

CENTRAL MONOAMINERGIC RESPONSES TO SALINITY AND TEMPERATURE RISES IN COMMON CARP

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Summary

Juvenile common carp, *Cyprinus carpio*, were exposed to increased levels of salinity (1 % NaCl) at 25 °C and 30 °C. Levels of the monoamine neurotransmitters dopamine (DA) and serotonin (5-HT) and their metabolites dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid were determined in different brain parts. Whereas the elevated temperature only resulted in higher levels of the metabolites, increased salinity caused increased levels of DA and 5-HT as well. Increased levels appeared after the first day of exposure and most effects were further

enhanced after 1 week in 1 % NaCl. Increases in DA and 5-HT levels were most pronounced in the hypothalamus, which is the major integrative centre controlling the release of hormones. Thus, one possible role of these changes in neurotransmitter metabolism could be to control the release of prolactin and cortisol, two major hormones involved in the regulation of ion homeostasis in teleosts.

Key words: common carp, *Cyprinus carpio*, temperature, salinity, serotonin, dopamine.

Introduction

Although teleosts are predominantly stenohaline fish, physiological responses to increased salinity in fish have mainly been studied in anadromous species such as eels, salmonids and tilapia, while comparatively few studies have focused on stenohaline fish (Balment *et al.* 1987). In some areas, however, stenohaline fish might be challenged with increasing salinity due to desertification or superfluous irrigation programmes. The common carp (*Cyprinus carpio*) is a well-known example of a stenohaline fish (Gupta and Hanke, 1982) and, although it displays some tolerance to salinity (Kulijev and Agayarova, 1984; Schildhauer *et al.* 1992) and diluted sea water (0.03–0.30 % salinity) has been reported to enhance survival, growth and development of carp larvae (Lam and Sharma, 1985), exposure to higher levels of salinity appears to have adverse effects on carp. Exposure to diluted artificial sea water (1.5 % salinity) results in elevated levels of glucose and cortisol in the blood (Abo Hegab and Hanke, 1984) and increased plasma osmolarity and ion levels (Abo Hegab and Hanke, 1982) in juvenile common carp. In carp larvae, exposure to diluted sea water showed clear unfavourable effects at 1 % salinity or higher, with lower growth rates and increased mortality (Schildhauer, 1983). Earlier experiments in our laboratory showed that juvenile carp could survive a sudden salinity change from 0 to 1 % NaCl for several weeks, whereas they all died within 24 h when transferred to 1.1 % NaCl. Long-term exposure (>3 weeks) to

1 % NaCl at both 25 °C and 30 °C, however, stopped growth and at 30 °C susceptibility to bacterial infections also increased (G. De Boeck, unpublished results). The narrow range of salinity between the iso-osmotic value of 0.9 % NaCl where fish are submitted to a minimal osmotic challenge and 1.1 % NaCl where death occurs appears to be critical in the osmoregulation of common carp.

The brain is likely to play a coordinating role in the physiological responses to changes in ambient salinity. Neural control of the osmoregulatory processes is at present poorly understood, although both serotonin (5-HT) and dopamine (DA) have been suggested to be involved in neurally mediated adaptation to changes in osmotic conditions (Mazeaud *et al.* 1985; Abo Hegab and Hanke, 1981). The monoamine neurotransmitters 5-HT and DA probably regulate the release of a number of hypothalamic–hypophyseal hormones, including prolactin, which is thought to control salt retention in teleost fish (see Wendelaar Bonga, 1993, for a review). In tilapia, *Tilapia mossambica*, 5-HT has been found to stimulate, and DA to inhibit, prolactin secretion *in vitro* (Nagahama *et al.* 1975; Grau and Helms, 1990). The release of cortisol, which plays a more important role for life in hyperosmotic salt water (Henderson and Garland, 1980) is, at least in mammals, partially under serotonergic control (see Chaouloff, 1993, for a review).

However, only two studies on the effects of environmental

salinity changes on brain monoamine neurotransmitter systems in fish are known to us. Mazeaud *et al.* (1985) found that transferring rainbow trout *Salmo gairdneri* to artificial sea water resulted in a fall in the levels of 5-hydroxyindoleacetic acid (5-HIAA), the main serotonin (5-HT) metabolite, suggesting a decrease in the activity of the 5-HT system. In carp, *Cyprinus carpio*, and tilapia, *Sarotherodon mossambicus*, hypersalinity exposure (diluted artificial sea water, 1.5 ‰ salinity) has been reported to cause a fall in DA levels in the hypothalamus (Abo Hegab and Hanke, 1981), results that are more difficult to interpret since levels of DA metabolites were not measured.

Thus, the aim of the present study was to elucidate the effect of salinity stress on monoamine neurotransmitter metabolism. The following hypotheses were tested: (1) is 5-HT metabolism stimulated (possibly in order to stimulate cortisol secretion) or depressed (because stimulation of prolactin secretion becomes superfluous); and (2) is DA metabolism stimulated (in order to inhibit prolactin secretion)? Additionally, the effect of thermal stress on the levels of these neurotransmitters was tested since thermal stress has, besides general effects on metabolic and monoaminergic processes, the capacity to disturb osmotic and ionic regulation (Elliott, 1981). Therefore, the effects of increased salinity were studied at two temperatures: the first being within the optimal growth range of carp (25 °C), and the second being above the optimal growth range, and at which carp are clearly stressed but still feed (30 °C). Hence, the effects of short-term salinity exposure (1 day and 1 week) and temperature on the levels of 5-HT, DA and their main metabolites 5-HIAA and dihydroxyphenylacetic acid (DOPAC) were determined in different brain areas (telencephalon, hypothalamus and brain stem) in common carp.

Materials and methods

Animal holding and salinity exposure conditions

Juvenile (1-month-old) common carp, *Cyprinus carpio* L., were obtained from the fish hatchery at the Agricultural University of Wageningen, The Netherlands. They were raised at the University of Antwerp at the optimal temperature of 25 °C (Elliott, 1981) in softened Antwerp city tap water (Ca^{2+} , 0.87 mmol l⁻¹; Mg^{2+} , 0.15 mmol l⁻¹; Na^+ , 1.38 mmol l⁻¹; K^+ , 0.06 mmol l⁻¹, pH 7.0–8.0). Water was filtered using a trickling filter and water quality was checked weekly using Visicolor test kits (Macherey-Nagel, Düren) for ammonia, nitrite and nitrate levels. 50 % of the water was changed when levels exceeded 0.1 mg l⁻¹, 1.0 mg l⁻¹ or 20 mg l⁻¹, respectively.

Fish were transferred to the standardised laboratory conditions 2 weeks before starting the experiments. Two groups of 24 carp weighing 18–23 g were transferred into two 150 l aquaria filled with standard, moderately hard fresh water (FW) composed according to standard methods (American Public Health Association, 1989) of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.35 mmol l⁻¹; MgSO_4 , 0.50 mmol l⁻¹; NaHCO_3 , 1.14 mmol l⁻¹; KCl , 0.05 mmol l⁻¹ (pH 7.8–8.0). The FW was well aerated for at least 24 h before use. The photoperiod was

set at 14 h:10 h L:D and during the first week one group was slowly adapted to 30 °C (at a 1 °C increase per day) while the other group remained at 25 °C. Carp were fed until satiation once a day (1 h after the light period started) with 'Pond Sticks' (Tetrapond, Henckel). Water was filtered using trickling filters filled with activated charcoal (Calgon Carbon) and Rivalon synthetic filter wadding. Water quality was checked daily for pH, ammonia, nitrite and nitrate levels, and 50 % of the water was changed twice a week. While following this procedure, levels of excretory products never exceeded the levels noted above and pH was 7.7 ± 0.1 (mean \pm 1 S.D.) for all groups.

After 2 weeks in the experimental tanks, salinity exposure was started by replacing 90 % of the water with standard fresh water containing 10 g l⁻¹ NaCl (SW, 1 ‰ salinity) and adding the appropriate amount of NaCl to adjust for the water that was not replaced. Water from the control group was replaced in the same way with FW. Water quality was checked daily for pH, ammonia, nitrite and nitrate levels and 50 % of the water was changed twice a week.

Tissue sampling

Fish were transferred to the experimental aquaria 2 weeks before salinity exposure began. As for previous studies (Winberg and Nilsson, 1992, 1993a; Winberg *et al.* 1993) in which a period of 1 week of acclimation was considered sufficient for the fish to recover from the transfer from the rearing tank to the experimental aquaria, brain tissue of eight fish was sampled 1 day and 1 week after exposure to salinity began for both groups. Brain tissue of eight fish in each group was also sampled the day before exposure to salinity began; any disturbance that this caused to the remaining fish was considered to be minor compared with the effects of salinity exposure. On the day the brain tissue was sampled, fish were not fed in the morning and sampling started at their normal feeding time (1 h after the light period started). Each fish was killed by decapitation. The brain (excluding the olfactory bulbs) was rapidly removed and dissected into three parts, telencephalon, hypothalamus and brain stem (i.e. the remaining parts of the brain). The mean mass (\pm 1 S.D., $N=48$) of each brain region was: telencephalon, 0.0223 ± 0.0018 g; hypothalamus, 0.0231 ± 0.0023 g; brain stem, 0.1675 ± 0.0113 g. The brain regions were frozen in liquid nitrogen within 1 min of decapitation and stored at -80 °C. Remaining fish were fed after samples had been taken.

HPLC assay of monoamines

After being weighed, the frozen brain samples were sonicated or homogenized at 0 °C in 0.2–1.2 ml of 4 % (w/v) ice-cold perchloric acid containing 2 mg ml⁻¹ EDTA, 0.5 mg ml⁻¹ sodium bisulphite and 40 ng ml⁻¹ epinine (deoxyepinephrine, the internal standard) using an MSE 100 W ultrasonic disintegrator (for telencephalon and hypothalamus samples) or a Potter–Elvehjem homogenizer (for brain stem samples). The amounts of monoamines present in 100 µl samples of the supernatants obtained after centrifugation (14 000 g for 10 min at 4 °C) were quantified using reversed-

phase ion-pair high-performance liquid chromatography (HPLC) with electrochemical detection as described by Nilsson (1989). Briefly, the HPLC system consisted of a 6000 A solvent delivery system and a U6K injector (both from Waters Associates Inc., Milford, MA, USA), a reverse-phase column (4.6 mm×125 mm, Nucleosil 120, C18, 3 µm, from Macherey-Nagel, Düren, Germany) kept at 40 °C, and an LC-3 electrochemical detector with a glassy carbon working electrode (which was set at +750 mV) and a Ag/AgCl reference electrode (all from Bioanalytical Systems, West Lafayette, IN, USA). The flow rate was 1.1 ml min⁻¹ and the mobile phase consisted of 100 mmol l⁻¹ NaH₂PO₄, 0.2 mmol l⁻¹ EDTA, 0.63 mmol l⁻¹ sodium octylsulphate and 9% (v/v) methanol, pH 3.6. Monoamines, their metabolites and epinine used for HPLC standards were obtained from Sigma Chemicals (St Louis, MO, USA). The monoamine contents are given in relation to the wet mass of the tissues.

Statistics

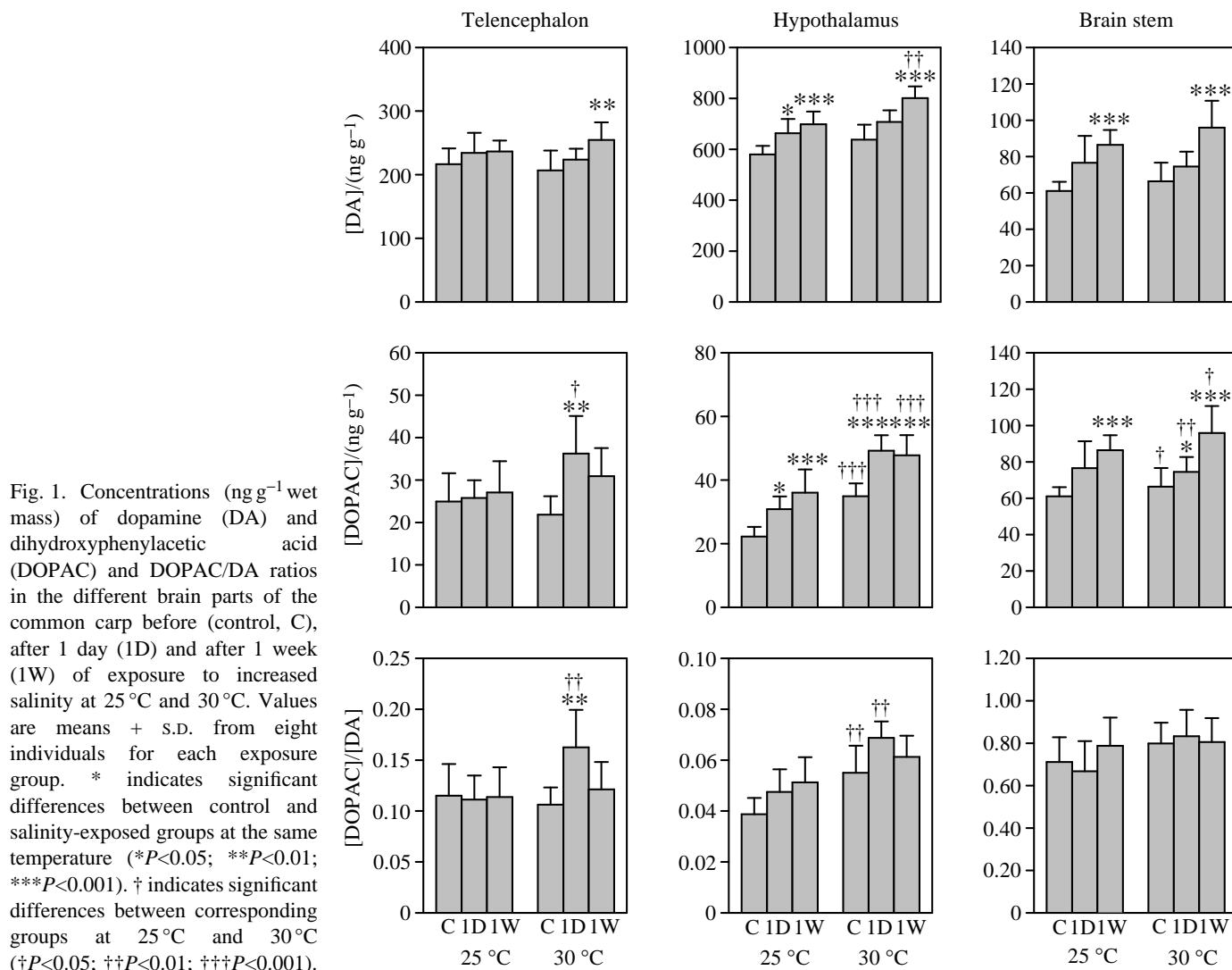
All values are given as means ± S.D. Statistics were

performed with GraphPad InStat. One-way analysis of variance (ANOVA) was used, followed by Tukey–Kramer multiple comparisons tests if significant differences ($P < 0.05$) were indicated by the ANOVA.

Results

Effect of temperature on dopamine levels

Elevating the temperature of carp kept in FW (controls) from 25 °C to 30 °C resulted in significantly higher levels of DOPAC in the hypothalamus, with an increase of 57% ($P < 0.001$), and also in the brain stem (21% increase, $P < 0.05$), while DA concentrations remained unchanged (Fig. 1). Consequently, a 42% increase in the DOPAC/DA ratio was seen in the hypothalamus ($P < 0.01$) of the 30 °C FW group. The DA metabolite homovanillic acid was found to be below the detection limit (10 ng g⁻¹) of the HPLC system. The effects of temperature on the dopaminergic systems appeared to be superimposed on the changes induced by exposure to SW.



Effect of saltwater-exposure on hypothalamic dopamine levels

At 25 °C, hypothalamic DA and DOPAC levels were increased by 14 and 39 %, respectively, after 24 h of SW-exposure ($P<0.05$), and after 1 week this effect was even more pronounced (20 and 62 %, respectively, $P<0.001$) (Fig. 1). At 30 °C, the rise in the DA concentration (11 %) was not significant after the first day in SW, but was significant after 1 week (25 %, $P<0.001$), also being significantly higher than the value after 1 week at 25 °C ($P<0.01$). The higher temperature seemed to enhance the effect of SW on DA turnover, since the increase in levels of the DA metabolite DOPAC was significant after 24 h (41 %, $P<0.001$) and appeared to have reached a maximum level. At 30 °C, DOPAC levels were higher than levels at 25 °C ($P<0.001$) for the whole exposure period. Since both DA and DOPAC concentrations increased simultaneously, the DOPAC/DA ratio displayed no significant changes in response to SW-exposure, although a general tendency towards an increase in these ratios was seen in the hypothalamus, especially at 25 °C.

Effect of saltwater-exposure on brain stem dopamine levels

In brain stem samples, DA levels were significantly increased after 1 week ($P<0.001$) of SW-exposure at both temperatures, with increases of 42 and 45 %, respectively (Fig. 1). At 25 °C, the 55 % rise in DOPAC levels was significant after 1 week ($P<0.001$), while at 30 °C, a significant difference was found after 24 h ($P<0.05$), the concentration of DOPAC having risen by 18 %; this increase was even more pronounced after 1 week (45 %, $P<0.001$). No changes were found in the DOPAC/DA ratio.

Effect of saltwater-exposure on telencephalic dopamine levels

SW-exposure appeared to affect the dopaminergic system in the telencephalon less than in the other brain parts (Fig. 1). However, after 1 week at 30 °C, a 23 % rise in DA concentration was seen ($P<0.01$) and DOPAC levels had increased after 24 h at 30 °C (41 %, $P<0.01$), which resulted in an elevated DOPAC/DA ratio (53 %, $P<0.01$).

Effect of temperature on 5-HT levels

The serotonergic systems appeared to be activated by a higher temperature in a similar way to the DA systems, since 5-HIAA levels in the freshwater group (controls) were elevated by 29 % in the hypothalamus ($P<0.001$) and by 33 % in the brain stem ($P<0.05$) (Fig. 2). For the brain stem, this resulted in a 25 % higher 5-HIAA/5-HT ratio ($P<0.001$). Moreover, as for DA, these effects of temperature on the serotonergic systems tended to persist during SW-exposure, although no effects of temperature were seen in the telencephalon of the freshwater group.

Effect of saltwater-exposure on hypothalamic 5-HT levels

SW-exposure induced an increase in the hypothalamic levels of both 5-HT and 5-HIAA (Fig. 2). The level of 5-HT was

significantly increased by 26 % after 24 h of SW-exposure at 25 °C ($P<0.01$) and remained high after 1 week of exposure ($P<0.001$). At 30 °C, the increase (37 %) was only significant after 1 week ($P<0.001$). However, in contrast to the DA metabolite, which already showed elevated levels after 24 h, increased 5-HIAA levels were only seen after 1 week of exposure to 1 % salinity with a 35 % increase at 25 °C ($P<0.001$) and a 29 % increase at 30 °C ($P<0.001$).

Effect of saltwater-exposure on brain stem 5-HT levels

In brain stem samples, a 35 % elevation of the 5-HIAA level could be seen after 1 week of SW-exposure at 25 °C ($P<0.01$), while no changes were observed in 5-HT levels (Fig. 2). This resulted in a 22 % higher 5-HIAA/5-HT ratio after 1 week of SW-exposure at 25 °C.

Effect of saltwater-exposure on telencephalic 5-HT levels

Unlike DA, telencephalic 5-HT showed significant changes at the lower temperature (Fig. 2). Thus, at 25 °C, elevated levels of 5-HT were observed after 24 h (a 30 % increase, $P<0.01$), and they remained high after 1 week ($P<0.01$). At 30 °C, the rise was only significant after 1 week (36 %, $P<0.001$). Because the 5-HIAA level remained unchanged in response to SW, a general tendency towards a reduction in the 5-HIAA/5-HT ratio was seen: a significant 39 % reduction after 24 h in SW at 25 °C ($P<0.001$) and a 26 % decrease ($P<0.05$) after 1 week of exposure to SW at 30 °C.

Discussion

As expected, the fish held at the higher temperature displayed a general elevation of the levels of the monoamine metabolites, suggesting a higher level of activity of DA and 5-HT metabolism. The most substantial change occurred in the DOPAC levels in the hypothalamus, the brain region thought to be involved in temperature selection (Smith, 1984). Previous studies on fish brain have shown that monoamine oxidase (MAO) activity is temperature-dependent with a Q_{10} of 2 (Olscese and De Vlaming, 1979, 1980; Hall *et al.* 1982; Khan and Joy, 1990). Changes in DA and 5-HT levels in those studies were, however, not consistent and depended on the combination of temperature and photoperiod.

A temperature of 30 °C is clearly above the optimal temperature for carp (Elliott, 1981) and it is therefore likely that this was a stressful situation. However, the temperature rise reinforced the effects of salinity stress on the levels of DOPAC in the hypothalamus and brain stem. At this higher exposure temperature, significant changes occurred after only 24 h of SW-exposure instead of after 1 week at 25 °C. It is possible that the fish had already acclimated to 30 °C before salinity-exposure began, since elevation of the temperature was carried out gradually, starting 2 weeks before SW-exposure. Therefore, it appears likely that the increases in DOPAC and 5-HIAA levels should be seen as responses to elevated temperature, reflecting Q_{10} effects rather than stress.

When examining the effects of salinity on monoamine

neurotransmitters in the teleost brain, the most pronounced effects can be expected in the hypothalamus, since it is the major integrative centre controlling the release of a whole range of hormones. Several hormones are involved in the regulation of both salt and water balance in teleost fish, including growth hormone, prolactin and cortisol (Wendelaar Bonga, 1993). Prolactin is usually considered to promote the mechanisms required for the fish to achieve osmoregulatory homeostasis when in fresh water. In fact, while prolactin-producing cells appear very early in the embryonic development of freshwater teleosts, the development of similar cells in seawater teleosts is delayed until several days after hatching (Jobling, 1995). The major function of prolactin is in the retention of ions, particularly in the gills but also in the epithelia of the skin, the intestine, the renal tubules and the bladder, at a level required for survival in water of low salinity (Wendelaar Bonga, 1993). In mammals (Brownstein, 1987) as well as in fish (Nishioka *et al.* 1988), DA is a potent inhibitor of prolactin secretion, and it has been suggested that DA is the prolactin-release-inhibiting factor. The results of our

experiments indicate a considerable increase in DA metabolism after exposure to SW, particularly in the hypothalamus. After 1 week of salinity stress at 25 °C, the DOPAC level had increased by 62 % in the hypothalamus. Thus, it is possible that when the carp were transferred from FW to SW, increased DA activity played a role in the osmoregulatory response by down-regulation of prolactin release, an effect possibly more potent than the stimulatory effect of 5-HT on prolactin release.

In contrast to prolactin, growth hormone and cortisol are the main osmoregulatory hormones under saline conditions and the control of ion-regulatory processes is presumably to a great extent dependent on corticosteroids in virtually all teleost fishes (Henderson and Kine, 1987). Whereas growth hormone stimulates chloride cell development and Na⁺/K⁺-ATPase activity in gills (Madsen, 1990), cortisol evokes a broad range of stress responses, including hyperglycaemia, through a stimulation of glycolysis and gluconeogenesis from protein and lipid sources (Wendelaar Bonga, 1993). When Abo Hegab and Hanke (1984) exposed common carp to sea water (1.5 %

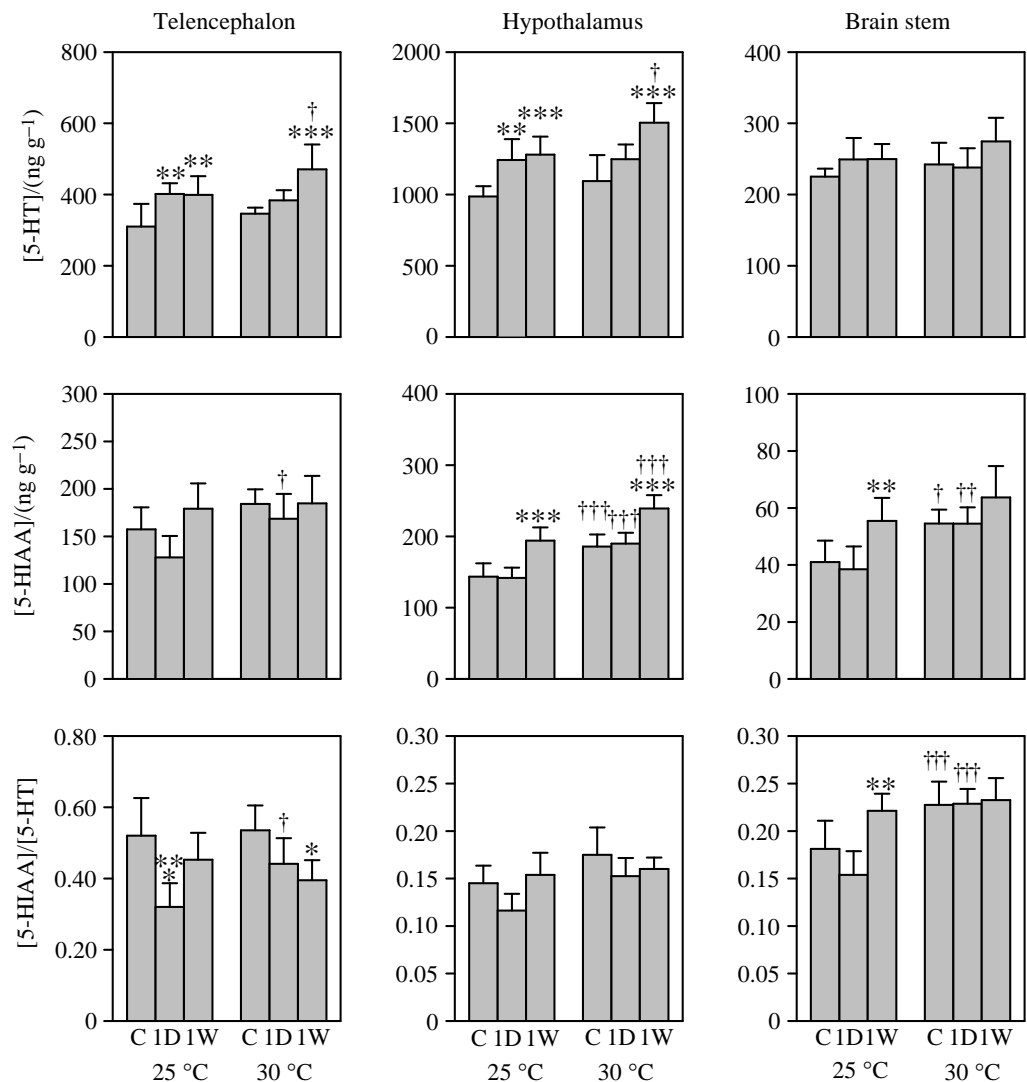


Fig. 2. Concentrations (ng g⁻¹ wet mass) of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) and 5-HIAA/5-HT ratios in the different brain parts of the common carp before (control, C), after 1 day (1D) and after 1 week (1W) of exposure to increased salinity at 25 °C and 30 °C. Values are means + S.D. from eight individuals for each exposure group. * indicates significant differences between control and salinity-exposed groups at the same temperature (**P* < 0.05; ***P* < 0.01; ****P* < 0.001). † indicates significant differences between corresponding groups at 25 °C and 30 °C (†*P* < 0.05; ††*P* < 0.01; †††*P* < 0.001).

salinity), they did indeed find elevated levels of glucose and cortisol which lasted for several days. When exposing another cyprinid fish, the goldfish *Carassius auratus*, to saline stress, Singley and Chavin (1975) observed elevated levels of cortisol as well as of the adrenocorticotrophic hormone (ACTH).

The release of cortisol in fish, as in other vertebrates, is controlled by ACTH, which itself is activated by the corticotropin releasing factor (CRF) (Brownstein, 1987). In mammals, 5-HT has been shown to increase hypothalamic CRF release in a dose-dependent way, and evidence exists for a direct interaction between 5-HT and ACTH, as a number of pharmacological studies indicate that 5-HT receptors may directly control ACTH release from the pituitary (see Chaouloff, 1993, for a review). Our experiments reveal an increased rate of 5-HT metabolism following salinity exposure, again especially in the hypothalamus where 5-HIAA levels were increased by 35 % after 1 week. This makes it tempting to speculate that there may also be a serotonergic control of cortisol release in fish.

Besides the changes seen in the hypothalamus, a considerable rise in the 5-HT content of the telencephalon also occurred after exposure to SW. As the major function of the telencephalon in fish is thought to be related to olfaction (Smith, 1984), it seems possible that this elevation is correlated with olfactory detection of the changing salinity conditions.

In spite of the apparent elevation of the rates of DA and 5-HT metabolism, indicating high conversion rates of DA to DOPAC and 5-HT to 5-HIAA, the carp were able to maintain and even increase their levels of DA and 5-HT in all brain parts, suggesting that an up-regulation of synthesis paralleled the increased degradation of the monoamines. Because of this up-regulation, the DOPAC/DA and 5-HIAA/5-HT ratios did not show any consistent significant increases, although there was a tendency for the hypothalamic DOPAC/DA ratio to increase with salinity exposure at 25 °C. The usual application of the metabolite/monoamine ratio as an index for monoaminergic activity (see Winberg and Nilsson, 1993b, for a review) is therefore only of little value in this situation.

In conclusion, the results show that exposure of common carp to 1 % NaCl has substantial effects on the levels and metabolism of brain monoamine neurotransmitters. Rises in DA and 5-HT levels and increases in their metabolic rates, especially in the hypothalamus, could be related to regulation of levels of prolactin and cortisol, two important hormones in teleost osmoregulatory homeostasis, although further experiments are of course needed to prove such a connection. Rises in 5-HT levels in the telencephalon could be related to olfactory perception of the changed environment. Increasing the temperature only increased the levels of the monoamine metabolites, suggesting higher activities of DA and 5-HT metabolism. This is likely to be a Q₁₀ effect.

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