spot comigrating with the fetal LC_1 in labeled human fibroblast cultures (Fig. 3B) shows that it is not synthesized by fibroblasts.

Finally, a significant amount of FLC_3 is synthesized in cultures of fetal human tissue (Fig. 3A and Table 1). Of the total light chains 15 to 17 percent can be attributed to FLC_3 synthesis. In contrast, Whalen *et al.* (*9*) show very little FLC_3 in cultures of rat thigh muscle and L6 cells and Keller and Emerson (*12*) show from 1 to 4 percent in primary cultures of chicken and quail pectoralis muscle and quail clonal cultures. Blau and Webster (*13*) report only four light chains in clonal cultures of adult human muscle but do not clearly identify them, and it is unclear whether FLC_3 or a fetal light chain is present. Synthesis and accumulation of FLC_3 are normally associated with the early adult stage in both avian and mammalian muscle (*14–18*). Its relatively high concentration in our human cultures suggests that these culture conditions for human muscle will support, at least, a partial expression of adult myosin phenotype. Complete adult expression in cultured fibers originating from myogenic cells of normal and diseased muscle has not yet been obtained.

The presence of a fetal-specific myosin light chain in human muscle provides a marker with which to detect shifts from fetal to adult myosin gene expression. This is especially important for work in cell culture where it is highly desirable to define conditions that will allow the expression of adult gene patterns as opposed to embryonic or fetal patterns. In the search for a gene product associated with inherited, X-linked, Duchenne muscular dystrophy, for example, human muscle culture could be extremely useful. Usefulness is now limited because cultured muscle expresses a phenotype that is predominantly fetal (*19*). Where there are muscle-specific isoform shifts involving an embryonic (or fetal) to adult or a neonatal to adult transition in vivo, these shifts do not occur in vitro (*20*). For example, even when cultured chicken muscle fibers are maintained for 30 to 50 days, they express an embryonic pattern of myosin heavy and light chains and apparently completely repress the activation of the adult configuration (*16*). This is also true for patterns of tropomyosin and myosin expression in cultured muscle fibers derived from satellite cells of adult chicken muscle (*21*). Cultured rat muscle fibers synthesize a fetal light chain and fetal myosin heavy chains and, like cultured chicken muscle, apparently do not make the transition to expression of adult isoforms of myosin and perhaps other muscle-specific proteins (*22*). If genes responsible for diseases like Duchenne dystrophy are associated with the modification of adult gene expression as suggested by Fitzsimmons and Hoh (*23*), then cell culture studies should be valuable provided that we can devise conditions supportive of a full range of gene expression. The presence of a fetal-specific myosin light chain in human muscle and its synthesis in human muscle cultures provides a reliable marker for determining whether variations in culture conditions can induce shifts from fetal to adult modes of muscle-specific protein synthesis.

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Identification of Pro-Opiomelanocortin–Derived Peptides in the Human Adrenal Medulla

Abstract. *Extracts from adult human adrenals contained high concentrations of immunoreactive β-endorphin and α-melanotropin. Lower quantities of immunoreactive adrenocorticotropic hormone could also be detected. Distribution studies showed the presence of pro-opiomelanocortin fragments in the adrenal medulla. No α-melanotropin, β-endorphin, or adrenocorticotropic hormone could be found in adrenal extracts from several other mammalian species. Analysis of the β-endorphin-like immunoreactivity using region specific radioimmunoassays interfacing with gel filtration and reverse-phase high-performance liquid chromatography showed the majority of the β-endorphin-like material to exist as nonacetylated β-endorphin-(1–31) with a small percentage of lipotropin-sized molecules. The α-melanotropin-like immunoreactivity cochromatographed on gel filtration and reverse-phase high-performance liquid chromatography with desacetyl α-melanotropin. The data suggest that pro-opiomelanocortin is expressed in the adrenal medulla of humans but is not detectable in the adrenal glands of many other mammalian species.*

Since the characterization of Met- and Leu-enkephalin by Hughes *et al.* (*1*) in 1975, numerous other endogenous opiate active peptides have been detected (*2*). All endogenous opioid peptides thus far isolated from mammalian tissue are derived from three separate precursors, namely, pro-opiomelanocortin (*3*), pro-enkephalin (*4, 5*), and pro-neoendorphin/dynorphin (*6*). One of the richest sources

of endogenous opioids in the mammalian organism is the adrenal medulla, which contains high concentrations of opioid peptides derived from proenkephalin (*7–12*). In fact, the messenger RNA that codes for this precursor was isolated, cloned, and sequenced from bovine adrenal medulla and human pheochromocytoma tissue (*4, 5*). There is also some evidence that the adrenal may contain peptides derived from the second major opioid peptide precursor, pro-neo-endorphin/dynorphin (*13, 14*). There is considerable variability in the amount of opioid peptides found in adrenals from different species: the bovine and human adrenal medulla, for example, contain high concentrations of opioid activity whereas the rat adrenal medulla is almost devoid of opioid peptides (*11, 15*). The functional significance of the presence of opioid peptides in adrenal medulla is unclear, but it is of interest that enkephalins have been shown to be stored with adrenalin in chromaffin cells of the adrenal medulla and that they are released together with adrenalin during stress (*11, 16*). We now report that the human adrenal medulla unexpectedly contains high concentrations of β-endorphin, the major opioid peptide derived from the third mammalian opioid peptide precursor, pro-opiomelanocortin (*3*). We also present evidence that human adrenal extracts contain desacetyl α-melanocyte–stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) immunoreactive material, the other two major bioactive substances besides β-endorphin that are derived from pro-opiomelanocortin.

Human adrenal glands obtained less than 24 hours after death were extracted by homogenization in five volumes (by weight) of acetone containing HCl and thiodiglycol (0.1 percent). The extracts were then spun at 30,000*g* for 25 minutes and the supernatant was evaporated to dryness by a stream of nitrogen. After reconstitution of the residue in buffer, radioimmunoassay (RIA) with antibodies to the middle region of the β-endorphin molecule (*17*) detected considerable amounts of immunoreactive material (39.0 ± 6.26 pmole/g; $N = 5$). Moreover, RIA with antibodies directed to the COOH-terminal region of β-endorphin (*17*) also detected considerable amounts of immunoreactive material, suggesting that the immunoreactive substance had considerable homology to authentic β-endorphin (Table 1).

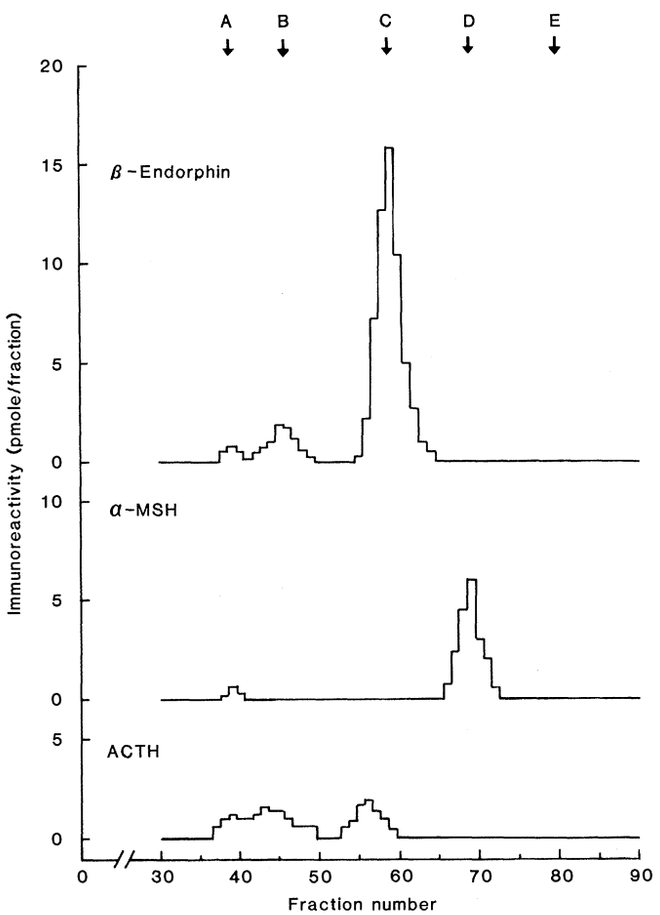

Fig. 1. β-Endorphin, α-MSH, and ACTH immunoreactivity profiles of a human adrenal extract chromatographed on a Sephadex G-75 column (0.9 by 110 cm) eluted with 25 percent acetic acid. Markers were (A) exclusion (B) ^{125}I-labeled human β-lipotropin, (C) [^{125}I]β_h-endorphin, (D) [^{125}I]α-MSH, and (E) [^{125}I]Leu^5-enkephalin. Fractions of 1.5 ml were collected.

The presence of β-endorphin-like immunoreactive material raises the question as to whether ACTH or its processing fragments are also present in this tissue, since both in brain and pituitary β-endorphin is derived from a precursor that also contains ACTH and α-MSH. The crude whole adrenal extracts were therefore reassayed with an RIA specific for ACTH (*18*), and lower levels of immunoreactive material were detected (7.58 ± 1.7 pmole/g; $N = 5$). We also measured α-MSH in the crude adrenal extracts using a COOH-terminal–directed α-MSH RIA (*19*). The concentration of α-MSH-like immunoreactivity in the adrenal extract was 12.5 ± 1.59 pmole/g ($N = 5$). In contrast to the human adrenal, extracts from cow, sheep, rabbit, rat, dog, and guinea pig adrenals did not contain detectable quantities of β-endorphin or ACTH-related peptides. The possibility was therefore considered that the pro-opiomelanocortin fragments found in the human adrenal were postmortem artifacts. To investigate this possibility, a guinea pig was killed by cervical dislocation and left for 24 hours at 4°C (mimicking postmortem conditions). We then removed and extracted the adrenals but, as found with the fresh tissue, no β-endorphin or α-MSH-like material could be detected.

To determine the distribution of the pro-opiomelanocortin fragments in the human adrenal gland, the medulla was dissected from the cortex by using catecholamine levels to control the completeness of the dissection (*20*). These experiments showed the pro-opiomelanocortin–like immunoreactivity to be concentrated in the medulla extract (Table 1).

The β-endorphin antibodies used in this study cross-react equally with β-lipotropin and α-*N*-acetylated β-endorphin, both of which are endogenous opiate inactive peptides that possess the β-endorphin sequence. Furthermore, the β-endorphin-like immunoreactivity could represent COOH terminally shortened variants such as β-endorphin-(1–27) or β-endorphin-(1–26), which are present in the pituitary but display considerable less potency than β-endorphin-(1–31) in some bioassays in vivo (*21*). With regard to α-MSH, the variants that have been characterized involve changes in acetylation at the NH_2-terminal serine (*19, 22*), a modification shown to enhance bioactivity (*23*).

To identify which molecular forms of α-MSH and β-endorphin were present in human adrenals, we characterized the immunoreactivity by interfacing gel fil-

tration column chromatography and reverse-phase high-pressure liquid chromatography (HPLC) with RIA's. To determine the molecular weight of the immunoreactive peptides, crude extracts were chromatographed on Sephadex G-75 eluted in 25 percent acetic acid. A single immunoreactive peak was detected by the α-MSH RIA that coeluted with [^{125}I]α-MSH (Fig. 1). However, two immunoreactive peaks were detected with the β-endorphin RIA (Fig. 1). The majority of the immunoreactivity (≥ 85 percent) eluted with [^{125}I]β-endorphin-(1–31) and the remainder with ^{125}I-labeled human lipotropin. The question as to whether the β-endorphin–sized immunoreactive peptides were α-*N*-acetylated was resolved by using antibodies to α-*N*-acetyl β-endorphin. The RIA we developed with these antibodies required the α-*N*-acetyl group at the NH_2-terminal tyrosine as part of the recognition site (*17, 24*). No immunoreactive material was detected by using this acetyl-specific RIA, showing that the endogenous β-endorphin-like peptides were not α-*N*-acetylated and were potentially opiate-active.

Further characterization of the immunoreactivity was achieved by reverse-phase HPLC. We analyzed crude extracts as well as the α-MSH and β-endorphin immunoreactive peaks from the gel filtration column. The results from three human adrenal extracts were consistent. All the α-MSH-like immunoreactivity chromatographed at the position of the desacetyl α-MSH standard (Fig. 2). The majority of the β-endorphin immunoreactive material ran in the position of β-endorphin-(1–31), and no immunoreactivity eluted in the position of β-endorphin-(1–27) (Fig. 2). In all the human adrenal extracts we studied, the sum of the α-MSH and ACTH-like immunoreactive peptides did not equal the β-endorphin-like immunoreactivity, as might be expected from fragments of a common precursor. One explanation for this is that a portion of the ACTH-derived peptides may not be recognized by our antibodies. This could occur, for instance, if ACTH were cleaved at the double basic processing recognition site but the NH_2-terminal fragments were not amidated, leaving ACTH-(1–14), which would not be recognized by our α-MSH RIA (*19*).

The results of this study show that pro-opiomelanocortin fragments are present in adult human adrenal glands. Although the major products have been shown to be desacetyl α-MSH and β-endorphin-(1–31), these fragments may represent postmortem processing products and not necessarily those found in the normal healthy gland in vivo. However, the presence of high concentrations of three major products of pro-opiomelanocortin in adrenal medulla does suggest that this tissue is capable of synthesizing this precursor. A previous immunohistochemical study has shown that ACTH-like material is present during week 23 of human fetal gestation (*25*). In that study the ACTH was not accompanied by β-endorphin or β-MSH immunoreactive material, contrary to what we observed in extracts from adult human adrenals.

Our findings, together with results reported by others (*7–14*), suggest that the human adrenal gland expresses the gene products of all three mammalian opioid peptide precursors and may therefore be an ideal model in which to study the regulation and interaction of these three closely related precursor molecules. Moreover, our findings may be of considerable significance with regard to the classical hypothalamopituitary adrenal axis. Although in autopsy adrenal extracts ACTH is not a prolific processing product, it is obvious that if ACTH were released in such close proximity to the adrenal cortex, its target organ, one may expect pronounced effects on blood steroid levels as well as on the regulation of

Table 1. Distribution of pro-opiomelanocortin fragments in human adrenal glands. Samples of human adrenal cortex (N = 9) and adrenal medulla (N = 9) were divided and weighed. Each portion was then extracted in 0.2*M* perchloric acid or a solution of 75 percent acetone, 25 percent 0.2*M* HCl, and 0.1 percent thiodiglycol. The perchloric acid extracts were used directly for adrenalin assay by reverse-phase HPLC with electrochemical detection (*20*). The acid-acetone extracts were centrifuged at 30,000*g* for 25 minutes, the pellet was discarded, and the supernatant was evaporated to dryness by a stream of nitrogen. The residue was then redissolved in 5 percent acetic acid and semipurified with a Sep-Pak (*28*). Greater than 85 percent of iodinated α-MSH, ACTH, and β-endorphin was recovered in the acid-acetone eluate from the Sep-Pak. The eluate from the Sep-Pak was evaporated to dryness, resuspended in buffer, and assayed by RIA.

Assay	Medulla	Cortex
Middle region β-endorphin RIA	144 ± 14.3 pmole/g	2.6 ± 0.4 pmole/g
COOH-terminal β-endorphin RIA	104 ± 12.0 pmole/g	1.0 ± 0.3 pmole/g
α-MSH RIA	53 ± 8.0 pmole/g	Below detection limit
ACTH RIA	25 ± 2.7 pmole/g	Below detection limit
Adrenalin content	2628 ± 452 μg/g	65 ± 16 μg/g

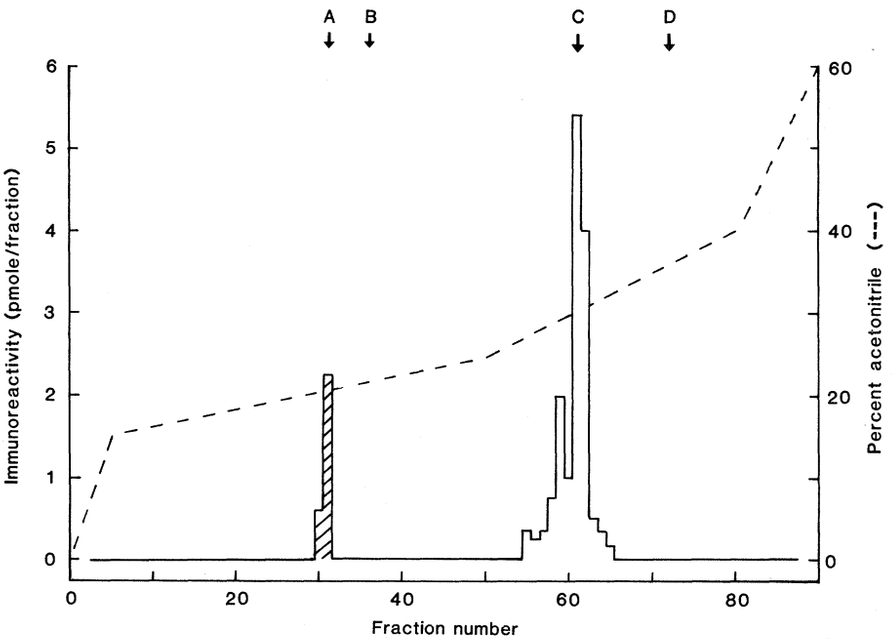

Fig. 2. Reverse-phase HPLC analysis of the α-MSH (shaded) and β-endorphin (unshaded) immunoreactivity from human adrenal tissue. An Altex Ultrasphere 5 μ*M* octadecyl silane column (0.46 by 25 cm) was used for the separations. The buffer was 50 m*M* monosodium phosphate, 5 percent methanol, and phosphoric acid (*p*H 2.7) (1 mg/ml) and the column was eluted at 1.5 ml/min with an increasing concentration of acetonitrile. Calibration standards were (A) desacetyl α-MSH, (B) α-MSH, (C) β_h-endorphin (1–31), and (D) β_h-endorphin (1–27).

epinephrine formation. It remains to be seen if the pro-opiomelanocortin fragments found in the human adrenal medulla do have a physiological role and if this role involves controlling the formation (*26*) or release of catecholamines or steroids from the adrenal gland.

Note added in proof: After submitting this report, we learned of a study in which ACTH-, β-endorphin-, and γ-MSH-like immunoreactive material were found in human adrenal glands.

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Normalizing Effect of an Adrenocorticotropic Hormone (4–9) Analog ORG 2766 on Disturbed Social Behavior in Rats

Abstract. *Short-term isolation increased the frequency of social interactions in rats tested in pairs, while pairs of rats placed in an unfamiliar test cage and subjected to a high level of illumination spent less time in active social contact. These changes in social behavior elicited by environmental manipulations were counteracted by treatment with the adrenocorticotropic hormone (4–9) analog ORG 2766. The peptide's normalizing effect may be mediated by endogenous opioid systems.*

It is usually in a social setting that people take psychopharmacological agents intended to alter behavior and feelings. The interactions between drug effects and social variables should thus be analyzed in detail (*1*). Social behavior can be affected by drugs, and social variables can influence the effects of medications. Furthermore, pathophysiological changes in social behavior and distressing symptoms associated with social interactions may indicate that medication is needed. However, any investigation in sociopharmacology is hampered by the fact that few animal models are available for social behaviors under standardized conditions. More information about changes in social behavior in response to environmental manipulation could improve our understanding of the pathophysiological aspects of social behavior.

Short-term isolation increases the frequency of social interactions in rats (*2*), while the time spent in active social contact decreases when rats are exposed to an unfamiliar test cage and a high level of illumination (*3*). We used these two test situations to analyze the influence of the adrenocorticotropic hormone (4–9) analog ORG 2766 on social behavior. Initially, this peptide was shown to be 1000 times more potent than ACTH-(4–10) in delaying extinction of pole jumping avoidance behavior (*4*). This increased potency could be generalized to other behaviors, including passive avoidance behavior and self-stimulating behavior elicited from the medial septal area (*5*). It was recently reported that the hippocampal morphological correlates of brain aging and the age-dependent behavioral impairment of rats were reduced by long-term treatment with ORG 2766 (*6*). As had been predicted from animal experiments, ORG 2766, like ACTH-(4–10), beneficially influenced motivational and attentional processes in clinical studies. In addition, ORG 2766 ameliorated the mood and increased the sociability of mentally retarded and elderly people (*7*).

In our first series of experiments (*8*), rats that had been housed alone for 7 days and group-housed rats were placed together in pairs and their social behavior was observed for 5 minutes. Most of the social interactions of the rats appeared to be amicable explorative behavior—biting, kicking, and fighting rarely occurred. The frequency of social interactions was significantly greater in isolated rats than in their group-housed partners (Fig. 1A). This was probably due to a specific effect on gregariousness, since ambulation was no different for the two rats, whether it was measured during or outside the social interaction test. Subcutaneous treatment of group-housed rats with ORG 2766 (0.1 μg/kg) 1 hour before the test did not significantly change the frequency of social interactions. However, identical treatment of isolated rats decreased the frequency of social interactions to the level seen in group-housed rats (Fig. 1A). The frequency of social interactions among the test partners of rats treated with ORG 2766 did not differ from that of control rats. Intraperitoneal injections of ORG 2766 (0.1, 5, or 50 μg/kg) 5 minutes before the test had a similar effect, that is, the frequency of social interactions in isolated rats was decreased to the level measured in group-housed rats. The ORG 2766 did not appear to affect nonsocial activities.

In our second series of experiments (*9*), pairs of rats tested in an unfamiliar environment under intense light (group 1) spent less time in active social contact than did rats tested in a familiar environment under low lighting (group 2) (Fig. 1B). The more explorative social behaviors, such as exploration of the partner,