Ann. Rev. Pharmacol. Toxicol. 1986. 26:311–32

PHARMACOLOGY OF THYROTROPIN-RELEASING HORMONE

A. Horita[1,2], M. A. Carino[1], and H. Lai[1]

Departments of Pharmacology[1] and Psychiatry & Behavioral Sciences[2], University of Washington School of Medicine, Seattle, Washington 98195

INTRODUCTION

Thyrotropin-releasing hormone (TRH) was identified as L-pyroglutamyl-L-histidyl-L-proline amide in 1973, and its eventual synthesis enabled researchers to investigate its physiological and pharmacological properties. During the past ten years it has become clear that TRH and its receptors are ubiquitously distributed in the central nervous system (CNS) as well as in several peripheral organs. Studies in which TRH was administered to experimental animals have revealed some intriguing and potentially important clinical actions, most of which are unrelated to its thyrotropin-releasing property. In fact, this property in some instances is looked upon as a side effect, and attempts have been made to synthesize analogues that retain the central, but not the thyrotropin-releasing, properties of TRH (1, 2).

Because of space considerations, the scope of this review has been limited to selected aspects of the pharmacology of TRH. The review of the literature has also been selective and is devoted mostly to papers published after 1982. For a more comprehensive background on the extrapituitary properties of TRH the reader is referred to several earlier publications (3–7).

TRH RECEPTORS

The first studies of extrapituitary TRH binding sites were made as early as 1975 (8), but the lack of a ligand with high specific activity prevented accurate description of their properties. The later availability of ^{3}H-3MeHis-TRH, a

0362-1642/86/0415-0311$02.00

TRH analogue with higher binding affinity, enabled characterization not only of the properties, but also of the distribution of the receptors. It is now generally agreed that TRH receptors in brain are ubiquitously distributed, but are found in highest densities in limbic structures, especially the amygdala and hypothalamus, and in lower densities in brain stem and cerebellum (9–11). TRH receptors also occur in the spinal cord, with the highest densities in the dorsal and ventral gray matter and lower densities in the dorsal root and ganglia (12, 13). The receptors in the brain and pituitary are very similar (14, 15), but may differ in some ways in their interaction with certain drugs (16).

The receptor is membrane bound, digestible by trypsin and phospholipase, and inactivated by various thiol reagents and metal salts (17, 18). Various drugs have been tested for ability to compete with the radioactive ligand, but thus far only two classes of drugs have been identified that compete at micromolar concentrations. Their pharmacology might be related to TRH-receptor interactions: the dihydrogenated ergot alkaloids and the benzodiazepines. The several components of dihydroergotoxin (DHET) have been found to range in IC_{50} between 10 and 50 μM. Fourteen days of DHET treatment induced an up-regulation of TRH receptors in the cerebral cortex of aged rats, but not in other brain areas (19). The benzodiazepines, particularly chlordiazepoxide, were reported to compete at micromolar concentrations with ^{3}H-3MeHis-TRH binding to brain and pituitary membranes (20, 21). No relationship was observed between antianxiety potency/benzodiazepine receptor binding and ability to displace the TRH ligand. In fact, flunitrazepam and diazepam, both potent agonists of the benzodiazepine receptor, were considerably weaker than chlordiazepoxide in displacement ability. More recent work demonstrated the presence of regional differences in inhibition of TRH binding by the benzodiazepines (16). The IC_{50}s of chlordiazepoxide and diazepam for ^{3}H-3MeHis-TRH displacement in membranes from hippocampus, spinal cord, and hypothalamus were 2–25 times greater than in those from the amygdala, retina, and pituitary. Although the significance of these differences is as yet unclear, they suggest the possible existence of multiple classes of TRH receptors.

Some beginnings have been made in solubilizing the TRH receptor from brain. Ogawa et al (22) solubilized the ^{3}H-TRH-bound receptor with Triton X-100. The supernatant was passed through a Sepharose 6B column, and from the elution profiles the molecular weight of the TRH-receptor complex was approximated at 300,000 and the Stoke's radius at 5.8 nm. Johnson et al (23) found that, among a variety of detergents, digitonin alone can solubilize the unbound receptor. They found comparable molecular weights and Stoke's radii as described for the bound receptor, but also demonstrated that the solubilized receptor retains most of its original binding characteristics and therefore must also retain its native conformation. The solubilized receptor demonstrated

excellent stability, showing no loss in binding activity even after two months of storage at −20°C.

For a finer localization of receptor sites, several investigators have utilized autoradiographic techniques on rat brain (24–28) and rabbit spinal cord (29). In general these have confirmed results of binding experiments but have made evident differences in density distribution over discrete brain areas, e.g. higher densities in parts of the amygdala, hippocampus, diagonal band of Broca, stria terminalis, and superior colliculus; and lower densities in cortex, brain stem, and spinal cord. High receptor densities were reported in laminae II and substantia gelatinosa in human spinal cord (30).

The various factors that influence brain and spinal cord TRH receptors are now receiving considerable attention. As with many receptor systems, chronic exposure of animals to TRH or its analogues causes down-regulation. Ogawa et al (31) administered 6 mg/kg/day of TRH for 14 days and demonstrated a 20–25% loss of TRH receptor binding sites in hippocampus, hypothalamus, and cerebral cortex, but not in other brain regions investigated. Recovery from down-regulation was seen within one week of discontinuation of TRH treatment. Simasko & Horita (32) also observed down-regulation of TRH receptors in all brain regions investigated after the intracerebroventricular (i.c.v.) infusion of the stable TRH analogue MK-771 continuously via a miniosmotic pump for seven days (5 μg/μl/hr) or periodical administration of it. They found development of tolerance of certain behaviors, such as shaking or large motor movements, which were related temporally to the down-regulation of brain TRH receptors. Other responses, such as tremors, exhibited much less tolerance development. These studies indicate that chronic exposure of the CNS to TRH results in rapid and reversible down-regulation of its receptors, accompanied by development of functional tolerance, the degree of tolerance differing among the responses.

Various toxins and lesioning procedures have also been employed in the functional localization of TRH receptors. Microinjection of kainic acid into the medial septum produced a 35% decrease in density of septal TRH receptors, whereas injections of kainic acid or 6-OHDA into the lateral ventricles or electrolytic lesioning of the fimbria or medial forebrain bundle were ineffectual (33). Manaker et al (34), employing quantitative autoradiographic techniques, found that pretreatment with 6-OHDA would effect variable decreases (25–75%) in TRH receptors in some parts of the brain. From these data and a finding of increased regional TRH levels, the investigators suggested that some TRH receptors are located in presynaptic terminals and that the TRH receptors down-regulate in response to elevated levels of TRH.

In addition to effects on its own receptors, TRH may influence the regulation of other receptors as well. Pirola et al (35, 36) found in both brain and isolated

intestine increased densities of muscarinic receptors after a single exposure to TRH. Concomitantly, enhanced responsiveness of both systems to acetylcholine was observed, although TRH itself exerted no direct effect of its own. In contrast to these results, chronic administration of TRH was without effect on central muscarinic receptors (31).

PHARMACOLOGY OF TRH

Behavioral Effects

CONDITIONED BEHAVIOR The effects of TRH on conditioned behavior in animals have been studied under a variety of performance conditions. In most of the studies TRH was given peripherally (i.p., i.m., or i.v.).

In a reward situation, with FR or FI schedule of reinforcement for food or saccharin solution, TRH and MK-771 decreased the response rate in squirrel monkeys, rabbits, and pigeons (37). Both drugs were equipotent in the squirrel monkeys, whereas MK-771 was twenty times more potent than TRH in rabbits and pigeons. However, under conditions in which responding was maintained by shock termination or presentation, both TRH and MK-771 increased the response rate in the squirrel monkeys. At the same doses of the drugs, responding to food presentation was not affected (38). Thus, the effect of TRH on schedule-controlled behavior is dependent on the consequence (reward or punishment) of the behavior. This conclusion was further confirmed by an experiment in which intramuscular TRH or MK-771 caused a dose-dependent decrease in punished responses maintained by food presentation and a dose-dependent increase in punished responses maintained by shock termination (39). An increase in "punished responding" behavior by a drug is usually indicative of anxiolytic activity; thus, the result would suggest that under certain conditions, TRH has an anxiolytic effect. Related to this effect is a finding (40) of potentiation by TRH (0.03 mg/kg, i.m.) of the rate-increasing effect of pentobarbital, chlordiazepoxide, and ethanol on "punished responding" behavior seen at low doses of these drugs, but it did not affect the rate-decreasing effect observed at higher doses of these drugs.

TRH given intraperitoneally enhanced the acquisition of shuttle-box avoidance behavior (41), apparently by increasing motor activity, and thus intertrial responses. In the same study, the resistance to extinction of the avoidance response was unaffected. A recent study also demonstrated potentiation by TRH (20 mg/kg i.p.) of the conditioned flavor aversion induced by pentobarbital in the rat (42). In a two-choice visual discrimination test, TRH (1 and 50 μg, i.c.v) was found to exert no significant effect on performance (43), but by the signal-detection technique it was found to produce response preservation in the

task in a way similar to that produced by peripherally administered amphetamine.

LOCOMOTOR ACTIVITY Early studies of TRH and locomotor activity have likewise revealed a potentiating effect in animals (44, 45). More recent investigations have dealt, however, with the neural substrates and pharmacology of the locomotor activity-inducing effect of TRH. Both the nucleus accumbens and the hypothalamus are main sites of action, and dopamine appears to play a mediating role. Support for this view has come from several studies. In a recent study (46) in mice, TRH-induced motor activity was inhibited and enhanced by small doses of apomorphine and haloperidol, respectively. These data suggest that TRH-induced motor activity depends on release of dopamine from presynaptic terminals. Other investigators (47, 48) found the response in the rat blocked by pretreatment with the dopamine antagonists haloperidol and α-flupenthixol, as well as by the narcotic antagonist naloxone, and the α_2-adrenergic antagonist yohimbine (47). Narumi & Nagawa (49) observed that haloperidol blocked the locomotor response produced by TRH or DN-1417 injections into the nucleus accumbens, the brain site most sensitive in initiating the response. Sharp et al (50) also reported success in inducing the response by microinjection of TRH into the septum or nucleus accumbens, but not with similar injection into the striatum. Masserano & King (51) observed the response, however, after injection into the hypothalamus, but not after administration into the septum and caudate. The discrepancy in results may proceed from the differences in methods of measuring activity used.

Breese et al (52, 53) recently showed that TRH injections into the medial septum antagonized the locomotor depressant effect of ethanol but did not affect locomotor activity in normal animals even at a dose of 5 μg. These studies are of interest because the septum is a highly sensitive site for the analeptic effect of TRH, and intraseptal TRH injection reverses the effect of pentobarbital on hippocampal acetylcholine turnover but has no significant effect on turnover in conscious animals. Andrew & Sahgal (48) reported on a circadian variation of TRH effects on locomotor activity; the peptide increased locomotor activity when it was injected in the afternoon (13:00–17:00 h) but not when injected in the morning (09:00–12:00 h). This property could be due to the known circadian variation in dopamine activity in the brain. Furthermore, TRH-induced locomotor activity was enhanced in frontal decorticated rats, which indicates an inhibiting role of the frontal cortex (54). This may involve the glutamate innervation from the cortex to the dopamine system of the nucleus accumbens. Thus, it is clear that dopaminergic mechanisms in the nucleus accumbens play a major role in mediating the locomotor effects of TRH.

Biochemical and cellular studies of dopamine functions also support the

conclusion that TRH has a selective effect on the mesolimbic dopamine system. For example, Yamada et al (55) reported a TRH enhancement of dopamine turnover rate and synthesis in the nucleus accumbens but not in the olfactory tubercle and striatum. However, it was strange that the powerful TRH analogue MK-771 was without effect on dopamine turnover in the nucleus accumbens. Sharp et al (56), using the in vivo voltammetry technique, also found that TRH and its analogue CG 3509 selectively increased dopamine activity in the mesolimbic system. Further evidence that TRH increases dopamine activity in the nucleus accumbens is given by Kalivas (reported in 7), who found that i.c.v. or intra-accumbens injection of TRH increased the ratio of DOPAC/DA in the nucleus accumbens.

The exact cellular site of action of TRH in the nucleus accumbens is not known. Pinnock et al (57) reported that TRH, when applied iontophoretically, did not affect the activities of neurons in the caudate and nucleus accumbens. In contrast, Hashimoto et al (58) found that TRH and DN-1417 produced both inhibition and excitation on nucleus accumbens neurons, both of which were blocked by haloperidol. However, TRH released ^{3}H-DA from accumbens tissue only at high concentrations (10^{-2} M) (49). These data suggest that TRH does not act directly on dopamine nerve terminals or postsynaptic dopamine receptors to facilitate dopamine mechanisms in the nucleus accumbens.

Other evidence also indicates that TRH could modify dopaminergic function in the striatum. Oki et al (59) found that specific ^{3}H-spiroperidol binding to striatal membrane was reduced if TRH was given 15–150 min before sacrifice. However, TRH did not affect binding in vitro. TRH caused body turning to the lesioned side in unilateral 6-OHDA nigral-lesioned animals, and enhanced apomorphine- and L-DOPA-induced body turning in animals with unilateral caudate lesions (49). These findings could imply either a direct action of TRH on the intact nigrostriatal dopamine system or a secondary effect on that system via an effect of TRH on the nucleus accumbens, since the dopamine systems of accumbens and striatum are known to modify each other's activities. It is of interest that TRH also increased DA-stimulated-c-AMP formation in super-sensitized caudate but not in normal tissue. Thus, TRH could affect striatal dopamine functions in certain pathological conditions, such as Parkinson's disease and tardive dyskinesia, when the striatal dopamine system is super-sensitive.

OTHER EFFECTS A number of other behavioral effects have been attributed to TRH, such as rearing (60), body shakes (61–63), head turning (64), stereotypy (65), and inhibition of feeding and drinking (66). Most of these responses appear to involve dopamine mechanisms, although other neurotransmitters may play secondary or indirect roles.

Analeptic Effect

Although many pharmacological properties have been ascribed to TRH, its analeptic action in shortening the duration of various depressant drugs remains one of its most intriguing effects. The recent review by Nemeroff et al (7) provides excellent background information on this property of TRH; therefore, only a brief summary of the literature through 1981 will be presented here, and more detailed attention will be given to subsequent publications.

The original observation of the analeptic effect of TRH was made by Breese et al (67) in mice and rats. Subsequently the effect was observed in several other species (67, 68). The suggestion of a cholinergic involvement in its mechanism of action was supported by the fact that it was antagonized by anticholinergic drugs (67, 69). The rat proved to be an exception in that atropine or scopolamine, even in high doses, did not block the analeptic effect (70), although Miyamoto et al (71) showed sensitivity to cholinergic blockade. Kalivas & Horita (72) localized the analeptic effect to several brain sites, with the greatest sensitivity being in the area of the medial septum and nucleus of the diagonal band of Broca (MS/DBB), areas that were subsequently shown to contain high densities of TRH receptors (27). It is of interest that whereas the analeptic effect of i.c.v.-administered TRH was resistant to cholinergic blockade, when TRH was injected into the medial septum it was readily blocked by atropine (73); it would thus appear that the septohippocampal cholinergic pathway was involved in part in the analeptic response. That TRH injected into the septum of animals anesthetized with pentobarbital activated hippocampal cholinergic innervation was subsequently demonstrated neurochemically (74). Miyamoto et al (71) also localized multiple brain sites mediating the analeptic effect in rats. They found that i.c.v. or intrahypothalamic injections of atropine methyl bromide blocked the analeptic effect of TRH administered i.p. or into the hypothalamus, but not when the peptide was administered into other brain sites.

It appears that in the rat the analeptic effect may be mediated by multiple neural substrates and, depending upon the route of administration, different systems are activated to arouse the animal. For instance, when the authors determined the diffusion of ^{14}C-DN-1417, a TRH analogue, after its injection i.c.v. or into lateral hypothalamus, they found different patterns of ^{14}C distribution. After i.c.v. injection the highest density of grains was found in periventricular structures, including the septal regions, hippocampus, central gray substance, etc. As expected, after injection into the lateral hypothalamus, the greatest density of radioactivity was retained at and near the site of injection.

Sharp et al (75) recently reported on the analeptic, respiratory, and shaking effects of TRH and several of its analogues. They selected the lateral septum, nucleus accumbens, and striatum of the rat as sites for microinjection because they contain TRH and its receptors. As reported by others, these investigators

showed analeptic activity in pentobarbitalized rats when TRH was microinjected into the septum and nucleus accumbens, but not into the striatum. They also noted that whereas their data were qualitatively similar to those reported by Kalivas & Horita, it required some 200 times the dose of TRH microinjected into the septum to produce a similar analeptic response.

It should be pointed out that Kalivas & Horita found a marked difference in sensitivity to TRH between the medial and lateral septal areas. The MS/DBB exhibited far greater sensitivity than the lateral septum to TRH in evoking the analeptic response. According to the description of the stereotaxic coordinates and the diagrams presented in their article, Sharp et al did not microinject into the medial septum, and this may explain the apparent discrepancy in sensitivity to TRH.

These differences in results raise an interesting question regarding the neural substrate(s) mediating the analeptic effect of TRH. Several investigators have reported on the presence of high concentrations of TRH in the lateral, but not in the medial, septum. Recently, Ishikawa et al (76) demonstrated by immunohistochemical techniques the presence of large numbers of TRH-containing nerve terminals in the lateral septum, but not in the medial. In contrast, the density of TRH receptors is greater in the MS/DBB (27). A recent study clearly demonstrated that neurons of the MS/DBB were highly sensitive to TRH (77). Iontophoretic application of TRH onto these sites produced an atropine-insensitive excitatory response. Most of these same cells could be excited by cholinergic agonists. From these results Lamour et al (77) suggested that among the septohippocampal neurons examined the cholinergic neurons were the most sensitive to TRH. Their data also suggest that TRH exerts a direct effect on the cholinergic cell bodies of the septohippocampal neurons and not by a presynaptic release of acetylcholine.

The neurochemical evidence for a role of the cholinergic system in the analeptic effect was first suggested by Schmidt (78), who found that the reduction of sodium-dependent high affinity ^{3}H-choline uptake in hippocampus and cortex by pentobarbital anesthesia was attenuated or prevented by TRH and MK-771. Similar results with TRH and its analogue DN-1417 were also shown in rat brain slices (79). It is interesting to note that under in vitro conditions TRH (10^{-4} M) and DN-1417 (10^{-5} M) were also inactive in increasing ^{3}H-choline uptake in brain slices, but they attenuated or blocked pentobarbital's ability to lower ^{3}H-choline uptake. These peptides also increased O_2 consumption and cyclic AMP formation in brain slices and reversed the depression of these responses produced by pentobarbital.

From these results the authors suggested possible relationships between the biochemical effects in vitro and the analeptic effect produced by these agents in intact animals. It is important, however, to recall that the pentobarbital-induced reduction of choline uptake in the hippocampus in vivo was not produced by a

direct action of the barbiturate at that brain site, but was initiated at some other site that sent projections to the hippocampus via the septum (80). Thus, the effects of pentobarbital on choline uptake in hippocampal or cortical slices in vitro may not represent the same mechanism affected by anesthesia in the intact animal. It is nevertheless important with respect to the TRH antagonism of pentobarbital effects that there is a biochemical correlate with the interaction in vivo.

The role of TRH in natural arousal (i.e. not from drug-induced CNS depression) is not known. The interesting work of Stanton et al (81) on the arousal of animals from hibernation supports the view that TRH may act as a natural arousal agent. Doses as low as 0.1 ng of TRH microinjected into the dorsal hippocampus of the hibernating ground squirrel *(Citellus lateralis)* not only aroused, but also reversed the depressed metabolism and temperature states associated with hibernation. In later studies they demonstrated that the TRH effect varied with the initial arousal state of the animal. In awake animals it produced effects opposite to those seen in the hibernating animals—hypothermia, behavioral quieting, and decreased metabolic and EMG activity. The magnitude of these responses was greater during periods when animals were behaviorally active (82). Regional brain levels of TRH in these animals varied during different seasons of euthermia and during hibernation. While several regions exhibited seasonal variations in brain TRH levels, the most pronounced change was seen in the pineal, in which TRH levels rose over threefold between the early and late hibernation periods. The authors suggested that the change in pineal TRH might be associated with the activation of neuronal mechanisms necessary for arousal (83).

Body Temperature Effects

Peripherally and centrally administered TRH has been reported to increase body temperature in different species of animals. However, negative results have also been reported in the rat. In conscious animals, the increase in body temperature may be a secondary effect of the increased locomotor activity and body shaking behavior induced by TRH. Thus far, no attempt has been made to isolate the influence of these factors on the temperature effects of TRH.

Effects of TRH on thermoregulation in the conscious rat have been studied by Lin et al (84). Intracerebroventricular TRH produced hypothermia at lower ambient temperatures from 8–20°C, but hyperthermia at an ambient temperature of 30°C. These data indicate a loss of thermoregulatory response after i.c.v. TRH injection. However, TRH injected into the preoptic-anterior hypothalamus (85) produced only hyperthermia at all ambient temperatures studied (8–30°C); thus a change of body temperature set point had been initiated by TRH. The hyperthermia caused by TRH injection into the preoptic-anterior hypothalamus was due either to cutaneous vasoconstriction or to an increase in

metabolism, depending upon the ambient temperature, and was blocked by intrahypothalamic injections of yohimbine, phentolamine, or DL-propranolol; thus, involvement of adrenergic mechanisms is suggested (86).

Although many studies have been undertaken of the interaction of TRH with other drugs on body temperature, few have focused on the brain mechanisms of this response. Kalivas & Horita (61) investigated the brain loci of this thermal effect of TRH. In agreement with their finding that the anterior hypothalamus/preoptic area of the rat was the most sensitive site for TRH in reversing pentobarbital-induced hypothermia, Salzman & Beckman (87) showed that the warm-sensitive cells in the anterior hypothalamus/preoptic area were inhibited by iontophoretically applied TRH, whereas cold-sensitive cells in that same area were relatively less sensitive. Thus, the effect of TRH on the warm-sensitive cells could conceivably lead to a hyperthermic response.

That the thermogenic effect of TRH has a specific brain locus of action is further confirmed by a recent multiple-dissociation study of Sharp et al (75) on TRH and its analogues RX 77368 and CG 3509. Pentobarbital-induced hypothermia was reversed after microinjection of these drugs into the nucleus accumbens and septum, but no significant effect was seen after intrastriatal injection. It was also interesting that the analeptic effect of these drugs seemed to correlate with the thermogenic effect, i.e. analeptic effect of TRH was seen after intra-accumbens and intraseptal injections but not with intrastriatal injection. It is possible that the analeptic and thermal effects of TRH could interact with each other.

Other recent studies have been devoted to the interactions of TRH and other peptides on body temperature. In conscious rats i.c.v. TRH (10 μg) antagonized hypothermia induced by naloxone and somatostatin, but not that produced by bombesin and neurotensin (88). However, in mice, hypothermia induced by intracisternally administered neurotensin was antagonized by TRH or its analogues (89). The discrepancy between the above two studies could reflect differences in animal species used or the routes of drug administration.

Effects on Autonomic Functions

The central administration of TRH in experimental animals produces effects associated with increased activity of the peripheral sympathetic and parasympathetic nervous systems. The sympathetic responses observed include vasopressor activity, increased heart rate, piloerection, pupillary dilation, hyperthermia, and hyperglycemia. Most of these effects are probably the consequence of catecholamine release from adrenal medulla and sympathetic nerve endings (90). Concomitantly, one observes increased gastrointestinal (GI) motor activity (91), intestinal transit and diarrhea (92), gastric acid secretion (93), pancreatic secretion (94), and activation of superior laryngeal nerve to the thyroid gland (95, 96). All of these responses were antagonized by

atropine and/or vagal transection. These observations, together with the fact that TRH is found in high concentrations in several discrete areas in the medulla (dorsal motor nucleus of vagus, nucleus tractus solitarius, nucleus ambiguus) and preganglionic autonomic neurons in several species, strongly support the growing view that TRH serves as a central regulator of peripheral autonomic function (97, 98).

CARDIOVASCULAR EFFECTS Prior to 1981 little attention was paid to the cardiovascular actions of TRH. Microinjections of TRH into cisterna magna or into the cerebral ventricle of rabbits and rats (99) produced pressor responses. In rabbits the response was not totally abolished by α-adrenergic or ganglionic blockers, nor by thoracic cord transection (100). These results prompted the search for other potential vasopressor agents released by TRH. Vasopressin was considered as a possible candidate when blood levels were found to be increased in animals after central administration of TRH (101). It may play a part in the TRH reversal of drug-induced hypotension. This was suggested by the finding that the pressor response of TRH in clonidine- or α-methyldopa–pretreated animals was blocked by a vasopressin antagonist, but not by prazosin or hexamethonium (102).

A most important discovery was the finding that TRH improved cardiovascular function and survival of animals exposed to experimental endotoxic or hemorrhagic shock (103, 104). This pressor effect of i.c.v. TRH was absent in endotoxic rats with demedullated adrenals, but the response persisted when TRH was given i.v. The TRH-induced respiratory stimulation persisted under all experimental conditions. The ability of TRH to reverse hypotension appears to be relatively nonspecific, for it is effective against hypotension produced by anaphylactic shock (105, 106), leukotriene (107), and platelet-activating factor (108), as well as endotoxin and hemorrhage.

Feuerstein et al (109) found that microinjection of 0.8–80 nM TRH into the medial preoptic nucleus (POM) of conscious rats elicited dose-related pressor and cardiac-stimulant responses accompanied by increased plasma levels of norepinephrine and epinephrine. These cardiovascular effects of TRH were also produced in adrenal demedullated animals, but were abolished in adrenal demedullated + bretylium-pretreated animals. These results suggested that the vasopressor effect of TRH injected into POM was largely mediated by catecholamine release, especially of norepinephrine, from sympathetic nerves. The injections of TRH (30–150 nM) into the nucleus tractus solitarius produced responses of lesser magnitude and duration.

Diz & Jacobowitz (110) further delineated the brain site(s) for the cardiovascular actions of TRH to specific preoptic and hypothalamic nuclei. Microinjection of doses as low as 1.4 pmol (0.5 ng) into medial and suprachiasmatic preoptic nuclei increased blood pressure and heart rate. Both

responses were also seen with TRH injections into the posterior hypothalamic nucleus, but only tachycardia was seen after injection into anterior and dorsomedial hypothalamic nuclei. Changes in regional blood flow may also contribute to the TRH effect in experimental shock. Koskinen & Bill (111) demonstrated in rabbits that i.v. injections of 2 mg/kg of TRH produced approximately a 70% increase in cerebral blood flow. Mean arterial blood pressure and arterial $PaCO_2$ were also increased, but these effects could not account for the increases in cerebral blood flow. Peripheral organs showed mainly a reduced blood flow after TRH administration; in some the effect was blocked in sympathectomized animals. It would thus appear that the effect was mediated via sympathetic innervation.

It is clear from this discussion that TRH can produce dramatic improvement of cardiovascular function in animals exposed to various forms of shock and traumatic injury. The high sensitivity of the POM to TRH, and the known presence of TRH nerve cells in preoptic-hypothalamic areas, strongly suggest a role of this peptide in central control of the cardiovascular system. The current status of its possible mechanism of action has been discussed in recent reviews (112, 113).

GASTROINTESTINAL EFFECTS One of the most profound autonomic effects of TRH is that produced on the GI tract in rabbits. Even in the intact anesthetized animal the GI response to TRH is recognizable as a massive vermiform movement of the abdominal region. In conscious animals a watery diarrhea is consistently observed, and with larger doses it is reminiscent of the diarrhea described after administration of cholera toxin. With the long-acting compounds, such as MK-771, the diarrhea lasts for over 24 hours. TRH, in doses as low as 0.1 μg i.c.v. to anesthetized rabbits, initiated increased smooth muscle contractions of the entire GI tract. It was evident that these effects were mediated via central vagal mechanisms, for they were completely blocked by bilateral vagotomy, hexamethonium, and atropine (91).

In other studies in which motility and rate of colonic transit were compared, it became clear that the two were unrelated and that only the latter was related to the development of the diarrhea. Atropine or bilateral vagotomy blocked the former, whereas only vagotomy attenuated the transit and diarrhea responses. Examination of the intestinal tract showed massive fluid accumulation, which fluidized the fecal material and enhanced its transit in an aboral direction. That serotonin release was involved was suggested by the findings that (*a*) antiserotonin drugs blocked the colonic transit and diarrhea effects of TRH (but not the hypermotility), and (*b*) TRH administered centrally produced a dose-related increase in levels of portal blood serotonin. High doses of TRH (e.g. 100 μg i.c.v.) produced prolonged hyperserotonemia (>2 hr). Like the diarrhea response, the hyperserotonemia was attenuated by bilateral vagotomy but not by

atropine (92). Therefore, centrally administered TRH produced not only a cholinergically mediated hypermotility, but also a vagally mediated release of intestinal serotonin; the latter was responsible for the production of diarrhea.

TRH effects on the GI system have been reported in other species, although they appear not to be identical with those in the rabbit. For instance, rats exhibit vagally mediated hypermotility (114), gastric acid secretion (95) leading to gastric erosion (115), hyperserotonemia, and intestinal fluid accumulation, but even with high doses there was no evidence of watery diarrhea as was seen in the rabbit (A. Horita and M. A. Carino, unpublished). In contrast, Metcalf & Myers (116) reported frequent salivation, vomiting, and defecation in cats given small quantities of TRH into the lateral ventricles. The response to TRH in mice and dogs represents a departure from that described in most other species in showing a decrease in GI transit (117) and acid secretion (118), respectively.

The GI effects produced by i.c.v. TRH in rabbits have shown some interesting drug interactions. Naloxone (2.5 mg/kg i.p.) or naltrexone (1.0 mg/kg i.p.) blocked the charcoal transit, fluid accumulation, and diarrhea formation induced by i.c.v. TRH, but not the cholinergically mediated hypermotility. The TRH-induced portal hyperserotonemia was also not affected by these antagonists (119). The authors suggested that this TRH-opiate antagonist interaction was probably not associated with specific blockade of mu-opiate receptors because of the large doses required. Some indirect evidence suggested that naloxone and naltrexone blocked serotonin on the secretory and/or smooth muscle receptors. In contrast, the TRH-mediated inhibition of GI transit in mice was blocked by 0.1 mg/kg of naloxone, which suggested that opiate receptors may be involved in this response in mice (117).

Other drug interactions of interest on the GI effects of TRH have been reported. Clonidine (120) or a combination of 6-hydroxydopamine (i.c.v.) and α-methyltyrosine (121) abolished TRH-induced acid secretion, as did dopamine agonists (122), and corticotropin-releasing factor (123). These interactions occurred centrally and add further evidence that central monoaminergic and peptidergic systems modulate vagal responses to the GI tract.

Effects on Spinocerebellar Functions

After having observed beneficial effects of TRH on hypotensive shock produced in animals with spinal cord injury, Faden et al (124, 125) examined its effects on the neurological symptoms produced by the injury. The neurologic function scores of TRH-treated animals were higher than those of dexamethasone- or saline-treated animals over the six-week period of the study. Whereas the latter animals displayed spasticity and/or ataxia, the TRH animals exhibited normal motor function. In addition, all six animals given TRH

survived for six weeks, whereas four animals died in each of the dexamethasone and saline groups. Surprisingly, histopathologic evaluation of the area of injury showed no differences between treated and control animals; thus, other more specific histological methods are necessary in order to differentiate degrees of spinal cord injury. In subsequent studies, the authors found a dose-response relationship (0.02–2.0 mg/kg/hr i.v.) in which even the lowest dose produced better motor recovery than saline controls. An especially important finding was that the high dose of TRH given 1 or 24 hr after injury produced essentially the same degree of improvement of neurological function (126). This finding is of considerable clinical significance since it is generally assumed that 4–8 hr after spinal cord injury the neuropathological effects are irreversible.

Partly as a result of these successful animal studies, Engel et al (127) employed TRH and found its favorable effects in patients with amyotrophic lateral sclerosis (ALS). Large doses of TRH (up to 500 mg in 2–5 hr/day) were infused intravenously into twelve patients. Moderate to marked improvement of muscle weakness and spasticity were observed, although all affected muscle areas were not equally improved. Some of the improved responses included increased vital capacity, speech clarity and volume, and improved walking agility. All of the improvements persisted during the infusion and for 0.5–1 hr afterwards. Side effects included shivering, tachypnea, sweating, urinary urgency, and abdominal cramps. With higher doses increased blood pressure and heart rate were noted. The high doses used in these studies may be necessary to produce therapeutic effects since other workers using 4 mg i.m. for two weeks failed to demonstrate improved muscle strength in nine patients with ALS (128).

While Engel was the first to report on the efficacy of TRH in ALS, similar observations were made by Sobue et al (129), who employed the peptide in the treatment of ataxia of spinocerebellar degeneration (SCD). TRH was given i.m. in doses of 2 mg, 0.5 mg, or placebo once a day for two weeks. Global improvement and ataxia improvement ratings showed that both doses of TRH were significantly superior to placebo in patients with predominantly cerebellar forms of SCD, and this effect persisted for a week after cessation of treatment. The 2-mg dose was also more effective than placebo in improving standing, speech, and writing.

The mechanism of TRH activity in spinocerebellar injury is not known. It has been suggested that TRH might exert effects opposite to those produced by some of the endogenous opioids released after trauma (103). Numerous investigators have demonstrated not only pharmacologic activity, but also the localization and distribution of TRH and its receptors in spinal cord. More recent studies on the distribution of TRH in monkey (98) and human (130) spinal cord confirm earlier rat data that TRH is present in motoneurons and preganglionic autonomic neurons. ALS patients display lowered TRH levels in

their anterior horn (131). Lesion studies indicate that the perikarya of TRH neurons in spinal cord originate in the ventral medulla, and that many of these also contain 5-hydroxytryptamine (5HT), suggesting that they are cotransmitters (132). Motoneuron damage induced by neurectomy or virus infection was associated with redistribution of fibers containing 5HT and TRH. Treatment with TRH normalized this redistribution response (133). TRH infusions also antagonized motor deficits produced by reserpine, but not those produced by strychnine; thus monoaminergic systems in descending motor pathways might be important for this TRH effect (134). TRH administered to chick embryo enhanced survival of spinal cord motoneurons that would normally have died during development. This effect may be mediated by c-GMP activation by TRH (135). Also, treatment of cultured spinal ventral horn neurons from rat embryos with TRH for 2–5 weeks enhanced growth of cells and produced a 16-fold increase over controls of choline acetyltransferase activity (136).

Alterations in TRH receptors may be involved in genetically or experimentally induced spinocerebellar dysfunctions. Administration of TRH to ataxic mice has been reported to cause instantaneous recovery from ataxia. In the ataxic mutant Rolling Mouse Nagoya, the number of TRH receptors was significantly lower in cerebellum and higher in spinal cord than in normal mice (137). Newborn mice showed decreased numbers of TRH receptors in spinal cord at 2 weeks, but not at 4 weeks, after inoculation with murine leukemia virus. The change in receptor density preceded the neurological signs, since motor dysfunction appeared 4–5 weeks after exposure to the virus (138). In contrast, Hawkins & Engel (139) hypothesize that the effects of TRH and its analogues on the spinal cord might be mediated via non-TRH receptors.

CONCLUSIONS

In this review we have discussed the current status of some aspects of the pharmacology of TRH. The presence of TRH and its receptors in various regions of brain and spinal cord and the many diverse effects associated with its administration into specific brain sites strongly support the growing contention that it serves as an endogenous regulator of neural function. It appears that many of the extrapituitary effects of TRH are mediated by other neurotransmitters, notably acetylcholine and the monoamines, so that this peptide is delegated the role of a neuromodulator of those systems.

Some important beginnings as to possible clinical utility of TRH have been made. In addition to its use in spinocerebellar disorders as discussed above, its potential application in some forms of cardiovascular shock seems imminent. Speculations and suggestions as to these and other possible clinical uses of TRH have also been made (140–142). If any of the pharmacological properties of TRH becomes important in clinical medicine, it is unlikely that TRH itself will

be selected as the drug of choice. Because of its transient action due to rapid inactivation and the need for high doses in parenteral form, synthetic analogues with the appropriate potency, specificity, and pharmacokinetic properties will be sought. Indeed, several compounds with some of these attributes have been developed. TRH and its analogues are becoming a unique class of drugs that may be useful in certain clinical disorders previously treated with little or no success. Continued neuropsychopharmacological and neurochemical research of TRH mechanisms is necessary for a full understanding of the basis for its clinical efficacy.

ACKNOWLEDGMENT

The authors' research has been supported in part by a grant from the US Department of Health and Human Services, NS-19210.

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