Purification of a material immunoreactive to an antiserum against the C-terminal part of the oxytocin (Pro-Leu-Gly-amide) and present in the central nervous system of the Pharyngobdellid leech Erpobdella octoculata was performed by reversed-phase high performance liquid chromatography combined with both enzyme-linked immunosorbent and dot immunobinding assays for oxytocin. The amino acid sequence of the purified peptide (Ile-Pro-Glu-Pro-Tyr-Val-Trp-Asp) was established by Edman degradation and confirmed by electrospray mass spectrometry measurement. When injected in leeches, purified or synthetic peptides exert an anti-diuretic effect, the most effective ranged between 10 pmol and 1 nmol. They provoked an uptake of water 1-2 h post-injection. Furthermore, electrophysiological experiments conducted in the leech Hirudo medicinalis revealed an inhibition of the potency of Na⁺ conductances of leech skin by this peptide. Immunocytochemical studies with an antiserum against synthetic oxytocin-like molecule provided the cytological basis for existence of a neuropeptide, since large amounts of immunoreactive neurons were detected in the central nervous systems of E. octoculata. The purified molecule is both different to peptides of the oxytocin/vasopressin family and is a novel neuropeptide in the animal kingdom. It was named the leech osmoregulator factor (LORF).

An identification of the proteins immunoreactive to an antiserum against oxytocin performed at the level of both central nervous systems extracts and in vitro central nervous system-translated RNA products indicated that in the two cases, a single protein was detected. These proteins with a molecular masses of, respectively, 34 kDa (homodimer of 17 kDa) for the central nervous systems extracts and 19 kDa for in vitro central nervous system-translated RNA products were not recognized by the antiserum against MSEL- and VLDV-neurophysin (proteins associated to oxytocin and vasopressin), confirming that LORF did not belong to the oxytocin/vasopressin family.

In annelids, the central nervous system (CNS) of Hirudinidae is known to influence water balance (Rosca et al., 1958; Czehowicz, 1968; Kulkarni and Nagabhushanam, 1978; Malecha, 1983). In the rhynchobdellid leech Theromyzon tessulatum genital maturity is concomitant with a phase of water retention reflected by an increase in mass of the animals and correlated to a cælomic accumulation of yolk proteins (Baert et al., 1991, 1992). This animal was used for a study of the control of water balance.

Angiotensin II-amide (Salzet et al., 1995), GDPFLRF-amide (Salzet et al., 1994), and lysine-conopressin (Salzet et al., 1993a) have been isolated from CNS of the Pharyngobdellid leech Erpobdella octoculata. They generated when injected in T. tessulatum a decrease in mass of the injected animals, expressing a diuretic effect. On the other hand, an injection of an antiserum directed against oxytocin (OT) provoked a loss of mass in the injected leeches (Malecha et al., 1989a), pointing to an anti-diuretic role for substance(s) immunoreactive to anti-OT.

In E. octoculata, it is known that an OT-like material is found in large amounts in the sex segmental ganglia (sex SG), and that an extract of sex SG exerts an anti-diuretic effect when injected in leeches (Salzet et al., 1993c). Cell counts of OT-like cells in immature and mature animals indicated that the number of immunoreactive cells was higher in immature specimens. Furthermore, radioimmunoassays showed an amount of OT-like material 3-fold higher in immature than in mature leeches. A biochemical study of an extract of sex SG of mature E. octoculata demonstrated the presence of two zones immunoreactive to a-OT. On the other hand, in immature E. octoculata, an additional zone bearing ~80% immunoreactivity to a-OT was detected. For this additional zone in immature animal, hypothesis of the presence of a possible fragment of the OT-like precursor was given (Salzet et al., 1993c). In supernumerary neurons of sex SG, OT-like material is colocalized with four RF-amide peptides, i.e. FMRF-amide, FM(O)RF-amide, FLMRF-amide, and GDPFLRF-amide. FMRF-amide and GDPFLRF-amide as OT-like substance(s) are involved in osmoregulation (Salzet et al., 1994).

In this study, we now report the isolation and the characterization of an OT-like peptide from CNS of the Pharyngobdellid leech E. octoculata using HPLC purification procedures, automatic Edman degradation and electrospray mass spectrometry analysis, electrophysiological experiments, and immunocytochemical studies performed with an antiserum against syn-

ELISA, enzyme-linked immunosorbent assay; HPGPC, high performance gel permeation chromatography; PLGα, Pro-Leu-glycinamide; OT, oxytocin; a-OT, anti-oxytocin; LORF, leech osmoregulator factor; VP, vasopressin; PAGE, polyacrylamide gel electrophoresis; SG, segmental ganglia.
thetic oxytocin-like peptide. Finally, an identification of CNS proteins immunoreactive to an antisera against OT at the level of both CNS extracts and in vitro CNS-translated products was also undertaken. This work demonstrated the existence of a novel neuropeptide involved in the process of osmoregulation. This molecule named leech osmoregulator factor (LORF) is unique in the animal kingdom.

**MATERIALS AND METHODS**

**Animals and Dissection Procedure**

Mature and immature Pharyngobdellid leeches E. octoculata, collected at Maches (Belgium) and kept in the dark at 15°C in pond water, were used for the isolation of the OT-like peptide. After anesthesia in 0.01% chloroethane, animals were pinned out, dorsal side up, in leech Ringer’s solution (Muller et al., 1981) and central nervous systems (CNS) were excised, immediately frozen in liquid nitrogen, and stored at −70°C until use.

Mature Rhynchobdellid leeches T. tessulatum were used in this study as a bioassay according to Salzet et al. (1995).

**Antiserum**

A polyclonal antisera used in ELISA and dot immunobinding assay (DIA) procedures was raised in our laboratory by immunizing rabbits with synthetic oxytocin (Interchim) coupled to thyroglobulin with glutaraldehyde. It was characterized by Salzet et al. (1993b). Briefly, it did not cross-react with arginine-vasopressin and lysine-vasopressin but presented 40% cross-reactivity with isotocin in ELISA. As regards the molecule of oxytocin (OT), it was only directed against its C-terminal fragment (Pro-Leu-Gly-amide, PLGa: 80.7% cross-reactivity with PLGa, 30% with the dipeptide Pro-Leu and 20% with the dipeptide Pro-Ile in competitive ELISA, and 78.8% cross-reactivity with PLGa in radioimmunoassay. It did not recognize the N-terminal fragment (tocinoic acid) of OT: 0.06% cross-reactivity with tocinoic acid in competitive ELISA and in radioimmunoassay.

**Immunoassays**

DIA and ELISAs based on the protocols of Salzet et al. (1992, 1993a) were used to follow the OT-like activity during the purification procedures. Quantification of the OT-like peptide in CNS extracts was done in direct ELISA. As control, preadsorption of a-OT was carried out using homologous peptide at a concentration of 100 μg/ml undiluted a-OT.

**Purification of the OT-like Peptide**

A four-step procedure was used for this purification.

**Step I: Sep-Pak Prepurification—4000 CNS were needed. Batches of 400 CNS were homogenized at 4°C in 400 μl of 1% acetic acid and sonicated (30 s) twice. Homogenates were centrifuged at 12,000 rpm for 30 min at 4°C. After reextraction of the pellet, the two supernatants were combined and loaded onto Sep-Pak C2 cartridges (500 μl of extract). The column was washed with the extractives (5 ml of 50% acetonitrile in acidified water from 0 to 60% in 60 min). The column effluent was monitored by absorbance at 226 nm and the presence of OT-like material detected as above. The OT-like material was finally purified by reverse-phase HPLC on a Beckman Gold HPLC system equipped with a photodiode array detector Beckman 168.

**Amino Acid Sequence Analysis**

Automated Edman degradation of the purified peptide and detection of phenyl-thiodydantoin amino acids derivatives were performed on a pulse-liquid automatic sequenator (Applied Biosystems, model 473A).

**Electrospray Mass Spectrometry**

The purified peptide was dissolved in water/methanol (50/50, v/v) containing 1% acetic acid and analyzed on a VG Biotech BioQ mass spectrometer (Manchester, United Kingdom) and treated according to Salzet et al. (1995).

**Biological Assay**

The bioassay was conducted on T. tessulatum, rhynchobdellid leeches bred in the laboratory (Malecha, 1989b). Leeches at stage 3B, stage which corresponds to an important water retention phase according to Malecha et al. (1989a), were distributed in lots of 20 animals having an identity with the mean body mass before being injected subepidermally (10 μl of solution/leech).

**Step I—Leeches received an aequorin solution of each immunoreactive fraction to a-OT eluted from the HPGPC column. Controls received deionized water.**

**Step II—Leeches received an aequorin solution of either synthetic peptide (1 pmol; Neosystem) corresponding to the isolated LORF from E. octoculata or purified peptide (1 pmol), or either synthetic LORF at four different doses (1–100 fmol, 1–100 pmol, and 1–100 nmol). Controls received deionized water.**

All injected animals were kept at room temperature. To estimate the effect of an injection, leeches blocked on tissue paper were weighed to the nearest 0.1 mg at various time intervals following the injection (1, 2, 4, and 6 h). The change in body mass of the animals between the beginning of the experiment and the time of weighing was registered. Responses were expressed as percentages of mass variation (means ± S.D.). The efficiency of the product was determined by its capacity to elicit a variation of mass significantly different from that registered in controls.

**Statistical analysis of data was done according to Salzet et al. (1993a). The confidence limit of the relative mean variation of mass was obtained according to Cochran (1977).**

**Electrophysiological Experiments: Ussing Chamber Experiments**

The methods and materials were similar to those used and described in detail in a previous study (Weber et al., 1995).

**Immunocytochemistry—A polyclonal antiserum raised against synthetic LORF used in immunocytochemistry was generated in a rabbit using synthetic LORF coupled to human serum albumin via glutaraldehyde. Parts of E. octoculata at the level of the nerve cord and brain were fixed overnight at 4°C in Bouin-Hollande fixative (+10% HgCl₂ saturated solution), they were then embedded in paraffin and serially sectioned at 7 μm. After removal of paraffin with toluene, the sections were successively treated with the primary antibody (a-LORF) diluted 1:200 and with goat anti-rabbit IgG conjugated to horseradish peroxidase as described elsewhere (Verger-Bocquet et al., 1992).**

The specificity of the antiserum (a-LORF) was tested on consecutive sections mounted on different slides by preadsorbing this antisera overnight at 4°C with the homologous antigen at a concentration of 500 μg/ml pure antiserum.

**OT-like Protein Identification**

**Protein Purifications—CNS in batches of 400 were homogenized at 4°C in 400 μl of Tris-buffered saline (50 mM Tris-HCl, pH 7.4, 150 mM NaCl) supplemented with 2% EDTA and 1 mM phenylmethylsulfonyl fluoride and sonicated (30 s) twice. Each homogenate was centrifuged at 12,000 rpm for 30 min at 4°C.**

**The pellets were reextracted a second time, and the supernatants were combined and subjected to a HPGPC on a SEC2000 (Ultraspherogel, 7.5 mm × 300 mm, Beckman) column and**
tration in 1 M acetic acid at pH 2. ELISA revealed the presence of acetonitrile in water acidified by 0.1% trifluoroacetic acid. Was eluted by 50% acetonitrile in acidified water (0.1% trifluoroacetic acid), containing the oxytocin-like material, was loaded onto a HPGPC (Ultrasphere- gel, 7.5 × 300 mm, SEC200, Beckman) column. Elution was performed with 30% acetonitrile at an elution rate of 1 ml/min. The oxytocin-like material was detected on aliquots of each fraction by the oxytocin-RIA. Bars indicate the immunoreactive material, and arrowheads, the eluted positions of molecular mass markers (a, hirudin; b, adrenocorticotropic hormone; c, angiotensin II; d, tryptophan).

RESULTS

OT-like Peptide Isolation

4000 CNS of E. octoculata were subjected to a peptide extraction in 1 M acetic acid at pH 2. ELISA revealed the presence in the crude extract of CNS of a quantity of OT-like material estimated at 8 ± 1.2 pmol/CNS. The crude extract was purified using Sep-Pak C18 cartridges. The fraction eluted by 50% acetonitrile in water acidified by 0.1% trifluoroacetic acid was reduced 20-fold by freeze-drying and applied to a HPGPC. DIA results indicated that the OT-like substances eluted from the column in three zones with molecular mass less than 1 kDa for Z1, 1–4.5 kDa for Z2, and 5–10 kDa for Z3 (Fig. 1). Results obtained after using a-OT preadsorbed by synthetic OT established the specificity of the immunodetection. Quantification by ELISA at this step of purification indicated 0.6 ± 0.12 pmol of OT-like material/CNS for Z1, 2.05 ± 0.65 pmol of OT-like material/CNS for Z2, and 4.35 ± 1.12 pmol of OT-like material/CNS for Z3. Before separated in reversed-phase HPLC, peptides contained in Z1, Z2, and Z3 were injected in vivo to T. tessulatum (Fig. 2). Only fraction Z2, which has an anti-diuretic potency (Fig. 2), was applied to a C18 reversed-phase HPLC column. A heterologous immunoreactive peak to a-OT (Fig. 3) eluted at a retention time of 25.1 min (corresponding to 37.6% acetonitrile). The immunoreactive peak containing the OT-like material was analyzed on the same column with a more resolutive gradient. A peak immunoreactive to a-OT, with a retention time of 27 min (corresponding to 32% acetonitrile), was resolved. At this step of purification, quantification by ELISA indicated an amount of 1.5 ± 0.75 pmol of OT-like material/CNS (recovery ~33% of the starting material). This peak was then purified to homogeneity on an ODS C18 reversed-phase column and gave a peptide at a retention time of 29.52 min (Fig. 4). Quantification by ELISA at this step of purification indicated 1.05 ± 0.25 pmol of OT-like material/CNS (final recovery of ~20%). The retention time of this peptide did not correspond to the ones found in this system for OT (native, 24.4 min; reduced, 21.3 min; oxidized, 24.7 min), OT fragments (tocinoic acid, 20.5 min; PLGa, 15.6 min), and peptides of the OT/VP family (arginine-vasopressin, 24.1 min; arginine-vasotocin, 23.7 min; lysine-vasopressin, 23.1 min; lysine-vasopressin, 29 min; isotocin, 23.5 min).

OT-like Peptide Characterization

After the final purification step, a fraction aliquot of the immunoreactive purified material was submitted to Edman degradation. The sequence, established on 43.33 pmol of purified OT-like peptide with a sequencing yield of 91.5%, was...
Ile-Pro-Glu-Pro-Tyr-Val-Trp-Asp (Table I). Measurement of the molecular mass of the OT-like peptide by electrospray mass spectrometry gave an m/z of 1018.29 ± 0.68 Da, which was in good agreement with monoisotopic molecular mass (1018.6 Da) calculated from the amino acid sequence determined by Edman degradation. The primary structure (IPEPYVWD) of the E. octoculata OT-like peptide has no sequence similarity with peptides of the OT/VP family.

Biological Activity of the Leech OT-like Peptide

In Vivo Experiments—Comparative analyses of repercussion on the leech mass variation after injection of OT-like peptides were performed (Figs. 5 and 6). Injection of either synthetic or purified OT-like peptide at a same concentration of 1 pmol shown a very close response (Fig. 5). The two peptides exert, compared to controls, an anti-diuretic effect when injected to T. tessulatum (Fig. 5). Moreover, dose-response experiments with synthetic LORF indicated compared to controls, and in contrast to the lowest doses assayed (1–100 fmol), that the highest doses assayed (1–100 pmol and 1 nmol) were found to be effective in injected leeches. The optimal doses were 100 pmol and 1 nmol, which provoked an increase in mass in injected leeches 1, 2, and 4 h post-injection, reflecting an uptake of water (Fig. 6).

Electrophysiological Experiments—Pieces of the dorsal integument of the medicinal leech Hirudo medicinalis could be maintained in modified Ussing chambers under stable conditions for several hours. After an equilibration period of about 30 min, the electrical parameters reached a stable phase for at least 4–5 h. With a transepithelial resistance of about 1 kΩ, leech skin belongs to the class of tight epithelia. After the equilibration period all preparations were routinely tested for amiloride inhibition. When amiloride (100 μM) was added to the apical side of the short-circuited skin, I_{sc} began to drop instantaneously because amiloride-sensitive Na^{+} channels are the main pathway for ionic transport across leech skin (Weber et al., 1993). The short circuit current showed a further decrease when after amiloride application (100 μM) sodium was removed from the apical solution. Readdition of sodium resulted in a strong overshoot in short circuit due to activation of silent Na^{+} channels under Na^{+}-free conditions and their immediate
down-regulation by increasing \( \text{Na}^+ \) concentrations (Fig. 7). Shortly after serosal application of oxytocin-like peptide (5 \( \mu \text{M} \)), \( I_{\text{Sc}} \) decreased by 18.15 ± 0.94% (n = 3), 28.19 ± 2.74% of the amiloride-sensitive portion of the short circuit was inhibited by oxytocin-like peptide, and 34.8 ± 9.5% of the whole \( \text{Na}^+ \)-mediated currents were blocked.

**Immunocytochemical Study**

Large amounts of neurons immunoreactive to an antiserum against a-LORF were detected at the level of the segmental ganglia of the nerve cord (Fig. 8b). Preadsorption of a-LORF with the homologous antigen abolished the staining capacity of the antiserum, reflecting the specificity of the immunodetection (Fig. 8, a and c). Among the LORF neurons, several are also immunoreactive to a-OT (data not shown), confirming the previous results obtained at the level of the supernumerary neurons of sex segmental ganglia (Salzet et al., 1993c).

**Identification of Proteins Immunoreactive to a-OT**

Identification in CNS Extracts—Extracts of CNS with Tris-buffered saline were fractionated on HPGPC. The collected fractions were assayed with a-OT (Fig. 9). Two specific immunoreactive zones (Za and Zb), corresponding to proteins with a molecular mass ranging from 20 to 45 kDa for Za and to peptides with a molecular mass less than 10 kDa for Zb, were observed. Peptides contained in Zb corresponded to immunoreactive material present in Z1, Z2, and Z3, previously detected in the acidic extraction method. Proteins contained in Za were further subjected to Western blot analysis with a-OT. After SDS-PAGE in reducing conditions, a single immunoreactive band was detected at 17 kDa (Fig. 9A). In contrast, in non-reducing conditions, an immunoreactive band at 34 kDa was detected (Fig. 9B), indicating that the OT-like protein extract from CNS is an homodimer protein with a molecular mass of 17 kDa.

Identification in CNS RNA-translated Products—After extraction of total RNA and transcription in rabbit reticulocyte lysate, translated proteins were treated in the same way as the proteins extracts from CNS. After HPGPC, a single immunoreactive zone (Zc) corresponding to proteins with molecular masses of 10–25 kDa was detected (Fig. 10). The proteins contained in Zc were further subjected to a Western blot analysis with a-OT in reducing conditions. A protein with a molecular mass of 19 kDa was detected (Fig. 10), slightly larger than the one detected in reducing conditions (–17 kDa) in CNS extracts (Fig. 9B).

**DISCUSSION**

After reversed-phase HPLC purification, the sequence of an OT-like peptide (IPEPYVWD) was established by a combina-
tion of automated Edman degradation and electrospray mass spectrometry measurement. Our data revealed that the 8-residue peptide present in the CNS of the leech E. octoculata is a novel neuropeptide, structurally different from peptides of the OT/VP family. This result constitutes the first report of such a peptide in the animal kingdom.

The isolation of this peptide with an antiserum against OT could be explained by the antiserum used. It was directed against the C-terminal part of OT (PLG-amide) and more precisely against dipeptides PL (30%) or PI (20%). PI is present in the purified OT-like peptide (IPEPYVWD) at the level of the N-terminal sequence. Similar results were also obtained in the mollusk Lymnaea stagnalis with an anti-lysine-vasopressin antiserum. The lysine-vasopressin antiserum directed against the sequence PKG-amide recognized the SKPFLRF-amide, peptide of the RF-amide family (De With et al., 1993). Although these results led us to think that the peptide isolated from E. octoculata CNS is not really an OT-like peptide, three lines of evidence established our results.

The use of another a-OT characterized by Tramu et al. (1983), which recognized both the N-terminal and the C-terminal parts of OT, gave identical results (data not shown). Moreover, from preliminary experiments conducted on the biological activity of the purified peptide from E. octoculata, it appears that this purified peptide is involved in the control of the hydric balance. Injection of 10 pmol, 100 pmol, or 1 nmol of synthetic OT-like peptide provoked an uptake of water in injected T. tessulatum. Furthermore, electrophysiological experiments conducted in H. medicinalis establish the inhibition of potency sodium conductance of leech skin by the OT-like peptide. Leech skin has proven to be a valuable model for the study of ion transport in invertebrate tight epithelia (Weber et al., 1993, 1995). It is known that Na\(^+\) transport in leech skin can be modulated by the second messenger cAMP via an increase in the number of Na\(^+\) channels. However, in leech skin oxytocin-like peptide failed to activate the cAMP second messenger system, as could be seen by a decreasing effect on transepithelial short circuit current. Oxytocin-like peptide inhibited the currents through amiloride-sensitive or amiloride-insensitive Na\(^+\) conductances. At present, it remains unclear by which mechanism oxytocin-like peptide enters its inhibiting potency on Na\(^+\) conductances of leech skin. Two mechanisms seem to be possible: 1) a direct action of the peptide on the channel protein from the outside of the cell, or 2) coupling of oxytocin-like peptide to a membrane bound receptor and subsequent signal pathways.
transduction to the inside of the cell, followed by a regulation of Na⁺ channels from within the cell. Finally, immunocytochemical studies performed with an antiserum raised against synthetic oxytocin-like peptide indicated a high specific immunostaining of neurons in segmental ganglia of the nerve cord. For all these reasons, the purified oxytocin-like peptide was named the leech osmoregulator factor (LORF).

The LORF sequence (IPEPYVWD) is included within the N-terminal part of a respiratory pigment, the myohemerythrin (protein of –14 kDa) of the sipunculid Themiste zostericola (Klippenstein et al., 1976): GWDIPEPYVWDESFRV... It also presents 77% sequence homology with a fragment of the N-terminal part of a yolk protein (–14 kDa), the ovohemerythrin of the leech T. tessulatum: YDIPEPFRWDESFV... (Baert et al., 1976): GWDIPEPYVWDESFRV... It also appears that LORF is a novel neuropeptide; 2) it is a contaminant from hemerythrin present in blood and cœlomic fluid. However, several findings argue in favor of the first hypothesis, i.e. LORF is a novel neuropeptide. First, immunocytochemical studies performed with an antiserum against synthetic LORF revealed neurons immunoreactive to a-LORF in the nerve cord. Moreover, some of these neurons reached projections in direction to neurohemal area, indicating a neurohormonal role (Hagadorn, 1958; Orchard and Webb, 1980) of LORF. Moreover, most of supernumerary neurons of the sex segmental ganglia that were immunoreactive to a-OT (Salzet et al., 1993c) are also immunoreactive to a-LORF. Second, a-LORF and a-OT did not recognize the ovohemerythrin in Western blot or in ELISA and a-ovohemerythrin, the LORF. Third, LORF is active on osmoregulation of leeches. Our experiments, conducted at the protein level with CNS extracts or RNA-translated products, indicated that the OT-like precursor is a homodimer protein of –17 kDa in CNS extract and a protein of –19 kDa in translated RNA. These proteins were not recognized with a-MSEL and VLDV)-neurophysin, associated proteins in the case of OT or VP (Acher et al., 1985), confirming that LORF is not a peptide of the family. No recognition of these proteins by a-RF-amide was found, although these molecules are colocalized with LORF in supernumerary neurons of sex SG (Salzet et al., 1993c). Fourth, molecular mass of ovohemerythrin (14.4 kDa) is different to OT-like proteins.

According to its localization in sex segmental ganglia of the nerve cord, it could be postulated that this peptide could also act on reproduction. Selective ablation of either sex segmental ganglia or cerebropleura ganglia of T. tessulatum at stage 3A, stage just before water retention phasis, indicated that only the lateral cerebropleura ganglia blocks the water phasis retention and oogenesis. LORF could exert different biological activities in function of its localization in T. tessulatum CNS. However, further experimental testing are needed to conclude.

The whole of our results establish that LORF is a novel neuropeptide, never previously isolated in the animal kingdom. Further experimental testing with a-LORF would be performed either in other phyla of invertebrates or in vertebrates, in order to investigate the presence of this neuropeptide in course of evolution.

Acknowledgments—We are indebted to Dr. A. Van Dorsselaer, (Laboratoire de Spectrométrie de Masse bioorganique, UA 31 CNRS, Strasbourg, France), for the mass spectrometry determination. We appreciate the technical assistance of A. Desmons and N. Theesse.

REFERENCES

Acher, R., Chauvet, J., Chauvet, M. T., and Hurpet, D. (1985) in Current Trends in Comparative Endocrinology (Lofts, B., and Holmes, W. N., eds) pp. 1147–1152, Hong Kong University Press, Hong Kong

Baert, J. L., Britel, M., Slomianny, M. C., Delbart, C., Fournet, B., Sautière, P., and Malecha, J. (1993a) Eur. J. Biochem. 209, 563–569

Baert, J. L., Britel, M., Sautière, P., and Malecha, J. (1992) Eur. J. Biochem. 209, 563–569

Baert, J. L. (1988) J. Morphol. 102, 55–90

Klippenstein, G. L., Cote, J. L., and Ludlam, S. E. (1976) Biochemistry 15, 1128–1136

Kulkarni, G. K., and Nagabhushanam, R. (1978) Hydrobiologia 59, 197–201

Laemmli, U. K. (1970) Nature 227, 680–685

Malecha, J. (1983) Comp. Biochem. Physiol. 74, 443–451

Malecha, J., Verger-Bocquet, M., and Tramu, G. (1989a) Can. J. Zool. 67, 636–640

Malecha, J., Verger-Bocquet, M., Leprière, A., and Tramu, G. (1989b) C. R. Acad. Sci. Paris 309, 127–130

Muller, K. J., Nicholls, J. G., and Stent, G. S. (1981) Neurobiology of the Leech, p. 529, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Orchard, I., and Webb, R. A. (1989) J. Neurobiol. 11, 229–242

Rosa, D., I., Wittenberger, C., and Rudseide, D. (1958) Stud. Cercet. Biol. Cluj 9, 113–136

Salzet, M., Wattez, C., Slomianny, M. C., Léu, B., and Siegenthaler, J. (1992) Comp. Biochem. Physiol. 102C, 483–487

Salzet, M., Bulet, P., Van Dorsselaer, A., and Malecha, J. (1993a) Eur. J. Biochem. 217, 897–903

Salzet, M., Wattez, C., and Slomianny, M. C. (1993b) Comp. Biochem. Physiol. 104A, 75–81

Malecha, J., Verger-Bocquet, M., Boulavand, J. C., and Malecha, J. (1993a) Brain Res. 601, 173–184

Salzet, M., Bulet, P., Wattez, C., and Malecha, J. (1994) Eur. J. Biochem. 221, 269–275

Salzet, M., Bulet, P., Wattez, C., Verger-Bocquet, M., and Malecha, J. (1995) J. Biol. Chem. 270, 1575–1582

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Vol. I, pp. 1–20, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Sawyer, R. T. (1986) Leech Biology and Behavior, Vol. I, Academic Press, New York

Sawyer, R. T. (1986) Leech Biology and Behavior, Vol. I, Academic Press, New York

Weber, W.-M., Dannenmaier, B., and Clauss, W. (1993) J. Comp. Physiol. B 163, 579–589

Weber, W.-M., Blank, U., and Clauss, W. (1995) Am. J. Physiol. 268, R605–R613
Structural Characterization of a Novel Neuropeptide from the Central Nervous System of the Leech *Erpobdella octoculata*: THE LEECH OSMOREGULATOR FACTOR

Michel Salzet, Phillipe Bulet, Wolf-Michael Weber, Wolfgang Clauss, Martine Verger-Bocquet and Jean Malecha

*J. Biol. Chem. 1996, 271:7237-7243.
doi: 10.1074/jbc.271.12.7237*

Access the most updated version of this article at [http://www.jbc.org/content/271/12/7237](http://www.jbc.org/content/271/12/7237)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/271/12/7237.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 24 references, 1 of which can be accessed free at [http://www.jbc.org/content/271/12/7237.full.html#ref-list-1](http://www.jbc.org/content/271/12/7237.full.html#ref-list-1)