

Neurohypophyseal peptide inhibition of adenylate cyclase activity in fish gills

The effect of environmental salinity

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Adenylate cyclase activity was measured in plasma membranes prepared from gill epithelium of the rainbow trout (*Salmo gairdneri*) adapted to a large range of salinity (deionized water to seawater). Fish neurohypophyseal peptides (arginine-vasotocin and isotocin) elicited a dose-dependent inhibition (maximum for 10^{-12} – 10^{-10} M which corresponds to physiological blood concentrations) of both basal and 10^{-9} M glucagon-stimulated enzyme activity. While basal activity was inhibited by up to 40% only in high salt media, glucagon-stimulated activity was lowered by 60–100% for the same concentrations, in both freshwater and seawater.

Adenylate cyclase; Neurohypophyseal peptide; Arginine-vasotocin; Isotocin; Salt adaptation; (Trout gill)

1. INTRODUCTION

In tetrapod vertebrates, neurohypophyseal hormones are very potent in the regulation of water and ion transport by epithelia (such as kidney tubules, amphibian skin or bladder) by stimulating adenylate cyclase via so-called V_2 receptors [1–4]. Other effects of these peptides (vasoconstriction, glycogenolysis, platelet aggregation) appear to be mediated by stimulation of phosphoinositide breakdown and/or calcium mobilization, via V_1 -type receptors [2,5–9].

In teleost fishes, osmoregulation depends upon transport activity of several organs, the gills in particular. Their neurohypophysis produces vasotocin (AVT) and isotocin (IT) but the physiological

functions of these hormones are still poorly defined and no studies have been carried out concerning relationships between them and adenylate cyclase activity. Recently, however, we have characterized this enzyme in gills by in vitro assay [10] and shown the importance of high salt media in modulating this activity and the potency of hormonal and pharmacological substances in stimulating the enzyme [11,12].

This lack of information led us to analyze the effect of arginine-vasotocin and of isotocin on adenylate cyclase in the gills of trout and to examine the changes in this enzyme associated with fish adaptation to various salinities.

Our data suggest that neurohypophyseal peptides in fishes do not behave in the same way as in tetrapods, since they inhibit adenylate cyclase in gills (rather than the stimulation seen in other ion transporting epithelia). Moreover, we showed in membrane fractions that these peptides are able to reduce basal adenylate cyclase activity, a fact rare-

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ly reported in tetrapods. Finally, we found that the ability to inhibit this enzyme is sensitive to the environmental salinity of the animals.

2. MATERIALS AND METHODS

2.1. Chemicals

Cyclic 3',5'-[2,8-³H]adenosine monophosphate (cAMP, 50 Ci/mmol) was obtained from the Commissariat à l'Energie Atomique (Saclay, France) and [α -³²P]ATP 50 Ci/mmol) was supplied in dry ice by New England Nuclear (via Dupont de Nemours, Paris). Dowex AG-50W-X8 cation-exchange resin (200–400 mesh, H⁺ form) was obtained from Bio-Rad (Vitry/Seine, France). Arginine-vasotocin and isotocin were purchased from Bachem (Torrance, USA). Scintillation fluid (Aqualuma) was obtained from Kontron (Trappes, France). All other products were purchased from Sigma (USA).

2.2. Animals

Rainbow trout, *Salmo gairdneri* (average weight 220 g), were purchased from a local fish farm and maintained at 12–15°C in the laboratory, with a constant photoperiod (12 h light per day). Mullet, *Mugil* sp. (average weight 70 g), were supplied by the Musée Océanographique, Monaco. Salt adaptation of these animals was carried out stepwise as described [12]. Controls corresponding to each condition of salinity were run in parallel.

2.3. Preparation of gill plasma membranes

Gill membranes were prepared as described in [10,12] and the pelleted membranes, obtained after centrifugation at 25000 × g for 10 min, were suspended in an appropriate volume of 0.3 M sucrose to obtain a concentration of approx. 6 mg protein per ml. Aliquots of 1 ml were immediately frozen and stored at –30°C to be used as the source of adenylate cyclase. Protein content of the membranes was measured by the dye-binding procedure of Bradford [13].

2.4. Assay of gill adenylate cyclase activity

Typical assay medium contained: 4 mM MgCl₂; 1 mM EDTA; 75 mM Tris-HCl, pH 7.4; 8 mM theophylline; an ATP regenerating system (10 mM creatine phosphate; 0.5 mg/ml creatine phosphokinase, 200 U/mg; 0.05 mg/ml myokinase,

2000 U/mg); 0.5 mM cAMP and 50000 dpm [³H]cAMP; 0.75 mM [α -³²P]ATP (50–80 dpm/pmol); 10^{–5} M GTP. Routinely, the final volume of assay mixture was 50 μ l, containing 10 μ l of adenylate cyclase membrane preparation (average 50–60 μ g protein). The reaction was initiated by addition of labelled and non-labelled nucleotides, and after incubation for 10 min at 20°C under slow shaking, it was terminated by the addition of 200 μ l stopping solution (10 mM CaCl₂; 10 mM Tris-HCl, pH 7.4) and heating for 3 min in boiling water.

Adenylate cyclase activity was determined by measuring [α -³²P]cAMP formation from [α -³²P]ATP as in [10,12]. When neurohypophyseal peptides were present, the activity was expressed by its absolute value (pmol cAMP produced/mg protein per 10 min) or as a stimulation ratio with respect to the corresponding basal value (test/basal ratio). For statistical purposes, each type of experiment was repeated 3 times and each determination was made in separate quadruplicates. Statistical analysis was made by desk computer (Sirius Victor) using Student's test for unpaired values.

3. RESULTS

In previous observations [11,12], we measured adenylate cyclase activity in trout adapted to a large range of salinities and in the mullet, a marine species. In trout, this activity declined by a factor of 2 between deionized water and full strength sea water (pmol cAMP/mg protein per 10 min): 530 in deionized water (DW), 440 in fresh water (FW), 340 in 3/4 sea water (3/4 SW), 250 in sea water (SW). For mullet in sea water it was very low: 35. The effects of neurohypophyseal peptides have been considered at these various salinities in the present work.

Fig.1 represents the effects on basal (or control) activity of both AVT and IT. All the ratios (test/basal) were below 1, indicating an inhibition of the adenylate cyclase activity. In addition, the maximum inhibition was obtained for 10^{–10}–10^{–12} M concentrations of the hormones, while the IC₅₀ was around 10^{–13} M. Strikingly, however, the inhibition was only evident in high salt media (40% for AVT, 30% for IT in trout; 50% with AVT or IT in mullet).

This inhibitory effect was also studied when the

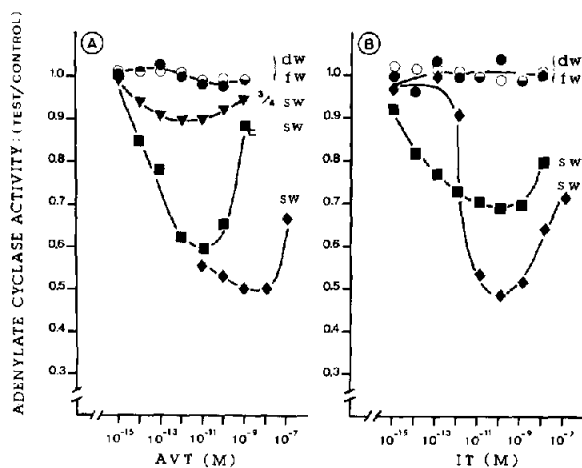


Fig.1. Effect of (A) AVT and (B) IT concentrations on basal adenylate cyclase activity in trout adapted to (○) DW, (●) FW, (▼) 3/4 SW, (■) SW and in (◆) SW-adapted mullet. Results are expressed as a ratio (test/control). SE less than 5% of means.

enzyme activity was pre-stimulated by the addition of 10^{-9} M glucagon.

Fig.2 illustrates the results in FW-adapted fish. The inhibition was now demonstrable in this medium since the stimulatory effect of glucagon was completely abolished by AVT and was reduced by 60% by IT.

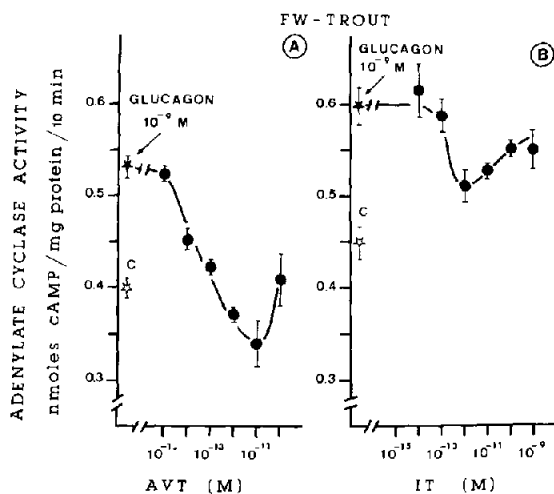


Fig.2. Effect of (A) AVT and (B) IT concentrations on 10^{-9} M glucagon-pre-stimulated adenylate cyclase activity in FW-adapted trout. Results are expressed as means $\pm$ SE ($n = 3$ separate experiments).

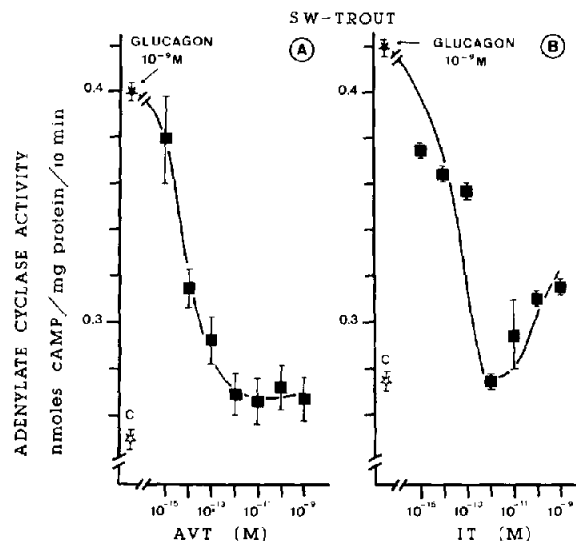


Fig.3. Effect of (A) AVT and (B) IT concentrations on 10^{-9} M glucagon-pre-stimulated adenylate cyclase activity in SW-adapted trout. Results are expressed as means $\pm$ SE ($n = 3$ separate experiments).

Fig.3 presents similar experiments in SW-adapted animals. Again, the glucagon-stimulated activity was completely inhibited when AVT or IT was present in the same concentration range as above.

The shape of the curves indicates that desensitization occurred when higher hormonal concentrations were used.

4. DISCUSSION

The present data show for the first time that AVT and IT at low concentrations (10^{-10} – 10^{-12} M) are capable of producing a large inhibition of adenylate cyclase activity in fish gills. These results are biologically meaningful since recent measurements of circulating AVT levels in trout by radioimmunoassay indicate that these concentrations are within the physiological range [14].

The inhibitory effect is therefore opposite to the well established stimulatory action of vasopressin on ion and water transporting epithelia of tetrapods, in which there is an activation of the adenylate cyclase-cyclic AMP system linked to the V_2 receptor type [1].

Reduction of cyclic AMP accumulation by vasopressin has been demonstrated in a number of

cellular types in mammals, e.g. rat hepatocytes [15], rat brain [16], rat cultured aortic smooth muscle [17], and human platelets [9]. It has also been shown in our laboratory [18] to occur in fish hepatocytes. This effect was obtained, however, with much higher doses of hormone (up to 10^{-6} M). It has been ascribed to Ca^{2+} mobilization and/or to phosphoinositide breakdown and has been associated with the V_1 receptor type [9,15,19–24]. However, circumstantial evidence suggests that the V_1 receptors may comprise 2 subtypes or more [25] and that a third class (V_3) of receptor may exist [16,26,27]. In addition, Morgan [15] has demonstrated in rat hepatocytes that the inhibition of cAMP accumulation was not solely the consequence of cellular Ca^{2+} mobilization but may also involve direct inhibition of adenylate cyclase.

Our experiments, performed on membrane fractions rather than intact cells, involved direct measurements of adenylate cyclase activity and Ca^{2+} intervention was ruled out in our assay system. Moreover, not only did neurohypophyseal peptides reduce or suppress the glucagon stimulatory effect, but the basal activity was also inhibited, a fact rarely shown previously to our knowledge (15–25% in platelets [9]). In other systems, vasopressin has been found only to prevent partially the cyclic AMP accumulation induced by glucagon (20% in hepatocytes [15] or by noradrenalin, dopamine and forskolin (50%, 45% and 40% in rat brain [16]).

In other experiments, to be reported in detail elsewhere, we made use of additional tools (pertussis toxin, guanine nucleotides) to investigate further the properties of the enzyme. Together with the present results, they strongly suggest that the neurohypophyseal receptors of fish gills are negatively coupled to the adenylate cyclase, presumably by way of G_i protein. Current investigations using agonist and antagonist analogues of vasopressin should enable us to define the receptor subtypes present in this tissue.

Another outcome of the present work is that the stability to which fish were acclimated had a large effect on the basal and hormone-sensitive enzyme activity. This confirms our previous observations concerning stimulation by isoproterenol, glucagon, VIP and NaF [11,12].

Since the gill epithelium is heterogeneous, it is

essential to elucidate the cellular type(s) concerned in this regulation. In consideration of existing histological observations however, we have suggested that the variations affecting the gill adenylate cyclase activity and its responses reflect changes in the 'chloride cell' population [12]. This interpretation should apply to the present study of neurohypophyseal hormone action.

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