Hormonal Actions in Non-endocrine Systems

Walter B. Essman

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Edited by
Walter B. Essman
Departments of Psychology and Biochemistry
Queens College of the City University of New York
Flushing, New York

MTP PRESS LIMITED
International Medical Publishers

Published in the UK and Europe by
MTP Press Limited
Falcon House
Lancaster, England

Published in the US by
SPECTRUM PUBLICATIONS, INC.
175-20 Wexford Terrace
Jamaica, NY 11432

ISBN: 0-85200-612-8

Printed in the United States of America

Preface

The actions of hormones upon systems outside of the usual target sites for such molecules represents an area of increasing interest and growing clinical significance. This volume represents a cross-section of such actions of hormones upon several relevant sites.

In the first chapter of this volume Dr. Malick discusses the current status of endorphins as analgesic agents. It is now known that a more primary level of control exists for β-endorphin in that a 41-amino acid peptide has been isolated from ovine hypothalamus; this peptide stimulates β-endorphin release as well as the secretion of corticotropin (Vale et al., 1981). The analgesic properties of corticotropin and its immunoactive-like analogs are well known, so it does not come as a surprise that these two classes of analgesic peptides are regulated by a common hypothalamic control peptide. It may also be of interest to observe that an increase in β-endorphin concentration in the pituitary occurs in genetically obese mice and rats, and that such obesity can be attenuated through the administration of nalaxone (Margules et al., 1978). It has also been determined that genetically obese mice have a probable cholecystokinin deficiency in the cerebral cortex in that this peptide is a satiety-inducing agent (Saito, et al., 1981). The analgesic properties of the latter have also been observed.

The extra-pituitary actions of another pituitary peptide, as examined in the second chapter of this volume by Dr. Hirsch, while indicating the nature of TRH receptors in several brain regions and how these interact with barbiturates, concerns essentially sites, although outside of the pituitary, still within the brain. The feedback of peripheral hormone status upon the pituitary can in turn affect the pituitary response to TRH. A case in point is that males with primary testicular failure show an exaggerated response of prolactin release to the administration of TRH (Spitz et al., 1980), an ef-

fect that may be mediated by estrogen. The predictable release of prolactin by TRH under normal circumstances may play a role in the regulatory effects of TRH outside of the pituitary. In addition, it may be observed that specific membrane receptor sites for TRH have been identified in fetal sheep thyroid cells (Essman et al., 1980), which could well represent only one of several non-brain extra pituitary sites upon which TRH is capable of exerting a regulatory function.

Some of the functions of the gastrointestinal hormones were explored by Dr. Misra in a previous volume (1980) and in the present book he and Dr. Baruh consider some of the diagnostic and therapeutic roles of these agents. In this regard some attention may be called to a familial glucogonoma syndrome (Stacpoole et al., 1981) in which a pancreatic A cell neoplasm was documented in family members. There may also be note taken that there are a reasonably large number of nonfunctioning islet cell tumors in which there is no clinical evidence of hormone production. It may also be observed that hormones acting upon the gastrointestinal tract and secondarily upon G.I. hormones, can alter the gastric mucosa; glucagon is capable of protecting the gastric mucosa against aspirin-induced injury (Stachura et al., 1981) and adaptive mucosal hyperplasia has been observed from elevated levels of enteroglucagon. The changes in gastric and colonic smooth muscle in response to proinsulin, insulin, and motilin are well known, but the effects of the latter upon gastric emptying appear to be specific for glucose rather than fats (Chistofedes et al., 1981). There is a possibility that gastric fat content may serve to interfere with the gastric mucosal effects of motilin.

In Chapter 4 the issue of perinatal hypothyroidism and brain function is considered. It might be appropriate to consider that a resistance to thyroid hormone could serve independently as a basis for CNS effects, especially since a familial entity of this type has been reported (Bartato et al., 1981). An altered response of the pituitary to thyroxine or to TRH could account for a receptor-mediated hormonal failure, which if present during perinatal development could profoundly influence brain function.

The actions of thyroid hormones on the lung, as explored in Chapter 5 by Drs. Das and Steinberg, concerns a highly original relationship that has suggested important regulatory functions for thyroid hormones in pulmonary status. Changes in thyroid status, the dependence of phospholipid synthesis in the lung upon thyroid hormones and the requirement of specific phospholipids at those sites in the lung to which thyroid hormones bind suggest that specific lung cells may contain sites that autoregulate in response to the availability of thyroid hormones. This holds potential clinical significance, not only for the lung as a target site that may be affected in thyroid dysfunction, but also that alterations in pulmonary function may modify the response of specific lung sites to thyroid hormones during

critical periods of growth and development. The issue also brings into play a possible therapeutic role for thyroid hormones in pulmonary disorders, such that altered lung mechanics, gas exchange, or drug absorption may be modified.

In the final chapter of this volume, Drs. Chitkara and Khan have undertaken the ambitious task of discussing the hormonal status of the lung and also have pointed to some of the functions that these endogenous principles underlie. A very important role for some of the endogenous hormones of the lung is as markers for carcinomas of the lung. Recent important observations (Berger et al., 1981), measurement of L-dopa-decarboxylase, histaminase, calcitonin, and β-endorphin in samples of pulmonary neoplasms indicated a wide range of variability for different tumor types with only minimal selectivity shown; for example, adenocarcinomas or large cell carcinomas did not contain β-endorphin, whereas very high calcitonin levels were found in the former. At least two, or more markers were associated with small cell carcinomas. Whether hormone marker profiles will characterize the type of lung carcinoma or predict size, extent of metastases, or response to therapy remains to be studied, however, there can be little doubt that the lung is a significant site for hormone production.

The present volume is the result only of the cooperation and patience of its contributors, who took time from busy clinical and research activities to provide a current and usable guide to those subjects in which they are experts. Such expertise from fields as pharmacology, psychology, gastroenterology, metabolism, biochemistry, and pulmonary medicine, made my job, as editor, a most enjoyable one. It is my hope that the reader will find, as I did, that there is much to be learned about the influences of hormones upon most endocrine sites.

WALTER B. ESSMAN

Flushing, N.Y.
May, 1983

Contributors

Selim Baruh, M.D.
Division of Diabetes and Metabolism
Department of Medicine
Queens Hospital Center
Jamaica, N.Y.

Rajinder K. Chitkara, M.D.
Division of Pulmonary Medicine
Department of Medicine
Queens Hospital Center
Jamaica, N.Y.

Dipak K. Das, Ph.D.
Division of Pulmonary Medicine
Department of Medicine
Long Island Jewish-Hillside Medical Center
New Hyde Park, N.Y.

Walter B. Essman, M.D., Ph.D.
Departments of Psychology and Biochemistry
Queens College, of the City University of New York
Flushing, N.Y.

Michael D. Hirsch, Ph.D.
Roche Institute for Molecular Biology
Nutley, New Jersey

Faroque A. Khan, M.D.
Division of Pulmonary Medicine
Department of Medicine
Queens Hospital Center
Jamaica, N.Y.

Jeffrey B. Malick, Ph.D.
Division of Neuropharmacology
I.C.I.
Wilmington, Delaware

Prem Misra, M.D.
Division of Gastroenterology
Department of Medicine
Queens Hospital Center
Jamaica, N.Y.

Harry Steinberg, M.D.
Division of Pulmonary Medicine
Department of Medicine
Long Island Jewish-Hillside Medical Center
New Hyde Park, N.Y.

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edited by W. B. Essman.

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Analgesic Activity of Endorphins

JEFFREY B. MALICK

The demonstration that electrical stimulation of the mesencephalic gray in rats produced a profound analgesic response was one of the earliest reports providing experimental evidence suggestive of the presence of endogenous morphine-like substances in the brain (Reynolds, 1969). The analgesia produced by the electrical brain stimulation was sufficient to permit a laparotomy to be performed (Reynolds, 1969). Subsequent reports have demonstrated that electrical stimulation of medial brain stem regions, especially the periaqueductal gray (PAG), resulted in potent analgesic activity in the rat (Balagura and Ralph, 1973; Mayer and Libeskind, 1974), cat (Liebeskind et al., 1973; Oliveras et al., 1974), rhesus monkey (Goodman and Holcombe, 1976) and man (Adams, 1976; Richardson and Akil, 1973); Mayer and Price (1976) have written an excellent review on brain stimulation-induced analgesia.

Brain stimulation-induced analgesia is significantly antagonized by naloxone, a narcotic antagonist (Adams, 1976; Akil et al., 1976). In addition, microinjection of morphine into the PAG resulted in marked naloxone-reversible analgesic activity (Herz et al., 1970; Pert and Yaksh, 1974) and brain stimulation-induced analgesia exhibited both tolerance (Mayer and Hayes, 1975) and cross-tolerance to morphine administered at the same brain site (Mayer and Hayes, 1975; Mayer and Murphin, 1976). Therefore, it was believed that the electrical stimulation caused the release of some endogenous substance that resulted in an opiate-like analgesic response.

Goldstein and associates (1971) discovered a method for detecting the presence of opiate receptors in mouse brain. This discovery generated a flurry of research activity in this area and, as a result, within two years several laboratories had independently demonstrated the occurrence of stereospecific opiate receptor binding sites in brain (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973). Obviously, these opiate binding sites were not present in brain merely to accommodate the poppy alkaloids; therefore, several laboratories throughout the world intensified their search for the endogenous morphine-like substances.

Hughes and associates (1975) discovered two pentapeptides in extracts of porcine brain that produced morphinomimetic activity when tested on their guinea pig ileum and mouse vas deferens preparations. They named these peptides methionine-enkephalin and leucine-enkephalin, and noted that the methionine form was approximately four times as prevalent in whole pig brain as the leucine form (Hughes et al., 1975). These pentapeptides only differed from one another structurally in their terminal amino acid and they were identified as Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu, met-enkephalin and leu-enkephalin, respectively. In their original paper (Hughes et al., 1975) the authors pointed out the interesting and important observation that the sequence of met-enkephalin was present in the structure of β-lipotropin (β-LPH residues 61-65) (see Figure 1); this peptide hormone containing 91 amino acids had been isolated from pituitary glands as early as 1964. Three additional segments of β-LPH with opiate-like activity were isolated from brain and pituitary extracts (Bradbury et al., 1976; Cox et al., 1976; Lazarus et al., 1976; Ling et al., 1976; Ling and Guillemin, 1976): β-endorphin (β-LPH 61-76); γ-endorphin (β-LPH 61-77), and β-endorphin (β-LPH 61-91; C-fragment) (see Figure 1).

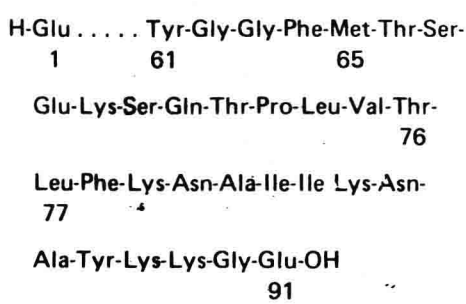

Fig. 1. The amino acid sequence of various endorphins in human β-lipotropin: met-enkephalin (β-LPH 61-65); α-endorphin (β-LPH 61-76); γ-endorphin (β-LPH 61-77); β-endorphin (β-LPH 61-91).

As in any area in which the research and discoveries proceed very rapidly, the nomenclature does not always evolve in a systematic manner. Hughes and co-workers (1975) named their peptides enkephalins. However, Eric Simon coined the term endorphins in 1975 and since then this has been used as a generic descriptor for the endogenous morphine-like substances.

The purpose of this chapter is to survey the literature on the analgesic activity of the endorphins and to speculate as to their possible relevance in pain mechanisms, especially in terms of pain syndromes observed clinically in humans.

EFFECTS OF ENDORPHINS IN TESTS PREDICTIVE OF ANALGESIC ACTIVITY

This section will summarize the results of the many experiments that have been performed to assess the analgesic potential of the naturally occurring opiate-like substances (endorphins); the intent is to discuss the endogenous compounds and to omit any discussion of the many synthetic endorphins, several of which appear to be extremely potent antinociceptive agents in animals.

The bulk of the discussion will deal with the activities of methionine-enkephalin, leucine-enkephalin, and β-endorphin. However, brief mention will be made of the activity of β-lipotropin and several of the other endorphins (e.g., α-endorphin, γ-endorphin) that have been discovered in animals and humans and that could have a physiologic role in the organism.

Methionine-Enkephalin

Table 1 summarizes the results of the analgesic studies performed on methionine-enkephalin in various laboratories. In April 1976 Belluzzi and co-workers (1976) were the first to report analgesic activity following the central administration of enkephalins to rats. In this study rats were implanted stereotaxically with permanently indwelling cannulae, the tips of which were located in the lateral ventricle. One week after surgery the rats were tested in the tail flick test (D'Amour and Smith, 1941) for analgesic activity following the intracerebroventricular infusion of methionine-enkephalin. The tail flick test has been used frequently to evaluate the analgesic potential of the endorphins since it readily detects opiate drugs (e.g., morphine) (Bloom et al., 1976). Follow-

Table 1. Effects of Methionine-Enkephalin in Various Analgesic Tests in Rodents and Cats.

Investigator(s)	*Species*	*Analgesic Model*	*Route of Administration*	*Dose (μg)*	*Injection Volume (μl)*	*Analgesic Activity*
Belluzzi et al. (1976)	Rat	Tail flick	i.c.v.[a]	100,200	10	Significant analgesia at both doses; onset (2-6 minutes); duration (10-12 minutes)
Büscher et al. (1976)	Mouse	Tail flick	i.v.[b]	Several[c]	—[d]	ED_{50} = 170 mg/kg; 15-second duration
	Mouse	Tail flick	i.c.v.	56-180	10-30	ED_{50} = 75 (52-100); peak activity (2 minutes); duration (5 minutes)
Chang et al. (1976)	Rat	Tail flick	i.c. (PAG)[e]	30-360	3	Sign. analgesia only at 120 μg/rat; duration <3 minutes
Feldberg and Smyth (1976)	Cat	Tail pinch	i.c.v.	50-400	40	No analgesic activity
	Cat	Tail pinch	i.v.	1 mg/kg	—[d]	No analgesic activity
Graf et al. (1976)	Rat	Tail flick	i.c.v.	670 nmoles	20	Slight analgesia; duration (8 minutes)

Table 1 (Continued)

Leybin et al. (1976)	Rat	Hot plate (44.5°C)	i.c.v.	100	10	No analgesia; apparent increase in pain perception (increased paw licking)
Loh et al. (1976)	Mouse	Tail flick Hot plate Acetic acid writhing	i.c.v.	50	5	Weak analgesic activity; duration <10 minutes
Malick and Goldstein (1976, 1977)	Rat	Tail flick	i.c. (PAG)	4.8-48	1	ED_{50} = 15.5 (9.9-24.2) μg/rat; peak activity (1 minute); short duration (dropping off by 7 minutes)
Ronai et al. (1976)	Rat	Tail flick	i.c.v.	400	20	Moderate prolongation of reaction times; duration: 4-6 minutes
Bradbury et al. (1977)	Rat	Hot plate (55.5°C)	i.c.v.	100	10	Transient analgesia; 10-minute duration; N = 1
Meglio et al. (1977)	Cat	Tooth pulp	i.c.v.	100-400	500	No analgesic activity
	Cat	Pinch test (tail, limbs, ears)	i.c.v.	100-400	500	No analgesic activity

Table 1 (Continued)

Investigator(s)	*Species*	*Analgesic Model*	*Route of Administration*	*Dose (μg)*	*Injection Volume (μl)*	*Analgesic Activity*
Roemer et al. (1977)	Mouse	Tail flick	i.c.v.	Several[c]	—[d]	ED_{50} = 68 μg/mouse at 2 minute postinfusion
Szekely et al. (1977a)	Rat	Tail flick	i.c.v.	Several[c]	20	Weak analgesic activity; impossible to calculate an ED_{50} as high as 6.7×10^{-7} mole/rat
Urca et al. (1977)	Rat	Tail flick	i.c.v.	200	10	8/13 drug naive rats exhibited significant analgesia; duration 1–40 minutes (median = 4 minutes)
Walker et al. (1977)	Rat	Tail flick	i.c.v.	200	10	Slight, significant analgesia; maximum increase in latency = 2.0 seconds; duration = 4 minutes

[a]Intracerebroventricular
[b]Intravenous
[c]Actual doses not reported
[d]Not reported
[e]Intracerebral; periaqueductal gray

ing the administration of methionine-enkephalin (100 and 200 μg/rat, i.c.v.), statistically significant, dose-related increases in tail flick response) were observed. The analgesia produced by the highest dose (200 μg/rat) of methionine-enkephalin appeared to be equivalent to that produced by a 10-μg/rat dose of morphine sulfate; thus, although methionine-enkephalin produced significant analgesia, it was approximately 20 times less potent than morphine when administered into the ventricles.

Methionine-enkephalin exhibited a rapid onset (2–6 minutes) of analgesic activity, which also rapidly disappeared by 10–12 minutes postdrug infusion. The delay between the time of administration and the onset of activity was most likely attributable to diffusion time to the active sites and the short duration of activity was probably due to the rapid catabolism of enkephalins by brain enzymes (Hughes, 1975). Since pretreatment with naloxone (2 mg/kg, s.c.), a pure narcotic antagonist, significantly antagonized the analgesic activity produced by methionine-enkephalin, it appeared to produce this activity by an interaction with opiate receptors.

At approximately the same time (April 1976) that the first publication in this area (Belluzzi et al., 1976) appeared, an abstract was submitted by Malick and Goldstein (1976) for presentation at the Fall Pharmacology Meeting (New Orleans, La., August 1976); the expanded manuscript appeared in early 1977 (Malick and Goldstein, 1977). This study was the first in which complete dose-response curves were generated in order to compare the analgesic potencies of methionine-enkephalin and morphine via the potency ratio analysis of Litchfield and Wilcoxon (1949). In this study rats were stereotaxically implanted with cannulae located in the dorsal border of the raphe nucleus in the midbrain periaqueductal gray (PAG). This site was chosen because it had been shown to be one of the primary sites in the brain at which morphine exhibits potent analgesic activity. The methodology used in this study is discussed briefly as an example of the procedures commonly utilized in many of the studies reported here.

In studies in which drugs are to be infused directly into the brain, rats are generally allowed at least a week to recover fully from the trauma associated with the surgical implantation of the guide cannula. All rats are then tested for their responsiveness to the noxious stimulus, which in the case of the tail flick procedure is radiant heat, following the infusion of the vehicle (sterile water in this study) in which the test drugs have been dissolved; such studies are used as control baselines and are absolutely necessary to assure that the rat does not respond to the vehicle alone. Any

animal that exhibited a significant alteration from control baselines following control (vehicle) infusion would have been eliminated from the study; in the study being discussed, none of the subjects exhibited a significant change following vehicle infusion. Infusions are accomplished by inserting an internal cannula into the permanently implanted guide cannula; the internal cannula extends 2 mm beyond the tip of the guide cannula. An infusion pump is used to deliver a small volume (1 μl or less into tissue) relatively slowly and at a constant rate; in the present study drugs were infused in a 1 μl volume over 24 seconds (infusion rate = 0.04 μl/second). The internal cannula should be left in place for a short period of time (e.g., 25 seconds) to prevent the infused fluid from immediately flowing back up the guide cannula. Rats were then placed in a standard Plexiglas restrainer so that only the tail protruded. Control sessions were performed on the morning of each day of drug testing and consisted of infusion of sterile water and testing for tail flick responses (latency to respond) at 1, 3, and 7 minutes postinfusion. After establishing same-day control thresholds for each rat, drugs were infused and tail flick latencies were measured once again at the same intervals postinfusion. Thus each rat served as its own control. Any rat exhibiting a 50% or greater increase in response latency as compared with the rat's own same-day control value was considered to have shown a significant analgesic response. If the rat did not respond within 20 seconds, the heat source was terminated in order to prevent tissue damage. When animals were used more than once, at least seven days were allowed between drug administrations in an attempt to avoid the development of tolerance. The results of this study are summarized in Table 2.

When infused directly into the PAG, both morphine and methionine-enkephalin exhibited potent, dose-related analgesic activity (Table 2). Methionine-enkephalin was approximately four times less potent than morphine on a weight basis; however, if the potency comparison were based on the molar ED_{50} ratio, the potency of met-enkephalin would be increased by a factor of 2 relative to morphine (i.e., molar ED_{50}s: met-enkephalin = 0.27 μmoles; morphine = 0.13 μmoles). Thus met-enkephalin was found to be much more potent in the study by Malick and Goldstein (1977) as compared with the results obtained by Belluzzi et al. (1976). This difference in potency most likely arises from the fact that Belluzzi and his associates utilized the intraventricular route of administration, which required diffusion to the site(s) of action. This could have dramatically reduced the activity of the peptide as enkephalins have been shown to be rapidly deactivated

Table 2. Analgesic Activity of Methionine-Enkephalin and Morphine in the Tail Flick Test in Rats Following Intracerebral Administration[a]

Treatment	*Dose (μg/rat)*	*N*[b]	*% Rats Exhibiting a Significant Increase in Response Latency*[c]	*ED_{50} (μg/rat) (95% CL)*[d]
Sterile water	1 μl	133	0.0	—
Morphine SO_4 (7 minutes)[e]	1.7	7	28.6	3.7
	3.0	7	28.6	(2.2-6.1)
	10.0	10	80.0	
		9	100.0	
Met-enkephalin (1 minute)[e]	4.8	19	21.1	15.5
	9.6	17	35.3	(9.9-24.2)
	16.0	19	47.4	
	48.0	16	81.3	

[a]Data from Malick and Goldstein (1977)
[b]Number of rats tested
[c]Any rat exhibiting a 50% or greater increase in response latency was considered to be significantly affected
[d]ED_{50} and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (1949)
[e]Data from time of peak activity

by peptidases in brain and other tissue (Hambrook et al., 1976). Following intracerebral infusion (PAG), the analgesic activity of met-enkephalin peaked by 1 minute postinfusion but had a very short duration of activity (its activity had significantly diminished by 7 minutes postinfusion); this is in agreement with the short duration of activity reported by Belluzzi and co-workers (1976). In contrast, the activity of morphine did not peak until the 7-minute observation time but persisted for as long as several hours postinfusion, which is consistent with previous observations (Yaksh et al., 1976). The analgesic activity of met-enkephalin was completely antagonized by pretreatment with the narcotic receptor antagonist, naloxone (see Table 3). The high dose of naloxone (20 mg/kg, i.p.) used in this antagonism study was chosen since previous studies had demonstrated that although much lower doses will antagonize parenteral doses of morphine, relatively high doses of the antagonist are necessary to antagonize morphine-induced analgesia following direct infusion into the periaqueductal gray.

In contrast to the studies of Malick and Goldstein (1977), Chang et al. (1976) reported that methionine-enkephalin was at least 20 times less potent than morphine in the tail flick test in