CHAPTER ONE

5-Hydroxytryptophan: A precursor of serotonin influences regional blood-brain barrier breakdown, cerebral blood flow, brain edema formation, and neuropathology

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Contents

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Abstract

5-Hydroxytryptophan (5-HTP), a precursor of serotonin, is therapeutically used for several psychiatric disorders such as anxiety and depression in the clinic. However, severe side effects, including abnormal mental functions, behavioral disturbances and intolerance are associated with this treatment. 5-HTP-induced elevation of plasma and brain serotonin levels may affect blood-brain barrier (BBB) breakdown, edema formation and regional cerebral blood flow (CBF) disturbances. Breakdown of BBB to serum proteins leads to vasogenic brain edema formation and cellular injuries. However, 5-HTP-neurotoxicity is still not well known. In this investigations 5-HTP induced elevation of endogenous plasma and brain serotonin levels and its effect on BBB breakdown, edema formation neuronal injuries was examined in a rat model. Furthermore, potential role of oxidative stress and nitric oxide (NO) was evaluated. In addition, several neurochemical agents such as *p*-CPA (5-HT synthesis inhibitor) indomethacin (prostaglandin synthase inhibitor), diazepam (ant stress drug), cyproheptadine, ketanserin (5-HT2 receptor antagonists) and vinblastine (inhibitor of microtubule function) were examined on 5-HT neurotoxicity. Our observations suggest that 4 h after 5-HTP administrations, the endogenous serotonin levels increased by fourfold (150 mg/kg) in the plasma and brain associated with profound hyperthermia (+3.86 ± 0.24 °C, oxidative stress and NO upregulation. Breakdown of the BBB to Evans blue albumin (EBA) in 8 brain regions and to $^{[131]}$Iodine in 14 brain regions was observed. The CBF exhibited marked reduction in all the brain regions examined. Brain edema and cellular injuries are present in the areas associated with BBB disruption. Drug treatments reduced the BBB breakdown, edema formation NO production and brain pathology. These observations are the first to point out that 5-HTP-neurotoxicity caused by BBB breakdown, edema formation and NO production is instrumental in causing adverse mental and behavioral abnormalities, not reported earlier.

1. Introduction

The blood-brain barrier (BBB) maintains the composition of extracellular fluid environment of the central nervous system (CNS) by strictly regulating the blood-brain interface in physiological conditions (Bradbury, 1979;

Rapoport, 1976; Sharma, 1982, 1999, 2009; Sharma & Westman, 2003, 2004). A slight alteration in the BBB function to blood-borne agents could adversely impair neuronal functions leading to long-term mental abnormalities (Bosma, van Noorden, Schlingemann, & Klaassen, 2018; Brightman, Klatzo, Olsson, & Reese, 1970; Erickson & Banks, 2018; Sharif et al., 2018; Sharma et al., 2016). This is further supported by the fact that in several neurological diseases and psychiatric disorders breakdown of the BBB and blood-cerebrospinal fluid barrier (BCSFB) is observed (Nation et al., 2019; Sweeney, Kisler, Montagne, Toga, & Zlokovic, 2018; Sweeney, Sagare, & Zlokovic, 2018; Sweeney, Zhao, Montagne, Nelson, & Zlokovic, 2019). Thus, dementia, depression, anxiety disorders, schizophrenia, autism as well as neurodegenerative disorders like Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) alteration in blood-brain or blood-CSF transport is quite common (Bradbury, 1979; Hurtado-Alvarado, Domínguez-Salazar, Pavon, Velázquez-Moctezuma, & Gómez-González, 2016; Montagne et al., 2016; Montagne, Zhao, & Zlokovic, 2017; Ozkizilcik et al., 2018; Rapoport, 1976; Sharma, Castellani, Smith, & Sharma, 2012; Sharma et al., 2019; Sharma & Westman, 2004; Sweeney et al., 2019). This reflects an abnormal state of the BBB and BCSFB function (see Bradbury, 1979; Rapoport, 1976; Sharma & Westman, 2003, 2004). This dysfunction ultimately leads to pathologies abnormalities in the CNS in these diseases (Bosma et al., 2018; Erickson & Banks, 2018; Nation et al., 2019; Sharma et al., 2016; Sweeney, Zhao, et al., 2019).

The exact molecular of neurochemical basis of these neurological diseases, however, remains unknown. Available evidences indicate an involvement of the serotonergic system in several psychiatric diseases (Chakraborty et al., 2019; Conio et al., 2019; Daut & Fonken, 2019; Kraus, Castrén, Kasper, & Lanzenberger, 2017; Reiche et al., 2018; Shelton, 2019; Sinopoli, Burton, Kronenberg, & Arnold, 2017; Vadodaria, Stern, Marchetto, & Gage, 2018; Yohn, Gergues, & Samuels, 2017). In particular depression, emotional disturbances, schizophrenia and dementia altered levels of serotonin, its receptors, and metabolizing enzymes are well documented (see (Reiche et al., 2018; Shelton, 2019; Yohn et al., 2017). On the basis of either low or high serotonin syndromes in these psychiatric diseases drugs modifying serotonin metabolism are prescribed for clinical use in young or adult patients (Kraus et al., 2017; Shelton, 2019; Yohn et al., 2017).

In general, drugs that interfere with serotonin system or its metabolism in the CNS induce changes in psychological mood and behavior of the patients (Reiche et al., 2018; Shelton, 2019). In these clinical conditions

of depression, anxiety or in mania cases, 5-HTP a precursor of serotonin is prescribed as a potent therapeutic agent (Birdsall, 1998; Fukuda, 2014, 2015; Jacobsen, Krystal, Krishnan, & Caron, 2016; Kaneko, Kumashiro, Takahashi, & Hoshino, 1979; Maes, Calabrese, Jayathilake, & Meltzer, 1997; Zmilacher, Battegay, & Gastpar, 1988). The drug is administered orally in doses varying from 50 to 150 mg/kg per day for extended periods that is continued the despite side effects involving disturbed mental function and abnormal behaviors (Jacobsen et al., 2016; Nakajima, Kudo, & Kaneko, 1978; Takahashi, Takahashi, Masumura, & Miike, 1976; van Vliet, Slaap, Westenberg, & Den Boer, 1996). Unfortunately, the mechanisms underlying 5-HTP induced abnormal behaviors and mental dysfunction are not well characterized (see Fahn, 1977; Martin, 1996; Sandyk, 2006).

Several lines of recent evidences indicate that serotonin is one of the powerful neurochemical agents that have the capacity to induce a reversible increase of the BBB permeability. This idea is further supported by the findings that application of serotonin on either the abluminal or luminal side of the cerebral microvessels lead to the opening of the BBB permeability. This effect of serotonin in the BBB permeability is mediated via specific 5-HT receptors (Sharma, 2000, 2004, 2007; Sharma & Dey, 1981; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1990a, 1995; Sharma, Westman, Cervós-Navarro, Dey, & Nyberg, 1995; Winkler, Sharma, Stålberg, Olsson, & Dey, 1995).

Furthermore, experimental conditions that invoke release of serotonin either following stress caused by immobilization, environmental heat stress or forced swimming, or that produced by mechanical trauma to the brain or spinal cord also elicit a breakdown of the BBB or blood-spinal cord barrier (BSCB) permeability (Dey & Sharma, 1983, 1984; Olsson, Sharma, Pettersson, & Cervos-Navarro, 1992; Sharma, 2004; Sharma & Cervós-Navarro, 1990; Sharma, Cervós-Navarro, & Dey, 1991a, 1991b; Sharma & Dey, 1981, 1984, 1986a, 1987, 1988; Sharma, Nyberg, Cervos-Navarro, & Dey, 1992; Sharma, Olsson, & Dey, 1990b; Sharma, Westman, Cervós-Navarro, et al., 1995; Sharma, Westman, Cervós-Navarro, Dey, & Nyberg, 1997; Sharma, Westman, Cervós-Navarro, & Nyberg, 1997; Sharma, Westman, & Nyberg, 1998; Sharma, Westman, Nyberg, Cervos-Navarro, & Dey, 1994). These observations suggest that elevation of endogenous serotonin levels influences the BBB Function (see Sharma, 2004; Sharma et al., 1990a).

Breakdown of the BBB to endogenous serum proteins into the brain fluid microenvironment lead of vasogenic edema formation (Sharma, 1999, 2004; Sharma et al., 1990b, 1992, 1994; Sharma, Dey, & Olsson, 1989). The magnitude and intensity of spread of edema fluid is one of the primary reasons for cell injury. Several lines of evidences suggest that exogenous elevation of serotonin in to the circulation causes disruption of the BBB, BCSFB and BSCB associated with brain edema formation.

5-HTP administrations induce significant increase in serotonin in the whole body, including plasma and the brain (Haberzettl, Bert, Fink, & Fox, 2013; Meltzer et al., 1982; Nakamura & Hasegawa, 2009; Udenfriend, Weissbach, & Bogdanski, 1957). Thus, it is quite likely that this treatment may also influence the BBB dysfunction, edema formation and cellular injuries (see Sharma, 2004). To our knowledge, there is no pervious information available on 5-HTP-induced BBB dysfunction. To expand our knowledge in this matter, the present investigation was undertaken to find out whether 5-HTP induced elevation of serotonin could induce a breakdown of the BBB permeability and induce edema formation associated with neuronal injures. Serotonin has powerful vasoconstrictor and/or vasodilation effects on the cerebral circulation (Bonvento, MacKenzie, Seylaz, & Lacombe, 1994; Edvinsson & MacKenzie, 1976; Kobayashi et al., 1985; Raskin, 1981). This effect of the amine is mediated through specific 5-HT receptors (Bonvento et al., 1994; Edvinsson & MacKenzie, 1976; Sharma, 2004; Sharma, Westman, Cervós-Navarro, et al., 1995). Thus, in this study the influence of 5-HTP on regional CBF was also investigated. Finally, to find out the receptor-mediated effect of serotonin on BBB function, brain edema and cellular injuries, several drugs modifying serotonin receptor or serotonin induced signal transduction mechanisms on the endothelial cell vesicular transports were also analyzed (Anon, 1989; Burnstock, 1985; MacKenzie & Scatton, 1987; Parsons, 1991; Saxena, Bom, & Verdouw, 1989; Wiernsperger, 1990). A brief description on our findings on 5-HT-induced neurotoxicity is discussed below.

2. Methodological considerations

2.1 Animals

Experiments were carried out on 164 inbred male Charles Foster Rats (110–150 g) housed at controlled room temperature (21 ± 1 °C) with 12 h light and 12 h dark schedule. Rat food pellets and tap water was provided ad libitum before the experiment.

2.2 Injection of 5-hydroxytryptophan

5-Hydroxytryptophan (5-hydroxy-L-tryptophan, Sigma Chemical Co., USA) was dissolved in sterile water and injected intraperitoneally (0.3 mL) in separate group of rats in a dose of 50 mg, 75 mg and 150 mg/kg. The animals were replaced in the cage after administration of the compound. The animals were kept alive 4 h after the injection.

2.2.1 Sham group

Saline treated animals (0.3 mL, i.p.) were used as sham group.

2.2.2 Control group

Normal intact rats served as controls.

2.3 Parameters measured

The following parameters were measured in control, saline treated and 5-HTP treated rats.

2.3.1 Stress and behavioral symptoms

Changes in rectal temperature were measured using a thermistor probe (Yellow Springfield, USA) connected to a 6-channel telethermometer (Aplab Electronics, USA). The other behavioral parameters such as salivation, apparent abnormal locomotion, jumping, circular motion and wet dog-shake activities were observed and scored (see Table 2). In addition, excretion of fecal pellets during the experiment and occurrence of gastric hemorrhages in the stomach wall was examined at autopsy (Sharma et al., 1991a; Sharma & Dey, 1986b, 1987).

2.3.2 Physiological variables

Changes in the mean arterial blood pressure (MABP), arterial pH, blood gases, heart rate and respiration were examined using standard procedures described earlier (Sharma, 2007; Sharma et al., 1990a; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). The MABP was monitored from a polythene cannula (PE 25) inserted into the right common carotid artery and connected to a strain gauge pressure transducer (Statham P 23, USA). The out-put from the transducer was connected to a chart recorder (Electromed, UK). Specific leads placed on the abdominal skin over the heart and a respiratory belt tied over the belly were used to record heart rate and respiration, respectively (Harvard Apparatus, USA). The outputs

were also recorded in the chart recorder (Electromed, UK) (Sharma, Ali, Hussain, Schlager, & Sharma, 2009; Sharma et al., 2009).

At the time of connecting the arterial cannula to the pressure transducer, a sample of arterial blood was withdrawn for the measurement of the arterial pH, PaO_2 and $PaCO_2$ using a Radiometer apparatus (Copenhagen) (Sharma, 1987).

2.4 Blood-brain barrier permeability

Four hours after saline or 5-HTP administration, animals were anesthetized with urethane (1 g/kg, i.p.). Evans blue (2% of a 0.5 mL solution) containing 10 μCi $^{[131]}$I-sodium (about 1 million counts per minute) were administered into the right femoral vein through a needle puncture (Sharma, 2007; Sharma, Dey, & Olsson, 1989; Sharma et al., 1990a; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). The tracer was allowed to circulate for 5 min. At the end of the experiment, the intravascular tracer was washed-out with a brief saline rinse via heart (45 s). Immediately before perfusion 1 mL arterial blood sample was withdrawn through a needle puncture from the left ventricle to determine whole blood radioactivity at the time of killing (Sharma, 2007; Sharma & Dey, 1986a, 1986b, 1987, 1988).

After perfusion, the brain was removed and bisected in the mid line. In one half of the brain extravasation of Evans blue dye was examined using a magnifying lens. The dye entered into the brain was measured calorimetrically as described earlier (Sharma, 1987; Sharma, Ali, Hussain, et al., 2009; Sharma, Ali, Tian, et al., 2009). The other half brain was dissected into the 14 brain regions that were weighed immediately and counted for radioactivity in a three-in well type gamma counter (Beckman, Germany). The extravasation of radioiodine was expressed as percentage over the blood radioactivity (see Sharma, 1987, 2007; Sharma & Dey, 1981; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995).

2.5 Cerebral blood flow

The CBF was measured once in different brain regions using carbonized tracer microspheres (OD 15 μm ± 0.6 μm) labeled to $^{[125]}$Iodine as described earlier (Sharma, 1987; Sharma et al., 1990b, 1992; Sharma & Dey, 1986b, 1987; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989; Sharma, Westman, Cervós-Navarro, Dey, & Nyberg, 1997). In brief, about 10^6 microspheres were injected near the left atrium through a cannula (PE 25)

implanted into the left common carotid artery. The tip of the cannula was advanced retrogradely toward the heart (see Sharma, 1987).

Timed arterial blood samples at every 30 s from the periphery were collected from the right femoral artery at the rate of 0.8 mL/min starting from 30 s before infusion and continued until 90 s after the infusion (Sharma, 1987; Sharma et al., 1990b; Sharma & Dey, 1986b, 1987) (Figs. 1 and 2).

After 90 s of bolus injection, the animals were decapitated and the brain and spinal cord was removed immediately and placed on filter paper wetted with ice-cold saline. The large superficial vessels were removed and discarded. The brain and spinal cord was then divided into 14 anatomical regions (Fig. 3), weighed immediately and counted in a three-in well type Beckman Gamma Counter (USA) at 50–80 keV energy window (Sharma, 1987; Sharma et al., 1990b; Sharma & Dey, 1986b, 1987).

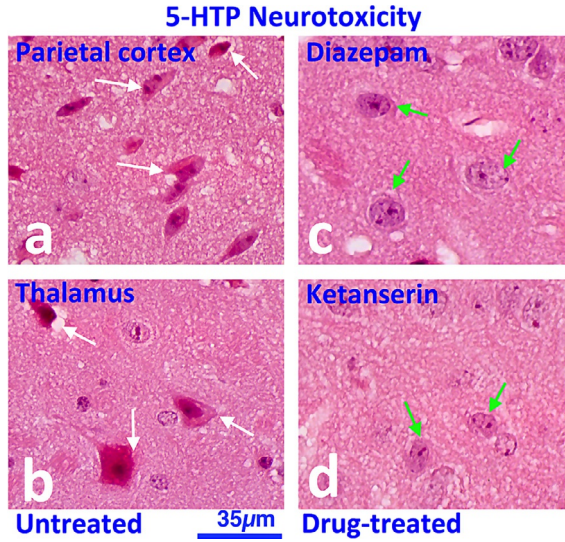

Fig. 1 High power light micrograph of Hematoxylin and Eosin (H&E) stained paraffin sections (3-μm thick) showing neuronal damages in 5-HTP (150 mg/kg, i.p.) treated (a and b) rat and neuroprotection induced by diazepam (c) and ketanserin (d). Several neurons (white arrows) showed distortion, perineuronal edema and sponginess in the neuropil of parietal cerebral cortex (a) and thalamus (b). Dark neuronal cell cytoplasm and karyoplasm in some neurons are clearly visible (a and b). Pretreatment with diazepam (c) or ketanserin (d) markedly attenuated neuronal damages in 5-HTP treated groups. Several neurons (green arrows) show clear neuronal cytoplasm and karyoplasm with compact neuropil. Perineuronal edema is much less distinct in drug-treated group after 5-HTP-administration. For details see text. Bar = 35 μm.

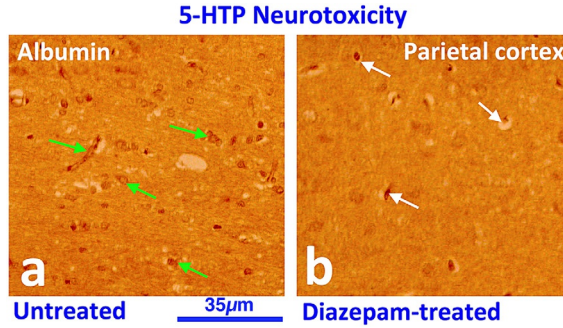

Fig. 2 Low power light micrograph of albumin immunostaining on 3-μm thick paraffin sections of rat brain parietal cerebral cortex after 4 h of 5-HTP (150 mg/kg, i.p.) treated (a) its modification with diazepam pretreatment (b). Albumin extravasation (green arrows) is clearly evident in the neuropil around microvessel and neurons. Perineuronal vacuolation showing albumin deposit is clearly visible (a). Pretreatment with diazepam (b) markedly reduced albumin leakage in 5-HTP administered rat. Few albumin positive cells (white arrows) are only visible in 5-HTP intoxicated diazepam treated group (b). Bar = 35 μm.

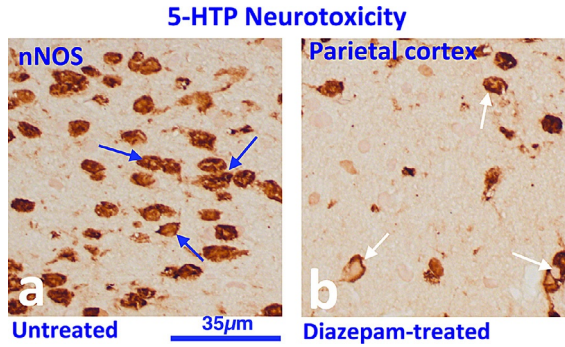

Fig. 3 Low power light micrograph of neuronal nitric oxide synthase (nNOS) immunoreactivity in the parietal cerebral cortex in the rat following 4 h after 5-HTP (150 mg/kg, i.p.) administration (a) and its reduction in diazepam pretreated (b) group. Several nNOS positive neurons could be seen (blue arrows) in the 5-HTP treated groups in the neuropil. Diazepam pretreatment in 5-TP intoxicated animal markedly reduced the nNOS up-regulation (b). Only a few nNOS positive cells could be seen in diazepam pretreated 5-HTP group (white arrows). Paraffin sections 3-μm thick, Bar = 35 μm. For details see text.

The CBF (mL/g/min) was calculated according to the formula: CPM Brain Tissue/Total counts in reference blood flow × 0.8 (reference blood flow) (Heistad, Marcus, & Mueller, 1977; Marcus, Busija, Bischof, & Heistad, 1981; Marcus, Heistad, Ehrhardt, & Abboud, 1977; Sharma, 1987).

2.6 Brain edema formation and volume swelling

After counting the radioactivity for measuring regional CBF, the tissue samples that are already weighed (wet weight) on a preweighed filter paper were placed in an oven maintained at 90 °C for 72 h to evaporate the water content of the tissue (Kiyatkin, Brown, & Sharma, 2007; Kiyatkin & Sharma, 2009; Sharma, 2007; Sharma et al., 1992, 1994, 2015; Sharma & Kiyatkin, 2009; Sharma, Westman, Cervós-Navarro, et al., 1995). After that, the dry weight of the samples was recorded on the same microbalance at three different occasions until the dry weight become constant. The brain water content representing edema formation was calculated in control, experimental and drug treated animals using the formula: Wet weight—dry weight/wet weight × 100 (Olsson et al., 1992; Sharma et al., 1991b; Sharma & Cervós-Navarro, 1990; Sharma & Olsson, 1990; Sharma, Olsson, & Cervós-Navarro, 1993).

Volume swelling from the changes in brain water between control and experimental group were calculated suing formula of Elliott and Jasper (1949). Roughly, 1% increase in brain water is equal to 4% increase in volume swelling (Dey & Sharma, 1983, 1984; Kiyatkin et al., 2007; Olsson et al., 1992; Sharma et al., 1991b, 1993, 1994; Sharma, Ali, Hussain, et al., 2009; Sharma & Olsson, 1990; Sharma, Westman, & Nyberg, 1998).

2.7 Measurement of serotonin

Separate group of rats were anesthetized with urethane 4 h after saline or 5-HTP treatment. Simultaneous measurement of 5-HT and the BBB permeability was not carried out in these animals due to obvious reasons that injection of Evans blue and brain perfusion may have interfered with the biochemical determination (Sharma et al., 1990a; Sharma, Westman, Cervós-Navarro, et al., 1995).

We measured serotonin in plasma and in brain using a sensitive and specific spectrophotofluorometric assay (Sharma, 1982; Sharma et al., 1991a; Sharma & Dey, 1981, 1986a, 1987, 1988; Sharma, Westman, Cervós-Navarro, et al., 1995; Snyder, Axelrod, & Zweig, 1965). In rats after experiments, about 1 mL of whole blood was withdrawn from the left ventricle and centrifuged immediately to obtain plasma. Plasma (0.5 mL) was diluted with perchloric acid (PCA) and protein was precipitated out, centrifuged, and the supernatant was collected (Sharma & Dey, 1986a, 1987, 1988). The brain was removed after decapitation and placed in ice cooled 0.4 N perchloric acid and stored at 4 °C overnight (Sharma, 1982; Sharma & Dey, 1981). On the second day, the brain was dissected into eight regions (see Table 2). Each region

was homogenized in PCA and centrifuged at room temperature (×900 g) and the supernatant was obtained (Sharma & Dey, 1981). 1 mL of aliquots from plasma and brain homogenates was used for serotonin measurement. The extraction of serotonin from these plasma and brain samples was carried out in butanol (extra pure grade, BDH, London, UK) in a salt saturated alkaline medium (pH 10). The purification of serotonin extract was done using *n*-heptane (Extra pure, Merck, Germany) (Snyder et al., 1965).

The fluorspars were develop by incubating the samples with ninhydrin (Sigma Chemical Co., St. Louis, USA) at 75 °C for 30 min (Sharma & Dey, 1986a, 1987, 1988; Snyder et al., 1965). The florescence was measured in triplicate in cooled samples at room temperature (21 ± 1 °C) in an AMINCO-Bowman Spectrophotofluorometer (Waltham, MA, USA) at excitation 385 nm and emission 490 nm wavelengths as described earlier (Sharma et al., 1991a; Sharma & Dey, 1986a, 1987, 1988; Sharma, Westman, Cervós-Navarro, et al., 1995; Snyder et al., 1965).

2.8 Brain pathology

In separate group of control, 5-HTP intoxicated and drug-treated groups after experiments were deeply anesthetized with urethane (1 mg/kg, i.p.) and perfused with 4% buffered paraformaldehyde after brief saline rinse (Sharma, 2007; Sharma et al., 1991b, 1993; Sharma & Cervós-Navarro, 1990; Sharma & Olsson, 1990). For this purpose, after anesthesia, the chest was rapidly opened and heart was exposed. The right auricle was excised and a perfusion butterfly cannula was inserted into the left ventricle connected with bottle filled with 0.9% sterile cold saline (4 °C) (see Sharma, 2007). About 50 mL of saline was perfused through heart to clear remaining blood from the circulation system. After the perfusion system was switched to bottle filled with cold paraformaldehyde in 0.1 M phosphate buffered saline (pH 7.0). About 250 mL fixative was perfused through heart at 90 mm Torr perfusion pressure (Sharma et al., 1993; Sharma & Olsson, 1990).

After completion of the perfusion, the animals were wrapped in aluminum foil and kept in a refrigerator at 4 °C overnight (Sharma et al., 1993; Sharma & Olsson, 1990). On the next day, the brain and spinal cord was dissected out and desired pieces from different areas were cut and placed in the same fixative at 4 °C for 1 week. After that coronal sections of the brain and spinal cord were embedded in paraffin using standard protocol and 3-μm thick sections were cut and processed for histological or immunohistochemical staining (see below) (Sharma et al., 1991b; Sharma & Cervós-Navarro, 1990; Sharma & Olsson, 1990).

2.8.1 Light microscopy

Neuronal injury and neuropil were examined using Nissl or Hematoxylin and Eosin (H&E) staining on 3-μm thick paraffin section using standard protocol (Sharma et al., 1993; Sharma & Olsson, 1990). The stained sections were examined under a Zeiss Bright field upright Microscope (Axio Imager. Z2m, Germany). The images were captured on a digital camera attached to it and processed in a Apple Macintosh Computer Mac OX X using software Adobe Photoshop 10.2 using identical color filters from various groups for comparison (Pettersson, Sharma, & Olsson, 1990; Sharma, Gordh, Wiklund, Mohanty, & Sjöquist, 2006; Sharma & Sjöquist, 2002).

2.8.2 Transmission electron microscopy

For ultrastructural analysis of vascular permeability using lanthanum-an electron dense tracer (molecular diameter 12 Å) was examined using standard protocol (see Olsson et al., 1992; Pettersson et al., 1990; Sharma et al., 2006; Sharma, Olsson, Persson, & Nyberg, 1995). For this purpose, in separate group of rats after experiments, were perfused with fixative containing lanthanum as described earlier. In brief, the lanthanum chloride (2.5%) was dissolved in 0.4% paraformaldehyde in 0.1 M phosphate buffer saline (pH 7.0) containing 0.2% picric acid and about 200 mL of this fixative was perfused in situ via cardiac puncture after rinsing the microvessels with 0.9% buffered saline (4 °C, 50 mL) at 90 Torr perfusion pressure (Olsson et al., 1992; Pettersson et al., 1990; Sharma et al., 2006).

Perfusion with lanthanum has advantages to examine the passage of the tracer across the endothelial cells at ultrastructural level using transmission electron microscopy (TEM) as black particles with without any specific processing (Pettersson et al., 1990; Sharma et al., 2006; Sharma, Olsson, Persson, & Nyberg, 1995). Thus, after perfusion, small tissue pieces were postfixed in OSO_4 and embedded in Epon 812. Semithin sections (about 1 μm) were cut and stained with toluidine blue staining to identify the area for ultrathin sections. Ultrathin sections were cut using diamond knife at LKB Ultramicrotome (Stockholm, Sweden) and serial sections were collected on a one hole copper grid (Sharma, 2007; Sharma, Westman, & Nyberg, 1998). Some sections were examined unstained whereas; some sections were stained for contrast with lead citrate and uranyl acetate and examined under a Phillips 400 TEM. The images were captured on a digital camera and processed using identical filters in a Macintosh computer using Adobe Photoshop software 10.2 (Olsson et al., 1992; Pettersson et al., 1990; Sharma et al., 2006; Sharma, Olsson, Persson, & Nyberg, 1995).

2.8.3 Immunohistochemistry for albumin and neuronal nitric oxide synthase

Immunohistochemistry (IHC) was performed on 3-μm thick paraffin sections for endogenous albumin leakage and neuronal nitric oxide synthase (nNOS) according to standard protocol (Gordh, Chu, & Sharma, 2006; Sharma et al., 1998, 2015; Sharma, Alm, & Westman, 1998; Sharma, Badgaiyan, Alm, Mohanty, & Wiklund, 2005; Sharma, Miclescu, & Wiklund, 2011; Sharma, Westman, Olsson, & Alm, 1996).

(a) *Albumin immunohistochemistry*

IHC for albumin was done on paraffin sections using a sheep polyclonal ant-rat albumin antibody (Sigma, USA) and the streptavidin-horse radish peroxidase (HRP)-biotin techniques (Gordh et al., 2006; Sharma et al., 2011; Sharma, Patnaik, et al., 2015). In brief, the endogenous peroxidase activity was blocked with 3% H_2O_2 and 5% normal goat serum. After that, the sections were incubated with the primary antibodies (1:500) for overnight with shaking at 4 °C. This was followed by incubation with biotinylated linking antibody and HRP (DAKO, Germany) with brief rinses in phosphate buffer saline (PNS) between incubations. The reaction was visualized using 3-amino-9-ethylcarbazole (Vector Lab, Burlingame, USA) and some of the sections were counterstained with Hematoxylin and Eosin (HE). For technical control sections were omitted with primary antibody incubation or substituted with nonimmune serum for the primary antibody in the staining protocol simultaneously to confirm the specificity of the primary antibody used (Gordh et al., 2006; Sharma et al., 2011).

The number of albumin positive cells was counted in blinded fashion by three observers and median values are used for calculations in various groups.

(b) *Neuronal nitric oxide synthase (nNOS) immunohistochemistry*

The nNOS IHC was dome on 3-μm thick paraffin sections using commercial nNOS antiserum (1:5000 in PBS) using DAB technique (Sharma et al., 1996, 2005; Sharma, Alm, & Westman, 1998). In brief, the sections were incubated with primary nNOS antibodies under agitation at room temperature for 36 h together with normal swine serum (1:30 in PBS). After that the sections were rinsed 10 min six times in PBS and incubated with swine antirabbit immunoglobulin (1:30 in PBS) for 60 min at room temperature with several rinses in PBS. Thereafter, the sections were incubated with rabbit peroxidase-antiperoxidase complex (PAP) (1:20 in PBS) and rinsed several times with PBS (see Sharma et al., 1996, 2005; Sharma, Alm, & Westman, 1998).

The immunoreactivity was developed on sections by incubating them for 6–7 min in a solution containing 75 mg of DAB and 30 μL of 30% H_2O_2/100 mL of Tris–HCl buffer. After that the sections were washed with sodium cacodylate buffer and processed for light microscopy using commercial protocol (Sharma et al., 1996). The number of nNOS positive cells were counted and identified for comparison as mentioned above.

3. Pharmacological treatments

In separate groups of rats, the following drugs were given as pre-treatment (Table 2). In these drug treated rats, BBB permeability, CBF, 5-HT, brain edema and cell injury were examined according to the standard protocol.

(a) *p-Chlorophenylalanine* (*a 5-HT synthesis inhibitor*): *p*-CPA was dissolved in sterile saline and administered intraperitoneally in animals (100 mg/kg) daily for 3 days. On the 4th day, animals received 5-HTP injections. This dose and time schedule depletes serotonin content of blood as well and in the CNS for long-time (Andén & Modigh, 1972; Bhagavan & Coursin, 1973; Sicuteri, Anselmi, & Fanciullacci, 1970).

(b) *Indomethacin* (*a prostaglandin synthetase inhibitor*): The drug was dissolved in saline with addition of a pinch of sodium bicarbonate at 60 °C. The pH was adjusted to 7.4 before administration of indomethacin (10 mg/kg) in animals through intraperitoneal route. These animals received 5-HTP injection 30 min after indomethacin treatment. This dose of the drug induces inhibition of prostaglandins in the CNS for several hours (Crook & Collins, 1977; Gafni, Schwartzman, & Raz, 1978; Sharpe, Thalme, & Larsson, 1974).

(c) *Diazepam* (*an anxiolytic drug*): Diazepam ampoule was diluted in saline and injected subcutaneously (4 mg/kg) 30 min before 5-HTP administrations. This dose of the compound is known induce anxiolytic effects in rats (Dailly, Hascoët, Colombel, Jolliet, & Bourin, 2002; Kerry & Jenner, 1962; Yeung, Treit, & Dickson, 2012).

(d) *Ketanserin* (*a 5-HT2 receptor antagonist*): The drug was dissolved in 30% ethanol and administered 30 min (1 mg/kg, i.p.) before 5-HTP administration. This dose of the drug effectively blocks 5-HT2 receptors in the CNS (de Clerck, David, & Janssen, 1982; Leysen et al., 1981; Van Nueten, Xhonneux, Vanhoutte, & Janssen, 1981).

(e) *Cyproheptadine* (*a 5-HT2-receptor antagonist with weak effects on Histamine H1 receptors*): The drug was dissolved in saline and injected intraperitoneally (5 mg/kg) 30 min before 5-HTP administrations. This dose of compound mainly inhibits 5-HT2 receptors. The pre synaptic 5-HT receptors or auto receptors are not blocked with this dosage used (Doggrell, 1992; Krstić & Katusić, 1982; Reiche & Frey, 1983).

(f) *Vinblastine* (*an inhibitor of vesicular transport*): Vinblastine (0.8 mg/kg) was administered intravenously 30 h before 5-HTP challenge. The drug in this dose is known to inhibit mitosis in microtubules thereby effectively able to block formation of channels or vesicular transport in endothelial cells (Dustin, 1963; Florian & Mitchison, 2016; Johnson, Armstrong, Gorman, & Burnett, 1963).

3.1 Statistical analysis

The unpaired student's t-test was applied to evaluate statistical significance between control and experimental groups. ANOVA followed by Dunnett's test was applied for multiple comparison involving drug-treatments. A P value <0.05 was considered to be significant.

4. Our observations on 5-HTP neurotoxicity

4.1 Effect of 5-HTP on stress and behavioral symptoms

Administration of 5-HTP in conscious rats resulted in development of stress symptoms, which is dose dependent (Table 1). At the end of 4 h after 5-HTP injections, animals exhibited a dose dependent hypothermia with 50 mg (-0.54 ± 0.04 °C) and 75 mg (-1.18 ± 0.02 °C) respectively. However, with further increase in doe to 150 mg, significant hyperthermia ($+3.46 \pm 0.23$ °C, $P < 0.001$, compared to control) was noted. The hyperthermic animals exhibited signs of salivation, a feature not observed in animals, which received lower dosage of the compound. Animals with 5-HTP 150 mg dose showed hyperlocomotor activity compared to untreated or saline treated rats. This effect was dose dependent. The other behavioral symptoms such as jumping, wet dog shake and circular motion exhibited a dose dependent response compared to normal animals (Table 1). Animals received highest dose of 5-HTP showed diarrheic stool. Postmortem examination revealed microhemorrhages in the stomach wall in animals with 150 mg dose of 5-HTP.

Table 1 Effect of 5-hydroxytryptophan on stress symptoms and physiological variables.

			5-HTP treatment		
Parameters measured	**Control**	**Saline**	**50 mg/kg**	**75 mg/kg**	**150 mg/kg**
Stress symptoms	$n=6$	$n=5$	$n=5$	$n=6$	$n=8$
Δ°C Rectal T	$+0.40 \pm 0.30$	$+0.44 \pm 0.21$	-0.54 ± 0.04^{b}	-1.18 ± 0.02^{a}	$+3.46 \pm 0.23^{a}$
Salivation	–	–	–	–	+++
Locomotion	+	+	++	++	++++
Defecation pellets (total)	5 ± 2	4 ± 1	8 ± 2	14 ± 4^{a}	$20 \pm 6\#^{a}$
Wet dog shake	–	–	–	4 ± 2	8 ± 3
Jumping	–	–	+/–	++	+++
Circular motion	–	–	–	+/–	++
Grooming	–	–	+	+	++
Gastric hemorrhages	–	–	–	4 ± 2	20 ± 4^{c}
Physiological variables	$n=6$	$n=5$	$n=5$	$n=5$	$n=5$
MABP torr	104 ± 6	108 ± 4	120 ± 8^{b}	128 ± 6^{b}	82 ± 8^{a}
Arterial pH	7.38 ± 0.04	7.38 ± 0.06	7.37 ± 0.04	7.36 ± 0.08	7.36 ± 0.05
Arterial PaO_2 torr	80.23 ± 0.21	80.24 ± 0.18	79.25 ± 0.34	78.34 ± 0.56	81.23 ± 01.6
Arterial $paCO_2$ torr	34.36 ± 0.23	34.56 ± 0.43	33.28 ± 0.18	33.23 ± 0.23	36.13 ± 1.15
Heart rate beats/min	64 ± 4	66 ± 3	68 ± 5	70 ± 6	74 ± 4
Respiration cycle/min	35 ± 5	33 ± 4	38 ± 4	40 ± 6	44 ± 6

a = significantly different from control; b = significantly different from 5-HTP 150 mg dose; c = significantly different from 5-HTP 75 mg dose. # In few cases ($n=3$) partial diarrheic stool; – = absent, + = very mild, ++ = mild, +++ = moderate, ++++ = extensive; * = $P<0.05$, *** = $P<0.001$, Student's unpaired t-test.

4.2 Effect of 5-HTP on physiological variables

Administration of 5-HTP induced widespread changes in physiological variables (Table 1). Lower dosage of 5-HTP (50 and 75 mg) induced a mild hypertension while 150 mg dose of the compound resulted in hypotension (20 Torr). The arterial pH did not show significant difference from the control group irrespective of the dose used. There was a trend of increase PaO_2 as well $PaCO_2$ at the end of 4 h after 5-HTP 150 mg administration. However, this difference was not statistically significant. With low dose of the compound, a slight insignificant decrease in PaO_2 and $PaCO_2$ were noted. 5-HTP treatments on the other hand resulted in dose dependent changes in heart rate. Low dose of the compound induced bradycardia whereas, 75 and 150 mg dose induced tachycardia compared to saline treated animals. Low dose of 5-HTP resulted in slight hypopnea, 75 and 150 mg dose resulted in hyperpnoea (Table 1).

4.3 Effect of 5-HTP on blood-brain barrier permeability

Administration of 150 mg dose of 5-HTP significantly increased the permeability of the BBB to Evans blue and $^{[131]}$Iodine compared to normal control and sham treated group (Table 1). Evans blue dye was seen in the cerebral cortex superficial layers as well as on the dorsal surface of the hippocampus and caudate nucleus. The cerebellar vermis and occasionally lateral cerebellar cortex were also stained. Extravasation of radioiodine was further extended to many other regions (Table 3). Lower doses of the compound did not induce extravasation of either tracer.

In general, the magnitude and severity of radiotracer extravasation was much higher than the Evans blue dye permeability.

This increase in the permeability of the BBB following 5-HTP (150 mg) dose was reversible in nature. Thus, in separate group of rats, when the tracer substances were administered 10 h after the 5-HTP administration, no significant increase in the permeability to either Evans blue or radio-iodine was noted (Table 2).

Regional BBB permeability showed wide range of variation in the magnitude of radiotracer extravasation. Thus cortical regions exhibited a minimum of 175% increase (frontal cortex) to a maximum of 722% higher (cingulate cortex) permeation of radioiodine compared from controls. The cerebellar cortices showed even further high extravasation (777–925% increase from the control). Hippocampus, caudate nucleus and brain stem showed 485–731% increase in tracer extravasation. On the other hand,

Table 2 Effect of 5-hydroxytryptophan on the blood-brain barrier permeability and cerebral blood flow.

			BBB permeability		Cerebral blood flow
Type of experiment	***n***	**Rectal T (°C)**	**EBA mg%**	**[131]Iodine %**	**Whole brain mL/g/min**
Control group	6	36.83 ± 0.23	0.24 ± 0.04	0.34 ± 0.03	1.12 ± 0.04
Saline	5	37.04 ± 0.18	0.22 ± 0.06	0.38 ± 0.06	1.03 ± 0.05
		+0.21	−8.3	+10.5	−8.03
5-HTP 50 mg/kg	5	36.01 ± 0.04*	0.28 ± 0.10	0.42 ± 0.10	1.34 ± 0.08*
		−0.82	+16.67	+23.52	+19.64
5-HTP 75 mg/kg	6	35.67 ± 0.23*	0.26 ± 0.08	0.38 ± 0.06	0.94 ± 0.06
		−1.16	+8.33	+11.76	−16.07
5-HTP 150 mg/kg	8	40.48 ± 0.24***	1.34 ± 0.65***	2.34 ± 0.65***	0.76 ± 0.10***
		+3.65	+458.33	+588	−32.14
5-HTP 150 mg/kg[a]	6	37.64 ± 0.34	0.54 ± 0.23	0.68 ± 0.28	0.96 ± 0.08
		+0.81	+58.82	+100	−14.28

[a]Values measured 10 h after 5-HTP injection.

Animals received intraperitoneal injection of saline or 5-HTP in sterile water (0.3 mL). Four hours after administration of drug or saline body temperature, BBB permeability and CBF were measured.

Values are mean ± SD; Figures below the mean values indicate % change from control. − = below, + = above the control values. BBB = blood-brain barrier; CBF = cerebral blood flow.

* = $P < 0.05$, *** = $P < 0.001$, Student's unpaired t-test.

Table 3 Effect of 5-HTP on plasma and regional brain serotonin levels.

Serotonin content	Control	Saline	5-HTP treatment		
			50 mg/kg	75 mg/kg	150 mg/kg
Brain serotonin (μg/g)	$n=6$	$n=5$	$n=5$	$n=6$	$n=8$
Cerebral cortex	0.67 ± 0.21	0.72 ± 0.43	0.56 ± 0.23	0.89 ± 0.32[a]	2.36 ± 1.23[b]
		−7.46	−16.41	+32.83	+525.72
Hippocampus	0.34 ± 0.15	0.38 ± 0.08	0.89 ± 0.21[a]	1.15 ± 0.34[a]	1.33 ± 0.34[a]
		+11.76	+161.76	+238.23	+291.17
Cerebellum	0.28 ± 0.07	0.33 ± 0.06	0.56 ± 0.08[a]	0.89 ± 0.08[a]	0.95 ± 0.06[a]
		+17.85	+100	+217.85	+239.28
Thalamus	0.54 ± 0.12	0.48 ± 0.18	0.38 ± 0.17[b]	0.86 ± 0.17	1.67 ± 0.23[a]
		−11.11	−29.62	+59.25	+209.25
Hypothalamus	0.43 ± 0.12	0.46 ± 0.16	0.67 ± 0.14[b]	0.76 ± 0.18[b]	1.87 ± 0.23[a]
		+6.97	+55.81	+76.74	+334.88
Medulla	0.35 ± 0.11	0.36 ± 0.08	0.66 ± 0.12[b]	0.68 ± 0.14[b]	1.24 ± 0.18[a]
		+2.85	+88.57	+94.28	+254.28
Brain stem	0.48 ± 0.08	0.54 ± 0.10	0.68 ± 0.12[b]	0.76 ± 0.08[a]	0.89 ± 0.14[a]
		+12.5	+41.66	+58.33	+85.41
Plasma μg/mL	0.24 ± 0.04	0.26 ± 0.03	0.34 ± 0.06[a]	0.58 ± 0.08[a]	0.87 ± 0.10[a]
		+8.33	+41.66	+141.66	+262.5

[a] $P<0.001$.
[b] $P<0.05$.
Values are mean ± SD; Figures below mean values indicate % change from control group. − = below, + = above the control value.
Student's unpaired t-test compared from control group.

thalamus, hypothalamus, pons, medulla and frontal cortex exhibited only 175–293% increase. The inferior colliculus showed an insignificant increase of 7% only (Table 3).

4.4 Effect of 5-HTP on cerebral blood flow

Subjection of animals to 4 h after 5-HTP injection (150 mg) resulted in a marked 32% decrease in the cerebral blood flow. This decrease in CBF was not

seen after 10 h 5-HTP administration. Thus, at this time period, the CBF showed a nonsignificant decline by 16% only (Table 2). Administration of 50 mg dose in fact increased the CBF by 20% after 4 h. The 75 mg dose did not significantly influence the CBF changes.

Analysis of regional CBF showed widespread reduction in flow in 150 mg treated rats at 4 h. The cerebral and cerebellar cortices, hippocampus, caudate nucleus showed 21–24% decline in the CBF. Thalamus, hypothalamus, pons, medulla and brain stem showed a 2–17.5% decline in the regional CBF. Whereas, colliculi exhibited 8–13% decrease in the CBF compared to control group.

The magnitude and severity of CBF reduction were in no way comparable to the increase in the regional BBB permeability.

4.5 Effects of 5-HTP on brain edema formation and volume swelling

Regional brain edema was measured in eight brain regions (Table 4) after saline or 5-HTP administration in selected doses. Our observations showed a dose related increase in the brain water content and volume swelling in 5-HTP treated rats. The cerebral cortex showed 3%, 5% and 11% increase in volume swelling after 5-HTP 50, 75 and 150 mg doses, respectively. Interesting cerebellum was the top region that showed highest volume swelling that was higher than the cerebral cortex in 5-HTP treated rats. Thus, 4%, 9% and 11% volume swelling was seen in the cerebellum after 5-HTP 50, 75 and 150 mg doses, respectively. The other brain regions namely hippocampus, thalamus, hypothalamus, medulla and brain stem showed volume swelling of 4%, 6% and 7–8% after 5-HTP administrations in doses 50, 75 and 150 mg (Table 4).

4.6 Effect of 5-HTP on plasma and brain 5-HT concentration

We measured 5-HT concentration in the plasma and brain 4 h after 5-HTP administrations. The plasma 5-HT showed a dose dependent increase from 41% to 262.5% following 50 to 150 mg doses of the 5-HT precursor. In brain there is a regional variation in serotonin concentration after 5-HTP administrations. In 50 mg group, hippocampus and cerebellum showed 161% and 100% increase in 5-HT concentration, respectively. Hypothalamus, medulla and brain stem exhibited about 41% to 88% increase in the amine level. The cerebral cortex and thalamus in fact showed a mild 16–22% decreases in the 5-HT level.

Table 4 Effect of 5-HTP on brain water content and volume swelling.

			5-HTP treatment		
Brain edema	**Control**	**Saline**	**50 mg/kg**	**75 mg/kg**	**150 mg/kg**
Brain water %	$n=6$	$n=5$	$n=5$	$n=6$	$n=8$
Cerebral cortex	74.34 ± 0.21	74.56 ± 0.32	75.12 ± 0.13[a]	75.67 ± 0.21[a]	77.12 ± 0.35[b]
			+3.12	+5.32	+11.12
Hippocampus	68.34 ± 0.12	68.53 ± 0.09	69.34 ± 0.21[a]	69.98 ± 0.11[a]	70.56 ± 0.21[b]
			+4.00	+6.56	+8.96
Cerebellum	71.23 ± 0.06	71.32 ± 0.10	72.34 ± 0.22[b]	73.41 ± 0.22[b]	74.10 ± 0.21[b]
			+4.44	+8.72	+11.48
Thalamus	67.34 ± 0.32	67.28 ± 0.18	68.23 ± 0.16[a]	68.97 ± 0.23[b]	69.04 ± 0.14[b]
			+3.56	+6.52	+6.80
Hypothalamus	65.23 ± 0.08	65.67 ± 0.21	66.23 ± 0.16[b]	66.74 ± 0.21[b]	67.34 ± 0.18[b]
			+4.00	+6.04	+8.44
Medulla	65.34 ± 0.21	65.67 ± 0.31	66.28 ± 0.25[b]	66.87 ± 0.29[b]	67.23 ± 0.30[b]
			+3.76	+6.12	+7.56
Brain stem	64.39 ± 0.25	64.45 ± 0.52	65.32 ± 0.25[b]	65.87 ± 0.12[b]	66.46 ± 0.15[b]
			+3.73	+5.92	+8.28

[a] $P<0.05$.
[b] $P<0.01$.
Values are Mean ± SD; Figures below mean values indicate % f (volume swelling) from control group. −=below, +=above the control value.
Student's unpaired t-test compared from control group.

With 75 mg dose, all brain regions showed a significant increase. The most pronounced increase (two- to threefold) was seen in the hippocampus and cerebellum. The other regions exhibited 30–100% increase. With further increase in dose to 150 mg, the cerebral cortex showed fivefold increase in 5-HT level. Thalamus, hypothalamus and medulla also exhibited two- to threefold increase of the amine. Hippocampus and cerebellum was still two- to threefold higher in their 5-HT content. Brain stem showed only 85% increase in the amine level.

4.7 Effects of 5-HTP on brain pathology

Neuronal injuries were examined in 5-HTP treated rats using Nissl or H&E staining on paraffin sections (Table 5). In general, neuronal injuries were seen in almost all eight-brain regions examined in a dose dependent manner. Interestingly the number of damaged neurons was highest in the cerebellum followed by hippocampus and cerebral cortex (Table 5). Cerebellum showed mean 34, 44 and 63 damaged neurons in the cerebellar cortical areas following 5-HTP administrations of 50, 75 and 150 mg doses. On the other hand hippocampus exhibited 54, 78 and 89 damaged neurons in the area

Table 5 Effect of 5-HTP on neuronal injury using Nissl or Hematoxylin and Eosin (H&E) staining on 3-μm thick paraffin sections.

			5-HTP treatment		
Neuronal Injury Nrs.	**Control**	**Saline**	**50 mg/kg**	**75 mg/kg**	**150 mg/kg**
Neuronal injury	$n=6$	$n=5$	$n=5$	$n=6$	$n=8$
Cerebral cortex	3 ± 4	2 ± 3	24 ± 6^{a}	46 ± 8^{a}	78 ± 8^{a}
Hippocampus	2 ± 1	2 ± 3	34 ± 8^{a}	44 ± 10^{a}	63 ± 12^{a}
Cerebellum	3 ± 2	3 ± 4	54 ± 12^{a}	78 ± 14^{a}	89 ± 18^{a}
Thalamus	4 ± 2	4 ± 5	18 ± 5	26 ± 8	43 ± 12^{a}
Hypothalamus	2 ± 1	2 ± 2	12 ± 6^{a}	18 ± 8^{a}	34 ± 7^{a}
Medulla	3 ± 1	3 ± 2	8 ± 2^{b}	12 ± 6^{b}	20 ± 4^{a}
Brain stem	2 ± 2	2 ± 3	6 ± 2^{b}	8 ± 3^{b}	13 ± 4^{b}

[a] $P<0.01$.
[b] $P<0.05$.
Neuronal injury is characterized by deform, swollen or shrunken neurons associated with perineuronal edema, loss of nucleolus or eccentric nucleolus within the nucleus of nerve cells in anatomical defined areas. The number of such neurons is examined in a blinded fashion by three observers and median values are used for statistical analysis.
Values are Mean ± SD; Student's unpaired t-test compared from control group.

following 50, 75 and 150 mf doses of 5-HTP within 4 h. Cortex showed mean 24, 46, 78 damaged neurons after 5-HTP 50, 75 and 150 mg doses. Thalamus and hypothalamus showed moderate damage in nerve cells and medulla and brainstem showed significant but mild neuronal damages (Table 5). These damaged neurons are present in the edematous brain areas exhibiting BBB breakdown.

4.8 Effect of 5-HTP on lanthanum exudation across the endothelial cells

Ultrastructural studies showed lanthanum extravasation across the endothelial cells in 5-HTP treated rats after 4 h. The magnitude and intensity of lanthanum exudation within the endothelial cell cytoplasm and in basal lamina occasionally is dose dependent (results not shown). Lanthanum could be sometimes seen within the microvesicular profiles in the endothelial cell cytoplasm as well as in the basal lamina while tight junctions are closed to this tracer in all the microvessels examined (Fig. 2). Interestingly, one vessel could show lanthanum exudation in some part of the endothelial cell while the adjacent endothelial cell is completely free of lanthanum exudation within the cell cytoplasm or across the endothelial cell. In this regard, the vessel diameter or number of endothelial cells affected by lanthanum exudation in one capillary is not consistent. However, those capillaries showing exudation of lanthanum across the cell cytoplasm and in basal lamina perivascular edema id often seen in 5-HTP treated rats. The number of microvascular showing leakage of lanthanum following 5-HTP treatments is related to the doses of the compound used. However, the pattern of lanthanum exudation and mode of tracer transfer is not altered by the dose used. Tight junctions in all cases and in all doses of 5-HTP remained intact to lanthanum (results not shown).

Normal saline treated animals showed lanthanum as black particles totally confined within the lumen of the microvessels.

4.9 Effects of 5-HTP on albumin and neuronal nitric oxide synthase immunohistochemistry

5-HTP treatment exhibited albumin leakage in the neuropil of cerebral cortex, cerebellum, hippocampus, thalamus and hypothalamus in the areas showing edematous swelling and sponginess. The magnitude and intensity of albumin leakage is dose dependent in 5-HTP treated groups. Medulla and brainstem showed fewer but significantly higher number of albumin positive cells as compared to control group or saline treated animals (Figs. 4 and 5).

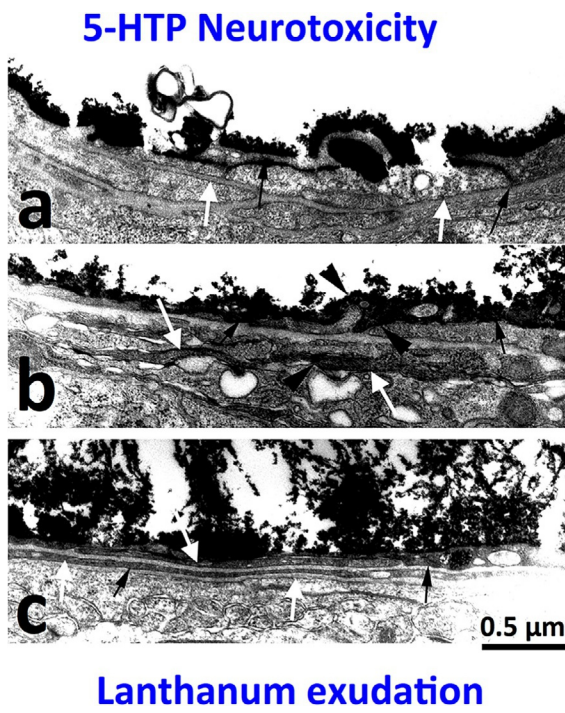

Fig. 4 High power transmission electron micrograph (TEM) showing lanthanum exudation across the cerebral endothelial cells from parietal cerebral cortex of one 5-HTP (150 mg/kg, i.p.) administered rats after 4 h (a–c). Lanthanum is largely confined within the lumen of the cerebral endothelium and stopped at the tight junctions (black arrow, a) and presence of lanthanum in the basal lamina (white arrow) is not seen (a). Lanthanum is seen into the basal lamina (white arrow, b) whereas; the tight junction (black arrow) is still impermeable to the electron-dense tracer (b). Lanthanum infiltration into the basal lamina of one capillary (black arrows) whereas the tight junctions are still intact (white arrow, c). Bar = 500 nm.

Interestingly, upregulation of nNOS is also seen in the vicinity of albumin exudation in the neuropil. This upregulation of nNOS is most prominent in the cerebellum followed by hippocampus and cerebral cortex. Thalamus and hypothalamus also showed significant nNOS upregulation in the edematous region. Very few nNOS positive ells are seen in the medulla and brainstem. The nNOS immunoreactivity is most prominent in the neuronal cell cytoplasm and occasionally karyoplasm also showed nNOS immunoreaction in 5HTP treated group. The magnitude and intensity of nNOS upregulation showed a close parallelism with the dose of the compound used.

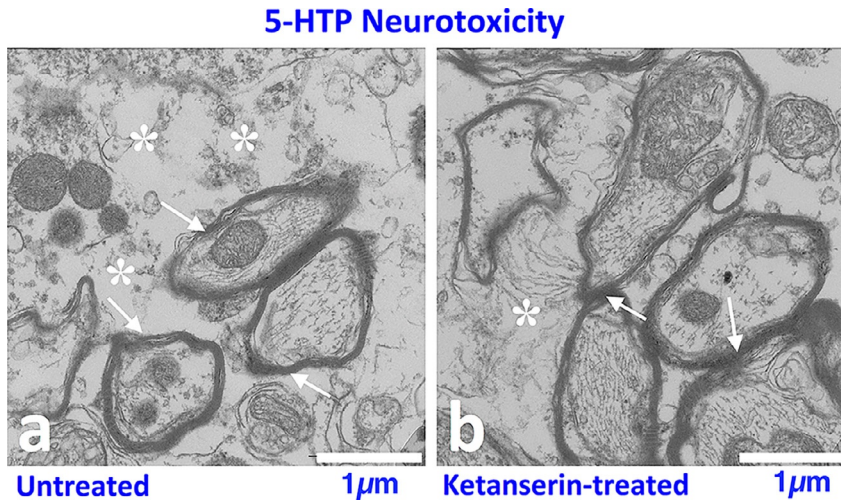

Fig. 5 High power transmission electron micrograph of neuropil from IIIrd cellular layer of parietal cerebral cortex in one 5-HTO (150 mg/kg, i.p.) administered rats after 4 h (a) and neuroprotection by ketanserin (b). Vacuolation in neuropil, edema (*) and axonal distortion is clearly seen in untreated 5-HTP administered rats (a). In ketanserin pretreated rat 5-HTP administration induced changes in the neuropil is markedly less evident (*). The neuropil appears to be compact (b) than untreated (a) group after 5-HTP administrations. Bar = 1 μm.

5. Effect of drug treatments on 5-HTP induced alteration in behavioral, physiological and cerebrovascular function

Since, 150 mg 5-HTP produced the most increase in the 5-HT levels in the plasma, BBB permeability, CBF alterations, stress symptoms, physiological variables, the influence of several drugs on these parameters were observed in this group only. The following drugs were used.

(a) *Effect of tryptophan hydroxylase inhibitor, p-CPA*

Pretreatment with *p*-CPA significantly reduced the BBB permeability increase to Evans blue and radioactive iodine (Table 6). The regional BBB permeability was also significantly attenuated. The regional CBF was almost restored near normal levels. This treatment significantly thwarted the 5-HT increase in the brain and spinal cord (Table 7).

There was a significant reduction in core body temperature from the untreated group. The 5-HTP induced tachycardia was mainly absent. Microhemorrhages in the stomach wall and diarrheic stool were significantly reduced by *p*-CPA treatment. The other behavioral symptoms were considerably reduced (Table 6).

Table 6 Effect of 5-HTP (175 mg/kg) on regional blood-brain barrier permeability and regional cerebral blood flow.

	rBBB permeability $^{[131]}$Iodine %			rCBF mL/g/min		
Brain regions	**Saline**	**5-HTP**	**% Change**	**Saline**	**5-HTP**	**% Change**
1. Cingulate cortex	0.18 ± 0.03	1.48 ± 0.08**	+722	1.04 ± 0.04	0.82 ± 0.06**	−21
2. Frontal cortex	0.24 ± 0.06	0.66 ± 0.10*	+175	1.16 ± 0.06	0.80 ± 0.04**	−31
3. Parietal cortex	0.30 ± 0.04	1.76 ± 0.08**	+486	1.23 ± 0.04	0.84 ± 0.08**	−32
4. Occipital cortex	0.32 ± 0.04	1.46 ± 0.11***	+356	1.18 ± 0.06	0.85 ± 0.06***	−28
5. Temporal cortex	0.28 ± 0.05	1.64 ± 0.12***	+485	1.09 ± 0.10	0.82 ± 0.10*	−25
6. Hippocampus	0.26 ± 0.08	1.76 ± 0.08***	+576	0.96 ± 0.04	0.78 ± 0.08**	−20
7. Caudate nucleus	0.28 ± 0.04	1.64 ± 0.06***	+485	0.94 ± 0.06	0.72 ± 0.08**	−23
8. Cerebellar vermis	0.18 ± 0.02	1.58 ± 0.04***	+777	0.98 ± 0.05	0.74 ± 0.05**	−24
9. Cerebellar cortex	0.16 ± 0.04	1.64 ± 0.08***	+925	0.92 ± 0.04	0.72 ± 0.02***	−21
10. Colliculus superior	0.25 ± 0.05	1.45 ± 0.10***	+480	1.21 ± 0.08	0.83 ± 0.10*	−31
11. Colliculus inferior	0.20 ± 0.07	0.34 ± 0.08	+7	1.05 ± 0.06	0.96 ± 0.08	−8.5
12. Thalamus	0.44 ± 0.05	1.68 ± 0.08**	+281	0.87 ± 0.04	0.76 ± 0.06*	−13
13. Hypothalamus	0.48 ± 0.08	1.89 ± 0.06**	+293	0.92 ± 0.06	0.80 ± 0.08***	−13
14. Pons	0.23 ± 0.04	0.78 ± 0.08***	+239	1.01 ± 0.03	0.86 ± 0.08**	−15
15. Medulla	0.18 ± 0.06	0.56 ± 0.05**	+211	1.14 ± 0.08	0.94 ± 0.06*	−17.5
16. Brain stem	0.19 ± 0.04	1.58 ± 0.10*	+731	1.18 ± 0.04	1.04 ± 0.10	−12
Average	0.28 ± 0.09	1.37 ± 0.49	+451	1.05 ± 0.11	0.83 ± 0.08	−22
		+389			−21	

Values are mean ± SD, * $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$, compared from control group; Student's unpaired t-test.
Figures below the values represent % change from control group. + = increase, − = decrease.

(b) *Effect of cyclooxygenase inhibitor, indomethacin*

Pretreatment with indomethacin significantly reduced the stress symptoms and physiological variables. However, indomethacin did not reduce the rise in rectal temperature caused by 5-HTP administrations. Increase in heart rate and respiration, occurrence of gastric hemorrhages, diarrheic stool, wet dog shakes, jumping behavior, motor behavior were considerably attenuated by indomethacin treatment, Blood gases and blood pressure were not statistically different from 5-HTP-treated group.

The BBB permeability was significantly reduced in indomethacin treated animals. The CBF decline in this group was absent.

There was a slight but significant decrease in 5-HT content in this drug treated group. Thus, hippocampus, cerebellum and thalamus showed only mild increase in 5-HT (18–139%). Hypothalamus did not show any change in 5-HT content. The plasma 5-HT level showed only 50% increase compared to untreated group.

(c) *Effect of anxiolytic drug, diazepam*

Pretreatment with diazepam significantly inhibited stress and behavioral symptoms without affecting 5-HTP induced rise in the body temperature (Table 6). This drug markedly inhibited 5-HTP induced gastric hemorrhages. Alterations in motor behavioral pattern were completely absent in diazepam treated rats. Tachycardia was absent in these animals without affecting respiration. The MABP continued to remain depressed like untreated rats and no significant difference in the physiological variables were seen compared to 5-HTP administered rats.

Diazepam pretreated rats did not exhibit 5-HTP induced breakdown of the BBB permeability to Evans blue or radioactive iodine. The CBF values were also restored to normal levels. Regional BBB permeability showed significant increase in radiotracer in two out of 16 brain regions (medulla and inferior colliculus) from the control group. The decline in regional CBF was mild (ranging from +1% to −17%). In one out of 16 regions, the decline in regional CBF was statistically significant (occipital cortex).

(d) *Effect of a potent 5-HT2 and a weak Histamine H1 receptor antagonist, cyproheptadine*

Cyproheptadine pretreatment influenced stress and behavioral symptoms mildly. Thus, behavioral symptoms and hyper motor activity were less evident in this drug treated rats following n 5-HTP administration. Mild diarrheic stool was noted in this group. The incidence of wet dog shakes was considerably reduced. This drug treatment

prevented tachycardia and respiratory changes caused by 5-HTP. However, 5-HTP induced hypotension and changes in blood gases or arterial pH were not affected significantly (Table 6).

Cyproheptadine pretreatment significantly reduced the extravasation of Evans blue and radioactive iodine in the brain following 5-HTP injections. The CBF also did not exhibit significant decline from the untreated control group. Changes in the regional BBB and CBF were also markedly influenced by cyproheptadine pretreatment. In this drug treated group 5-HTP administration was able to retain significant increase in the permeability of the radiotracer in medulla only. In all 15 other brain regions, the mild increase in the BBB permeability by 9–125% was not found statistically significant. The CBF showed significant decline in parietal cortex and in the superior colliculus. In other 14 regions, changes in rCBF (+1% to −17%) were not significant.

Cyproheptadine pretreatment in general did not influence regional 5-HT increase following 5-HTP administrations. In cerebellum and in the spinal cord, a significantly higher serotonin content was noted in this drug treated animals compared to 5-HTP treatments alone (Table 7).

(e) *Effect of a potent and specific 5-HT2-receptor antagonist, ketanserin*

Pretreatment with ketanserin significantly attenuated most of the stress and behavioral symptoms without affecting 5-HTP induce rise in the core body temperature. Tachycardia and hyperpnoea were absent in this drug treated rats. However, state of 5-HTP induced hypotension and changes in the blood gases or arterial pH were not significantly different (Table 6). Behavioral symptoms and hyper motor activity were less marked in this drug treated animals following 5-HTP challenges. Gastric hemorrhages were significantly reduced without any significant effect on diarrhea or soft stool.

The BBB permeability to Evans blue and radiotracer were markedly attenuated. The CBF decline was much less evident and insignificant compared to control group. The region changes in the BBB and CBF were also less intense. Only spinal cord exhibited 89% increase in iodine extravasation compared to control group. In all other 15-brain regions the increase in radiotracer extravasation from 0% to 100% were not found statistically significant. The regional CBF changes ranged from +2% to −12% from the control group and were insignificant.

Changes in regional serotonin content were not significantly affected by ketanserin in this group caused by 5-HTP injections. In spinal cord, 5-HT content was significantly much higher in ketanserin treated group compared to untreated rats (Table 6).

Table 7 Pharmacological manipulation of 5-HTP (150 mg/kg) induced changes in the rat brain.

Prototype	Known function	Dose/route (mg/kg)	Schedule	Source
1. 5-Hydroxytryptophan	Serotonin precursor	50, 75, 150, i.p.	4 h before	Sigma Chemicals, USA
2. *p*-Chlorophenylalanine	Serotonin synthesis inhibitor	100/day for 3 days, i.p.	1 day before	Sigma Chemicals, USA
3. Indomethacin[b]	Prostaglandin synthesis inhibitor	10, i.p.	30 min before	Sigma Chemicals, USA
4. Diazepam (Valium)[a]	Anxiolytic drug	4, s.c.	30 min before	ICI, UK
5. Cyproheptadine	5-HT2 receptor antagonist	5, i.p.	30 min before	Merck, Sharp & Dhome, UK
6. Ketanserin	Selective 5-HT2 receptor antagonist	1, i.p.	30 min before	Lysen, Beerse, Belgium
7. Vinblastine[a]	Antimitotic and vesicular transport inhibitor	0.8, i.v.	30 min before	Eli, Lilly & Co., USA

[a]Ampoule.
[b]Dissolved in water at 60 °C with a pinch of sodium bicarbonate and pH adjusted to 7 before administration.
The choice of drug-dosage, route and time schedule were determined according to the standard protocol from literature (for details see text); Other drugs in powder form were dissolved in sterile water and injected i.p. (0.3 mL), i.v. (0.5 mL), s.c. (0.05 mL).

(f) *Effect of a vesicular transport inhibitor and antimitotic drug, vinblastine*

Pretreatment with vinblastine in general did not influence 5-HTP induced stress and behavioral symptoms markedly. The body temperature continues to remain high and the tachycardia and hyperpnoea were present. Diarrheic stool with hyper locomotion and other motor behavior was apparent. The arterial pH and blood gases were very similar to that of untreated 5-HTP injected rats.

The extraction of dye and radiotracer were, however, significantly reduced from the untreated group. The absolute extravasations of

tracers were significantly different from control group. However, the CBF continued to decline like untreated 5-HTP challenged group (Table 7). The regional changes in the BBB were significantly less than the untreated 5-HTP administered groups except in medulla, where the extravasation of radiotracer was 55% higher than the control group. Changes in BBB permeability in other regions ranged from −14% to 137% from the control, but the differences were not found statistically significant. The regional decline in CBF was significant (−17% to −28%) in 10 out of 16 regions. In remaining six regions a decline in 2–17% was not significant from control group.

The serotonin content was very similar to that of untreated 5-HTP administered group. In hippocampus, the 5-HT content was significantly higher in vinblastine treated group compared to untreated 5-HTP administered rats.

6. Effects of drugs on 5-HTP induced brain pathology and immunohistochemistry

Morphological and ultrastructural studies were done in 150 mg dose of 5-HTP treated animals only and its modulation by few-selected drug such as *p*-CPA, diazepam and ketanserin.

Our observations showed that pretreatment with these drug significantly reduced neuronal changes in the cerebral cortex, hippocampus and cerebellum caused by 5-HTP administrations. Also albumin immunoreactivity and nNOS upregulation was significantly reduced in these brain areas in drug treated group after 5-HTP administrations. The most superior neuroprotective effects on cell changes and albumin or nNOS immunoreaction in 5-HTP treated groups was induced by diazepam followed by *p*-CPA and ketanserin (results not shown).

Ultrastructural changes on lanthanum exudation as also reduced by these drugs after 5-HTP administration. Thus, lanthanum was largely confined within the lumen in these drug treated animals after 5-HTP administrations. However, only a few endothelial cells in sporadic capillaries exhibited vesicular transport of lanthanum within the endothelial cell cytoplasm. Present of lanthanum in the basal lamina is not seen in these drug treated animals after 5-HTP administrations.

7. Possible mechanisms of 5-HTP induced neurotoxicity

The salient new findings of the present investigation show that acute 5-HTP administration is capable of inducing marked breakdown of the BBB

permeability. This observation is the first to point out that this breakdown of the BBB could be one of the most important factors in causing disturbed mental function and mood alterations on the first day of the drug therapy (Das, Bagchi, Bagchi, & Preuss, 2004). Altered BBB function to serum factors is the hallmark of several psychiatric diseases (Montagne et al., 2016, 2017; Ozkizilcik et al., 2018; Sharma et al., 2012; Sweeney, Montagne, et al., 2019). However, it is still not known whether this increased permeability of the BBB seen in depression, mental retardation, schizophrenia, and autism will adversely affect 5-HTP administration induced BBB breakdown as compared to normal healthy brain (see Birdsall, 1998). This is an important novel subject that requires additional investigation.

Fortunately, this increase in BBB permeability to proteins is time-dependent and appears to be reversible in nature. This is evident from the finding that no further evidence of extravasation of Evans blue or radioactive iodine was apparent when the BBB was examined 12 h after 5-HTP administration. However, it is unclear whether the BBB is still open to small molecules as compared to serum protein complex (MW 28–68 kDa) (Bradbury, 1979; Rapoport, 1976). There are evidences that hyperosmotic solutions induced BBB breakdown to Evans blue is repaired within 2 h period whereas, the BBB breakdown to $Na+$ is still open for 4 days after the initial insult (see Rapoport, 1976). This suggests that 5-HTP induced breakdown of the BBB could have long lasting effects leading to potentially long-term brain dysfunction.

The altered BBB permeability appears to be related with its ability to enhance endogenous serotonin concentrations in the plasma and brain (Udenfriend et al., 1957). Increased circulating serotonin level markedly affects CBF (Bonvento et al., 1994; Burnstock, 1985; Edvinsson & MacKenzie, 1976; Kobayashi et al., 1985; MacKenzie & Scatton, 1987; Raskin, 1981). This idea is further supported by the fact that 5-HTP-administration resulted in widespread alterations in the regional cerebral blood flow (rCBF) in present investigation. Although, elevation of endogenous serotonin levels following 5-HTP administrations has been known for decades, to our knowledge, this is the first report that demonstrates widespread alterations in the BBB permeability and the CBF in several brain regions after its systemic administration.

To examine breakdown of the BBB permeability, we used two different protein tracers (Sharma, 2007; Sharma et al., 1990a; Sharma & Dey, 1981; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). Both the tracers bind to endogenous serum proteins when administered in the systemic circulation (Bradbury, 1979; Rapoport, 1976; Sharma, 1999, 2009;

Sharma & Westman, 2003, 2004). Thus, leakage of these tracers in brain in fact represent extravasation of tracer-protein complex (Bradbury, 1979; Brightman et al., 1970; Rapoport, 1976; Sharma et al., 1990a). One molecule of Evans blue (molecular weight 960), in particular, will bind to approximately 12 molecules of albumin (combined molecular wt. 68,000) (Bradbury, 1979; Rapoport, 1976; Sharma, 1982). About two-thirds of serum albumin will be bound to Evans blue in the doses that is used in this study (Sharma, 2007; Sharma et al., 1990a; Sharma & Dey, 1981; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). Likewise, iodine largely binds to albumin by 50% (mol. wt. about 28,000) (Bradbury, 1979; Rapoport, 1976). Thus, the wide permeation of iodine compared to Evans blue may be due to differences in the molecular sizes and diameter of the tracer-protein or complexes and/or due to the differences in the proteins to which they bind in circulation (Sharma, 2007; Sharma et al., 1990a; Sharma & Dey, 1981; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). Obviously, smaller molecular size tracers could pass through the barrier more readily compared to larger sized tracers at the BBB interface.

Leakage of plasma proteins into the cerebral microenvironment leads to vasogenic edema (Sharma et al., 1991a, 1992, 1994). This is clearly evident with our findings by 5-HTP induced brain edema formation. Brain edema and volume swelling is present in almost all brain regions exhibiting BBB breakdown to proteins. Leakage of endogenous albumin using immunohistochemical techniques further confirms this hypothesis in present investigation. Thus, albumin leakage is evident in areas showing sponginess and edematous expansion. Furthermore, distorted neurons, swollen or shrunken with perineuronal edema is present in areas showing albumin immunoreaction in the neuropil after 5-HTP administrations. When the BBB breakdown of Evans blue and radioiodine is no longer observed after 12 h of 5-HTP administrations, neuronal damages, leakage of serum albumin is till visible within the neuropil. These observations clearly suggests that 5-HTP induced BBB dysfunction leads to long-term neuronal damages and may lead to neurodegeneration over time (H.S. Sharma, unpublished observations).

The possibility that 5-HTP induced BBB breakdown, edema formation and cell injury could also be associated with increased oxidative stress and free radical formation (Kayacan, Yazar, Cerit, & Ghojebeigloo, 2019; Reyes-Gonzales et al., 2009). This idea is supported by the fact that we have

observed upregulation of neuronal nitric oxide synthase (nNOS) in various parts of the brain associated with neuronal injuries (Chae et al., 2009; Henry, Ishimura, & Peisach, 1976). The nNOS upregulation suggests generation of nitric oxide $(NO)^-$ the free radical that induces membrane damage directly (Dalkara & Moskowitz, 1994; Dawson, Dawson, & Snyder, 1992; Dawson, Zhang, Dawson, & Snyder, 1994;Sharma et al., 1996; Sharma et al., 2005; Sharma, Alm, & Westman, 1998; Sharma, Nyberg, et al., 1998). This damage to endothelial cells leads to BBB breakdown (Sharma et al., 1996; Sharma et al., 2005; Sharma, Alm, & Westman, 1998; Sharma, Nyberg, et al., 1998). Massive upregulation of nNOS by 5-HTP in a dose dependent manner suggest that 5-HTP-induced neurotoxicity could be mediated through NO production (see Sharma, Alm, & Westman, 1998). High level of circulating serotonin could also one of the primary reasons to induce oxidative stress and production of free radical NO (Bedrosian & Nelson, 2014; Dhir & Kulkarni, 2011; Lee et al., 2013; Zhou, Zhu, Nemes, & Zhu, 2018). This fact is well reflected in upregulation of nNOS immunoreactivity in 5-HTP administered group.

That 5-HTP induced nNOS upregulation could induce endothelial cell membrane disruption leading to BBB breakdown is further supported by our ultrastructural investigation of microvascular permeability using the electron dense tracer lanthanum (12 Å diameter). 5-HTP administrations in a dose dependent manner induce vesicular transport of lanthanum across the endothelial cell in several places in the brain exhibiting nNOS upregulation. Lanthanum is also seen in the basal lamina. However, the tight junctions remain intact. This suggests that serotonin, NO and oxidative stress could somehow stimulate vesicular transport at the endothelial cells leading to the breakdown of the BBB following 5-HTP administrations (Sharma, 2007; Sharma et al., 1990a, 1990b, 1991a, 1992, 1994; Sharma & Dey, 1981, 1987, 1988; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995).

The mechanisms of BBB breakdown following 5-HTP administrations appear to be related with several other factors stress associated with 5-HTP administrations. Stress is one of the main reasons to enhance plasma and brain serotonin level, hyperthermia and free radical formation (Dey & Sharma, 1983, 1984; Sharma et al., 1990b; Sharma & Dey, 1984, 1986a, 1986b, 1987, 1988).

Administration of 5-HTP also resulted in marked stress symptoms and behavioral alteration. These changes are most prominent at the time of increased BBB permeability. However, it is unclear whether altered

behavioral and symptoms are due to effects of the drug alone or drug-induced elevation of 5-HT level. Since several drugs in this study influence symptoms and behavioral alterations, it seems most likely that behavioral symptoms are related with both the peripheral and central actions of the 5-HT (Musumeci et al., 2015).

However, prevention of BBB permeability by several drugs in this investigation, irrespective of their effects on 5-HT level, modified the behavioral alterations and stress symptoms. Thus, it seems quite likely that breakdown of the BBB is somehow interrelated with behavioral alterations and/or stress symptoms (Dey & Sharma, 1983, 1984; Sharma, 2007; Sharma et al., 1990a, 1990b, 1991a, 1992, 1994; Sharma & Dey, 1981, 1984, 1986a, 1987, 1988; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). Alternatively, symptoms associated with 5-HTP administrations such as hyperthermia and stress caused by elevation of serotonin may also contribute to BBB permeability (Dey & Sharma, 1983, 1984; Sharma et al., 1990b, 1991a, 1992, 1994; Sharma & Dey, 1984, 1986a, 1987, 1988). Obviously, serotonin induced stress response can be modulated by drugs modulating serotonergic transmission and/or its receptor function.

5-HTP administration results in profound alterations in the rCBF. Although, those brain areas showing regional BBB (rBBB) breakdown also exhibit reduction in the rCBF, however, rCBF is not directly responsible for rBBB breakdown. This is evident with the fact that the magnitude of reduction in rCBF is unrelated with the intensity of the rBBB breakdown.

This is further apparent from the fact that drugs that reduced serotonin levels in plasma and brain prevented BBB breakdown, edema formation, nNOS expression and cell injuries. Whereas, drugs that are receptor blocker or inhibitor of vesicular transport did not prevent serotonin elevation after 5-HTP administrations but blocked the BBB breakdown, edema formation cell injury and albumin extravasation (H.S. Sharma, unpublished observation). Obviously, blockade of serotonin receptors could not stimulate cascades of molecular signaling leading to BBB breakdown (Olsson et al., 1992; Sharma, 2007; Sharma et al., 1990a, 1990b; Sharma, Dey, & Olsson, 1989; Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). Inhibition of microtubule function or vesicular transport can also prevent BBB leakage because the 5-HTP induced BBB breakdown is mediated through enhanced vesicular transport and not by widening of the tight junctions (Sharma, 2007; Sharma et al., 1990a; Sharma & Dey, 1981; Sharma, Dey, & Olsson, 1989;

Sharma, Olsson, & Dey, 1989, 1995; Sharma, Westman, Cervós-Navarro, et al., 1995; Winkler et al., 1995). Obviously, when BBB breakdown is prevented it will reduce edema formation and subsequently nNOS upregulation and cellular injury.

8. Conclusions and future perspectives

In conclusion, our observations suggest that acute administration of 5-HTP induces breakdown of the BBB to serum proteins leading to edema formation and neuronal injuries. These changes are associated with enhanced plasma and brain serotonin level associated with nNOS upregulation. Leakage of proteins although reduced over 12 h but the deposition of plasma protein and cell injury could continue for longer periods after 6-HTP administrations. Further research is needed to understand 5-HTP induced acute and chronic neurotoxicity. Since oxidative stress appears to play key roles in 5-HTP neurotoxicity adjunct therapies using neurotrophic factors and related strategies are needed to contain acute brain damage. Our laboratory is engaged in finding suitable adjunct therapy to minimize 5-HTP neurotoxicity by coadministration of cerebrolysin-a balanced composition of several neurotrophic factors and active peptide fragment either alone or its nanodelivery. These strategies could expand our knowledge and help cases in psychiatric diseases where 5.HTP therapy is needed.

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