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The Binding of ^{3}H-(3-MeHis^2) Thyrotropin Releasing Hormone to Brain and Pituitary Membranes of Morphine Tolerant-Dependent and Abstinent Rats

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BHARGAVA, H N , S DAS, M BANSINATH AND R PRASAD *The binding of ^{3}H-(3-MeHis^2) thyrotropin releasing hormone to brain and pituitary membranes of morphine tolerant-dependent and abstinent rats* PHARMACOL BIOCHEM BEHAV **34**(1) 7–12, 1989 —The effect of chronic administration of morphine and subsequent withdrawal on brain and pituitary receptors for thyrotropin releasing hormone (TRH) was investigated in Sprague-Dawley rats The rats were implanted subcutaneously with four morphine pellets (each containing 75 mg of morphine free base) during a 3-day period Placebo pellets, which contained all the excipients of morphine pellets except the morphine, were implanted in rats which served as controls Both tolerance and physical dependence on morphine have been shown to develop as a result of this procedure TRH receptors were labeled with ^{3}H-(3-MeHis^2) TRH (MeTRH) ^{3}H-MeTRH bound to brain membranes at a single high affinity site with B_{max} (receptor density) value of 24 6 ± 2 2 fmol/mg protein and K_d (apparent dissociation constant) value of 3 7 ± 0 4 nM The binding of ^{3}H-MeTRH to five regions of the brain namely, hypothalamus, cortex, striatum, midbrain and pons + medulla, as well as pituitary was also investigated The binding of ^{3}H-MeTRH to pituitary membranes was increased during the development of tolerance, wheres the binding to membranes prepared from different brain regions was unaffected Serum concentration of triiodothyronine (T_3) and thyroxine (T_4) were found to be lower in chronic morphine-treated rats when compared to placebo-treated rats, however, serum TSH level remained unaltered Twenty-four hours after the removal of morphine pellets (natural withdrawal), the binding of ^{3}H-MeTRH to pons + medulla membranes was greater than in placebo control group Naloxone-precipitated withdrawal produced results which were qualitatively similar to those obtained in rats from which pellets had been removed The results suggest that the development of tolerance to morphine may be associated with changes in the pituitary-thyroid axis

Morphine tolerance-dependence TRH receptors Pituitary, brain regions Naloxone-precipitated withdrawal Thyroid hormones T_4, T_3, T_3U, TSH

CONSIDERABLE evidence suggests that acute and chronic treatment with opiates affect the hypothalamic-pituitary-thyroid axis Acute administration of morphine and endogenous opiates depresses the secretion of thyroid stimulating hormone (TSH) (23, 27, 30, 31, 33) Chronic administration of morphine by multiple daily injections is not associated with the development of tolerance to the TSH inhibiting effect of morphine (16) Using lesion studies, it has been demonstrated that the inhibitory effect of morphine on thyroid is mediated via the caudal hypothalamus in the region of medial mammary nuclei (16,25) Chronic administration of morphine also decreases thyroid weight and pituitary TSH content (1,20)

The hypothalamus contains the tripeptide thyrotropin releasing hormone (TRH), which has an important role in the maintenance of pituitary TSH secretion In chronic opioid-addicted patients the normal TSH response to large doses of TRH remains unaltered (16) TRH as well as its receptors are also important constituents of extrahypothalamic neuronal tissues and much attention has been focused in recent years towards understanding the nonendocrinological role of TRH in the central nervous system (CNS) (11,34) The interactions of TRH with both acute and chronic effects of endogenous as well as exogenous opiates are well recognized

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(11) TRH antagonizes opioid-induced hypothermia, catalepsy, respiratory depression (10, 21, 36), inhibits gastrointestinal transit involving stereospecific opioid receptors (2,12) and modifies the chronic effects of morphine (5, 6, 9) Behaviorally, TRH is capable of producing a syndrome similar to "wet dog shake" of morphine withdrawal (38) Brain areas where naloxone precipitates withdrawal in morphine-dependent animals parallel the sites of TRH-stimulated shaking behavior and the endogenous sites of TRH distribution (19,39) Naloxone-precipitated morphine withdrawal produces a fall in cerebral cortical TRH content (32) Such evidences suggest that endogenous TRH may be involved in the chronic effects of morphine

The mechanisms by which TRH interacts with opiates is not well understood At the receptor level, it has been shown that TRH does not displace the binding of ^{3}H-naloxone (36) or of ^{3}H-dihydromorphine (21) to brain membranes It must be noted that both naloxone and dihydromorphine label mu opiate receptors predominantly To date, at least five opiate receptor types have been postulated They are mu (preferring morphine), delta (preferring enkephalin), kappa [preferring dynorphin (1–13) or ethylketocyclazocine], sigma (preferring N-allylnormetazocine) and epsilon (preferring β-endorphin) Studies from this laboratory have shown that TRH does not affect the binding of ligands for mu, delta and kappa opiate receptors to brain membranes (15) However, delta and kappa opiates inhibit the binding of ^{3}H-(3-MeHis^2) TRH (MeTRH) to brain membranes (7,14) and this effect appeared to be stereoselective (8) Thus, the interaction between opiates and TRH appeared to be unidirectional at the level of their brain receptors

Recent studies demonstrate that some of the opioid receptors are upregulated in rodents treated chronically with morphine (17) Since our earlier studies have shown that the tolerance to and dependence on morphine could be blocked by repeated injections of TRH (5, 6, 9), it was of interest to determine the effect of chronic administration of morphine to rats by subcutaneous implantation of morphine pellets, and of abrupt and naloxone-precipitated withdrawal on the TRH receptors in the brain regions and pituitary In addition, the effect of chronic morphine treatment on serum concentrations of T_3, T_4, TSH and T_3 uptake have also been determined

METHOD

Animals

Male Sprague-Dawley rats weighing 225–250 g obtained from King Animal Company, Oregon, WI were acclimated to the laboratory environment for at least four days before being used They were housed three to a cage in rooms with controlled temperature (23 ± 1°C), humidity (50 ± 10%) and artificial lighting (L 0600–1800 hr) Food and water were provided ad lib

Drugs

Morphine pellets, each containing 75 mg free base, were supplied by the Research Technology Branch, National Institute on Drug Abuse, Rockville, MD through the courtesy of Drs Richard Hawks and Rao S Rapaka Naloxone was a gift from Endo Laboratories, New York through the courtesy of Dr Alan Rubin ^{3}H-MeTRH (specific activity 70 4 Ci/mmol) was purchased from New England Nuclear Corporation, Boston, MA Cold TRH was a gift from the American Hoechst Corporation, Somerville, NJ through the courtesy of Mr Val R Wagner

Induction of Tolerance to and Physical Dependence on Morphine and Withdrawal Syndrome

Rats were made tolerant to and dependent on morphine by the procedure described previously (3,4) Briefly, each rat was implanted with one pellet on the morning of day 1, under light ether anesthesia The second pellet was implanted in the afternoon of day 1 Two additional pellets were implanted in the afternoon of day 2 Rats implanted similarly with placebo pellets served as controls The pellets were left in place for 72 hr after the implantation of the first pellet The studies were carried out in rats with pellets intact or from which pellets had been removed for 24 hr To determine the effects of naloxone-precipitated withdrawal, rats were divided into two groups One group was implanted with placebo pellets while the other with morphine pellets as described above Seventy-two hours after the implantation of the first pellet, animals of each group were divided into two subgroups Rats in one subgroup were injected with saline and the other with naloxone HCl (5 mg/kg, IP) The rats were sacrificed 30 min after the injection of naloxone

Determination of the Binding of ^{3}H-MeTRH to Brain Membranes

Whole brain without the cerebellum was homogenized in 20 ml of 0 32 M sucrose with a Polytron homogenizer (setting 5, 8 seconds) The homogenate was centrifuged for 10 min at 1,500 × g The pellet (crude nuclear fraction, P_1) was discarded and the supernatant suspension was centrifuged at 17,500 × g for 30 min in a Sorvall RC-5B refrigerated centrifuge The second pellet (P_2 fraction) was suspended in 20 ml of 20 mM sodium phosphate buffer (pH 7 4) using the Polytron (setting 5, 45 seconds) and was used for the binding studies. The binding assay was performed essentially based on the method described previously (7) using 0 2 ml of the homogenate in a total volume of 0 5 ml and containing 0 1 ml of ^{3}H-MeTRH ^{3}H-MeTRH was dissolved in the sodium phosphate buffer (pH 7 4) containing 0 1% bovine serum albumin to limit its loss to the glassware Incubations were carried out in triplicate in a shaking ice bath for five hours The incubation was terminated by the addition of 4 ml of ice-cold saline to the tubes The contents of the tube were rapidly filtered under reduced pressure through a glass fiber filter (GF/F) using Millipore filtration manifolds The filters were washed twice with 4-ml aliquots of ice-cold physiological saline. The filters were transferred to liquid scintillation vials containing 10 ml of 3a70 cocktail (Research Products International Corp , Elk Grove Village, IL) After an overnight equilibration period, the radioactivity in the samples was determined in a Packard liquid scintillation spectrometer (Model 4640) with a counting efficiency of 54% The specific binding of ^{3}H-MeTRH was defined as the difference in binding obtained in the absence and presence of 10 μM TRH All assays were performed in triplicate Generally, the specific binding accounted for 60–70% of the total binding The concentration of protein in the samples was determined by the method of Lowry *et al.* (26) The amount of ^{3}H-MeTRH specifically bound was expressed as fmoles of ligand bound per mg protein For the determination of B_{max} and K_d values, the concentration of the radioligand used was 1 to 8 nM The resulting data were subjected to Scatchard analyses The binding constants were determined after subjecting the data to linear regression analysis The binding of ^{3}H-MeTRH was also determined at 2 nM concentration to membranes of brain regions and pituitary which were prepared in a manner analogous to whole brain membranes described above The differences in the treatment and control groups of rats were determined by Student's *t*-test A value of $p<0$ 05 was considered to be statistically significant

Determination of Serum Concentration of Thyroid Hormones

Serum concentration of triiodothyronine (total T_3), T_3 uptake

TABLE 1

EFFECT OF CHRONIC ADMINISTRATION OF MORPHINE BY PELLET IMPLANTATION, ABRUPT WITHDRAWAL (PELLET REMOVED FOR 24 HR) AND NALOXONE-PRECIPITATED WITHDRAWAL ON THE BINDING OF ^{3}H-MeTRH TO RAT BRAIN MEMBRANES

	Specific Binding of [^{3}H]-MeTRH† Mean ± S E M (N=5)	
Treatment*	B_{max} (fmol/mg protein)	K_d (nM)
Pellet intact		
Placebo	24 6 ± 2 2	3 71 ± 0 43
Morphine	25 3 ± 1 7	3 46 ± 0 16
Pellet removed (24 hr)		
Placebo	31 4 ± 1 3	3 21 ± 0 07
Morphine	33 7 ± 1 7	3 38 ± 0 22
Pellet intact + naloxone		
Placebo + vehicle	33 1 ± 1 3	3 60 ± 0 13
Placebo + naloxone	34 0 ± 1 7	4 00 ± 0 31
Morphine + vehicle	33 4 ± 1 1	3 65 ± 0 12
Morphine + naloxone	35 7 ± 0 8	3 77 ± 0 17

*Rats were implanted subcutaneously, under light ether anesthesia, with either four morphine or four placebo pellets during a three-day period Animals were sacrificed with either the pellets intact or removed for 24 hr For studies on naloxone-precipitated withdrawal, animals were injected with naloxone HCl (5 mg/kg, IP) or vehicle and sacrificed 30 min later

†Binding was performed as described in the Method section

(T_3U), and thyroxine (T_4) were measured by using commercial radioimmunoassay (RIA) kits Total T_3 and T_3U kits were procured from Amersham Corporation, Arlington Heights, IL and T_4 kits were obtained from Diagnostic Products Corporation, Los Angeles, CA TSH kit was obtained from NIAMDD. The differences in the mean values of total T_3, T_3U, T_4 and TSH concentration in the serum of various groups of morphine and placebo pelleted rats were compared by using ANOVA followed by the Student's *t*-test A value $p<0$ 05 was considered to be significantly different

RESULTS

Effect of Chronic Administration of Morphine by Pellet Implantation, Abrupt and Naloxone-Precipitated Withdrawal in Morphine-Dependent Rats on the Binding of ^{3}H-MeTRH to Brain and Pituitary-Membranes

^{3}H-MeTRH bound to rat brain membranes of placebo pellet implanted rats at a single high affinity site with B_{max} value of 24 6 ± 2 2 fmol/mg protein and a K_d value of 3.71 ± 0 43 nM As shown in Table 1, implantation of morphine pellets did not alter the binding constants of ^{3}H-MeTRH The abrupt withdrawal of morphine by removal of the pellets also did not change the B_{max} or the K_d value of ^{3}H-MeTRH Similarly, naloxone-precipitated withdrawal in morphine-dependent rats had no effect on the binding constants of ^{3}H-MeTRH to whole brain membranes (Table 1)

The binding of ^{3}H-MeTRH at 2 nM concentration to rat brain regions of morphine tolerant-dependent rats and rats undergoing abrupt or naloxone-precipitated withdrawal is shown in Table 2 Among the brain regions studied, the highest density of ^{3}H-MeTRH binding sites was present in the hypothalamus which was followed by midbrain, pons + medulla, cortex and striatum The binding of ^{3}H-MeTRH to any brain region under any condition did not show any change except the binding was increased in pons + medulla of 24 hr abruptly withdrawn morphine-dependent rats (Table 2)

Chronic administration of morphine to rats by pellet implantation increased the binding of ^{3}H-MeTRH to pituitary membranes

TABLE 2

EFFECT OF CHRONIC ADMINISTRATION OF MORPHINE BY PELLET IMPLANTATION, ABRUPT WITHDRAWAL (PELLET REMOVED FOR 24 HR) AND NALOXONE-PRECIPITATED WITHDRAWAL ON THE BINDING OF ^{3}H-MeTRH TO MEMBRANES OF BRAIN REGIONS AND PITUITARY

	Specific Binding of [^{3}H]-MeTRH (fmol/mg protein)† Mean ± S E M (N=5)					
	Brain Regions					
Treatment*	Cortex	Midbrain	Striatum	Hypothalamus	Pons + Medulla	Pituitary
Pellet intact						
Placebo	10 53 ± 0 55	13 22 ± 0 81	7 44 ± 0 62	20 38 ± 1 20	12 35 ± 0 40	5 90 ± 0 64
Morphine	10 49 ± 0 57	13 42 ± 0 78	7 95 ± 1 22	20 23 ± 1 05	12 04 ± 1 14	9 11 ± 0 79‡
Pellet removed (24 hr)						
Placebo	12 53 ± 0 49	11 35 ± 0 36	7 62 ± 0 35	18 27 ± 1 38	10 49 ± 0 72	6 67 ± 0 98
Morphine	11 24 ± 0 65	12 67 ± 0 56	8 22 ± 0 21	18 20 ± 1 08	13 02 ± 0 79[c]	5 75 ± 0 47
Pellet intact + naloxone						
Placebo + vehicle	11 83 ± 0 16	12 35 ± 0 43	7 61 ± 0 15	18 03 ± 0 18	12 52 ± 0 26	4 61 ± 0 73
Placebo + naloxone	11 77 ± 0 14	12 71 ± 0 32	8 38 ± 0 20	17 84 ± 0 13	13 49 ± 0 87	5 22 ± 1 11
Morphine + vehicle	11 67 ± 0 17	11 95 ± 0 33	7 71 ± 0 19	18 03 ± 0 26	12 72 ± 0 69	9 00 ± 0 67‡
Morphine + naloxone	11 92 ± 0 21	12 17 ± 0 33	7 98 ± 0 17	17 83 ± 0 24	12 79 ± 0 73	9 19 ± 0 89

*Rats were implanted subcutaneously, under light ether anesthesia, with either four morphine or four placebo pellets during a three-day period Animals were sacrificed with either the pellets left intact or removed for 24 hr For studies on naloxone-precipitated withdrawal, animals were injected with naloxone HCl (5 mg/kg, IP) or vehicle, and sacrificed 30 min later

†Binding was performed as described in the Method section using 2 nM concentration of ^{3}H-MeTRH

‡$p<0$ 05 vs the placebo control group

TABLE 3

EFFECT OF CHRONIC ADMINISTRATION OF MORPHINE AND ITS WITHDRAWAL ON THYROID FUNCTION IN THE RAT

Treatment Group*	Serum Concentration of Thyroid Hormone T_4 (μg/dl)	T_3 (ng/ml)	TSH	T_3 Uptake Index
	Mean ± S E M (N=5)			
Placebo pellets intact	3 54±0 26	0 25 ±0 02	3 09±0 14	1 50±0 01
Morphine pellets intact	1 34±0 17‡	0 092±0 004†	3 24±0 11	1 53±0 002
Placebo pellets removed	3 26±0 34	0 18 ±0 02	3 24±0 08	1 49±0 01
Morphine pellets removed†	2 74±0 41‡	0 096±0 004†	2 87±0 10	1 52±0 01

*Rats were implanted with four morphine or four placebo pellets during a 3-day period as described in the text

†The pellets were removed and 24 hr later the rats were sacrificed, and the serum collected for determination of the concentration of thyroid hormones

‡$p<0$ 05 vs placebo pellets intact group, §$p<0$ 05 vs morphine pellets intact group

TABLE 4

EFFECT OF NALOXONE-PRECIPITATED WITHDRAWAL ON THYROID FUNCTION IN MORPHINE-DEPENDENT RATS

Treatment Group*	Serum Concentration of Thyroid Hormone T_4 (μg/dl)	T_3 (ng/ml)	TSH	T_3 Uptake Index
	Mean ± S E M (N=5)			
Placebo pellet + vehicle	4 74±0 37	0 24±0 04	3 09±0 16	1 50±0 01
Placebo pellet + naloxone	3 80±0 22†	0 26±0 04	3 07±0 17	1 51±0 01
Morphine pellets + vehicle	2 18±0 25†	0 13±0 01†	2 92±0 08	1 50±0 01
Morphine pellets + naloxone	1 28±0 19‡	0 10±0 004	2 75±0 08	1 54±0 01

*Rats were implanted with four morphine or four placebo pellets during a 3-day period as described in the text The animals were injected with either saline (vehicle) or naloxone (5 mg/kg, SC) and sacrificed 10 min later The serum was collected for determination of the concentration of thyroid hormones

†$p<0$ 05 vs vehicle-injected placebo pelleted rats, ‡$p<0$ 05 vs vehicle-injected morphine pelleted rats

As can be seen from Table 2, the pituitary membranes of morphine pellet implanted rats exhibited a 54% higher binding in comparison to placebo pellet implanted rats When the pellets were removed for 24 hr, i e , after abrupt withdrawal of morphine, the binding of ^{3}H-MeTRH had returned to normal since there was no significant difference between the placebo and morphine pellet implanted groups Administration of naloxone to placebo or morphine pellet implanted rats did not alter the binding of ^{3}H-MeTRH to pituitary membranes (Table 2)

Effect of Chronic Administration of Morphine and its Abrupt Withdrawal on Serum Concentration of Thyroid Hormone in the Rat

Chronic administration of morphine to rats by subcutaneous implantation of morphine pellets resulted in a decrease in the serum concentration of T_4 and T_3, but the concentration of TSH and the T_3 uptake index did not change (Table 3) Twenty-four hours after the removal of placebo pellets, the serum concentration of thyroid hormones was similar when compared to serum levels in which the placebo pellets were left intact Twenty-four hours after the removal of morphine pellets, a slight recovery of serum T_4 levels was observed, but T_3 levels remained depressed During the abrupt withdrawal of morphine, serum TSH levels did not differ from rats with morphine pellets intact (Table 3)

Effect of Naloxone-Precipitated Withdrawal in Morphine-Dependent Rats on the Serum Concentration of Thyroid Hormones

As shown above, implantation of morphine pellets resulted in decreases in serum concentration of T_3 and T_4 and no change in TSH and T_3 uptake index A dose of naloxone (5 mg/kg, SC) given to placebo pellet implanted rats significantly decreased serum concentration of T_4, but the concentration of T_3, TSH and T_3 uptake index was not affected (Table 4) Administration of naloxone to morphine-dependent rats caused a further decrease in the serum concentration of T_4 but T_3 and TSH levels were not affected (Table 4)

DISCUSSION

Results of the present study suggest that in rats rendered tolerant to the analgesic effects of morphine by the pellet implantation method, neither the affinity nor the number of TRH receptors appear to change in different regions of brain including hypothalamus, an area rich in TRH receptors However, specific binding of TRH to pituitary membranes was increased during the development of morphine tolerance Furthermore, tolerance development did not alter the inhibitory effect of morphine on circulating T_3 and T_4 However, there was no effect on T_3 uptake index and serum concentration of TSH Following the abrupt withdrawal by removal of morphine pellets, there was a tendency of serum T_4 levels to return to normal, but there was no change in serum T_3 levels even 24 hr after the removal of pellets Furthermore, naloxone caused a lowering of serum T_4 concentration, but did not affect the concentration of T_3, TSH and T_3 uptake index The lowering of T_4 concentration by naloxone in nontolerant and morphine-tolerant rats was of similar magnitude

The actions of opiates on the thyroid function are rather complex Divergent results are available in the literature regarding the acute effects of opiates on the thyroid functions Some studies indicate an inhibitory effect of opiates on TRH and/or on TSH secretion (13, 22, 30), while others support either lack of any effect (18,35) or a stimulatory effect (23,24) In vitro evidence suggests that TRH release, but not TSH release, is inhibited by endogenous opiates (22) Furthermore, depending on the site of central administration, morphine has been shown to have both stimulatory and inhibitory effects on TSH secretion (27) The site(s) and mechanism(s) of action of opiates involved in the modulation of serum TSH levels is far from being clear Some studies suggest that the opiates act on specific opiate receptors at the TRH

terminals of hypothalamus (22,37), while others suggest that the opiates directly act at the pituitary (24). It is noteworthy that in contrast to the studies after acute opiate administration, relatively few investigations appear to have been done as related to the effects of opiate-induced tolerance and dependence on TRH release and thyroid function

Acute administration of morphine (16) as well as opioid peptides (13, 28, 29) to rodents has been shown to decrease the serum TSH levels presumably through a hypothalamic mechanism In the present study chronic administration of morphine decreased circulating levels of T_3 and T_4 but not TSH levels This observation could indicate that tolerance develops to the TSH lowering effect of morphine In contrast, when tolerance in the rat was produced by multiple injections, the serum levels of TSH were reported to be lower compared to the control rats (16,32) The present observation of lack of changes in serum TSH levels in morphine-tolerant and-dependent animals may reflect an inhibition of hypothalamic TRH secretion Such a reduction in TRH secretion would lead to upregulation of TRH receptors on the pituitary and account for increased binding of ^{3}H-MeTRH observed in this study Under normal conditions, lower T_3 and T_4 levels are expected to result in elevated TSH levels. In our study, in chronic morphine-treated animals, although T_3 and T_4 levels were lower, TSH levels were not elevated Hence, it appears that the secretion and clearance studies of TSH, T_4 and T_3 would help to further clarify if the decrease in T_4 and T_3 levels is due to hyporesponsivity of TSH release mechanism and/or due to increased rate of clearance of the circulating hormones in this setting After chronic injections of morphine in rats, lack of tolerance to morphine induced inhibition of T_4 has been reported earlier (32) Our results confirm and extend these results to T_3 and T_4 in rats rendered morphine tolerant by implanting pellets Furthermore, present results also indicate that withdrawal does not appear to alter the levels of TSH significantly In light of the in vitro evidence of unidirectional interaction between opiates and TRH receptor agonists (8), a lack of up- or down-regulation of TRH receptors in different areas in the brain found in the present study suggests that 'nonneuroendocrinological' sites of TRH may not be implicated as the sites of action of TRH, for the inhibition of opiate tolerance and withdrawal, however, pituitary TRH receptors appear to be involved Clearly, more studies on alteration of neuroendocrine axis of TRH in hypophysectomized animals during tolerance and dependence may help to clarify the significance of pituitary receptor proliferation and its role in opiate tolerance-dependence and withdrawal processes

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REFERENCES

1 Bakke, J L , Lawrence, N L , Robinson, S The effect of morphine on pituitary-thyroid function in the rat Eur J Pharmacol 25 402–406, 1974
2 Bansinath, M , Bhargava, H N Evidence for a peripheral action of thyrotropin relasing hormone on gastrointestinal transit in mice Neuropharmacology 27 433–437, 1988
3 Bhargava, H N Rapid induction and quantitation of morphine dependence in the rat by pellet implantation Psychopharmacology (Berlin) 52 55–62, 1977
4 Bhargava, H N Quantitation of morphine tolerance induced by pellet implantation in the rat J Pharm Pharmacol 30 133–135, 1978
5 Bhargava, H N The effects of thyrotropin releasing hormone on the central nervous system responses to chronic morphine administration Psychopharmacology (Berlin) 68 185–189, 1980
6 Bhargava, H N Dissociation of tolerance to the analgesic and hypothermic effects of morphine by using thyrotropin releasing hormone Life Sci 29 1015–1020, 1981
7 Bhargava, H N , Das, S Evidence for opiate action at the brain receptors for thyrotropin releasing hormone Brain Res 368 262–267, 1986
8 Bhargava, H N , Das, S , Gulati, A Stereoselectivity of kappa opiate receptor ligands in inhibiting the binding of ^{3}H-(3-MeHis^2) thyrotropin releasing hormone to brain membranes J Pharm Pharmacol 40 70–72, 1988
9 Bhargava, H N , Matwyshyn, G A Comparative effects of thyrotropin releasing hormone and its synthetic analogs, MK-771 and DN-1417 on morphine abstinence syndrome Psychopharmacology (Berlin) 87 141–146, 1985
10 Bhargava, H N , Matwyshyn, G A , Currie, B L , Goebel, R J Structure activity relationship studies with hypothalamic peptide hormones II Effect of thyrotropin realsing hormone analogs on morphine-induced responses in mice Life Sci 30 711–718, 1982
11 Bhargava, H N , Yousif, D J , Matwyshyn, G A Interactions of thyrotropin releasing hormone its metabolites and analogues with endogenous and exogenous opiates Gen Pharmacol 14 565–570, 1983
12 Bhargava, H N , Pillai, N P Stereospecific opiate receptors in the actions of thyrotropin releasing hormone and morphine on gastrointestinal transit Life Sci 36 83–88, 1985
13 Bruni, J F , Van Vugt, D , Marshall, S , Meites, J Effects of naloxone, morphine and methionine enkephalin on serum prolactin, luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone Life Sci 21 461–466, 1977
14 Das, S , Bhargava, H N Inhibition of ^{3}H-(3-MeHis^2) thyrotropin releasing hormone recognition sites in the brain by tifluadom, a kappa opiate agonist Neuropharmacology 26 1141–1146, 1987
15 Das, S , Bhargava, H N Unidirectional interaction between thyrotropin releasing hormone and opiates at the level of their brain receptors Gen Pharmacol 18 99–102, 1987
16 George, R , Kokka, N The effects of narcotics on growth hormone, ACTH and TSH secretion In Ford, D H , Clouet, D H , eds Tissue responses to addictive drugs New York Spectrum Publications Inc , 1976 527–540
17 Gulati, A , Bhargava, H N Up-regulation of brain and spinal cord kappa opioid receptors in morphine tolerant-dependent mice FASEB J 2 A368, 1988
18 Hart, I C , Cowie, A T Effect of morphine, naloxone and an enkephalin analogue on plasma prolactin, growth hormone, insulin and thyroxine in goats J Endocrinol 77 16p, 1978
19 Herz, A , Teschemacher, H J , Albus, K , Zieglgansberger, S Morphine abstinence syndrome in rabbits precipitated by injection of morphine antagonists into the ventricular system and restricted parts of it Psychopharmacologia 26 219–235, 1972
20 Hohlweg, W , Knappe, G , Doerner, G Experimental animal studies on the effect of morphine on gonadotropic and thyrotropic pituitary gland function Endokrinologie 40 152–159, 1961
21 Holaday, J W , Tseng, L F , Loh, H H , Li, C H Thyrotropin releasing hormone antagonizes beta endorphin hypothermia and catalepsy Life Sci 22 1537–1544, 1978
22 Jordan, D , Veisseire, M , Borson-Chasot, F , Mornex, R In vitro effects of endogenous opiate peptides on thyrotropin function Inhibition of thyrotropin releasing hormone release and absence of effect on thyrotropin release Neurosci Lett 67 289–294, 1986
23 Judd, A M , Hedge, G A The roles of the opioid peptides in controlling thyroid stimulating hormone release Life Sci 31 2529–2536, 1982
24 Judd, A M , Hedge, G A Direct pituitary stimulation of thyrotropin secretion by opioid peptides Endocrinology 113 706–710, 1983
25 Lomax, P , George, R Thyroid activity following administration of morphine in rats with hypothalamic lesions Brain Res 2 361–367, 1966
26 Lowry, O H , Rosebrough, N J , Farr, A L , Randall, R J Protein

measurement with the Folin phenol reagent J Biol Chem 193 265–27, 1951
27 Mannisto, P T , Rauhala, P , Tuominen, R , Mattila, J Dual action of morphine on cold-stimulated thyrotropin secretion in male rats Life Sci 35 1101–1107, 1984
28 May, P , Mittler, A , Manougian, A , Ertel, N TSH release inhibiting activity of leucine-enkephalin Horm Metab Res 11 30–33, 1979
29 Mess, B , Ruzsas, C , Rekasi, Z Central monoaminergic and opioidergic regulation of thyroid function and its ontogenic differentiation, Mongr Neurol Sci 12 117–127, 1986
30 Mitsuma, T , Nogimori, T , Chaya, M β-Casomorphin inhibits thyrotropin secretion in rats Exp Clin Endocrinol 84 324–330, 1984
31 Mitsuma, T , Nogimori, T Effects of leucine-enkephalin on hypothalamic-pituitary-thyroid axis in rats Life Sci 32 241–248, 1983
32 Morely, J E , Yamada, T , Walsh, J H , Lamers, C B , Wong, H , Shulkes, A , Damassa, D A , Gordon, J , Carlson, H E , Hershman, J M Morphine addiction and withdrawal alters brain peptide concentrations Life Sci 26 2239–2244, 1980
33 Muraki, T , Nakadate, T , Tokunaga, Y , Kato, R Effect of morphine on the release of thyroid stimulating hormone stimulated by exposure to cold, thyroidectomy and the administration of thyrotropin releasing hormone in male rats J Endocrinol 86 357–362, 1980
34 Sharif, N A Diverse roles of thyrotropin-releasing hormone in brain, pituitary and spinal function Trends Pharmacol Sci 6 119–122, 1985
35 Sharp, B , Morely, J E , Carlson, H E , Gordon, J , Briggs, J , Melmed, S , Hershman, J M The role of opiates and endogenous opioid peptides in the regulation of rat TSH secretion Brain Res 219 335–344, 1981
36 Tache, Y , Lis, M , Collu, R Effects of thyrotropin-releasing hormone on behavioral and hormonal changes induced by β-endorphin Life Sci 21 841–847, 1977
37 Tapaia-Arabcibia, L , Astier, H Opiate inhibition on K^+-induced TRH release from superfused mediobasal hypothalamic slices in rats Neuroendocrinology 111 1181–1191, 1983
38 Wei, E , Sigel, S , Loh, H , Way, E L Thyrotropin-releasing hormone and shaking behaviour in rat Nature 253 739–740, 1975
39 Winokur, A , Utiger, R D Thyrotropin-releasing hormone regional distribution in rat brain Science 185 265–266, 1974