as methadone extends beyond their analgesic properties and, therefore, that they may exert their effects through the same spectrum of neurochemical actions.

10. We have obtained similar effects with chlorpromazine (25 mg/kg, subcutaneously).
11. J. Blasig and A. Herz, *Naunyn Schmiedeberg's Arch. Pharmacol.* **294**, 297 (1976); H. Teschemacher, J. Blasig, W. Kromer, *ibid.*, p. 293.
12. Bilateral cannulas (separated by 1 to 2 mm) were stereotaxically implanted approximately 1 mm above the dorsal surface of the PAG. Injection needles, when in place, extended 2 mm beyond the tips of the guide cannulas. The injection volume was 1 μl per side administered at a rate of 0.1 μl per 15 seconds.
13. Cannula placements were confirmed by injection of methylene blue dye in a volume of 1 μl per side at a rate of 0.1 μl per 15 seconds. Examination of 40-μm sections were made through the extent of dye spread.
14. Others [L. G. Sharpe, J. E. Garnett, T. J. Cicero, *Behav. Biol.* **11**, 303 (1974)] have found that injections of morphine into the dorsolateral PAG produced hyperresponsivity, whereas after ventromedial injections animals became hyporesponsive.
15. Haloperidol as well as other neuroleptics have been shown to enhance conversion of tyrosine to dopamine in caudate synaptosomes [R. Kuczenski, in *Neurobiological Mechanisms of Adaptation and Behavior*, A. J. Mandell, Ed. (Raven, New York, 1975), p. 109; ———, D. Schmidt, N. Leith, *Brain Res.* **126**, 117 (1977)]. This effect has been interpreted as reflecting a compensatory response to the dopamine receptor blockade produced by neuroleptics. We have found that, 45 minutes after injection, haloperidol (4 mg/kg) produced a 33 percent increase ($P < .02$) in caudate synaptosomal conversion of tyrosine to dopamine, but that neither methadone at 10 mg/kg nor 50 μg of β-endorphin (doses that produced profound muscular rigidity) affects this measure of dopamine biosynthesis. These results appear to indicate that neither the effects of β-endorphin nor methadone are mediated through inactivation of dopamine receptors in the caudate.
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17. We previously suggested that β-endorphin and related neuropeptides might be involved in the etiology of schizophrenic symptoms, and therefore that opiate antagonists might be therapeutically effective in the treatment of schizophrenia (*1*). In fact, it has been recently reported that 0.4 mg of naloxone, given intravenously, temporarily reduced auditory hallucinations in four long-term schizophrenics [L.-M. Gunne, L. Lindstrom, L. Terenius, *J. Neural Transm.* **40**, 13 (1977)]. In contrast, we have found, using a coded crossover design, that 1.2 mg of naloxone administered intravenously to eight long-term schizophrenics was ineffective in relieving psychotic symptoms (D. S. Janowsky, D. S. Segal, A. Abrams, F. Bloom, R. Guillemin, *Psychopharmacology*, in press). However, our current animal studies (Table 2) demonstrate that doses of naloxone much higher than those used in the clinical studies produce only a transient reversal of the effects induced by β-endorphin. Therefore, relatively high doses of an opiate antagonist may be required to produce observable antipsychotic effects.
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19. It has been shown that naloxone antagonized *d*-amphetamine-induced increases in avoidance responding, locomotor activity and intracranial self-stimulation in rats [E. Holtzman, *J. Pharmacol. Exp. Ther.* **189**, 51 (1974); *Psychopharmacologia* **46**, 223 (1976)]. Similarly, we have found that doses of naloxone as low as 0.5 mg/kg (subcutaneously) significantly reduced the locomotor activating effects of *d*-amphetamine (0.5 mg/kg) and methylphenidate (10 mg/kg).
20. We thank J. Cahill and S. Hines for technical assistance. Supported in part by PHS grant DA-01568-01 and research scientist award MH-70183-04 (to D.S.S.), by PHS research fellowship DA-01242-02 (to R.G.B.), by grants from the Alfred B. Sloan Foundation (to F.E.B.), by NIH grants HD-09690 and AM 19911 (to N.L. and R.G.), and by the William Randolph Hearst Foundation.

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Thyrotropin-Releasing Hormone: Abundance in the Skin of the Frog, *Rana pipiens*

Abstract. *Thyrotropin-releasing hormone, a hypothalamic tripeptide that stimulates the secretion of pituitary thyroid-stimulating hormone in mammalian species and is widely distributed throughout the brain of vertebrates, is present in the skin of the frog (*Rana pipiens*) in concentrations twice that found in the hypothalamus of this amphibian. A skin extract shows biologic activity appropriate to its immunoreactive content. Apart from the brain and spinal cord, immunoreactive thyrotropin-releasing hormone is found only in the blood and retina in significant concentrations. The results imply that frog skin is a huge endocrine organ that synthesizes and secretes this hormone.*

Thyrotropin-releasing hormone, pyroglutamyl-histidyl-prolinamide, is synthesized in the hypothalamus of mammalian species and plays an important role in regulating the secretion of thyroid-stimulating hormone (TSH) from the adenohypophysis (*1*). In mammals more than 70 percent of thyrotropin-releasng hormone (TRH) in the central nervous system lies outside the hypothalamus in brain and spinal cord (*2*), and none has been reported in other organs. Although TRH has no thyroidal action in species lower than Aves (*3*), large quantities of immunoreactive (IR) TRH are present in the extrahypothalamic brain tissues of the snake, frog, and fish (*4*). That the material found is, in fact, TRH is supported by the finding that extracts of these tissues give inhibition curves parallel to those for standard TRH in the TRH radioimmunoassay and that an extract of extrahypothalamic frog brain releases TSH when injected into rats (*4*). The anatomic and phylogenetic distribution of TRH and behavioral (*5*) and neurophysiologic (*6*) studies in mammalian species support a role for TRH in neuronal function, possibly as a neurotransmitter.

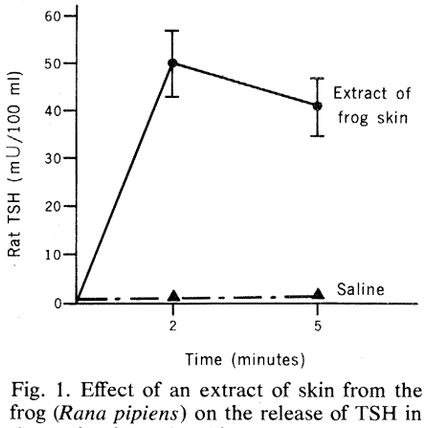

Fig. 1. Effect of an extract of skin from the frog (*Rana pipiens*) on the release of TSH in the rat in vivo. The skin was extracted in 90 percent methanol and the dried supernatant, reconstituted in buffer, was assayed for IR-TRH content. Skin extract containing 100 ng of IR-TRH, made up to 1 ml with saline solution, was injected intravenously into each of five Sprague-Dawley male rats, under Nembutal anesthesia, and blood was sampled at 2 and 5 minutes for TSH measurement. Each of the five control rats received saline solution alone. The results (means ± standard errors of the means) show the rise in serum TSH. The skin extract exhibited biologic potency appropriate to its content of IR-TRH. Saline-treated controls showed no TSH rise.

During the course of investigations of the physiologic role of TRH in the amphibian, we found large quantities of IR-TRH in the circulation of the leopard frog (*Rana pipiens*) in concentrations of 100 ng or more per milliliter of whole blood (*7*). For comparison, blood levels in humans and rats are estimated to be less than 30 pg/ml (*8*). Since the whole brain of the frog weighs only 100 to 150 mg and contains approximately 100 ng of TRH (*7*), we thought it unlikely that TRH secretion from brain tissue could be the source of the bulk of blood TRH. Accordingly, we examined the tissue distribution of TRH in *R. pipiens* to determine whether there was an extraneural site of TRH.

Four adult male frogs (*9*) were decapitated. The blood was collected in chilled tubes, 0.1-ml samples were quickly added to 1-ml portions of ethanol, and the resulting samples were extracted for TRH immunoassay (*10*). Hypothalamus, extrahypothalamic brain, spinal cord, splanchnic nerves, and fragments of heart, lung, tongue, stomach, intestine, liver, spleen, kidney, gonad, muscle, and skin were placed in 90 percent methanol for subsequent extraction for TRH assay, as described previously (*4*). Retinal tissue from another group of six frogs was similarly extracted. The protein in each sample was determined by the method of Lowry *et al.* (*11*). Table 1 shows the TRH concentrations in various organs. The concentration in blood is given for reference. Apart from the hypothalamus, brain, and spinal cord, the only organs with elevated TRH concentrations are the skin and retina. The concentration in the skin is much higher than that in the hypothalamus. The TRH levels in the thoracic and abdominal organs can probably be accounted for by contained blood, with the possible exception

of the tongue, which has a TRH concentration at least six times greater than that of any other gastrointestinal tissue.

To determine the biologic activity of the skin IR-TRH, all the skin from a decapitated frog was removed and extracted with 90 percent methanol. The dried extract was dissolved in saline solution, and a portion diluted in phosphate-buffered saline (*p*H 7.5) was assayed for IR-TRH. An extract of skin containing 100 ng of IR-TRH given intravenously to a group of five male rats (Charles River) produced a marked rise in serum TSH at 2 and 5 minutes after injection. Animals injected with saline solution alone showed no increase in serum TSH (Fig. 1). The rise in serum TSH with the skin extract is comparable to that obtained with 100 ng of synthetic TRH. Since skin accounts for about 8 percent of the body weight in *R. pipiens* (*7*), it is probable that circulating TRH reflects mainly secretion from the skin.

We examined the possibility that the skin might trap TRH from the circulation. After administration of [^{3}H]TRH, the concentration gradient from skin to blood was 0.29 in one frog and less than 0.10 in another (*12*). Since the concentration of endogenous TRH in the skin is 91 times that in the blood (Table 1), it seems likely that most of the TRH present in the skin is synthesized in situ. Uptake or reuptake of TRH from the blood is not excluded, however, and may contribute a small proportion to the overall skin TRH content.

The retina, which develops from the optic stalk, part of the primitive forebrain, is an extension of the central nervous system. It is thus noteworthy that significant quantities of TRH are present in the retina, which has important anatomic and functional connections with, as well as histological similarities to, the pineal (*13*), for we have shown that photoillumination affects the TRH content of the frog pineal (*14*).

The presence of large quantities of TRH in the skin, which is embryologically related to the brain, suggests a possible role for this peptide in skin function and metabolism and implies that the skin of the frog is a huge TRH-secreting organ.

Pearse (*15*) has pointed out that a number of frog skin peptides share amino acid sequences with peptides found in mammalian brain. These include caerulein and phyllocaerulein (both related to gastrin) and physalaemin (related to substance P). Interestingly, substance P, which is located in the gastrointestinal tract as well as the central nervous system, has been reported to be immunohistochemically stainable in sensory nerves of mammalian skin (*16*). Bombesin, another amphibian skin peptide known to be present in the human gastrointestinal tract, has been detected in the rat brain, where it may have a physiological role in its interaction with TRH (*17*).

Table 1. Concentration of TRH in various frog (*Rana pipiens*) tissues removed from a group of four animals. The blood TRH level is given for comparison. Values are means ± standard errors of the means.

Organ	TRH (micrograms per gram of protein)
Hypothalamus	14.9 ± 1.0
Extrahypothalamic brain	7.7 ± 1.2
Spinal cord	2.3 ± 1.1
Splanchnic nerve	0.072 ± 0.03
Skin*	26.1 ± 15.4
Retina†	3.3 ± 0.4
Heart	0.057 ± 0.009
Lung	0.058 ± 0.018
Tongue	0.28 ± 0.13
Stomach	0.041 ± 0.007
Intestine	0.023 ± 0.004
Liver	0.019 ± 0.005
Spleen	0.025 ± 0.009
Kidney	0.019 ± 0.007
Gonad	0.015 ± 0.006
Muscle	0.021 ± 0.010
Blood	0.045 ± 0.007‡

*The protein content of the skin is 15.7 ± 0.4 percent, wet weight (N = 6). The mean skin-blood concentration gradient of TRH is 91 : 1. †Tissue obtained from a separate group of six frogs. ‡Micrograms per milliliter of whole blood.

Anuran cutaneous glands that synthesize the skin peptides are considered to arise from specialized ectoderm related to neural crest tissue (*15*). We suggest that TRH in frog skin may be derived similarly. Further studies are required to determine the phylogenetic distribution as well as the factors regulating skin TRH, but clearly the role of TRH, like that of another hypothalamic releasing-hormone, somatostatin (*18*), is not limited to the central nervous system.

Note added in proof: After this report had been submitted for publication, we learned of the work of Yasuhara and Nakajima (*19*), who described the occurrence of a tripeptide, chemically characterized as pyroglutamyl-histidyl-prolinamide, in an extract of skin from the Korean frog, *Bombina orientalis* Boulenger. This finding provides further support for the authenticity of the TRH, present in frog skin, that we report here.

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9. Obtained from the Connecticut Valley Biological Supply Company, Southampton, Mass.
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