Quaternary Andean lavas are compatible with an increase in crustal Sr with increasing crustal thickness (*8, 9*). Andean Pb data are much less extensive, and adequate comparisons between the two decay systems are thus not possible. In principle, data for lavas from southern Peru of varying ages might resolve the problem. The Arequipa and Barroso lavas have been extruded recently when the crust had attained its present thickness of 60 to 70 km in the Arequipa district (*17*). On the other hand, for calc-alkaline lavas of the Toquepala Group, near the Toquepala mine, 300 km southeast of Arequipa, James reports a Rb-Sr age of 70 × 10^6 years and an initial $^{87}Sr/^{86}Sr$ ratio of 0.7042 (*18*). This ratio is much lower than those for the Arequipa and Barroso lavas and is in general agreement with ratios for similar lavas from oceanic settings. Since thickening of the Andean crust has probably occurred during the Cenozoic era (*17*), the crust should have been much thinner, perhaps of normal thickness at the time the Toquepala lavas were extruded. Two Pb isotope analyses from the Toquepala ore (*19*) show a general similarity to the Disputada mine Pb rather than the Arequipa and Barroso volcanic Pb. The mineralization at the mine has not been dated directly, but porphyry intrusives associated with the ore have yielded K-Ar ages of 57 × 10^6 years (*20*). These data suggest that crustal thickness has exerted a strong control on the isotopic ratios; however, broader sampling is needed to ascertain whether the lack of an imprint of granulite or other crustal rocks is due to a local peculiarity of the Toquepala data rather than to crustal thickness.

The petrogenetic arguments presented here are derived mainly from Pb isotopic data and in a narrow sense apply solely to that element. In fact, if one uses the concentration data in Table 1 and reasonable values of mantle-derived magmas to calculate lava compositions on the basis of simple bulk assimilation, it is difficult to account for the major element chemistry of the rocks. James (*21*) has reviewed this problem in detail for major and trace element data of the Barroso and Arequipa lavas. We believe that the granulite component in the lavas reflects selective contamination with Pb, Sr, and probably other trace elements as well. Carter *et al.* (*22*) reached a similar conclusion from Sr and Nd isotopic studies on Tertiary volcanics from northwestern Scotland and the basement rocks of the associated Precambrian continental crust.

Although our data give clear indication of the presence of a granulitic Pb component in the young lavas, the observations do not in themselves indicate the environment in which the mixing occurred. In view of the gross tectonic dissection of the Andes in the study area, the mixing could have happened at higher levels in the crust than are required to produce granulite. The Pb must, however, have been derived from rocks that were once in the lower crust.

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Evidence for Homologous Actions of Pro-Opiocortin Products

Abstract. *α-Melanocyte-stimulating hormone (α-MSH), a modified fragment of adrenocorticotropic hormone, derives from the same biosynthetic route as β-endorphin and is stored by the same arcuate neurons. Microinjection of α-melanocyte-stimulating hormone and several related peptides into the periaqueductal gray matter significantly reduced responsiveness to pain and had a behavioral profile similar to that produced by β-endorphin.*

Some neurons contain at least two putative neurotransmitters or neuromodulators. In particular, neurons arising from the hypothalamic arcuate nucleus contain substances with α-melanocyte-stimulating hormone (α-MSH) and β-endorphin immunoreactivities. Whereas β-endorphin is a known opioid with well-characterized receptors and numerous potent behavioral effects (*1*), α-MSH [*N*-acetyl-ACTH(1-13)-amide], which contains the behaviorally active sequence ACTH(4-10) (*2*), is less well characterized as a peptide with a unique central nervous system function.

The arcuate β-endorphin-containing neurons appear to synthesize active peptides from a common precursor, pro-opiocortin. This glycoprotein, characterized primarily in the pituitary by Mains *et al.* (*3*) and Roberts and Herbert (*4*), contains the full structure of β-lipotropin (β-LPH), β-endorphin, and ACTH(1-39), as well as a 16,000-dalton peptide of unknown function. The structure of pro-opiocortin in bovine pituitary has been obtained by complementary DNA (cDNA) sequencing (*5*). Within the brain, immunohistochemical studies with light microscopy (*6*) and electron microscopy (*7*) have demonstrated that all of the components of pro-opiocortin are found within the same neuron. The final products in the brain show a preponderance of β-endorphin over its precursor, β-LPH, and of α-MSH over its precursor, ACTH (*8*). Furthermore, the immunohistochemical and biochemical studies show that these peptides are packaged in a fashion consistent with their ability to be released (*6, 7, 9*). Studies in human subjects have demonstrated

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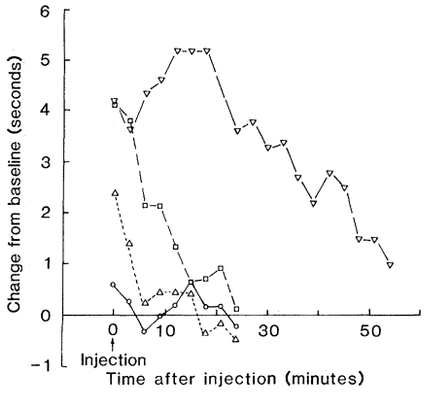

Fig. 1. Effects of MSH-related peptides on tail-flick latency, expressed as change from baseline. The peptide (35 nmole) was injected into the periaqueductal gray, via permanently implanted cannulas, in 2-μl doses; (○) saline, (△) α-MSH, (□) [des-acetyl]α-MSH, and (▽) ORG 2766.

the release of both α-MSH (*10*) and β-endorphin immunoreactivity during electrical stimulation for pain relief (*11*).

The presence of two potentially active substances in the same neuron raises questions about their postsynaptic effects, their regulation, their interaction, and how they modulate behavioral events. The question of whether such substances are functionally agonistic or antagonistic is critical (*12, 13*). We now report that α-MSH and other ACTH-related peptides are powerful analgesics on microinjection into the periaqueductal gray (PAG). This effect parallels that observed with β-endorphin and other opiates, thus demonstrating that common pain-modulating effects can be obtained from two different substances that have the same neuronal origin. Furthermore, the α-MSH analgesia seems to result from the activation of a specific nonopiate receptor.

Long-term indwelling cannulas aimed for the PAG (*14*) were implanted in 34 male rats (250 to 350 g) under deep barbiturate anesthesia. After 1 week of recovery, the animals were tested for analgesia in response to one of several peptides related to α-MSH, administered in one or more 2-μl doses.

Four peptides containing the critical α-MSH sequence ACTH(4-9) were used in the experiments. An analog of ACTH(4-9), ORG 2766 ($N = 8$) (*15*), was initially studied because of its ability to resist enzymatic degradation (*16*). The effects of various doses of ACTH(1-24) ($N = 8$) were also assessed, and the potency of α-MSH ($N = 18$) was compared to that of [des-acetyl]α-MSH because both are possible release products from pro-opiocortin-containing neurons (*8*).

Analgesia was measured with the tail-flick test (*17*); that is, the amount of time required for the rat to withdraw its tail from a source of radiant heat was used as the index of pain sensitivity. For each test session, after baseline tail-flick latencies were established (range, 3 to 5 seconds), the peptide (diluted daily) or a control solution was injected. Testing for analgesia continued at 3-minute intervals for a minimum of 30 minutes or until responses returned to baseline values. Peptide and control administration were counterbalanced by use of a Latin-square experimental design.

After the experiments were completed, the animals were killed with sodium pentobarbital. Frozen sections were obtained, and placement of cannulas was verified from the stereotaxic atlas of de Groot (*18*). For animals tested with ORG 2766, seven of eight implants had been clearly placed in the PAG. All animals tested with ACTH(1-24) had cannulas placed in the PAG, although one of the cannulas pierced the aqueduct. Of the 18 rats tested with α-MSH and [des-acetyl]α-MSH, histology was lost on 4, cannulas pierced the aqueduct in 3, and the cannulas were located throughout the PAG in the others.

The results indicated that all substances produced reliable analgesia. Analysis of variance was used to analyze each experiment. For ORG 2766, the approximate integral of the time function of analgesia was analyzed (*14*). The effects easily reached statistical significance ($P < .0001$) (Fig. 1). Further, studies of the naturally occurring MSH-like substances showed that all produced statistically reliable effects on pain sensitivity [for ACTH(1-24), $P < .05$ (Fig. 2); for [des-acetyl]α-MSH, $P < .001$; and for α-MSH, $P < .001$]. Even though analysis of [des-acetyl]α-MSH and α-MSH revealed no significant difference, [des-acetyl]α-MSH consistently appeared to be the more potent (Fig. 1).

Behavioral observations indicate generalized analgesia in the peptide-treated animals, since pinches and other noxious stimuli did not evoke escape responses. While several motor effects were readily apparent in some animals, significant analgesia was often observed in animals that appeared normal in other respects. The motor effects brought about by these ACTH-like compounds included "straub tail," jumping, and bursts of locomotor activity (*19*). Such behaviors are often observed after central administration of β-endorphin or stabilized enkephalin analogs (*20*). These results are consistent with a conclusion that ACTH-related substances produce behavioral effects similar to those of β-endorphin when directed at terminal fields of pro-opiocortin-containing neurons.

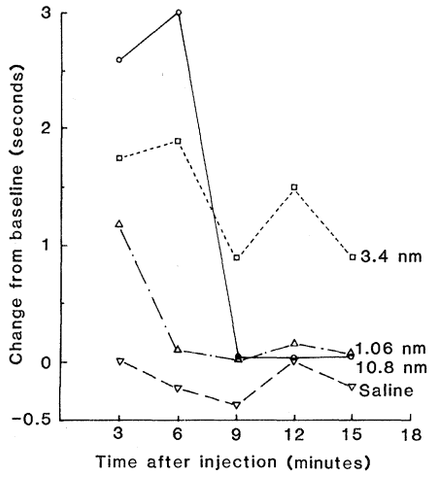

Fig. 2. Change from mean baseline tail-flick latency after microinjection of various doses of ACTH(1-24) into the periaqueductal gray.

Naloxone (2 mg) did not diminish the analgesia induced by ORG 2766. Morphine tolerance, induced by two 75-mg pellets, also failed to inhibit the analgesia. Finally, ORG 2766, at concentrations up to 10 μM, did not displace [^{3}H]naloxone binding (*13*). Thus, the analgesic effects of ORG 2766 are not due to activation of the opiate receptor. One possibility is that a proposed ACTH or α-MSH receptor mediates the effect (*21*).

Our data illustrate a novel and potent effect of α-MSH and ACTH in vivo. The analgesic effects occur in doses nearly equimolar to those necessary for morphine analgesia. This is noteworthy because the naturally occurring peptides are often subject to enzymatic breakdown that leads to underestimates of their analgesic potency. The enkephalins, for example, are rapidly degraded and exhibit modest and rapidly disappearing analgesic effects (*20, 22*), and α-MSH has a short half-life in plasma and brain (*16*), consistent with its brief effects in our behavioral experiments (10 to 15 minutes). Like stabilized enkephalin analogs, ORG 2766 exhibits long-lasting effects. Similarly, β-endorphin is relatively stable against breakdown (*23*) and dissociates from its binding site relatively slowly (*24*); this may account for its extraordinary analgesic potency in vivo (more than 1 hour). It is therefore possible that the simultaneous release of α-MSH and β-endorphin results in differential short- and long-term changes in neuronal excitability.

It is unclear whether the same terminal releases both α-MSH and β-endorphin, or whether these peptides are differentially released at different sites or

under different physiological states. Furthermore, a second group of neurons with α-MSH immunoreactivity has been discovered (*25*) and shown not to contain β-endorphin, a finding that would lead us to expect differential effects between the two peptides in other brain regions.

Nevertheless, our findings have implications for the understanding of endogenous mechanisms of pain modulation. Both opiate and nonopiate mechanisms of pain inhibition endogenous to the brain have been suggested (*26*). Our results demonstrate that these mechanisms can be produced by the action of two substances from the same neurons. Recent findings also indicate that analgesia arising from short-term stress is mediated by nonopiate mechanisms and that analgesia resulting from longer periods of stress is reversed by naloxone treatment (*27*). It is thus conceivable that α-MSH may participate in the former type of pain inhibition and β-endorphin in the latter. Finally, stimulation-produced analgesia is only partially reversed by naloxone (*28*). Since such electrical stimulation of pro-opiocortin-rich areas appears capable of releasing both α-MSH and β-endorphin (*11*), the residual analgesia may be due, in part, to the continued effects of α-MSH released from the same neurons.

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Transformation by Cloned Harvey Murine Sarcoma Virus DNA: Efficiency Increased by Long Terminal Repeat DNA

Abstract. *The coding sequences for the transforming (*src*) protein (p21) of Harvey murine sarcoma virus have been localized to a 1.3-kilobase pair segment near the 5′ end of the viral genome. Ligation of the viral long terminal repeat DNA to the left end of the* src *region DNA markedly enhanced the low transforming efficiency of the* src *region DNA.*

We have been studying the structural and functional organization of the genome of Harvey murine sarcoma virus (Ha-MuSV), a C-type mammalian retrovirus that is highly oncogenic in vivo, induces focal transformation of fibroblasts in tissue culture, and is defective for replication. Infection by Ha-MuSV or other retroviruses results in the formation of infectious linear and circular viral DNA's. The unintegrated and integrated linear viral DNA's have direct long terminal repeat (LTR) sequences, which means that sequences at the left end of the viral DNA are identical to those at the right end (*1*). The LTR of the viral DNA is composed of sequences from both ends of the viral RNA genome; in each LTR, sequences that are derived from the 3′ end of the viral RNA genome are located to the left of sequences that are derived from the 5′ end of the viral RNA. The supercoiled viral DNA's, which represent circularly permuted forms of the unintegrated linear viral DNA, contain single or tandem copies of the LTR (*1, 2*). The function of the LTR's remains to be determined, but it has been speculated that they participate in the integration, transcription, and replication of the viral genome (*1*). In the studies reported here, we have employed transfection of Ha-MuSV DNA onto NIH 3T3 fibroblasts to show that the LTR enhances the efficiency of cellular transformation induced by the transforming (*src*) region of Ha-MuSV, although the 0.65-kbp LTR of Ha-MuSV is not part of the *src* region.

The Ha-MuSV was originally isolated from a tumor that developed in a rat inoculated with Moloney murine leukemia virus (Mo-MuLV) (*3*); it is a recombinant between Mo-MuLV and rat cell nucleic acid sequences (*4*). The 5.5-kb RNA genome of Ha-MuSV is composed of an approximately 4.5-kb insert of rat sequences flanked on the 5′ and 3′ ends, respectively, by about 0.1 and 0.9 kb of Mo-MuLV sequences (*5*). The rat sequences of Ha-MuSV have a dual origin: (i) most are derived from endogenous retrovirus-like sequences that are expressed in some rat cells as 30*S* RNA (30*S* sequences) (*6*); (ii) those about 1 kb near the 5′ end of Ha-MuSV are derived from sequences that are not found as 30*S* (*7*). Transformation by Ha-MuSV is associated with the production of a virus-encoded 21,000-dalton phosphoprotein (p21) which specifically binds guanosine diphosphate (GDP) and is the *src* protein of Ha-MuSV (*8*). Lower levels of an antigenically related p21 are present in normal cells from many species, as is also true of the *src* protein of Rous sarcoma virus (RSV) (*9*).

For a detailed examination of the Ha-MuSV genome, supercoiled Ha-MuSV DNA's have been molecularly cloned in *Escherichia coli* at the single viral Eco RI site, with λ and pBR322 vectors (*10-12*). Since the viral Eco RI site is located near the middle of the linear viral DNA,

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