Neurohypophysial hormone regulation of Cl⁻ secretion: physiological evidence for V1-type receptors in sea bass gill respiratory cells in culture

M E Guibbolini¹ and M Avella

Laboartoire de Physiologie Cellulaire et Moléculaire, UMR CNRS 6548, Université de Nice–Sophia Antipolis, Faculté des Sciences, Parc Valrose, 06108 Nice Cedex 2, France

¹Laboratoire de Physiologie, Réponse des Organismes aux Stress Environnementaux, UMR INRA 1112, Université de Nice–Sophia Antipolis, Faculté des Sciences, Parc Valrose, 06108 Nice Cedex 2, France

(Requests for offprints should be addressed to M E Guibbolini; Email: marielle.guibbolini@unice.fr)

Abstract

Neurohypophysial hormone receptors were studied in primary cultures of sea bass (Dicentrarchus labrax) gill respiratory-like cells grown on permeable supports. This preparation was previously shown to provide a functional model for investigating the hormonal regulation of Cl⁻ secretion.

Under control conditions, the cultured monolayered epithelium had a short-circuit current (I_SC) of 3.5 ± 1.1 µA cm⁻². This current had previously been identified as an active Cl⁻ secretion. The addition of increasing concentrations of the fish neurohypophysial hormones, arginine vasotocin (AVT) or isotocin (IT), elicited a concentration-dependent stimulation of the I_SC. Maximal increases of 60.9 ± 12.1% and 117.7 ± 28.0% above the basal I_SC value were obtained for 10⁻⁷ M AVT and IT respectively. Half-maximal effects were obtained for 3.1 × 10⁻⁹ M AVT and for 1.4 × 10⁻⁹ M IT. Mucosal application of 1.0 mM diphenylalnine-2-carboxylic acid (a specific blocker of Cl⁻ channels) after serosal addition of 5 × 10⁻⁸ M AVT or IT inhibited not only the basal but also the stimulated current, revealing a correlation with a hormone-dependent Cl⁻ transport.

Specific V1 or V2 receptor analogues of vasoressin (mammalian hormone) were used to characterize the type of neurohypophysial hormone receptors pharmacologically. While the V1 agonist [Phe²,Orn⁸]-oxytocin stimulated the basal Cl⁻ secretion with a similar profile to that of AVT or IT, the V2 agonist [Deamino¹,Val⁴, d-Arg⁵]-vasopressin had no effect. The V1 antagonist [d(CH₂)₅¹,O-Me-Tyr²,Arg⁹]-vasopressin used at a concentration of 5 × 10⁻⁷ M totally reversed the 10⁻⁸ M AVT-stimulated Cl⁻ secretion, whereas the V2 antagonist [d(CH₂)₅¹,d-Ile²,Ille⁴,Arg⁸,Ala⁸]-vasopressin used at the same concentration had no significant effect. In contrast, similar experiments carried out in the presence of 10⁻⁸ M IT showed that both antagonists significantly reduced the IT-stimulated Cl⁻ secretion, with the efficiency of the V1 receptor antagonist being significantly greater than that of the V2.

This study provides evidence for neurohypophysial hormone control of Cl⁻ secretion in fish cultured gill respiratory cells. It suggests that on a physiological basis the hormonal effect is shared by the two peptides present in fish neurohypophysis (AVT and IT), acting by means of two distinct, although pharmacologically similar, V₁-type receptors (according to the mammalian classification). These specific receptors are expected to play an important role in controlling ion homeostasis in seawater fish.


Introduction

In teleost fishes, faced with a wide range of environmental ion concentrations, osmoregulation depends mainly on the transport activity of the gills. Direct exploration of ion transport pathways through the branchial epithelium has long been impeded by the anatomical complexity of this tissue. Primary gill cell cultures designed from a freshwater (FW) fish, the rainbow trout (Oncorhynchus mykiss) and a seawater (SW) fish, the European sea bass (Dicentrarchus labrax) by Pärt et al. (1993) and Avella et al. (1994) respectively have provided a new approach.

Primary cultures of sea bass gill cells exhibit the morphological (Avella et al. 1994) and functional (Avella & Ehrenfeld 1997) characteristics of a Cl⁻ secretory epithelium. Gill cells grown on permeable supports form a confluent, highly polarized (serosal positive) tight epithelium composed of a single cell type exhibiting the morphological characteristics of gill respiratory cells which have therefore been termed ‘respiratory-like cells’ (Avella &...
et al. 1994, Avella & Ehrenfeld 1997). The first exhaustive electrophysiological study of this epithelium demonstrated a short-circuit current (I_{sc}) that correlated with an active Cl\(^-\) secretion as measured by \(^{36}\)Cl fluxes (Avella & Ehrenfeld 1997). Pharmacological evidence led us to establish a new model defining separately basolateral and apical components of this transepithelial Cl\(^-\) secretion (Avella & Ehrenfeld 1997). Recent research carried out on this model provided strong evidence for a hormonal control of the Cl\(^-\) secretion (Avella et al. 1999).

In mammals, vasopressin, although displaying a wide variety of physiological properties, has been shown to play a major role in water and ion transport. Three typical vasopressin (AVP) receptors have been characterized by their tissue localization, signal transduction pathways, genetic structure, and functional and pharmacological parameters. In short, activation of V1a receptors increases inositol 1,4,5 triphosphate (IP\(_3\)) and Ca\(^{2+}\) mobilization, that of V1b increases Ca\(^{2+}\) mobilization (and cAMP in the apical components of this transepithelial Cl\(^-\)) and that of V2 leads to cAMP synthesis.

In teleost fish, the neurohypophysis produces arginine vasotocin (AVT) and isotocin (IT), peptides that differ from those of mammals. AVT is considered to be the ancestral peptide in the vertebrate neurohypophysial family and IT occurs in teleost fish only. The physiological functions controlled by these hormones remain uncertain, although AVT in particular is believed to play a key role in salt and water regulation (Maetz & Lahlou 1974, Takei et al. 1986). However, most of these observations were obscured by concurrent haemodynamic effects of these hormones. In vivo studies on fish, a number of physiological functions, such as vasopressor and diuretic effects, and hormonal control of water and ion exchanges across the gills, have been ascribed to these peptides (Babiker & Rankin 1972, Henderson & Wales 1974, Maetz & Lahlou 1974, Henderson et al. 1986). Most of these observations were obscured by concurrent haemodynamic effects of these hormones. In vitro studies showed that labelled AVT binds to specific receptors on eel gill cells (Guibbolini et al. 1988). In rainbow trout gill plasma membranes, the AVT or IT binding was followed by a strong inhibition of the adenylate cyclase activity (in the absence of Ca\(^{2+}\)) mediated by a G\(_i\) protein sensitive to pertussis toxin and guanine nucleotides (Guibbolini & Lahlou 1987, 1992). The use of specific neurohypophysial analogues indicated that the effects of AVT and IT were mediated by a new type of receptor, functionally similar to the mammalian neurohypophysial V1a type (Guibbolini & Lahlou 1990). Although the latter results favoured a direct effect of these peptides on gill cells, they could not define the exact target cells (gill chloride, respiratory, pillar or mucous cells), the function of these peptides, or the different types of neurohypophysial peptide receptors present in the branchial epithelium.

The present work represents a detailed study on primary cultures of sea bass gill cells which shows that (1) the respiratory-like cells are effective targets for the neurohypophysial peptides and (2) both AVT and IT stimulate Cl\(^-\) secretion, and (3) suggests that this response is mediated by two different types of specific V1-like receptors. This investigation proposes that neurohypophysial hormones participate directly in the control of ion transport across fish gills.

Materials and Methods

Fish

European sea bass (D. labrax) of 35 g average weight were obtained from a local sea farm (Société 3A, Antibes, France). They were kept at ambient temperature (14–18 °C) and natural lighting in a semi-open circuit (water completely renewed every 6 h) in 1 m\(^3\) tanks containing Mediterranean SW. Fish were fed daily with Aqualin pellets (Biomar, Nersac, France) at 2-5% of their body weight.

Primary cell culture

Cell culture protocol Before culture preparation, fish were kept for 2 h in a 10 litre tank of aerated SW containing antibiotics and fungicides: Furalatadone 0-02% (Sigma, St Louis, MO, USA) and Temerol 0-02% (Francodex, Carros, France). They were killed by a blow to the head and decerebrated. They were then decapitated and the gills excised. The cartilage of the gill arches was removed and the filaments remaining were dipped into a ‘washing medium’ (see below). They were washed under gentle automatic shaking (5 × 10 min, 100 agitations/min). Single cell suspensions were prepared by a technique modified from that of Pärt et al. (1993), as described by Avella & Ehrenfeld (1997), which consists of successive steps of trypsinization, filtration, centrifugation and resuspension in the ‘culture medium’ (see below). Finally, cells were seeded in Costar-Transwell 0-45 µm pore inserts (25 mm diameter, 4·8 cm\(^2\) surface; Corning Costar Corporation, Cambridge, MA, USA) in six-well Costar plates at a high density (2·5 × 10\(^5\) cells/cm\(^2\)) and maintained in a low-temperature incubator (Jouan, St Nazaire, France) at 18 °C, in humidified air (i.e. atmospheric pCO\(_2\)). After 24 h, cells reached confluency. The medium was changed every second day and the cells were used in the present experiments from days 6 to 9.

Solutions The solutions and culture conditions used have been described by Avella et al. (1994). In brief, the ‘washing medium’ consisted of Leibovitz L15 (ICN Pharmaceuticals, Orsay, France) supplemented with NaCl (20 mM), fungizone (0·1 µg/ml), penicillin (200 IU/ml), streptomycin (200 µg/ml) and gentamycin (400 µg/ml). All antibiotics were supplied by Sigma. The ‘culture medium’ consisted of Leibovitz L15 supplemented with


www.endocrinology.org

Downloaded from Bioscientifica.com at 02/17/2019 08:25:27PM via free access
10% sea bass serum (see Avella & Ehrenfeld 1997 for preparation), NaCl (20 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml) and gentamycin (200 μg/ml). The final pH of both washing and culture media was adjusted to 7.8.

**Electrical measurements** The inserts containing the gill cell monolayers were placed in a modified Ussing chamber (Avella & Ehrenfeld 1997) in order to measure the transepithelial electrical potential (Vt), ISC and resistance (Rt) of gill cells in culture. The volumes of the apical and basolateral bathing solutions were 2 ml and 4 ml respectively. The spontaneous Vt was measured with Agar–KCl salt bridges and was clamped at 0 V, through platinum electrodes, using an automatic voltage clamp (Model DVC-1000; World Precision Instruments, Sarasota, FL, USA). The sign of the transepithelial potential difference was determined by referring the basal side to the apical side (the latter taken as reference). The ISC was continuously recorded on a chart paper recorder (SEFRAM, Metrix Electronics Ltd, Basingstoke, UK).

The cell culture medium (antibiotics and serum free) was used on both sides of the monolayer to avoid possible intracellular ion and volume changes due to different experimental and culture media. In order to prevent electrical artifacts in the chambers, media were not withdrawn between the addition of agents and increasing concentrations of each agent were successively added to the appropriate side. Blockers or hormone antagonists were applied in the presence of the initial hormone. Hormones and pharmacological substances were prepared in the cell culture medium described above. Membranes were given vehicle alone (cell culture medium) before starting experiments.

**Hormones and drugs** Diphenylamine-2-carboxylic acid (DPC) was purchased from Sigma. Pure AVT, IT and vasopressin analogues were obtained from Bachem (Torrance, CA, USA). The following analogues were selected for their high pharmacological specificity in mammals, as established by Manning & Sawyer (1984) and Manning et al. (1993): [Phe2,Orn8]-oxytocin (V1a agonist), [Deamino1,Val4,D-Arg8]-vasopressin (V2 agonist), [d(CH2)5,1-O-Me-Tyr2,Arg8]-vasopressin (V1a antagonist) and [d(CH2)5,1-D-Ile2,IIe3,Arg8,Ala9]-vasopressin (V2 antagonist).

**Statistics** Experimental data correspond to ISC values taken at stabilization (steady state). For the sake of uniformity, results are presented in the Figures as ISC ratios (test/basal, i.e. after compared with before the addition of the agent). Data variability is expressed as the S.E. of the mean. The n values given in the Figure legends and Table 1 represent the number of filters used from at least three independent fish cell cultures. Considering the relatively high variability observed in ISC control levels, the effects of hormones, analogues and blocker were analysed by Student’s paired t-test to compare individual data differences (test minus basal). Statistical comparisons between V1 and V2 antagonist effects were determined with experimental data treated as ratios (hormone/hormone+V1 antagonist versus hormone/hormone+V2 antagonist) using Student’s unpaired t-test.

**Results**

In control conditions, the ISC of the cultured monolayered epithelium decreased slightly in the first 5–10 min and then remained stable for hours (ISC = 3.5 ± 1.1 μA × cm−2, 49 filters from 13 independent fish). The Vt after the stabilization period was 12.8 ± 1.7 mV (49 filters, serosal side positive) and the Rt was 7925 ± 878 Ω/cm² (49 filters). The stability of the cell spontaneous electrophysiological parameters facilitated the assessment of the neurohypophysial peptide effects and receptors involved in Cl− secretion.

**Effects of AVT and IT on Cl− secretion**

Figure 1 illustrates ISC changes as a function of time in the presence of 5 × 10−9 M AVT (Fig. 1A) or IT (Fig. 1B). The increase in the ISC was quasi-immediate after the addition of the peptides. The maximum was reached 10 min after the addition of AVT and 12 min after that of IT. In the presence of AVT or IT, these effects remained stable for at least 16 and 40 min respectively. The stabilization of ISC obtained in the presence of the peptides permitted us to carry out the experiments with blocker and antagonists. In the following experiments, the stimulated ISC values were recorded 15 min after the addition of the peptide when the effect had stabilized at its maximum.

Dose–response curves of AVT (Fig. 2A) and IT (Fig. 2B) on basal ISC were established over a wide range of concentrations, from 10−10 to 5 × 10−8 M. We did not use peptide concentrations higher than 5 × 10−8 M, considered less physiological (see Discussion). All ratios (test/basal) were above 1, indicating a stimulation of the ISC. Application of increasing concentrations of hormones elicited dose–response curves marked by a significant ISC increase for concentrations as low as 5 × 10−9 M for AVT and 5 × 10−10 M for IT. The current increased to a maximum of 60.9 ± 12.1% and 117.7 ± 28.0% above the basal level for 10−7 M AVT (n=6) and 10−7 M IT (n=5) respectively. Half-maximal ISC stimulation was observed at AVT and IT concentrations of 3.1 × 10−9 M and 1.4 × 10−9 M respectively.

Apical application of 1.0 mM DPC (a specific blocker of Cl− channels) on 5 × 10−8 M AVT- or IT-treated cells totally inhibited the peptide-stimulated ISC and reduced the basal ISC (control) by approximately 75% (Fig. 3).
Effects of neurohypophysial analogues on Cl⁻ secretion

Effects of agonists The effects of synthetic vasopressin analogues were next considered to characterize pharmacologically the receptors involved in the gill response to the fish peptides. The V1 agonist (Fig. 4A), used over a wide range of concentrations (10⁻¹⁰ to 10⁻⁷ M), increased the basal $I_{sc}$ in a dose-dependent manner, yielding an effect similar to those obtained with AVT and IT. In these conditions, maximal (25.6 ± 6.5% above basal, $n=4$) and half-maximal stimulatory effects were obtained at V1 agonist concentrations of 10⁻⁷ M and approximately 5 x 10⁻⁹ M respectively (Table 1). While the V1 agonist stimulated basal Cl⁻ secretion, the V2 agonist, used within the same range of concentrations, produced no significant effect (Fig. 4B and Table 1).

Effects of antagonists Specific V1 and V2 receptor antagonists were used to relate the effects of AVT and IT to the presence of receptors functionally close to V1 and/or V2 type(s).

The effects of the antagonists were considered on 10⁻⁸ M AVT- (Fig. 5) or IT- (Fig. 6) treated cells. The V1 or V2 antagonists used at 5 x 10⁻⁷ M were added to the serosal side of the preparation after the effects of AVT or IT had stabilized at their maximum.

In the presence of AVT, the V1 antagonist was able to reverse totally the stimulation induced by AVT (Fig. 5A). The V2 antagonist, used in the same conditions did not
produce any significant effect on the peptide-stimulated Cl⁻ secretion (Fig. 5B).

A similar study was carried out in the presence of 10⁻⁸ M IT (Fig. 6). Under these conditions, although the V1 antagonist used at a concentration of 5 × 10⁻⁷ M significantly reduced the IT-stimulated I_sc by 65·36 ± 3·4%, the V2 antagonist used at the same concentration was able to reduce it by 38·7 ± 5·2% only. Moreover, these antagonistic effects were significantly different from each other (P<0·01).

Discussion

Analysis of the electrophysiological parameters found in the present work reveals that the I_sc of the cell monolayer was lower than that observed in previous studies (Avella & Ehrenfeld 1997, Avella et al. 1999). The tissue resistance found here, a little higher than the one described previously, is characteristic of a very tight epithelium. The slight variations observed between the studies were probably due to different fish populations, and periods of the year for culture and fish serum preparations, the latter being essential for cell differentiation as shown by Avella & Ehrenfeld (1997).

AVT and IT

Since the present work is concerned with physiological experiments, it is relevant to compare the peptide concentrations used in the present study with the circulating AVT and IT levels and with previous results obtained in gills. The concentrations used in our work are within the range of the circulating peptide levels reported in fish (Henderson et al. 1986, Perrott et al. 1991, Warne et al. 1994, Pierson et al. 1995). As for the gills of SW-adapted rainbow trout, Guibbolini & Lahlou (1987) have shown that AVT and IT produced a maximal inhibition of membrane adenylate cyclase activity at 10⁻¹¹ to 10⁻⁷ M. Sainsbury & Balment (1991) confirmed, on the same material, a similar maximal inhibitory effect of AVT at 10⁻¹⁰ to 10⁻⁹ M on cellular cAMP content. The present results obtained with sea bass gill cells showed a significant stimulation of I_sc starting at 5 × 10⁻⁹ M of AVT and 5 × 10⁻¹⁰ M of IT, which compare approximately with the doses producing a maximal inhibitory effect in rainbow trout gill preparations. Our study also shows that half-maximal I_sc stimulation was observed with AVT and IT concentrations of 3·1 × 10⁻⁹ M and...
The increasing concentrations of 

<table>
<thead>
<tr>
<th>V1 agonist</th>
<th>I沈 ratio (test/basal)</th>
<th>I沈 (μA x cm⁻²)</th>
<th>I沈 difference (V1 agonist–basal) (μA x cm⁻²)</th>
<th>V2 agonist</th>
<th>I沈 ratio (test/basal)</th>
<th>I沈 (μA x cm⁻²)</th>
<th>I沈 difference (V2 agonist–basal) (μA x cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal I沈</td>
<td>(a) 1:000</td>
<td>2:03 ± 0:43</td>
<td></td>
<td>(a) 1:000</td>
<td>2:37 ± 0:33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agonist</td>
<td>5 x 10⁻¹⁰ M (b)</td>
<td>1:108 ± 0:091</td>
<td>0:11 ± 0:06 (b–a)</td>
<td>1:023 ± 0:013</td>
<td>2:44 ± 0:37</td>
<td>0:06 ± 0:04 (b–a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10⁻⁹ M (c)</td>
<td>1:117 ± 0:092</td>
<td>0:12 ± 0:06 (c–a)</td>
<td>1:032 ± 0:018</td>
<td>2:46 ± 0:38</td>
<td>0:09 ± 0:06 (c–a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 x 10⁻⁸ M (d)</td>
<td>1:142 ± 0:094</td>
<td>0:17 ± 0:07 (d–a)*</td>
<td>1:073 ± 0:041</td>
<td>2:57 ± 0:46</td>
<td>0:20 ± 0:13 (d–a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10⁻⁷ M (e)</td>
<td>1:166 ± 0:091</td>
<td>0:23 ± 0:08 (e–a)*</td>
<td>1:101 ± 0:047</td>
<td>2:64 ± 0:86</td>
<td>0:27 ± 0:15 (e–a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 x 10⁻⁶ M (f)</td>
<td>1:234 ± 0:072</td>
<td>0:40 ± 0:08 (f–a)*</td>
<td>1:139 ± 0:057</td>
<td>2:74 ± 0:52</td>
<td>0:37 ± 0:19 (f–a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 x 10⁻⁵ M (g)</td>
<td>1:256 ± 0:065</td>
<td>0:45 ± 0:08 (g–a)*</td>
<td>1:131 ± 0:060</td>
<td>2:72 ± 0:53</td>
<td>0:35 ± 0:20 (g–a)</td>
<td></td>
</tr>
</tbody>
</table>

The increasing concentrations of hormone receptors differed between chloride and oxygen uptake (Verrey et al. 1999, Kizer et al. 1995).

In the present study, the physiological significance of AVT- and IT-stimulated Cl⁻ secretion is consistent with the necessity for fish living in SW to excrete ions through the gills.

**Pharmacological characterization of receptors**

The use of vasopressin analogues for pharmacological characterization of neurohypophysial peptide receptors in mammals was popularized by Manning & Sawyer (1984) who designed a large number of these compounds. Several investigators have utilized this approach in amphibians (Larcher et al. 1992, Butlen et al. 1993) and fishes (Pang & Furspan 1984, Guibbolini & Lahlou 1990, Uchiyama & Murakami 1994, Pierson et al. 1996, Guibbolini et al. 2000). The present study is the first attempt to characterize pharmacologically the neurohypophysial peptide receptors in the gill respiratory cells of a marine fish.

**Agonists**

In our experiments, while the V1a agonist [Phe²,Orn⁸]-oxytocin stimulated Cl⁻ secretion over the same range of concentrations as did AVT or IT, the V2 agonist [Deamino¹,Val⁴,n-Arg⁸]-vasopressin, a potent antidiuretic, produced no effect. This agrees with the findings obtained in rainbow trout gill adenylate cyclase by Guibbolini & Lahlou (1990). In the present study, the rank order of potency (affinity and efficacy) was as follows: IT>AVT>V1a agonist>V2 agonist. Taken together, these results suggest the presence of V1-type receptors in sea bass gill respiratory-like cells. Previous studies on gill plasma membranes from SW-adapted rainbow trout revealed a different sequence: AVT>IT>V1a agonist>V2 agonist (Guibbolini & Lahlou 1987, 1990). Thus, comparison between sea bass and rainbow trout shows differences in the potency order of AVT and IT. This contrast may result from the differences in fish species, and from the presence of hormone receptors differentially sensitive to AVT and IT and/or differently distributed between chloride and respiratory cells. This latter point may be explained by
variations between the two fish gill preparations: the preparation of trout gill plasma membrane was obtained from both populations of chloride and respiratory cells while the culture of sea bass gill respiratory cells excludes the population of chloride cells. Moreover, work carried out in eel isolated gill cells showed that increasing external salinity produces parallel changes between chloride cell number and size and AVT-binding capacity (Guibbolini et al. 1988).

In sea bass, our present results suggest that the V1-type receptors located in gill respiratory cells are predominant over V2 receptors and are more sensitive to IT than to AVT. More generally, the difference in the relative potential effects between the two peptides in various fish (rainbow trout, eel and sea bass) preparations as described above might indicate that, in SW conditions, the V1-type receptors are predominantly more sensitive to AVT in gill chloride cells and to IT in gill respiratory cells. However, this hypothesis cannot rule out the possibility that the two types of receptor can be present in both cell types and that they may display different pharmacological characteristics.
in various species or biological preparations. Further experiments using a preparation of homogeneous chloride cells are required to confirm the present hypothesis.

Antagonists and fish neurohypophysial peptide receptors

The effects of antagonists on AVT- and IT-induced stimulation of Cl\(^-\) secretion reveal the presence of V1-type receptors, but with pharmacological differences depending on the peptide used. Indeed, while the V1a antagonist [d(CH2)\(^5\)O-Me-Tyr\(^2\),Arg\(^8\)]-vasopressin was able to suppress the AVT-stimulated Cl\(^-\) secretion totally, it reduced that of IT by 65% only. Moreover, while the V2 antagonist [d(CH2)\(^5\),p-Ile\(^2\),Ile\(^4\),Arg\(^8\),Ala\(^9\)]-vasopressin produced no effect on AVT-stimulated Cl\(^-\) secretion, it reduced by 39% that of IT. Considering the magnitude of the respective actions of the antagonists on the AVT- or IT-induced effect, the present data are in line with the results obtained previously on rainbow trout gill plasma membranes (Guibbolini & Lahlou 1990). Indeed, in rainbow trout, while the V1a antagonist totally suppressed the effects of AVT and IT on the adenylate cyclase activity, the V2 antagonist showed no effect on either peptide (Guibbolini & Lahlou 1990). Nevertheless, in contrast to the work performed on the heterogeneous population of rainbow trout gill cells, the present study carried out on the homogeneous population of sea bass gill cells in culture reveals some differences of interest. The results suggest the presence of two specific and pharmacologically similar V1-type receptors (according to the mammalian classification) – AVT and IT receptors – and permit us to localize them in a specific cell type, the respiratory-like cells. The presence of two types of neurohypophysial hormone receptors has already been described in fish gills (Mahlmann et al. 1994, Hausmann et al. 1995). Indeed, the molecular structure of the vasotocin receptor in the teleost fish Catostomus commersoni gills was established by Mahlmann et al. (1994) and that of the IT receptor by Hausmann et al. (1995). In their studies, the AVT receptor presented a sequence homology with mammalian neurohypophysial hormone V1-type receptors and the IT receptor displayed the greatest similarity to the mammalian oxytocin receptors. The studies of Mahlmann et al. (1994) and Hausmann et al. (1995), using the same V1 antagonist as in our work, are also in line with our physiological results. These authors showed that (1) xenopus oocytes injected with the cloned AVT or IT receptor-encoding cRNA respond to AVT or IT by an induction of membrane chloride currents and (2) the AVT-induced responses are blocked particularly by the V1 specific antagonist, [d(CH2)\(^5\),O-Me-Tyr\(^2\),Arg\(^8\)]-vasopressin.

The present investigation demonstrates clearly that primary cultures of sea bass gill cells are effective targets for the fish neurohypophysial peptides, AVT and IT, and that both stimulate branchial Cl\(^-\) secretion. This study provides pharmacological evidence and suggests, in a SW fish, that the physiological effects of AVT and IT in the gills are mediated by two distinct, although pharmacologically similar, V1-type receptors, both located in gill respiratory-like cells. However, we cannot rule out the possibility of the presence of V1 receptor also located in chloride cells.

Previous observations have described complex interactions between cellular signalling pathways involved in the effects of the neurohypophysial hormone in gill respiratory cells. Indeed, complex features between second messengers were found in sea bass with AVT used together with db-cAMP on ISC (Avella et al. 1999) and in rainbow trout where AVT inhibited cAMP and stimulated IP3 production depending on the concentration used (Guibbolini & Pärt 1997, Guibbolini et al. 1997). Further investigation is required to analyse the neurohypophysial hormonal signalling pathways in gill cells.

This work, together with earlier results involving other hormones or fish species (Guibbolini & Pärt 1997, Guibbolini et al. 1997, Avella et al. 1999), emphasizes the participation of gill respiratory cells as a site for the regulation of ion transport, enlarging the earlier views attributing this transport chiefly to gill chloride cells at the organismic scale.

Acknowledgements

The authors are greatly indebted to Professor B Lahlou (Université de Nice–Sophia Antipolis, France) for invaluable advice and extensive comments throughout this work. They also express their gratitude to Dr P Poujeol (Laboratoire de Physiologie Cellulaire et Moléculaire, Université de Nice–Sophia Antipolis) for his scientific comments. They are also grateful to Dr B Maetz for her critical comments on this manuscript.

References


Peptides 13 865–871.


Received in final form 1 October 2002
Accepted 1 October 2002