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# A new neurohypophysial peptide, seritocin ([Ser$^5$,Ile$^8$]-oxytocin), identified in a dryness-resistant African toad, *Bufo regularis*

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From the pituitary neurointermediate lobe of the African toad *Bufo regularis*, vasotocin, hydrin 2 (vasotocinyl-Gly) and a mesotocin-like peptide have been isolated by HPLC and characterized by mass spectrometry, amino acid sequence and chromatographic coelution with synthetic peptides. The mesotocin-like peptide has been identified as [Ser$^5$,Ile$^8$]-oxytocin in place of mesotocin ([Ile$^8$]-oxytocin) found in all other amphibians investigated to date. The name *seritocin* is suggested. The molecule is virtually devoid of oxytocic activity on rat uterus in contrast to mesotocin. On the other hand, the molar ratio of hydrin 2 to vasotocin in the pituitary reaches 2, whereas it is about 1 in toads and frogs from temperate regions. *B. regularis* is an anuran species able to withstand a hot and dry season by burrowing. The possible relationship between occurrence of seritocin and adaptation to arid environment remains to be demonstrated. © Munksgaard 1995.

*Key words:* African toad; amphibian neuropeptide; *Bufo regularis*; neurohypophysial hormone evolution; oxytocin-like peptide

Amphibia, as other nonmammalian tetrapods such as reptiles and birds, possess two neurohypophysial hormones, mesotocin ([Ile8]-oxytocin) and vasotocin ([Ile3]-vasopressin), in contrast to mammals, that have oxytocin and vasopressin(s) (1, 2). Whereas vasotocin promotes reabsorption of water through the kidney nephron (antidiuretic activity) and, in anuran amphibia, through the urinary bladder and skin (3), the role of mesotocin in osmoregulation is less certain (4). In addition to mesotocin and vasotocin, in anuran species, intermediates derived from differential processing of provasotocin and termed hydrins, namely hydrin 1 (vasotocinyl-Gly-Lys-Arg) in *Xenopus laevis*, and hydrin 2 (vasotocinyl-Gly) in frogs and toads, have been identified (5). Hydrins stimulate water transport through the frog skin *in vivo* or the urinary bladder membrane *in vitro* (5) but, in contrast to vasotocin, seem devoid of antidiuretic action on the frog kidney (6). Furthermore, in amphibians able to adapt to an arid environment, the molar ratio of hydrin 2 to vasotocin is about 2, rather than 1 for anurans living in temperate regions (6).

The African toad *Bufo regularis* faces the hot and dry season by burrowing in the ground. Vasotocin and hydrin 2 have been identified in this species by coelution with synthetic peptides in high-performance reversed-phase liquid chromatography (HPLC), pharmacological properties (vasotocin) and microsequencing (7). An additional peptide has been detected which had a retention time in HPLC different from that of mesotocin, the hormone characterized in all other species of frogs and toads investigated to date (6, 7). We describe here experiments performed in order to identify the oxytocin-like peptide of the toad *B. regularis*.

## EXPERIMENTAL PROCEDURES

*Animals.* Toads (40–90 g) were caught during the dry season (November) near Ouagadougou (Burkina-Faso). A first batch (211 animals) was maintained one night after capture under humid conditions and sacrified. Anterior and posterior lobes of the pituitary were dissected and desiccated in cold peroxide-free acetone. A second batch (98 animals) was kept 3 days after capture in the dry and hot (25–30 °C) open air. The decrease of body weight due to evaporation was about 20%. Glands were taken out and dried in the same way as for the first batch. A third batch (25 animals) was flown to the laboratory and maintained for several months at room temperature with free access to water. These animals were mainly used for measuring water uptake activity (8).

*Gland extraction.* Acetone-desiccated neurointermediate lobes (average weight 0.12 mg) were extracted with 0.1 M HCl (20 $\mu$L/gland) for 4 h at 4 °C under stirring. The oxytocic activity was 0.13 U/mg and the pressor activity 0.18 U/mg for animals killed one night after capture. After centrifugation for 1 h at 4 °C, the supernatant was subjected to analytical (1 or 2 glands) or preparative (12 glands) reversed-phase high-performance liquid chromatography (HPLC). Individual fresh posterior pituitaries (average weight 0.6 mg) were extracted in the same way using 60 $\mu$L of 0.1 M HCl per gland.

*High-performance liquid chromatography (HPLC).* HPLC was performed on a Waters model 204 chromatograph equipped with a model U6K manual injector, a model 680 solvent programmer, a model 441 UV absorbance detector and a model 730 data module. A Delta-Pak Waters 300 A column (3.9 × 150 mm, particle size 5 $\mu$m, pore size 300 Å) was employed.

Absorbance was monitored at 214 nm. Four acetonitrile systems were used: the first with a linear acetonitrile gradient (0–60%) containing 0.05% trifluoroacetic acid (TFA) during 60 min, the second with a three-step linear acetonitrile gradient (0–7% for 7.5 min, 7–22% for 20 min and 22–60% for 32.5 min) containing 0.05% TFA, the third with a linear acetonitrile gradient (0–60%) containing 0.155 M NaCl for 60 min, the fourth with a concave gradient (Waters curve 9) of acetonitrile 9–60% containing 0.05% TFA.

Preparative chromatographies were performed using 200 $\mu$L of 0.1 M HCl extract corresponding to 12 neurointermediate lobes, for injection. Fractions (0.35 mL) were collected with a flow rate of 0.7 mL/min. Fractions concentrated in a Speed-Vac concentrator were used for microsequencing (6, 7).

*Bioassays.* Oxytocic (uterotonic) activity was determined on isolated rat uterus according to Holton (9) and pressor activity on anaesthetized rats according to Dekanski (10). Water uptake activity was measured on *Rana esculenta in vivo* according to Bentley (8).

*Microsequencing.* Microsequencing of peptides was carried out in an Applied Biosystems model 470 A gas-phase protein sequencer under the conditions described by Hewick *et al.* (11). Phenylthiohydantoin derivatives were identified and measured by reversed-phase HPLC in an on-line Applied Biosystems model 120 A.

*Molecular mass determination.* Matrix-assisted laser desorption mass spectrometric analysis was performed on HPLC-purified peptides according to Beavis & Chait (12).

*Synthetic peptides.* Synthetic vasotocin and hydrin 2 (vasotocinyl-Gly) were kindly given by Prof. M. Manning (Ohio University, USA). [Ser5,Ile8]-oxytocin was prepared by Dr. Carl-Johan Aurell (Ferring Research Institute, Malmö, Sweden). Peptide homogeneity was checked by HPLC and mass spectrometry.

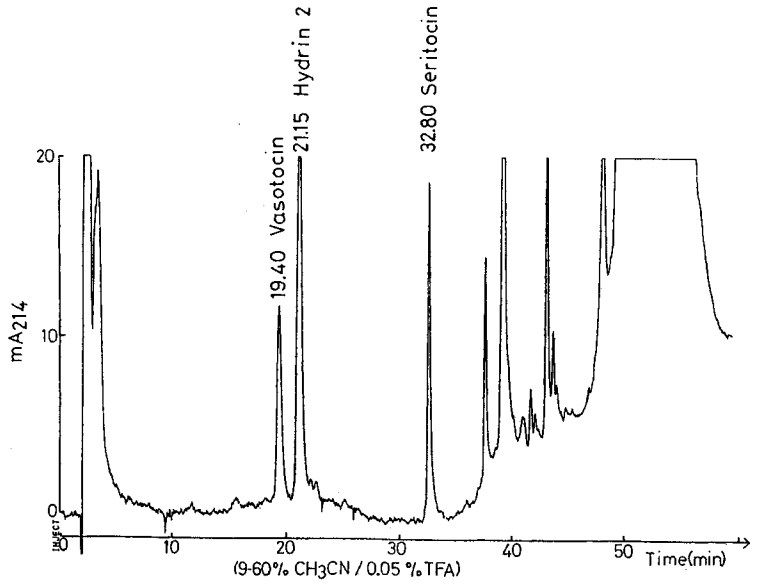


FIGURE 1

Separation by HPLC of neurohypophysial peptides from a chlorhydric extract (0.1 M HCl) of acetone-desiccated neurointermediate pituitaries of *B. regularis*. Retention times are indicated in minutes. A concave acetonitrile gradient (9–60%, Waters curve 9) containing 0.05% trifluoroacetic acid for 60 min was used.

TABLE 1

*Amino acid sequences of hydrin 2 and mesotocin-like peptide of B. regularis*[a]

| Hydrin 2 (vasotocinyl-Gly) (RT 21.15) | | | Mesotocin-like peptide (RT 32.80) (80 pmol) | | |
|---|---|---|---|---|---|
| Cycle | Residue identified | pmol | Cycle | Residue identified | pmol |
| 1 | – | – | 1 | – | – |
| 2 | Tyr | 69 | 2 | Tyr | 10.6 |
| 3 | Ile | 110 | 3 | Ile | 11.7 |
| 4 | Gln | 44 | 4 | Gln | 6.9 |
| 5 | Asn | 82 | 5 | Ser | 2.5 |
| 6 | – | – | 6 | – | – |
| 7 | Pro | 90 | 7 | Pro | 5.8 |
| 8 | Arg | 40 | 8 | Ile | 5.0 |
| 9 | Gly | 29 | 9 | Gly | 6.0 |
| 10 | Gly | 22 | 10 | – | – |

[a] Half-cystines (cycles 1 and 6) are not detected when intact peptides are subjected to sequencing.

## RESULTS

*Purification of neurohypophysial peptides*

30 acetone-desiccated posterior pituitaries (3.72 mg) from animals caught during the hot season and sacrified one night after capture were extracted with 0.5 mL of 0.1 M HCl for 4 h at 4 °C. The supernatant (0.49 mL) was separated by centrifugation. Two preparative HPLC were performed under the conditions described above, 0.2 mL of the supernatant being injected in each experiment. A concave gradient (Waters curve 9) of acetonitrile 9–60% containing 0.05% TFA was applied for 60 min. Fractions (0.35 mL) were collected with a flow rate of 0.7 mL/min. Absorbance was monitored at 214 nm. Bioassays were performed on aliquots. Separation of peptides is shown in Fig. 1. Tubes corresponding to each peak were pooled, and solutions were concentrated in a Speed Vac concentrator to small volumes.

*Identification of neurohypophysial peptides*

*Vasotocin.* The peptide with a retention time 19.40 min (Fig. 1) was identified as vasotocin by coelution with the synthetic peptide in HPLC and by its pharmacological properties (7), in particular the ratio of pressor activity to oxytocic activity of about 2, a ratio typical of vasotocin (13, 14).

*Hydrin 2.* The peptide with a retention time 21.15 min (Fig. 1) was characterized as vasotocinyl-Gly by coelution with the synthetic peptide in HPLC and by amino acid sequence determination (7) (Table 1). The amount of hydrin 2 is about twice that of vasotocin, assuming the two peptides have the same molecular absorbance.

*Mesotocin-X.* The peptide with a retention time of 32.80 min occupies a position in HPLC that is close but not identical to that of mesotocin ([Ile8]-oxytocin), the oxytocin-like peptide identified in all the amphibian species investigated to date (5, 6). When synthetic mesotocin is added to the extract, distinct peaks are observed for mesotocin and *B. regularis* peptide (Fig. 2). Furthermore, the *B. regularis* peptide does not display

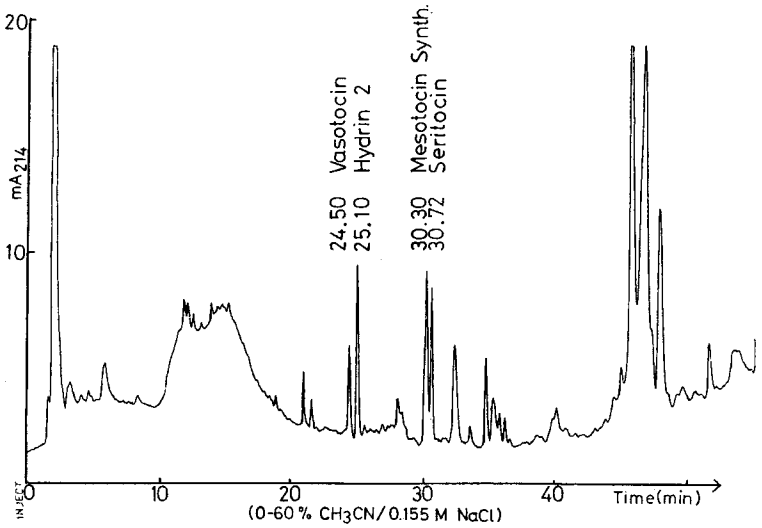


FIGURE 2

Separation by HPLC of the mesotocin-like peptide (seritocin) of *B. regularis* and synthetic mesotocin added to the chlorhydric extract of a fresh neurointermediate pituitary. Retention times are indicated in min. A linear acetonitrile gradient (0–60%) containing 0.155 M NaCl for 60 min was used.

oxytocic activity, in contrast to mesotocin, the molecular oxytocic activity of which is 291 mU/nmol (13) (Table 2).

Amino acid sequencing reveals that the peptide has a similar structure to that of mesotocin, but position 5 is occupied by Ser instead of Asn found in all oxytocin-like peptides identified up to now (1, 2) (Table 1). Mesotocin-X therefore appears to be [Ser5,Ile8]-oxytocin, assuming an amidated *C*-terminal residue.

Synthetic [Ser5,Ile8]-oxytocin (Ferring Research Institute) has been compared with the oxytocin-like peptide isolated from *B. regularis* neurointermediate pituitary. Figure 3 shows chromatographic results for the purified peptide (seritocin) and the mixture of natural peptide and synthetic [Ser5,Ile8]-oxytocin in a three-step linear acetonitrile gradient.

The molecular mass of mesotocin-X has been determined by matrix-assisted laser desorption mass spectrometry (12). Two determinations carried out on two distinct HPLC-purified samples gave 979.6 and 979.6 ± 0.5 Da. Independent mass spectrometry measurements performed on the synthetic peptide by Dr. C.J. Aurell (Ferring Research Institute) gave 979 Da. The calculated molecular mass for [Ser5,Ile8]-oxytocin is 978, and there is good agreement between the values. Mesotocin has recently been identified in the Australian lungfish, *Neoceratodus forsteri* (15). The molecular mass determined by laser desorption mass spectrometry was 1006.5 ± 0.5 for an expected value of 1007.2. So the two oxytocin-like peptides can be clearly distinguished.

## DISCUSSION

*Substitutions in natural oxytocin-like peptides*

When the 13 vertebrate neurohypophysial hormones and the 5 invertebrate neurohypophysial hormone-like peptides known to date (1, 2) are compared, it appears that whereas some positions, such as positions 4 and 8, are rather variable, others are strictly invariant (Table 2). These are positions 1 and 6, that are occupied by half-cystines building the disulfide bridge, and 5, 7 and 9. Position 9 is occupied by a glycinamide, a residue resulting from the precursor processing through the action of the peptidyl-glycine α-amidating enzyme complex (16, 17). Position 7 is a proline, a residue essential in the orientation of the tripeptide tail of the

TABLE 2

*Naturally occurring "neurohypophysial" peptides and their pharmacological activities (from refs. 13, 28 and 29)*

| | Amino acid sequences[a] | Activities[b] | | | |
|---|---|---|---|---|---|
| | | Rat pressor | Rat antidiuretic | Rat uterotonic | Rabbit galactagogic |
| | *Oxytocin-like peptides* | | | | |
| *Vertebrates* | 1 2 3 4 5 6 7 8 9 | | | | |
| Oxytocin | Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly($NH_2$) | 5 | 5 | 450 | 450 |
| Mesotocin | Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Ile-Gly($NH_2$) | 6.3 | 1 | 291 | 330 |
| Seritocin | Cys-Tyr-Ile-Gln-Ser-Cys-Pro-Ile-Gly($NH_2$) | 0.009 | | 1.2 | |
| Isotocin | Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly($NH_2$) | 0.06 | 0.18 | 145 | 290 |
| Glumitocin | Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Gln-Gly($NH_2$) | – | 0.41 | 8 | 53 |
| Valitocin | Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Val-Gly($NH_2$) | 9 | 0.8 | 199 | 308 |
| Aspargtocin | Cys-Tyr-Ile-Asn-Asn-Cys-Pro-Leu-Gly($NH_2$) | 0.13 | 0.04 | 107 | 298 |
| Asvatocin | Cys-Tyr-Ile-Asn-Asn-Cys-Pro-Val-Gly($NH_2$) | | | 80 | |
| Phasvatocin | Cys-Tyr-Phe-Asn-Asn-Cys-Pro-Val-Gly($NH_2$) | | | 5 | |
| *Invertebrates* | | | | | |
| Cephalotocin | Cys-Tyr-Phe-Arg-Asn-Cys-Pro-Ile-Gly($NH_2$) | | | | |
| Annetocin | Cys-Phe-Val-Arg-Asn-Cys-Pro-Thr-Gly($NH_2$) | | | | |
| | *Vasopressin-like peptides* | | | | |
| *Vertebrates* | | | | | |
| Arg-vasopressin | Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly($NH_2$) | 435 | 435 | 17 | 70 |
| Lys-vasopressin | Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Lys-Gly($NH_2$) | 285 | 260 | 5 | 63 |
| Phenypressin | Cys-Phe-Phe-Gln-Asn-Cys-Pro-Arg-Gly($NH_2$) | 130 | 375 | 0.2 | 3 |
| Vasotocin | Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly($NH_2$) | 255 | 260 | 120 | 220 |
| *Invertebrates* | | | | | |
| Locust hormone | Cys-Leu-Ile-Thr-Asn-Cys-Pro-Arg-Gly($NH_2$) | 0.02 | 8.6 | | |
| Arg-conopressin-S | Cys-Ile-Ile-Arg-Asn-Cys-Pro-Arg-Gly($NH_2$) | 1.14 | 16 | | |
| Lys-conopressin-G | Cys-Phe-Ile-Arg-Asn-Cys-Pro-Lys-Gly($NH_2$) | 2.91 | 0.90 | | |

[a] Residues different from those from oxytocin are underlined.

[b] International units per μmol.

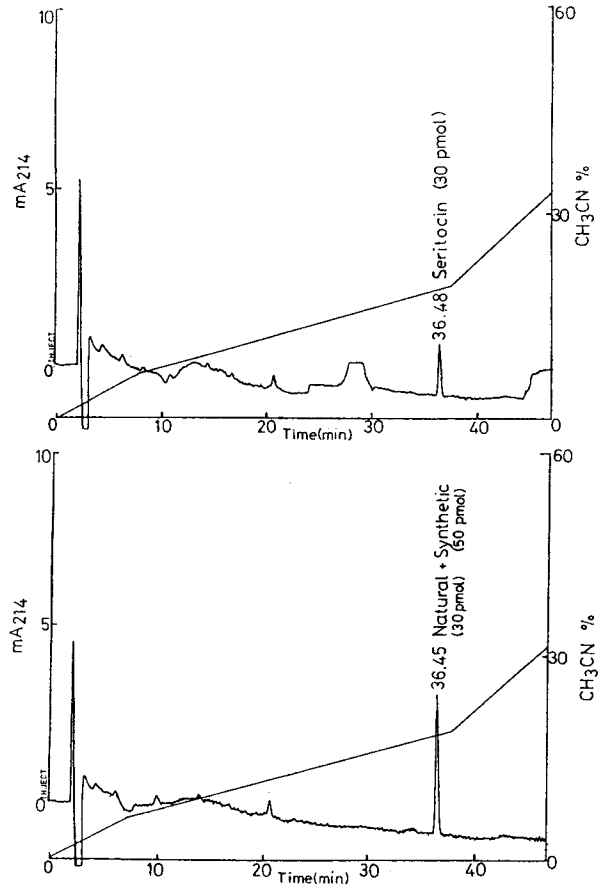


FIGURE 3

HPLC of purified mesotocin-like peptide of *B. regularis* (top) and of the mixture of natural peptide with synthetic [Ser5,Ile8]-oxytocin (bottom). Retention times are indicated in min. A three-step linear acetonitrile gradient (0–60%) containing 0.05% trifluoroacetic acid for 60 min was used.

molecule. Position 5 is occupied by asparagine in all neurohypophysial peptides identified up to now whatever their possible function. There is no evident explanation for its evolutionary stability. When asparagine is replaced by serine, as in synthetic [Ser5]-oxytocin, rat uterotonic activity, rabbit galactagogic activity and chicken blood depressor activity of oxytocin are virtually lost (13). The same result is observed with substitutions by alanine, glutamine (13) or valine (14). In the same way, rat blood pressure and rat antidiuretic activities of lysine vasopressin, a hormone found in pig and some marsupials (1, 2), are lost in [Ser5,Lys8]-vasopressin (13). A strict fit to the respective receptors could be involved, prohibiting a change in position 5. Neurohypophysial hormone-like peptides have been found in invertebrates: locupressin in insects [*Locusta migratoria* (18)], conopressins in mollusc gasteropods [*Conus geographicus*, *Conus striatus* (19), *Lymnea stagnalis* (20), *Aplysia kurodai* (21)], cephalotocin in mollusc cephalopods [*Octopus vulgaris* (22)], annetocin in annelids [*Eisenia foetida* (23)]. Although their possible functions are poorly known and different from those of vertebrate neurohypophysial hormones, the invariability of position 5 is strictly respected (Table 2).

TABLE 3

*Variations of the neurohypophysial hydrin 2/vasotocin ratio in anuran amphibian species with environmental conditions*

| Origin | Species | Ratio hydrin 2/ vasotocin |
|---|---|---|
| *Temperate regions* | | |
| Europa | *R. esculenta* | 1.19 |
| France | *R. temporaria* | 1.14 |
| North America | *R. pipiens* (1967) | 0.82 |
| North America | *R. pipiens* (1991) | 0.83 |
| North America | *R. catesbeiana* | 0.87 |
| France | *B. bufo* | 1.43 |
| North America | *B. marinus* | 1.27 |
| South America | *B. ictericus* | 1.25 |
| *Arid regions* | | |
| Africa | *B. regularis* | 2.00 |
| Near-east | *B. viridis* | 2.20 |

*Neutral or selective evolution*

The finding of [Ser5,Ile8]-oxytocin in a particular species of anuran amphibia, whereas ten species of this group possess mesotocin, as all the non-mammalian tetrapods investigated to date, is puzzling. Mesotocin has been identified in lungfish (15), amphibians (24), reptiles (25), birds (26) and marsupials (27), so that its evolutionary stability is striking. Although no unquestionable role has been ascribed to mesotocin in amphibians, in contrast to vasotocin, the evolutionary stability and the relative large amounts stored in neurohypophysis suggest an important function.

Apparently it seems that the substitution was determined by a neutral mutation in the promesotocin gene, since *B. regularis* looks quite similar to other toads, as suggested by its name. Assuming seritocin is as active as mesotocin on the putative amphibian mesotocin receptor, the latter should be different from the mammalian uterus receptor of oxytocin, since [Ser5,Ile8]-oxytocin has virtually no rat uterotonic activity. It remains to explain in this case why variations in position 5 of mesotocin are so rare in amphibians. Another particular feature of *B. regularis* is the molar ratio of hydrin 2 (vasotocinyl-Gly) to vasotocin stored in the neurohypophysis, which reaches 2 rather than 1 for toads living in temperate areas (6) (Table 3).

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## REFERENCES

1. Acher, R. (1988) *Progress in Endocrinology 1988* (Imura, H., Shizume, K. & Yoshida, S., eds.) Elsevier, Excerpta Medica, Amsterdam, Vol. 2, pp. 1505–1526
2. Acher, R. (1993) *Regul. Peptides* **45**, 1–13
3. Acher, R. (1993) *Progress in Comparative Endocrinology* (Mornex, R., Jaffiol, C. & Leclere, J., eds.) Parthenon Publishing Group, pp. 187–191
4. Pang, P.K.T. & Sawyer, W.H. (1978) *Am. J. Physiol.* **235**, F151–155
5. Rouillé, Y., Michel, G., Chauvet, M.T., Chauvet, J. & Acher, R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5272–5275
6. Michel, G., Ouedraogo, Y., Chauvet, J., Katz, U. & Acher, R. (1993) *Neuropeptides* **25**, 139–143
7. Chauvet, J., Ouedraogo, Y., Michel, G. & Acher, R. (1993) *Comp. Biochem. Physiol.* **104A**, 497–502
8. Bentley, P.J. (1957) *J. Endocrinol.* **16**, 126–134
9. Holton, P. (1948) *Br. J. Pharmacol.* **3**, 328–334
10. Dekanski, J. (1952) *Br. J. Pharmacol.* **7**, 567–572
11. Hewick, R.M., Hunkapiller, M.W., Hood, L.E. & Dreyer, W.J. (1981) *J. Biol. Chem.* **256**, 7990–7997
12. Beavis, R.C. & Chait, B.T. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6873–6877
13. Berde, B. & Boissonnas, R.A. (1968) *Handbook of Experimental Physiology New Series*, (Berde, B., ed.) Springer, Berlin, vol. 23, pp. 802–870
14. Lebl, M., Jost, K. & Brtnik, F. (1987) *Handbook of Neurohypophyseal Hormone Analogs* (Jost, K., Lebl, & Brtnik, F., eds.), CRC Press, Boca Raton, Fl, vol. II, Part 2, chap. 6, pp. 122–267
15. Michel, G., Chauvet, J., Joss, J.M.P. & Acher, R. (1993) *Gen. Comp. Endocrinol.* **91**, 330–336
16. Rouillé, Y., Chauvet, J. & Acher, R. (1991) *J. Neuroendocrinol.* **3**, 15–20
17. Rouillé, Y., Chauvet, J. & Acher, R. (1992) *Biochem. Int.* **26**, 739–746
18. Proux, J.P., Miller, C.A., Li, J.P., Carbey, R.L., Girardie, A., Delaage, M. & Schooley, D.A. (1987) *Biochem. Biophys. Res. Commun.* **149**, 180–186
19. Cruz, L.J., De Santos, V., Zafaralla, G.C., Ramilo, C.A., Zeikus, R., Gray, W.R. & Olivera, B.M. (1987) *J. Biol. Chem.* **262**, 15821–15824
20. Van Kesteren, R.E., Smit, A.B., Dirks, R.W., De With, N.D., Geraerts, W.P.M. & Joosse, J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4593–4597
21. Mc Master, D., Kobayashi, Y. & Lederis, K. (1992) *Peptides* **13**, 413–421
22. Reich, G. (1992) *Neurosci. Lett.* **134**, 191–194
23. Oumi, T., Ukena, K., Matsushima, O., Ikeda, T., Fujita, T., Minakata, H. & Nomoto, K. (1994) *Biochem. Biophys. Res. Commun.* **198**, 393–399
24. Acher, R., Chauvet, J. & Chauvet, M.T. (1969) *Nature (London)* **221**, 759–760
25. Acher, R., Chauvet, J. & Chauvet, M.T. (1969) *Gen. Comp. Endocrinol.* **13**, 357–360
26. Acher, R., Chauvet, J. & Chauvet, M.T. (1970) *Eur. J. Biochem.* **17**, 509–513
27. Hurpet, D., Chauvet, M.T., Chauvet, J. & Acher, R. (1982) *Int. J. Peptide Protein Res.* **19**, 366–371
28. Chauvet, J., Rouillé, Y., Chauveau, C., Chauvet, M.T. & Acher, R. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 11266–11270
29. Kruszynski, M., Manning, M., Wo, N.C. & Sawyer, W.H. (1990) *Experientia* **46**, 771–773

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