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In vitro release of [^{3}H]5-hydroxytryptamine from fetal and maternal brain by drugs of abuse

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Abstract

Cortical synaptosomes were prepared from pregnant dams (GD-17) and rat fetuses (ED-17), loaded with [^{3}H]5-HT and assayed to evaluate release mediated by cocaine (COC), fenfluramine (FEN) and 3,4-methylenedioxymethamphetamine (MDMA). COC and FEN elicited a high-affinity (10^{-9} M) release response in fetal tissue which was not apparent in the dam. MDMA-induced release was similar in magnitude in both tissue types. Consequently, the release of 5-HT from developing neurons may be one mechanism by which COC and FEN elicit their teratogenetic effects in utero.

Key words: Synaptosome; Inhibition; Cocaine; Fenfluramine; 3,4-Methylenedioxymethamphetamine; Transporter

In utero exposure to several psychoactive drugs of abuse are known to produce transient neurobehavioral and neurochemical alterations in the progeny of treated laboratory animals [17]. Previous work in our laboratory has shown that gestational exposure (GD 13-parturition) to COC and FEN inhibits cortical serotonin (5-HT) fiber outgrowth measured during the early postnatal period (PND 1–7) in rats [1,2].

Cocaine, fenfluramine and the substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA) have been shown to both inhibit the reuptake of and/or promote the release of 5-HT, DA and NE (in vitro) from a variety of neuronal preparations [4,6]. Other neurochemical effects include stimulation of tyrosine hydroxylase activity, inhibition of tryptophan uptake and reduction of serotonin synthesis [13,16,17]. However, these effects have never been clearly demonstrated in fetal rats making it difficult to determine how these drugs may be damaging to the developing brain.

Serotonin acts as a potent teratogen/embryotoxin during development due to 5-HT's influence on uterine/umbilical blood flow [9]. Furthermore, extracellular 5-HT has the capability to autoregulate the growth of fetal 5-HT neurons in culture [20]. All three compounds share the potential to increase the extraneuronal 5-HT concentration, which could then facilitate the aforementioned inhibition of cortical fiber outgrowth and maturation. In this study, we examined the relative potencies of COC, FEN and MDMA to promote the release of [^{3}H]5-HT from loaded fetal (ED-17) synaptosomes and to compare these results to those obtained in maternal tissue (GD-17). These observations may then help to elucidate the mechanism by which these drugs mediate their effects in utero.

Pregnant Sprague–Dawley rats (GD-17) were placed under deep anesthesia and the fetuses were removed by cesarian section. Crown-rump lengths (CRL's) were recorded for all fetuses to insure accuracy of gestational age. Fetuses measured between 16–17 mm (average 16.6 mm; $n = 12$) according to the methods of Lauder and Bloom [11]. The brains of the mother and all litter mates were rapidly removed and the cerebral cortex was carefully prepared following removal of the cerebellum. Cortices were weighed and placed in ice-cold (4°C) 0.32 M sucrose at 10 × volume/wet weight. Samples were homogenized using a Cole Palmer glass/teflon homogenizer and centrifuged for 10 min at 1700 *g* in a Sorvall centrifuge. The supernatant (S1) was decanted and centrifuged at 12,500 *g* for 10 min The

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resultant pellet, representing the crude synaptosomal preparation, was resuspended in 10 × v/w Krebs–Ringers buffer (KR) containing (mM) NaCl 145, KCl 1.86, $CaCl_2$ 1.2, $MgSO_4$ 1.2, Hepes 25, KH_2PO_4 1.2, glucose 11.1 and pargyline 0.1, a monoamine oxidase inhibitor (pH 7.4). The Ca^{2+} dependency of drug-mediated release was assessed in a buffer where Ca^{2+} was replaced with 1.5 mM ethyleneglycoltetraacetate (EGTA).

Synaptosomes were loaded by incubation for 15 min at 37°C with [^{3}H]5-HT (50 nM; 28.2 Ci/mmol) New England Nuclear, Boston, MA. Uptake was terminated by centrifugation for 5 min at 2400 *g*, resuspension of the pellet in KR and recentrifugation for 5 min at 2400 *g*. The final pellet was resuspended in 10 × vol./wet wt. of buffer. Experimental plates (Nunc, Denmark) were filled with or without 20 μl of releasing drugs (KCl, (+)-MDMA-HCl and Cocaine-HCl (NIDA; Washington, DC), fenfluramine (Sigma; St. Louis, MO), followed by 40 μl of loaded synaptosomes (approx. 45.0 μg protein/fetal condition and 160.0 μg protein/maternal condition; as tested by the Lowry method [12]) and enough KR to bring the final volume to 200 μl. Plates were incubated at 37°C for 20 min to allow for the full releasing effect. Plates were placed on ice for 1 min to halt release and were harvested onto glass-fiber filter paper with normal KR (pH 7.4) using a Titertek cell harvester. Filters were placed in scintillation vials along with 3 ml of Liquiscint (National Laboratories) and counted in a Beckman liquid scintillation counter (efficiency 40%) for 1 min CPM data was converted to synaptosomal [^{3}H]5-HT retention. Results are expressed as % control of the average of four determinations performed over three experiments. Statistical significance was determined on the raw data using the two-tailed Student's *t*-test respective to control conditions.

KCl. In both tissue types, KCl produced a significant and dose-dependent release of loaded [^{3}H]5-HT beginning at 10 mM. K^+-stimulated [^{3}H]5-HT release was found to be dependent on the presence of extracellular Ca^{2+}. In Ca^{2+}-free buffer ($CaCl_2$ replaced by 1.5 mM EGTA), 20 mM KCl produced a small decrease in synaptosomal [^{3}H]5-HT retention in fetal tissue (94.6% versus 74.5% in normal buffer). KCl-mediated release in fetal tissue did not differ significantly from that measured in maternal tissue (data not shown).

Cocaine. Fetal (ED-17) synaptosomes: in vitro cocaine produced a biphasic release of [^{3}H]5-HT from loaded synaptosomes. The first release response occurred at 10^{-9} M (41.8% of total release) and the second at 10^{-4} M (70% of total release). Maternal (GD-17) synaptosomes: in vitro treatment produced significant release of [^{3}H]5-HT beginning at 10^{-6} M (23.1% of total release) and this response remained dose-dependent through 10^{-3} M (100% of total release). Although COC produces release at relatively low concentrations in fetal tissue, COC releases a greater amount of the available [^{3}H]5-HT pool in maternal tissue (40.0% vs. 22.4%) (Fig. 1).

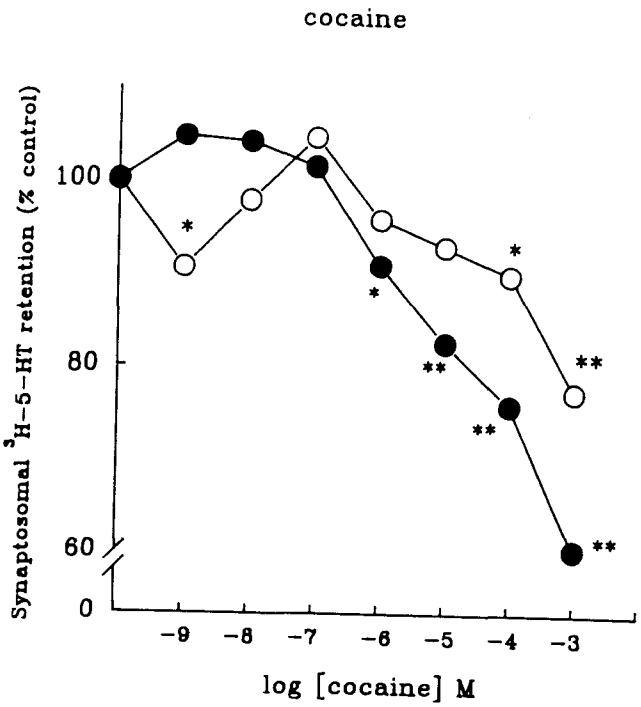

Fig. 1. The effect of cocaine (concentrations indicated) on the release of [^{3}H]5-HT (50 nM) from loaded fetal (ED-17) (open circles) and maternal (GD-17) (closed circles) synaptosomes. Each data point is the average ± SD of four determinations performed over three experiments ($n = 3$) of synaptosomal [^{3}H]5-HT retention as a percentage of control (560.0 ± 23.3 cpm/well for fetal synaptosomes and 26,343.3 ± 150.1 cpm/well for maternal synaptosomes). All SD's were less than 8.0% of the calculated mean for all data points. Student's *t*-test showed: * $P < 0.05$ and ** $P < 0.01$.

Fenfluramine. Fetal (ED-17) synaptosomes: FEN also produced a biphasic release of [^{3}H]5-HT from loaded synaptosomes. Significant release ($P < 0.05$) was first observed at 10^{-9} M (42.8% of total release) and a second response beginning at 10^{-6} M FEN (26.8% of total release) which then remained concentration-dependent through 10^{-3} M (100% of total release). Maternal (GD-17) synaptosomes: FEN induced a dose-dependent release of [^{3}H]5-HT from synaptosomes over the range of concentrations from 10^{-7} M (11.6% of total release) through 10^{-3} M (100% of total release). FEN also has the capability of releasing a greater amount of the available [^{3}H]5-HT from maternal versus fetal synaptosomes (91.2% vs. 39.9%) (Fig. 2).

3,4-Methylenedioxymethamphetamine. Fetal (ED-17) synaptosomes: exposure to MDMA, in vitro, produced a concentration-dependent release of [^{3}H]5-HT beginning at 10^{-7} M (16.8% of total release). Maternal (GD-17) synaptosomes: as with fetal tissue, exposure to MDMA induced a dose-dependent efflux of loaded

[^{3}H]5-HT at concentrations of 10^{-7} M (9.9% of total response) through 10^{-3} M. Interestingly, MDMA shows a potent inhibition of [^{3}H]5-HT release at 10^{-9} M ($P < 0.01$), which was not apparent in fetal tissue. In contrast with the other two drugs, in vitro exposure to MDMA induces a similar release profile in both fetal and maternal tissue, appears effective over the same range of concentrations in both tissue types and releases comparable amounts of serotonin at its highest dose (Fig. 3).

All three compounds are known to induce the release of ^{3}H-5-HT from loaded adult synaptosomes via an interaction with the 5-HT plasma-membrane transporter [6,14]. Our results indicate that at ED-17 the machinery is present for both the uptake of, as well as the release of [^{3}H]5-HT. These results compare favorably with earlier studies showing that the enzymes that control monoamine synthesis, degradation, uptake and K^+-induced, Ca^{2+}-dependent release are available by ED-18 [7,8,19]. This indicates that the young neurons have matured to a point where they can begin to seek out their targets. We have previously described that gestational COC and FEN exposure (GD 13-parturition) delays the maturation of the serotonergic innervation of the cortex and hippocampus [1,2]. It is feasible that chemical agents may have a greater impact on subsequent development during this time of 'neural organization' than by altering neurogenesis directly.

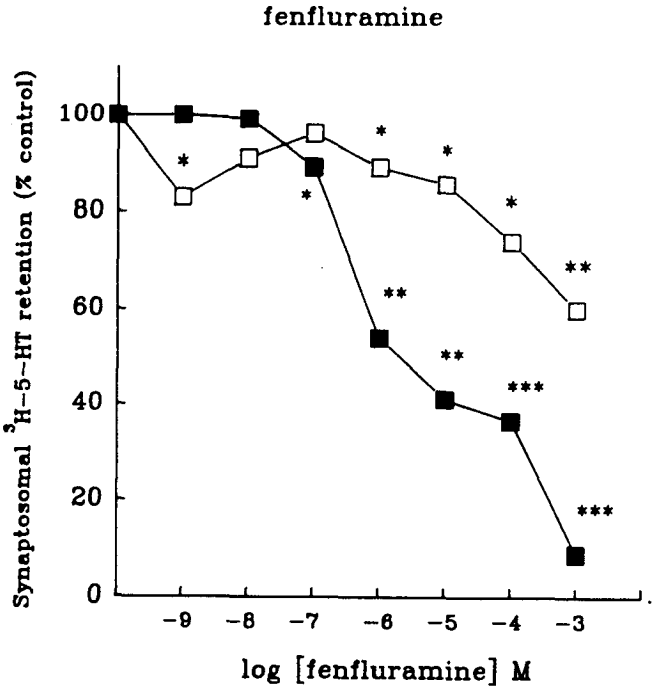

Fig. 2. The effect of fenfluramine (concentrations indicated) on the release of [^{3}H]5-HT (50 nM) from loaded fetal (ED-17) (open squares) and maternal (GD-17) (closed squares) synaptosomes. Each data point is the average ± SD of four determinations performed over three experiments ($n = 3$) of synaptosomal [^{3}H]5-HT retention as a percentage of control (485.3 ± 1.53 cpm/well for fetal synaptosomes and 21,315.3 ± 988.1 cpm/well for maternal synaptosomes). All SD's were less than 8.0% of the calculated mean for all data points. Student's t-test showed: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

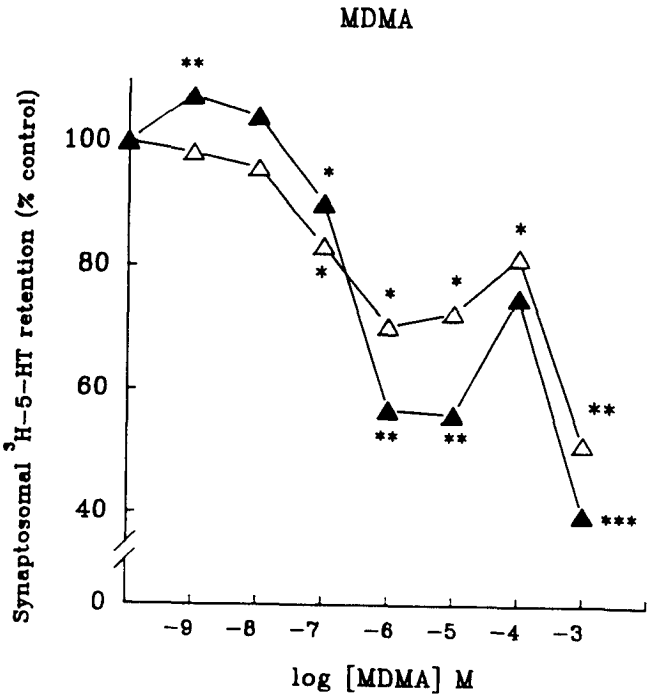

Fig. 3. The effect of MDMA (concentrations indicated) on the release of [^{3}H]5-HT (50 nM) from loaded fetal (ED-17) (open triangles) and maternal (GD-17) (closed triangles) synaptosomes. Each data point is the average ± SD of four determinations performed over three experiments ($n = 3$) of synaptosomal [^{3}H]5-HT retention as a percentage of control (552.7 ± 8.1 cpm/well for fetal synaptosomes and 23,675 ± 325.6 cpm/well for maternal synaptosomes). All SD's were less than 8.0% of the calculated mean for all data points. Student's t-test showed: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

It appears that both COC and FEN activate a high affinity release response in the fetal brain which is absent from the maternal brain. This effect may be due, in part, to the physical immaturity of the functional 5-HT transporter and over time, this release mechanism appears to be lost or masked [15]. Differences between the 5-HT transporter proteins (in fetal and maternal tissue) may also explain why COC and FEN released a greater percentage of the available [^{3}H]5-HT from maternal synaptosomes.

MDMA had a similar release profile in both tissue types compared to the differences observed with COC and FEN. Prenatal MDMA exposure was found to produce only minor behavioral alterations in developing rats, but left the density of 5-HT uptake sites, 5-HT and 5-hydroxyindolaceticacid (5-HIAA) levels unchanged compared to untreated controls [15]. Conversely, gestational MDMA caused significant de-

creases in 5-HT and 5-HIAA levels throughout the maternal brain [15]. These findings are in contrast to the anatomical and biochemical deficits reported after in utero COC and FEN exposure in newborn rats.

The ultimate action of all the substances tested is to increase the extraneuronal concentration of 5-HT within the fetal environment. The source of this excess transmitter can either be from the mother or as indicated within this report, from fetal neurons directly. Studies performed by Woods et al. [23], reported that cocaine (2.0 mg/kg i.v.) administered to pregnant ewes increased maternal blood pressure by 50%, increased uterine vascular resistance by 168% and decreased uterine blood flow by 50%. These effects are due to constriction of the uteroplacental vasculature and results in diminished O_2 delivery to the fetus. The uterine ateries have been found to be highly sensitive to vasoactive amines like norepinepherine (NE) and presumably, 5-HT [10]. Evidence has been presented for an imipramine-sensitive 5-HT transporter in human placental brush-boarder membranes [5]. Placental 5-HT uptake proteins inhibited by COC, FEN and MDMA could allow excess 5-HT (and/or the test compounds) to pass through the placenta and into the embryonic brain via the underdeveloped blood–brain barrier.

Such a mechanism could explain the potent teratogenetic effects of 5-HT on fetal brain development. Excess systemic 5-HT causes constriction of the placental vasculature and the restriction of fetal/maternal blood flow, which can induce acute hypoxia within the fetal environment [9,23]. Furthermore, 5-HT itself has the ability to autoregulate the growth of fetal serotonergic neurites in culture [20]. This latter effect is interesting because it may occur in response to a disruption in the synthesis, storage and release of the serotonergic trophic factor S-100ß; whose activity is believed to be mediated through interactions with the glial-5-HT_{1A} receptor during development [21,22].

Our results are consistent with the theory that both the uptake and release of neurotransmitters are important during normal fetal neuronal development [3]. Our observations indicate that the release of 5-HT from growing serotonergic fibers may be one putative mechanism by which drugs of abuse modulate the development of the 5-HT innervation of the brain.

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