

Atrial Natriuretic Peptide in Thermal Acclimation in the Catfish, *Silurus asotus*

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Responses of immunoreactive atrial natriuretic peptide (ir-ANP) to environmental temperature were studied in the freshwater catfish, *Silurus asotus*, acclimated at various temperatures for one week. According to increase in environmental temperature, plasma sodium and chloride concentrations, and osmolality were significantly increased, while hematocrit showed a marked decrease. When the catfishes were acclimated at cold (3°C) or warm (18°C) temperatures, ir-ANP contents in the atrium and bulbus arteriosus were significantly lower than those at 9°C. However, ventricular contents of ir-ANP were not different among the three groups. Levels of plasma ir-ANP in the catfish increased in response to the increment of acclimated temperature. On the basis of these results, we suggest that the environmental temperature may modulate synthesis and secretion of ir-ANP in the fish. We also suggest that changes of plasma ir-ANP levels may be associated with the control of body fluid homeostasis.

KEY WORDS: Catfish, ANP, Thermal acclimation

It is well known that naturally occurring stress from the change of environmental temperature in ectothermic animals elicits compensatory changes in morphology and/or physiology. During thermal acclimation of the fish, the thermal compensation appears to be physiological changes related to oxygen consumption (Eccles, 1985), locomotory ability (Loughna *et al.*, 1983), development (Wang *et al.*, 1985), cardiovascular function (Barron *et al.*, 1987; Farrell, 1984), and electrolyte composition (Houston and Koss, 1984; Kuhn *et al.*, 1986). Especially hormonal changes against thermal stress in ectothermic animals can be explained on the basis of an adaptation processes related with body fluid balance, metabolism, reproduction, and hibernation (Pancak and Taylor, 1982; Licht *et al.*, 1983; Jolivet-Jaudet *et al.*, 1984; Cho *et al.*, 1987; Kim *et al.*, 1987a).

Atrial natriuretic peptide (ANP), eliciting potent natriuresis and diuresis, has been isolated from mammalian atrial tissue and has been shown to be

implicated in the control of body fluid volume and vascular function. Recently immunoreactive atrial natriuretic peptide (ir-ANP) in the fish has been found and characterized by immunohistochemistry and radioimmunoassay (Hirohama *et al.*, 1988; Takei *et al.*, 1989, 1990; Kim *et al.*, 1989; Price *et al.*, 1990; Kim *et al.*, 1991). In several species of the fishes it was reported that this peptide has some biological functions associated with salt adaptation (Westenfelder *et al.*, 1988), renal function (Duff and Olson, 1986; Lee and Malvin, 1987; Benyajati and Yokota, 1990), gill hemodynamics (Evans *et al.*, 1989), and vascular resistance (Olson and Meisheri, 1989). Thus it was supported that physiological role of ir-ANP in the fishes was the regulation of extracellular fluid volume via its diuretic and natriuretic actions. Despite of important physiological functions of ANP, however, no attempt has been made to determine ir-ANP contents in the heart and plasma of the fish as well as other ectothermic animals under

conditions of changing temperature.

In the present study, we measured ir-ANP contents in the atrium, ventricle, bulbus arteriosus, and plasma of catfish in different thermal conditions.

Materials and Methods

Experimental animals

Sixty six freshwater Far Eastern Catfishes, 120-200 g, of the species *Silurus asotus* were obtained commercially. They were first held in an aquarium at 9°C under a simulated natural photoperiod condition for one week. To study the effect of temperature on the concentration of ir-ANP they were randomly divided into three groups, and acclimated in aquaria under the same condition except different temperatures (3, 9 and 18°C) for one week.

Preparation of tissues and plasma

To obtain blood and tissue, the fishes were anesthetized in 0.02% MS-222 (ethyl-*m*-aminobenzoate methanesulfonate). Blood were taken from the bulbus arteriosus with a heparinized polyethylene tubing and put into prechilled tubes containing ethylenediaminetetraacetic acid (EDTA, 1 mg/ml of blood), phenylmethylsulfonyl fluoride (PMSF, 0.4 mg %), soybean trypsin inhibitor (SBTI, 50 N α -benzoly-*L*-arginine ethyl ester/ml), and aprotinin (200 KIU/ml). Plasma was obtained after centrifugation at 10,000 g for 15 min at 4°C. After blood was obtained, the atria, ventricles and bulbus arteriosus were immediately removed, and put into cold 0.1 N acetic acid containing aprotinin (200 KIU/ml) and PMSF (0.4 mg %). Tissues were weighed and boiled for 10 min to inactivate proteolytic enzymes followed by homogenizing with a Polytron homogenizer. The homogenate was centrifuged at 10,000 g for 15 min at 4°C, and the supernatant was collected.

Radioimmunoassay of ir-ANP

Ir-ANP was extracted from the supernatants and plasma using Sep-Pak C18 cartridge (Waters Associates, Milford, MA, U.S.A.) as described previously (Cho *et al.*, 1988a). In brief, the supernatants and plasma were applied on a Sep-Pak C18 cartridge previously activated with 4 ml of

acetonitrile followed by 4 ml of 0.1% trifluoroacetic acid (TFA). The cartridge was washed with 4 ml of 0.1% TFA, and the adsorbed peptide was eluted with 3 ml of 60% acetonitrile in 0.1% TFA. The eluants were subsequently lyophilized, and stored at -70°C until assayed.

Ir-ANP was measured by radioimmunoassay as described previously (Cho *et al.*, 1988a, b, c). The lyophilized samples were reconstituted with 100 μ l of Tris-acetate buffer (pH 7.4) and incubated with antibody for 24 hr at 4°C. Following the additional incubation with I¹²⁵-ANP for 24 hr at 4°C, the bound form was separated from the free form by second antibody.

Analytical methods

Sodium and potassium concentrations in plasma were determined by flame photometry, and chloride concentration was measured by chloridometer. Plasma osmolality was measured by osmometer using freezing point depression. Hematocrit was measured by a microhematocrit procedure.

Statistical analysis

All results were expressed as the mean \pm SE. Statistical comparisons were made using Student's *t*-test. Values of $p < 0.05$ were considered significant.

Results

Changes in plasma electrolyte concentration, osmolality and hematocrit

In the catfish, levels of plasma electrolyte, osmolality and hematocrit were shown in Table 1. At 9°C, plasma sodium, potassium, and chloride concentrations were 133.83 ± 0.83 , 3.27 ± 0.18 , and 115.67 ± 1.63 mEq/L, respectively. When the catfish was acclimated at various temperatures, plasma sodium and chloride concentrations decreased significantly at cold (3°C), and slightly increased at warm (18°C) temperature. Plasma osmolality of the catfish acclimated at 3, 9, and 18°C were 256.29 ± 2.04 , 271.67 ± 2.55 , and 277.67 ± 0.61 mOsm/kg, respectively. A positive relationship between plasma osmolality and sodium concentration in the catfish was observed ($p < 0.001$) (Fig. 1). According to increment of acclimated temperature, hematocrit signi-

Table 1. Effects of environmental temperature on plasma electrolyte concentrations, osmolality and hematocrit in the catfish.

Parameter	Temperature (°C)		
	3	9	18
sodium (mEq/L)	124.00 ± 1.49**	133.83 ± 0.83	137.00 ± 1.37
potassium (mEq/L)	2.43 ± 0.17**	3.27 ± 0.18	2.03 ± 0.11**
chloride (mEq/L)	100.14 ± 0.83**	115.67 ± 1.63	118.83 ± 2.46
osmolality (mOsm/kg)	256.29 ± 2.04**	271.67 ± 2.55	277.67 ± 0.61*
hematocrit (%)	29.81 ± 1.43*	23.63 ± 1.62	22.38 ± 1.31

Values are the mean ± SEM (n = 7). Asterisks indicate significant difference from values obtained the catfish acclimated at 9°C (*p < 0.05 and **p < 0.01).

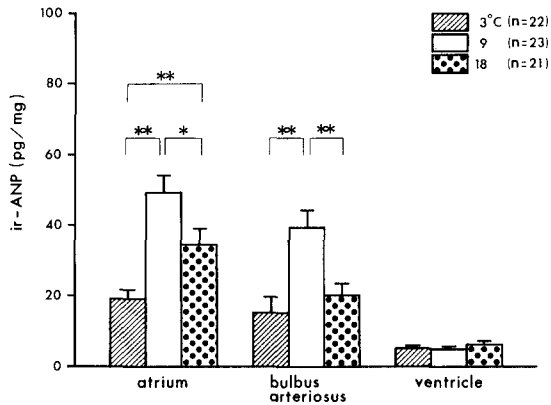


Fig. 1. Relationship between plasma sodium concentration and plasma osmolality at 3 (○), 9 (●) and 18°C (●). The regression equation for osmolality (y) against sodium concentration (x) was $y = 74.00 + 1.48x$ ($r = 0.9249$).

ificantly decreased ($p < 0.05$).

Changes of ir-ANP contents in tissues

In the catfish, ir-ANP was found to exist in the bulbus arteriosus as well as in the atria and ventricles.

Under different thermal conditions, the changes in ir-ANP contents of the atrium, bulbus arteriosus and ventricle in the catfish were presented in Fig. 2. At 9°C, ir-ANP contents of the atrium, bulbus arteriosus, and ventricle were 49.31 ± 4.82 , 39.50 ± 4.79 , and 4.80 ± 0.47 pg/mg, respectively. When the catfish was acclimated at cold temperature (3°C) ir-ANP contents in the atrium were 19.05 ± 2.55 pg/mg, which was significantly lower ($p < 0.01$) than at 9°C. The contents of ir-ANP in the bulbus arteriosus at cold tempera-

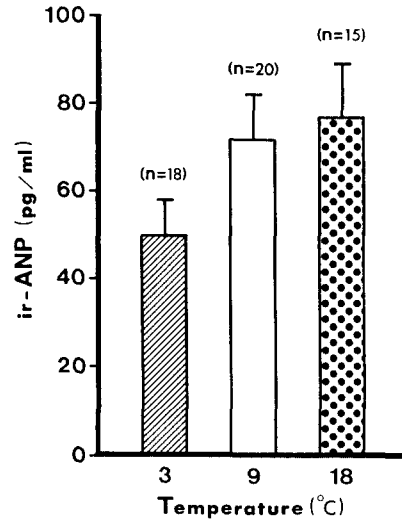


Fig. 2. Effects of changes in environmental temperature on the levels of immunoreactive atrial natriuretic peptide in atrium, bulbus arteriosus and ventricle of the catfish. *p < 0.05; **p < 0.01.

ture was 14.86 ± 4.94 pg/mg, and was also significantly lower than that at 9°C ($p < 0.01$). The ventricular ir-ANP contents at 3°C, however, was not different from that at 9°C. When the catfish was acclimated at warm temperature (18°C), ir-ANP contents in the atrial, bulbus arteriosus and ventricular tissues were 34.50 ± 4.78 , 20.02 ± 3.47 and 6.59 ± 0.93 pg/mg, respectively. Ir-ANP contents in the atria and bulbus arteriosus at 18°C were significantly lower than those at 9°C, while ir-ANP content of ventricular tissues made no difference among the two groups (9 and 18°C).

Changes of ir-ANP content in the plasma

Ir-ANP content of plasma in the catfish acclimated at 3, 9, and 18°C were 49.95 ± 8.79 , 71.08 ± 11.26 , and 77.23 ± 12.72 pg/ml, respectively (Fig. 3). Ir-ANP concentrations in plasma at 18°C were about one and half fold higher than that at cold (3°C) temperature.

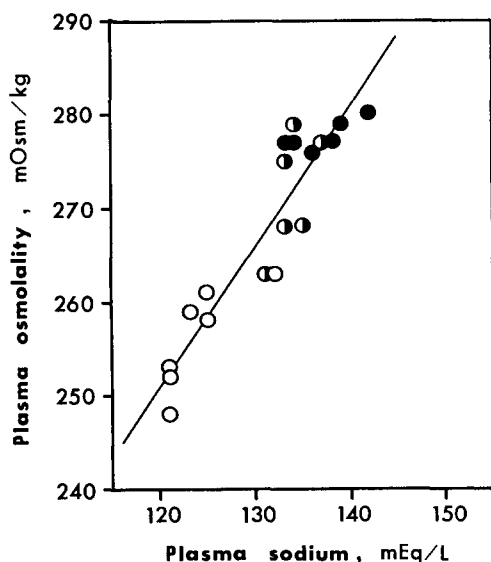


Fig. 3. Effects of changes in environmental temperature on the levels of plasma immunoreactive atrial natriuretic peptide in the catfish.

Discussion

To the best of our knowledge, this is the first study to examine the possible role of environmental temperature in the regulation of ANP biosynthesis and secretion in the ectothermic animals.

Environmental temperature is known to play an important role in the control of endocrine function in the ectothermic animals including the fish. It has been reported that in the ectotherms the synthetic and secretory responses of endocrine organs were changed by temperature, and the endocrine system has a seasonal variation (Balletto *et al.*, 1979; Porter and Licht, 1986; Kim *et al.*, 1987a; Licht *et al.*, 1989). Although the heart is now well known to be a new endocrine organ to synthesize, store and secrete the ANP, a hormone

that plays an important role in salt and body fluid homeostasis, no attempt has been made to characterize the temperature regulation of this hormone in ectotherms as well as endotherms.

It is well known that the cardiac atrium is the major site of ANP synthesis and secretion in the fishes (Chapeau *et al.*, 1985; Kim *et al.*, 1989; Takei *et al.*, 1989). Hirohama *et al.* (1988) have indicated the specific granules in ventricular cardiocytes to be sites for the storage of ir-ANP in the fishes. And it has been reported that fish ventricular cells secreted substantial quantities of ir-ANP (Baranowski and Westenfelder, 1989). Unlike normal mammalian ventricular cells, it indicates the ventricles of some lower vertebrates have the capacity to secrete ir-ANP. In several freshwater teleostean fishes, we have recently found that the bulbus arteriosus, a specialized structure for damping system of the pressure oscillation, has ANP with high as well as low molecular weight forms, and contains amounts of ANP comparable to that of atrial tissues (Kim *et al.*, 1991). Therefore, we believe that in teleostean fishes the bulbus arteriosus may act as an extracardiac site for ANP synthesis and secretion.

In the present study, we found that in the catfish, *Silurus asotus*, ir-ANP contents of heart tissues were changed by various environmental temperatures maintained for one week. Average contents of ir-ANP in the atrium and bulbus arteriosus at 9°C were significantly higher than those at 3 and 18°C. Ir-ANP contents of plasma at cold temperature (3°C) were lower than those at 9°C. Compared with plasma ir-ANP levels at 9°C, however, plasma levels of ir-ANP in the catfish acclimated at 18°C were slightly increased although average contents of ir-ANP in atrium and bulbus arteriosus were significantly lower than those of 9°C. Hormonal changes induced by environmental temperature have been reported in different species. In goldfish, the gonadotropin-releasing hormone content in the hypothalamus was higher at warm temperature than at cold one (Yu *et al.*, 1987). According to Delgado and Vivien-Roels (1989), a decrease of environmental temperature abolished the melatonin rhythm in the frog, while high temperature induced an increment of melatonin levels in the plasma. In reptiles, ACTH-induced stimulation of corticosterone and

aldosterone release was significantly reduced during hibernation (Dauphin-Villemant *et al.*, 1990). In ectotherms, therefore, it seems that environmental temperature may be an important factor affecting hormonal synthesis and/or secretion. It has been suggested by Licht *et al.* (1989) that reduced temperature may have major effects on processes requiring synthesis rather than on release of stored hormone in endocrine system of reptiles. In the cold-acclimated catfish, the decrease of ir-ANP contents in the atrium and bulbus arteriosus may be elicited by the suppression of processes for ir-ANP synthesis. The low levels of plasma ir-ANP may be due to suppression of ir-ANP synthesis. In the catfish acclimated at 18°C, however, the increase of ir-ANP levels in plasma may be deciphered as the reflection of the active release rather than the poor synthesis of this peptide. In contrast, the ventricular levels of ir-ANP showed no differences in response to temperature changes. It is, therefore, suggested that the temperature sensitivity of the ventricular tissue may be different from the other cardiac tissues in the catfish.

We have also observed that the environmental temperature may have profound effects on the levels of plasma electrolytes and osmolality of the catfish in this study. Both plasma sodium concentration and osmolality increased gradually with the

increment of acclimation temperature. These data are consistent with previous reports in freshwater fishes (Umminger, 1971; Catlett and Millich, 1976; Kim *et al.*, 1987b). Our results strongly suggest that the changes in plasma osmolality may be correlated directly with those of sodium concentrations, and also showed changes of hematocrit characterized by an increase in cold and decrease in warm temperature. Baustian (1988) reported that in the toad the decrement of hematocrit indicates a steady increase of blood volume caused by the input of lymphatic fluid. Thus, the variations of hematocrit may be associated with the changes in the extracellular fluid volume although the exact mechanisms are not clear.

As shown in Fig. 4, elevated concentrations of ir-ANP in the plasma of catfish acclimated at increasing temperatures are closely related with the concentration of plasma sodium and osmolality. Although a number of factors for the regulation of ANP synthesis and secretion in various animal groups were suggested, few reports are available in the fishes. Westenfelder *et al.* (1988) have reported that in the fishes ANP levels rise in parallel with water salinity, and they have suggested that ANP may be a mediator of salt tolerance in teleostean fish.

This study suggests that the environmental temperature may modulate ANP synthesis and

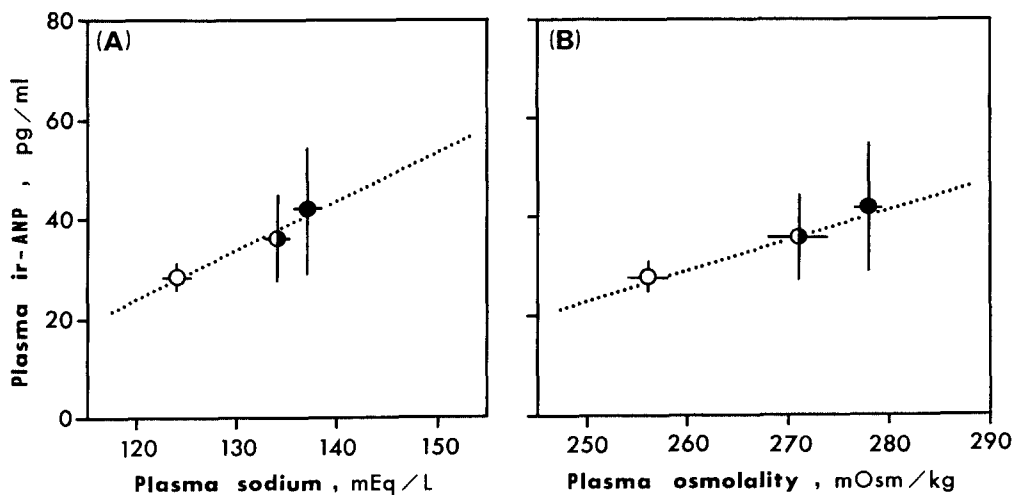


Fig. 4. (A) Relationship between concentration of plasma sodium and plasma ir-ANP concentration, and (B) relationship between plasma osmolality and plasma ir-ANP concentration at various temperature (○, 3°C; ◐, 9°C; ●, 18°C).

secretion in the cardiac tissues of the catfish. Additionally under different thermal conditions, changes in the plasma ANP levels may be related with the extracellular fluid homeostasis, although the mechanisms are not clear.

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메기(*Silurus asotus*)에서 수온의 변화에 의한 Atrial Natriuretic Peptide의 변동

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변온동물에서 주변 환경의 온도 변화에 따른 체액 조절기전을 구명하고자 하여, 메기에서 일주일 동안 수온의 변화를 가했을 때 체액조절 호르몬의 일종인 immunoreactive atrial natriuretic peptide(ir-ANP) 및 혈장 전해질과 삼투질 농도의 변동을 관찰하였다. 메기의 혈장 sodium과 chloride 및 삼투질 농도는 수온의 증가에 비례하여 유의하여 증가하였다. 반면에 hematocrit는 현저하게 감소하였다. 수온 3°C와 18°C에 노출된 메기의 심방 및 동맥구 조직내의 ir-ANP 농도는 9°C의 수온에 노출된 실험군에 비해 유의하게 낮게 측정되었으며, 혈장내 ir-ANP 농도는 수온의 증가에 비례하여 증가하였다. 따라서 수온의 변동은 메기의 ir-ANP의 합성과 분비에 영향을 미치고 있으며, 이러한 변화는 메기의 체액조절에 관여하리라 추정된다.