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Short-term erythrosine B-induced inhibition of the brain regional serotonergic activity suppresses motor activity (exploratory behavior) of young adult mammals

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ABSTRACT

Previous studies showed that repeated ingestion of erythrosine B (artificial food color) developed behavioral hyperactivity, but nothing is known about its single administration effect as well as the neurochemical (s) involvement. The present study provides evidence that a single higher dosage (10, 100 or 200 mg/kg, p.o.) of erythrosine administration to young adult male rats reduced motor activity (MA) maximally at 2 h and brain regional (medulla-pons, hippocampus and hypothalamus) serotonergic activity (measuring steady-state levels of 5-HT and 5-HIAA, pargyline-induced 5-HT accumulation and 5-HIAA declination rate and 5-HT receptor binding) under similar experimental condition. The degree of erythrosine-induced inhibition of both MA and brain regional serotonergic activity was dosage dependent. Lower dosage (1 mg/kg, p.o.) did not affect either of the above. Erythrosine (100 or 200 mg/kg, p.o.)-induced MA suppression was also observed in the presence of specific MAO-A inhibitor, clorgyline (5 mg/kg, i.p.) or MAO-B inhibitor, deprenyl (5 mg/kg, i.p.); but their co-application (5 mg/kg, i.p., each) effectively prevented the erythrosine-induced motor suppression. Altogether these results suggest that a single higher dosage of erythrosine (10–200 mg/kg, p.o.) may reduce MA by reducing serotonergic activity with modulation of central dopaminergic activity depending on the brain regions.

1. Introduction

Artificial food colors have been utilized over the century for esthetic that make the foods attractive and stimulate appetite. Feingold (1975) initially claimed that synthetic food dyes play a major role in etiology of behavioral disturbance. Recently, a number of studies including double-blinded, placebo-controlled trials have suggested a significant link between the long-term or repeated ingestion of synthetic food colors and behavioral hyperactivity (Bateman et al., 2004; Boris and Mandel, 1994; McCann et al., 2007; Schab and Trinh, 2004). Among the various synthetic food colors/dyes, erythrosine [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; Food additives in Europe, 2000) is a highly lipid soluble anionic dye and acts as an organic anion (Levitan, 1977) in biological system. Initially Levitan et al. (1984) and later Hirohashi et al. (1997) have reported that erythrosine, like other fluorescent compounds, crosses the blood-brain-barrier, though its brain uptake has been found to be restricted due to dye–plasma protein complex formation depending on the age and condition of the subject (Levitan et al., 1984). In experimental animal, long-term administration of erythrosine, like other synthetic food color (Tanaka, 2006), significantly increases the movement activity of exploratory behavior in a dosage dependent manner (Tanaka, 2001, 2006). It has also been observed that erythrosine increases Ca^{+2} permeability in neural membrane (Colombini and Wu, 1981; Heffron et al., 1984), the release of neurotransmitters like dopamine, GABA, serotonin, acetylcholine, norepinephrine etc. (Augustine and Levitan, 1983; Logan and Swanson, 1979; Wade et al., 1984) and inhibits brain Mg^{2+}- and Na^{+}–K^{+}-ATPase (Wade et al., 1984), Ca^{+2}-ATPase (Watson and Haynes, 1982), high affinity ouabain binding (Hnatowich and Labella, 1982; Silbergeld, 1981; Swann, 1982) and dopamine uptake in rat brain tissue (Lafferman and Silbergeld, 1979). Despite all these evidences which may suggest a significant link between the ingestion of artificial food color (erythrosine) and behavioral hyperactivity, the involvement of specific neurotransmitters and neural mechanisms in relation to behavioral alteration mediated by artificial food color(s) consumption (*in vivo*) are yet to be studied.

Several pharmacological evidences have implicated the involvement of the dopaminergic system in the etiology of behavioral conditions (including attention-deficit hyperactivity disorder) (Brennan and Arnsten, 2008) and hyper locomotor activity (Giros et al., 1996; Hechtman, 1994). Recent studies indicate that central serotonergic system has a positive modulating effect on the functional activities of the brain dopaminergic system (Alex and Pehek, 2007). Several recent studies have also been performed to characterize the potential role of central serotonin (5-HT) in the activation and modulation of the locomotor system and continue to be an area of major interest (Liu and Jordan, 2005; Schmidt and Jordan, 2000). Today, it is widely

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acknowledged that serotonin plays a potential role in the development of locomotor circuits and modulation of the locomotor central pattern generator (Branchereau et al., 2002; Cazalets et al., 2000; Pflieger et al., 2002) specifically the circuit involved in exploratory behavior (Grailhe et al., 1999). This role of serotonin depends upon neuroanatomical location of various classes of 5-HT receptors in brain areas related to motor control (striatum, medulla, hippocampus, frontal cortex and spinal cord). Thus, it is conceivable that brain regional 5-HT may have a role in erythrosine-induced disturbance in motor (rearing) activity. In the present study, the authors therefore investigated the effect of a single oral consumption of erythrosine with varying dosages on brain regional (medulla-pons, hypothalamus, hippocampus and corpus striatum) serotonergic activity and its pharmacological modulation by using specific monoamine oxidase inhibitor(s) [MAOI(s)] in relation to changes in motor (rearing) activity in young adult male albino rats.

2. Experimental procedures

2.1. Reagents

Erythrosine B (dye content 90%), serotonin (5-HT)-HCl, R-(−)-deprenyl-HCl (selegiline), clorgyline, ninhydrin, semicarbazide-HCl, and bovine serum albumin (BSA) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Pargyline-HCl was obtained from Abbott laboratories (North Chicago, Illinois, USA). [^{3}H]-5-HT (specific activity 1 Ci/mmol) was purchased from Board of Radiation and Isotope Technology (BRIT, Mumbai, India). Cocktail 'O' (liquid scintillation solution in toluene) was purchased from Spectrochem Pvt. Limited (Mumbai, India). All other reagents used in the study were of analytical grade.

2.2. Animals and animal care

Young adult male albino rats of Charles Foster strain weighing 130–140 g were taken as experimental subjects and 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 (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.

2.3. Experimental design

Young adult male albino rats were randomly divided into four groups (Groups I, II, III and IV). Each group was further divided into five (05) sub-groups (sub-group 1 was considered as control and sub-groups 2–5 were considered as experimental). Experimental sub-groups (2, 3, 4 and 5) were treated orally (p.o.) with a single dosage of erythrosine (1, 10, 100 and 200 mg/kg, p.o. respectively in 0.5 ml distilled water) and their corresponding control rats of sub-group 1 were treated with the equivalent volume of vehicle of erythrosine (distilled water) through the same route under similar condition.

Group I: 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 a single dosage of erythrosine (1, 10, 100 or 200 mg/kg, p.o. in 0.5 ml) and motor activity (MA) was measured at different time points (0 to 9 h). In the present investigation dosages of erythrosine (1 to 200 mg/kg, p.o.) for single oral exposure were selected considering the previous studies reported by Abdel Aziz et al. (1997), Sasaki et al. (2002), Tanaka (2001) and Tsuda et al. (2001).

Group II: Rats of Group II were used for neurobiochemical parameters. Each neurobiochemical parameter (using 4–6 rats in each sub-group of Group II) was measured following 2 h or 7 h of erythrosine consumption.

Group III: Animals of Group III (containing 12 rats in each sub-group and pretreated with a single dosage of erythrosine or its vehicle 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. Control rats of Group III were treated with an equal volume (0.5 ml) of vehicle of erythrosine (distilled water) and saline (when required) through the same route under similar experimental conditions.

Group IV: Animals of Group IV (containing 8–12 rats in each sub-group and pretreated with a single dosage of erythrosine or its vehicle as mentioned above) were treated with specific MAOIs [clorgyline (5 mg/kg, i.p.) and/or deprenyl (5 mg/kg, i.p.)] and used for behavioral (motor activity) study. Clorgyline and/or deprenyl (dissolved in normal saline) were administered to Group IV rats after 10 min of erythrosine treatment. MA was measured (0 to 9 h) at every 30 min interval. Control rats of Group IV were treated with an equal volume (0.5 ml) of vehicle of erythrosine (distilled water) and saline (when required) through the same route under similar experimental conditions.

2.4. Behavioral rating of rat motor activity (MA)

The number of vertical (rearing) motor activity of each animal was measured during 5 min observation period to monitor the MA as mentioned in Jamaluddin and Poddar (2003). The animals treated with either erythrosine or its vehicle (distilled water) considered as corresponding control or erythrosine in the presence of specific MAOI (s) or their corresponding vehicle(s) considered as corresponding control, were gently transferred to a transparent plastic chamber (24 × 24 × 20 cm3) 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) and then they were gently placed back in their home cages.

2.5. Collection of brain tissue

Rats of both control and experimental groups were sacrificed by cervical dislocation between 2:00 and 2:30 pm to avoid circadian effect, if any. After decapitation brains were immediately taken out and immersed 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) activity and 5-HT receptor binding studies. Immediately after collection of the brains, different brain regions (e g. medulla-pons, hypothalamus, hippocampus and corpus striatum) were dissected out following the method described by Poddar and Dewey (1980).

2.6. Estimation of neurobiochemical parameters

2.6.1. Steady-state levels of 5-HT and 5-HIAA

Brain regional (medulla-pons, hypothalamus, hippocampus and corpus striatum) steady-state levels of 5-HT and its metabolite 5-HIAA were estimated spectrophotofluorometrically according to the method of Scapagnini et al. (1969). Rat brain tissues were homogenate in 20 volume of 0.4 N perchloric acid (PCA). 1 ml of tissue homogenate was mixed with 2.5 mg of EDTA and 2.5 mg of ascorbic acid and then centrifuged at 4500 rpm for 5 min. The clear supernatant was used for determination of 5-HT and 5-HIAA. The pH of the supernatant was adjusted to 6.8–7.0 with solid K_2CO_3 10% $ZnSO_4$, and 1 M NaOH. Then mixed well with solid NaCl, 6 N HCl and 10 ml of butyl acetate and centrifuged for 5 min at 4500 rpm.

Aqueous phase was used for the determination of 5-HT and organic phase was used for 5-HIAA determination. In the organic phase 0.1 M phosphate buffer was added, shaken and centrifuged for 5 min at 4500 rpm. Concentrated HCl was then added to the buffer phase and the fluorescence was measured by spectrophotoflurometer (Hitachi F-3010) with an excitation and emission 305 and 540 nm respectively. The pH of the aqueous phase was increased by means of solid K_2CO_3. Then 0.5 M