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Serotonin release varies with brain tryptophan levels

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This study examines directly the effects on serotonin release of varying brain tryptophan levels within the physiologic range. It also addresses possible interactions between tryptophan availability and frequency of membrane depolarization in controlling serotonin release. We demonstrate that reducing tryptophan levels in rat hypothalamic slices (by superfusing them with medium supplemented with 100 μM leucine) decreases tissue serotonin levels as well as both spontaneous and electrically-evoked serotonin release. Conversely, elevating tissue tryptophan levels (by superfusing slices with medium supplemented with 2 μM tryptophan) increases both tissue serotonin levels and serotonin release. Serotonin release was found to be affected independently by tryptophan availability and frequency of electrical field-stimulation (1–5 Hz), since increasing both variables produced nearly additive increases in release. These observations demonstrate for the first time that both precursor-dependent elevations and reductions in brain serotonin levels produce proportionate changes in serotonin release, and that the magnitude of the tryptophan effect is unrelated to neuronal firing frequency. The data support the hypothesis that serotonin release is proportionate to intracellular serotonin levels.

INTRODUCTION

The rate at which serotonin (5-HT) is released from brain neurons is often assumed to vary with that of 5-HT synthesis, and thus with brain levels of its precursor tryptophan. This assumption arises from the known dependence of 5-HT synthesis on the local concentration of tryptophan[13,15,36]. It also derives from studies reporting modifications in certain behaviors, including aggression[26] and pain sensitivity[33], when brain tryptophan levels are altered in vivo; the tryptophan-sensitivity of these and other behaviors is thought to be due to precursor-dependent changes in 5-HT synthesis and subsequent release of the transmitter into brain synapses. If 5-HT synthesis and release do in fact covary, then one would expect to find that changes in 5-HT synthesis would produce parallel changes in 5-HT release. Indeed, numerous studies have demonstrated increases in 5-HT release in vivo[5,6,11,24,47,48] or in vitro[3,43] when brain tryptophan levels have been elevated; a few studies, however, failed to reveal this affect[14,34,50]. Conspicuously, no study has examined directly the effect of *reducing* brain tryptophan levels on the release of endogenous 5-HT. Furthermore, it is not known whether the firing activity of serotoninergic neurons influences the relationship between tryptophan availability and 5-HT release. The present study addresses these questions in vitro.

We now report that 5-HT release is decreased when brain tryptophan levels are lowered, and that the modulation of 5-HT release by tryptophan availability is independent of, and nearly additive with, the effects of varying the frequency of membrane depolarization.

MATERIALS AND METHODS

Slice preparation

Hypothalamic slices (300 μm) from male Sprague–Dawley rats (180–240 g) were prepared as described previously[43]. Some slices were retained, frozen over dry ice, and stored at −70 °C until biochemical assays were performed. Most slices were placed into parallel chambers and superfused (0.6 ml/min) with pre-warmed physiologic medium having the following composition (in mM): NaCl 130, KCl 3.5, $CaCl_2$ 1.3, $MgSO_4$ 1.5, NaH_2PO_4 1, $NaHCO_3$ 25, D-glucose 10. This medium also contained 2 μM fluoxetine (unless noted otherwise) to block the reuptake of synaptic 5-HT[31], and was gassed continuously with 95% O_2/5% CO_2 to sustain pH 7.4. Slices were equilibrated by superfusion for 50 min prior to fraction collection.

Experimental design

Effects of LNAAs on indole levels and release. In experiments designed to examine the effects of reducing or elevating brain tryptophan levels on 5-HT release, each chamber contained the equivalent of one hypothalamus. Slices in one chamber were superfused with fluoxetine-containing medium supplemented with 100 μM leucine, 2 μM tryptophan, or 100 μM leucine plus 2 μM tryptophan. Slices in the other chamber were superfused with amino acid-unsupplemented medium, and thus served as control. Four experiments were performed using each of the 3 comparative conditions. Successive 5-min fractions (FC-80K Fractionator, Gilson Medical Electronics, Inc., Middleton, WI) were collected for 80 min

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(i.e., between 50 and 130 min) beyond the initial equilibration period. Each fraction of superfusion medium was collected into a glass tube containing 100 μl of 7 mM ascorbate and internal standards [5-hydroxy-N-methyltryptamine oxalate (5-HT-CH_3) and 5-hydroxy-2-indolecarboxylic acid (5-HICA)], mixed by vortex, and stored in the dark on ice, until further processing (within 1–2 h). Slices were electrically field-stimulated for 3 periods of 4.7 min each, starting 60, 85 and 110 min after the onset of superfusion. Electrical field-stimulation was induced by delivery of bipolar square-wave pulses (5 Hz, 2 ms, 100 mA·cm^2) using a stimulator (S88, Grass Instrument Co., Quincy, MA) in series with a 12 V relay; pulses were monitored by an oscilloscope (V-212, Hitachi Denshi Ltd., Tokyo, Japan). At the end of each experiment, the slices were removed from their chambers, quickly rinsed in distilled water, frozen over dry ice, and stored at −70 °C for subsequent biochemical assays.

In experiments addressing possible interactions between neuronal activity and altered tryptophan availability in controlling 5-HT release, each superfusion chamber contained the equivalent of a demi-hypothalamus. The fluoxetine-containing medium superfusing slices in one chamber was supplemented with 2 μM tryptophan, that superfusing the other chamber was amino acid-unsupplemented (control). Ten-minute fractions were collected over a 70-min period (i.e., between 50 and 120 min) beginning at 50, 60, 75, 85, 100 and 110 min from the onset of superfusion. These fractions were collected and handled as described above. Slices were electrically field-stimulated for 3 periods of 4 min each, starting at 60, 85 and 110 min from the onset of superfusion. Electrical field-stimulation was induced as described above, although the frequency of the stimulator was set at either 1, 3 or 5 Hz, which delivers 240, 720 or 1200 pulses respectively, over a constant (4-min) period of electrical field-stimulation. Seven or eight experiments were performed at each of the 3 stimulation frequencies tested.

Time-course of tissue indole levels. Eight experiments were conducted in which slices (6 per time-point) were retrieved after 0, 15, 30, 60, 90 and 120 min of superfusion with fluoxetine-free physiologic medium. These slices were quickly rinsed with distilled water, blotted, frozen over dry ice and stored at −70 °C until assays were performed. We tested whether exposing slices to those additional conditions applied during the release experiments altered the time-courses obtained with superfusion alone. Thus, slices were delivered 3 periods of electrical field-stimulation (4.7 min, 5 Hz, 2 ms, 100 mA·cm^2) and/or superfused with medium containing 2 μM fluoxetine. 4 experiments were performed under each of these conditions.

Time-course of tryptophan concentration in superfusion medium. Each superfusion chamber contained the equivalent of one hypothalamus and was superfused with fluoxetine-containing physiologic medium. Slices in one chamber were electrically field-stimulated (5 Hz, 2 ms, 100 mA·cm^2) for 3 periods of 4.7 min each starting at 60, 85 and 110 min; those in the other were just superfused throughout. Five experiments were conducted in which fractions (15 s; 150 μl) of superfusion medium were collected from the latter chamber starting at 5, 15, 30, 60, 90 and 120 min from the onset of superfusion; fractions (15 s, 150 μl) from the electrically-stimulated chamber were collected starting at 5, 15, 30, 60, 65, 85, 90, 110 and 115 min. Ten microliters of 0.75 mM ascorbate containing the internal standard 5-HICA were added to each fraction; samples were stored on ice until assay (usually within 20 min of collection).

Biochemical analysis

Indole concentrations in superfusion medium. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were extracted from each fraction of superfusion medium using the newly developed preparative reverse-phase method described previously[43]. This method was modified slightly to extract these 5-hydroxyindoles from either the 5-min (3.1 ml) or 10-min (6.1 ml) fractions: columns containing 100 mg (for a 3.1-ml fraction) or 200 mg (for a 6.1-ml fraction) of C_{18} sorbent (40 μM, Analytichem International, Harbor City, CA) were prepared in Pasteur pipettes (9 inch). Each column was conditioned with methanol (1.5 ml) and then 0.1 M NaH_2PO_4 (pH 3.0, 0.75 ml) before loading a sample or standard fraction (pH 2.8–3.0). After passage of a fraction, the aqueous phase in a 100-mg column was displaced by 125 μl of 70% methanol/30% acetic acid and that in a 200-mg column by 200 μl of this organic solution. The 5-hydroxyindoles were eluted into Eppendorf tubes with 300 μl of this methanol/acetic acid solution, and the solvent was evaporated under a stream of nitrogen. The dried eluates were reconstituted with 50 μl of 0.15 M HCl containing 0.25 mM ascorbate. Recoveries of 5-HT and 5-HIAA were generally 80–90% based on calculations using the internal standards in standard fractions.

5-HT and 5-HIAA in the reconstituted samples were assayed by high-performance liquid chromatography with electrochemical detection (HPLC-ED). Samples (45 of 50 μl) were injected automatically (712; Waters Intelligent Systems Program, Milford, MA) over a reverse-phase C_{18} column (5 μm, 25 cm, Beckman Instrument Co., San Ramon, CA). The mobile phase was pumped (110A, Altex Scientific Inc., Berkeley, CA) over this column at a rate of 1.2 ml/min, and was of the following composition (in mM): NaH_2PO_4, 200; Na_2EDTA, 0.1; octyl sodium sulfate, 0.17; with 13% methanol (vol/vol) and having a final pH of 4.3. 5-HT and 5-HIAA were detected electrochemically (LC-4A; Bioanalytical Systems Inc., West Lafayette, IN) at 2 nA/V when the potential of the glassy carbon electrode was set at 0.55 V against the Ag/AgCl reference electrode.

Tryptophan in fraction aliquots (50 μl) was assayed using the HPLC-ED conditions as described above, but with the applied potential set at 0.9 V.

Tissue indole levels. Frozen tissue samples were homogenized by ultrasonication in 0.2 M $HClO_4$ (ca. 0.4 ml/mg protein) containing 0.5 mM ascorbate and the internal standards 5-HT-CH_3 and 5-HICA. These homogenates were centrifuged (35 000 × g, 10 min) and aliquots (50 μl) of these tissue supernatants were assayed for their contents of tryptophan, 5-HT and 5-HIAA by HPLC-ED, with the applied potential set at 0.85 V and the sensitivity at 5 nA/V. Tissue pellets were assayed for protein using the method of Lowry et al.[35], with bovine serum albumin serving as the protein standard.

Data analysis

Amounts of the indoles in each sample of superfusion medium or tissue supernatant were estimated by correcting the recorded peak height for its recovery, using the designated internal standard (5-HT-CH_3 for 5-HT and tissue tryptophan, 5-HICA for 5-HIAA and medium tryptophan), followed by linear regression analysis based on standard curves run in parallel with each set of samples. These amounts were normalized to the amount of protein in the tissue pellet.

For each experiment examining the effects of leucine and/or tryptophan on hypothalamic 5-hydroxyindole release, calculations were made of the average rates of 5-HT release (pmol/g protein/min) over the 4 rest periods (spontaneous 5-HT release) and the 3 periods of electrical field-stimulation. Evoked 5-HT release was calculated by subtracting the 5-HT released spontaneously from that released with stimulation. The rate of 5-HIAA efflux (pmol/g protein/min) was taken as the average across the 80-min collection period. Tissue indole levels and release are reported here as means or mean percents ± S.E.M. relative to paired control slices. The fractions of intracellular 5-HT released (%) per min were calculated by dividing the average rate of 5-HT released spontaneously or with stimulation (pmol/g protein/min) by the final tissue 5-HT levels (nmol/g protein), and then multiplying by 100. Data were evaluated by the 2-tailed Student paired *t*-test. Statistically significant differences were noted for these and all other analysis when the probability value was less than 0.05.

For experiments addressing the effect of tryptophan availability on the release of 5-HT evoked at varying frequencies of electrical field-stimulation, we calculated 5-HT release per pulse (pmol/g protein/pulse) and total 5-HT release (pmol/g protein). These data were evaluated by a 2-way analysis of variance, followed by the Duncan multiple range post-hoc test.

One-way or two-way analysis of variance, as appropriate, followed by the Duncan multiple range test, was used to evaluate the effects of treatments (i.e., superfusion time, electrical field-stimulation, fluoxetine) on tryptophan concentrations in superfusion medium and on tissue indole levels.

Materials

Fluoxetine HCl was generously provided by Eli Lilly Laboratories (Indianapolis, IN). All L-amino acids (tryptophan and leucine) and standard compounds (5-HT, 5-HIAA and albumin) were purchased from Sigma Chemical Company (St. Louis, MO). The internal standards 5-HT-CH_3 and 5-HICA were obtained from Aldrich Chemical Company (Milwaukee, WI). Glass-distilled methanol used for HPLC and for the extraction procedure was obtained from EM Science (Gibbstown, NJ); acetic acid for extraction use was of buffer grade, and was purchased from Pierce Chemical Company (Rockford, IL). All other reagents were of analytic grade.

RESULTS

Levels of tryptophan, 5-HT and 5-HIAA in hypothalamic slices prior to superfusion were 184.6 ± 10.3, 71.3 ± 1.7 and 41.6 ± 2.5 nmol/g protein, respectively. When slices were superfused with physiologic medium (unsupplemented with amino acids or fluoxetine) time-dependent changes in tissue levels of tryptophan ($F_{6,42} = 7.35$, $P < 0.01$), 5-HT ($F_{6,36} = 2.97$, $P < 0.05$) and 5-HIAA ($F_{6,13} = 44.91$, $P < 0.01$) were observed (Fig. 1). Tissue tryptophan levels increased with the onset of superfusion, and then remained relatively constant before declining gradually over the second hour. Tissue 5-HT levels also rose with superfusion, peaking after those of tryptophan and then remained relatively unchanged for the remainder of the experimental period. Tissue 5-HIAA levels fell precipitously with superfusion and remained low thereafter. Exposing slices to those additional conditions present during the release experiments (i.e., 3 periods of electrical field-stimulation; 2 μM fluoxetine) did not modify these time-courses observed with superfusion alone (data not shown). Tissue levels of tryptophan, 5-HT and 5-HIAA in slices retrieved at the end of each release experiment (i.e., after 130 min of superfusion during which time slices were exposed to electrical field-stimulation and fluoxetine) were 239.9 ± 12.1, 89.7 ± 2.6 and 3.8 ± 0.4 nmol/g protein, respectively.

We examined the possibility that the time-dependent changes in tissue tryptophan levels could be accounted for by changes in the tryptophan concentration of the extracellular medium. We found that under control conditions the amino acid-unsupplemented medium actually contained endogenous tryptophan, in concentrations (ca. 0.06–0.12 μM) which varied with time ($F_{5,36} = 4.99$, $P < 0.01$); they peaked 15–30 min after the onset of superfusion, and then declined gradually over the next 90 min (Fig. 2). This time-course was not affected by delivery of 3 periods of electrical field-stimulation.

Slices superfused with control medium released 5-HT spontaneously at a rate of 51 ± 2 pmol/g protein/min; this rate increased to 320 ± 10 pmol/g protein/min with each of the 3 periods of electrical field-stimulation. These rates were relatively consistent over the 4 rest periods and the 3 periods of electrical field-stimulation. The fraction of intraneuronal 5-HT released spontaneously was 0.06% per min; this increased with electrical field-stimulation to 0.36 ± 0.02% per min. The rate of 5-HIAA efflux during the 80-min collection period was 659 ± 30 pmol/g protein/min.

Superfusing slices with medium containing 100 μM leucine reduced final tissue tryptophan levels to 60.7 ± 1.0% ($P < 0.01$) and 5-HT levels to 88.7 ± 2.0% ($P < 0.05$) of control levels (Fig. 3). The addition of 2 μM tryptophan to the superfusion medium caused final slice tryptophan and 5-HT levels to be elevated to 183.1 ±

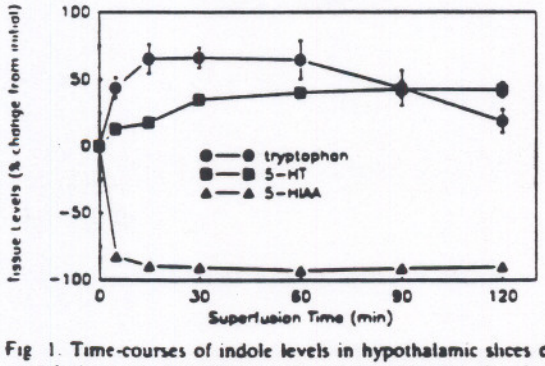

Fig. 1. Time-courses of indole levels in hypothalamic slices during superfusion with physiologic medium (unsupplemented with amino acids or fluoxetine). Time-dependent changes in tissue levels of tryptophan ($F_{6,42} = 7.35$, $P < 0.01$), 5-HT ($F_{6,36} = 2.97$, $P < 0.05$), and 5-HIAA ($F_{6,13} = 44.91$, $P < 0.01$) were observed. Data are expressed as mean percents ± S.E.M., relative to levels in pre-superfusion ($t = 0$) slices.

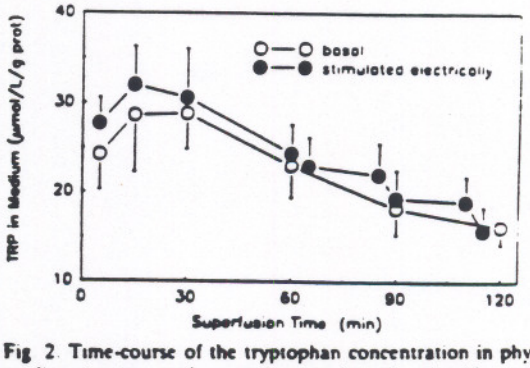

Fig. 2. Time-course of the tryptophan concentration in physiologic medium (amino acid-unsupplemented medium) with superfusion and electrical field-stimulation of hypothalamic slices. Slices in one of the parallel chambers were electrically field-stimulated (4.7 min, 5 Hz, 2 ms, 100 mA/cm²) for 3 periods starting at 60, 85 and 110 min after the onset of superfusion. Values are group means ± S.E.M. Two-way analysis of variance detected a significant effect of time ($F_{5,36} = 4.99$, $P < 0.01$), but not of treatment.

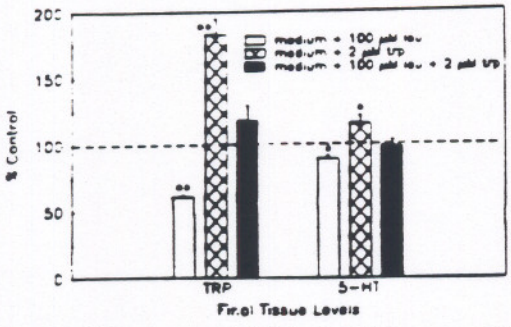

Fig. 3. Effects on tissue indole levels of decreasing and increasing tryptophan availability. Paired rat hypothalamic slices were superfused with amino acid-unsupplemented medium (control) or medium to which LNAAs leucine (100 μM, leu) and or tryptophan (2 μM, trp) were added. Slices were retrieved at the end of each release experiment (i.e., after 130 min of superfusion) and were assayed for their contents of tryptophan and 5-HT. Data are expressed as mean percents ± S.E.M., relative to control, for n = 4 pairs for each treatment. $^{*}P < 0.05$, $^{**}P < 0.01$ differs from control by the Student paired t-test.

12.4% ($P < 0.01$) and 115.0 ± 5.9% ($P < 0.05$) of control, respectively. Co-supplementing the medium with 100 μM leucine plus 2 μM tryptophan resulted in final tryptophan and 5-HT levels of 118.1 ± 10.5% and 97.7 ± 5.4% of control, respectively.

Slices superfused with medium containing 100 μM leucine decreased their spontaneous release of 5-HT to 72.6 ± 5.0% ($P < 0.05$) and their electrically-evoked release to 79.7 ± 2.6% ($P < 0.01$) of control rates (Fig. 4). 5-HIAA efflux decreased to 70.3 ± 2.1% of control ($P < 0.01$). Superfusing slices with medium containing 2 μM tryptophan increased spontaneous and electrically-evoked 5-HT release to 138.1 ± 3.9% ($P < 0.01$) and 125.9 ± 3.9% ($P < 0.01$) of control, respectively.

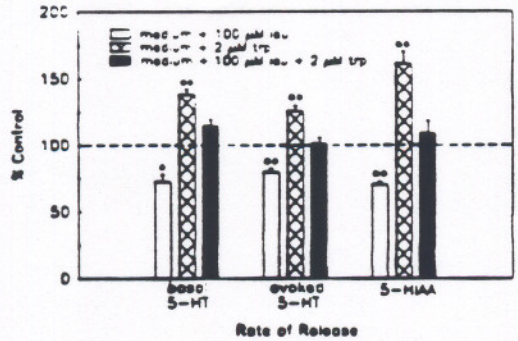

Fig. 4. Effects on 5-hydroxyindole release of decreasing and increasing tryptophan availability. Paired rat hypothalamic slices were superfused with amino acid-unsupplemented medium (control) or medium to which LNAAs leucine (100 μM, leu) and/or tryptophan (2 μM, trp) were added. Data are expressed as mean percents ± S.E.M., relative to control, for n = 4 pairs of each treatment. $^{*}P < 0.05$, $^{**}P < 0.01$ differs from control by the Student paired t-test.

TABLE 1

Fractional rates of 5-HT released per min from hypothalamic slices superfused with medium containing LNAAs

Rat hypothalamic slices were superfused with either control medium (−) or medium supplemented with 100 μM leucine (Leu), 2 μM tryptophan (Trp), or 100 μM leucine plus 2 μM tryptophan (+). 5-HT released basally and with electrical field-stimulation (4.7 min, 5 Hz, 2 ms, 100 mA/cm²) were monitored; tissue 5-HT levels were assayed in slices retrieved at the end of each release experiment (i.e., after 130 min of superfusion). Fractional rates of 5-HT release (%) per min were calculated. Values are mean percents ± S.E.M. (where greater than 0) for n = 4 pairs for each experimental condition. The Student paired t-test did not detect significant differences between fractional rates of basal or evoked 5-HT release.

LNAA		Fractional rate of 5-HT release (%)	
		Basal	Evoked
Leu	−	0.06	0.36 ± 0.03
	+	0.05	0.32 ± 0.03
Trp	−	0.05	0.35 ± 0.04
	+	0.06	0.39 ± 0.03
Leu + Trp	−	0.06	0.37 ± 0.02
	+	0.07	0.39 ± 0.02

5-HIAA efflux increased to 161.3 ± 9.0% of control ($P < 0.01$). Co-supplementing the medium with both of the large neutral amino acids (LNAAs) suppressed the changes induced by either LNAA individually.

The fractional rates of 5-HT released spontaneously or with electrical field-stimulation were not modified by supplementing the medium with leucine, tryptophan, or leucine plus tryptophan (Table 1).

Total 5-HT release increased ($F_{2,36} = 74.47$, $P < 0.01$) as a function of stimulation frequency (1 Hz: 753 ± 69

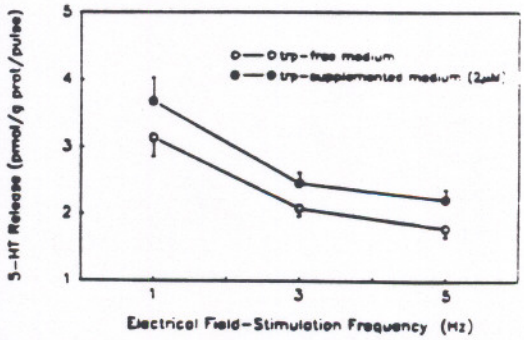

Fig. 5. Effect of electrical field-stimulation frequency (1, 3 or 5 Hz) on 5-HT released per pulse from slices superfused with tryptophan (trp)-free or tryptophan-supplemented (2 μM) medium. Data are expressed as group means ± S.E.M. Two-way analysis of variance detected a significant effect of tryptophan supplementation ($F_{1,36} = 6.67$, $P < 0.05$) which was independent of stimulation frequency, a significant effect of stimulation frequency was also detected ($F_{2,36} = 24.41$, $P < 0.01$) with 5-HT release evoked by 1 Hz different from that evoked by 3 or 5 Hz, as assessed by the Duncan multiple range post-hoc test.

pmol/g protein; 3 Hz: 1496 ± 87 pmol/g protein; 5 Hz: 2142 ± 154 pmol/g protein). However, 5-HT release per pulse decreased with frequencies greater than 1 Hz ($F_{2,38}$ = 24.41, $P < 0.01$; Fig. 5). Superfusing slices with medium containing 2 μM tryptophan augmented, by 21.4 ± 4.5%, total 5-HT release ($F_{1,38}$ = 8.20, $P < 0.01$) and 5-HT release per pulse ($F_{1,38}$ = 6.67, $P < 0.05$). The magnitude of the tryptophan-induced enhancement in 5-HT release was independent of stimulation frequency (1–5 Hz). Moreover, increasing both tryptophan availability and stimulation frequency produced nearly additive increases in 5-HT release (e.g., 2 μM tryptophan increased spontaneous 5-HT release by 21 ± 5% and evoked release by 19 ± 5%; 3 Hz stimulation increased 5-HT release by 284 ± 21%; and 2 μM tryptophan plus 3 Hz stimulation increased 5-HT release by 357 ± 24%).

DISCUSSION

These data provide the first direct evidence that reducing brain tryptophan levels produces proportionate decreases in 5-HT release, both spontaneous and electrically-evoked (Figs. 3 and 4). Earlier in vivo studies provided only indirect evidence that local tryptophan levels can limit 5-HT release: It had been shown that supplemental tryptophan could restore the decrements in 5-HT release caused by prolonged stimulation[21,31]; and the stress-induced elevations in brain tryptophan levels[28] and 5-hydroxyindole release[25] could be blocked by pretreating with valine, a LNAA which competes with tryptophan for uptake into brain[40]. Another in vivo study failed to reveal a precursor-dependence of 5-HT release: In cats fed a tryptophan-free meal which lowered their brain levels of tryptophan and 5-HT, the release of [^{3}H]-5-HT, synthesized from [^{3}H]tryptophan injected intraventricularly, was reportedly not different from that released in cats fed the control, tryptophan-containing meal; it was concluded that diet-induced changes in brain tryptophan levels do not modify 5-HT release[50]. However, since total brain levels of 5-HT were reportedly lower in the tryptophan-deficient brains than in control brains, the specific activity of [^{3}H]5-HT was probably higher relative to control. While the release of [^{3}H]5-HT was observed to be unaffected by diet, total 5-HT release in the tryptophan-deficient brain may thus have diminished (see ref. 39).

In the present study, superfusing rat hypothalamic slices with medium containing the LNAA leucine, at a concentration which approximates the summed concentrations of LNAAs in cerebrospinal fluid[1,17,35] reduced tissue tryptophan levels. This observation is consistent with other in vitro studies demonstrating that LNAAs competitively inhibit tryptophan's influx across the plasma membrane of brain cells[41,51] and also facilitate its efflux[20,41]. Our data suggest that serotoninergic neurons can couple LNAA-induced decrements in net tryptophan uptake to transmitter release.

These data confirm our earlier finding that elevating tryptophan levels in rat hypothalamic slices causes proportionate increase in tissue 5-HT levels and release[43]. Final tissue tryptophan and 5-HT levels were altered in parallel over ranges of 122% (−39% to +83%) and 26% (−11% to +15%), respectively, by superfusing slices with medium to which 100 μM leucine and/or 2 μM tryptophan were added. These variations in tissue indole levels are quantitatively similar to those which occur physiologically in rat brain after consumption of certain meals[10,16,45]; with time of day[37,42]; or after particular stressors[8,30]. Thus, our data suggest that physiologic elevations or reductions in brain tryptophan levels cause parallel changes in brain 5-HT levels and 5-HT release. Within the physiological dynamic range for brain 5-HT levels, the absolute amounts of released 5-HT changed proportionately; and this proportionality is evidenced by the invariant fractional rates of 5-HT release when tissue 5-HT levels were raised, lowered, or unaltered (Table 1).

Our data suggest that intracellular 5-HT stores and monoamine oxidase activity may buffer precursor-dependent changes in 5-HT release. This interpretation is based on the finding that the molar changes in 5-HT release were significantly smaller than those in tissue 5-HT levels (ca. one-quarter) and in 5-HIAA efflux (ca. one-seventh). The precursor-dependent increases or decreases in 5-HT release were apparently partially suppressed by more pronounced elevations or reductions, respectively, in tissue 5-HT levels and 5-HIAA efflux. Thus, compensatory alterations in 5-HT storage pools and degradation may serve to dampen increases in 5-HT release when synthesis exceeds immediate demands for release, and to offset decreases in 5-HT release when synthesis contributes less to the pool of releasable 5-HT than immediate demands.

Levels of tryptophan, 5-HT and 5-HIAA in hypothalamic slices changed over the experimental period when superfused with physiologic medium (unsupplemented with amino acids or fluoxetine; Fig. 1). The time-dependent changes in tissue tryptophan levels probably resulted from changes in the net uptake of free tryptophan (likely to have derived from protein breakdown in association with slice preparation). This view is supported by the parallel changes in the endogenous tryptophan concentrations of the superfusion medium (Fig. 2). A similar time-course of tryptophan accumulation has been described for slices prepared from rat cerebral cortex[29].

The changes in tissue 5-HT levels with superfusion in unsupplemented (Fig. 1) or LNAA-supplemented (Fig. 3) medium paralleled those in tissue tryptophan levels.

and thus probably reflect changes in 5-HT synthesis, inasmuch as tryptophan hydroxylase is known to be unsaturated with its substrate at physiologic levels of brain tryptophan[13,15,36]. 5-HT levels of rat hippocampal slices have similarly been shown to change with incubation time[2]. The rapid decline in tissue 5-HIAA levels with the onset of superfusion probably reflects the efficient transport of the acid metabolite into the extracellular space.

We observed that supplemental tryptophan enhanced 5-HT release by an amount which was invariant across the frequency range tested (Fig. 5). Thus, the influence of tryptophan availability on 5-HT release was independent of the frequency of membrane depolarization. This finding is consistent with earlier suggestions that the effect on 5-HT synthesis of raising brain tryptophan levels was independent of the firing rate of serotoninergic neurons. The enhancement in 5-HT synthesis which occurred when rats received a tryptophan dose or consumed a carbohydrate-rich meal was not attenuated by drugs [i.e., a monoamine oxidase inhibitor; chlorimipramine; 8-hydroxy-2 (di-*n*-propylamine) tetralin] which suppress the flow of impulses along serotoninergic raphe neurons[23,45]. Additionally, 5-HT synthesis in resting brain synaptosomes[9,27] and slices[36] was proportionate to the tryptophan concentration of the incubation medium, and thus apparently not highly dependent on neuronal activity. The apparent independence of tryptophan's effect on firing frequency differs from that described for the precursors of catecholamine and acetylcholine: By and large, tyrosine and choline affect the production and release of their transmitter products only when neurons are firing rapidly[34].

We also found that varying the frequency of electrical field-stimulation (over a constant period of time) affected 5-HT release. Other in vivo studies have similarly demonstrated that 5-HT release is decreased by pharmacological agents (i.e., 5-HT_{1A} agonists) which reduce impulse flow along serotoninergic neurons[18,44,45], and is increased by delivery to raphe perikarya electrical pulses which accelerate impulse flow[22,45]. Increasing the frequency of electrical field-stimulation increased, in a pulse-dependent manner, total 5-HT release but decreased 5-HT release per pulse (Fig. 5). This apparent paradox has been described previously in vivo[22,45] and in vitro[4,19], and is likely to have resulted from the activity of release-modulating serotoninergic autoreceptors present on hypothalamic nerve terminals[7]. This hypothesis is supported by the finding of others that incubating rat cerebrocortical slices with the 5-HT autoreceptor antagonist methiothepin eliminated the frequency-dependent differences in [^{3}H]5-HT efflux[4]. In our experimental system, it is possible that the presence of fluoxetine in the superfusion medium (which was necessary for us to measure reliably released 5-HT) may have potentiated autoreceptor-mediated effects on 5-HT release (see ref. 43).

In summary, this study suggests that the final output of a serotoninergic neuron, reflected in the amounts of 5-HT released per unit time, is determined by processes occuring both at the somatodentritic region and at its nerve terminals. Events at the somatodentritic region regulate the pattern of neuronal firing, and thus influence the rate at which 5-HT molecules residing in nerve terminals are released. The amounts of 5-HT available for release from nerve terminals are regulated by the activity of tryptophan hydroxylase, and thus by the availability of tryptophan. Action potentials invading serotoninergic nerve terminals at a certain frequency are likely to release amounts of 5-HT proportionate to the levels of intracellular 5-HT.

This study further suggests that serotoninergic neurons may release their transmitter by a nonquantal process. We have shown that the amounts of 5-HT released per unit time, at a given level of membrane depolarization, are not constant. Rather, 5-HT release is responsive to changes in intracellular 5-HT levels. Thus, the dynamics of 5-HT release from the hypothalamic nerve terminals appear to differ from the classic quantal model of neurotransmitter release[12].

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