concentrations, taurine and β-alanine showed stimulated influxes of the same magnitude in the presence of isoproterenol, whereas leucine, serine, or aminoisobutyric acid (a transportable but nonmetabolizable amino acid) showed no change. Furthermore, α-amino acids did not influence the influx of taurine when perfused simultaneously with taurine at concentrations fivefold higher. β-Alanine under the same conditions inhibited taurine influx by 85 percent. The lack of competition between α- and β-amino acids indicates that transport occurs in the heart at separate sites, one site being specific for β-amino acids. Similarly, specific transport sites for β-amino acids have been shown to exist in Ehrlich ascites cells (*6*), and brain (*10*). Other workers have shown that increased influx of nonutilizable α-amino acids occurs in hearts under work stress (*11*). These increases were not observed until after 15 minutes of increased work load in isolated working hearts. The stimulation in β-amino acid influx reported here occurs within 20 seconds of exposure of the heart to isoproterenol, the first time interval at which we are able to determine influx rate.

The major pharmacological action of isoproterenol is that of β-adrenergic agonism. That the effect observed on the β-amino acids was indeed an adrenergic action was verified by the finding that propranolol blocked isoproterenol-induced stimulation of taurine influx (Fig. 3). However, perfusion of propranolol in the absence of isoproterenol did not result in a measurable change in influx of taurine. This suggests that there is one component of taurine influx in the heart which is independent of adrenergic influences and another component that is adrenergically modulated. Additional evidence for adrenergic involvement is shown in Table 2. β-Adrenergic effects are generally considered to be mediated intracellularly by the formation of adenosine 3′,5′-monophosphate (cyclic AMP). Perfusion of hearts with dibutyryl cyclic AMP resulted in a stimulation of taurine uptake of the same magnitude observed with isoproterenol. Theophylline, a phosphodiesterase inhibitor that decreases the rate of enzymatic hydrolysis of cyclic AMP, elevating endogenous levels, produced a similar result.

On the basis of our observations, we propose the following: A high-affinity uptake system for taurine exists in the heart which has a basal rate in the absence of adrenergic influences. In the presence of β-adrenergic stimulation this system is stimulated; the level of stimulation depending on the degree of β-adrenergic activation. At maximum stimulation, the system has 20 to 30 percent higher transport capacity than the noninduced system. The induction is cyclic AMP–dependent and possibly involves the phosphorylation of a membrane transport site. The elevated taurine concentrations found in the affected ventricles in congestive failure are explained by the presence of a modulated uptake system for taurine, maximally stimulated in a stressed heart in a state of chronic β-activation. In addition, this system provides a potentially important link between two agents that modulate calcium flux in the heart cell: β-adrenergic activation stimulates both calcium and taurine influx into the heart cell, and taurine modulates the pool size of free intracellular calcium.

Table 2. Effect of various agents on the rate of taurine influx. The concentrations were as follows: taurine, $1 \times 10^{-4} M$; isoproterenol $4 \times 10^{-7} M$; theophylline $1 \times 10^{-3} M$; and dibutyryl cyclic AMP $9.5 \times 10^{-4} M$. The control rate for the dibutyryl cyclic AMP experiment was 20.47 ± 0.24.

| Treatment | Influx [nanomole per gram (dry weight) per minute] | Percentage of control |
|---|---|---|
| Control | 20.01 ± 0.40 | 100 ± 2 |
| Isoproterenol | 23.96 ± 0.80* | 119 ± 4 |
| Theophylline | 22.67 ± 0.03* | 112 ± 1 |
| Dibutyryl cyclic AMP | 25.21 ± 1.73* | 123 ± 8 |

RYAN HUXTABLE
JAMES CHUBB
*Department of Pharmacology, University of Arizona, Health Sciences Center, Tucson 85724*

**References and Notes**

1. R. Huxtable, in *Taurine*, R. Huxtable and A. Barbeau, Eds. (Raven, New York, 1976), p. 99.
2. J. G. Jacobsen and L. H. Smith, *Physiol. Rev.* **48**, 424 (1968).
3. R. Huxtable and R. Bressler, *Science* **184**, 1187 (1974); *Life Sci.* **14**, 1353 (1974); M. B. Peterson, R. J. Mead, J. D. Welty, *J. Mol. Cell. Cardiol.* **5**, 139 (1973).
4. S. Duprè and C. De Marco, *Ital. J. Biochem.* **13**, 386 (1964); R. Huxtable and R. Bressler, in *Taurine*, R. Huxtable and A. Barbeau, Eds. (Raven, New York, 1976), p. 45.
5. E. J. Peck, Jr., and J. Awapara, *Biochim. Biophys. Acta* **141**, 499 (1967).
6. D. L. Oxender and H. N. Christensen, *J. Biol. Chem.* **238**, 3686 (1963).
7. Z. N. Gaut and C. B. Nauss, in *Taurine*, R. Huxtable and A. Barbeau, Eds. (Raven, New York, 1976), p. 91.
8. R. E. Hruska, R. J. Huxtable, R. Bressler, H. I. Yamamura, *Proc. West. Pharmacol. Soc.* **19**, 152 (1976).
9. R. Huxtable, *Circ. Res.* **38**, I-112 (1976).
10. P. Lähdesmäki and S. S. Oja, *J. Neurochem.* **20**, 1411 (1973).
11. K. Ahrén, Å. Hjalmarson, O. Isaksson, *Acta Physiol. Scand.* **86**, 257 (1972).
12. Statistical analyses were performed by unpaired Student's *t*-test. Differences were considered significant at a probability of 5 percent.
13. We thank M. Rowley for technical assistance. This work was supported by grants HL 20087 and HL 19394 from the Public Health Service.

31 May 1977

## β-Endorphin: Endogenous Opiate or Neuroleptic?

Abstract. *The opiatelike neuropeptide β-endorphin produces a spectrum of effects that contrasts with that induced by the neuroleptic haloperidol. Rats injected intraventricularly or directly into the periaqueductal gray with β-endorphin (0.5 to 50 micrograms) exhibited rigid immobility accompanied by the loss of righting reflex; the period of rigidity was preceded or followed (depending upon dose) by a state of hyperactivity. In contrast, no dose of haloperidol tested (0.5 to 12 milligrams per kilogram) produced rigidity, loss of righting reflex, or behavioral excitation. Furthermore, whereas animals injected with haloperidol remained stationary on a vertical grid, rats injected with β-endorphin typically slid off the grid. Moreover, combined β-endorphin and haloperidol treatment produced flaccidity in most animals. These results do not support the contention that this opiatelike peptide may be a naturally occurring neuroleptic.*

Recently we reported that intraventricular administration of β-endorphin, which is β-lipotropin, residues 61 to 91 [β-LPH-(61-91)], induces in rats a profound state of immobilization characterized by the absence of movement, loss of righting response, and extreme generalized muscular rigidity (*1*). Similar results have since been obtained by others (*2*). However, Jacquet and Marks (*3*) reported that injections of β-endorphin into the periaqueductal gray (PAG) elicit a "cataleptic-like" state similar to that produced by most neuroleptics (antipsychotic drugs). On the basis of these observations, they suggested that β-endorphin may be an endogenous neuroleptic and that its reduced availability may be etiologically significant in certain forms of psychopathology. Differentiation of the effects of β-endorphin as rigid immobility or neuroleptic-like catalepsy may be crucial, both with respect to mechanistic considerations as well as possible clinical implications. Therefore, we have extended our earlier studies to character-

ize more completely the state of immobilization produced by β-endorphin and to compare it with the behavioral profile produced by the neuroleptic agent haloperidol.

Adult male Wistar rats (350 to 400 g) were obtained from Hilltop Laboratories and housed for 1 week under standard laboratory conditions. For intraventricular injections, the rats were stereotaxically implanted with a stainless steel cannula (21 gauge) over the right lateral ventricle (DeGroot coordinates: A, 5.4; ML, 2.5; H, +4.0). Injections and behavioral testing took place more than 1 week after surgery. The injection volume was 10 μl, administered over a 1-minute interval through an injection needle (27 gauge) which extended 1 mm beyond the tip of the guide cannula. After they were tested, animals were infused with methylene blue dye, and the cannula placements were verified by gross dissection of the brain. Only data from animals with accurate placements are reported. β-Endorphin was prepared by solid phase synthesis and purified (*4*). Test solutions were prepared in isotonic saline from dry powders and used 1 day only. Haloperidol (Haldol) was purchased from McNeil Laboratories, Inc., and was injected subcutaneously in unoperated rats.

Animals were placed individually into observation chambers (54 by 32 by 15 cm) for about 30 minutes prior to injection and were then tested for rigidity, immobility on a vertical grid, and righting response every 15 to 30 minutes for periods of 1 to 4 hours. Rigidity was evaluated by three tests: (i) subjective assessment during handling of the animals, (ii) trunk rigidity (*5*), and (iii) bridge test (*1*). The vertical grid measure is the only test [of those commonly used to characterize catalepsy (*6*)] that distinguishes behavioral immobilization associated with rigidity or flaccidity from neuroleptic-induced catalepsy. The righting reflex was monitored since it is retained after neuroleptic-induced catalepsy, but not after other drug-induced states of immobilization (*6*). In addition to these tests, animals were also regularly observed for gross behavioral and physiological changes.

A dose-response analysis of β-endorphin and haloperidol revealed a marked difference in their respective behavioral profiles (Table 1). Within 30 minutes after injection, most rats responded to the doses of β-endorphin tested with a brief period of wet-dog shakes followed by general muscular rigidity (accompanied by stiffness of the tail) and immobility. The rigidity persisted for up to 4 hours after injection with the highest dose (50 μg). Within minutes after injection of 5 μg, animals exhibited marked hyperactivity accompanied by oral stereotypies, periodically interrupted by episodes of wet-dog shakes (*7*). With this low dose, rigidity lasted for about 20 minutes, after which hyperactivity (but not the wet-dog shaking) again emerged. During the period of rigidity (especially that produced by the lower doses of β-endorphin, 5 and 10 μg), rats could be provoked into moving by mild auditory, visual, or tactile stimuli. Furthermore, the intensity of rigidity appeared to be reduced during tests at night, a time when rats are normally active. These results indicate that during the rigidity phase animals were capable of coordinated motor activity and that the behavioral immobility may have been partially due to an impaired ability to initiate voluntary movement.

In contrast to the effects of β-endorphin, no dose of haloperidol produced rigidity. In fact, with the higher doses of haloperidol (8 and 12 mg/kg) most animals were moderately flaccid, especially during the first 30 minutes after injection. The effects of β-endorphin and haloperidol were also opposite on the vertical grid test. Thus, animals injected with β-endorphin would quickly climb off the grid before and after the period of rigidity and typically would slide off of the grid during the rigidity phase. In contrast, rats that received haloperidol grasped the grid tightly and remained stationary for relatively long periods (at least 60 seconds). In addition, these two substances had different effects on the righting response: doses of β-endorphin as low as 5 μg always abolished this reflex during the rigidity phase, while the righting response was retained after injection of haloperidol at doses as high as 12 mg/kg. Furthermore, after injection of

Table 1. Behavioral characterization of β-endorphin and haloperidol. The rigidity score (0 to 4) represents a composite measure derived from three tests: (i) body stiffness, assessed during handling (scored 0 to 4); (ii) trunk rigidity; based on the time (up to 4 seconds) that the animal remained in an upright posture when held above the knee joints of the hind limbs (scored 0 to 4); and (iii) bridge test; a positive score assigned when the animal remained self-supporting for 10 seconds when placed across metal bookends. The vertical grid test was scored on a 0 to 3 scale based on the time (up to 60 seconds) that the rat remained immobile on the grid. A minus righting reflex was designated when the rat stayed up in a supine position for 10 seconds. Values (expressed as mean ± S.E.M.) indicate effects at 1 hour after injection.

| Treatment | Dose | *N* | Rigidity | Vertical grid | Righting reflex |
|---|---|---|---|---|---|
| β-Endorphin | None | 10 | | 0 | + |
| | 5.0 μg/10 ml | 12 | 1.5 ± 0.3 | Slides | − |
| | 10.0 μg/10 ml | 7 | 2.6 ± 0.4 | Slides | − |
| | 25.0 μg/10 ml | 4 | 3.4 ± 0.4 | Slides | − |
| | 50.0 μg/10 ml | 20 | 3.4 ± 0.2 | Slides | − |
| Haloperidol | None | 10 | 0 | 0 | + |
| | 0.5 mg/kg | 12 | 0 | 1.8 ± 0.3 | + |
| | 1.0 mg/kg | 12 | 0 | 2.9 ± 0.1 | + |
| | 2.0 mg/kg | 15 | 0 | 2.6 ± 0.2 | + |
| | 4.0 mg/kg | 10 | 0 | 2.5 ± 0.2 | + |
| | 8.0 mg/kg | 15 | 0 | 2.3 ± 0.2 | + |
| | 12.0 mg/kg | 6 | 0 | 2.6 ± 0.2 | + |

Table 2. Comparison of β-endorphin and haloperidol: pharmacological interactions. Successive injections were made at 1-hour intervals (see Table 1 and text for further details.)

| Prior treatment | Treatment | *N* | Rigidity | Vertical grid | Righting reflex |
|---|---|---|---|---|---|
| None | Naloxone (2 mg/kg) | 4 | 0 | 0 | + |
| None | β-Endorphin (50 μg/10 μl) | 20 | 3.4 ± 0.2 | Slide | − |
| β-Endorphin (50 μg/10 μl) | Naloxone (0.1 to 2.0 mg/kg) | 15 | 0 | 0 | + |
| None | Haloperidol (2 mg/kg) | 15 | 0 | 2.6 ± 0.2 | + |
| Haloperidol (2 mg/kg) | Naloxone (2 mg/kg) | 5 | 0 | 2.8 ± 0.1 | + |
| β-Endorphin (50 μg/10 μl) | Haloperidol (2 mg/kg) | 14 | Flaccid | Slide | − |
| β-Endorphin (50 μg/10 μl) + haloperidol (2 mg/kg) | Naloxone (2 mg/kg) | 7 | 0 | 2.1 ± 0.5 | + |

haloperidol (in contrast to β-endorphin), animals displayed a hunched posture with abducted limbs, vocalized when touched, and frequently urinated when placed on the vertical grid (*8*).

The effects of β-endorphin and haloperidol can also be differentiated on the basis of pharmacological interactions (Table 2). Thus, for example, although naloxone (2 mg/kg, injected subcutaneously) did not alter the effects of haloperidol, doses of naloxone as low as 0.1 mg/kg appeared to reverse all the actions of β-endorphin. At this dose of naloxone, recovery was transient and rigidity again emerged within 30 minutes after injection. Furthermore, animals made rigid with β-endorphin and later injected with haloperidol (2.0 mg/kg) became flaccid (*9*). In rats rendered flaccid by this combined treatment, naloxone (2 mg/kg) administration resulted in the emergence of the typical haloperidol effects (Table 2). Thus, naloxone, by selectively antagonizing the action of β-endorphin, unmasked the behavioral pattern induced by haloperidol (*10*).

Our studies and those of others indicate that β-endorphin and other opiate-like neuropeptides produce a multiplicity of effects, each of which may be mediated by the action of these substances at different sites in the brain (*1–3, 11*). Therefore, although intraventricular administration of β-endorphin results in a state of immobility that can be readily dissociated from the cataleptogenic action of haloperidol, the behavioral pattern elicited by β-endorphin may be different after intraventricular, as opposed to PAG, administration. Thus, the apparent discrepancy between our results and those of Jacquet and Marks (*3*) might be due to different patterns of neurochemical activation resulting from the different sites of administration.

In order to test this possibility, we injected either isotonic saline or 2, 4, or 8 μg of β-endorphin bilaterally into the PAG (*12*). The results revealed a dose-dependent pattern of effects similar to that produced by intraventricular administration of β-endorphin, with the most effective PAG site being the ventromedial region, encompassing the dorsal raphe (*13*). Therefore, in order to characterize these effects further, we used 15 additional rats to unilaterally implant cannulas for injection of β-endorphin into the ventromedial region of the PAG (Konig and Klippel coordinates: A, 0.1 to 0.5; ML 0.0; H, −0.6). In those animals ($N = 9$) that were subsequently confirmed to have accurate placements, 8 μg of β-endorphin produced a behavioral profile identical, in most respects, to that observed after intraventricular administration. Wet-dog shakes, although observed frequently, were typically more delayed than was the case after intraventricular injections. A rigidity score of 2.2 ± 0.2 [mean ± standard error of the mean (S.E.M.)] was achieved with β-endorphin 30 minutes after injection into the PAG. During the rigidity period, the righting reflex was lost and rats slid or fell off of the grid. All these actions were reversed within minutes after subcutaneous injection of naloxone (2 mg/kg). Furthermore, as with intraventricular administration, most animals became flaccid after injection of haloperidol (2 mg/kg). Nevertheless, injections of β-endorphin into the ventromedial PAG did not produce rigidity as intense as that resulting from ventricular administration (Table 1). Therefore, although the ventromedial region of the PAG may be implicated in the β-endorphin-induced rigidity syndrome, additional sites activated by ventricular administration of β-endorphin must also be involved. Those rats ($N = 6$) found to have cannula placements in the lateral or dorsal aspects of the PAG displayed marginal or no effects after injection of β-endorphin over the same dose range; however, morphine (10 μg in 1 μl) induced a naloxone-reversible, intensely violent responsivity in those animals with dorsolateral PAG placements (*14*). Regardless of PAG placement, no animal exhibited a haloperidol-like behavioral syndrome after injection with either β-endorphin or morphine.

Thus, β-endorphin injected intraventricularly or directly into the PAG produced a state of immobility that contrasted significantly with that produced by haloperidol or other neuroleptics. Furthermore, whereas β-endorphin produced an amphetamine-like state of hyperactivity before or after (or both) the rigidity phase, no such pattern of effects was observed after injection of haloperidol. Thus, extended behavioral characterization of the response to β-endorphin does not support the contention that this opiate-like neuropeptide may be a naturally occurring neuroleptic. Moreover, unlike haloperidol, β-endorphin does not elevate synaptosomal conversion of tyrosine to dopamine within the caudate (*15*), the site at which haloperidol is alleged to exert its cataleptogenic effects (*6, 16*).

We previously considered the possible clinical implications of the catatonic-like rigid immobility induced by β-endorphin (*17*). Our studies reported here indicate that β-endorphin, like other opiates, also produces periods of hyperactivity that resemble the behavioral response to amphetamine (*6, 18*). The behavioral effects of amphetamine are blocked by the neuroleptic haloperidol and are at least partially antagonized by naloxone (*19*). Therefore, the mechanisms underlying the amphetamine response may be similar to those responsible for the β-endorphin-induced behavioral excitation. It is conceivable therefore that psychological disturbances similar to those produced by amphetamine may result as a consequence of excessive endorphin receptor activation.

DAVID S. SEGAL
RONALD G. BROWNE
*Department of Psychiatry, University of California, San Diego, La Jolla 92093*

FLOYD BLOOM
*Arthur V. Davis Center for Behavioral Neurobiology, Salk Institute, San Diego, California 92112*

NICHOLAS LING
ROGER GUILLEMIN
*Laboratory of Neuroendocrinology, Salk Institute*

### References and Notes

1. F. Bloom, D. Segal, N. Ling, R. Guillemin, *Science* **194**, 630 (1976).
2. L. F. Tseng, H. H. Loh, C. H. Li, *Biochem. Biophys. Res. Commun.* **74**, 390 (1977); K. Izumi, T. Motomatsu, M. Chretien, R. F. Butterworth, M. Lis, N. Seidah, A. Barbeau, *Life Sci.* **20**, 1149 (1977).
3. Y. F. Jacquet and N. Marks, *Science* **194**, 632 (1976).
4. N. Ling and W. Vale, *Biochem. Biophys. Res. Commun.* **63**, 801 (1975); N. Ling, J. Rivier, M. Monahan, W. Vale, *J. Med. Chem.* **19**, 937 (1976).
5. A. Barnett, J. Goldstein, E. Fiedler, R. Taber, *Eur. J. Pharmacol.* **30**, 23 (1975).
6. R. Fog, *Acta Neurol. Scand.* **48** (Suppl. 50), 11 (1972).
7. A similar pattern of behavioral activation has been observed in rats after injection of β-endorphin into the third ventricle [W. W. Gispen, V. M. Wiegant, A. F. Bradbury, E. C. Hulme, D. G. Smyth, C. R. Snell, D. DeWied, *Nature (London)* **260**, 794 (1976)] and after administration of Met$^5$-enkephalin into the lateral ventricle [L. Leybin, C. Pinsky, F. S. Labella, V. Havlicek, M. Rezek, *ibid.* **264**, 458 (1976)]. In addition, A. Pert and C. Sivit [*ibid.* **265**, 645 (1977)] showed that 10 μg of [D-Ala$^2$]-Met-enkephalin injected into the nucleus accumbens induced hypermotility in rats.
8. Similar results were obtained after intraventricular administration of [D-Ala$^2$]-Leu-enkephalinamide (100 μg/10 μl). We also found that the synthetic opiate methadone produced a pattern of effects closely resembling the β-endorphin-induced syndrome. Thus, methadone (5.0 to 10 mg/kg, subcutaneously) produced rigidity, with dose-related increases in magnitude and duration. All eight animals injected with methadone (10 mg/kg) were rated as achieving the highest possible score (4.0). During the period of methadone-induced rigidity, most animals lost their righting reflex and slid off of the vertical grid, as they did when given β-endorphin. Furthermore, low doses of methadone (1.0 to 2.5 mg/kg) produced an amphetamine-like behavioral excitation within 2 hours after injection. A pattern of activation, including intense stereotypy, was evident in animals emerging from the prolonged state of rigidity produced by higher doses of methadone.
9. A further resemblance between methadone and β-endorphin is that haloperidol (4 mg/kg) significantly reduced the rigidity produced by methadone (10 mg/kg) from a score of 4.0 to 1.2 ± 0.2 ($N = 6$; $P < .01$). These results indicate that the similarity between β-endorphin and opiates such

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. Oth ➡ 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.
16. B. Costall and J. E. Olley, *Neuropharmacology* **10**, 297 (1971); B. Costall and R. J. Naylor, *Arzneim. Forsch.* **23**, 674 (1973).
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.
18. I. H. Ayhan and A. Randrup, *Psychopharmacologia* **27**, 203 (1972); M. Babbini and W. M. Davis, *Br. J. Pharmacol.* **46** 213 (1972); W. M. Davis and C. C. Brister, *J. Pharmacol. Sci.* **62** 974 (1973); L. Ahtee, *Eur. J. Pharmacol.* **39**, 203 (1976); E. F. Domino, M. R. Vasko, A. E. Wilson, *Life Sci.* **18**, 361 (1976).
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.

16 May 1977

# 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.

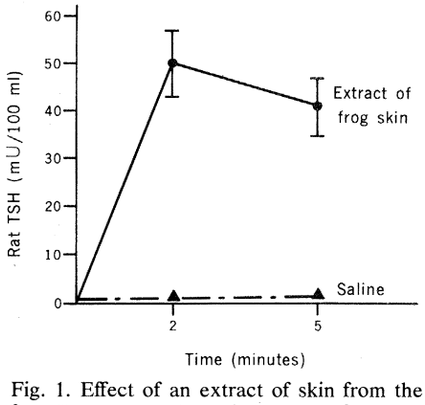


Fig. 1. Effect of an extract of skin from the frog (*Rana pipiens*) on the release of TSH in the rat in vivo. The skin was extracted in 90 percent methanol and the dried supernatant, reconstituted in buffer, was assayed for IR-TRH content. Skin extract containing 100 ng of IR-TRH, made up to 1 ml with 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