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An Analysis of the Proopiomelanocortin Systems in the Pituitary of the Squamate Reptile *Lacerta galloti*

ANA LANCHA,* M. A. P. BATISTA,* AND ROBERT M. DORES†,[1]

*Departamento de Microbiologia y Biologia Celular, Universidad de La Laguna, 38206 La Laguna, Tenerife, Canary Islands, Spain; and †Department of Biological Sciences, University of Denver, Denver, Colorado 80208

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Acid extracts of the pars intermedia of the squamate reptile *Lacerta galloti* were screened for immunoreactive forms of proopiomelanocortin (POMC)-related end products following Sephadex G-50 column chromatography. α-MSH-sized end products were detected with a Val-NH_2, C-terminal-specific RIA, and β-endorphin-sized end products were detected with a separate C-terminal-directed RIA. Five peaks of α-MSH-related immunoreactivity were isolated following fractionation by reversed-phase HPLC. Based on a comparison of the reversed-phase HPLC properties and the net positive charges (pH 2.75) of the *Lacerta* forms of α-MSH to those of the mammalian forms of α-MSH and *Anolis carolinensis* ACTH(1–13)NH_2, it appears that the N-acetylation of α-MSH is a major post-translational processing event in the pars intermedia of *L. galloti*. Although multiple forms of β-endorphin were detected in the pars intermedia of *L. galloti* following cation-exchange chromatography, the low levels of N-acetylated β-endorphin detected with an *N*-acetyl-specific β-endorphin RIA indicate that the N-acetylation of β-endorphin in this species is a minor post-translational processing event. This pattern of POMC processing in the pars intermedia of *L. galloti* is similar to the processing events observed for the turtle *Pseudemys scripta*, but distinct from the processing events observed in the squamate reptile *A. carolinensis*.

In the intermediate pituitaries of most fish, amphibians, and mammals, the proopiomelanocortin (POMC) end products, α-MSH and β-endorphin, undergo N-terminal acetylation (Dores *et al.*, 1993a). Although N-acetylation enhances the biological activity of α-MSH in both *in vitro* and *in vivo* assay systems (Ramachandran and Li, 1967), this reaction greatly decreases the opiate receptor binding activity of β-endorphin (Akil *et al.*, 1981; Deakin *et al.*, 1981).

Since α-MSH and β-endorphin undergo N-acetylation in the intermediate pituitaries of amphibians and mammals, it would be reasonable to assume that reptiles also have POMC-specific N-acetylation mechanisms. Recent studies on the turtle *Pseudemys scripta* (Dores and Harris, 1993) support this assumption. However, an apparent exception to this generalization is the lizard *Anolis carolinensis*. Although β-endorphin and α-MSH are produced as major end products in the pars intermedia of *A. carolinensis* (Dores, 1982a,b), the N-acetylation of these end products does not occur (Dores, 1983; Dores *et al.*, 1991). Therefore, it appears that the POMC-specific N-acetylation mechanism in the pars intermedia of *A. carolinensis* has been secondarily lost. The objective of this study was to determine whether the absence of a POMC-specific N-acetylation mechanism is restricted to the pituitary of *A. carolinensis* or whether it is a feature common to other squamate reptiles. To this end, the following studies were conducted on the pituitary of the Old World lizard *Lacerta galloti* (Family: Lacertidae).

[1] To whom reprint requests should be addressed.

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The fossil record supports the conclusion that the modern squamate reptiles have a monophyletic origin (Carroll, 1988). These reptiles can be segregated into two clades, Iguania and Scleroglossa, as reviewed by Zug (1993). *A. carolinensis* is a member of family Iguanidae within clade Iguania, and *L. galloti* is a member of family Lacertidae within clade Scleroglossa. The iguanid and the scleroglossid lines of lizard evolution diverged at the very latest during the mid-cretaceous period. Hence, the two species selected for analysis are not closely related, but represent diverse branches within the phylogeny of the squamate reptiles.

METHODS

Animals

Adult male (mean wet wt, 15 g) and female (mean wet wt, 12 g) *L. galloti* were collected on the island of Tenerife (Canary Islands, Spain). Immediately after capture the animals were sacrificed by decapitation, the dorsal surface of the skull was removed, and the brain and pituitary were dissected. Pituitaries were then separated into the pars distalis and the pars intermedia. Pools of pars distali and pars intermedia were separately extracted in 2 ml of 5 *N* acetic acid which contained 1 mg/ml bovine serum albumin and 0.3 ng/ml phenylmethylsulfonyl fluoride. The extract was mechanically homogenized, and the homogenate was centrifuged at 10,000g for 15 min at 4°. The supernatant was concentrated under vacuum (Speed Vac, Savant) in the presence of 2β-mercaptoethanol (0.01 ml/ml of supernatant) for RIA analysis.

Radioimmunoassay Procedure and RIA Specificity

RIAs were performed as described previously (Dores, 1982a). Heterologous RIAs specific for ACTH(1–39), α-MSH, and β-endorphin were used in this study. The interassay coefficient of variance for these RIAs averaged 10% and the intraassay coefficient of variance averaged 1%.

ACTH RIAs were done using antiserum 19. This antiserum was raised against synthetic human (ACTH(1–24) and is directed against residues 18–24 of ACTH(1–39). Antiserum 19 fully recognizes human ACTH(1–39) and has 10% molar cross-reactivity with synthetic human ACTH(18–39), but it has less than 0.1% molar cross-reactivity with α-MSH. This antiserum was used at a final dilution of 1:30,000.

α-MSH RIAs were done using antiserum 7a. This antiserum was used at a final dilution of 1:20,000. Specificity tests indicate that this RIA is C-terminal directed and fully recognizes ACTH(1–13)NH_2, *N*-acetyl-ACTH(1–13)NH_2 (monoacetylated α-MSH), and *N,O*-diacetyl-ACTH(1–13)NH_2 (diacetylated α-MSH). However, this antiserum has less than 0.1% molar cross-reactivity with *N*-acetyl-ACTH(1–13) free acid and ACTH(1–39) (Dores and Joss, 1988).

Two β-endorphin-specific RIAs were used in this study. Antiserum 2 is specific for the C-terminal of β-endorphin. This antiserum was raised against synthetic human β-endorphin(1–31) and was used at a final dilution of 1:10,000. Specificity tests indicate that this antiserum fully recognizes human β-endorphin(1–31), camel β-endorphin(1–27), and camel β-endorphin(1–26), but has less than 0.1% molar cross-reactivity with human α-endorphin(1–16) and methionine enkephalin (Steveson *et al.*, 1990).

N-Acetylated forms of β-endorphin were detected with antiserum 24. This antiserum was raised against synthetic N-acetylated salmon β-endorphin II(1–16). Specificity tests indicate that antiserum 24 is directed against the acetylated N-terminal of β-endorphin and fully recognizes N-acetylated human α-endorphin(1–16), N-acetylated camel β-endorphin(1–31), N-acetylated salmon β-endorphin I, and N-acetylated salmon β-endorphin II. However, this antiserum has less than 0.1% molar cross-reactivity with methionine enkephalin, human β-endorphin(1–16), camel β-endorphin(1–31), nonacetylated salmon β-endorphin I, and nonacetylated salmon β-endorphin II(1–16) (Dores and Harris, 1993). Antiserum 24 was used at a final dilution of 1:20,000.

Sephadex G-50 Column Chromatography Procedure

Acid extracts of lizard pars distali and pars intermedia were separately fractionated by gel filtration chromatography on a calibrated Sephadex G-50 column (1 × 40 cm) equilibrated in 10% formic acid. The flow rate of the column was 6 ml/hr and 0.6-ml fractions were collected. The void volume (V_o) and the total volume (V_t) of the column were marked by adding 1 mg of bovine serum albumin and 0.075 ml of 2β-mercaptoethanol, respectively, to the sample. Both internal markers were detected spectrophotometrically at A_{280}.

Reversed-Phase HPLC Procedure

The α-MSH-related immunoreactivity detected in extracts of the pars intermedia was further analyzed by reversed-phase HPLC. This material was fractionated on a Beckman C-18 ODS ultrasphere 5-μm column (4.6 mm × 25 cm) which was equilibrated in 0.1%

trifluoroacetic acid. The column was eluted with an increasing acetonitrile gradient. After each unknown run, the column was eluted with a linear acetonitrile gradient (blank program: 5 to 80% acetonitrile/0.1% trifluoroacetic acid) over a 60 min period, and reequilibrated to 5% acetonitrile/0.1% trifluoroacetic acid over the next 30-min period. Upon completion of the blank program, the following synthetic standards were chromatographed: ACTH(1–13)NH_2, *N*-acetyl-ACTH(1–13)NH_2 (α-MSH), and *N,O*-diacetyl-ACTH(1–13)NH_2 (diacetylated α-MSH) (all purchased from Bachem, Torrance, CA). These standards were detected spectrophotometrically at an absorbance of 220 nm. After the standards were chromatographed, the column was eluted with 80% acetonitrile for 30 min.

Cation-Exchange Chromatography Procedure

The immunoreactive β-endorphin-related material detected in extracts of the pars intermedia was further fractionated by cation-exchange chromatography on a Bio-Sil TSK CM-2SW column (4.6 mm × 25 cm). This column was equilibrated in 22 m*M* ammonium formate/30% acetonitrile and eluted with a linear ammonium formate gradient (22–450 m*M*) at pH 2.75. Aliquots of column fractions were screened with either the N-terminal-specific β-endorphin RIA or the C-terminal-specific RIA. The retention times of the forms of lizard β-endorphin detected by RIA were compared to the retention times of the following standards: N-acetylated human α-endorphin(1–16) (+1), ACTH(1–13)NH_2 (+4), and human β-endorphin(1–31) (+6) (all purchased from Bachem).

The net positive charge of each form of lizard α-MSH-related immunoreactivity isolated by reversed-phase HPLC was also determined on the CM-2SW column at pH 2.75.

RESULTS

POMC Products Detected in the Pars Intermedia

Extracts of adult male and female pars intermedia were separately fractionated by gel filtration on a Sephadex G-50 column to determine whether there were any gender differences in the production of POMC-related products in this tissue. A typical profile for an extract of male pars intermedia is shown in Fig. 1. For this gel filtration analysis, 13 adult male pars intermedia were pooled. The C-terminal-directed α-MSH RIA detected a major peak of immunoreactivity which eluted at a K_{av} value of 0.81. This immunoreactive material had an apparent molecular weight of 1.5K which is identical to that of synthetic α-MSH. The recovery of α-MSH-sized im-

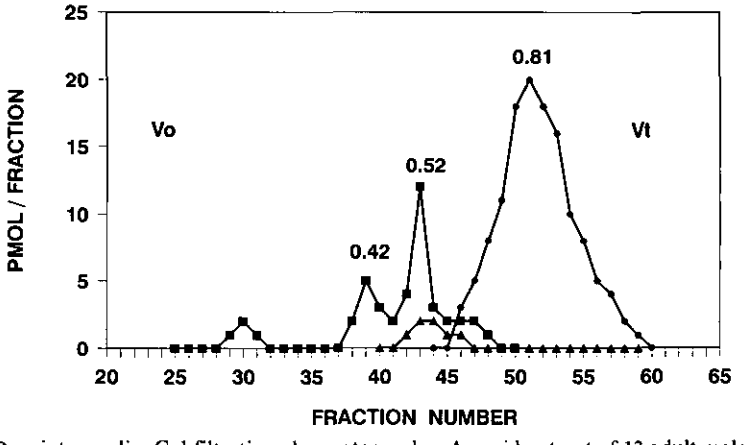

FIG. 1. Pars intermedia: Gel-filtration chromatography. An acid extract of 13 adult male lizard pars intermedia was fractionated by gel filtration on a calibrated Sephadex G-50 column (1 × 40 cm) which was equilibrated in 10% formic acid. Aliquots of column fractions were screened with the α-MSH RIA (●), the C-terminal-specific β-endorphin RIA (■), or the *N*-acetyl-specific β-endorphin RIA (▲). The K_{av} values for the major immunoreactive peaks are shown. In addition, the elution of the void volume (V_o) and total volume (V_t) markers is shown.

munoreactivity from this extract was 122 pmol.

Two peaks of immunoreactivity were detected with the C-terminal β-endorphin RIA. The first peak eluted with a K_{av} value of 0.42 and had an apparent molecular weight of 5K. The second peak eluted with a K_{av} value of 0.52 and had an apparent molecular weight of 3.5K. The identity of the former immunoreactive peak is unclear. However, this form may be an N-terminally truncated form of lizard β-LPH. The latter peak of immunoreactivity is within the molecular weight range for β-endorphin-sized material. Overall, the C-terminal β-endorphin RIA detected 37 pmol of immunoreactivity in the lizard extract.

In order to determine whether N-acetylated forms of β-endorphin were present in the lizard pars intermedia extracts, column fractions (Fig. 1) in a molecular weight range from 3.5K to 1.0K (fractions 39 to 59) were screened with the *N*-acetyl-specific β-endorphin RIA. N-Acetylated β-endorphin-related immunoreactivity was detected in fractions 41 to 47. However, only a total of 7 pmol of immunoreactivity was detected with the *N*-acetyl-specific β-endorphin RIA in these fractions.

Gel filtration analysis of pools of adult female pars intermedia yielded similar profiles of α-MSH-related and β-endorphin-related immunoreactivity (data not shown). However, the content of α-MSH-related immunoreactivity in the male pars intermedia was approximately eightfold higher than the content of α-MSH-related immunoreactivity in the female pars intermedia. This observation is of interest since the average wet weight of the pars intermedia was approximately 5 mg for both sexes.

Upon completion of several analyses of male and female pars intermedia extracts, the following pattern emerged: (1) α-MSH-sized immunoreactivity was the major POMC-related product detected; (2) the content of α-MSH-sized immunoreactivity was fourfold higher than the β-endorphin-related immunoreactivity detected; and (3) N-acetylated forms of β-endorphin accounted for only 19% of the total β-endorphin-related material isolated by gel filtration.

In order to determine if multiple forms of β-endorphin were present in the lizard pars intermedia, the β-endorphin-sized material detected in fractions 41 to 48 (Fig. 1) was fractionated by cation-exchange chromatography (Fig. 2). The C-terminal β-endorphin RIA detected peaks of immunoreactivity which eluted with retention times of 15, 25, 40, 54, 61, and 73 min. In addition, N-acetylated β-endorphin-related material was detected in the 25-min peak and the 40-min peak. Finally, a minor peak of immunoreactivity was detected with the *N*-acetyl-specific β-endorphin RIA at a retention time of 7 min. This peak was not detected with the C-terminal-specific β-endorphin RIA. As indicated in Table 1, the 40- and the 54-min peaks were the major forms of β-endorphin detected and represented, respectively, 51 and 36% of the total β-endorphin detected. This analysis confirmed that multiple forms of β-endorphin are present in the lizard pars intermedia. However, as indicated in Fig. 2, N-acetylated forms of β-endorphin are produced as minor end products in the lizard pars intermedia.

The α-MSH-sized immunoreactivity detected in Fig. 1 was further fractionated by reversed-phase HPLC to determine whether multiple forms of α-MSH were present in the lizard pars intermedia extracts. As shown in Fig. 3, five peaks of immunoreactivity were detected. Peaks 1, 3, and 4 eluted with retention times very similar to mammalian ACTH(1–13)NH_2, mammalian *N*-acetyl-ACTH(1–13)NH_2, and mammalian *N,O*-diacetyl-ACTH(1–13)NH_2, respectively. Peak 2 had reversed-phase HPLC properties very similar to those of purified *A. carolinensis* ACTH(1–13)NH_2. Finally, the most hydrophobic form, Peak 5, had reversed-phase HPLC properties

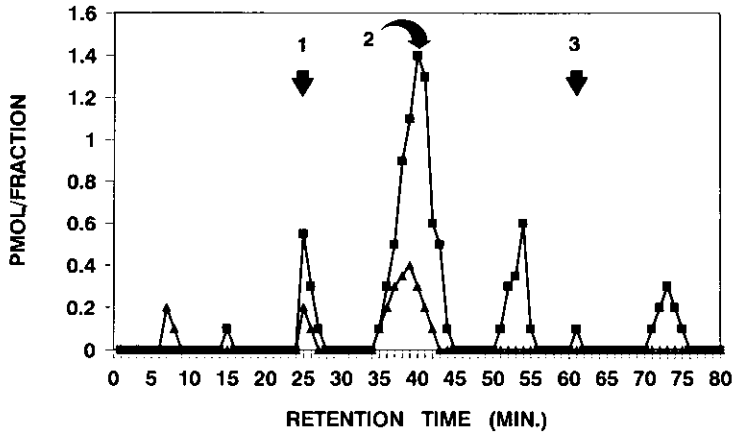

FIG. 2. Pars intermedia: Cation-exchange chromatography. The fractions of β-endorphin-sized immunoreactivity detected in Fig. 1 (fractions 40 to 48) were pooled, concentrated, and fractionated by cation-exchange chromatography as described under Methods. Aliquots of column fractions were screened with either the C-terminal-specific β-endorphin RIA (■) or the *N*-acetyl-specific β-endorphin RIA (▲). The following standards were chromatographed separately: (1) α-MSH (+3); (2) ACTH(1–13)NH_2; and (3) human β-endorphin (+6).

distinct from those of any of the currently characterized forms of vertebrate α-MSH.

In an effort to clarify the identities of some of the peaks of α-MSH-sized material detected in Fig. 3, peaks 2,3,4, and 5 were separately analyzed by cation-exchange chromatography. As shown in Table 2, Peak 2 had a net positive charge of +4 at pH 2.75, whereas Peaks 3,4, and 5 each had a net charge of +3. The quantity of Peak 1 recovered from the HPLC analysis was insufficient for screening by the cation-exchange procedure. It is noteworthy that nonacetylated forms of α-MSH [ie. ACTH(1–13)NH_2] have a net charge of +4 at this pH, whereas acetylated forms of α-MSH (either monoacetylated or diacetylated) have a net charge of +3 at pH 2.75.

POMC Products Detected in the Pars Distalis

Since both α-MSH-related and β-endorphin-related end products are derived from

TABLE 1
FORMS OF LIZARD β-ENDORPHIN ISOLATED BY CATION-EXCHANGE CHROMATOGRAPHY

Peak[a]	Net positive charge[b]	Detected with C-terminal β-endorphin RIA	Detected with *N*-acetyl β-endorphin RIA	Percentage of total immunoreactivity detected
7	+1	No	Yes	3
15	+2	Yes	No	>1
25	+3	Yes	Yes	5
40	+4	Yes	Yes	51
54	+5	Yes	No	36
61	+6	Yes	No	>1
73	+7	Yes	No	4

[a] Retention time (minutes).
[b] pH 2.75.

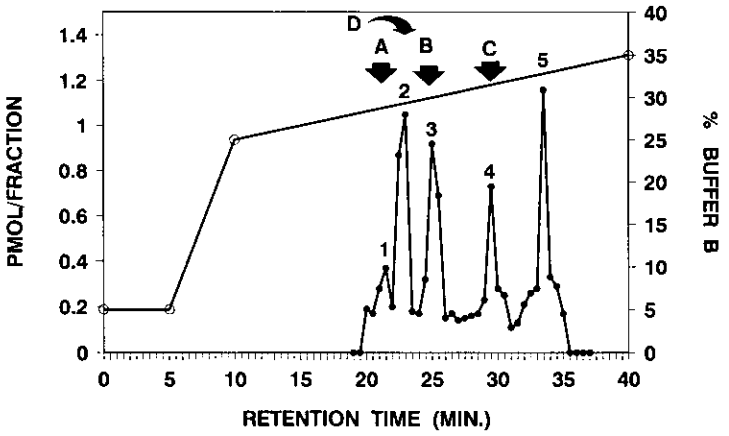

FIG. 3. Pars intermedia: Reversed-phase HPLC analysis. The fractions of α-MSH-related immunoreactivity detected in Fig. 1 (fractions 46 to 56) were pooled, concentrated, and fractionated by reversed-phase HPLC on a Beckman C-18 ODS column. The column was equilibrated in 0.1% trifluoroacetic acid (buffer A) and was eluted with increasing acetontrile gradient. Buffer B was 0.1% trifluoroacetic acid/80% acetonitrile. Aliquots of column fractions were screened with the α-MSH RIA (●). Each immunoreactive peak is designated by number (1 through 5). The retention times of the following standards (marked by arrows) are shown: (A) mammalian ACTH(1–13)NH_2; (B) mammalian monoacetylated α-MSH; (C) mammalian diacetylated α-MSH; and (D) *Anolis carolinensis* ACTH(1–13)NH_2.

a common precursor, these end products should be present in roughly equimolar amounts in extracts of the lizard pars intermedia. As shown in Fig. 1, this was not the case. The low levels of β-endorphin-related material detected in the pars intermedia relative to the levels of α-MSH-related products detected could be due to: (a) changes in the primary sequence of the epitope within the lizard β-endorphin which is recognized by the C-terminal β-endorphin RIA or (b) novel proteolytic processing events which generate C-terminally shortened forms of nonacetylated β-endorphin. The first possibility has been observed in studies on forms of salmon and dogfish β-endorphin (Kawauchi *et al.*, 1980; Lorenz *et al.*, 1986). The latter possibility has been observed in the pars intermedia of the toad *Bombina orientalis*, where the major form of β-endorphin has a molecular weight of 1.2K (Dores *et al.*, 1992).

TABLE 2
FORMS OF LIZARD α-MSH ISOLATED BY REVERSED-PHASE HPLC

Peak	Retention time (min)	Net positive charge at pH 2.75	Percentage of total immunoreactivity detected
1	21.5	Not determined	11
2	22.5	+4	24
3	25.0	+3	25
4	29.5	+3	16
5	33.5	+3	24

To evaluate these two possibilities, extracts of the female lizard pars distalis were examined. As shown in Fig. 4, although a prominent peak of ACTH-sized immunoreactivity could be detected in this extract at a K_{av} value of 0.45, the levels of β-LPH-sized immunoreactivity (K_{av} value of 0.21) and β-endorphin-sized immunoreactivity (K_{av} value of 0.50) were significantly lower. Similar observations were made for male pars distalis extracts (data not shown). Since β-endorphin does not usually undergo C-terminal cleavage in the pars distalis of most gnathostomes (Loh, 1992), it appears that the C-terminal β-endorphin RIA used in this study is underestimating the total amount of β-endorphin present in these tissues.

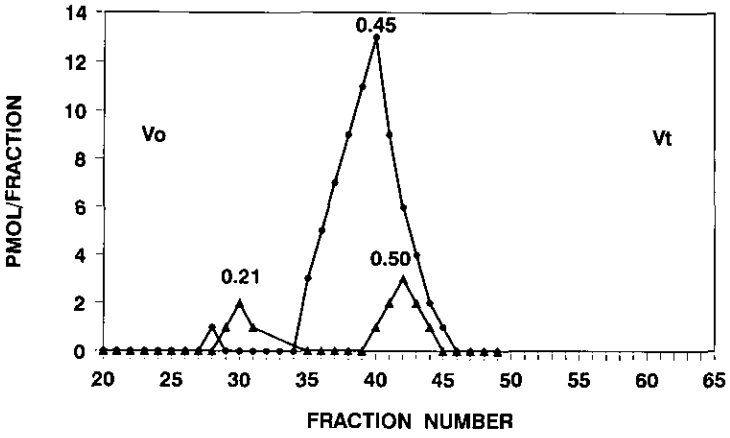

FIG. 4. Pars distalis: Gel filtration chromatography. An acid extract of eight female adult lizard pars distali was fractionated by gel filtration on a calibrated Sephadex G-50 column as described in the legend to Fig. 1. Aliquots of column fractions were separately screened with a C-terminal-specific β-endorphin RIA (▲) or a middle-region-specific ACTH(1–39) RIA (●). Abbreviations: V_o, void volume; V_t, total volume.

DISCUSSION

A characteristic feature of the pars intermedia of gnathostomes is the generation of N-terminally acetylated forms of α-MSH and β-endorphin as a result of the post-translational processing of the polypeptide hormone precursor, POMC (Dores *et al.*, 1993a). The detection of N-acetylated forms of α-MSH and β-endorphin in gnathostomes ranging from cartilaginous fish to mammals strongly suggests that the POMC-specific N-acetylation mechanisms appeared early in the evolution of the vertebrates. What might be construed as the ancestral gnathostome scheme for the N-acetylation of α-MSH and β-endorphin has been retained in mammals, where N-acetylated forms of α-MSH and β-endorphin are the major POMC-derived end products produced by the pars intermedia (Eipper and Mains, 1980; Zakarian and Smyth, 1982). This processing scheme also has been observed in the cartilaginous fish *Hydrolagus colliei* (Dores *et al.*, 1993a), the cladistian fish *Calamoichthys calabaricus* (Dores *et al.*, 1993b), two species of holostean fishes (Dores *et al.*, manuscript in preparation), two species of lungfish (Dores and Joss, 1988; Vallarino *et al.*, 1992), and several species of anuran amphibians (Vaudry *et al.*, 1984; Dores *et al.*, 1992).

A minor deviation from the general gnathostome N-acetylation scheme has been observed in the pars intermedia of the turtle *P. scripta* (Dores and Harris, 1993). Although the N-acetylation of α-MSH is a major post-translational processing event in this species, the N-acetylation of β-endorphin is only a minor processing event; approximately 22% of the total β-endorphin in the pars intermedia is in the N-acetylated state. On the other hand, a major deviation from the general gnathostome N-acetylation scheme has been observed for the pars intermedia of the lizard *A. carolinensis* (Dores, 1983; Dores *et al.*, 1991). In this species, the POMC-specific N-acetylation mechanisms are absent, and the major end products produced in the pars intermedia are ACTH(1–13)NH_2 and nonacetylated forms of β-endorphin. The objective of this study was to determine whether the absence of POMC-specific N-acetylation mechanisms in the pars intermedia of *A. carolinensis* is unique to this species or if it is a common trait among squamate reptiles. Since *A. carolinensis* is a New World

iguanid, the Old World scleroglossid lizard *L. galloti* was selected for analysis. The working hypothesis of this study was that *L. galloti* would either resemble *A. carolinensis* and produce nonacetylated forms of α-MSH and β-endorphin or resemble the turtle *P. scripta* (Dores and Harris, 1993) and product N-acetylated forms of α-MSH and, to a lesser extent, N-acetylated forms of β-endorphin.

As expected, both α-MSH-sized and β-endorphin-sized immunoreactive forms were detected in extracts of the pars intermedia of *L. galloti* following gel filtration chromatography analysis. The screening of β-endorphin-sized forms with the *N*-acetyl-specific β-endorphin RIA indicated that only 19% of the total β-endorphin isolated was in the N-acetylated state. However, this value is clearly inflated due to the fact that the C-terminal directed β-endorphin RIA used in this study underestimated the total content of β-endorphin in extracts of both the pars intermedia and the pars distalis of *L. galloti*. Hence, the N-acetylation of β-endorphin is, at best, a minor post-translational processing event in the pars intermedia of *L. galloti*. In this regard, the post-translational processing of POMC in the pars intermedia of *L. galloti* resembles the processing events observed in the turtle.

Reversed-phase HPLC analysis of the α-MSH-sized immunoreactivity revealed the presence of multiple forms of α-MSH in the *L. galloti* pars intermedia extracts. However, the profile of immunoreactive forms was far more complicated than anticipated. The five immunoreactive forms detected in Fig. 3 share several properties. Based on the gel filtration analysis presented in Fig. 1, all of these forms have an apparent molecular weight similar to that of synthetic mammalian α-MSHs. Furthermore, based on the specificity of the α-MSH RIA, each of these immunoreactive forms has a C-terminal valine-NH_2 residue.

Based on comparisons with the reversed-phase HPLC retention times of mammalian forms of α-MSH and *A. carolinensis* ACTH(1–13)NH_2, the *L. galloti* immunoreactive forms could be separated into three groups. The first group consists of immunoreactive forms which eluted with HPLC retention times similar to those of the mammalian forms of α-MSH. Peaks 1, 3, and 4 eluted with retention times nearly identical to those of mammalian ACTH(1–13)NH_2, monoacetylated α-MSH, and diacetylated α-MSH, respectively. It is noteworthy that Peaks 3 and 4 both had net positive charges of +3 at pH 2.75 (Table 2). Both the monoacetylated and diacetylated forms of mammalian α-MSH also have a net charge of +3 at this pH.

Peak 2 did not co-elute with any of the mammalian forms of α-MSH. Instead, this peak had reversed-phase HPLC properties identical to those of *A. carolinensis* ACTH(1–13)NH_2. Furthermore, this immunoreactive peak had a net positive charge of +4 at pH 2.75, the same as *A. carolinensis* ACTH(1–13)NH_2.

Peak 5 did not co-elute with any of the available α-MSH standards and must be placed into a third group of α-MSH-related substances. Since this peak had a net positive charge of +3, it may be the acetylated form of Peak 2. The hydrophobic nature of Peak 5 would be consistent with this conclusion.

Based on the preceding observations, it would appear that in *L. galloti*, as in the turtle, the N-acetylation of α-MSH is a major processing event in the pars intermedia. This conclusion is based on the assumption that N-acetylated forms of α-MSH will have a net positive charge of +3. However, in the absence of primary sequence data, these conclusions are only tentative. In spite of this caveat, the detection of multiple forms of α-MSH in the pars intermedia of *L. galloti* with chromatographic properties similar to those of the forms of mammalian α-MSH and at least one form of α-MSH with a chromatographic profile sim-

ilar to that of *A. carolinensis* ACTH(1–13)NH_2 is unique to this species. These observations may reflect the expression of more than one POMC gene in the pars intermedia of *L. galloti*. However, the possibility that *L. galloti* α-MSHs undergo novel post-translational modifications cannot be dismissed at this time.

Even with the preceding reservations, it is clear from the data presented that the processing of POMC in the pars intermedia of *L. galloti* is distinct from the processing events observed for *A. carolinensis*. The latter species clearly lacks POMC N-acetylation mechanisms, and this may be a characteristic shared by other members of genus *Anolis*, or perhaps it is unique to the iguanids.

In many respects, the processing of POMC in the pars intermedia of *L. galloti* resembles the processing events observed in the pars intermedia of the turtle *P. scripta* (Dores and Harris, 1993). The low levels of N-acetylated β-endorphin and the apparently higher levels of acetylated forms of α-MSH represent a shift away from the dual N-acetylation mechanisms for α-MSH and β-endorphin observed in anuran amphibians (Dores *et al.*, 1991) and mammals (Glembotski, 1982; Zakarian and Smyth, 1982). At this stage, it is unclear whether a single N-acetyltransferase which favors α-MSH over β-endorphin as a substrate has evolved in some reptiles or whether there is differential expression of α-MSH-specific and β-endorphin-specific *N*-acetyltransferases in the pars intermedia of some reptiles.

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