THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS Vol. 202, No. 3

ACUTE CHANGES IN CENTRAL SEROTONIN METABOLISM INDUCED BY THE BLOCKADE OR STIMULATION OF SEROTONINERGIC RECEPTORS DURING ONTOGENESIS IN THE RAT[1]

S. BOURGOIN, F. ARTAUD, A. ENJALBERT, F. HÉRY, J. GLOWINSKI AND M. HAMON

Groupe NB, INSERM U. 114, Laboratoire de Neurophysiologie and Laboratoire de Physiologie Cellulaire, Collège de France, Paris, France

Accepted for publication May 11, 1977

ABSTRACT

BOURGOIN, S., F. ARTAUD, A. ENJALBERT, F. HÉRY, J. GLOWINSKI AND M. HAMON: Acute changes in central serotonin metabolism induced by the blockade or stimulation of serotoninergic receptors during ontogenesis in the rat. J. Pharmacol. Exp. Ther. 202: 519–531, 1977.

Both the synthesis and utilization of serotonin (5-HT) are accelerated in the brain of the adult rat after the administration of the 5-HT antagonist methiothepin. In contrast, the reverse is observed when the central 5-HT agonist, LSD, is administered. The present study was undertaken in order to determine if these metabolic events also occur in the newborn rat. Serotoninergic receptors are linked to an adenylate cyclase in the brain of the newborn rat. The effects of methiothepin and LSD on this enzyme demonstrated that methiothepin and LSD could be also considered, respectively, as an antagonist and a partial agonist of 5-HT in the central nervous system of the neonate. The administration of methiothepin induced an acceleration in the rate of 5-HT synthesis in the brain of newborn rats as it does in adult animals. In contrast, LSD treatment resulted in a partial inhibition of 5-HT synthesis only in rats older than 8 days of age. These results were confirmed by estimating the rate of 5-HT synthesis *in vitro* in brain slices prepared from rats treated with methiothepin or LSD. The addition of 30 mM K^+ to the incubating medium of brain stem slices from adult or young rats (12 hours and 7 days old) induced a doubling in the output of [^{3}H]5-HT newly synthesized from [^{3}H]tryptophan. The releasing effect of K^+ was enhanced in the presence of methiothepin. In contrast, LSD partially inhibited the K^+-induced release of [^{3}H]5-HT. These changes induced by methiothepin and LSD were also observed in experiments with tissues from newborn rats. Therefore, it could be concluded that the feedback mechanisms triggered by (presynaptic) serotoninergic receptors could regulate 5-HT release immediately after birth. The delayed occurrence of the negative feedback process controlling 5-HT synthesis (after LSD treatment) might suggest that the regulation of 5-HT synthesis is not related to 5-HT release during the early life period.

Received for publication December 4, 1976.

Send reprint requests to: Dr. S. Bourgoin, Groupe NB-INSERM U114, College de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05-France.

[1]This work was supported by grants from INSERM (ATP 6.74.27 and CRL 76.4041.6), DGRST, DRME and la Société des Usines Chimiques Rhône-Poulenc.

The biosynthesis and release of monoamines in the central nervous system of adult rodents are controlled at least partially by feedback mechanisms triggered by specific receptors. The first experimental evidence of such a regulation was reported by Carlsson and Lindqvist (1963) who observed that the administration of dopamine antagonists, *i.e.*, neuroleptics, resulted in an enhanced turnover of dopamine in the brain of the mouse. Similar observations were then made for other monoaminergic neurotransmitters, notably serotonin (5-HT). Thus, the stimulation of central serotoninergic receptors by LSD (Andén *et al.*, 1968; Hamon *et al.*, 1974), by 5-methoxy-N,N-dimethyltryptamine (Fuxe *et al.*, 1972), by ergocornine (Corrodi *et al.*, 1975) and by some hallucinogenic phenylethylamines (Andén *et al.*, 1974) initiates a compensatory negative feedback mechanism which slows down the turnover of 5-HT in the brain. In contrast, the blockade of central serotoninergic receptors by methysergide (Sofia and Vassar, 1975), by methergoline (Fuxe *et al.*, 1975) and by methiothepin (Monachon *et al.*, 1972; Hamon *et al.*, 1975) induces an increase in the rates of synthesis and utilization of the indoleamine. These feedback mechanisms are still operating in brain slices to control the release of [^{3}H]5-HT induced by K^+ or electrical field stimulation. Thus, LSD significantly decreases the rate of [^{3}H]5-HT efflux from brain slices induced by K^+ (Hamon *et al.*, 1974) or electrical field stimulation (Katz and Kopin, 1969). The same effect has been observed with ergocornine, another 5-HT agonist (Farnebo and Hamberger, 1974). In contrast, when a 5-HT antagonist like methiothepin is added to the incubating medium, the evoked release of [^{3}H]5-HT is largely increased (Farnebo and Hamberger, 1974; Hamon *et al.*, 1975).

In spite of these numerous data, little is yet known about the molecular mechanisms involved in these feedback regulatory processes. In particular, we recently observed that the rate-limiting enzyme in the synthesis of 5-HT (tryptophan hydroxylase) is, as is tyrosine hydroxylase (Zivcovic *et al.*, 1974), an allosteric enzyme which can be activated by the blockade of specific receptors *in vivo* (Hamon *et al.*, 1976b). The role of neuronal loops and/or local synaptic events in triggering these feedback regulatory mechanisms has not been satisfactorily assessed. Since the central innervation is immature at birth in the rat, one might suppose that the regulatory mechanisms which can be detected at this age do not involve complex neuronal loops but rather local synaptic processes. The present paper reports our studies on the onset of feedback mechanisms regulating the release and the synthesis of 5-HT in the rat brain during ontogenesis. The negative and positive feedback processes were examined in young animals by analyzing the acute effects of LSD and methiothepin administrations, respectively, on 5-HT metabolism in brain.

Methods

Adult male Sprague Dawley rats (Charles River strain) weighing 250 to 300 g were used. Pregnant females of the same strain (IFFA CREDO, France) were obtained 7 days before the delivery. The litters were limited to nine newborns. Before weaning (on the 21st postnatal day), both males and females were used, whereas only males were used for experiments in older animals.

All rats were maintained in the same environmental conditions (24°C; 60% humidity; alternate cycles of 12 hours darkness and 12 hours light). Food and water were freely available.

The following compounds were used: *d*-lysergic acid diethylamide (LSD, Sandoz Ltd., Basel, Switzerland), methiothepin (1-[10, 11-dihydro-8 (methylthiol) (b,f) thiepin-10-yl]-4-methylpiperazine maleate, Hoffmann-La Roche Inc., Basel, Switzerland), benserazid (Ro 4-4602, Hoffmann-La Roche Inc.) and chlorimipramine (Ciba-Geigy Corp., Summit, N.J.).

G-[^{3}H]L-tryptophan (4.5 to 9.8 Ci/mmol), 5 (n) L-[^{3}H]tryptophan (29 Ci/mmol) and G-[^{3}H]5-HT (13.5 Ci/mmol) were purchased from Radiochemical Centre (Amersham, England). Each compound was purified just before each experiment by ion-exchange and adsorption chromatography as described previously (Hamon et al., 1974). [α-^{32}P]adenosine triphosphate ([α-^{32}P]ATP, 10–20 Ci/mmol) was from New England Nuclear, Boston, Mass.

***In vivo* experiments.** Drugs were administered intraperitoneally except in rats younger than 14 days old which were injected subcutaneously on the back. All injections were performed in the middle of the light period, around 2 PM. Doses refer to the bases in all cases. At the times indicated under "Results," animals were killed by decapitation and their brains were removed in the cold room in less than 30 seconds. Generally, biochemical analyses were conducted on the whole brain minus the cerebellum. In some cases, the brain stem and the forebrain were separated by a transverse midcollicular cut. Tissues were then homogenized in an ethanol-water (74:16 v/v) solution containing ascorbic acid and EDTA (0.02% each) as antioxidants. 5-HT was selectively extracted from the clear supernatant by ion-exchange chromatography on an Amberlite CG

50 column. Tryptophan and 5-hydroxytryptophan (5-HTP) in the Amberlite effluent were then retained on a Dowex AG-WX4 column, and 5-hydroxyindoleacetic acid (5-HIAA) was finally adsorbed on a Sephadex G-10 column. Each isolated compound was estimated in corresponding eluates using spectrofluorimetric methods. Detailed descriptions of the techniques used can be found in preceding papers (Hamon *et al.*, 1973; Bourgoin *et al.*, 1975).

***In vitro* experiments.** Brain stem slices (0.3 mm thick) of control or treated rats were prepared with a McIlwain tissue chopper. They were incubated at 37°C for 30 minutes using a Dubnoff metabolic shaker in a physiological medium (5 ml for 65-110 mg of tissues) containing (in millimolar concentrations): NaCl, 136; KCl, 5.6; $MgCl_2$, 1.2; $CaCl_2$, 2.2; NaH_2PO_4, 1.2; $NaHCO_3$, 16.2; and glucose 5. The pH was maintained at 7.35 by a constant atmospheric mixture of O_2–CO_2 (95:5%) in the incubating flasks. When the K^+ concentration was increased to 30 mM, the isoosmolarity of the medium was maintained by decreasing the concentration of NaCl to 112 mM. [^{3}H]tryptophan (generally labeled or the 5(n) [^{3}H]amino acid) was added to the incubating medium just before the incubation period. At the end, tissues and medium were separated by rapid filtration through Whatman paper (no. 3) under a slight vacuum. [^{3}H]5-HT, [^{3}H]5-HIAA, [^{3}H]tryptophan and cold tryptophan were then estimated in each sample as previously described (Hamon *et al.*, 1973).

For the uptake experiments, the crude synaptosomal fraction (P2) from brain stem sucrose homogenates was used. The effects of methiothepin on [^{3}H]5-HT uptake in brain stem synaptosomes were analyzed under conditions described in detail elsewhere (Hamon *et al.*, 1976a).

Enzymatic assays. *Adenylate cyclase activity.* Adenylate cyclase activity was estimated by measuring the rate of conversion of [α-^{32}P]ATP into [^{32}P]cyclic AMP according to Bockaert *et al.* (1976). The enzyme source was a whole homogenate of colliculi from newborn rats (less than 24 hours old) made in 20 volumes (v/w) of an isotonic buffer containing 2 mM Tris-maleate (pH 7.2), 2 mM ethyleneglycol - bis - (β-aminoethylether) - *N,N'* - tetraacetic acid (EGTA, Sigma Chemical Company, St. Louis, Mo.) and 300 mM sucrose. The whole procedure has been described in detail previously (Hamon *et al.*, 1976a).

MAO activity. The enzyme source was a whole homogenate of brain stem from adult rats made in 10 volumes of 0.1 M phosphate buffer, pH 7.4. Monoamine oxidase activity was estimated as proposed by Kraml (1965) with kynuramine (Sigma Chemical Company) as the substrate.

Statistical calculations (Student's *t* test) were done according to Snedecor and Cochran (1967). When P was higher than .05, a difference was not considered to be significant. Linear regression analysis was applied to Dixon plots for the determination of the K_i of methiothepin inhibition of [^{3}H]5-HT uptake in synaptosomes.

Results

Effects of LSD and methiothepin on 5-HT-sensitive adenylate cyclase activity. The conversion of [α-^{32}P]ATP into cyclic [^{32}P]AMP by a crude homogenate of colliculi from newborn rats was accelerated when 5-HT was added to the assay mixture. The largest increase of adenylate cyclase activity (about 100%) was observed when the concentration of 5-HT reached 10^{-5} M (fig. 1, a and b). As shown in figure 1a, LSD also increased the activity of adenylate cyclase in colliculi homogenates of newborn rats. However, the effect of LSD was much less striking than that of 5-HT and occurred mainly in the presence of low concentrations of the drug. In contrast, when 5-HT was included in the assay mixture, higher concentrations of LSD induced a partial inhibition of the activated enzyme (fig. 1a). A much more pronounced inhibition was observed when methiothepin instead of LSD was added to the assay mixture. As shown in fig. 1b, the stimulating effect of 5-HT was decreased by more than 80% in the presence of 10^{-5} M methiothepin. However, in contrast to LSD, methiothepin (up to

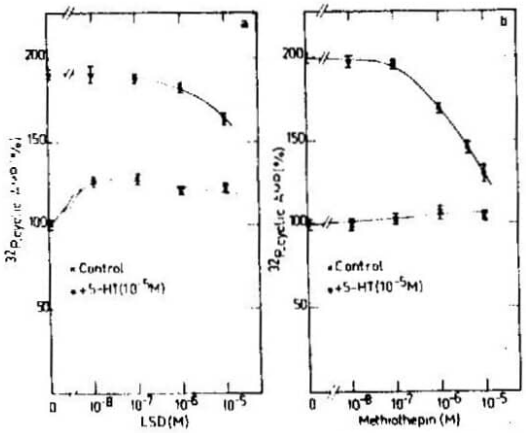

FIG. 1. Effects of LSD (a) or methiothepin (b) on 5-HT-sensitive adenylate cyclase activity in colliculi homogenates from newborn rats. The effect of each drug was determined in absence (x) or in presence (●) of 10^{-5} M 5-HT. Each point is the mean ± S.E.M. of triplicate determinations of cyclic [^{32}P] AMP formed in assay conditions described under "Methods." The basal activity (100%) corresponds to 4.50 ± 0.15 and 5.62 ± 0.19 pmol of cyclic [^{32}P] AMP formed per min and per mg protein in experiments a and b, respectively.

10^{-5} M) did not significantly alter the basal adenylate cyclase activity (fig. 1b).

Effects of methiothepin and/or LSD administration on tryptophan, 5-HT and 5-HIAA in the brain of rats at various ages. Obvious behavioral changes were observed after the administration of methiothepin (20 mg/kg) or LSD (1 mg/kg) even in 12-hour-old rats. General sedation (catalepsy in adults) occurred within 5 minutes after the injection of methiothepin regardless of the age of the rats. In contrast, the administration of LSD resulted in a hyperactive syndrome beginning 2 to 3 minutes after the injection. The behavioral effects of the psychotomimetic agent was particularly striking in 14- to 21-day-old rats which jumped and ran continuously for more than 30 minutes after the treatment. Whereas the normal behavior was recovered within 45 minutes in LSD-treated rats, animals were still deeply sedated 5 hours after methiothepin administration. The latter observations applied to adult, young and newborn rats.

As shown in figures 2 and 3, LSD and methiothepin altered the levels of indoles in the brain. Thus, 40 minutes after the injection of LSD (1 mg/kg), the concentration of 5-HT in the brain was significantly increased (by 20–40%) in newborn as well as in adult rats (fig. 2). The reverse was true for 5-HIAA levels which were significantly reduced by this treatment in rats as young as 7 days (fig. 2). Tryptophan levels were not altered by the administration of LSD in these conditions (fig. 2).

As shown in figure 3, the administration of methiothepin (20 mg/kg) 2 hours before sacrifice also resulted in an increase in 5-HT levels in the whole brain of 12-hour- and 7-day-old rats. However, in contrast to LSD treatment, this effect was no longer detected in older animals. Both tryptophan and 5-HIAA levels were significantly increased in the brain of adult rats after methiothepin treatment. These changes were also detected in young animals except that the increase in tryptophan concentration in the brain of 14-day-old rats was too small to reach significance. In another experiment, the brain stem and the forebrain were separated for biochemical analyses. Similar changes to those observed in the whole brain were detected in both regions after methiothepin administration. However, increases in 5-HT and 5-HIAA levels were more pronounced in the brain stem (+35–80%) than in the forebrain (+8–52%). In contrast, tryptophan levels were altered to the same extent in both regions.

In another set of experiments, the effects of combined treatments with LSD and methiothepin were analyzed. When LSD (1 mg/kg) was administered twice, 10 minutes before and 10 minutes after methiothepin, the changes induced by the latter drug were largely prevented. Thus, in adult (fig. 4) and in newborn rats (fig. 5), the increases both in the 5-HIAA levels and in the 5-HIAA/5-HT ratio occurring

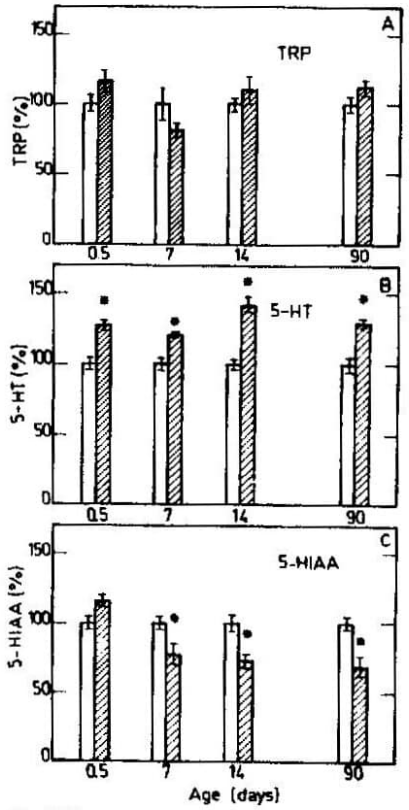

FIG. 2. Effects of LSD treatment on tryptophan (TRP) (A), 5-HT (B) and 5-HIAA (C) levels in the brain of 0.5, 7-, 14- or 90-day-old rats. LSD (1 mg/kg) or saline was administered 40 minutes before death. Each indolic compound was measured in the whole brain as described under "methods." Each bar is the mean ± S.E.M. of eight determinations. *$P < .05$ when compared with respective control values. Empty bars: control values taken as 100%; hatched bars: values from LSD-treated rats as a percentage of control values. The absolute levels (in micrograms per gram) of tryptophan in control rats were equal to 21.5 ± 1.8, 9.1 ± 0.2, 6.2 ± 0.3 and 4.8 ± 0.2 (means ± S.E.M.) at 12 hours, 7 days, 14 days and 90 days of age, respectively. Those of 5-HT were equal to 0.20 ± 0.01, 0.35 ± 0.01, 0.44 ± 0.01 and 0.56 ± 0.01 μg/g. 5-HIAA levels were equal to 0.58 ± 0.03, 0.65 ± 0.03, 1.05 ± 0.07 and 0.66 ± 0.03 μg/g, respectively.

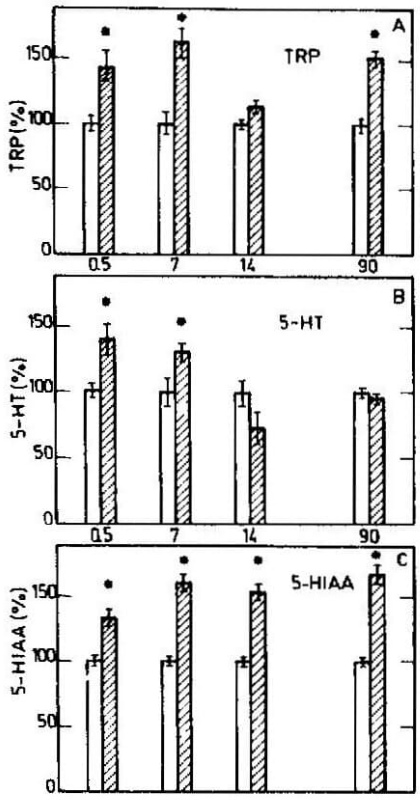

FIG. 3. Effects of methiothepin treatment on tryptophan (TRP) (A), 5-HT (B) and 5-HIAA (C) levels in the brain of 0.5-, 7-, 14- or 90-day-old rats. Methiothepin (20 mg/kg) or saline was administered 120 minutes before death. Tryptophan, 5-HT and 5-HIAA levels were determined in the whole brain. Empty bars: control values taken as 100%; hatched bars: values from methiothepin-treated rats as a percentage of control values. Each bar is the mean ± S.E.M. of eight determinations. * $P < .05$ when compared with respective control values. Absolute levels of tryptophan, 5-HT and 5-HIAA were not significantly different from those in the legend of figure 2.

normally after methiothepin injection were no longer observed in LSD pre- and post-treated animals.

Effects of tryptophan loading on the levels of 5-HT, 5-HIAA and tryptophan in the rat brain. Comparisons among methiothepin, LSD- and saline-treated rats. As shown in tables 1 and 2, tryptophan loading (300 mg/kg) 1 hour before death resulted in large increases in tryptophan, 5-HT and 5-HIAA levels in the brain of adult rats. Previous administration of LSD (1 mg/kg i.p., 10 minutes before the amino acid injection) induced a partial inhibition of the effect of tryptophan loading on 5-HIAA levels in the brain (table 1). Indeed, the 5-HIAA/5-HT ratio was significantly reduced by LSD treatment in tryptophan-loaded rats compared with control animals (table 1). In contrast, the effects of methiothepin treatment on tryptophan and 5-HIAA levels (table 2) were completely masked in tryptophan-loaded rats. Thus, neither 5-HIAA levels nor the 5-HIAA/5-HT ratio was significantly altered by methiothepin administration 30 minutes before the tryptophan load (table 2).

Effects of LSD treatment on the synthesis of 5-HT *in vivo* and *in vitro* in brain. *In vivo synthesis.* The rate of 5-HT synthesis was estimated as proposed by Carlsson and Lindqvist (1970) by measuring the rate of 5-HTP accumulation in the brain after the blockade of 5-HTP decarboxylase by benserazid (800 mg/kg i.p.). As shown in figure 6, LSD administration (1 mg/kg, 40 minutes before death) resulted in a large decrease in the rate of 5-HTP accumulation in the brain of adult rats. Such an effect did not occur in 12-hour- and 7-day-old rats but became highly significant at the end of the 2nd postnatal week.

In vitro synthesis of [^{3}H]5-HT from [^{3}H]-tryptophan after in vivo treatment with LSD. In adult rats, LSD treatment (1 mg/kg, 20 minutes before death), partially inhibited the formation of [^{3}H]5-HT from [^{3}H]tryptophan in brain stem slices (table 3). As shown by the reduction in the conversion index, *in vivo* LSD treatment resulted in a slight decrease (−17%) in the rate of 5-HT synthesis in brain stem slices (table 3). In contrast, this treatment failed to alter the rate of 5-HT synthesis in tissues from 6-hour-old rats.

In vitro addition of LSD. The addition of LSD (10 μM) to the incubating medium of brain stem slices of adult rats altered neither the accumulation of [^{3}H]tryptophan nor the synthesis of [^{3}H]5-hydroxyindoles in the tissues. Similar findings were obtained with tissues of newborn rats (data not shown).

Effects of methiothepin treatment on the synthesis of 5-HT *in vivo* and *in vitro* in brain. *In vivo synthesis.* In rats treated with methiothepin (20 mg/kg), the accumulation of 5-HTP after the blockade of 5-HTP decarboxylase with Ro 4-4602 (800 mg/kg) was significantly more rapid than in saline-treated animals. As shown in figure 6, this effect was detected in adult as well as in newborn rats.

In vitro synthesis after in vivo treatment with methiothepin. Adult or 12-hour-old rats were

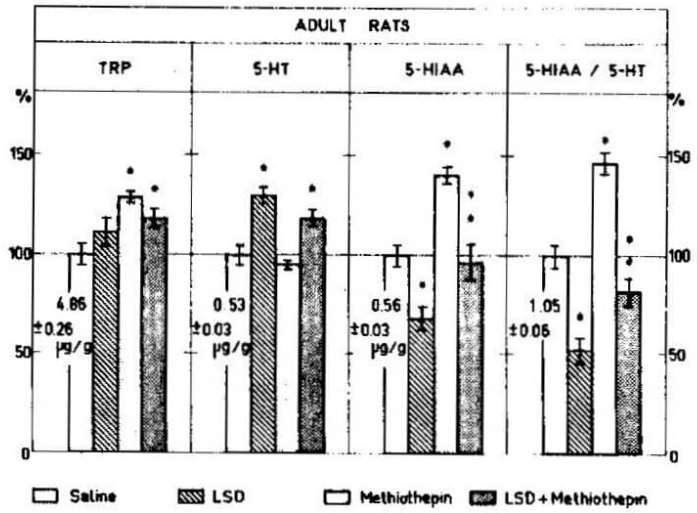

FIG. 4. Reversal by LSD of changes induced by methiothepin administration on indole levels in the whole brain of adult rats. Adult (3-month-old) rats were treated with LSD (2 × 1 mg/kg, 120 and 100 minutes before death), methiothepin (20 mg/kg, 110 minutes before death) or both. Using the same time conditions, saline was injected in control rats. Results are expressed as a percentage of control values. Each bar is the mean ± S.E.M. of six determinations. * $P < .05$ when compared with saline-treated rats; ** $P < .05$ when compared with rats treated with LSD alone.

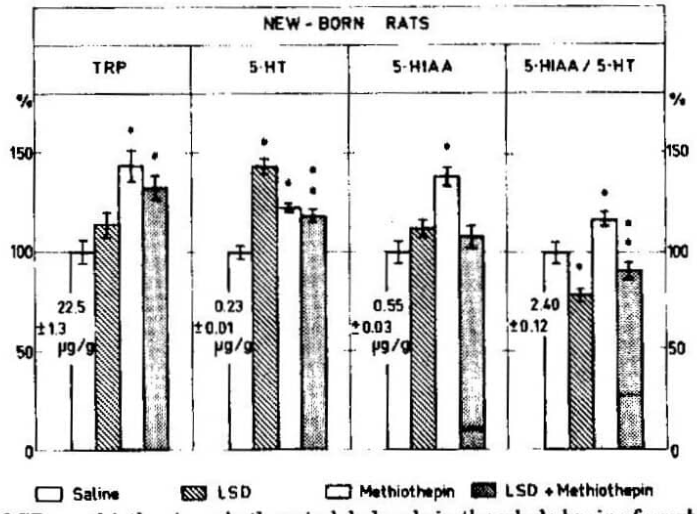

FIG. 5. Effects of LSD, methiothepin or both on indole levels in the whole brain of newborn rats. The same experiment as that described in the legend of figure 4 was performed on 12-hour-old rats. Each bar (percentage of control values) is the mean ± S.E.M. of six determinations. * $P < .05$ when compared with saline-treated newborn rats; ** $P < .05$ when compared with animals treated with LSD alone.

treated with methiothepin (20 mg/kg) or saline 90 minutes before sacrifice. Brain stem slices were then prepared and incubated for 30 minutes with [^{3}H]tryptophan. As shown in table 4, the administration of methiothepin to adult rats induced an increased accumulation of [^{3}H]tryptophan in the tissues and accelerated the formation of [^{3}H]5-HT and [^{3}H]5-HIAA from the labeled amino acid. In tissues of methiothepin-treated newborn rats, only the formation of [^{3}H]5-HIAA was significantly more rapid than in slices from respective control animals. Since the conversion index of tryptophan into 5-HT was significantly increased in tissues from methiothepin-treated rats (table 4), we can conclude that the actual synthesis rate of 5-HT was enhanced by this treatment. This conclusion applies to adult as well as to newborn rats (table 4).

In vitro addition of methiothepin. When

TABLE 1

Effects of tryptophan loading on the levels of 5-hydroxyindoles in the brain of control or LSD-treated adult rats

LSD (1 mg/kg i.p.) was administered 10 minutes before L-tryptophan (300 mg/kg i.p.) or saline. Other rats received an injection of L-tryptophan or saline alone. All animals were killed 60 minutes after tryptophan or 70 minutes after LSD treatment. Each value is the mean level (in micrograms per gram of whole brain) ± S.E.M. of six to eight determinations.

	Saline		Tryptophan	
	Control	LSD	Control	LSD
Tryptophan (μg/g)	5.09 ± 0.26	5.47 ± 0.27	110.7 ± 6.6	106.8 ± 4.7
5-HT (μg/g)	0.54 ± 0.04	0.69 ± 0.02[a]	0.86 ± 0.07	0.95 ± 0.04
5-HIAA (μg/g)	0.47 ± 0.03	0.34 ± 0.02[a]	1.03 ± 0.03	0.74 ± 0.07[a]
R[b]	0.87 ± 0.06	0.50 ± 0.03[a]	1.19 ± 0.09	0.78 ± 0.06[a]

[a] $P < .05$ when compared with respective control values.

[b] R corresponds to the ratio of 5-HIAA over 5-HT levels (mean ± S.E.M. of six to eight determinations).

TABLE 2

Effects of tryptophan loading on 5-hydroxyindoles levels in the brain of control or methiothepin-treated adult rats[a]

Methiothepin (20 mg/kg i.p.) was administered 30 minutes before L-tryptophan (300 mg/kg i.p.). Other rats received tryptophan or methiothepin alone and control animals were injected simultaneously with the same volumes of solvents. Sacrifice occurred 90 minutes after methiothepin or 60 minutes after tryptophan administration

	Saline		Tryptophan	
	Control	Methiothepin	Control	Methiothepin
Tryptophan (μg/g)	4.83 ± 0.26	6.69 ± 0.27[b]	99.5 ± 6.3	95.3 ± 8.6
5-HT (μg/g)	0.51 ± 0.02	0.50 ± 0.04	0.96 ± 0.05	0.94 ± 0.10
5-HIAA (μg/g)	0.47 ± 0.03	0.74 ± 0.05[b]	1.15 ± 0.08	1.09 ± 0.08
R[c]	0.92 ± 0.06	1.49 ± 0.11[b]	1.20 ± 0.09	1.16 ± 0.11

[a] Each value (in micrograms per gram of whole brain) is the mean ± S.E.M. of 8 to 14 determinations.

[b] $P < .05$ when compared with respective control values.

[c] R is the ratio of 5-HIAA over 5-HT levels (mean ± SEM of 8 to 14 determinations).

methiothepin (20 μM) was added to the incubating medium of brain stem slices prepared from 12-hour-old or adult rats, it induced a significant increase in the accumulation of ^{3}H-5-HIAA newly synthesized from [^{3}H]- tryptophan (table 5). In slices from newborn animals, this change was counterbalanced by a significant decrease in the amount of newly synthesized ^{3}H-5-HT finally recovered from tissues and incubation medium (−48.8%) so that the conversion index of tryptophan into 5-HT remained unaltered by the addition of methiothepin. The latter observation also applied to slices from adult rats indicating that methiothepin had no direct effect on the synthesis of 5-HT in the brain stem whatever the age of the rats.

In vitro addition of methiothepin (table 5) as well as *in vivo* treatment with this drug (table 4) significantly increased the rate of formation of ^{3}H-5-HIAA from newly synthesized ^{3}H-5-HT in brain stem slices from adult or newborn rats. Therefore, a possible activating effect of methiothepin on monoamine oxidase activity was examined. Neither *in vivo* treatment (20 mg/kg, 30, 60 or 90 minutes before death) nor *in vitro* addition of the drug (10 μM, 0.1 mM) resulted in a significant change in MAO activity measured in whole brain stem homogenates with kynuramine as the substrate (data not shown).

Effects of LSD or methiothepin on the K^+-induced release of newly synthesized [^{3}H]5-HT from brain stem slices. Regardless of the age of the rats, 30 mM K^+ (instead of 5.6 mM) in the incubating medium increased the output of newly synthesized [^{3}H]5-HT by about 100% (figs. 7 and 8).

Although LSD (10 μM) did not alter the spontaneous release of newly synthesized [^{3}H]5-HT, it significantly depressed the K^+-induced

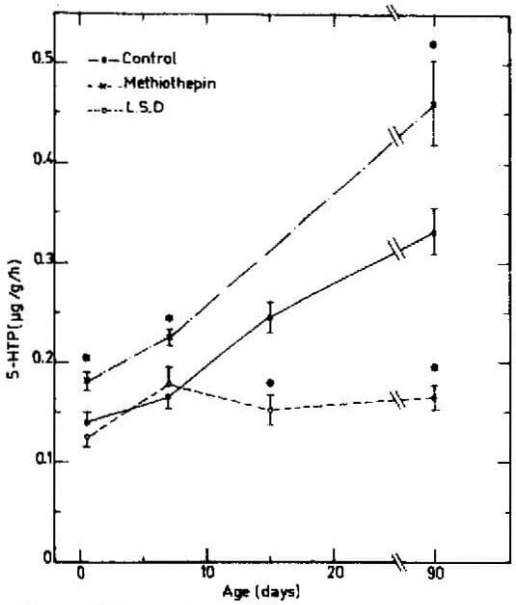

FIG. 6. Effects of methiothepin or LSD treatment on the rate of 5-HT synthesis in the brain of rats at various ages. The rate of 5-HT synthesis was determined by measuring the accumulation of 5-HTP in the whole brain 30 minutes after the injection of a 5-HTP decarboxylase inhibitor, benserazid (800 mg/kg). In all cases, methiothepin (20 mg/kg) was administered 90 minutes before benserazid whereas LSD (1 mg/kg) was injected 10 minutes before the 5-HTP decarboxylase inhibitor. When saline was injected 90 or 10 minutes before benserazid, no change in the rate of 5-HTP accumulation could be detected by comparison with that occurring in rats receiving the 5-HTP decarboxylase inhibitor alone. The rate of 5-HT synthesis is expressed in micrograms of 5-HTP accumulated per gram of fresh tissue and per hour. Each point is the mean ± S.E.M. of six to eight determinations. * $P < .05$ when compared with values found in control rats of same age.

release of the labeled amine (fig. 7). This inhibitory effect of LSD was observed with tissues from adult as well as with those from 12-hour-old rats. In experiments with brain stem slices from 7-day-old rats, the effect of LSD was not significant.

When methiothepin (10 µM) instead of LSD was added to the incubating medium, the releasing effect of K^+ was considerably increased (fig. 8). In fact, as shown by the effect of methiothepin on the spontaneous release of newly synthesized [^{3}H]5-HT (fig. 8), the increase observed might be the consequence of partial inhibition of [^{3}H]5-HT reuptake by the drug. Studies with synaptosomes from adult brain stem actually showed that methiothepin is a competitive inhibitor (K_i = 1.30 µM) of [^{3}H]5-HT uptake. However, when [^{3}H]5-HT uptake was inhibited by chlorimipramine (5 µM) in order to increase the spontaneous release of [^{3}H]5-HT to the same extent as that observed in the presence of methiothepin (fig. 8), the K^+-evoked release of the labeled indolamine in the presence of the 5-HT uptake inhibitor was markedly less pronounced than that seen in the presence of the 5-HT antagonist (fig. 8). As shown in figure 8, the stimulating effect of methiothepin (10 µM) on the K^+-induced release of newly synthesized [^{3}H]5-HT was obvious at all ages studied.

Discussion

The interaction of 5-HT with specific receptors in the central nervous system of the newborn rat results in the activation of adenylate

TABLE 3

Effect of LSD treatment on the synthesis of [^{3}H]5-hydroxyindoles from [^{3}H]tryptophan in brain stem slices of adult or newborn rats[a]

LSD (1 mg/kg) or saline (control animals) was administered to adult (3-month-old) or newborn (6-hour-old) rats 20 minutes before death. Their brain stems were dissected and incubated for 30 minutes with [^{3}H]tryptophan (4 µCi/ml, 0.14 µM) and [^{3}H]- and cold tryptophan in slices, [^{3}H]5-HT and [^{3}H]5-HIAA in slices and incubating medium were estimated.

	Adults		Newborns	
	Control	LSD	Control	LSD
[^{3}H]Tryptophan (µCi/g)	10.3 ± 0.4	9.9 ± 0.5	15.0 ± 1.2	16.1 ± 0.7
[^{3}H]5-HT (µCi/g)	0.93 ± 0.06	0.81 ± 0.03[b]	0.72 ± 0.05	0.71 ± 0.05
[^{3}H]5-HIAA (µCi/g)	0.30 ± 0.02	0.29 ± 0.02	0.57 ± 0.04	0.53 ± 0.04
CI[c]	3.97 ± 0.27	3.28 ± 0.21[b]	1.91 ± 0.13	1.77 ± 0.14

[a] Each value is the mean ± S.E.M. of eight determinations.

[b] $P < .05$ when compared with respective control values.

[c] CI, the conversion index, is the ratio of ([^{3}H]5-HT + [^{3}H]5-HIAA) over the specific activity of [^{3}H]tryptophan (microcuries per nanomole) in tissues.

TABLE 4

Effect of methiothepin treatment on the synthesis of [^{3}H]5-hydroxyindoles from [^{3}H]tryptophan in brain stem slices of adult or newborn rats[a]

Methiothepin (20 mg/kg) or saline (control animals) was administered to adult (3-month-old) or newborn (12-hour-old) rats, 90 minutes before death. Their brain stem was dissected, sliced and incubated with [^{3}H]tryptophan (4.4 μCi/ml corresponding to 0.98 μM) for 30 minutes. [^{3}H] and cold tryptophan in slices, [^{3}H]5-HT and [^{3}H]5-HIAA in slices and their incubating medium were then determined.

	Adults		Newborns	
	Control	Methiothepin	Control	Methiothepin
[^{3}H]Tryptophan (μCi/g)	11.3 ± 0.9	13.9 ± 0.9[b]	15.6 ± 0.6	17.2 ± 0.8
[^{3}H]5-HT (μCi/g)	0.72 ± 0.06	0.95 ± 0.08[b]	0.53 ± 0.04	0.61 ± 0.05
[^{3}H]5-HIAA (μCi/g)	0.25 ± 0.02	0.39 ± 0.02[b]	0.34 ± 0.04	0.45 ± 0.02[b]
CI[c]	3.46 ± 0.22	4.70 ± 0.38[b]	1.54 ± 0.10	1.96 ± 0.13[b]

[a] Each value is the mean ± S.E.M. of eight determinations.

[b] $P < .05$ when compared with respective control values.

[c] CI (conversion index) is equal to the ratio of ([^{3}H]5-HT + [^{3}H]5-HIAA) (in microcuries per gram) over the specific activity of [^{3}H]tryptophan (in microcuries per nanomole) in tissues.

TABLE 5

Changes in the metabolism of [^{3}H]5-HT newly synthesized from [^{3}H]tryptophan induced by the addition of methiothepin (20 μM) to the incubating medium of brain stem slices of adult or newborn rats[a]

Brain stem slices from control adult (3-month-old) or newborn (12-hour-old) rats were incubated with [^{3}H]tryptophan (4.2 μCi/ml, 0.93 μM) in presence or absence of 20 μM methiothepin. At the end of the 30-minute incubation period, [^{3}H]tryptophan and cold tryptophan in slices and [^{3}H]5-HT and [^{3}H]5-HIAA in tissues and medium were determined.

	Adults		Newborns	
	Control	Methiothepin	Control	Methiothepin
[^{3}H]Tryptophan (μCi/g)	10.8 ± 0.8	12.4 ± 0.5	15.1 ± 0.8	13.6 ± 0.9
[^{3}H]5-HT (μCi/g)	0.81 ± 0.06	0.77 ± 0.06	0.60 ± 0.05	0.31 ± 0.03[b]
[^{3}H]5-HIAA (μCi/g)	0.26 ± 0.02	0.51 ± 0.03[b]	0.43 ± 0.03	0.56 ± 0.04[b]
CI[c]	3.58 ± 0.25	4.19 ± 0.30	1.68 ± 0.14	1.47 ± 0.11

[a] Each value is the mean ± S.E.M. of eight determinations.

[b] $P < .05$ when compared with respective control values.

[c] CI is the conversion index of tryptophan into 5-HT (same definition as in the legend of table 4).

cyclase (Von Hungen *et al.*, 1975; Hamon *et al.*, 1976a). This effect is particularly striking on the adenylate cyclase in synaptic membranes from colliculi of newborn rats (Von Hungen *et al.*, 1975) but can be also observed with synaptic membranes from the whole brain of adult rats (Pagel *et al.*, 1976). In agreement with previous observations (Von Hungen *et al.*, 1975; Hamon *et al.*, 1976a), the present data indicate that LSD is a partial agonist of this 5-HT-sensitive adenylate cyclase in colliculi from the newborn rat. In contrast, methiothepin blocked the stimulating effect of 5-HT on this enzyme, thus confirming previous studies (Monachon *et al.*, 1972; Tebećis, 1972; Jacoby *et al.*, 1975) suggesting that it can be considered as a central 5-HT antagonist.

Alterations of behavior by LSD or methiothepin were obvious even when the drug was administered immediately after birth. Thus, methiothepin treatment almost completely abolished locomotor activity whereas LSD induced a disorganized general activation in newborns. Recently, Jacobs (1976) also reported that changes in 5-HT receptor activity in 1- to 3-day-old rats after 5-HTP, *p*-chlorophenylalanine or 5-methoxy-N,N-dimethyltryptamine administration induced dramatic behavioral consequences.

Several authors (Katz and Kopin, 1969; Schubert *et al.*, 1970; Hamon *et al.*, 1974) already reported that the stimulation of serotoninergic receptors by LSD induced a significant decrease in the synthesis and the release of 5-

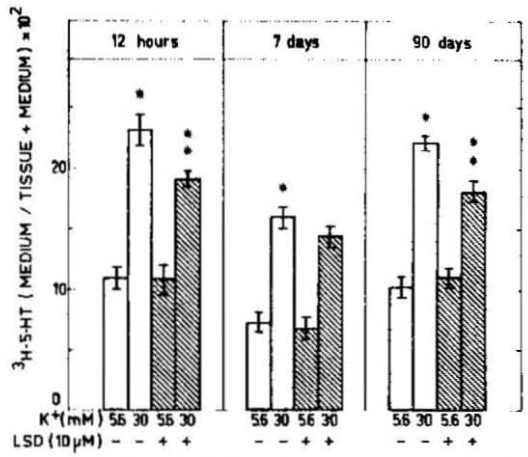

FIG. 7. Effects of LSD on the spontaneous and K^+-induced release of newly synthesized [^{3}H]-5-HT in brain stem slices of 12-hour-, 7-day- or 90-day-old rats. Brain stem slices were incubated in the presence of [^{3}H]tryptophan (5.5 μCi/ml corresponding to 0.56 μM) with or without LSD (10 μM) as indicated under the figure. The concentration of K^+ was equal to 5.6 or 30 mM. The incubation was stopped 30 minutes later and newly synthesized [^{3}H]5-HT was determined separately in slices and their incubating medium. The [^{3}H]5-HT release is expressed as the ratio (in percentage) of [^{3}H]5-HT in the medium over the total [^{3}H]5-HT accumulated in tissues + medium. Each bar is the mean ± S.E.M. of six to eight determinations. * $P < .05$ when compared with control values. ** $P < .05$ when compared with values found with 30 mM K^+ conditions.

HT from the adult rat brain (fig. 9). As shown by the present study, the inhibitory effect of LSD on the K^+-induced release of [^{3}H]5-HT could be detected immediately after birth. In contrast, the negative feedback process reducing 5-HT synthesis after LSD treatment did not seem to occur before the 2nd postnatal week (fig. 9). This result was recently confirmed by analyzing the effects of quipazine on 5-HT synthesis in rats at various ages. Since this drug is able to release 5-HT from presynaptic terminals and to inhibit its reuptake (Hamon *et al.*, 1976a), its administration leads to increased stimulation of the serotoninergic receptors by 5-HT itself. Then, quipazine, as LSD treatment, induced a reduction of 5-HT synthesis by triggering the negative feedback process in adult rats (Hamon *et al.*, 1976a). During ontogenesis, this effect of quipazine treatment could not be detected during the 1st postnatal week (Bourgoin, 1976).

In contrast to the inhibitory effects of LSD or quipazine treatment on 5-HT synthesis, the acceleration of 5-HT synthesis after methiothepin administration could be detected immediately after birth. As LSD, methiothepin had no direct effect on 5-HT synthesis either in brain stem slices (table 5) or in the distal part of the cut spinal cord (Jacoby *et al.*, 1975). Since brain tryptophan levels increased after methiothepin administration, this might well be the single reason why the rate of 5-HT synthesis was accelerated by this treatment. However, in 12-hour-old rats, tryptophan hydroxylase is already saturated by its amino acid substrate (Bourgoin *et al.*, 1974; Hoff *et al.*, 1976) so that increasing the concentration of tryptophan in brain at this age does not alter the rate of 5-HT synthesis. This result strongly suggests that the accelerated synthesis of 5-HT occuring in 12-hour-old rats treated with methiothepin was not related to alterations in brain tryptophan levels. Indeed, we demonstrated recently (Hamon *et al.*, 1976b) that the stimulating effect of methiothepin treatment on 5-HT synthesis was the consequence of an activation of soluble tryptophan hydroxylase. Therefore, the effect of methiothepin on 5-HT synthesis resulted likely from a positive feedback triggered by the blockade of serotoninergic receptors (fig. 9) rather than from alterations in tryptophan levels.

The stimulating effect of methiothepin on the K^+-induced release of [^{3}H]5-HT from brain stem slices could not be entirely explained by its inhibitory action on the reuptake process for [^{3}H]5-HT. As already proposed by Farnebo and Hamberger (1974), the antagonistic properties of methiothepin towards 5-HT were also likely involved in this effect (fig. 9). Therefore, the present data indicate that the positive feedback processes which lead to increases in both the synthesis rate and the K^+-induced release of 5-HT after the blockade of central serotoninergic receptors can already be detected at birth.

From these results, several conclusions can be drawn:

1. The regulatory mechanism triggered by serotoninergic receptors and controlling [^{3}H]5-HT release matures very soon during development in the rat, well before the negative feedback process regulating 5-HT synthesis. This suggests that these mechanisms could act independently during the first 2 weeks after birth. In adult rats, Héry *et al.* (1972) reported that the spontaneous release and the synthesis of 5-HT fluctuated in opposite directions according to a nycthemeral rhythm in physiological conditions. This would suggest that regulatory processes controlling these metabolic events

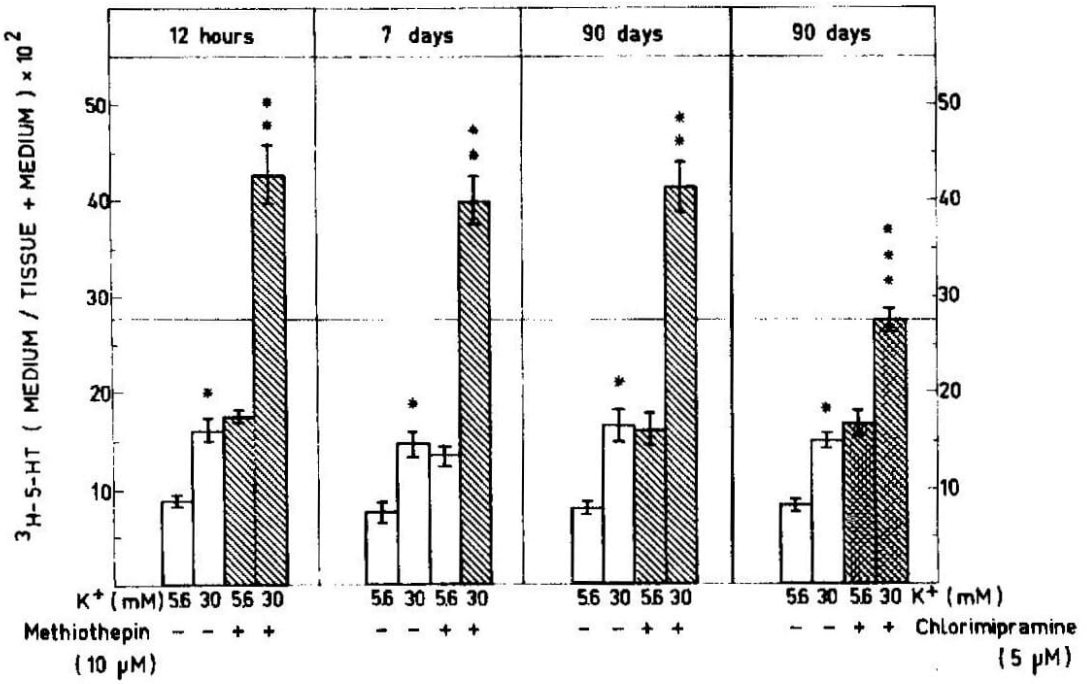

FIG. 8. Effects of methiothepin on the spontaneous and K^+-induced release of newly synthesized [^{3}H]5-HT in brain stem slices of 12-hour-, 7-day- or 90-day-old rats. Comparison with the effects of chlorimipramine. The same experiment as that described in the legend of figure 7 was performed except that methiothepin (10 μM) instead of LSD was added to some samples. The experiment with slices from adult rats was repeated by adding chlorimipramine (5 μM) instead of methiothepin (10 μM). The concentration of [^{3}H]tryptophan was equal to 4.9 μCi/ml corresponding to 0.50 μM. Each bar represents the mean value ± S.E.M. of eight determinations of the ratio (in percentage) of [^{3}H]5-HT in the medium over the total [^{3}H]5-HT accumulated in tissues + medium. * $P < .05$ when compared with control values; ** $P < .05$ when compared with values found with K^+-enriched medium; *** $P < .05$ when compared with values found with adult brain stem slices incubated with 30 mM K^+ and 10 μM methiothepin.

might still be largely independent in adult life.

2. Since the negative feedback process reducing 5-HT synthesis after LSD administration occurred later than the positive one induced by methiothepin treatment, one could propose that these two mechanisms are independent. In fact, as shown in figure 4, even when LSD did not reduce 5-HT synthesis in normal animals *i.e.*, in 12-hour-old rats, this drug was able to prevent the stimulatory effect of methiothepin on 5-HT metabolism. This result would suggest that the negative feedback process could act at birth but only if the turnover of 5-HT exceeded the normal rate. Another possibility might be that this negative feedback process was fully acting yet in newborn rats so that additional stimulation of serotoninergic receptors by LSD would be unable to induce a stronger inhibition of 5-HT synthesis; in particular, this might occur if serotoninergic receptors were hypersensitive to the indoleamine during the early life period. In this case, 5-HT at a submaximal concentration would be more efficient to stimulate 5-HT receptors in the neonatal brain than in the adult one. Indeed, the stimulating effect of the indoleamine on the specific adenylate cyclase in the neonatal brain is much higher than that occurring in adult tissues (Von Hungen *et al.*, 1975). In addition, if 5-HT receptors were already hypersensitive, this could explain why midbrain raphe lesions (Bourgoin *et al.*, 1977) or intracisternal 5-7-dihydroxytryptamine injections (Bennett and Snyder, 1976) failed to change the characteristics of these receptors in the newborn rat. However, Bennett and Snyder (1976) did not detect any change in the affinity of the receptors for 5-HT during ontogenesis, so that the presence of hypersensitive 5-HT receptors in the newborn rat is still an open question. In addition, all these studies (Von Hungen *et al.*, 1975; Bennett and Snyder, 1976; Bourgoin *et al.*, 1977) concern the postsynaptic 5-HT receptors, and nothing is known yet about the possible changes during development in the

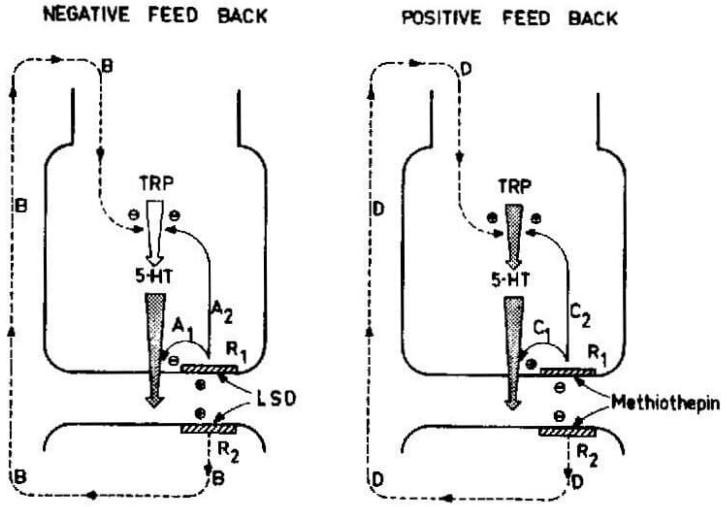

FIG. 9. Schematic diagram illustrating the negative and postive feedback processes triggered by the stimulation and the blockade of serotoninergic receptors. *Negative feedback:* In adult rats, the stimulation of serotoninergic receptors (R_1,R_2) by LSD induced a partial inhibition of the rates of 5-HT release (A_1) and synthesis (A_2). The possible involvement of a neuronal loop in the latter effect is indicated by a dotted line (B). *Positive feedback:* The blockade of serotoninergic receptors (R_1,R_2) by methiothepin induced both an increase of the 5-HT release (C_1) and an accelerated 5-HT synthesis (C_2). The role of a neuronal loop in the latter effect cannot be excluded (dotted line D). In newborn rats the mechanisms A_1, C_1 and C_2 are present. The metabolic steps which are controlled by serotoninergic receptors already at birth are represented by the grey arrows.

sensitivity of the presynaptic 5-HT receptors toward 5-HT.

3. The other possibility which would explain the delay between the first detection of the negative feedback process and that of the positive one might be that both regulatory processes mature independently. Indeed, several observations support the idea that these mechanisms are independent. Thus, tryptophan loading could completely mask the activation induced by methiothepin treatment whereas it did not antagonize the partial inhibition evoked by LSD administration. In the case of methiothepin treatment, this effect of tryptophan was not surprising since the acceleration of 5-HT synthesis after the administration of the 5-HT antagonist is due to an enhanced affinity of tryptophan hydroxylase for tryptophan with no change in its V_{max} (Hamon *et al.*, 1976b). Therefore, saturating the enzyme with its substrate might completely abolish the effect of methiothepin on 5-HT synthesis. In contrast to methiothepin, acute LSD treatment failed to alter the activity of soluble tryptophan hydroxylase (Hamon *et al.*, 1976b).

The present study revealed that the metabolism of 5-HT in brain is regulated early in ontogenesis. The delay between the first occurrence of the negative and the positive feedback processes controlling 5-HT synthesis suggests that these two processes are in fact independent. Experiments with other 5-HT agonists and antagonists (*e.g.*, 5-methoxy-*N*,*N*-dimethyltryptamine and methergoline), probably more specific than LSD and methiothepin, should offer a solution to this problem.

Acknowledgments. The authors thank Hoffmann-La Roche Laboratories (Basel, Switzerland) for generous supplies of methiothepin and benserazid (Ro 4-4602).

References

ANDÉN, N. E., CORRODI, H., FUXE, K. AND HÖKFELT, T.: Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. Brit. J. Pharmacol. 34: 1-7, 1968.

ANDÉN, N. E., CORRODI, H., FUXE, K. AND MEEK, J. L.: Hallucinogenic phenylethylamines: Interactions with serotonin turnover and receptors. Eur. J. Pharmacol. 25: 176-184, 1974.

BENNETT, J. P., JR. AND SNYDER, S. H.: Serotonin and lysergic acid diethylamide binding in rat brain membranes: Relationship to postsynaptic serotonin receptors. Mol. Pharmacol. 12: 373-389, 1976.

BOCKAERT, J., PREMONT, J., GLOWINSKI, J., THIERRY, A. M. AND TASSIN, J. P.: Topographical distribution of dopaminergic innervation and of dopaminergic receptors in the rat striatum. II. Distribution and characteristics of dopamine adenylate cyclase. Interaction of d-LSD with dopaminergic receptors. Brain Res. 107: 303-315, 1976.

BOURGOIN, S.: Evolution du métabolisme cérébral de la sérotonine chez le rat au cours du développement, Thèse de Doctorat esSciences Naturelles, Universite de Paris VII, 1-303, 1976.

BOURGOIN, S., ENJALBERT, A., ADRIEN, J., HÉRY, F. AND HAMON, M: Midbrain raphe lesion in the newborn rat. II. Biochemical alterations in serotoninergic innervation. Brain Res. 127:111-126, 1977.

BOURGOIN, S., FAIVRE-BAUMAN, A., BENDA, P., GLOWINSKI, J. AND HAMON, M: Plasma tryptophan and 5-HT metabolism in the CNS of the newborn rat. J. Neurochem. 23: 319-327, 1974.

BOURGOIN, S., TERNAUX, J. P., BOIREAU, A., HÉRY, F. AND HAMON, M.: Effects of halothane and nitrous oxide anaesthesia on 5-HT turnover in the rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 288: 109-121, 1975.

CARLSSON, A. AND LINDQVIST, M: Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. Toxicol. 20: 140-144, 1963.

CARLSSON, A. AND LINDQVIST, M., Accumulation of 5-hydroxytryptophan in mouse brain after decarboxylase inhibition. J. Pharm. Pharmacol., 22: 726-727, 1970.

CORRODI, H., FARNEBO, L. O., FUXE, K. AND HAMBERGER, B.: Effect of ergot drugs on central 5-hydroxytryptamine neurons: Evidence for 5-hydroxytryptamine release or 5-hydroxytryptamine receptor stimulation. Eur. J. Pharmacol. 30: 172-181, 1975.

FARNEBO, L. O. AND HAMBERGER, B.: Regulation of (^{3}H)5-hydroxytryptamine release from rat brain slices. J. Pharm. Pharmacol. 26: 642-644, 1974.

FUXE, K., AGNATI, L. AND EVERITT, B.: Effects of methergoline on central monoamine neurons. Evidence for a selective blockade of central 5-HT receptors. Neurosci. Letters, 1: 283-290, 1975.

FUXE, K., HOLMSTEDT, B. AND JONSSON, G.: Effects of 5-methoxy-N,N-dimethyltryptamine on central monoamine neurons. Eur. J. Pharmacol. 19: 25-34, 1972.

HAMON, M., BOURGOIN, S., ENJALBERT, A., BOCKAERT, J. HÉRY, F., TERNAUX, J. P. AND GLOWINSKI, J.: The effects of quipazine on 5-HT metabolism in the rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 294: 99-108, 1976a.

HAMON, M., BOURGOIN, S. AND GLOWINSKI, J.: Feedback regulation of 5-HT synthesis in rat striatal slices. J. Neurochem. 20: 1727-1745, 1973.

HAMON, M., BOURGOIN, S., HÉRY, F., TERNAUX, J. P. AND GLOWINSKI, J.: Changes in 5-HT metabolism in the rat brain following blockade of central 5-HT receptors by methiothepin. Vth International Meeting of International Society for Neurochemistry, Abstract 423, 1975.

HAMON, M., BOURGOIN, S., HÉRY, F., TERNAUX, J. P. AND GLOWINSKI, J.: In vivo and in vitro activation of soluble tryptophan hydroxylase from rat brainstem. Nature (London) 260: 61-63, 1976b.

HAMON, M., BOURGOIN, S., JAGGER, J. AND GLOWINSKI, J.: Effects of LSD on synthesis and release of 5-HT in rat brain slices. Brain Res. 69: 265-280, 1974.

HÉRY, F., ROUER, E. AND GLOWINSKI, J.: Daily variations of serotonin metabolism in the rat brain. Brain Res. 43, 445-465, 1972.

HOFF, K. M., BAKER, P. C. AND BUDA, R. E.: Precursor and end product effects upon indoleamine maturation in mouse brain. Biol. Neonate 29: 360-367, 1976.

JACOBS, B. L.: An animal behavior model for studying central serotonergic synapses. Life Sci. 19: 777-786, 1976.

JACOBY, J. H., SHABSHELOWITZ, H., FERNSTROM, J. D. AND WURTMAN, R. J.: The mechanisms by which methiothepin, a putative serotonin receptor antagonist, increases brain 5-hydroxyindole levels. J. Pharmacol. Exp. Ther. 195: 257-264, 1975.

KATZ, R. I. AND KOPIN, I. J.: Effect of D-LSD and related compounds on release of norepinephrine-^{3}H and serotonin-^{3}H evoked from brain slices by electrical stimulation. Pharmacol. Res. Commun. 1: 54-62, 1969.

KRAML, M: A rapid microfluorimetric determination of monoamine oxidase. Biochem. Pharmacol. 14: 1683-1685, 1965.

MONACHON, M. A., BURKARD, W. P., JALFRE, M. AND HAEFELY, W.: Blockade of central 5-hydroxytryptamine receptors by methiothepin. Naunyn-Schmiedeberg's Arch. Pharmacol. 274: 192-197, 1972.

PAGEL, J., CHRISTIAN, S. T., QUAYLE, E. S. AND MONTI, J. A.: A serotonin sensitive adenylate cyclase in mature rat brain synaptic membranes. Life Sci. 19: 819-824, 1976.

SCHUBERT, J., NYBACK, H. AND SEDVALL, G.: Accumulation and disappearance of ^{3}H-5-hydroxytryptamine formed from ^{3}H-tryptophan in mouse brain; Effect of LSD 25. Eur. J. Pharmacol. 10: 215-224, 1970.

SNEDECOR, G. W. AND COCHRAN, W. G.: Statistical Methods, 6th ed., Iowa State University Press, Ames, 1967.

SOFIA, R. D. AND VASSAR, H. B.: The effect of ergotamine and methysergide on serotonin metabolism in the rat brain. Arch. Int. Pharmacodyn. Thér. 216: 40-50, 1975.

TEBEĆIS, A. K.: Antagonism of 5-hydroxytryptamine by methiothepin shown by microiontophoretic studies of neurons in the lateral geniculate nucleus. Nature (London) 238: 63-64, 1972.

VON HUNGEN, K., ROBERTS, S. AND HILL, D. F.: Serotonin-sensitive adenylate cyclase activity in immature rat brain. Brain Res. 84: 257-267, 1975.

ZIVCOVIC, B., GUIDOTTI, A. AND COSTA, E.: Effects of neuroleptics on striatal tyrosine hydroxylase: Changes in affinity for the pteridine cofactor. Mol. Pharmacol. 10: 727-735, 1974.