Toxicology Mechanisms and Methods, 2010; 20(6): 287–297

informa
healthcare

RESEARCH ARTICLE

Involvement of high plasma corticosterone status and activation of brain regional serotonin metabolism in long-term erythrosine-induced rearing motor hyper activity in young adult male rats

Arindam Dalal, and Mrinal K. Poddar

Department of Biochemistry, University of Calcutta, 35 B. C. Road, Kolkata 700 019, India

Abtract

Long-term consumption of artificial food color(s) can induce behavioral hyperactivity in human and experimental aminals, but no neurobiochemical mechanism is defined. This study investigates the role of brain regional serotonin metabolism including its turnover, MAO-A activity, and plasma corticosterone status in relation to behavioral disturbances due to an artificial food color, erythrosine. Long-term (15 or 30 consecutive days) erythrosine administration with higher dosage (10 or 100 mg/kg/day, p.o.) produced optimal hyperactive state in exploratory behavior (rearing motor activity) after 2 h of last erythrosine administration, in young adult male albino rats. Erythrosine-induced stimulation in brain regional (medulla-pons, hypothalamus, hippocampus, and corpus striatum) serotonin metabolism (measuring steady state levels of 5-HT and 5-HIAA, MAO-A activity), including its turnover (pargyline-induced 5-HT accumulation and 5-HIAA declination rate), as well as plasma corticosterone were also observed depending on dosage(s) and duration(s) of erythrosine administration under similar experimental conditions. The lower dosage of erythrosine (1 mg/kg/day, p.o.) under similar conditions did not affect either of the above. These findings suggests (a) the induction as well as optimal effect of long-term erythrosine (artificial food color) on behavioral hyperactivity in parallel with increase in 5-HT level in brain regions, (b) the activation of brain regional serotonin biosynthesis in accordance with plasma corticosterone status under such behavioral hyperactivity, and (c) a possible inhibitory influence of the enhanced glucocorticoids–serotonin interaction on erythrosine-induced rearing motor hyperactivity in young adult mammals.

Keywords: *Erythrosine (artificial food colour); consecutive intake; motor activity; plasma corticosterone; brain regional serotonin metabolism*

Introduction

Erythrosine (CAS No. 16423-68-0, Chemical name/synonyms: Disodium 2-(2',4',5',7'-tetraiodo-3-oxido-6-oxoxanthen-9-yl) benzoate monohydrate/CI Acid Red 51; CI Food Red 14; D&C Red No. 3; FD & C Red No. 3; Erythrosine B or BS) (Budavari 1989; The Nordic Council of Ministers 2002), an artificial food color, is a highly lipid soluble fluorescein compound. Like other fluorescein compounds, it can cross the blood–brain-barrier (Levitan et al. 1984; Hirohashi et al. 1997) though the amount in the brain is restricted due to dye-plasma protein complex formation depending on the age and condition of the subject (Levitan et al. 1984). It also acts as an organic anion in the biological system and a fairly potent membrane active agent (Levitan 1977). Moreover, erythrosine increases the Ca^{+2} permeability in neural membrane (Colombini and Wu 1981; Heffron et al. 1984) and inhibits brain Mg^{2+}-ATPase, Na^{+}-K^{+}-ATPase (Wade et al. 1984), Ca^{+2}-ATPase (Watson and Haynes 1982), and high affinity ouabain binding (Silbergeld 1981; Hnatowich and Labella 1982; Swann 1982). This artificial food dye also potentiates the release of neurotransmitters (dopamine, GABA, serotonin, acetylcholine, norepinephrine, etc.) (Logan and Swanson 1979; Augustine and Levitan 1983; Wade et al. 1984) and inhibits dopamine uptake (Lafferman and Silbergeld 1979) in rat brain tissue. Long-term administration of erythrosine, like other artificial food colors, stimulates the exploratory behavior in juvenile as well as

Address for Correspondence: Professor Mrinal K. Poddar, PhD, DSc, Department of Biochemistry, University of Calcutta, 35, B. C. Road, Kolkata – 700 019, India. Tel: 91-33-24614959. Fax: 91-33-24614849. E-mail: mrinalkp@yahoo.com

(Received 01 February 2010; revised 29 March 2010; accepted 30 March 2010)

ISSN 1537-6516 print/ISSN 1537-6524 online © 2010 Informa UK Ltd
DOI: 10.3109/15376516.2010.483070

in adult animals (Tanaka 2001; 2006). The artificial food color(s)-induced hyperactive behavior in experimental animals supports the proposed hypothesis regarding the onset of behavioral hyperactivity including attention deficit hyperactivity disorders (ADHD) in human (particularly in young of age group 3–12 years) due to long-term consumption of artificial food additives (including artificial food colors), as reported by McCann et al. (2007) and others (Boris and Mandel 1994; Bateman et al. 2004; Schab and Trinh 2004); but possible neurochemical mechanisms have little been defined (Bateman et al. 2004; Schab and Trinh 2004; McCann et al. 2007). Studies (Hechtman 1994; Giros et al. 1996; Brennan and Arnsten 2008) over the past decades have led to the development of specific theories regarding the role of the dopaminergic system in the etiology of behavioral conditions and hyper (locomotor) activity. Recent past observations of Alex and Pehek (2007) suggest a positive modulatory role of central serotonin over the central dopaminergic activity. Cazalets et al. (2000), Branchereau et al. (2002), and Pflieger et al. (2002) also reveal that endogenous 5-HT is directly involved in the development and regulation of central locomotor pattern generation. Moreover, various classes of serotonergic receptors located in brain areas related to the motor control system (like striatum, medulla, hippocampus, frontal cortex, or spinal cord) modulate the neural circuit involved in exploratory behavior (Grailhe et al. 1999). Based on this information as well as our recent past observation (Dalal and Poddar 2009), the present authors aim to investigate the effect of long-term erythrosine administration on brain regional serotonin metabolism in relation to changes in exploratory behavior (rearing motor activity) in young adult mammals.

Materials and methods

Reagents

Erythrosine B (dye content 90%), serotonin (5-HT)-HCl, ninhydrin, semicarbazide-HCl corticosterone, and bovine serum albumin (BSA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Pargyline-HCl was obtained from Abbott laboratories (North Chicago, IL, USA). All other reagents used in the study were of analytical grade.

Animals and animal care

Young adult male albino rats (130–140 gm body wt.; 12–14 weeks of age) of Charles Foster strain were taken as experimental subjects. Animals were housed in a room having constant temperature (28 ± 0.5°C) and relative humidity (85 ± 5%) with 12 h light–dark cycle, and were maintained with standard laboratory food and water ad libitum. In the present study, the guidelines of the animal ethical committee of Department of Biochemistry, University of Calcutta (Registration no. 797/CPCSEA) were followed and all efforts were made to minimize the number of animals used and their suffering.

Experimental design

Rats were randomly divided into three groups (Groups I, II, and III) (Scheme 1). Each group was further divided into four sub-groups (sub-groups 2–4 were considered as experimental and sub-group 1 was considered as corresponding control). Experimental sub-groups 2, 3, and 4 were orally (p.o.) treated with erythrosine (1, 10, and 100 mg/kg/day, p.o., respectively, in 0.5 ml distilled water) for 15 or 30 consecutive days. Control rats (sub-group 1) of corresponding experimental groups were treated with the equivalent volume of vehicle of erythrosine (distilled water) through the same route under similar conditions. In the present study erythrosine is administered by the oral gavaging method so that a more precise amount of test compound can be delivered and the oral administration was achieved by using an animal intubation needle (18 G × 3" curved) (Gad 2007).

In order to observe the optimum time required for the maximum erythrosine-induced behavioral response, rats of Group-I (containing 8–12 rats in each sub-group) were treated with erythrosine (1, 10, or 100 mg/kg/day, p. o. in 0.5 ml) or its vehicle for 15 or 30 consecutive days, and motor activity (MA) was measured at different time points (0–9 h) after last erythrosine administration. In the present investigation dosages of erythrosine for consecutive days treatment were selected on the basis of our recent study (Dalal and Poddar 2009) and the studies of others (Abdel Aziz et al. 1997; Tanaka 2001; Tsuda et al. 2001; Sasaki et al. 2002).

Rats of Group II were used for estimation of neurobiochemical parameters and plasma corticosterone concentration. Neurobiochemical parameter and plasma corticosterone concentration was measured (using 4–6 rats in each sub-group of Group II) following 15 or 30 consecutive days of erythrosine administration.

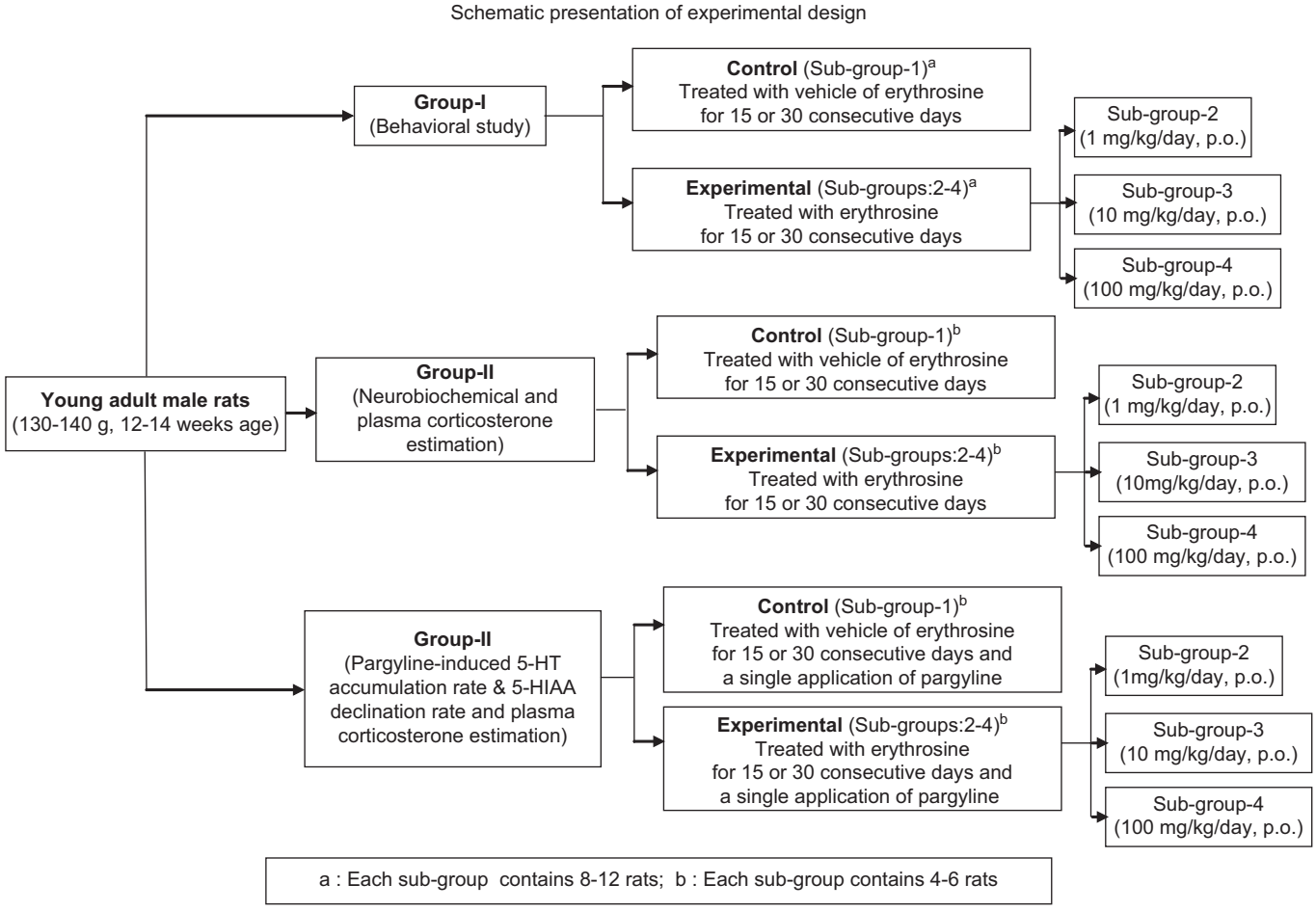
Animals of Group III (containing 12 rats in each sub-group and pre-treated with erythrosine (1, 10, and 100 mg/kg/day, p.o.) or its vehicle for 15 or 30 consecutive days as mentioned above) were used for determination of pargyline (75 mg/kg, i.p. in 0.2 ml saline)-induced 5-HT accumulation and 5-HIAA declination rates and pargyline-induced plasma corticosterone concentration. Control rats corresponding to Group III were treated with an equivalent volume (0.5 ml) of vehicle of erythrosine (distilled water) and/or saline (when required) through the same route under similar experimental condition(s).

Behavioral rating of rat motor activity (MA)

Vertical rearing motor activity of each animal was measured during a 5 min observation period to monitor the MA, as described elsewhere (Jamaluddin and Poddar 2003). The animals treated with either erythrosine or their respective controls (treated with distilled water) were gently transferred to a transparent plastic chamber ($24 \times 24 \times 20\ cm^3$) illuminated with an electrical lamp at the top. The vertical rearing frequency was measured by an electrical device based on the capacitance change proportional to the distance between the animal's head and probe following the method of Keenan and Johnson (1972).

Collection of brain tissue

Rats of both control and experimental groups were sacrificed by cervical dislocation between 9:00–9:30 am to avoid



Scheme 1. The schematic presentation of the experimental design.

circadian effect (if any), following 2 h of last erythrosine administration. After decapitation, brains were immediately taken out and were collected either in liquid nitrogen for estimation of steady state levels of 5-HT, 5-HIAA, or kept in ice-cold condition (0–4°C) for the enzyme (MAO-A) assay. Immediately after collection of the brains, different brain regions (including medulla-pons, hypothalamus, hippocampus, and corpus striatum) were rapidly dissected out following the method described by Poddar and Dewey (1980).

Estimation of neurobiochemical parameters

Steady state levels of 5-HT and 5-HIAA

Steady state levels of 5-HT and its metabolite 5-HIAA were estimated spectrophotofluorometrically in different brain regions following the method of Scapagnini et al. (1969). Rat brain tissues were homogenized (20 Vol) with 0.4 N perchloric acid (PCA) and EDTA (2.5 mg) and ascorbic acid (2.5 mg) were mixed with 1 ml of tissue homogenate. The homogenate was then centrifuged for 5 min at 2500 × g, the clear supernatant was collected and pH was adjusted to 6.8–7.0 with the addition of 40% K_2CO_3, 10% $ZnSO_4$, and 1 M NaOH. Then solid NaCl, 6 N HCl, and 10 ml of butyl acetate were added, mixed well, and centrifuged at 2500 × g for 5 min. Aqueous phase was used for the determination of 5-HT and organic phase was used for 5-HIAA determination.

Phosphate buffer (0.1 M) was added in the organic phase, shaken, and centrifuged again at 2500 × g for 5 min. Then concentrated HCl was added to the buffer phase and the fluorescence of 5-HIAA was spectrophotoflurometrically (Hitachi F-3010) measured with excitation and emission of 305 nm and 540 nm, respectively.

The pH of the aqueous phase was increased (pH 10) with 40% K_2CO_3 and then borate buffer (0.5 M, pH 10), n-butanol, and NaCl were added, mixed well, and centrifuged at 2500 × g for 5-min. The organic layer was then transferred to another centrifuged tube containing *n*-haptane and phosphate buffer, mixed well, and centrifuged at 2500 × g for 5 min. For 5-HT determination a known amount buffer layer was taken, mixed with ninhydrin solution (0.1 M), and incubated at 75°C for 30 min. Then fluorescence was measured spectrophotofluorometrically (Hitachi F-3010) at room temperature with an excitation and emission of 385 nm and 490 nm, respectively. Steady-state level of 5-HT and 5-HIAA were expressed as ng/ mg protein.

Assay of monoamine oxidase-A (MAO-A) activity

The pure mitochondria were obtained from different brain regions by sucrose density gradient centrifugation method, as described by Gray and Whittaker (1962). In brief, brain tissues were homogenized (10 Vol) in 0.32 M sucrose (pH 7.4)

and then centrifuged at 600 × g for 10 min. The supernatant was collected and centrifuged again at 8500 × g for 20 min. Then the crude mitochondrial pellet was collected and suspended in sucrose (0.32 M). After density gradient centrifugation, the pellet at the bottom containing mitochondria was re-suspended in 0.25 M sucrose and used as mitochondrial enzyme source.

MAO-A activity was spectrophotometrically measured using serotonin as substrate, according to the method of Green and Haughton (1961) and Guha (1966). The standard incubation mixture of a total volume of 2 ml consisting of phosphate buffer (0.1 M, pH 7.0), mitochondrial enzyme source (containing 0.2 mg protein), semicarbazide (0.125 M), and serotonin (0.1 M) was incubated for 30 min at 37°C. The reaction was then terminated with the addition of 0.1 M 2,4 Dinitro phenyl hydrazine (DNPH) in 2 N HCl. Enzyme blank was prepared by adding 2,4 DNPH before addition of substrate serotonin (0.1 M). The products formed were then extracted with benzene and the organic layer was then added into NaOH (0.4%), mixed well, and centrifuged at 2800 × g for 5 min. The benzene layer was removed and the aqueous layer was kept at 80°C for 10 min, then cooled at room temperature, and the color developed was read spectrophotometrically (Hitachi U-2010) at 450 nm against a reagent blank. All values were corrected against enzyme blanks and the MAO-A activity was expressed as ΔO.D./mg protein/h.

Estimation of plasma corticosterone

Immediately after sacrifice, trunk blood was collected in heparinized tubes and plasma was prepared by centrifuging at 800 × g for 10 min under cold (0–4°C) conditions. Corticosterone level of plasma was assayed according to the method of Purves and Sirett (1965). Briefly, plasma was mixed with distilled water and chloroform, shaken vigorously, and centrifuged (Sorvall RC-5B) at 800 × g for 5 min. The chloroform layer was taken and NaOH (0.1 N) was added, shaken vigorously, and the aqueous layer was removed by centrifugation as described above. Then the chloroform layer was taken and mixed with 2 ml of fluorescent reagent, shaken vigorously, and centrifuged (Sorvall RC-5B) at 800 × g for 5 min. The chloroform layer was removed completely. After 30 min at room temperature, the fluorescence was measured (Hitachi F-3010) with an excitation and emission of 470 nm and 520 nm, respectively. The corresponding blank and standard were prepared using water and pure corticosterone, respectively, in place of plasma. The plasma corticosterone level was expressed as μg corticosterone/100 ml plasma.

Estimation of accumulation rate of 5-HT and declination rate of 5-HIAA in brain regions

Accumulation rate of 5-HT and declination rate of 5-HIAA in the brain regions were determined by following the method of Tozer et al. (1966) using pargyline as a monoamine oxidase (MAO) inhibitor. Pargyline (75 mg/kg, i.p.) or its vehicle (saline) was injected into rats at various time intervals (30, 60, and 90 min) after the last erythrosine (1–100 mg/kg/day, p.o.) or its vehicle (distilled water) administration. Rats of both experimental and corresponding control were then sharply decapitated after 2 h of last erythrosine or its vehicle (control) administration and steady state levels of 5-HT and 5-HIAA in brain regions were determined according to the method of Scapagnini et al. (1969) (as described above). 5-HT accumulation rate and 5-HIAA declination rate were calculated from graphical presentation (not shown) of both control and experimental data (three separate observations) of 5-HT contents in rat brain regions obtained after every pargyline application in an individual set of rats (containing three rats in each set) at different time points (30, 60, and 90 min after last erythrosine administration) following the method of Tozer et al. (1966).

Protein estimation

Protein content of tissues was estimated spectrophotometrically following the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

Statistical analysis

Statistical significance between the mean values was assessed by analysis of variance (ANOVA) using Scheffe's multiple comparison *F*-test unless otherwise mentioned. Statistical significance between mean values was considered as $p < 0.05$.

Results

Erythrosine-induced changes in MA

Figure 1 depicts that lowest erythrosine dosage (1 mg/kg/day, p.o.) did not significantly influence the motor (rearing) activity (MA) of young adult male rats irrespective of duration of exposure. Only higher dosages of erythrosine increased MA with the increase of dosage (10 or 100 mg/kg/day, p.o.) and duration (15 or 30 consecutive days). The erythrosine-induced stimulation of MA was maximum at 2 h after last dosage of erythrosine administration under both 15 consecutive ($F(3, 34) = 25.111$, $p < 0.01$) and 30 consecutive ($F(3, 34) = 46.347$, $p < 0.01$) days and then gradually restored to basal (control) value with time. No significant difference was observed in MA following 9 h of last erythrosine administration in any of the dosage group with respect to their corresponding control.

Erythrosine-induced changes in steady state levels of 5-HT, 5-HIAA, and MAO-A activity

Figure 2(A–C) represents that lowest dosage (1 mg/kg/day, p.o.) of erythrosine was ineffective to alter the steady-state levels of 5-HT and 5-HIAA and MAO-A activity in any of the brain region studied (medulla-pons, hypothalamus, hippocampus, and corpus striatum). Higher dosage of erythrosine (10–100 mg/kg/day, p.o.), on the other hand, significantly increased brain regional steady-state level of 5-HT (Figure 2A) following 2 h of last dosage of erythrosine administration for 15 consecutive days (medulla-pons, $F(3, 18) = 14.497$, $p < 0.01$; hypothalamus, $F(3, 18) = 5.005$, $p < 0.01$; hippocampus, $F(3, 18) = 5.991$, $p < 0.01$; corpus striatum, $F(3, 18) = 3.436$, $p < 0.05$) as well as 30 consecutive days (medulla-pons,

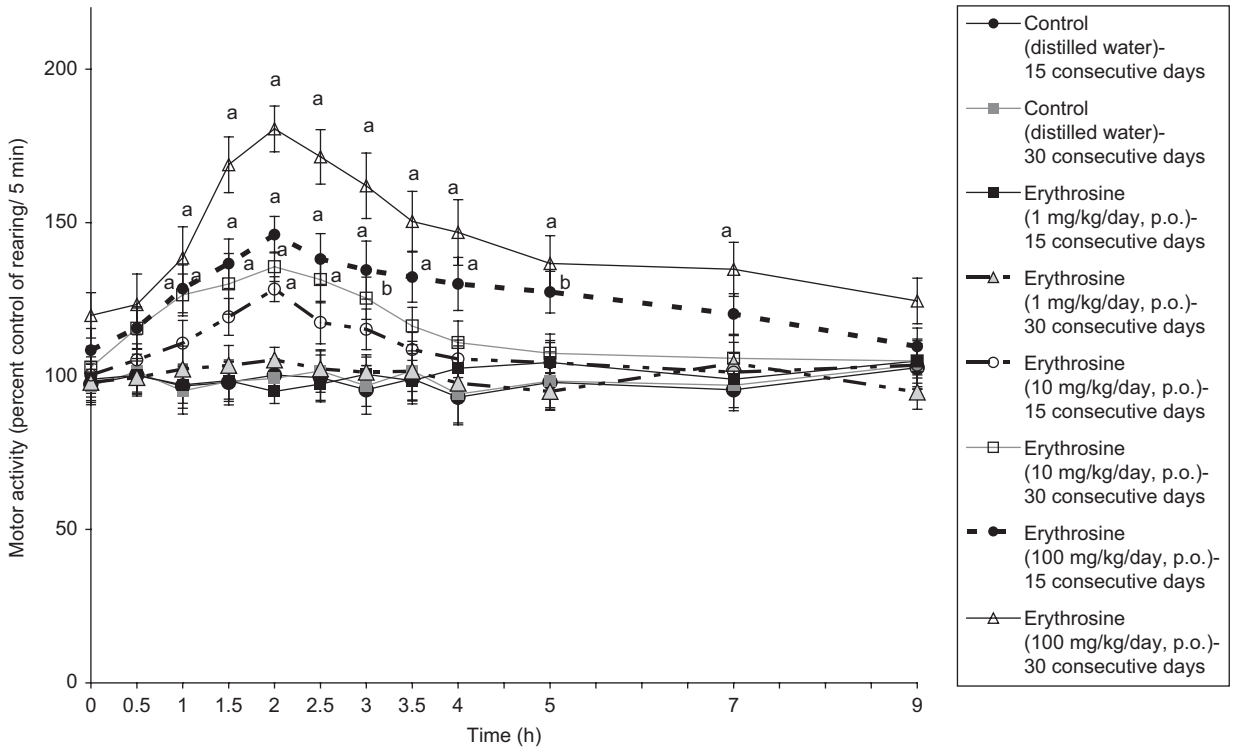


Figure 1. Effect of consecutive (15 or 30) days of erythrosine administration (1, 10, or 100 mg/kg/day, p.o.) on motor activity (MA) of young adult male rats at different time intervals. Each point represented as percentage control mean ± SEM of 8–12 separate observations (each observation was made from a single rat). Vertical line represents ± SEM. *X*-axis indicates time (h) after administration of erythrosine. MA (20.68 ± 1.46) of control rats (without any vehicle treatment) was considered as 100.0 ± 6.23 and the results were calculated accordingly. No significant change was observed between the control values corresponding to time of erythrosine exposure. Significantly different from corresponding control (treated with vehicle of erythrosine (distilled water)) $^{a}p<0.01$ and $^{b}p<0.05$.

F (3, 18) = 33.853, $p<0.01$; hypothalamus, F (3, 18) = 32.10, $p<0.01$; hippocampus, F (3, 18) = 14.611, $p<0.01$; corpus striatum, F (3, 18) = 11.255, $p<0.01$) with respect to their corresponding control. The degree of increase in brain region of 5-HT content was dependent on both dosages and duration of erythrosine exposure (Figure 2A). Unlike 5-HT, no significant change was observed in brain regional steady-state level of 5-HIAA with higher dosage of erythrosine (10–100 mg/ kg/ day, p.o.), irrespective of duration of exposure, in comparison to their corresponding control (Figure 2B). It is evident from Figure 2C that MAO-A activity was significantly decreased following long-term (30 consecutive days) exposure of erythrosine with only highest dosage (100 mg/kg/day, p.o.) in all the brain regions studied (medulla-pons, F (3, 18) = 15.09, $p<0.01$; hypothalamus, F (3, 18) = 5.283, $p<0.01$; hippocampus, F (3, 18) = 5.495, $p<0.01$; and corpus striatum, F (3, 18) = 3.274, $p<0.05$).

Erythrosine-induced changes in 5-HT accumulation rate and 5-HIAA declination rate

Tables 1 and 2 represent that lowest experimental dosage of erythrosine (1 mg/kg/day, p.o.) did not significantly affect pargyline-induced 5-HT accumulation rate (Table 1) and 5-HIAA declination rate (Table 2) in any of the brain region studied following 15 or 30 consecutive days of exposure. Higher dosage of erythrosine (10–100 mg/kg/ day, p.o.) significantly increased pargyline-induced 5-HT accumulation rate without affecting pargyline-induced 5-HIAA declination rate at 2 h after the last erythrosine administration following 15 consecutive (medulla-pons, F (3, 8) = 11.07, $p<0.01$; hypothalamus, F (3, 8) = 5.190, $p<0.05$; hippocampus, F (3, 8) = 4.705, $p<0.05$; and corpus striatum, F (3, 8) = 4.6780, $p<0.05$) as well as 30 consecutive (medulla-pons, F (3, 8) = 17.122, $p<0.01$; hypothalamus, F (3, 8) = 19.412, $p<0.01$; hippocampus, F (3, 8) = 9.817, $p<0.01$; and corpus striatum, F (3, 8) = 6.367, $p<0.05$) days with respect to their corresponding control (Tables 1 and 2).

Erythrosine-induced changes in plasma corticosterone concentration

It is evident from Figure 3 that erythrosine at its lowest experimental dosage (1 mg/kg/day, p.o.) was ineffective to produce any significant change in plasma corticosterone concentration following 15 or 30 consecutive days. Higher dosage of erythrosine (10–100 mg/kg/day, p.o.) administration, on the other hand, significantly increased plasma corticosterone level at 2 h of last erythrosine administration following both 15 consecutive (F (3, 18) = 5.276, $p<0.01$) and 30 consecutive (F (3, 18) = 10.511, $p<0.01$) days of administration with respect to their corresponding controls. Figure 3 also depicts that a single application of pargyline (non-specific MAO inhibitor) to erythrosine vehicle treated group increased plasma corticosterone concentration following 15 consecutive (F (1, 10) = 11.59, $p<0.01$) and 30 consecutive (F (1, 10) = 15.26,

$p < 0.01$) days of with respect to their corresponding control. In long-term erythrosine (1–100 mg/kg/day, p.o.) pre-treated rats, a single application of pargyline also produced a further significant increase in plasma corticosterone concentration under both 15 consecutive (F (3, 18) = 15.17, $p < 0.01$) and 30 consecutive (F (3, 18) = 25.28, $p < 0.01$) days of erythrosine administration with respect to their corresponding control treated with distilled water along with pargyline (as mentioned above).

Discussion

The present study investigates both vertical spontaneous rearing motor activity (vertical exploration; Keenan and Johnson 1972; Kelley 1993) and the brain regional neurobiochemical action of long-term erythrosine administration as observed under acute conditions of erythrosine treatment (Dalal and Poddar 2009) in the young adult male rats. Long-term (15 or 30 consecutive days) administration of erythrosine at lowest experimental dosage (1 mg/kg/day,

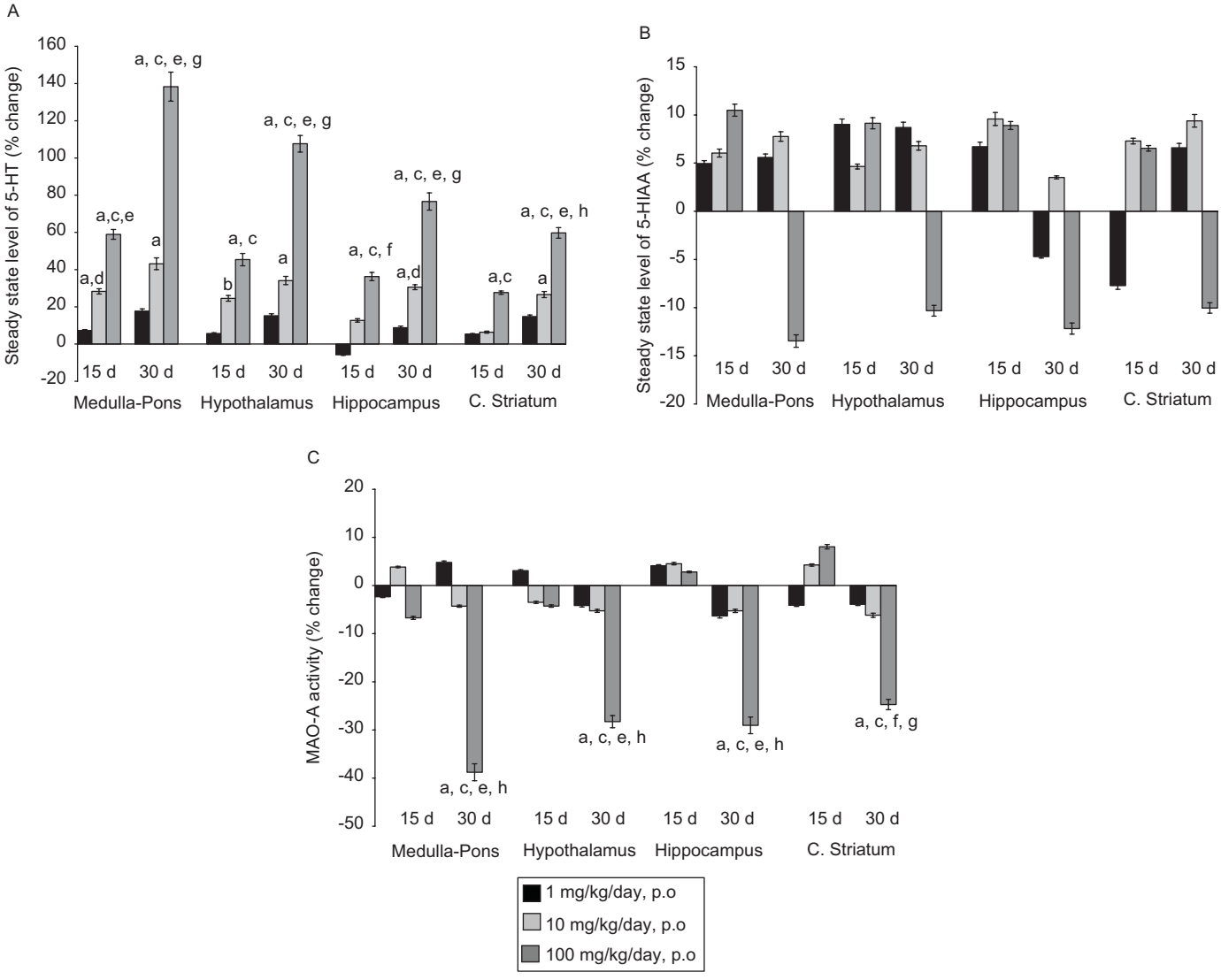


Figure 2. Effect of consecutive (15 or 30) days of erythrosine administration (1, 10, and 100 mg/kg/day, p.o.) on brain regional stead-state levels of 5-HT (A), 5-HIAA (B), and mitochondrial MAO-A activity (C) of young adult male rats. Results are expressed as percentage change (mean ± SEM) of 4–6 separate observations (each observation was made from a single rat) with respect to their corresponding controls. Vertical bars represent the percentage change and vertical lines shown on top of each bar represent ± SEM. Percentage change was calculated with respect to the corresponding 15 or 30 consecutive days control values. The control values of steady-state levels of 5-HT (ng/mg protein) in medulla-pons, hypothalamus, hippocampus, and corpus striatum were 11.476 ± 0.638, 9.5817 ± 0.52, 3.9712 ± 0.276, and 4.9575 ± 0.254, respectively, following 15 consecutive days, and 11.5213 ± 0.651, 9.4207 ± 0.476, 4.0591 ± 0.282, and 5.0784 ± 0.385, respectively, following 30 consecutive days of vehicle treatment. The control values of steady-state levels of 5-HIAA (ng/mg protein) in medulla-pons, hypothalamus, hippocampus, and corpus striatum were 5.7086 ± 0.30, 4.8064 ± 0.362, 3.0441 ± 0.23, and 4.6851 ± 0.24, respectively, following 15 consecutive days, and 5.5494 ± 0.345, 4.7491 ± 0.264, 3.0583 ± 0.184, and 4.7805 ± 0.25, respectively, following 30 consecutive days of vehicle treatment. The control values of mitochondrial MAO-A activity (ΔOD/mg protein/h) in medulla-pons, hypothalamus, hippocampus, and corpus striatum were 0.1457 ± 0.009, 0.1264 ± 0.006, 0.0463 ± 0.003, and 0.0609 ± 0.003, respectively, following 15 consecutive days, and 0.1443 ± 0.007, 0.1252 ± 0.008, 0.0475 ± 0.002, and 0.0615 ± 0.004, respectively, following 30 consecutive days of vehicle treatment. Significantly different from corresponding (i) control $^{a}p < 0.01$, $^{b}p < 0.05$; (ii) 1 mg/kg/day, p.o group $^{c}p < 0.01$, $^{d}p < 0.05$; (iii) 10 mg/kg/day, p.o group $^{e}p < 0.01$, $^{f}p < 0.05$; and (iv) 15 consecutive days treatment group $^{g}p < 0.01$, $^{h}p < 0.05$.

p.o.) though fails to produce any significant effect on motor activity (MA) in young adult male rats, higher dosage of erythrosine (10 or 100 mg/ kg/day, p.o.) produces behavioral (rearing) hyperactivity in a dosage and duration-dependent manner (Figure 1). The motor hyperactivity is maximized at 2 h after last erythrosine dosage, but diminishes with time and restores to base value depending on the conditions (dosage and duration) of erythrosine administration (Figure 1). The behavioral activity in the mammalian system is potentially regulated by central neurotransmitters—serotonin and dopamine (Cazalets et al. 2000; Branchereau et al. 2002; Pflieger et al. 2002; Dalley et al. 2008). Moreover, central serotonin (5-HT), by modulating its several classes of 5-HT receptors as well as the activities of brain dopaminergic neurons, regulates locomotor activities (Bankson and Cunningham 2001; Alex and Pehek 2007). In mammalian brain, functional output of 5-HT neuron again varies in different brain regions (Waterhouse et al. 1986; Jacobs et al. 2002). Therefore, in the present investigation brain regions (including medulla-pons, hypothalamus, hippocampus, and corpus striatum) are selected to study the serotonin metabolism including its turnover at 2 h (maximum behavioral hyperactivity) after last dosage of long-term (15 or 30 consecutive days) erythrosine (1–100 mg/kg/day, p.o.) administration in young adult mammals.

Long-term (15 or 30 consecutive days) erythrosine administration at lowest experimental dosage (1 mg/ kg/ day, p.o.) fails to produce any significant alteration in brain regional serotonin metabolism (Figure 2, Tables 1 and 2). Unlike lowest dosage, comparatively higher dosage of erythrosine

Table 1. Effect of consecutive days of oral administration of erythrosine on pargyline-induced accumulation rate of 5-HT in rat brain regions.

Dosage of Erythrosine (mg/kg/day, p.o.)	Duration of treatment (consecutive days)	Pargyline-induced accumulation rate of 5-HT (percent control)			
		Medulla-pons	Hypothalamus	Hippocampus	Corpus striatum
1 mg	15	104.62 ± 7.787	103.72 ± 9.5207	97.07 ± 7.88	105.62 ± 5.051
	30	118.64 ± 6.417	110.44 ± 5.657	103.39 ± 6.53	111.63 ± 9.349
10 mg	15	129.07 ± 7.148^{a,d}	125.29 ± 8.98^{b}	117.29 ± 10.967	106.70 ± 5.695
	30	138.70 ± 3.352^{a}	129.48 ± 8.458^{a}	126.40 ± 5.1937^{a,d}	130.23 ± 4.953^{a}
100 mg	15	152.11 ± 7.226^{a,c}	140.68 ± 8.772^{a,c}	136.32 ± 5.4675^{a,c}	129.83 ± 6.671^{a,c,f}
	30	186.34 ± 14.972^{a,c,e,g}	189.91 ± 8.16^{a,c,e,g}	144.63 ± 7.62^{a,c}	138.40 ± 6.28^{a,d}

Results are expressed as mean ± SEM of three separate observations (each observation was made from three rats). Pargyline (75 mg/kg) dissolved in 0.2 ml of normal saline (0.9% NaCl) was administered interperitonially (i.p.) at various time intervals (30, 60, and 90 min) following last erythrosine (1, 10, or 100 mg/kg/day, p.o.) or its vehicle (distilled water) administration. Rats treated with distilled water (vehicle of erythrosine) were considered as the control of the corresponding experimental groups. All rats (experimental or corresponding control) were sacrificed following 2 h of last erythrosine or its vehicle (distilled water) administration.

Accumulation rates were calculated from graphical presentation (not shown) of both control and experimental data (three separate observations) of 5-HT contents in rat brain regions obtained after every pargyline application in individual set of rats (containing three rats in each set) at different time points (30, 60, and 90 min after last erythrosine administration) following the method of Tozer et al. (1966). Percent controls were calculated with respect to the corresponding control (treated with distilled water) which was considered as 100, and results were calculated accordingly. The control values (vehicle treatment) of pargyline-induced accumulation rate of 5-HT (ng/mg protein/h) in medulla-pons, hypothalamus, hippocampus, and corpus striatum were 10.3380 ± 0.699, 9.222 ± 0.528, 3.1458 ± 0.256, and 4.197 ± 0.286 following 15 consecutive days, and 10.3785 ± 0.701, 9.0679 ± 0.556, 3.2154 ± 0.229, and4.30 ± 0.271 following 30 consecutive days, respectively.

Significantly different from corresponding (i) control $^{a}p < 0.01$, $^{b}p < 0.05$; (ii) 1 mg/kg/day, p.o group $^{c}p < 0.01$, $^{d}p < 0.05$; (iii) 10 mg/kg/day, p.o group $^{e}p < 0.01$, $^{f}p < 0.05$; and (iv) 15 consecutive days treatment group $^{g}p < 0.01$.

Table 2. Effect of consecutive days of oral administration of erythrosine on pargyline-induced declination rate of 5-HIAA in rat brain regions.

Dose of Erythrosine (mg/kg/day, p.o.)	Duration of treatment (consecutive days)	Pargyline-induced declination rate of 5-HIAA (percent control)			
		Medulla-pons	Hypothalamus	Hippocampus	Corpus striatum
1 mg	15	93.71 ± 5.46	95.04 ± 6.26	97.09 ± 5.39	102.91 ± 5.26
	30	97.47 ± 7.08	96.19 ± 5.57	97.54 ± 3.90	90.09 ± 7.20
10 mg	15	96.62 ± 6.33	94.26 ± 5.89	95.79 ± 4.04	96.08 ± 5.34
	30	86.74 ± 3.78	88.40 ± 5.52	91.33 ± 7.02	95.60 ± 6.58
100 mg	15	84.71 ± 4.96	87.95 ± 4.79	93.51 ± 4.38	103.19 ± 6.30
	30	79.98 ± 5.80	84.13 ± 5.24	83.37 ± 5.07	89.03 ± 5.84

Results are expressed as mean ± SEM of three separate observations (each observation was made from three rats). Pargyline (75 mg/kg) dissolved in 0.2 ml of normal saline (0.9% NaCl) was administered interperitonially (i.p.) at various time intervals (30, 60, and 90 min) following last erythrosine (1, 10, or 100 mg/kg/day, p.o.) or its vehicle (distilled water) administration. Rats treated with distilled water (vehicle of erythrosine) were considered as the control of the corresponding experimental groups. All rats (experimental or corresponding control) were sacrificed following 2 h of last erythrosine or its vehicle (distilled water) administration.

Declination rates were calculated from graphical presentation (not shown) of both control and experimental data (three separate observations) of 5-HIAA contents in rat brain regions obtained after every pargyline application in individual set of rats (containing three rats in each set) at different time points (30, 60, and 90 min after last erythrosine administration) following the method of Tozer et al. (1966). Percentage controls were calculated with respect to the corresponding control (treated with distilled water) which was considered as 100, and results were calculated accordingly. The control values of pargyline-induced declination rate of 5-HIAA (ng/mg protein/h) in medulla-pons, hypothalamus, hippocampus, and corpus striatum were 9.291 ± 0.57, 8.7569 ± 0.52, 3.1066 ± 0.190, and 3.4431 ± 0.225 following 15 consecutive days, and 9.3273 ± 0.432, 8.6106 ± 0.433, 3.1753 ± 0.178, and 3.5276 ± 0.219 following 30 consecutive days, respectively.

No significant difference was observed with respect to their corresponding controls.

(10 mg/ kg/ day, p.o. or more) significantly increases steady-state level of 5-HT in medulla-pons and hypothalamus without affecting the other two brain regions studied following 15 consecutive days of exposure (Figure 2A). Prolonged (30 consecutive days) administration of erythrosine with the same dosage not only increases medulla-pons and hypothalamic steady state level of 5-HT but also enhances 5-HT level of hippocampus and corpus striatum either by increasing 5-HT synthesis and/or by decreasing its catabolism in these brain regions (Figure 2A). This thought can be supported by a significant increase in pargyline-induced 5-HT accumulation rate (Table 1) without affecting its 5-HIAA content (Figure 2B), 5-HIAA declination rate (Table 2), or MAO-A activity (Figure 2C), and a significant decrease in the ratio of steady-state level of 5-HIAA over 5-HT (Table 3) in brain regions. The degree of potency of erythrosine (10 mg/ kg/ day, p.o.)-induced effect on brain regional serotonin metabolism further enhances with greater erythrosine dosage (100 mg/ kg/ day, p.o.) (Figure 2, Tables 1–3). Therefore, long-term erythrosine (10–100 mg/ kg/ day, p.o.) administration produces such an alteration in neurochemical mechanisms where system demands more and more accumulation of 5-HT in rat brain regions with an increase of erythrosine dosage (Figure 2, Tables 1 and 2). This thought may be supported by the significant decrease in brain regional MAO-A activity (Figure 2C) following 30 consecutive days of erythrosine (100 mg/kg/ day, p.o.) administration. Such conditions in brain regional MAO-A (either no change or significant reduction, Figure 2C) activity may potentially increase the 5-HT receptor activity by promoting more release of 5-HT in the extracellular region (Celada and Artigas 1993; Dringenberg et al. 1995). This increase in brain regional serotonin content in long-term erythrosine treated rats may be explained as stress-induced changes, because plasma corticosterone concentration (Figure 3) is enhanced under similar experimental conditions. Studies also show that repeated exposure to a stressor enhances the corticotropin-releasing factor (CRF)-related sensitization of serotonergic neurons as well as their neural transmission in brain regions (Chamas et al. 2004; Berton and Nestler 2006; McEuen et al. 2008) and plasma glucocorticoid (corticosterone in rodents, cortisol in humans) level (Lowry et al. 2001; Lora et al. 2007; McEuen et al. 2008)—one of the major prevalences of stress-induced behavioral changes in mammalian system (Maier and Watkins 2005). As stress-induced increase in corticosterone level elevates the activity of tryptophan hydroxylase-2 (TPH2, the rate-limiting enzyme of serotonin synthesis) (McEuen et al. 2008), the present, high plasma corticosterone level in long-term erythrosine (10–100 mg/kg/day, p.o.) treated-rats (Figure 3) may influence brain regional serotonin content (Figure 2) as well as 5-HT synthesis rate (Table 1). Such increases in TPH2 may serve to meet a higher 5-HT demand (Tan et al. 2004) in brain regions necessary during long-term erythrosine administration, which may be further supported by enhanced brain regional 5-HT synthesis rate (Table 1; Korte-Bouws et al. 1996) under the present experimental conditions. Comparing the accumulation of 5-HT (index of 5-HT synthesis) particularly in medulla-pons and hypothalamus (Table 1) with linear increase in plasma corticosterone concentration (Figure 3) under a single application of pargyline in long-term erythrosine-treated rats may indicate a shift of CRF tone (as indicated by increase in plasma corticosterone status, Figure 3) that in turn may elevate the 5-HT content and its synthesis (Figure 2, Table 1) in brain regions.

The present study, therefore, first time evidentially represents the induction as well as optimal effect of long-term erythrosine (artificial food color) administration on behavioral hyper activity (Figure 1) in parallel with activation of brain regional serotonin metabolism including its synthesis (Figure 2, Table 1), possibly mediated by high plasma corticosterone status (Figure 3) in young adult male rats. Unlike our previous report of short-term erythrosine exposure that shows low serotonergic neural transmission reduces exploratory behavior (Dalal and Poddar 2009); repeated (long-term) erythrosine administration to young adult male rats displays hyperactivity in exploratory behavior (Figure 1) with stimulation in brain regional serotonin metabolism (Figure 2) including its synthesis (Table 1). Such discrepancy between short- and long-term erythrosine-induced effect on behavioral activity in relation to brain regional serotonin in young adult mammals can be explained by the plasma corticosterone status (McEuen et al. 2008), which is increased following a single administration of highest erythrosine dosage (data not presented) and repeatedly activated by consecutive erythrosine administration (Figure 3) and, thereby, may have the ability to activate 5-HT synthesis rate (Table 1) and 5-HT

Table 3. Effect of consecutive days of erythrosine administration on 5-HIAA/5-HT ratio in rat brain regions.

Dose of Erythrosine (mg/kg/day)	Duration of treatment (consecutive days)	5-HIAA/5-HT ratio (% change)			
		Medulla-pons	Hypothalamus	Hippocampus	Corpus striatum
1 mg/kg/day	15	(–)2.17 ± 0.107	(+)3.25 ± 0.17	(+)13.19 ± 0.834	(–)12.42 ± 0.885
	30	(–)10.28 ± 0.59	(–)5.61 ± 0.35	(–)12.40 ± 0.94	(–)7.15 ± 0.484
10 mg/kg/day	15	(–)17.37 ± 0.90	(–)15.97 ± 1.09	(–)2.77 ± 0.172	(+)0.875 ± 0.049
	30	(–)27.10 ± 2.05a	(–)20.37 ± 1.28	(–)22.06 ± 0.96[b]	(–)13.59 ± 0.589
100 mg/kg/day	15	(–)30.48 ± 1.090a	(–)24.92 ± 1.84[b]	(–)20.09 ± 1.05	(–)16.54 ± 1.136
	30	(–)63.69 ± 4.97a	(–)56.81 ± 4.25[a]	(–)50.27 ± 3.09[a]	(–)43.67 ± 2.60[a]

Results are expressed as mean ± SEM of 4–6 separate observations. Percentage changes were calculated with respect to the corresponding control treated with distilled water. The control values of 5-HIAA/5-HT ratio in medulla-pons, hypothalamus, hippocampus, and corpus striatum were 0.4974 ± 0.021, 0.5016 ± 0.029, 0.7665 ± 0.046, and 0.9450 ± 0.060 following 15 consecutive days, and 0.4817 ± 0.023, 0.5041 ± 0.027, 0.7534 ± 0.044, and 0.9413 ± 0.056 following 30 consecutive days, respectively. The ratios of 5-HIAA/5-HT in brain regions were calculated from the respective steady-state values as represented in figures 2a and b.

Significantly different from corresponding controls [a]$p < 0.01$, [b]$p < 0.05$.

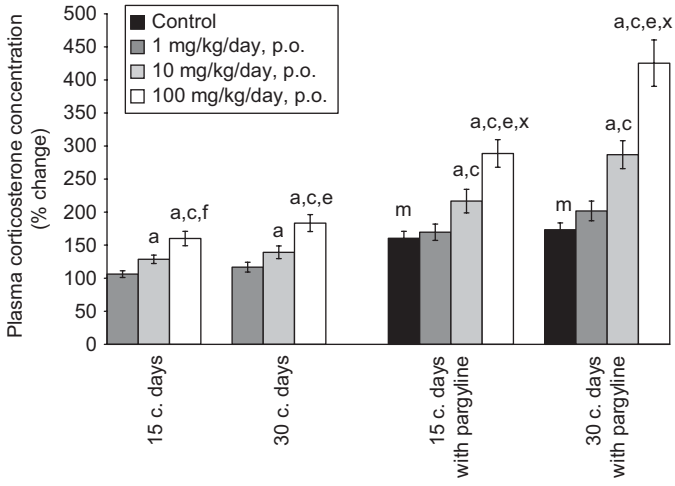


Figure 3. Effect of long-term (15 or 30 consecutive days) erythrosine (1–100 mg/kg/day, p.o) administration with or without pargyline (75 mg/ kg, i.p.) application on plasma corticosterone concentration in young adult male albino rats. Pargyline (75 mg/kg) dissolved in 0.2 ml of normal saline (0.9% NaCl) was administered interperitonially (i.p.) 10 min after last erythrosine (1, 10, or 100 mg/kg/day, p.o.) or its vehicle (distilled water) administration. All rats (experimental or corresponding control) were sacrificed following 2 h of last erythrosine or its vehicle administration. Results are expressed as percentage change (mean ± SEM) of 4–6 separate observations (each observation was made from a single rat) with respect to their corresponding controls. Vertical bars represent the mean and vertical lines shown on top of each bar represent ± SEM. The control values of plasma corticosterone concentration (μg/100 ml plasma) were 20.163 ± 1.01 and 20.76 ± 1.06 following 15 and 30 consecutive days of vehicle administration, respectively, and 32.35 ± 1.15 and 34.95 ± 1.16 following 15 and 30 consecutive days of vehicle of erythrosine along with pargyline administration, respectively. Significantly different with respect to corresponding control rats $^{a}p < 0.01$, treated with either distilled water or distilled water + single application of pargyline, as applicable; and corresponding (i) 1 mg/kg/day treatment group $^{c}p < 0.01$; (ii) 10 mg/kg/day treatment group $^{e}p < 0.01$, $^{f}p < 0.01$; and (iii) 15 consecutive days treatment group $^{x}p < 0.01$ treated with either erythrosine or erythrosine + single application of pargyline, as applicable. Significant difference between control groups treated with distilled water and distilled water + single application of pargyline $^{m}p < 0.01$.

content (Figure 2A) in brain regions (McEuen et al. 2008). The reduction in motor activity of young adult mammals following single erythrosine exposure (Dalal and Poddar 2009) correlates with the hypothesis that, in certain environments, reduction in brain serotonergic activity reduces behavioral activity, particularly exploratory behavior (Traversa et al. 1985; Dringenberg et al. 1995). Studies, on the other hand, show that activation of brain 5-HT inhibits behavioral hyperactivity in mammals (Gainetdinov et al. 1999). Therefore, in long-term erythrosine-treated rats higher glucocorticoids-serotonin interaction (Figures 2 and 3, Table 1), if not directly involved in the onset of behavioral hyperactivity, may have an inhibitory influence on erythrosine-induced rearing motor hyperactivity (Figure 1). Such inhibitory influence of serotonin may prevent the prolongation of erythrosine-induced behavioral hyperactive state and restore the behavioral activity to the baseline with time (Figure 1) (Gainetdinov et al. 1999).

Conclusion

Long-term erythrosine administration at (i) low dosage (1 mg/kg/day, p.o.) is not effective to change normal motor behavior, brain regional serotonin metabolism, as well as plasma corticosterone concentration, (ii) higher dosage (≥ 10 mg/kg/day, p.o.) stimulates brain regional serotonin metabolism in accordance with plasma corticosterone concentration in behavioral hyperactivity in young adult male albino rats. Our present investigation, therefore, positively throws some light on possible neurobiochemical mechanisms involving brain serotonin and plasma corticosterone status, behind such artificial food color(s)-induced behavioral hyperactivity as reported earlier (Weiss 1986; Boris and Mandel 1994; Tanaka 2001; Bateman et al. 2004; Schab and Trinh 2004; McCann et al. 2007; Stein et al. 2007). Altogether the present study may indicate the importance of these glucocorticoid-serotonin interactions in the behavioral hyperactivity resulting from long-term erythrosine exposure. The physiological effects and the significance of any artificial food additive(s) depends on a number of factors including the amount of any particular additive(s) present in food, the consumed-amount of that particular food (containing artificial additive(s)) and the sensitivity of the consumer to that particular food additive(s) (Larsen 2006). Of course the present effective dosage of erythrosine (10 mg/kg/day, p.o) that initiates behavioral hyperactivity in young adult rats is much higher than the present ADI of erythrosine (0.1 mg/ kg/b.wt) (JECFA 1991; Larsen 2006) and may apparently indicate no attributable behavioral disturbance with consumption of erythrosine within ADI. Again, studies show that human subjects are more sensitive to erythrosine rather than experimental mammals (Larsen 2006). Therefore, the implications of the present results could be substantial for future human trials (Bateman et al. 2004; Schab and Trinh 2004; McCann et al. 2007). Again, serotonin is perhaps best known as a neurotransmitter that modulates virtually all human behavioral processes (Berger et al. 2009). Therefore, future study at the level of specific 5-HT receptors activity in the regulation of motor behavior along with their interaction with plasma corticosterone or other central neurotransmitter system(s) following long-term consumption of erythrosine will throw additional light.

Acknowledgements

Arindam Dalal is a Senior Research Fellow of ICMR, New Delhi, India. The authors are also thankful to Professor S. Sengupta, Department of Statistics, University of Calcutta, for helping to evaluate the data by statistical analysis.

Declaration of interest

The present work was supported by the Indian Council of Medical Research, New Delhi, India, University Grants Commission (UGC), New Delhi, India, and University of Calcutta, Kolkata, India. The authors report no conflicts of

interest. The authors alone are responsible for the content and writing of the paper.

References

Abdel Aziz AH, Shouman SA, Attia AS, Saad SF. 1997. A study on the reproductive toxicity of erythrosine in male mice. Pharmacol Res 35:457–462.

Alex KD, Pehek EA. 2007. Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol Ther 113:296–320.

Augustine GJ, Levitan H. 1983. Neurotransmitter release and nerve terminal morphology at the frog neuromuscular junction affected by the dye Erythrosin B. J Physiol 334:47–63.

Bankson MG, Cunningham KA. 2001. 3,4-Methylenedioxymethamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. J Pharmacol Exp Ther 297:846–852.

Bateman B, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald C, Stevenson J. 2004. The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch Dis Child 89:506–511.

Berger M, Gray JA, Roth BL. 2009. The expanded biology of serotonin. Annu Rev Med 60:355–366.

Berton O, Nestler EJ. 2006. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137–151.

Boris M, Mandel FS. 1994. Foods and additives are common causes of the attention deficit hyperactive disorder in children. Ann Allergy 72: 462–468.

Branchereau P, Chapron J, Meyrand P. 2002. Descending 5-hydroxytryptamine raphe inputs repress the expression of serotonergic neurons and slow the maturation of inhibitory systems in mouse embryonic spinal cord. J Neurosci 22:2598–2606.

Brennan AR, Arnsten AF. 2008. Neuronal mechanisms underlying attention deficit hyperactivity disorder: the influence of arousal on prefrontal cortical function. Ann N Y Acad Sci 1129:236–245.

Budavari S. 1989. The Merck Index: An encyclopedia of chemicals, drugs and biologicals. Rahway, NJ: Merck & Co.

Cazalets JR, Gardette M, Hilaire G. 2000. Locomotor network maturation is transiently delayed in the MAOA-deficient mouse. J Neurophysiol 83:2468–2470.

Celada P, Artigas F. 1993. Monoamine oxidase inhibitors increase preferentially extracellular 5-hydroxytryptamine in the midbrain raphe nuclei. A brain microdialysis study in the awake rat. Naunyn-Schmiedeberg's Arch Pharmacol 347:583–590.

Chamas FM, Underwood MD, Arango V, Serova L, Kassir SA, Mann JJ, Sabban EL. 2004. Immobilization stress elevates tryptophan hydroxylase mRNA and protein in the rat raphe nuclei. Biol Psychiatry 55: 278–283.

Colombini M, Wu CY. 1981. A food dye, erythrosine B, increases membrane permeability to calcium and other ions. Biochim Biophys Acta 648:49–54.

Dalal A, Poddar MK. 2009. Short-term Erythrosine B-induced inhibition of the brain regional serotonergic activity suppresses motor activity (exploratory behavior) of young adult mammals. Pharmacol Biochem Behav 92:574–582.

Dalley JW, Mar AC, Economidou D, Robbins TW. 2008. Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. Pharmacol Biochem Behav 90:250–260.

Dringenberg HC, Hargreaves EL, Baker GB, Cooley RK, Vanderwolf CH. 1995. p-Chlorophenylalanine-induced serotonin depletion: reduction in exploratory locomotion but no obvious sensory-motor deficits. Behav Brain Res 68:229–237.

Gad SC. 2007. Aminal model in toxicology. 2nd Ed. Broken Sound Parkway, NW, Boca Raton: CRC press.

Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG. 1999. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 283:397–401.

Giros B, Jaber M, Jones S, Wightman R M, Caron M. 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606–612.

Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, Geyer MA, Hen R. 1999. Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. Neuron 22:581–591.

Gray EG, Whittaker VP. 1962. The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. J Anat 96:79–88.

Green AL, Haughton TM. 1961. A colorimetric method for the estimation of monoamine oxidase. Biochem J 78:172–175.

Guha SR. 1966. Inhibition of purified rat liver mitochondrial monoamine oxidase by trans-2-phenyl-cyclopropylamine. Biochem Pharmacol 15:161–168.

Hechtman L. 1994. Genetic and neurobiological aspects of attention deficit hyperactive disorder: a review. J Psychiatry Neurosci 19:193–201.

Heffron JJ, O'Callaghan AM, Duggan PF. 1984. Food dye, erythrosin B, inhibits ATP-dependent calcium ion transport by brain microsomes. Biochem Int 9:557–562.

Heisler LK, Pronchuk N, Nonogaki K, Zhou L, Raber J, Tung L, Yeo GS, O'Rahilly S, Colmers WF, Elmquist JK, Tecott LH. 2007. Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. J Neurosci 27:6956-6964.

Hirohashi T, Terasaki T, Shigetoshi M, Sugiyama Y. 1997. In vivo and in vitro evidence for nonrestricted transport of 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein tetraacetoxymethyl ester at the blood-brain barrier. J Pharmacol Exp Ther 280:813–819.

Hnatowich M, Labella FS. 1982. Light-enhanced inhibition of ouabain binding to digitalis receptor in rat brain and guinea-pig heart by the food dye erythrosine. Mol Pharm 22:687–692.

Jacobs BL, Martín-Cora FJ, Fornal CA. 2002. Activity of medullary serotonergic neurons in freely moving animals. Brain Res Rev 40:45–52.

Jamaluddin S, Poddar MK. 2003. Brain regional gamma-aminobutyric acid (GABA) and locomotor activity: effect of long-term aldrin exposure. Pol J Pharmacol 55:371–382.

JECFA, WHO Food Additives Series 28. 1991. Toxicology evaluation of certain food additives and contaminants. Joint FAO/WHO Expert Committee on Food Additives. International Programme on Chemical Safety (IPCS). Geneva: WHO. p 171–181.

Keenan A, Johnson FN. 1972. Development of behavioural tolerance to nicotine in rat. Experientia 28:428–429.

Kelley AE. 1993. Locomotor activity and exploration. In: Sahgal A, ed. Behavioral neuroscience. A practical approach. Vol. 2. New York: IRL Press. p 1–4.

Korte-Bouws GA, Korte SM, De Kloet ER, Bohus B. 1996. Blockade of corticosterone synthesis reduces serotonin turnover in the dorsal hippocampus of the rat as measured by microdialysis. J Neuroendocrinol 8:877–881.

Lafferman JA, Silbergeld EK. 1979. Erythrosin B inhibits dopamine transport in rat caudate synaptosomes. Science 205:410–412.

Larsen JC. 2006. Risk assessment of chemicals in European traditional foods. Trends Food Sci Technol 17:471–481.

Levitan H. 1977. Food, drug, and cosmetic dyes: biological effects related to lipid solubility. Proc Natl Acad Sci USA 74:2914–2918.

Levitan H, Ziylan Z, Smith QR, Takasato Y, Rapoport SI. 1984. Brain uptake of a food dye, erythrosin B, prevented by plasma protein binding. Brain Res 322:131–134.

Logan WJ, Swanson JM. 1979. Erythrosin B inhibition of neurotransmitter accumulation by rat brain homogenate. Science 206:363–364.

Lowry CA, Burke KA, Renner KJ, Moore FL, Orchinik M. 2001. Rapid changes in monoamine levels following administration of corticotropinreleasing factor or corticosterone are localized in the dorsomedial hypothalamus. Horm Behav 39:195–205.

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. 1951. Protein measurement with Folin-phenol reagent. J Biol Chem 193:265–275.

Maier SF, Watkins LR. 2005. Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev 29:829–841.

McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, Kitchin E, Lok K, Porteous L, Prince E, Sonuga-Barke E, Warner JO, Stevenson J. 2007. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. Lancet 370:1560–1567.

McEuen JG, Beck SG, Bale TL. 2008. Failure to mount adaptive responses to stress results in dysregulation and cell death in the midbrain raphe. J Neurosci 28:8169–8177.

Pflieger JF, Clarac F, Vinay L. 2002. Postural modifications and neuronal excitability changes induced by a short-term serotonin depletion during neonatal development in the rat. J Neurosci 22:5108–5117.

Poddar MK, Dewey WL. 1980. Effect of cannabinoids on catecholamine uptake and release in hypothalamus and striatal synaptosomes. J Pharmacol Exp Ther 214:63–67.

Purves HD, Sirett NE. 1965. Assay of corticotrophin in dexamethasone-treated rats. Endocrinology 77:366–374.

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. 2002. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res 519:103–119.

Scapagnini U, Vandenbroeck R, De Schaepdryver A. 1969. Simultaneous estimation of 5-hydroxytryptamine and 5-hydroxyindol-3-acetic acid in rat brain. Biochem Pharmacol 18:938–940.

Schab DW, Trinh NH. 2004. Do artificial food colors promote hyperactivity in children with hyperactive syndromes? A meta-analysis of double-blind placebo-controlled trials. J Dev Behav Pediatr 25:423–434.

Silbergeld EK. 1981. Erythrosin B is a specific inhibitor of high affinity 3H-ouabain binding and ion transport in rat brain. Neuropharmacy 20: 87–90.

Stein DJ, Fan J, Fossella J, Russell VA. 2007. Inattention and hyperactivity-impulsivity: psychobiological and evolutionary underpinnings of ADHD. CNS Spectr 12:190,193–196.

Swann, AC. 1982. Brain (Na+-K+)-ATPase: biphasic interaction with erythrosin B. Biochem Pharm 31:2185–2190.

Tan H, Zhong P, Yan Z. 2004. Corticotropin-releasing factor and acute stress prolongs serotonergic regulation of GABA transmission in prefrontal cortical pyramidal neurons. J Neurosci 24:5000–5008.

Tanaka T. 2001. Reproductive and neurobehavioural toxicity study of erythrosine administered to mice in the diet. Food Chem Toxicol 39: 447–454.

Tanaka T. 2006. Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. Food Chem Toxicol 44:179–187.

The Nordic Council of Ministers 2002. Food additives in Europe 2000—Status of safety assessments of food additives presently permitted in the EU. Copenhagen: The Nordic Council of Ministers, Tema Nord. Available online at: www.norden.org

Traversa U, Puppini P, Jacquot C, Vertua R. 1985. Effect of an atypical barbiturate, the 2-allophanyl-2-allyl-4-valerolactone (valofan), on exploratory behaviour and brain serotonin concentrations in mice. J Pharmacol 16:279–290.

Tozer TN, Neff NH, Brodie BB. 1966. Application of steady state kinetics to the synthesis rate and turnover time of serotonin in rat brain of normal and reserpine-treated rats. J Pharmacol Exp Ther 153:177–182.

Tsuda S, Murakami M, Matsusaka N, Kano K, Taniguchi K, Sasaki YF. 2001. DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicol Sci 61:92–99.

Wade PD, Marder E, Siekevitz P. 1984. Characterization of transmitter release as a response of vertebrate neural tissue to erythrosin B. Brain Res 305:259–270.

Waterhouse BD, Mihailoff GA, Baack JC, Woodward DJ. 1986. Topographical distribution of dorsal and median raphe neurons projecting to motor, sensorimotor and visual cortical areas in the rat. J Comp Neurol 249: 460–476.

Watson BD, Haynes DH. 1982. Structural and functional degradation of Ca^{2+}:Mg^{2+} -ATPase rich sarcoplasmic reticulum vesicles photosensitized by erythrosin B. Chem Biol Interact 41:313–325.

Weiss B. 1986. Food additives as a source of behavioral disturbances in children. Neurotoxicology 7:197–208.