Thyroid hormone modulation of brain *in vivo* tyrosine hydroxylase activity and kinetics in the female catfish *Heteropneustes fossilis*

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Abstract

In the female catfish *Heteropneustes fossilis*, administration of thyroxine (T_4), 1 μg/g body weight, i.p., in both gonadal resting and preparatory phases for 7, 14 and 21 days caused hyperthyroidism, as evidenced from a duration-dependent significant increase in serum triiodothyronine (T_3), and of tyrosine hydroxylase (TH) activity in telencephalon, hypothalamus–pituitary and medulla oblongata (Newman–Keuls' test; $P<0{\cdot}05$). Hypothyroidism induced by adding 0·03% thiourea to aquarium water holding the catfish for 7, 14 and 21 days decreased serum T_3 levels in a duration-dependent manner (Newman–Keuls' test; $P<0{\cdot}05$) and inhibited TH activity in the brain regions. T_4 replacement in 21-day thiourea-treated fish restored and even elevated significantly serum T_3 levels as well as brain TH activity in a duration-dependent manner. In general, the changes in enzyme activity were higher in the forebrain regions than medulla oblongata and in the resting phase than preparatory phase. Kinetic studies by Lineweaver–Burk plots showed that the stimulatory effect following T_4 administration and T_4 replacement on TH activity was due to increased affinity of the enzyme for its cofactor (6,7-dimethyl-2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine), as evident from a significant decrease in apparent Michaelis–Menten constant (K_m) and an increase in apparent velocity maximum (V_{max}). The TH inhibition due to the thiourea treatment can be related to decreased affinity of the enzyme for its cofactor, as evident from a significant increase in apparent K_m value and a significant decrease in V_{max}. These data clearly show that circulating levels of T_4/T_3 modulate brain TH activity by altering the kinetic properties of the enzyme, which, in turn, influence catecholaminergic activity and dependent functions.

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Introduction

Thyroid hormone (T_3) is a primary epigenetic factor influencing multiple events in neural development such as axonal maturation, neurite outgrowth, cell migration, myelin formation, etc. (Denver 1997, Oppenheimer & Schwartz 1997). Apart from their well known feedback actions in the brain related to the regulation of thyrotrophin (TSH) secretion from the pituitary, thyroid hormones (thyroxine (T_4) and triiodothyronine (T_3)) exert a crucial role in the overall neural activity of both central and peripheral nervous systems in vertebrates (Rastogi & Singhal 1976, Ruiz-Marcos *et al.* 1994, Claustre *et al.* 1996). T_4 is transported into the brain, concentrated, retained and metabolised in discrete neural systems (Dratman *et al.* 1987, Schreiber *et al.* 1990). T_3, the functional thyroid hormone, accounts for the major part of iodocompounds in the brain (80%) and its concentration is generally maintained at a relatively stable level despite thyroid hormone deficiency or excess (Dratman *et al.* 1987). T_3 receptors (α and β subtypes) are generally distributed in neurons, glial and ependymal cells (Puymirat *et al.* 1991).

Thyroid hormones have been demonstrated to influence the maturation and maintenance of the brain catecholamine (CA)-ergic system (Rastogi & Singhal 1976, Claustre *et al.* 1996). Noradrenergic activity in the peripheral sympathetic nervous system and CA-ergic activity in the adrenal medulla are also dependent on the thyroid status; plasma noradrenaline increases in hypothyroid subjects (Valens & Gripois 1990). Immunocytochemical studies have demonstrated the coexistence of both thyronergic (T_3-containing) and noradrenergic systems (locus coeruleus neurons and their targets) and T_3 can act as a neurotransmitter/neuromodulator (Rozanov & Dratman 1996, Gordon *et al.* 1999). Tyrosine hydroxylase (TH) is the rate-limiting step in CA biosynthesis and is influenced by the thyroid status (Kizer *et al.* 1978, Wang *et al.* 1989, Claustre *et al.* 1996, Evans *et al.* 1999); surgical thyroidectomy increases TH activity and T_4 replacement restores it. However, hypothyroidism induced by propylthiouracil (PTU) decreases TH activity in the anterior

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part of the locus coeruleus and adrenal medulla and hyperthyroidism by T_4 injection elevates it (Claustre *et al.* 1996).

In teleosts, investigations on brain TH are largely related to its use as a phenotypic marker for the CA-ergic system (Hornby & Piekut 1990). In rainbow trout, TH activity was demonstrated in forebrain regions (Linard *et al.* 1996). Further, cDNA cloning and sequencing of TH have been demonstrated in the rainbow trout (Linard *et al.* 1998) and eel (Boularand *et al.* 1998). In our earlier studies, we have demonstrated seasonal, diurnal, regional and sexual differences in, and effects of environmental factors (photoperiod and temperature) as well as oestrogens on brain TH activity and kinetics in the catfish (Chaube & Joy 2002, 2003). To the best of our knowledge, there are no studies relating to the role of thyroid hormones in the regulation of TH activity in non-mammals. In teleosts, the role of thyroid hormones in morphogenesis, development, growth, osmoregulation, migration, metabolism and reproduction are broadly defined (Eales 1993), but their involvement in specific functions of the brain is not demonstrated. T_3 is the functional thyroid hormone and is formed extrathyroidally, while T_4 is involved in the feedback regulation of TSH secretion (Eales *et al.* 1993). As in mammals, the teleost brain is also a site for T_4 and T_3 deiodination (Plate *et al.* 2002).

In the present study, we demonstrated the effects of hyperthyroidism (by T_4 administration), hypothyroidism (induced by thiourea) and T_4 replacement on *in vivo* TH activity and kinetics in the brain regions of the female catfish *Heteropneustes fossilis*.

Materials and Methods

Chemicals

Catalase, L-tyrosine, 6,7-dimethyl-2-amino-4-hydroxy-5,6,7,8-tetrahydropteridine ($DMPH_4$), BSA, Sephadex G-25 and T_4 were purchased from Sigma Chemical Company, St Louis, MO, USA. Sodium molybdate, 2-mercaptoethanol, sodium nitrite, thiourea and Folin–Ciocalteu reagent were purchased from E-Merck, Mumbai, India. RIA kits for T_3 were purchased from Bhaba Atomic Research Centre, Mumbai, India.

Fish collection and acclimatisation

H. fossilis is a freshwater air-breathing catfish whose reproductive cycle can be divided into five phases: resting (September–January); preparatory or early vitellogenic (February–April); prespawning or late vitellogenic (May–June); spawning (July–August); and post-spawning (September–October). The study was conducted in gonadal resting (December) and early vitellogenic (March) phases. Fish were collected from local fish markets in Varanasi. Female fish weighing 35–45 g were selected and acclimatised in flow-through aquarium tanks under normal photoperiod and ambient temperature (resting phase: 10·5 h light:13·5 h darkness, 18 ± 2 °C; vitellogenic phase: 11·5 h light:12·5 h darkness, 22 ± 2 °C). They were fed minced goat liver daily. After 15 days of acclimatisation, the fish were used for various experiments as follows.

Induction of hyperthyroidism

Fish were divided into two groups of 25 each. Group 1 was injected i.p. on alternate days with T_4 in a dose of 1 μg/g body weight (BW). T_4 was dissolved in alkaline saline (0·65% NaCl containing 5 M NaOH to adjust the pH to 8·1) as vehicle. Group 2 was treated with the vehicle as control. After 7, 14 and 21 days, five fish from each of the two groups were sampled at 1100–1200 h. Blood was collected by caudal puncture. The samples were centrifuged at 2000 ***g*** to collect serum, which was stored at −70 °C for T_3 assay. The fish were weighed, killed by decapitation and brains dissected out and stored at −70 °C for TH assay. Ovaries were weighed and the gonadosomatic index (GSI = ovary weight (g)/BW × 100) calculated.

Induction of hypothyroidism

Since surgical thyroidectomy is not feasible in catfish due to the diffuse distribution of thyroid follicles in the pharyngeal floor, hypothyroidism was induced by thiourea, a thyroid hormone inhibitor and goitrogen. Fish were divided into two groups of 25 each. Group 1 was maintained in water containing 0·03% thiourea, which was replenished every day after feeding. Group 2 fish were maintained in dechlorinated tap water as control. At intervals of 7, 14 and 21 days, five fish each from the two groups were sampled at 1100–1200 h. Blood was collected for serum separation and stored at −70 °C for T_3 assay. The fish were weighed, killed by decapitation and brains dissected out and stored at −70 °C for 24 h for TH assay. Ovaries were weighed for calculation of GSI.

Reversal of hypothyroidism by T_4 replacement

Fish were maintained in thiourea (0·03%) for 21 days, as described above, and then divided into two groups and maintained in dechlorinated water. Group 1 was injected with T_4 (1 μg/g BW), i.p., on alternate days. Group 2 was given vehicle only as control. Five fish from each of the two groups were sampled at intervals of 7, 14 and 21 days. Blood was collected for serum separation and serum stored at −70 °C for T_3 assay. The fish were weighed,

killed by decapitation and brains dissected out and stored at −70 °C for 24 h for TH assay. Ovaries were weighed for calculation of GSI.

RIA of T_3

Serum T_3 level was assayed by RIA using the T_3 kit. Serum (50 µl) was incubated in duplicate with 0·1 ml ^{125}I-T_3 and 0·1 ml antiserum at 37 °C for 3 h at room temperature. After incubation, 1·0 ml of PEG–second antibody (polyethylene glycol, 6% w/v+goat anti-rabbit antibody) was added, mixed by vortexing and centrifuged at 2000 ***g*** for 20 min at 4 °C. The supernatant was decanted and the pellet dried. Radioactivity was measured in a gamma counter (Beckman DP5000; Beckman Instruments Inc., Fullerton, CA, USA). For standard curve preparations, different concentrations of T_3 (0·15, 0·3, 0·6, 1·2, 2·4 ng/ml) provided with the kit were processed in the same manner as the plasma samples. The percentage of B/B_0 (B = count rate for each sample, B_0 = count rate for non-specific binding sample) was calculated and the standard curve was plotted using different standard concentrations of T_3 vs percentage of B/B_0 on a logit–log scale. From the standard curve, T_3 concentration of the sample was determined and expressed in ng/ml. All the samples were assayed from a single RIA kit. The minimum sensitivity of the assay was 0·24 ng/ml. The intra-assay coefficient of variation (determined from five standard curve assays) was 6%.

TH activity

Brains were thawed and dissected out immediately on ice. The telencephalon (excluding olfactory tract and bulb), and hypothalamus along with pituitary and medulla oblongata were separated as described earlier (Chaube & Joy 2002). Tissues were homogenised in 1 ml 30 mM sucrose containing 10 mM Tris–HCl buffer (pH 7·3) in a Potter–Elvehjem homogeniser with a loose-fitting Teflon pestle. The rotor speed was 300–500 r.p.m. and the pestle was taken up and down four or five times. The homogenate was centrifuged at 105 000 ***g*** for 1 h and passed through Sephadex G-25 column (1 ml column, flow rate 1 ml/40 min) at 4 °C to remove endogenous CAs. The eluate containing TH activity was stored up to 1 week at −20 °C and used as the enzyme preparation for TH assay. Enzyme activity was not affected by storage up to 1 week (data not shown).

TH activity was measured by the method of Shiman *et al.* (1971). The incubation mixture contained 0·25 ml L-tyrosine (2 mM), potassium phosphate-buffered saline (PBS, 2·0 M, pH 6·2), 0·01 ml catalase (1 mg/3 ml in PBS buffer), 0·05 ml 0·28 M 2-mercaptoethanol, 0·05 ml 6 mM $DMPH_4$. The reaction mixture was incubated in a test tube at 30 °C for 25 min. The reaction was stopped by adding 0·5 ml 0·5 M HCl. Freshly prepared nitrite-molybdate reagent (1 ml) was added to the mixture and allowed to stand for 5 min. The colour was stable for 30 min. Half a millilitre of a 2 M NaOH solution was quickly added and mixed. Absorption was immediately determined at 510 nm in a UV-VIS 118 spectrophotometer (Systronics, Ahmedabad, India). To express enzyme activity, tissue protein content in each aliquot was measured by the method of Lowry *et al.* (1951) using BSA as standard. Enzyme activity was expressed as nmol L-dopa formed/mg protein per h.

Table 1 Effects of T_4, thiourea and T_4 replacement on gonadosomatic index (means ± S.E.M. in g %) in the female catfish *Heteropneustes fossilis* in the vitellogenic phase. Comparisons were made with respective controls and duration groups

	7 days	14 days	21 days
Experiment			
Vehicle control (VC)	$1·46 \pm 0·08^1$	$1·50 \pm 0·12^1$	$1·44 \pm 0·09^1$
T_4	$1·74 \pm 0·18^2$	$2·16 \pm 0·24^3$	$2·44 \pm 0·18^4$
VC	$1·43 \pm 0·02^1$	$1·45 \pm 0·06^1$	$1·48 \pm 0·03^1$
Thiourea (TU)	$0·70 \pm 0·01^2$	$0·91 \pm 0·04^3$	$0·42 \pm 0·02^4$
TU+VC	$0·45 \pm 0·02^1$	$0·48 \pm 0·02^1$	$0·52 \pm 0·03^1$
TU+T_4	$1·34 \pm 0·15^2$	$1·78 \pm 0·02^3$	$1·92 \pm 0·12^4$

Values with the same superscripted numbers are not significant and those with different numbers are significant in each experiment (two-way ANOVA; Newman–Keuls' test).

Determination of kinetic parameters

The Michaelis–Menten constants (K_m) and maximum velocity (V_{max}) of TH were determined from the intercepts on the *x*- and *y*-axes respectively of double reciprocal Lineweaver–Burk plots with 1/[$DMPH_4$] (1–8 mM) or 1/[L-tyrosine] (0·1–0·5 mM) as independent variable and 1/TH as dependent variable.

Statistical analysis

All data are expressed as means ± S.E.M. The data were analysed by two-way ANOVA followed by Newman–Keuls' test ($P<0·05$).

Results

Effects of T_4 administration on GSI, serum T_3 levels and brain TH

The GSI registered an overall significant effect (two-way ANOVA; $P<0·001$) after T_4 administration in both vitellogenic (Table 1) and resting (data not shown) phases. The values increased significantly at all time points in both phases except on day 7 and 14 in the resting phase ($P<0·05$; Newman–Keuls' test). Serum T_3 levels showed an overall significant effect (two-way ANOVA; $P<0·001$) in both vitellogenic (Fig. 1B) and resting (data not shown) phases. The T_3 levels increased significantly at all time points ($P<0·05$; Newman–Keuls' test).

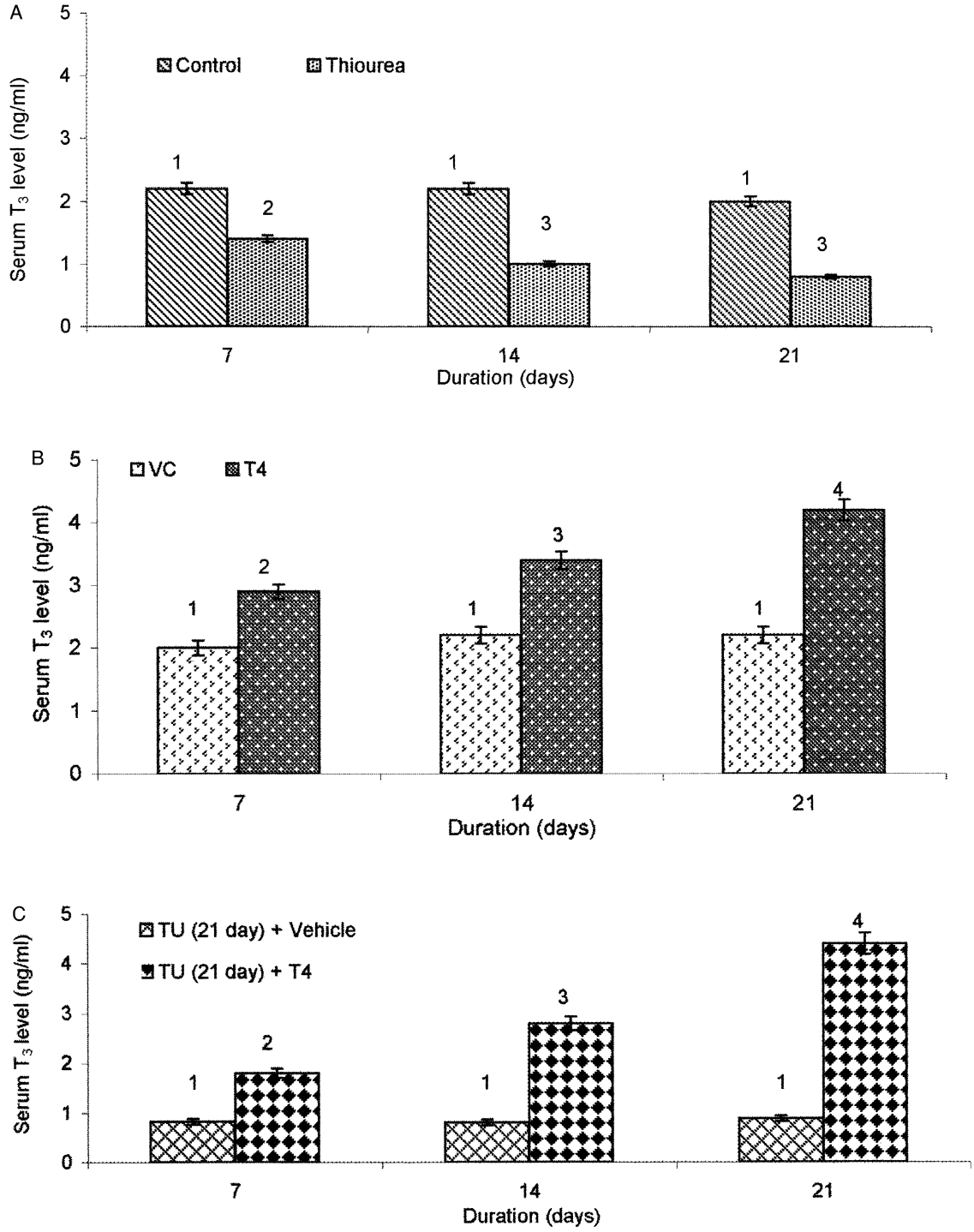

Figure 1 Effects of thiourea (TU) (A), T_4 (B) and T_4 replacement (C) on serum T_3 levels in the female catfish *Heteropneustes fossilis* (means ± S.E.M., $n=5$) in the vitellogenic phase. Data were analysed by two-way ANOVA ($P<0{\cdot}001$) and Newman–Keuls' test ($P<0{\cdot}05$). Comparisons were made with respective controls and duration groups. Values with the same number are not significant and those with different numbers are significant in each experiment. VC, vehicle control.

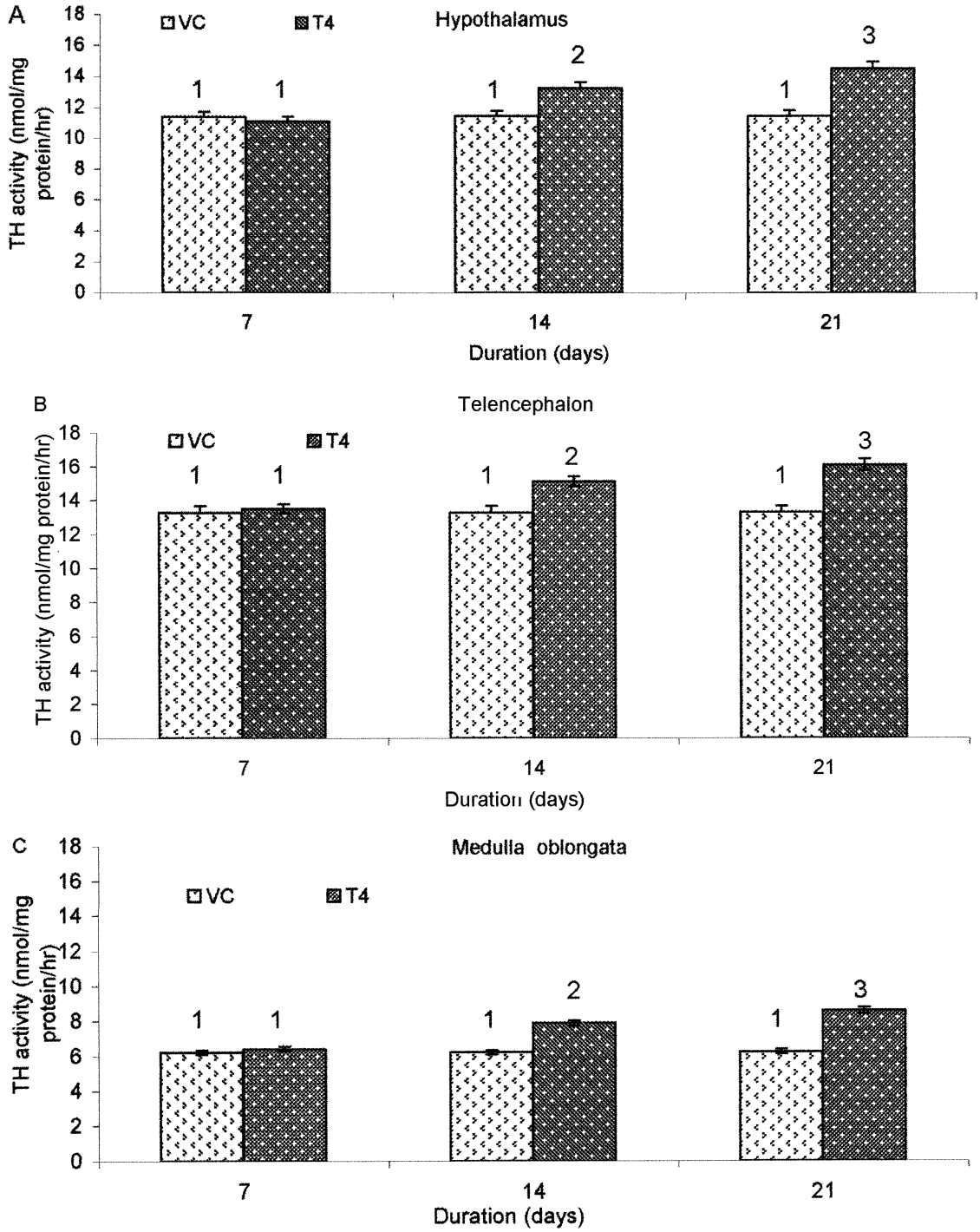

Figure 2 Effects of T_4 treatment on brain TH activity in the female catfish *Heteropneustes fossilis* (means ± S.E.M., $n=5$) in the vitellogenic phase. Data were analysed by two-way ANOVA ($P<0{\cdot}001$) and Newman–Keuls' test ($P<0{\cdot}05$). Comparisons were made with respective controls and duration groups. Values with the same number are not significant and those with different numbers are significant in each experiment. VC, vehicle control.

Table 2 Effects of T_4, thiourea and T_4 replacement on apparent K_m and V_{max} (means ± S.E.M.) of tyrosine hydroxylase for cofactor in brain regions of the female catfish *Heteropneustes fossilis* in the vitellogenic phase. The K_m and V_{max} values were calculated from Lineweaver–Burk plots. Data were analysed by two-way ANOVA ($P<0·001$) and Newman–Keuls' test ($P<0·05$). Comparisons were made with respective control groups and duration groups and were indicated by numbers (K_m) and letters (V_{max})

	7 days		14 days		21 days	
	K_m (mM)	V_{max} (nmol/mg protein/h)	K_m (mM)	V_{max} (nmol/mg protein/h)	K_m (mM)	V_{max} (nmol/mg protein/h)
Hypothalamus						
1. Vehicle control (VC)	$0·19 \pm 0·004^{1}$	$20·32 \pm 0·98^{a}$	$0·22 \pm 0·002^{1}$	$20·35 \pm 0·24^{a}$	$0·20 \pm 0·003^{1}$	$20·48 \pm 1·54^{a}$
T_4	$0·18 \pm 0·005^{1}$	$20·86 \pm 1·80^{a}$	$0·10 \pm 0·003^{2}$	$22·14 \pm 0·72^{b}$	$0·09 \pm 0·001^{2}$	$24·60 \pm 1·28^{c}$
2. Control	$0·18 \pm 0·03^{1}$	$20·26 \pm 0·92^{a}$	$0·19 \pm 0·01^{1}$	$20·30 \pm 1·86^{a}$	$0·17 \pm 0·02^{1}$	$20·42 \pm 0·75^{a}$
Thiourea (TU)	$0·20 \pm 0·01^{1}$	$20·12 \pm 1·08^{a}$	$0·58 \pm 0·03^{2}$	$14·65 \pm 1·05^{b}$	$0·82 \pm 0·08^{3}$	$12·02 \pm 0·98^{c}$
3. TU+VC	$0·78 \pm 0·12^{1}$	$11·62 \pm 1·42^{a}$	$0·80 \pm 0·15^{1}$	$11·60 \pm 1·68^{a}$	$0·82 \pm 0·08^{1}$	$11·65 \pm 1·72^{a}$
TU+T_4	$0·70 \pm 0·08^{1}$	$12·08 \pm 0·86^{a}$	$0·42 \pm 0·04^{2}$	$14·83 \pm 2·02^{b}$	$0·30 \pm 0·06^{3}$	$18·12 \pm 0·28^{c}$
Telencephalon						
1. VC	$0·15 \pm 0·004^{1}$	$22·61 \pm 1·70^{a}$	$0·14 \pm 0·003^{1}$	$22·42 \pm 0·24^{a}$	$0·14 \pm 0·001^{1}$	$22·32 \pm 1·42^{a}$
T_4	$0·14 \pm 0·002^{1}$	$22·86 \pm 1·82^{a}$	$0·11 \pm 0·002^{1}$	$24·42 \pm 0·62^{b}$	$0·05 \pm 0·001^{1}$	$26·45 \pm 1·18^{c}$
2. Control	$0·14 \pm 0·03^{1}$	$22·65 \pm 0·86^{a}$	$0·15 \pm 0·01^{1}$	$22·54 \pm 2·02^{a}$	$0·16 \pm 0·02^{1}$	$22·48 \pm 1·58^{a}$
TU	$0·35 \pm 0·05^{2}$	$22·58 \pm 0·40^{a}$	$0·98 \pm 0·08^{3}$	$18·62 \pm 1·80^{b}$	$1·28 \pm 0·15^{4}$	$10·25 \pm 1·02^{c}$
3. TU+VC	$1·16 \pm 0·84^{1}$	$10·64 \pm 0·48^{a}$	$1·10 \pm 0·02^{1}$	$10·66 \pm 0·78^{a}$	$1·12 \pm 0·03^{1}$	$10·68 \pm 1·14^{a}$
TU+T_4	$0·84 \pm 0·18^{2}$	$11·08 \pm 0·28^{a}$	$0·62 \pm 0·03^{3}$	$13·75 \pm 1·20^{b}$	$0·48 \pm 0·01^{4}$	$16·18 \pm 1·28^{c}$
Medulla oblongata						
1. VC	$0·26 \pm 0·04^{1}$	$12·88 \pm 1·12^{a}$	$0·24 \pm 0·003^{1}$	$12·65 \pm 1·26^{a}$	$0·22 \pm 0·003^{1}$	$12·78 \pm 1·12^{a}$
T_4	$0·20 \pm 0·01^{1}$	$13·24 \pm 0·61^{a}$	$0·15 \pm 0·002^{1}$	$16·24 \pm 0·78^{b}$	$0·12 \pm 0·001^{1}$	$18·68 \pm 0·82^{c}$
2. Control	$0·28 \pm 0·05^{1}$	$12·51 \pm 1·28^{a}$	$0·26 \pm 0·02^{1}$	$12·58 \pm 1·34^{a}$	$0·24 \pm 0·08^{1}$	$12·45 \pm 1·05^{a}$
TU	$0·30 \pm 0·03^{1}$	$11·22 \pm 1·01^{a}$	$0·45 \pm 0·05^{2}$	$8·42 \pm 0·75^{b}$	$0·58 \pm 0·06^{3}$	$5·42 \pm 0·86^{c}$
3. TU+VC	$0·60 \pm 0·07^{1}$	$5·63 \pm 0·81^{a}$	$0·56 \pm 0·08^{1}$	$5·61 \pm 1·06^{a}$	$0·58 \pm 0·10^{1}$	$5·68 \pm 0·80^{a}$
TU+T_4	$0·58 \pm 0·06^{1}$	$6·05 \pm 0·82^{a}$	$0·30 \pm 0·05^{2}$	$8·02 \pm 0·62^{b}$	$0·20 \pm 0·02^{2}$	$8·62 \pm 0·28^{c}$

Values with the same superscripted numbers or letters are not significant and those with different numbers and letters are significant.

Brain TH activity showed an overall significant effect (two-way ANOVA; $P<0·001$) in the vitellogenic phase (Fig. 2A–C) and resting phase (data not shown). In both phases, enzyme activity increased significantly on day 14 (except medulla oblongata) and on day 21 in all brain regions ($P<0·05$; Newman–Keuls' test). The percentage increase on day 21 was lower in the vitellogenic phase (hypothalamus 27%, telencephalon 21%, and medulla oblongata 37%) than resting phase (76, 69 and 53% respectively).

The T_4 administration produced overall significant effects on both apparent K_m and V_{max} of the enzyme for the cofactor in different brain regions (Table 2; two-way ANOVA; $P<0·001$). The K_m and V_{max} values did not vary significantly in the vehicle control groups over the duration in any of the brain regions. The K_m values decreased, and the V_{max} increased significantly in all brain regions on day 14 and 21 ($P<0·05$; Newman–Keuls' test). In the resting phase, similar changes were found (data not shown) (two-way ANOVA; $P<0·001$; Newman–Keuls' test; $P<0·05$).

Effects of thiourea treatment on GSI, serum T_3 levels and brain TH

The GSI showed an overall significant effect after thiourea treatment (two-way ANOVA; $P<0·001$) in the vitellogenic phase (Table 1) and in the resting phase (data not shown). Newman–Keuls' analysis indicated significant inhibition ($P<0·05$) at all times except on day 7 in the resting phase. The treatment resulted in an overall significant effect (two-way ANOVA; $P<0·001$) on serum T_3 levels in both vitellogenic (Fig. 1A) and resting (data not shown) phases. The T_3 levels decreased significantly at all time points compared with control values ($P<0·05$; Newman–Keuls' test). The inhibition on day 7, 14 and 21 was 36, 54·5 and 60% respectively in the vitellogenic phase and 47, 49 and 72% respectively in the resting phase.

The thiourea treatment produced an overall significant effect (two-way ANOVA; $P<0·001$) on TH activity in different regions of the brain in both vitellogenic (Fig. 3A–C) and resting (data not shown) phases. In both hypothalamus and telencephalon, enzyme activity decreased at all time points, but in the medulla oblongata, the decrease was significant only on day 14 and 21 ($P<0·05$; Newman–Keuls' test). The percentage decrease on day 21 was 54% (hypothalamus), 33% (telencephalon) and 20% (medulla oblongata) in the vitellogenic phase and 54·5, 61 and 21% respectively in the resting phase.

Apparent K_m and V_{max} of the enzyme for the cofactor showed overall significant effects (two-way ANOVA; $P<0·001$) in both vitellogenic (Table 2) and resting (data not shown) phases. Since the treatment influenced both K_m and V_{max}, the inhibition appeared to be of the mixed

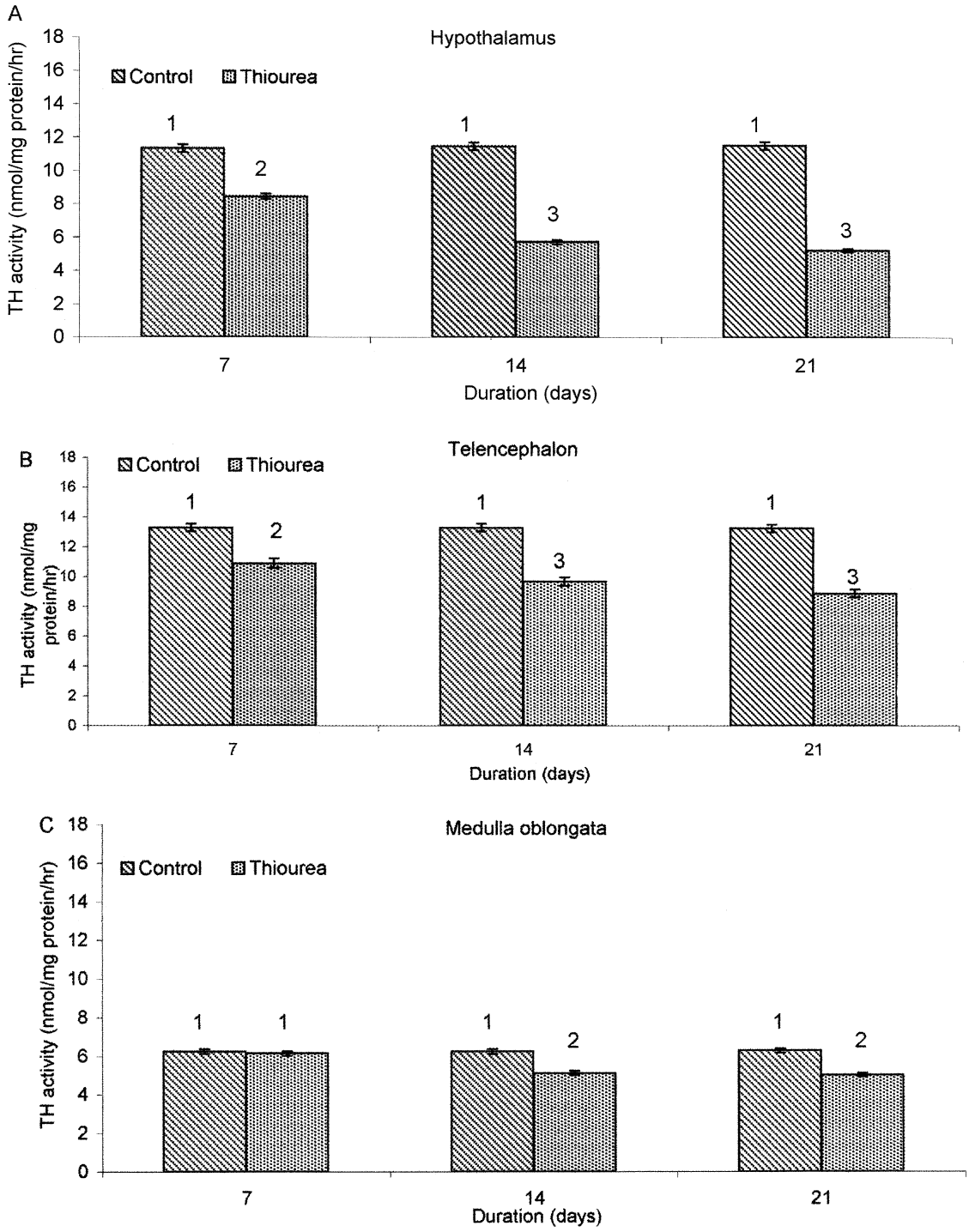

Figure 3 Effects of thiourea treatment on brain TH activity in the female catfish *Heteropneustes fossilis* (means ± S.E.M., $n=5$) in the vitellogenic phase. Data were analysed by two-way ANOVA ($P<0{\cdot}001$) and Newman–Keuls' test ($P<0{\cdot}05$). Comparisons were made with respective controls and duration groups. Values with the same number are not significant and those with different numbers are significant in each experiment.

or uncompetitive type. The K_m values increased, and the V_{max} decreased significantly over the duration of the treatment. In the thiourea-treated groups, the K_m and the V_{max} values did not change significantly in the hypothalamus and medulla oblongata on day 7 compared with the control groups ($P<0{\cdot}05$; Newman–Keuls' test). In the telencephalon, the V_{max} also did not vary significantly on day 7. In the resting phase, similar changes were noticed.

Effects of T_4 replacement on GSI, serum T_3 and brain TH

The administration of T_4 in 21-day thiourea-treated fish for 7, 14 and 21 days produced an overall significant effect (two-way ANOVA; $P<0{\cdot}001$) on GSI in the vitellogenic (Table 1; treatment $F=6{\cdot}25$, duration $F=16{\cdot}85$ and interaction of both $F=26{\cdot}65$) and resting (data not shown) phases. The GSI increased significantly at all time points ($P<0{\cdot}05$; Newman–Keuls' test). Serum T_3 levels showed a significant effect in both vitellogenic (Fig. 1C) and resting (data not shown) phases. The T_3 levels increased significantly on day 7, 14 and 21 and the percentage increase was 119·5, 250 and 400% respectively in the vitellogenic phase and 215, 335 and 418% respectively in the resting phase. In the control (thiourea+vehicle) groups, the T_3 levels remained low even up to 21 days of the withdrawal.

Brain TH activity showed an overall significant effect (two-way ANOVA; $P<0{\cdot}001$) after the replacement treatment in the vitellogenic and resting (data not shown) phases. The T_4 replacement caused a significant increase in TH activity in all brain regions at all times in the vitellogenic (Fig. 4A–C) and resting (data not shown) phases. The percentage increase on day 21 was much lower in the vitellogenic phase (124%, hypothalamus; 58·9%, telencephalon; 21%, medulla oblongata) than resting phase (213%, hypothalamus; 310%, telencephalon; and 50%, medulla oblongata).

T_4 replacement produced overall significant effects (two-way ANOVA; $P<0{\cdot}001$) on both apparent K_m and V_{max} values of the enzyme for the cofactor in the vitellogenic (Table 2) and resting (data not shown) phases. The values did not change significantly in the vehicle groups during the duration of the treatment. In the replacement groups, the values altered (K_m decreased and V_{max} increased) significantly in both phases on day 14 and 21 ($P<0{\cdot}05$; Newman–Keuls' test). On day 7, both the values are significantly different in the hypothalamus (resting phase) and the K_m values in the medulla oblongata (resting phase) and telencephalon (vitellogenic phase).

Discussion

The present study demonstrates clearly the involvement of thyroid hormones in the modulation of brain TH activity. The administration of T_4 evoked significant brain TH activation detected after 2 weeks of treatment. These observations are in agreement with studies in mammals of a stimulatory effect of T_4/T_3 on the enzyme in developing or adult brains and adrenals (Rastogi & Singhal 1976, Kato *et al.* 1982, Gripois & Valens 1984, Valens & Gripois 1990). However, conflicting reports of a lack of enzyme response, perhaps due to brain regional differences, short vs long treatment, developmental stage at which exposure was made, dosage, etc., are also available (Valens & Gripois 1990, Claustre *et al.* 1996). In the catfish, T_4 administration resulted in hyperthyroidic conditions, as evident from the duration-dependent rise in serum T_3 levels, which may be responsible for the elevated brain TH activity. The response varied with the brain region, duration of the treatment and season. The magnitude of the response was higher in the forebrain regions than medulla oblongata and in the resting phase than vitellogenic phase. These differences may be due to regional differences in enzyme activity (Chaube & Joy 2003) or the interplay of other factors like gonadal oestrogens on the brain–pituitary axis. The forebrain regions contain oestrogen feedback sites and the oestradiol (E_2) feedback is stronger in the vitellogenic phase than resting phase (Senthilkumaran & Joy 1995). The increase in GSI suggests that hyperthyroidism caused a stimulation of ovarian activity. Since thyroid hormones modulate gonadal steroidogenesis (Cyr & Eales 1988, Timmermans *et al.* 1997), the resulting strong E_2 feedback might have lessened the otherwise full-blown effect of T_4/T_3 in the vitellogenic phase (Chaube & Joy 2002).

Hypothyroidism has long been linked to retardation of development, maturation and functions of the nervous system and causes mental ailment, motor dysfunction, behavioural changes, early neurodegenerative activity, etc. – some of these disorders have been related to impairment of CA metabolism (Evans *et al.* 1999, Kincaid 2001). Hypothyroidism caused tyrosinaemia (tyrosine accumulation) in the brain, adrenal, heart, etc. (Diarra *et al.* 1989) and the genetically hypothyroid mouse (non-functional thyroid due to defective TSH receptor) showed significantly fewer (40%) dopamine (TH-positive) neurons in the substantia nigra and adjacent ventral tegmental area (Kincaid 2001). The reported effects of hypothyroidism on TH are at variance and seem to be influenced by several factors including its nature of induction. Hypothyroidism by surgical thyroidectomy resulted in an increase in TH function (activity and mass) in the median eminence (Wang *et al.* 1989) and in some hypothalamic nuclei (Kizer *et al.* 1978) but did not alter it in the preoptic nucleus (Kizer *et al.* 1978), substantia nigra (Nakahara *et al.* 1976) and superior cervical ganglia (Wang *et al.* 1989). Hypothyroidism induced by PTU treatment caused an increase in TH activity in the median eminence of the rat (Kizer *et al.* 1978), a decrease in the anterior locus coeruleus and adrenal, and no changes in the posterior locus coeruleus and substantia nigra (Claustre *et al.* 1996). In the rat,

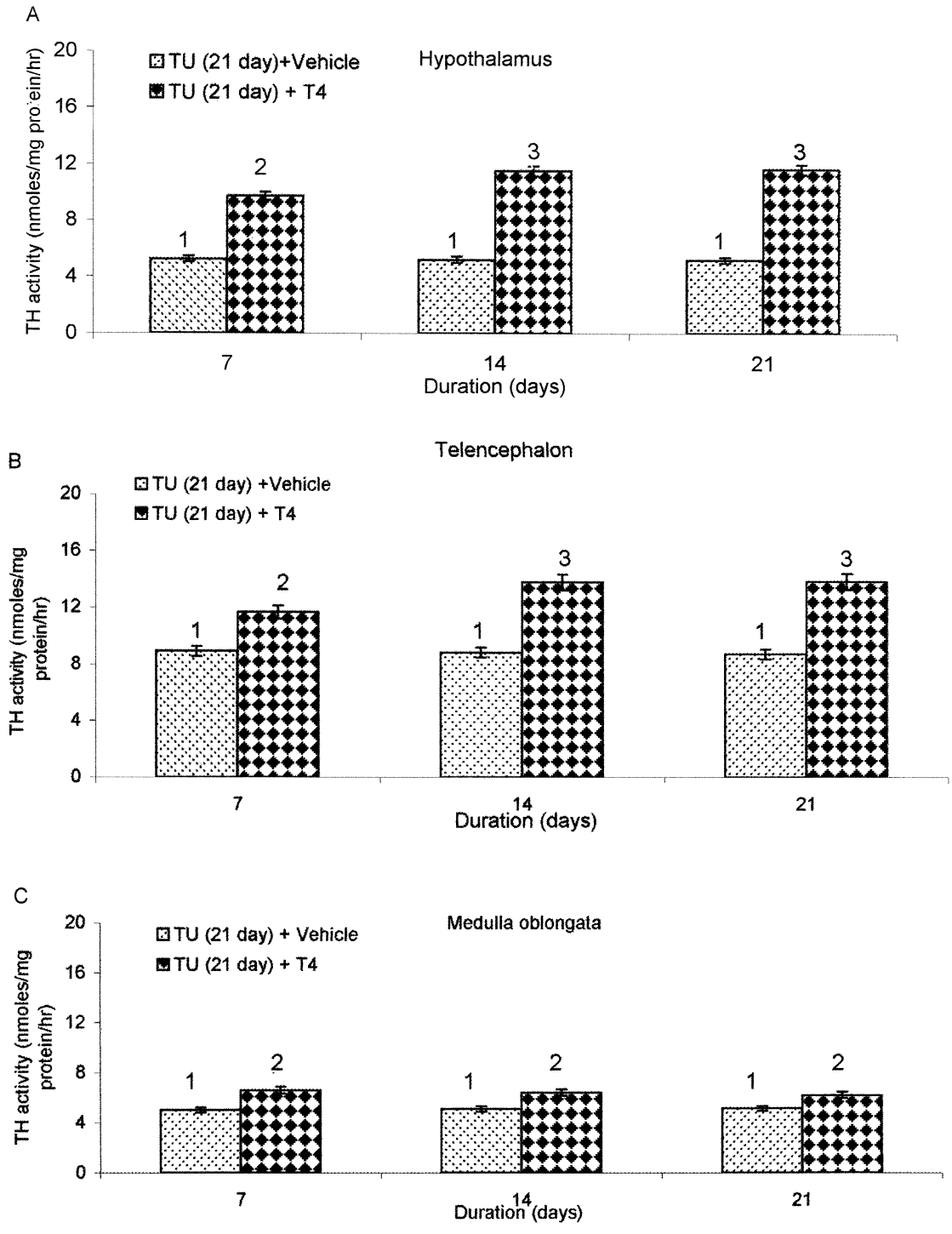

Figure 4 Effects of T_4 replacement in the 21-day thiourea (TU)-treated female catfish *Heteropneustes fossilis* (means ± S.E.M., $n=5$) in the vitellogenic phase. Data were analysed by two-way ANOVA ($P<0{\cdot}001$) and Newman–Keuls' test ($P<0{\cdot}05$). Comparisons were made with respective controls and duration groups. Values with the same number are not significant and those with different numbers are significant in each experiment.

neonatal hypothyroidism induced by PTU impaired TH activation in the adrenal and was reversed by T_3 replacement (Valens & Gripois 1990). The varied effects suggest that modulation of TH activity by T_4/T_3 is influenced by the interplay of other regulatory/modulatory signals as well. In the present study, catfish were rendered hypothyroidic by thiourea treatment, which resulted in a time-dependent significant decrease in serum T_3 levels. In the hypothyroidic catfish, a duration-dependent significant decrease in TH activity was noticed in all brain regions in both seasons. The inhibition was higher in the forebrain regions than medulla oblongata with the telencephalic activity showing marked seasonal difference. The decrease in the GSI following hypothyroidism suggests decreased gonadal and E_2 feedback activity and this may account for the higher inhibition of the enzyme in the vitellogenic phase. Claustre *et al.* (1996) reported decreased TH activity after PTU treatment, but Kizer *et al.* (1978) reported TH activation in rat brain nuclei. The thiourea-induced TH inhibition could be reversed by T_4 replacement, but not by withdrawal of the treatment alone (vehicle control group). The data also showed that forebrain TH activity was more sensitive to the replacement treatment.

The kinetic data presented in this study may explain the changes in TH activity in relation to thyroid hormone excess and deficiency. The stimulatory effect of T_4 appeared to be caused by a significant lowering of the apparent K_m value of the enzyme for the cofactor with concomitant increase in the apparent V_{max}, as reported in thyroidectomised rats (Kizer *et al.* 1978). Thus, the stimulatory effect of T_4 can be correlated to an increased affinity of the enzyme for the cofactor. In contrast, thiourea treatment (hypothyroidism) produced kinetic changes in the reverse manner and the TH inhibition could be due to a low affinity of the enzyme for the cofactor (high K_m and low V_{max}). The thiourea-induced changes in the enzyme kinetics could be reversed by T_4 replacement, resulting in a significant decrease in the K_m values and a significant increase in the V_{max}. In contrast, Kizer *et al.* (1978) reported that PTU treatment decreased the K_m value for the cofactor and increased the V_{max}, like surgical thyroidectomy. In hypothyroidic rat adrenal, the decreased TH activity was associated with an increase in K_m value in comparison with euthyroid animals (Blouquit *et al.* 1990). Thus, the stimulatory effect of thyroid hormones on TH activity may be mediated by the activation of the enzyme by kinetic changes. An increase in enzyme synthesis also may lead to increased TH activity. The thiourea treatment might have interfered with the enzyme activation and synthesis by decreasing the T_4/T_3 levels. Further studies are required to understand the molecular mechanisms involved in the modulation of TH activity by T_4/T_3.

In conclusion, brain TH is sensitive to the thyroid status; hormone excess activates and deficiency retards the enzyme activity by modifying its kinetic function. In turn, thyroid hormones can influence central CA-ergic activity and the dependent physiological processes such as osmoregulation, growth and reproduction in the catfish.

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