Neurohypophysial hormone receptors and second messengers in trout hepatocytes

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Abstract

Neurohypophysial hormone receptors and second messengers were studied in trout (Oncorhynchus mykiss) hepatocytes. Arginine vasotocin (AVT) and isotocin (IT) elicited a concentration-dependent inhibition of cAMP accumulation in the presence of 5 × 10⁻⁸ M glucagon (maximal effect for 4·5 × 10⁻⁷ M and 1·4 × 10⁻⁷ M, half-maximal effect for 2·1 × 10⁻⁸ M and 0·7 × 10⁻⁸ M, AVT and IT respectively). The effect of glucagon was inhibited up to 90% by AVT and 80% by IT. While AVT inhibited (up to 50%) the basal cAMP production, IT had no such action.

Specific V₁ or V₂ analogues (with reference to vasopressin in mammals) were used for pharmacological characterization of the type of neurohypophysial hormone receptor involved in this inhibition. The V₁ agonist [Phe², Orn¹]-oxytocin inhibited the glucagon-stimulated cAMP production with a maximal effect for 6 × 10⁻⁷ M and a half-maximal effect for 0·9 × 10⁻⁸ M concentrations of the analogue. While the V₁ agonist reduced the glucagon-stimulated cAMP level by 70%, it showed only a tendency to reduce the basal level. The V₂ agonist [deamino⁷, Val⁸, d-Arg⁸]-vasopressin had no effect either on basal or on glucagon-stimulated cAMP production. The V₁ antagonist [d(CH₂)₅¹, O-Me-Tyr², Arg⁸]-vasopressin totally reversed the 10⁻⁸ M AVT-induced inhibition of 5 × 10⁻⁸ M glucagon-stimulated cAMP production, whereas the V₂ antagonist [d(CH₂)₅¹, d-Ile², Ile⁴, Arg⁸, Ala³]-vasopressin had no such effect. In this particular case, maximal and half-maximal effects of the V₁ antagonist were obtained for 2·3 × 10⁻⁶ M and 1·2 × 10⁻⁶ M respectively.

Changes in intracellular calcium content were measured using the fluorescent probe FURA-2/AM. AVT and IT elicited a concentration-dependent increase in Ca²⁺ accumulation. The comparison of the effect of 10⁻⁸ M agonists versus AVT showed the following order of potency: AVT > IT > V₁ agonist > V₂ agonist. The V₁ antagonist reversed the AVT-induced Ca²⁺ accumulation whereas the V₂ antagonist had no such effect.

These results are taken as evidence for the presence in trout hepatocytes of neurohypophysial hormone receptors functionally close to the V₁₃-type linked to cAMP production and Ca²⁺ mobilization.

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Introduction

In mammals, vasopressin (AVP) possesses a glycogenolytic effect and its mechanism of action has been well defined in hepatocytes. In the rat, labelled AVP binds to specific receptors located on intact cells or isolated membranes and coupled to a G protein (Cantau et al. 1980, Fitzgerald et al. 1986). This receptor was pharmacologically characterized as a V₁₃-type (Fahrenholz et al. 1984, Sato et al. 1992); it was recently cloned and shown to possess seven transmembrane domains (Morel et al. 1992, 1993). The V₁₃ receptor was shown to mediate vasopressin-induced glycogenolytic responses by enhancing polyphosphoinositide breakdown and by increasing inositol trisphosphate (IP₃) and diacylglycerol (DAG) (Thomas et al. 1984, Cerpowicz & Ochs 1992). The IP₃ formed induces an increase in the concentration of intracellular calcium (Thomas et al. 1984) due to the liberation of calcium from intracellular stores (Hajnoczky & Thomas 1997) and sustained by an extracellular calcium influx (Garrison et al. 1979, Berven et al. 1994, Duszynski et al. 1995). DAG activates a protein kinase C (Tang & Houslay 1992). The intervention of protein kinases leads to protein phosphorylation (Morris et al. 1994) and to activation of mitogen-activated protein kinase (Romanelli & Van de Werve 1997). Another effect of vasopressin is to inhibit, in a dose-dependent manner, the cAMP accumulation induced by forskolin or glucagon whether cells are depleted in calcium or not (Morgan et al. 1983).

In amphibian hepatocytes, arginine vasotocin (AVT) or AVP (the heterologous hormone, generally used) produces a dose-dependent stimulation of glycogenolysis (Janssens et al. 1992). The IP₃ formed induces an increase in the concentration of intracellular calcium in amphibian liver (Janssens et al. 1992, 1993). The V₁₃ receptor was characterized pharmacologically as a V₁₃-type linked to CAMP production (Morel et al. 1992) and to the activation of a protein kinase C (Cantau et al. 1980). Inhibitors of protein kinases prevent the AVT-induced glycogenolysis (Morel et al. 1992). In amphibian liver, the IP₃ receptor induces elevation of intracellular Ca²⁺ whereas the V₁₃ receptor has no such action (Janssens et al. 1992). These results are in agreement with the characteristics of the V₁₃ receptor in mammals (Cantau et al. 1980) and with the presence of a V₁₃-type receptor in amphibian liver (Morel et al. 1992). The V₁₃ receptor is coupled to a G protein (Cantau et al. 1980) and couples to G protein-coupled receptors (Cantau et al. 1980) and couples to a heterologous hormone, generally used) produces a dose-dependent stimulation of glycogenolysis (Janssens et al. 1992). The IP₃ formed induces an increase in the concentration of intracellular calcium (Thomas et al. 1984) due to the liberation of calcium from intracellular stores (Hajnoczky & Thomas 1997) and sustained by an extracellular calcium influx (Garrison et al. 1979, Berven et al. 1994, Duszynski et al. 1995). DAG activates a protein kinase C (Tang & Houslay 1992). The intervention of protein kinases leads to protein phosphorylation (Morris et al. 1994) and to activation of mitogen-activated protein kinase (Romanelli & Van de Werve 1997). Another effect of vasopressin is to inhibit, in a dose-dependent manner, the cAMP accumulation induced by forskolin or glucagon whether cells are depleted in calcium or not (Morgan et al. 1983).
et al. 1983, Ade et al. 1995) mediated by cAMP accumulation (Janssens et al. 1986) and not by IP3 production (Kleineke & Janssens 1993). Kleineke and Janssens (1993) showed that AVT induced an increase in intracellular concentration of calcium, directly due to an extracellular calcium influx. They concluded that AVT stimulated cAMP production which not only induced glycogenolysis but also regulated the opening of an ionic channel allowing calcium influx. The stimulatory effect of AVT on cAMP accumulation and the pharmacological study carried out on primary cultures of Xenopus laevis hepatocytes led authors to classify this receptor as a V2-type receptor (Ade et al. 1995).

In contrast to other vertebrates, little is known in fish concerning the mechanism of action of neurohypophysial peptides on hepatocytes (Lahlou et al. 1988, Moon & Mommens 1990). Moon and Mommens (1990) showed that neurohypophysial peptides activated the glycochenolysis in eel and to a lesser extent in trout liver. In the same study, AVT induced in eel a dose-dependent stimulation of cAMP concentration. However, the concentrations of AVT used in their study were relatively high (10^{-7} to 10^{-5} M). In contrast, Lahlou et al. (1988) showed in the trout that neurohypophysial peptides at concentrations of 5 \times 10^{-7} to 5 \times 10^{-6} M (concentrations also relatively high) inhibited the cAMP accumulation stimulated by 4 \times 10^{-6} M glucagon (Lahlou et al. 1988).

Taken together, these data led us to investigate further the mechanism of action of neurohypophysial peptides in trout hepatocytes. The aim of the present study was (1) to analyse the nature of second messengers (cAMP and Ca^{2+}) involved in the transduction of the neurohypophysial peptide effect, and (2) to characterize pharmacologically the type(s) of peptide receptor(s) present in trout hepatocytes by using selected specific V1 or V2 analogues. Results are considered with regard to the phylogenetic evolution of neurohypophysial peptide receptors in the vertebrate series.

Materials and Methods

Animals

Rainbow trout, Oncorhynchus mykiss (average weight 220 g), were purchased from a fish farm and maintained in the laboratory at 12–15 °C, under constant photoperiod (12 h light per day).

Chemicals

Pure arginine vasotocin (AVT), isotocin (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 and colleagues (Manning & Sawyer 1984, Manning et al. 1993): [Phe^{2}, Orn^{8}]-oxytocin (V_{1a} agonist), [deamino^{8}, Val^{1}, d-Arg^{8}]-vasopressin (V_{2} agonist), [d(CH_{2})_{5}^{1}, O-Me-Tyr^{2}, Arg^{8}]-vasopressin (V_{1a} antagonist), [d(CH_{2})_{3}^{1}, p-Ile^{2}, Ile^{4}, Arg^{8}, Ala^{9}]-vasopressin (V_{2} antagonist). HANK’s minimum essential medium (HMEM), bovine serum albumin fraction V (BSA) and collagenase H were obtained from Boehringer Mannheim (Illkirch, France) and heparin LEO (5000 UI/ml) from LEO Laboratory (St-Quentin-les-Yvelines, France). Glucagon was supplied by NOVO Nordisk Pharmaceutique S.A. (Boulogne, France). Theophylline (cAMP phosphodiesterase inhibitor) was purchased from Sigma-Aldrich Chimie (L’Isle d’Abeau Chesnes, France). FURA-2/AM and pluronic acid were obtained from Molecular Probes (Interchim, France). The [^{3}H]cAMP radioimmunoassay kit was obtained from Amersham (Les Ulis, France).

Preparation of hepatocytes

The basic technique has been described previously (Porthé-Nibelle & Lahlou 1981). Briefly, the trout liver was perfused via the portal vein for 5 min with a perfusion buffer (in mM: NaCl 66, KCl 66, glucose 5:5, Hepes 10, pH 7-4) and then for 1 h with collagenase (0:8 mg/ml perfusion buffer). Hepatocytes were then collected after 2 passages through a 3-layer gauze, rinsed twice with perfusion buffer and re-suspended in HMEM (300 mosmol/l; pH 7-4). Under these conditions, the intracellular ion concentrations in the isolated cells are not in steady state, although they are viable at room temperature. Consequently, the cells were then preincubated for 40 min in HMEM prior to use. This treatment restores and stabilizes the cellular electrolyte concentrations for several hours (Lahlou et al. 1988). Cell viability was checked using 0:04% Trypan blue and preparations containing more than 15% stained cells were discarded.

Incubation procedure and cAMP determination

The basic technique has been described previously by Lahlou et al. (1988). Routinely, the typical incubation medium contained 10 mM theophylline and 5% (w/v) BSA in HMEM, pH 7-4. The final volume was 200 µl containing 100 µl equilibrated cell preparation (on average: 3:5 \times 10^{6} cells or 0:6 mg protein/100 µl cell preparation) in HMEM and 20 µl hormone(s). Because inhibitions are often larger or better revealed when the target system is stimulated by another hormone (Guibbolini & Lahlou 1987, Lahlou et al. 1988), 5 \times 10^{-8} M glucagon were added to the incubation medium. When hormones and analogues were considered together in the assay, they were added simultaneously in the incubation medium. The reaction was initiated by addition of the cells which were left to incubate for 10 min
at 20 °C under gentle shaking, and was terminated by 3 min heating in boiling water. Samples taken in duplicate were then centrifuged at 1600 g for 10 min at 4 °C, and the supernatants were collected for cAMP measurement. The levels of cAMP were determined in each sample using a [3H]cAMP radioimmunoassay kit as specified by the supplier. Protein quantities were determined in each sample by using the method of Bradford (1976).

Results were expressed as pmoles cAMP/mg protein per 10 min or as a ratio (test/basal, i.e. in the presence/absence of hormone). Because of variations between individual cell preparations, cAMP content was also expressed as a percentage of maximum (the 100% value was that obtained with 5 × 10⁻⁸ M glucagon alone).

Results were expressed as means ± s.e., for n independent liver preparations (with each test carried out in duplicate or in triplicate). Statistical significance was determined using the Student’s t-test applied to comparison between unpaired values, and comparison of means using ANOVA single factor-factorial test (Statview software, Brain Power Inc., Calabasas, CA, USA).

**Calcium measurement**

All subsequent operations were carried out at 20 °C in the dark and under slow shaking. Cell suspensions (2 × 10⁷ cells in 2 ml HMEM) were incubated for 1 h loading in the presence of FURA-2/AM (5 µM) and pluronic acid (0-05%). After this incubation period, cells were rinsed twice with HMEM (200 g, 20 s), re-suspended in 2 ml HMEM and left to stabilize for 45 min. Changes in intracellular concentration of calcium ([Ca²⁺]ᵢ) were measured in 1-5 ml hepatocyte suspension in the presence or absence of hormone(s) (15 µl added), using a Perkin-Elmer spectrofluorimeter. The fluorescence emission of FURA 2, induced by excitation at two wavelengths (340 and 380 nm), was recorded at 510 nm. The fluorescence ratio (F340/F380) was used as an indicator of the variations of [Ca²⁺]ᵢ. In these experiments, increasing hormone concentrations were successively added to the cell suspension. Therefore, the fluorescence ratios obtained represent cumulative responses. For each hormone concentration, the experiment was followed for 2:5 min (5 cycles of 30 s) at room temperature. Maximal effect was obtained between 2 and 2:5 min. Results were expressed as test vs basal ratio, means ± s.e. (n independent preparations).

Statistical significance of peptide effects on fluorescence ratios in cell preparations was assessed by using repeated measures ANOVA (SYSTAT 5-2-1 software, Systat Inc., NY, USA). This model was carried out since each cell preparation was submitted to increasing concentrations of AVT, IT or analogues. In addition, for AVT and IT analysis, since the sphericity assumption was not checked, P values were corrected using the Greenhouse–Geisser adjustment.

**Results**

**cAMP measurements**

**Effects of hormones** The basal cAMP content in hepatocytes was 2·9 ± 0·6 pmoles cAMP/mg protein per 10 min (n=14). The effects of AVT and IT in relation to their concentration are illustrated in Fig. 1. In this case, the level of cAMP, as expressed by the test vs basal ratio, was decreased by AVT in a dose-dependent manner. Maximal (Dₘₐₓ) and half-maximal (IC₅₀) inhibitions were obtained for 3·0 ± 1·4 × 10⁻⁶ M and 4·5 ± 2·3 × 10⁻⁷ M concentrations of the peptide (n=3). While AVT inhibited (up to 50%) basal cAMP production, IT had no significant effect.

The effects of AVT and IT were studied in the presence of 5 × 10⁻⁸ M glucagon and the results are shown in Fig. 2. We have previously shown that this concentration of glucagon was sufficient to yield an accurately measurable increase in cAMP (Lahlou et al. 1988). In the present experiments, 5 × 10⁻⁸ M glucagon stimulated cAMP accumulation by a factor of 3·9 ± 0·7 (n=14) for an average absolute cAMP level of 11·3 ± 3·2 pmoles cAMP/mg protein per 10 min (n=14). In these conditions, both peptides elicited a concentration-dependent inhibition of cAMP accumulation. For AVT, the maximum effect was obtained with 4·5 ± 2·1 ± 10⁻⁷ M and the half-maximum effect was obtained with 2·1 ± 3·4 ± 10⁻⁸ M (n=7); for IT, Dₘₐₓ was 1·4 ± 1·0 ± 10⁻⁷ M and IC₅₀ was 0·7 ± 0·35 ± 10⁻⁸ M (n=3). Stimulation by glucagon was inhibited up to 90% by AVT and 80% by IT.

**Effects of synthetic analogues** Specific V₁ and V₂ analogues were used to relate the AVT effect to the
Agonists The effects of the V1 and V2 agonists on basal (Table 1) and on 5 × 10^{-8} M glucagon-stimulated cAMP production (Fig. 3) revealed that the V1 agonist inhibited the glucagon-stimulated activity with maximal and half-maximal inhibitions obtained for 6·0 ± 2·7 × 10^{-7} M and 0·9 ± 0·4 × 10^{-8} M concentrations of the analogue (n=6).

While the V1 agonist reduced the glucagon-stimulated level of cAMP by 70%, it only showed a slight, not significant tendency to reduce the basal level. The V2 agonist produced no effect in any of the experimental conditions.

### Discussion

The results we obtained with cAMP led us to undertake experiments on Ca^{2+}, the other main second messenger. Data concerning the effect of the neurohypophysial peptides on Ca^{2+} mobilization are illustrated in Fig. 5. AVT and IT (in the range of 10^{-10} to 10^{-6} M) elicited an increase in intracellular Ca^{2+} content in hepatocytes. The analysis of the responses of these two peptides showed that AVT and IT exerted a similar dose-dependent effect on Ca^{2+} increase.

The use of the same neurohypophysial peptide analogues as in the cAMP measurements gave results summarized in Table 2. For the same concentration as AVT (10^{-8} M), the V1 agonist also increased the intracellular Ca^{2+} ratio significantly from the basal level. The V2 agonist had a less pronounced effect than the V1 agonist. The V1 antagonist reversed the AVT effect on Ca^{2+} accumulation whereas the V2 antagonist had no such effect.

### Table 1

<table>
<thead>
<tr>
<th>Agonist Type</th>
<th>V1 agonist (%)</th>
<th>V2 agonist (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10^{-9} M</td>
<td>94 ± 7</td>
<td>90 ± 21</td>
</tr>
<tr>
<td>10^{-8} M</td>
<td>78 ± 14</td>
<td>95 ± 11</td>
</tr>
<tr>
<td>10^{-7} M</td>
<td>78 ± 13</td>
<td>83 ± 15</td>
</tr>
<tr>
<td>10^{-6} M</td>
<td>75 ± 14</td>
<td>99 ± 32</td>
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</table>

Values were not statistically significantly different from basal (ANOVA).

Antagonists The concentration of 10^{-8} M AVT was chosen in this set of experiments because it corresponds to the IC_{50} of this hormone challenging the effect of 5 × 10^{-8} M glucagon (Fig. 2A). The V1 antagonist was able to reverse totally the inhibition caused by 10^{-8} M AVT on the 5 × 10^{-8} M glucagon-stimulated production of cAMP (Fig. 4A). Maximal and half-maximal effects of the V1 antagonist were obtained with 2·3 ± 0·8 × 10^{-6} M and 1·2 ± 0·6 × 10^{-6} M, respectively (n=6). In contrast, the V2 antagonist (Fig. 4B) did not produce this effect, and even showed a slight tendency to decrease further the glucagon-stimulated/AVT-inhibited cAMP accumulation. This tendency was noted for 10^{-8} M V2 analogue only and was not reproduced at higher concentrations.

### Ca^{2+} measurements

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cAMP accumulation in trout hepatocytes which is strikingly revealed in the presence of $5 \times 10^{-8}$ M glucagon. The stimulation by glucagon was decreased up to 90% by AVT and 80% by IT, for an AVT or IT concentration of $4 \times 10^{-7}$ and $1 \times 10^{-7}$ M ($n=7$) and $1 \times 10^{-7}$ and $1 \times 10^{-8}$ M ($n=3$), respectively. In rat liver, $10^{-8}$ M vasopressin used in similar conditions was only able to reduce by 16% the cAMP accumulation induced by $10^{-8}$ M glucagon (Morgan et al. 1983). In rat brain, vasopressin was found partially to prevent the cAMP accumulation induced by noradrenaline, dopamine and forskolin (50%, 45% and 40% by $3 \times 10^{-7}$, $5.5 \times 10^{-7}$ and $3 \times 10^{-7}$ M AVP respectively, Newman 1985). In our study, not only did the neurohypophysial peptides strongly inhibit the stimulatory effect, but the basal activity (Fig. 1) was also reduced by AVT and IT decrease cAMP production but they also behaved
Balment been estimated only recently in hormone levels, those of neurohypophysial peptides have tides in rat hepatocytes (Thomas & Thomas 1997). To our knowledge, the present study is like Ca2+-mobilizing hormones, as do homologous pep-

Figure 5 Effect of (A) AVT and (B) IT concentrations on intracellular Ca2+ content in trout hepatocytes. Results are means ± s.e. (n=6 for AVT and n=6 for IT) and are expressed as test vs basal ratio. The dose effect is statistically significant at 0.01 for AVT and at 0.05 for IT (repeated measures ANOVA, Greenhouse–Geisser P value of 0.011 for AVT and 0.027 for IT).

like Ca2+-mobilizing hormones, as do homologous pep-
tides in rat hepatocytes (Thomas et al. 1984, Hajnoczky & Thomas 1997). To our knowledge, the present study is the first showing an effect of IT on second messenger production in fish hepatocytes.

In the present study, neurohypophysial hormones inhibited glucagon-stimulated cAMP accumulation with IC50 values of around 10^{-8} M. With regard to circulating hormone levels, those of neurohypophysial peptides have been estimated only recently in fish (Perrott et al. 1991, Balment et al. 1993, Warne et al. 1994, Pierson et al. 1995a,b). Our present observations are consistent with our own estimates since, in plasma of trout adapted to various degrees of salinity, AVT and IT measured by ELISA were in the range of 8–26 nM and 3–5.5 nM respectively (Pierson et al. 1995a,b). Furthermore, neurohypophysial hormone IC50 values in the present study are in agreement with Ka values found in binding experiments in rat intact hepatocytes for AVP (15 nM, Cantau et al. 1980) and in eel isolated gill cells for AVT (3-2 to 1 nM in fresh- and sea-water-adapted fish respectively, Guibbolini et al. 1988).

The use of vasopressin analogues for pharmacological characterization of neurohypophysial peptide receptors may be extended with some caution to non-mammalian vertebrates. Several investigators used 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). The present study is the first attempt to characterize the neurohypophysial hormone receptor in fish hepatocytes by means of artificial analogues. The V1 agonist presented a slight tendency to act in a similar manner to AVT on basal cAMP levels, for concentrations within the range of those of AVT and IT. When cAMP accumulation was stimulated by glucagon, the Dmax for AVT, IT and V1 agonist decreased to a similar lower value of 1–6 × 10^{-7} M and the IC50 for AVT, IT and V1 agonist followed a similar pattern. When the V1 antagonist was added, it totally suppressed the AVT-induced inhibition of the glucagon-stimulated activity. In contrast, the V2 analogues did not show any effect. The slight further decrease only observed in the presence of 10^{-8} M V2 antagonist has already been noticed in mammals with the V2 antagonist behaving like a V1 agonist (Ellis et al. 1994). With regard to Ca2+ accumulation, neurohypophysial peptide agonists at 10^{-8} M revealed the following order of potency: AVT=IT>V1 agonist>V2 agonist. Concerning the antagonists, while the V1 antagonist reversed significantly the AVT effect, the V2 antagonist had no such effect.

Our results taken together (cAMP decrease, Ca2+ increase, pharmacological receptor characterization) are in favour of the presence of V1a-type receptors in trout hepatocytes. Some authors have proposed that V2-type receptors coupled with adenylyl cyclase stimulation may

| Table 2 Effect of the V1 and V2 vasopressin receptor agonists and antagonists on calcium content in trout hepatocytes. Results (means ± s.e. of n experiments) are expressed as test vs basal ratio. |
|---|---|
| **Intracellular Ca2+ ratio** | (test/basal) |
| Basal | 1 |
| AVT (10^{-8} M) | 1-187 ± 0.041 (n=6)** |
| V1 agonist (10^{-8} M) | 1-133 ± 0.027 (n=4)* |
| V2 agonist (10^{-6} M) | 1-070 ± 0.010 (n=3) |
| AVT (10^{-8} M)+V1 antagonist (10^{-6} M) | 1-036 ± 0.023 (n=3)# |
| AVT (10^{-8} M)+V2 antagonist (10^{-6} M) | 1-118 ± 0.010 (n=3) |

*P<0.05, **P<0.01 compared with basal; #P>0.05 compared with AVT (repeated measures ANOVA).
also exist in fish, namely in eel liver (Moon & Monnsmen 1990) and in trout kidney (Perrott et al. 1993), although the concentrations of hormone used in their experiments were high ($D_{max}$ between $10^{-6}$-$10^{-5}$ M). Our previous work on peripheral vasotocin receptors in lower vertebrates emphasized the ‘V₁’ feature of these receptors in such distinct organs as trout pituitary and gill (Guibbolini & Lahlou 1987, Guibbolini et al. 1988, Lahlou et al. 1988, Guibbolini & Lahlou 1990, 1992, Pierson et al. 1996). Recently, in the teleost fish Catosomus commersoni, the molecular structure of the vasotocin receptor was established by Mahlmann et al. (1994) and that of the isotocin receptor by Hausmann et al. (1995). Both AVT and IT receptors transcripts have been found in several bony fish organs (gills, liver, pituitary, lateral line). The AVT receptor presented a sequence homology with mammalian neurohypophysial hormone receptors, particularly with the rat vasopressin V₁a receptor (60-9% identity), and to a lesser extent with the human oxytocin receptor (48%) and rat and human vasopressin V₂ receptors (about 40%). The isotocin receptor displays the greatest similarity to the mammalian oxytocin receptor. It can be noted that the sequence homology between receptors may also explain the slight V₁ agonistic effect we observed with the V₂ antagonist on trout hepatocytes. Mahlmann’s study (Mahlmann et al. 1994) also showed that (1) Xenopus oocytes injected with the cloned AVT receptor-encoding cRNA respond to AVT by the induction of membrane chloride currents, (2) the AVT-induced responses are blocked, in particular, by the V₁ specific antagonist, [d(CH₂)₅O, O-Me-Tyr², Arg⁷]-vasopressin (the same as used in our study), and (3) the transduction of the hormonal message occurs via the phospholipase C and inositol phosphate/calcium pathway, usual characteristics of the V₁ receptor family.

In summary, trout hepatocytes display a neurohypophysial hormone receptor which is functionally close to the V₁a sub-family of vasopressin receptors, as results from pharmacological and second messenger production studies support. Interestingly, the cellular signalling machinery of these cells responds equally to the two peptides, arginine vasotocin and isotocin, present in the teleostean pituitary. The present results sustain the observation that, unlike the mammalian hormones vasopressin and oxytocin, the teleost fish peptides vasotocin and isotocin have not been shown up to now to act on separate target organs and their physiological effects are usually qualitatively similar, although displaying different hormonal potencies. Functional and structural binding studies are awaited to clarify this discrepancy, so as to determine the presence of one or more neurohypophysial peptide receptors within the same target organ.

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