/kg IV	30	5	delayed, then +1 for 60 min, then 3	1.0
Diazepam	7.5 mg/kg IV	30	5	+3	1.8 ± 0.3
Chlorpromazine	7.5 mg/kg IV	30	3	+3	1.2 ± 0.2

Rabbits were pretreated with the various drugs at the indicated times and routes of administration. TRH (100 μg ICV) was then administered and the subsequent behaviors and colonic temperatures were recorded. Temperature changes are designated as °C sem above that base line that existed at the time of TRH administration.

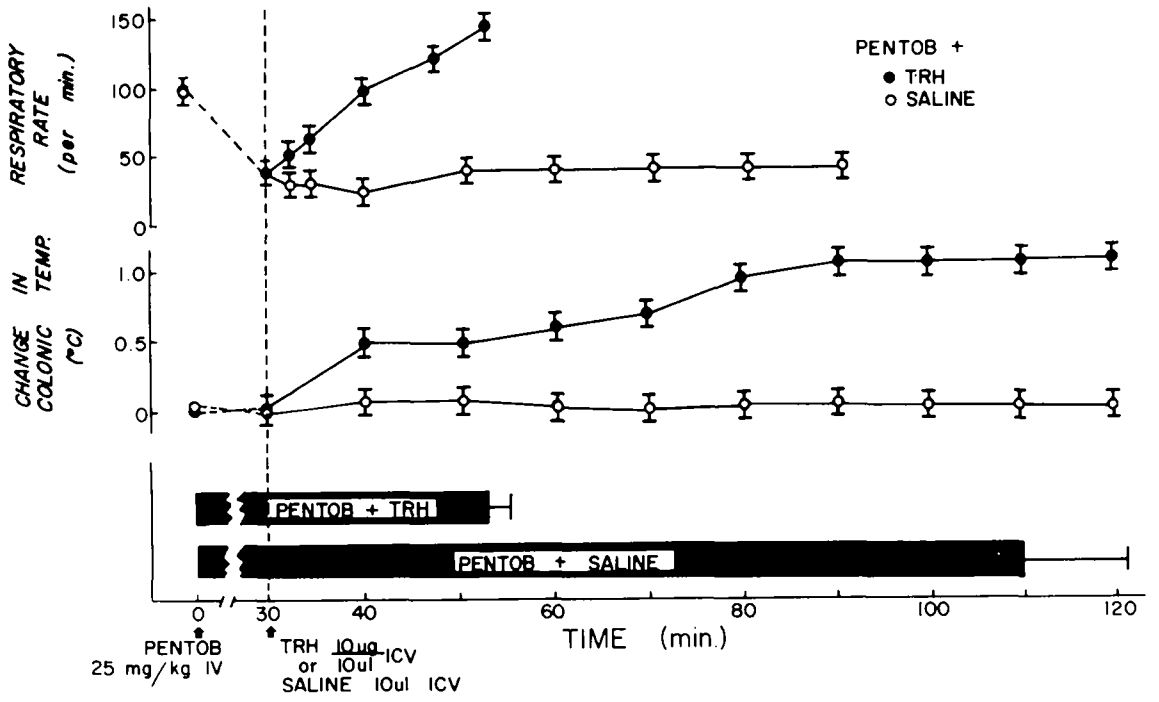

FIG. 1. The effect of TRH on changes in respiratory rate, colonic temperature and duration of narcosis produced by pentobarbital (25 mg/kg IV). Each line or bar represents mean responses in at least 5 animals. Variations are expressed as sem.

narcosis was delayed. Under this condition, TRH pretreatment lengthened the onset of ketamine narcosis but did not affect the induction time of halothane or methoxyflurane.

Effect of TRH in Pentobarbital- and Morphine-narcotized Rabbits

Pentobarbital (25 mg/kg IV) produced an immediate state of narcosis which lasted for 110 ± 12 min. When TRH (10 µg) was given 30 min after pentobarbital, the animals displayed a rapid increase in respiratory rate, hyperthermia and recovery of the righting reflex. Under these conditions the tachypnea and awakening responses were most dramatic (Fig. 1). Respiration rate was increased from 40 per min to 60 per min within 3 min after TRH administration and continued to increase beyond the time the animals regained their righting reflex. By the time TRH-treated animals had recovered from pentobarbital-induced narcosis (20–25 min after TRH), respiration rates had increased to 140 per min, as compared to 43 per min in pentobarbital-treated animals given saline ICV. Marked tachypnea continued for at least the following 2–3 hr. Hyperthermia was also present in these animals. This response to TRH also appeared rapidly, reached a peak of 1.0°C in 45–60 min, and gradually returned to base line over the following 2–3 hr.

Morphine (4 mg/kg IV) produced analgesia, marked sedation, hypothermia and respiratory depression. When given 30 min after morphine, TRH (100 µg) exerted no antagonism of the analgesia or sedation, but reversed the respiration and temperature responses (Fig. 2). Since the animals remained depressed, it was possible to follow respiratory changes during several hours. The morphine-induced hypothermia was also antagonized by TRH, and in 60–90 min the animals exhibited hyperthermia of 0.5°C above normal, after which they returned to their normal base-line temperatures, although sedation and analgesia still persisted.

Scopolamine-TRH Interactions in Conscious and Pentobarbital-Pretreated Rabbits

Breese *et al.* [3] demonstrated that anticholinergic drugs antagonized the awakening effect of TRH in pentobarbital-treated mice. We, therefore, determined whether scopolamine would modify the effects of TRH in conscious and pentobarbital-narcotized rabbits. In conscious animals scopolamine (30 µg ICV) produced slight excitation and hyperthermia in some animals whereas, in others, no changes were produced. No observable effects were present when 1 mg/kg was given IV. Upon administration of TRH to these animals a marked potentiation of behavioral excitation and hyperthermia was produced, and some animals succumbed from the extreme increases in body temperature.

As described above, TRH reduced the pentobarbital narcosis to about one-fifth of its normal duration. Scopolamine significantly ($p<0.05$) increased the duration of pentobarbital-induced narcosis (Table 2). However, in these animals TRH no longer exerted its awakening effect. The TRH-induced tachypnea and hyperthermia, however, were not antagonized in the scopolamine-pentobarbital pretreated animals. The hyperthermia was actually potentiated in that both peak intensity and duration of action were enhanced. All of these effects occurred while the animals were in the narcotized state. Several of these animals died during the subsequent 12 hr, presumably as a result of the prolonged hyperthermia.

DISCUSSION

TRH administered ICV to conscious rabbits produced a

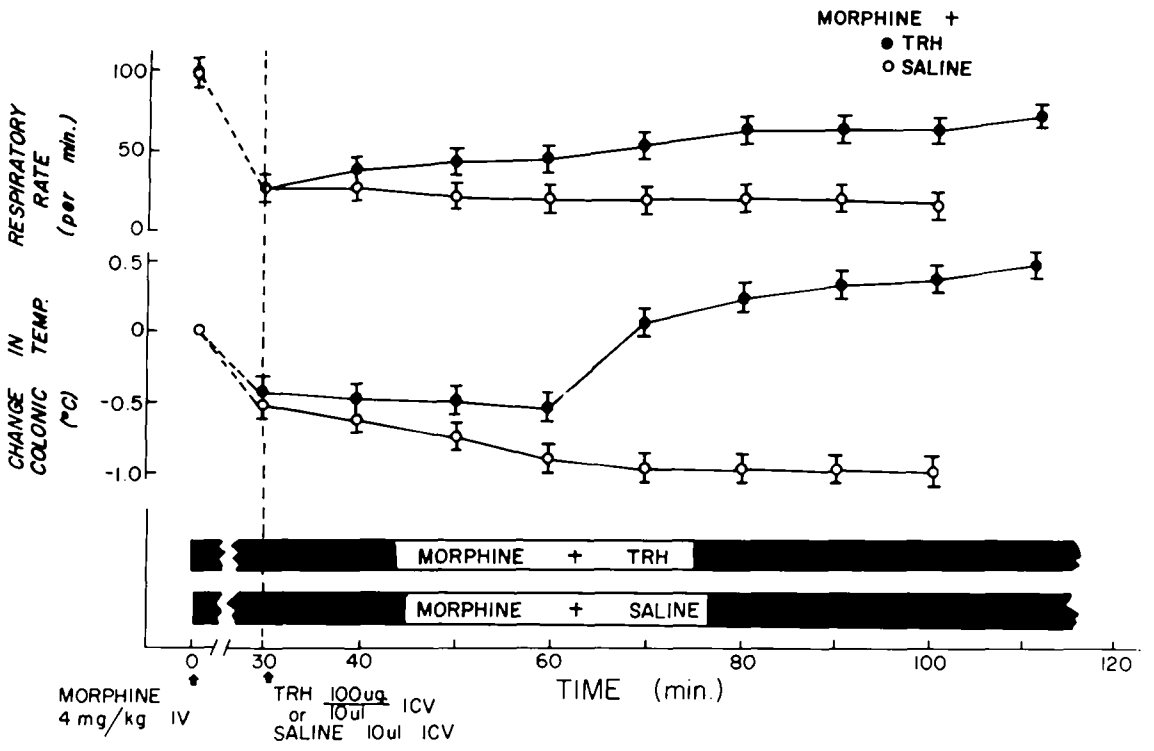

FIG. 2. The effect of TRH on changes in respiratory rate, colonic temperature and duration of narcosis produced by morphine (4 mg/kg IV). Each line or bar represents mean responses in at least 5 animals. Variations are expressed as sem.

TABLE 2

EFFECT OF SCOPOLAMINE ON THE TRH ANTAGONISM OF PENTOBARBITAL NARCOSIS

Drug Combination	Duration of Narcosis (min)*
Pentobarb + Saline	80 ± 9 (10)
Pentobarb + TRH	10 ± 5 (10)
Scopolamine + Pentobarb + Saline	111 ± 10 (5)
Scopolamine + Pentobarb + TRH	103 ± 14 (6)

*Time in min ± sem from the time of saline or TRH administration in pentobarbital-pretreated rabbits. Pentobarbital was given in a dose of 25 mg/kg IV. Scopolamine (30 μg) and TRH (25 μg) were administered ICV in a volume of 10 μl. A period of 30 min elapsed between the administration of each drug or saline. Figures in parentheses represent number of animals in each group.

dose-related stimulation of the central nervous system, primarily manifested by behavioral excitation, tachypnea, hyperthermia and, with higher doses, compulsive scratching. Some of these effects resemble those produced by amphetamine, but from antagonist studies, their mechanisms appear to be different. For instance, we find that the neuroleptics, such as chlorpromazine and haloperidol, block amphetamine-induced excitation and hyperthermia, but not those produced by TRH. Alpha-methyl-p-tyrosine and 6-hydroxydopamine (ICV), also antagonists of amphetamine, do not interfere with TRH. Of the many compounds we have thus far examined only phentolamine and morphine produce at least partial antagonism of the TRH-induced excitation. Because of its poor penetrability into the central nervous system, phentolamine injections were made directly into the lateral ventricles. Although its blocking action is transient, all effects of TRH are delayed for 10–15 min, after which TRH effects are evident. The phentolamine blockade of TRH is probably not associated with the α-adrenergic-blocking property of phentolamine, since phenoxybenzamine, a centrally-active α-antagonist, is ineffective against TRH. The phentolamine blockade of TRH may be similar to the antagonism of the cyclic AMP effect on barbiturate narcosis in rats [7].

By far the most intriguing aspect of this research is that of TRH antagonizing the narcosis produced by pentobarbital and other depressants, but not that produced by morphine. The arousal from pentobarbital is especially striking since animals regain their righting reflex within 10–15 min after TRH administration, while saline controls continue to sleep for a further 80 min. In earlier studies we found that large doses of TRH (50–100 μg) shortened the duration of pentobarbital narcosis to a few minutes. The lowest dose that produces an antipentobarbital effect is between 0.1–1.0 μg.

The fact that TRH exerts its awakening effect against a number of central nervous system depressants indicates that it is not a specific antagonist against a single group of structurally related agents, but rather is associated perhaps with the broader picture of wakefulness and narcosis. Cohn [6], who has shown an awakening action of dibutyryl cyclic AMP against many depressants, has postulated a

model for narcosis based on the "second messenger" hypothesis, in which cyclic AMP plays the key role in determining the level of arousal or narcosis. He includes TRH in this scheme as a possible inhibitor of neurohormones involved in the mechanism of sleep and narcosis. We have also found that dibutyryl cyclic AMP antagonizes not only pentobarbital narcosis, but also morphine-induced sedation in rabbits. Since TRH does not arouse rabbits from morphine sedation, it would appear that the TRH-induced arousal from barbiturates does not necessarily involve the cyclic AMP system.

Although the mechanism of TRH-induced arousal is as yet unresolved, it is clear that shortening of narcosis is unrelated to either altered distribution or metabolism of the depressant agent [3]. On the other hand, the blockade of arousal by scopolamine suggests a possible cholinergic involvement, as was suggested by Breese *et al.* [3]. Some of our preliminary data also indicate that serotonin may play a role in the TRH arousal since, after 5-HT depletion, the TRH reversal of pentobarbital narcosis is markedly attenuated [13]. However, both cholinergic and serotonergic mechanisms are not involved in the TRH effects in conscious animals. After scopolamine or p-chlorophenylalanine pretreatment, conscious animals respond to TRH in a normal or exaggerated manner.

The sedation produced by morphine represents an interesting exception in that it blocks the awakening effect of TRH. Morphinized animals given TRH exhibit tachypnea and hyperthermia, but in all other respects resemble control morphine animals. From these results we may conclude that the mechanism of morphine-induced sedation in rabbits is different from that produced by pentobarbital, benzodiazepines or the neuroleptics. Also, as mentioned earlier, morphine is one of the few antagonists of TRH-induced excitation in conscious animals. The antagonism is not merely because of the drugs possessing opposing actions. When small doses of morphine (4 mg/kg IV) are used to produce sedation, large doses of TRH (100–200 μg) do not visibly override the morphine sedation. Such results raise the question of whether morphine might produce its sedative effect by antagonizing endogenous TRH activity.

While most of this discussion has centered around the arousal properties of TRH, we feel that the hormone-induced stimulation of respiration represents more than an ancillary action to the arousal state. In the conscious animal tachypnea is the only observable effect seen with small doses of TRH (0.1 μg), while in pentobarbital-narcotized rabbits it always accompanies the arousal phenomenon. Even when arousal is blocked by scopolamine, tachypnea is present. The respiratory stimulation may contribute to the antagonism of pentobarbital narcosis; when rabbits are overdosed with lethal doses of pentobarbital, it is possible to restore respiration rapidly if TRH is administered immediately after the pentobarbital. Under these conditions the animals do not recover their righting reflexes until much later. A similar response is seen after morphine-induced depression of respiration. Such observations lead us to speculate on the possibility of TRH as a chemical mediator of respiratory regulation.

The present work has demonstrated several actions of TRH on the central nervous system which are unrelated to its hypophysiotropic function. Some of these, such as the arousal, tachypnea and hyperthermia, are produced with extremely small doses. Because of this it becomes extremely tempting to speculate on the possible physiological functions of TRH in the central nervous system. The question is even more relevant since the discovery of TRH in many regions of the brain [21], instead of only in the hypothalamo-hyophyseal area as previously thought. In addition, Burt and Snyder [5] recently reported on the presence of high-affinity-binding sites for TRH in brain-cell membranes. These, too, are distributed widely throughout the brain, and the authors look upon these binding sites as the possible receptors for TRH. All of these findings strengthen the idea that TRH and other neuropeptides may have important functions in the central nervous system.

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