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The Effects Produced by Prostaglandin D_2 on Serotonin Turnover and Release and Tryptophan Uptake[1]

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HOLLINGSWORTH, E. B. AND G. A. PATRICK. *The effects produced by prostaglandin D_2 on serotonin turnover and release and tryptophan uptake.* PHARMACOL BIOCHEM BEHAV 22(3) 371–375, 1985.—In earlier studies, it was proposed that there was a serotonergic involvement in the ability of prostaglandin D_2 (PGD_2) to potentiate pentobarbital sleeping time. The actions of PGD_2 on neuronal turnover and release of serotonin and uptake of tryptophan were examined in mice. The effect of PGD_2 administration on serum tryptophan levels was also investigated. PGD_2 (1 and 4 mg/kg) increased the concentrations in whole brain of endogenous tryptophan (TRYP) and of ^{3}H-tryptophan (^{3}H-TRYP) following an intravenous (IV) injection of ^{3}H-tryptophan. Formation of ^{3}H-5-hydroxyindoleacetic acid (^{3}H-5HIAA) was doubled after PGD_2 administration (1 and 4 mg/kg). Whole brain concentrations of endogenous serotonin (5HT) and ^{3}H-serotonin (^{3}H-5HT) were unchanged after the administration of the prostaglandin. PGD_2 (10^{-4} to 10^{-10} M) *in vitro* had no effect on spontaneous or K^+-evoked release of ^{3}H-5HT from whole brain synaptosomes. Uptake of ^{3}H-tryptophan in synaptosomes was neither stimulated nor depressed by (10^{-4} to 10^{-12} M) PGD_2. There was also no change in serum tryptophan levels after administration of this prostaglandin. Thus, PGD_2 administration does affect the serotonergic system but no direct neurochemical correlate of sedation can be shown.

Prostaglandin D_2 Serotonin Tryptophan 5-Hydroxyindoleacetic acid Synaptosomes

PROSTAGLANDIN administration has been reported to have an effect on the levels, turnover rates and release of biogenic amines in brain tissue. The norepinephrine level decreased in rat after intraventricular administration of PGE_1 and PGE_2 and increased after $PGF_{2\alpha}$. There was an increase in 5HT, 5-HIAA and "total acetylcholine" concentrations in brain after administration of $PGF_{2\alpha}$ [16]. Using synaptosomes from rat hypothalamus or slice preparations from rat cortex or neostriatum, $PGF_{2\alpha}$ has also been reported to both decrease norepinephrine (NE) release and also to have no effect [17,18]. PGE_2 administration results in a decrease in spontaneous or K^+-depolarized release of NE or dopamine [1, 12, 17, 18]. In slices of brain from rabbit cortex, caudate nucleus, or rat striatum, these actions of PGE_2 and $PGF_{2\alpha}$ are not seen. It has been reported that after inhibition of prostaglandin synthesis by indomethacin pretreatment, there is an increase in the overflow of NE from brain slices from the cerebral cortex [12].

Earlier studies (in press) reported on a possible involvement of the serotonergic system in PGD_2-induced potentiation of barbiturate sleeping time in mice. Pretreatment of animals with serotonergic modulators which increased the synthesis of serotonin increased the ability of PGD_2 to potentiate pentobarbital sleeping time, while drugs which decreased serotonin synthesis or neuronal firing of serotonergic neurons decreased the potentiating effect of PGD_2. The actions of PGD_2 on neuronal tryptophan uptake, serotonin turnover and release, and serum tryptophan levels were explored in this present study, with the hope of further defining the relationship between PGD_2 and the serotonergic system.

METHOD

Animals

Male CD/3 mice (Dominion Laboratories, Dublin, VA, 20–40 g) were kept on a 12-hour light/dark cycle and were given food and water ad lib.

Drugs

^{3}H-Tryptophan, ^{3}H-serotonin, and Aquasol-2 were purchased from New England Nuclear, Boston, MA. *L*-Tryptophan, serotonin, 5-hydroxyindoleacetic acid, ficoll, Sephadex resin (G-10-120), and Dowex resin were purchased from Amersham, Arlington Heights, IL. PGD_2 and $PGF_{2\alpha}$

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TABLE 1

THE EFFECT OF PGD_2 ON THE CONCENTRATIONS OF SEROTONIN, ITS PRECURSOR AND METABOLITE IN MOUSE BRAIN†

Treatment	5 HT (μg/g)	^{3}H-5HT (dpm/g)	TRYP (μg/g)	^{3}H-TRYP (dpm/g)	^{3}H-5-HIAA (dpm/g)
Control	0.56 ± 0.03	13300 ± 1030	2.5 ± 0.2	272000 ± 14000	6680 ± 1110
PGD_2 (0.4 mg/kg)	0.58 ± 0.03	14100 ± 1170	2.9 ± 0.2	337000 ± 22000	7850 ± 1690
PGD_2 (1 mg/kg)	0.65 ± 0.03	14500 ± 1500	3.3 ± 0.2*	343000 ± 19000*	12000 ± 2480
PGD_2 (4 mg/kg)	0.56 ± 0.02	13400 ± 1210	3.0 ± 0.2	347000 ± 22000*	13500 ± 3000

*Significantly different from control at $p<0.05$.

†Levels of radiolabelled or unlabelled neurochemicals per gram wet brain weight 10 min after ^{3}H-tryptophan administration; values are expressed as mean ± S.E.M. (n=19–21 per group).

‡^{3}H-tryptophan (5 μCi) and PGD_2 or ETOH were administered to mice 10 min before they were sacrificed.

*An overall F ($p<0.05$) calculated using ANOVA was considered significant. A pairwise t-test for all 6 pairs each at 0.05/6 (to account for Bonferonne Inequality) and the conclusions are reported at $p<0.05$.

were obtained from Upjohn Company, Kalamazoo, MI. Amberlite (CG-50) was purchased from Mallinckrodt, St. Louis, MO.

The prostaglandins were stored in stock solutions (1 mg/ml) in 95% ethanol at 4°C. The stock solution was diluted with an appropriate volume of isotonic saline solution prior to administration. PGD_2 was administered (4 mg/kg, 10 ml/kg) by injection into the lateral tail vein.

Serotonin Turnover Studies

1. ^{3}H-TRYP (0.5 μCi, 6.8 Ci/mM) was co-administered with PGD_2 intravenously and the mice were sacrificed 10 min later by decapitation. The brains were removed, homogenized in 0.4 N perchloric acid, and the homogenate was centrifuged (10,000 g × 10 min). The pellet was re-homogenized in 0.4 N perchloric acid and centrifuged again. The two supernatant fractions were combined. The procedures are a modification of a previously published method [5].

2. The samples were adjusted to pH 6.7–6.9 with NaOH and poured over an Amberlite resin (K^+-form) column (CG-50). The resin was washed with 0.05 M K_2HPO_4 and water, and the effluents were retained and later analyzed for 5-HIAA and tryptophan. 5HT was eluted with 3.0 N HCl. An aliquot of the eluate was counted in Aquasol-2 in a scintillation counter and another aliquot was analyzed spectrofluorometrically at an excitation wavelength of 305 nanometers (nm) and an emission wavelength of 540 nm.

3. The effluents from step No. 2 were adjusted to pH 1.5–2.0 with HCl and poured over a Dowex (200 mesh) resin (K^+-form) column. The resin was washed with water, and these effluents were kept for the determination of ^{3}H-5HIAA. The tryptophan was eluted with 0.5 N NH_4OH. An aliquot was collected in Aquasol-2 and counted for tritium in a scintillation counter, and the rest was cyclized to a norharman structure and measured spectrofluorometrically at an excitation wavelength of 380 nm and an emission wavelength of 445 nm.

4. Dowex effluents were adjusted to pH 1.5–2.0 with HCl and the samples were poured over Sephadex G10-120 resin. The columns were washed with 0.1 N HCl (containing 0.1% ascorbic acid) and water. The ^{3}H-5HIAA was eluted with 0.02 N NH_4OH and neutralized with 0.2 N HCl. Aliquots were added to Aquasol-2 and counted in a scintillation counter.

5. Standards for tryptophan, 5HT and ^{3}H-5HIAA were carried through the assay. The amount of the original material recovered and the amount of cross-contamination was taken into account in the final analysis.

Serotonin Release Studies

These methods are a modification of procedures that have been previously reported [8,15].

Preparation of synaptosomes. Four mice were sacrificed by decapitation and the brains were removed quickly. They were homogenized in a 0.32 M sucrose/hydroxyethyl piperazine-N'-2-ethanesulfonic acid (hepes) solution. This was centrifuged at 1000 g for 10 min and the supernatant was saved and recentrifuged at 18,000 g for 25 min. The pellet, consisting of the crude mitochondrial fraction, was dispersed in 16% ficoll solution in sucrose (0.32 M) and a 7.5% ficoll solution was layered on top of the 16% ficoll solution. This was centrifuged at 100,000 g for 100 min which resulted in the synaptosomes being isolated between the 16% and 7.5% ficoll layers.

Preparation of buffers. 5 mM KCl Incubation Medium—120 mM NaCl, 5 mM KCl, 1.0 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10.0 mM glucose, 20.0 mM hepes, 10^{-6} M tranylcypromine. This solution was adjusted to pH 7.5 with 1.0 M tris buffer. 115 mM KCl Incubation Medium—same as above, but NaCl was omitted and 115 mM KCl was added, and pH was adjusted to 7.5 with 1.0 m tris buffer. EGTA Terminating Buffer—same as 5 mM buffer, but $CaCl_2$ and $MgCl_2$ were omitted and 300 mM EGTA (10 ml) was added to 1000 ml of buffer.

Release procedure. The synaptosomal band was aspirated from the ficoll gradient and washed with 0.32 M sucrose/hepes and 5 mM KCl solutions. The synaptosomes were collected by centrifuging at 18,000 g for 10 min and the pellet was resuspended in 5 mM KCl medium. ^{3}H-5HT (20 μCi, 26.5 Ci/nmole) was added to this suspension, and this

TABLE 2

EFFECT OF PGD_2 ON ^{3}H-5HT RELEASE FROM SYNAPTOSOMES

Concentration of PGD_2 (M)	PGD_2	Vehicle Control
	Spontaneous Release (dpm/mg protein)*	
10^{-4}	27400 ± 1200	26700 ± 600
10^{-5}	25600 ± 600	
10^{-6}	29400 ± 1200	27300 ± 1600
10^{-8}	26100 ± 300	
	Depolarization Evoked Release (dpm/mg protein)*	
10^{-4}	42800 ± 2300	46700 ± 1300
10^{-5}	43800 ± 200	
10^{-6}	50800 ± 1800	47400 ± 1300
10^{-8}	45700 ± 200	

*Values are expressed as mean ± S.E.M. (N=4).

TABLE 3

EFFECT OF $PGF_{2\alpha}$ ON ^{3}H-5HT RELEASE FROM SYNAPTOSOMES

Concentration of $PGF_{2\alpha}$ (M)	$PGF_{2\alpha}$	Vehicle
	Spontaneous Release (dpm/mg protein)*	
10^{-4}	2400 ± 100	2500 ± 100
10^{-5}	2400 ± 100	
	Depolarization Evoked Release (dpm/mg protein)*	
10^{-4}	3900 ± 100	4200 ± 100
10^{-5}	3700 ± 100	

*Values are expressed as mean ± S.E.M. (N=4).

was incubated at 37°C for 15 min in order to preload the synaptosomes with ^{3}H-5HT. The ^{3}H-5HT loading was terminated with 5 mM KCl and the preparation was centrifuged at 18,000 g. The pellet was washed with 5 mM KCl, the centrifugation was repeated and the pellet was resuspended in 5 mM KCl buffer. Aliquots of the synaptosomal suspension plus test drug were preincubated at 37°C. Spontaneous release was initiated by adding prewarmed 5 mM KCl buffer. Depolarization-evoked release was begun by adding prewarmed 115 mM KCl buffer. These were incubated at 37°C for 1 min and the reaction was terminated with ice cold EGTA buffer. Then, the incubation mixture was centrifuged at 18,000 g for 10 min and the supernatant was transferred to scintillation vials in order to measure radioactivity in a liquid scintillation counter.

Tryptophan Uptake Studies

Preparation of Krebs-Ringer buffer. The buffer was aerated with O_2:CO_2 mixture (95:5) for 30 min, and then adjusted to pH 7.4.

Uptake procedure. Mice were decapitated and the brains were removed quickly. The brain was homogenized in 8 volumes of 0.32 M sucrose (2–4°C) and the homogenate was centrifuged at 1000 g for 10 min. The supernatant representing a crude synaptosomal suspension was saved and the volume was increased to 20 ml buffer per gram of brain. The uptake procedure is a modification of that described in an earlier report [3].

Total binding. The buffer/homogenate mixture was preincubated at 37°C with test drug or control vehicle. ^{3}H-Tryptophan (10^{-4} M, 5 μCi) was added and the uptake was stopped 5 min later with cold saline. The tubes were immediately centrifuged at 20,000 g for 10 min. The supernatant portion was discarded and the pellet was washed with saline and centrifuged again. The resulting pellet was solubilized in 0.5 N NaOH, neutralized with HCl, and an aliquot was counted by liquid scintillation spectroscopy.

Specific binding. The procedure was also performed at 0°C and the mean value of radioactivity was determined in these vessels as described above. This value, representing nonspecific binding, was subtracted from the value determined for total binding in order to determine the specific binding. Protein was determined according to a standard method [2].

Serum Tryptophan Study

Blood was drawn from the retro-orbital sinus of mice, using heparinized capillary (hematocrit) tubes, both prior to and at 10 min after IV administration of PGD_2 (0.1–4.0 mg/kg). The blood was separated in a hematocrit centrifuge, and the serum was analyzed for tryptophan content according to a fluorometric method [4].

Statistics

The serotonin turnover study was analyzed by Analysis of Variance followed by a pairwise t-test, taking into account the Bonferonne Inequality. The studies of synaptosomal uptake of tryptophan and release of serotonin were analyzed using the t-test for comparison of each experimental group with its appropriate control group. A two-way analysis of variance was used to evaluate the data on effects of PGD_2 on serum concentration of tryptophan. A p-value of less than 0.05 was the criterion for statistical significance in all cases.

RESULTS

Effects of PGD on Turnover of Serotonin in Whole Mouse Brain

There was no difference in the concentration of 5HT in the whole brain or in the quantity of ^{3}H-5HT formed from ^{3}H-TRYP after administration of PGD_2, when compared to vehicle-treated control animals (see Table 1). At a dose of 1 mg/kg, PGD_2 significantly increased endogenous tryptophan levels in whole brain. The ^{3}H-TRYP levels were also significantly elevated above controls at 1 and 4 mg/kg of PGD_2. At the same doses, the prostaglandin produced a 100% increase in the levels of ^{3}H-5HIAA, the major metabolite of 5HT, but the variance was so great in this case that the increase was not statistically significant.

TABLE 4

EFFECT OF PGD_2 ON ^{3}H-TRYPTOPHAN UPTAKE INTO SYNAPTOSOMES

Concentration of PGD_2 (M)	^{3}H-TRYP Uptake (dpm/mg protein)*	
	PGD_2	Control
10^{-4}	91000 ± 8000	95000 ± 6000
10^{-6}	89000 ± 9000	81000 ± 6000
10^{-8}	91000 ± 7000	82000 ± 4000
10^{-10}	85000 ± 7000	85000 ± 8000
10^{-12}	84000 ± 6000	79000 ± 3000

*Values are expressed as mean ± S.E.M. (N=9).

TABLE 5

EFFECT OF PGD_2 ON LEVEL OF TRYPTOPHAN IN SERUM

Dose of PGD_2 (mg/kg)	Serum Tryptophan (μg/ml)*	
	Control (before treatment)	PGD_2-Treated (after treatment)
0 (vehicle)	0.278 ± 0.030	0.343 ± 0.053
0.1	0.285 ± 0.039	0.291 ± 0.039
0.4	0.274 ± 0.030	0.275 ± 0.048
1.0	0.331 ± 0.062	0.351 ± 0.061
4.0	0.370 ± 0.068	0.399 ± 0.100

*Values are expressed as mean ± S.E.M. (N=6).

Effects of PGD_2 and $PGF_{2\alpha}$ on ^{3}H-5HT Release from Synaptosomes

The presence of PGD_2 (10^{-4} to 10^{-8} M) had no effect on spontaneous or K^+-evoked release of serotonin from synaptosomes as shown in Table 2. Prostaglandin $F_{2\alpha}$ at 10^{-4} or 10^{-5} M also had no effect on spontaneous or K^+-evoked release of ^{3}H-5HT (Table 3). The values for the ethanol vehicle controls (10^{-4} and 10^{-5} M) were no different from untreated control values.

Effect of PGD_2 on Tryptophan Uptake in Synaptosomes

PGD_2 (10^{-4} to 10^{-12} M) produced no significant changes from the control values on uptake of tryptophan into synaptosomes (see Table 4).

Effect of PGD_2 on Serum Tryptophan Levels

Serum tryptophan levels were not altered significantly upon administration of PGD_2 (0.1–4.0 mg/kg), as shown in Table 5.

DISCUSSION

The serotonergic system has been implicated in the ability of PGD_2 to potentiate barbiturate sleeping time (manuscript in review). In the present study, we have examined the effects of PGD_2 on the serotonergic system. At doses of 1 mg/kg and 4 mg/kg, PGD_2 increased tryptophan levels and ^{3}H-5HIAA levels, but we cannot definitely conclude that PGD_2 stimulated turnover of serotonin because the serotonin levels were unchanged after the administration of prostaglandin. From these data, one could postulate that PGD_2 was stimulating release of 5HT, stimulating uptake of tryptophan, or that PGD_2 administration resulted in an increase in the plasma level of tryptophan.

We could find no effect produced by PGD_2 *in vitro* on either serotonin release or tryptophan uptake. It has been reported that PGD_2 can be converted to $PGF_{2\alpha}$ in the periphery [6]. Therefore, the increase in formation of ^{3}H-5HIAA following administration of PGD_2 might have been due to conversion of PGD_2 to $PGF_{2\alpha}$, which might then act on the serotonergic neuron. We observed, however, that $PGF_{2\alpha}$ had no effect on 5HT release from synaptosomes (Table 3).

Using rat brain synaptosomes, other investigators [9] have found no effect on spontaneous or K^+-evoked release of NE by PGE_2 (0.5–4 μg/ml), whereas PGE_2 (2.8 and 5.6×10^{-6} M) reduced the K^+-evoked overflow to 70.6% and 77.0% of control values in slices of rat cerebral cortex [18]. Perhaps prostaglandin-induced actions can be demonstrated better with slice preparations than with homogenized tissue. In a recent study [10], it was noted that PGE_2 (0.1–10 μg/kg) inhibited sympathetically stimulated release of norepinephrine in the nictitating membrane preparation in a dose-dependent manner. PGD_2 (1 μmole/l), though, had no effect on ^{3}H-NE release from rabbit caudate or cortex slices, nor from rat striatum or cortex slices.

It has been demonstrated that brain serotonin level increases when the ratio of blood concentrations of large neutral amino acids to tryptophan decreases [19]. The results in Table 5 illustrate that PGD_2 administration does not increase serum tryptophan levels. However, there is a possibility, which has not been tested, that PGD_2 decreases the level of large neutral amino acids. In addition, insulin release is known to decrease the level in blood of large neutral amino acids, and several investigators have reported on the ability of other prostaglandins to stimulate insulin release [13,14]. Another mechanism by which PGD_2 might increase the entry of tryptophan into the brain is by increasing cerebral blood flow, as suggested by the recent finding of a cerebral arteriolar vasodilatory effect of prostaglandins D_2, E_2, G_2, and I_2 [7]. This effect may be causally related to the sedative action of the prostaglandins.

The data included in the present study indicate that PGD_2 administration does result in an increase in tryptophan and 5-HIAA levels in whole mouse brain. The depressant action of PGD_2, i.e., the increase in barbiturate sleeping time, may be linked to the increased level of 5-HIAA found in the synaptic cleft, as has been postulated for the depressant action of PGE_1 [9]. From the present studies, we can conclude that PGD_2 has no effect on release of 5HT or uptake of tryptophan in an *in vitro* synaptosomal preparation, and that PGD_2 administration does not change serum tryptophan levels. Thus, the mechanism of the effects on tryptophan and 5-HIAA remains unexplained. Other literature supports the view that prostaglandins exert more consistent effects in decreasing release in the noradrenergic system. Perhaps the alteration in the serotonergic system after PGD_2 administration is a secondary effect due to the action of PGD_2 on this neurotransmitter system.

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REFERENCES

1. Bergstrom, S., L. Farnebo and H. Fuxe. Effect of prostaglandin E_2 on central and peripheral catecholamine neurons. *Eur J Pharmacol* **21:** 362–368, 1973.
2. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:** 248–254, 1976.
3. Coyle, J. T. and S. H. Snyder. Catecholamine uptake by synaptosomes in homogenates of rat brain: Stereospecificity in different regions. *J Pharmacol Exp Ther* **170:** 221–231, 1969.
4. Denkla, W. D. and H. K. Dewey. The determination of tryptophan in plasma, liver and urine. *J Lab Clin Med* **69:** 160–169, 1967.
5. Diaz, J. L. and M. O. Huttunen. Persistent increase in brain serotonin turnover after chronic administration of LSD in the rat. *Science* **174:** 62–64, 1971.
6. Ellis, C. K., M. D. Smigel, J. A. Oates and B. J. Sweetman. Metabolism of prostaglandin D_2 in monkey. *J Biol Chem* **254:** 4152–4163, 1979.
7. Ellis, E. F., E. P. Wei and H. A. Kontos. Vasodilation of cat cerebral arterioles by prostaglandins D_2, E_2, G_2 and I_2. *Am J Physiol* **237:** H381–H385, 1979.
8. Gray, E. G. and V. P. Whittaker. The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. *J Anat* **96:** 79–88, 1962.
9. Haubrich, D. R., J. Perez-Cruet and W. E. Reid. Prostaglandin E_1 causes sedation and increases 5-hydroxytryptamine turnover in rat brain. *Br J Pharmacol* **48:** 80–87, 1973.
10. Hemker, D. P. and J. W. Aiken. Modulation of autonomic neurotransmitters by PGD_2: Comparison with effects of other prostaglandins in anesthetized cats. *Prostaglandins* **20:** 321–332, 1980.
11. Hiller, K., P. J. Roberts and W. W. Templeton. Prostaglandin and noradrenaline interactions in rat brain synaptosomes. *Br J Pharmacol* **66:** 102P–103P, 1979.
12. Hiller, K. and W. W. Templeton. Regulation of noradrenaline overflow in rat cerebral cortex by prostaglandin E_2. *Br J Pharmacol* **70:** 469–473, 1980.
13. Johnson, D. G., W. T. Fujimoto and R. H. Williams. Enhanced release of insulin by prostaglandins in isolated pancreatic islets. *Diabetes* **22:** 658–663, 1973.
14. Lefebvre, P. J. and A. S. Luyckx. Stimulation of insulin secretion after prostaglandin E_1 in anesthetized dog. *Biochem Pharmacol* **22:** 1773–1779, 1973.
15. McGovern, S., S. E. Maguire, R. S. Curd, H. R. Mahler and W. J. Moore. Separation of adrenergic and cholinergic synaptosomes from immature rat brain. *FEBS Lett* **31:** 193–198, 1973.
16. Poddubiuk, A. M. and A. Kleinrok. A comparison of the central actions of prostaglandins A_1, E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in the rat. *Psychopharmacology (Berlin)* **50:** 95–102, 1976.
17. Reimann, W., H. B. Steinnauer, L. Hedler, R. Starke and G. Hertting. Effect of prostaglandin D_2, E_2 and $F_{2\alpha}$ on catecholamine release from slices of rat and rabbit brain. *Eur J Pharmacol* **69:** 421–427, 1981.
18. Wendel, O. T. and J. W. Strandhoy. The effects of prostaglandins E_2 and $F_{2\alpha}$ on synaptosomal accumulation and release of ^{3}H-norepinephrine. *Prostaglandins* **16:** 441–449, 1977.
19. Wurtman, R. J., F. Hefti and E. Melamed. Precursor control of neurotransmitter synthesis. *Pharmacol Rev* **32:** 315–335, 1981.