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# CHANGES IN SEROTONIN METABOLISM MAY ELICIT OBSTRUCTIVE APNOEA IN THE NEWBORN RAT

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SUMMARY

1. Experiments were performed on anaesthetized newborn rats (aged 3–10 days) to know whether an increase in central serotonin levels might favour the occurrence of obstructive apnoeas as previously suggested by *in vitro* results from our group.

2. The levels of serotonin (5-HT), its precursor 5-hydroxytryptophan (5-HTP) and its metabolite, 5-hydroxyindole-acetic acid (5-HIAA), were analysed in cerebrospinal fluid samples collected at the level of the obex prior to and after intraperitoneal injection of L-tryptophan (50 mg $kg^{-1}$) in sixty-eight anaesthetized newborn rats (control rats prior to injection and injected rats 15, 30 and 45 min after the injection). A significant increase in 5-HT and 5-HTP levels occurred 30 min after the injection, attesting to the activation of 5-HT biosynthesis.

3. The EMG activity of both the genioglossus and the diaphragm was recorded before and after L-tryptophan load (50 mg $kg^{-1}$) in twenty-two newborn rats. After the injection of L-tryptophan, the amplitude of the integrated genioglossus activity decreased, or was even abolished, either during a few respiratory cycles or for long periods in twenty-one out of twenty-two animals. Recovery of the genioglossus activity occurred within 45–60 min.

4. The thoracic respiratory movements and the resulting upper airway pressure changes were recorded before and after L-tryptophan injection (50 mg $kg^{-1}$) in sixty-two animals. In some litters ($n = 7$), most of the animals (21/25) displayed, within 20–40 min of the injection, recurrent episodes of obstructive apnoea often followed by central ones. These respiratory difficulties became severe and drastic, and led in two instances to respiratory distress and death. Lower doses of L-tryptophan (10 mg $kg^{-1}$) did not induce any respiratory disorders unless these were potentiated by pargyline treatment (50 mg $kg^{-1}$, $n = 7$). The obstructive apnoeas liable to occur after an L-tryptophan load (50 mg $kg^{-1}$) were prevented by blocking the 5-HT receptor with methysergide (50 mg $kg^{-1}$, $n = 5$) or by blocking the 5-HT biosynthesis by applying *p*-chlorophenylalanine (PCPA) pretreatment at birth (300 mg $kg^{-1}$, $n = 7$). In other litters ($n = 6$), none of the eighteen newborn rats tested were affected by L-tryptophan, however. In five young adult rats, L-tryptophan again had no effect.

5. These *in vivo* results, which confirm that activation of 5-HT biosynthesis can elicit obstructive apnoeas in newborn rats, are discussed in the light of clinical

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observations which suggest that obstructive apnoeas and abnormal central 5-HT levels might occur in cases of sudden infant death syndrome.

## INTRODUCTION

The respiratory act cannot be achieved efficiently without the co-ordinated activation of numerous respiratory muscles controlling the chest wall and the upper airways. Any inability of the upper airway muscles to overcome the negative pressure generated by the chest pump will lead to obstructive apnoea with partial or total invagination of the upper airways (Remmers, de Groot, Sauerland & Anch, 1978; Guilleminault, Hill, Simmons & Dement, 1978; Iscoe, 1988). Although the exact site of the closure is still under debate and may vary from one patient to another (Kurtz, Krieger & Stierle, 1978; Remmers, 1984), a dysfunction of the genioglossus muscle is generally held to be responsible (Sauerland & Harper, 1976; Kurtz *et al.* 1978). These disorders occasionally occur even in healthy adults during sleep and lead to their arousal (Kurtz *et al.* 1978). When these occurrences are frequent, a pathology develops with chronic hypoxia, secondary cardiac disorders, insomnia, and interestingly, with elevated 5-hydroxyindole-acetic acid (5-HIAA) levels (Cramer, Warter, Renaud, Krieger, Marescaux & Hammers, 1981). In the newborn, obstructive apnoea during sleep may have drastic effects if no arousal ensues, since the work of the chest respiratory muscles against the closed airways is oxygen consuming and the resulting hypoxia may in turn aggravate the respiratory distress by centrally depressing the newborn infant's breathing (Jansen & Chernick, 1983).

Little is known about the intrinsic mechanisms which might elicit obstructive apnoeas. Acidification of the upper oesophageal tract after a gastro-oesophageal reflux is known to elicit obstructive apnoea in the newborn infant (Herbst, Minton & Book, 1979). Some substances such as alcohol, anaesthesia, diazepam have been reported to specifically depress the activity of the hypoglossal nerve (supplying the genioglossus) and to facilitate the occurrence of obstructive apnoea (see Iscoe, 1988, for a review). We have recently suggested that serotonin (5-HT), a widely distributed neurotransmitter, might be involved in the occurrence of obstructive apnoea. This hypothesis was based on *in vitro* data obtained on an experimental preparation from isolated respiratory centres of newborn rats. Adding exogenous 5-HT to the bathing medium (Monteau, Morin, Hennequin & Hilaire, 1990) and eliciting the release of endogenous 5-HT by electrical or pharmacological stimulation of the raphe nuclei (Morin, Hennequin, Monteau & Hilaire, 1990) depressed the hypoglossal respiratory motor output to the genioglossus muscle, while the motor output to the chest pump was not affected. Intracellular analysis of the hypoglossal motoneurons confirmed these results and suggested the involvement of presynaptic 5-$HT_2$ receptors impairing the central respiratory drive to the hypoglossal motoneurons (Morin, Monteau & Hilaire, 1992). It was therefore postulated that a 5-HT excess due to a dysfunction of the 5-HT biosynthesis mechanisms might set up conditions which favour obstructive apnoeas.

The aim of the present report was to check this hypothesis under *in vivo* conditions, i.e. in freely breathing anaesthetized newborn rats. The results obtained were in agreement with the previous *in vitro* results. Briefly, 5-HT synthesis was

activated in anaesthetized newborn rats by performing intraperitoneal injections of L-tryptophan; depression of the inspiratory activity of the genioglossus muscle and the occurrence of obstructive apnoeas were observed. Since some of the obstructive apnoeas were lethal, these results are discussed with reference to the hypothesis that abnormally high serotonin levels might be at least partly involved in the sudden infant death syndrome (SIDS).

## METHODS

Experiments were performed on 156 newborn Sprague–Dawley rats from pregnant females (Iffa Credo breeding centre) which arrived at the laboratory 1 week before delivery. The animals (age, 3–10 days; weight, 5–20 g.) were anaesthetized by intraperitoneal injection of low doses of sodium pentobarbitone (less than 10 mg $kg^{-1}$), which efficiently suppressed any responses to tail pinching (doses in the adult range, 45–50 mg $kg^{-1}$, were lethal). The animals were lying (dorsal decubitus) on a warming blanket to maintain the rectal temperature at around 38 °C and were spontaneously ventilating. Five experiments were also performed on anaesthetized young adult rats (150–200 g, I.P. sodium pentobarbitone, 50 mg $kg^{-1}$).

In the first set of sixty-eight newborn rats (ten litters), the levels of 5-HT, 5-hydroxytryptophan (5-HTP) and 5-HIAA in the cerebrospinal fluid were analysed by performing high-pressure liquid chromatography (HPLC) prior to and after intraperitoneal injections of L-tryptophan (50 mg $kg^{-1}$ in 0·2 ml saline). Cerebrospinal fluid was collected (at least 20 $\mu$l) at the level of the obex after midline incision of the dorsal skin, the neck muscles and the dura (any blood contamination of the sample was carefully avoided). Immediately after sampling, every sample was precipitated (perchloric acid, 2 M) and centrifuged (12000 *g*, 10 min at 4 °C), and the supernatant was analysed by HPLC (Waters 510 pump, 20 $\mu$l Rheodyne injection valve, RP18 Brownlee guard column and Ultrasphere ODS analytic column, both kept at 30 ± 0·5 °C; flow rate, 0·8 ml $min^{-1}$) coupled with electrochemical detection (Waters 460, glassy carbon working electrode at +0·75 V against Ag–AgCl reference electrode; sensitivity, 20 nA $V^{-1}$). The mobile phase contained 35 mM citric acid, 12·5 mM $Na_2HPO_4$, 0·25 mM sodium octyl sulphate, 0·05 mM EDTA and 8% acetonitrile and the pH was adjusted to 3·35. Reference solutions were prepared on the day of the analysis (by diluting stock solutions stored at −80 °C) and maintained in darkness at 2–4 °C in perchloric acid 0·1 M at concentrations of 45 ng $ml^{-1}$ in the case of 5-HT, 25 ng $ml^{-1}$ in the case of 5-HTP, 50 ng $ml^{-1}$ in the case of 5-HIAA and 100 ng $ml^{-1}$ in the case of *N*-acetyl-5-HT which was used as the internal standard. With the methods used it is possible to detect at least twelve bioamine compounds (see Fig. 1*A* and Caroff, Girin, Alix, Cann-Moisan, Sizun & Barthélémy, 1992), but we focused mainly on 5-HT, 5-HTP, 5-HIAA (Fig. 1*B*). Samples were collected either prior to (control, $n = 18$) or after L-tryptophan injection (15, 30 and 45 min on 17, 16 and 17 newborn rats, respectively). Since only one cerebrospinal fluid sample could be collected on each animal it was not possible to perform paired comparisons between control and post-injection values. The high variability of the levels under both control and test conditions (expressed in the Results section as means ± S.E.M.) makes it impossible to compare the data by a parametric method. A non-parametric Mann–Whitney *U* test was thus used in which the null hypothesis is that the two samples come from populations having the same distribution. The samples were considered as significantly different at least at $P < 0·05$.

In the second set of twenty-six newborn rats (7 litters), the EMG activity of the diaphragm and the genioglossus was monitored via two hooks of thin insulated copper wires (diameter 100 $\mu$m) implanted in the muscles through the skin with thin needles (diameter 500 $\mu$m). The location of recording electrodes was confirmed by postmortem examination. Electrical signals were filtered (10–3000 Hz), amplified (5–10000 times), fed into two identical leaky integrators (time constant 50 ms) and displayed on a screen and a paper recorder (Gould TA 2000).

In the third set of sixty-two newborn rats (13 litters), the chest respiratory movements and the resulting airway pressure changes were monitored via a strain gauge gently touching the lower ribs and a highly sensitive home-made pressure transducer (able to detect changes less than 1 $mmH_2O$) connected to a facial mask, respectively.

In both the latter protocols, after a 20–30 min control period, intraperitoneal injection of L-tryptophan was performed and the resulting changes in either the EMG amplitude or the

respiratory movements and air flow were analysed during a 60–90 min period. Sham experiments were performed in the same way with a 0·2 ml saline injection instead of L-tryptophan. In nineteen experiments, the animals were pretreated with drugs known to interact with 5-HT mechanisms: (i) pargyline (Sigma, I.P., 50 mg $kg^{-1}$, $n = 7$) to potentiate the effects of 5-HT by inhibiting 5-HT degradation by the monoamine oxidases; (ii) methysergide (Sandoz, I.P., 50 mg $kg^{-1}$, $n = 5$) to block the 5-HT receptors; and (iii) *p*-chlorophenylalanine (PCPA, Sigma, 300 mg $kg^{-1}$, $n = 7$) to block the serotonin biosynthesis chain by inhibiting tryptophan hydroxylase.

## RESULTS

### *Changes in 5-HT biosynthesis after intraperitoneal injection of L-tryptophan in the newborn rat*

Samples of cerebrospinal fluid collected at the level of the obex were analysed by HPLC coupled with electrochemical detection in sixty-eight anaesthetized newborn rats. In eighteen control animals, low levels of 5-HTP and 5-HT were detected ($3·0 \pm 1·3$ and $7·2 \pm 4·0$ ng $ml^{-1}$, respectively) with rather large amounts of 5-HIAA ($217 \pm 30$ ng $ml^{-1}$). Large individual variations were noted even between rats from the same litter and weight. Fifty further animals received an intraperitoneal injection of L-tryptophan (50 mg $kg^{-1}$) and the sampling was performed 15, 30 and 45 min after the injection on 17, 16 and 17 rats, respectively. Figure 1*D–F* summarizes the changes in the mean 5-HTP, 5-HT and 5-HIAA cerebrospinal fluid levels observed after the L-tryptophan injection ($5·8 \pm 1·5$, $18·8 \pm 8·5$ and $299 \pm 100$ ng $ml^{-1}$, respectively, within 30 min). At 30 min following L-tryptophan injection the distributions of 5-HTP and 5-HT values, but not those of 5-HIAA values were significantly different from the distributions of control values. No difference was observed in the distribution of 5-HT, 5-HTP and 5-HIAA as compared to control values at 15 and 45 min after the injection. The CSF levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured ($5·6 \pm 0·6$ and $49 \pm 15$ ng $ml^{-1}$, respectively) under control conditions and no changes were observed after L-tryptophan injection.

### *Depression of inspiratory genioglossus activity by L-tryptophan intraperitoneal injection in the newborn rat*

The EMG activities of the diaphragm and genioglossus muscles were recorded in twenty-six anaesthetized newborn rats (Fig. 2*A*). Under control conditions, the two muscles both fired during inspiration but the genioglossus started firing 50–100 ms before the diaphragm. Both muscles displayed slight spontaneous variations in their activities from one cycle to another: measuring the amplitude of the two integrated activities for thirty successive breaths and performing a linear regression between these two variables confirmed that the coefficient of correlation was not significant.

Among the twenty-six newborn rats, twenty-two were given an intraperitoneal injection of L-tryptophan (50 mg $kg^{-1}$). The amplitude of the integrated activity of the genioglossus always began to decrease in 15–20 min, while the activity of the diaphragm was not significantly affected in twenty-one out of twenty-two rats. The depression of the integrated EMG activity of the genioglossus reached 40% of the control value within 30 min. In some animals ($n = 5$), the genioglossus discharge disappeared completely during episodes lasting for several minutes. In sixteen rats, a slight inspiratory activity persisted in the genioglossus and the precession of

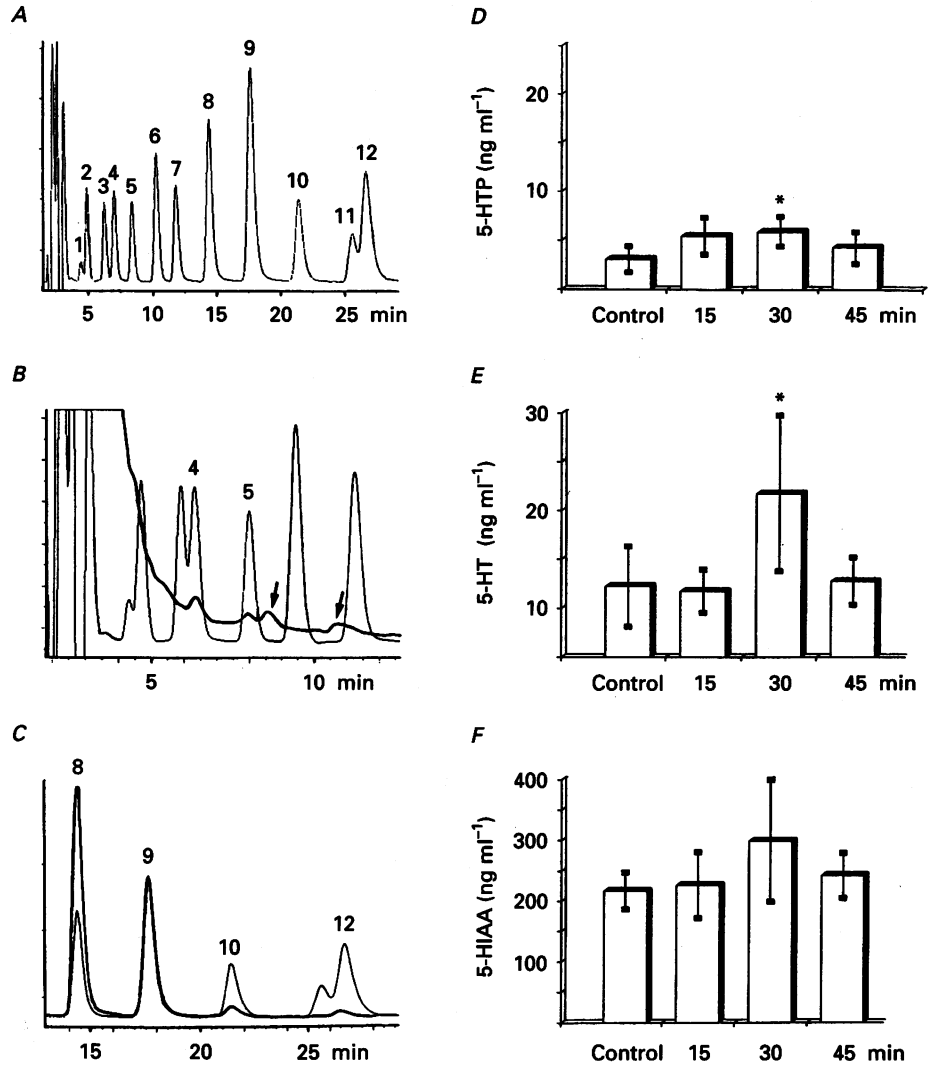


Fig. 1. L-Tryptophan load and 5-HT metabolism. *A*, chromatogram of a standard solution containing 3-methoxy-4-hydroxyphenylglycol (1, 40 ng ml$^{-1}$), noradrenaline (2, 25 ng ml$^{-1}$), adrenaline (3, 25 ng ml$^{-1}$), 5-hydroxytryptophan (4, 25 ng ml$^{-1}$), 3,4-dihydroxyphenylacetic acid (5, 25 ng ml$^{-1}$), 3,4-dihydroxyphenylalanine (6, 40 ng ml$^{-1}$), 5-hydroxytryptophol (7, 25 ng ml$^{-1}$), 5-hydroxyindole-acetic acid (8, 50 ng ml$^{-1}$), *N*-acetyl-5-hydroxytryptamine (9, 100 ng ml$^{-1}$), homovanillic acid (10, 100 ng ml$^{-1}$), 3-methoxytyramine (11, 40 ng ml$^{-1}$), 5-hydroxytryptamine (12, 45 ng ml$^{-1}$). *B* and *C*, typical chromatograms of cerebrospinal fluid sampled 30 min after I.P. tryptophan injection (heavy line) superimposed on the chromatogram of a standard solution (time base and amplification are different in *B* and *C*). *B*, first part of a chromatogram, 5-HTP, DOPAC and two unidentified components (arrows) are detected; *C*, last part of a chromatogram, 5-HIAA, *N*-acetyl-5-HT (internal standard), HVA and 5-HT can be detected. *D–F*, concentrations (means ± S.E.M.) of 5-HTP (*D*), 5-HT (*E*) and 5-HIAA (*F*) in CSF under control conditions and 15, 30 and 45 min after I.P. L-tryptophan injection; * according to the Mann–Whitney *U* test the distribution of these values is different from the distribution of control values at $P < 0.05$.

genioglossus firing over the diaphragm activity was sometimes reduced or abolished but this was not observed consistently. The genioglossus discharge occasionally stopped completely for five to ten successive breaths (Fig. 2*B*) or during a few minutes (2–3 min). Within 60–90 min, however, the inspiratory discharge of the

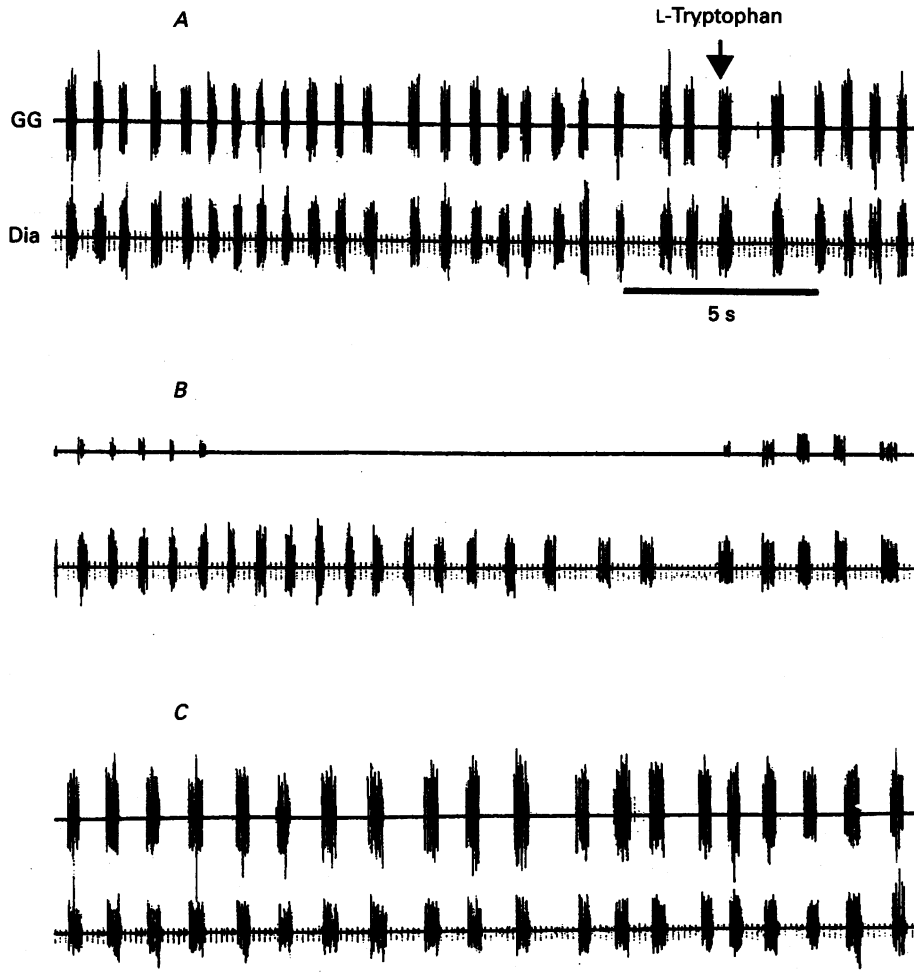


Fig. 2. Depression of genioglossus activity after L-tryptophan load in newborn rats. EMG activity of genioglossus (GG, upper trace) and diaphragm (Dia, lower trace) under control conditions (in *A*, I.P. injection of L-tryptophan, 50 mg $kg^{-1}$ at the arrow), 25 min (*B*) and 70 min (*C*) after L-tryptophan load; note in *B* the decrease in activity and even the total disappearance of the genioglossus discharge, but not of the diaphragm.

genioglossus had recovered in all the experiments but one, where respiratory arrest and death occurred 38 min after the injection. Three rats were given intraperitoneal injections of saline (prior to L-tryptophan load) which did not affect the genioglossus discharge.

Four animals were pretreated at birth with PCPA (300 mg $kg^{-1}$) in order to block L-tryptophan hydroxylase and therefore the synthesis of 5-HTP and 5-HT.

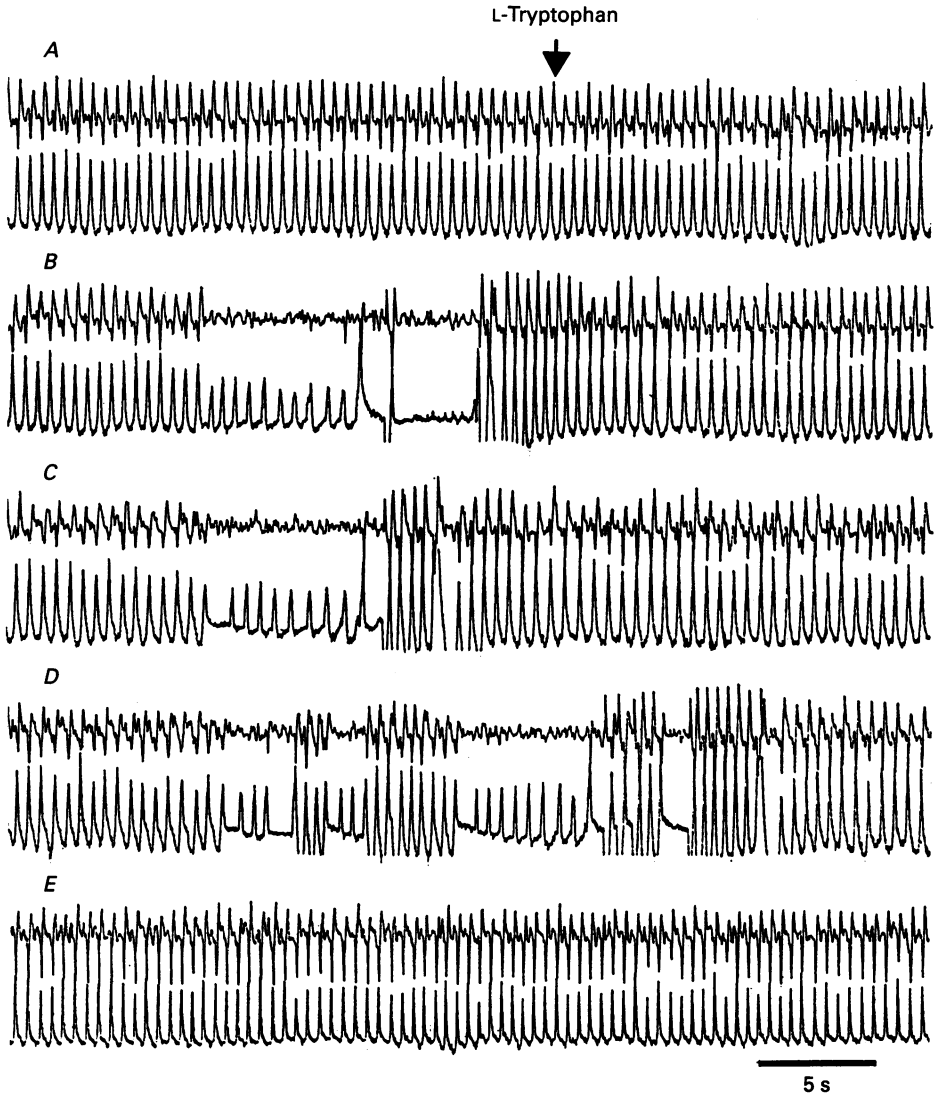


Fig. 3. Obstructive apnoeas elicited by L-tryptophan load in newborn rats. Upper airway pressure changes (upper trace) and respiratory chest movements (lower trace, inspiration upward) were recorded under control conditions (in *A*, I.P. injection of L-tryptophan, 50 mg $kg^{-1}$ at the arrow) and 25, 40, 45 and 90 min after L-tryptophan load (in *B*, *C*, *D* and *E*, respectively); note the occurrence of long episodes of obstructive apnoea ended by central apnoea in *B*, their shortening in *C* and *D* and the recovery in *E*.

Intraperitoneal injections of L-tryptophan performed two days later neither depressed the amplitude of the genioglossus discharge nor inactivated the muscle.

*Obstructive apnoeas induced by intraperitoneal L-tryptophan injection in the newborn rat*

In sixty-two newborn rats (13 litters), the lower rib movements and the resulting

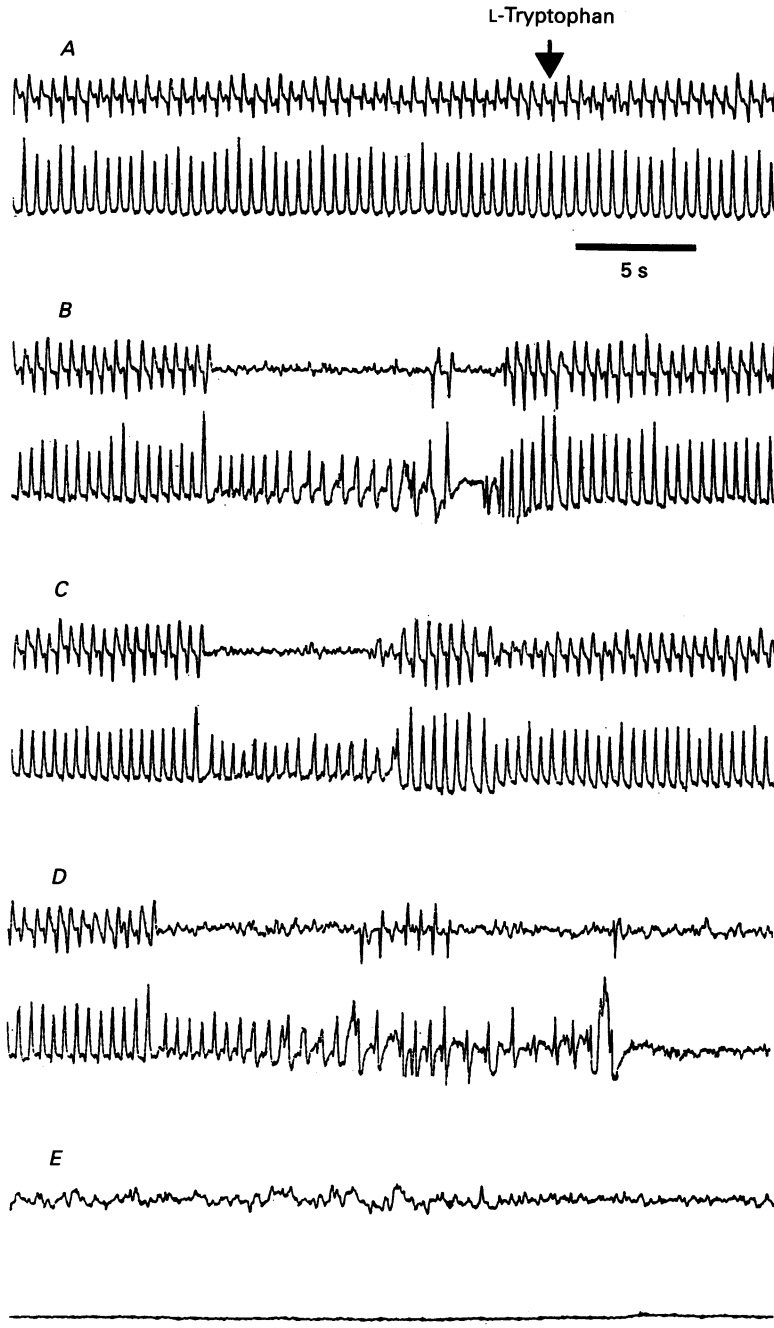


Fig. 4. Lethal obstructive apnoea elicited by L-tryptophan load in newborn rats. Same ordering as in Fig. 3 but *B*, *C*, *D* and *E* were recorded 30, 40, 49 and 50 min after I.P. injection of L-tryptophan at 50 mg $kg^{-1}$. Note the respiratory distress in *D* followed by lethal arrest of breathing in *E*.

upper airway pressure changes were monitored during a 20–30 min control period. Occasionally, one or two inspiratory chest movements could occur without any concomitant changes in upper airway pressure; these respiratory disorders which were very similar to spontaneous obstructive apnoeas, were observed in only five animals and recurred only infrequently (once or twice in 20–30 min).

In seven litters, twenty-one of the twenty-five newborn rats which were intraperitoneally injected with L-tryptophan (50 mg $kg^{-1}$) displayed within 20–40 min respiratory disorders characterized by the occurrence of frequent, long-lasting episodes of obstructive apnoea. In one newborn rat, this effect was rather limited since only three incidents were observed, each lasting around 5 s, but in all the others, obstructive apnoea recurred frequently (every 5–6 min), and became longer (10–30 s). In some newborn rats, a deep inspiration preceded every episode of obstructive apnoea but this was not the case in all the animals. Figure 3*A* illustrates a typical experiment: 25 min after L-tryptophan injection, obstructive apnoeas lasting for five to ten respiratory cycles (Fig. 3*B*) occurred every 3–4 min; a central apnoea lasting for several seconds often developed during the obstructive periods until efficient respiration restarted for a while with large inspiratory thoracic movements; obstructive apnoea then suddenly reappeared, followed by central apnoea, etc. The duration and frequency of the obstructive apnoea diminished 40–45 min after L-tryptophan injection (Fig. 3*C* and *D*). Total recovery was observed in nineteen cases but was not achieved before 45–60 min (Fig. 3*E*). In two newborn rats, episodes of obstructive apnoea followed by central apnoea and transient recovery (Fig. 4*A–C*) led to respiratory distress which worsened until death occurred (Fig. 4*D* and *E*). Only four animals (from 2 of these 7 litters) were not affected by the 50 mg $kg^{-1}$ L-tryptophan load.

In four other newborn rats, lower doses of L-tryptophan (10 mg $kg^{-1}$) did not elicit any respiratory disorders. These occurred however when an intraperitoneal injection of pargyline (50 mg $kg^{-1}$) was used to potentiate the effects of L-tryptophan. In three animals, pargyline was applied first and L-tryptophan (10 mg $kg^{-1}$) was injected 10 min later; drastic obstructive and central apnoeas appeared, leading to death in two out of three animals. In four animals pargyline was injected after L-tryptophan; 15 min after the injection, frequent obstructive and central apnoeas were observed in all the animals, leading in two out of four cases to drastic respiratory disorders and death.

Obstructive apnoea was never observed after intraperitoneal injections of (i) saline ($n = 5$), (ii) L-tryptophan given 10 min after intraperitoneal injection of the 5-HT antagonist, methysergide (50 mg $kg^{-1}$, $n = 5$) and (iii) L-tryptophan given to newborn rats pretreated at birth with PCPA (300 mg $kg^{-1}$, $n = 7$).

Eighteen newborn rats from six other litters were subjected to L-tryptophan injection (50 mg $kg^{-1}$); none of these however, displayed any significant respiratory changes. It is worth noting in particular that among the six newborn rats from one of these litters tested, none were affected by the L-tryptophan load, even when larger doses were used (100 mg $kg^{-1}$, $n = 3$). All these L-tryptophan-insensitive newborn rats were similar in age and weight to those which previously displayed obstructive apnoea. There was no obvious explanation for the failure of L-tryptophan to have a respiratory effect in these rats.

Furthermore, L-tryptophan (50 mg $kg^{-1}$) was tested on five young adult rats and found to induce no obstructive apnoea during recording sessions lasting at least 90–120 min.

## DISCUSSION

The experiments described here demonstrate that an intraperitoneal load of L-tryptophan in the newborn rat (1) activates 5-HT synthesis, (2) depresses, and sometimes even abolishes, the inspiratory activity of the genioglossus muscle, and (3) elicits drastic respiratory disorders. The depression of the inspiratory activity of the genioglossus observed confirms our previous *in vitro* results and the occurrence of severe obstructive apnoeas reinforces the hypothesis we previously put forward that 5-HT metabolism disorders might be involved in respiratory anomalies which might even lead to sudden infant death syndrome (Morin *et al.* 1992).

### *L-Tryptophan load and 5-HT biosynthesis*

In the adult rat, it is well known (for a review see Osborne, 1982) that (i) PCPA pretreatment blocks 5-HT biosynthesis by inhibiting tryptophan hydroxylase activity, (ii) pargyline potentiates endogenous 5-HT effects by inhibiting monoamine oxidase activity, (iii) methysergide is a broad 5-HT antagonist which blocks 5-HT effects and (iv) L-tryptophan intraperitoneal load activates 5-HT biosynthesis. In the newborn rat, we have already obtained results which confirm the first three findings listed above (Monteau *et al.* 1990; Morin *et al.* 1990), but we felt it was necessary to test the fourth since it was assumed that 'changes in tryptophan availability may be of less importance in the regulation of 5-HT synthesis in newborn animals' (Bourgoin, Faivre-Bauman, Benda, Glowinski & Hamon, 1974). Since the bioamine levels of the cerebrospinal fluid and those of the brain are correlated (Stanley, Traskman-Bendz & Dorivini-Zis, 1985), the increase in the 5-HTP and 5-HT levels which occurs after L-tryptophan load clearly shows that the 5-HT biosynthesis in the brain increases. The 5-HT biosynthesis processes in newborn rats have been reported to differ from those in the adult (Hamon & Bourgoin, 1982). Comparisons between the changes elicited by identical L-tryptophan loads (50 mg $kg^{-1}$) in newborn (present results) and adult rats (Hutson, Sarna, Kantamaneni & Curzon, 1985) shows that faster changes and recovery take place in the newborn (peak changes and recovery at 30 and 45 min in the newborn against 72 and 120 min in the adult, respectively). The fast return to control values is consistent with the idea that newly synthesized 5-HT is catabolized more rapidly in the newborn than in the adult rat (Bourgoin *et al.* 1974). The significant 5-HTP and 5-HT accumulation occurring soon after L-tryptophan load might be due to the absence of negative feedback control of 5-HT synthesis in the newborn rat (Bourgoin, Artaud, Enjalbert, Héry, Glowinski & Hamon, 1977).

The high variability we observed in the 5-HT and 5-HIAA levels under both control and test conditions might be due to the use of newborn rats of various ages (3–10 days old). This may have given rise to a large S.E.M. due to age-related or to individual differences. The brain 5-HT and 5-HIAA levels change considerably with age from day 0 to day 15 (see Fig. 1 from Hamon & Bourgoin, 1982). Age differences can only partly account for this variability however, since very different levels of 5-

HT and 5-HIAA were occasionally observed between newborn rats of the same age, weight and litter. This contrasts with the stable 5-HT and 5-HIAA values measured by other authors in the brain of newborn rats of a given age (Hamon & Bourgoin, 1982, as deduced from the low S.E.M.), and the proportionality between the monoamine levels in the brain and the cerebrospinal fluid (Stanley *et al.* 1985) might consequently be questioned. Hutson and co-workers (1985) analysed in adult rats the changes in the 5-HIAA levels of cerebrospinal fluid samples, dialysates and whole brain tissue elicited by L-tryptophan load. The changes were proportional and followed practically the same time courses, but considerable variations from one rat to another were noted, suggesting 'a remarkable degree of neurochemical individuality, especially if the similarity of the animals with respect to strain, age, diet, and environment is taken into account'. 5-HT metabolism at birth is influenced by pre- and postnatal nutritional factors (Hernandez, Manjarrez & Chagoya, 1989) which may define the individual degrees of maturation and functioning of the 5-HT mechanisms (nutrition of the female during pregnancy, gestational age at birth, number of pups per litter either at birth or on the day of the experiment, time of the last suckling, etc.). Although our data may not have been completely insensitive to these uncontrolled factors and the resulting variability, they clearly confirm that L-tryptophan load in the newborn rat does activate 5-HT biosynthesis and elicit a 5-HT accumulation in the cerebrospinal fluid.

*5-HT and respiratory changes*

The silencing of the genioglossus activity and the occurrence of severe obstructive apnoeas which are (1) concomitant with the increase in the cerebrospinal fluid 5-HT level, (2) potentiated by pargyline administration, (3) prevented by PCPA pretreatment at birth and (4) blocked by methysergide, are therefore likely to be due to the 5-HT newly synthesized in response to the L-tryptophan injections. The reason why obstructive apnoea was frequently observed in some litters but not in others is an important question which still remains to be elucidated, but a secondary effect of anaesthesia can be ruled out since the anaesthetic was prepared before each experiment and the possibility of deeper or lighter anaesthesia occurring for all the newborn rats of a given litter is highly unlikely. These different responses may again be related to uncontrolled conditions such as those put forward to explain the individual variability of 5-HT and 5-HIAA levels. If so, it will be of great importance in the future to correlate individual respiratory and metabolic responses to L-tryptophan load.

The 5-HT-induced depression of the genioglossus activity is consistent with our previous *in vitro* results which demonstrated that activation of presynaptic 5-$HT_2$ receptors depressed the central respiratory drive to the hypoglossal motoneurons (Morin *et al.* 1992). The presence of neuronal pathways from the raphe nuclei (the main serotonergic structures) to the hypoglossal nucleus (Card, Rinaman, Schwaber, Miselis, Whealy, Robbins & Enquist, 1990), and the existence of 5-HT profiles (Aldes, Marco & Chronister, 1989) and 5-HT receptor mRNA (Mengod, Pompeiano, Martinez-Mir & Palacios, 1990) within the hypoglossal nucleus provide further evidence that 5-HT may modulate genioglossus activity.

*Genioglossal activity and obstructive apnoea*

Our results confirm previous reports (Sauerland & Harper, 1976; Kurtz *et al.* 1978) which suggested that the silencing of the genioglossus may be involved in the occurrence of obstructive apnoea. The exact site of the closure is still a matter of debate, however (Kurtz *et al.* 1978; Remmers, 1984) and silencing of the genioglossus might be only one of several disorders responsible for the occurrence of obstructive apnoea: L-tryptophan silenced the genioglossus in almost all the newborn rats but elicited obstructive apnoea only in some. This may be due to dysfunctions of either the upper airways or the chest muscles. A dysfunction of other upper airway muscles might occur with high levels of 5-HT. In neonates, the laryngeal adductors seem to be active during most of expiration and sometimes occlude the airway so that expiratory flow is interrupted (Bartlett, 1989). 5-HT is known to excite cranial and spinal motoneurons (McCall & Aghajanian, 1979; Takahashi & Berger, 1992) and a sustained activation of expiratory motoneurons of the upper airway might occasionally occur along with genioglossal silencing, leading to a drastic obstructive apnoea. This suggestion still requires to be demonstrated, since access to these muscles is difficult in small-sized newborn rats and dissection may affect their activity by altering complex regulation loops. Furthermore, the chest muscles may be unable to overcome the genioglossal collapsus because most of the phrenic motoneurons are firing during normal breathing in young animals. As pointed out by Cameron, Jodkowsky, Fang & Guthrie (1991) 'the disadvantage is that there is less of a reserve of inactive motoneurons to be recruited in the event that much larger forces are required. Such manoeuvres may be required to overcome an obstruction of the airway'. In the adult, only half of the phrenic motoneurons are firing under normal breathing (Cameron *et al.* 1991) and large increases in the work of the chest pump are possible; the absence of any obstructive apnoea in young adult rats after L-tryptophan injection might relate to this phenomenon.

*Obstructive apnoea, enhanced 5-HT biosynthesis and non-respiratory effects*

Since all the influences from the periphery have been eliminated in the *in vitro* brainstem–spinal cord preparation, the 5-HT depression of the hypoglossal discharge reported *in vitro* can only have been due to central 5-HT effects. In the present *in vivo* study, the possibility that other effects besides the central ones may have been involved cannot be ruled out, however. 5-HT is known to be involved in the modulation of several activities (cardiovascular, digestive, sleep–awake states; see Osborne, 1982) which might at least partly contribute to the occurrence of obstructive apnoea. First, 5-HT may induce hypertension (Dedeoglu & Fisher, 1991) which in turn may depress genioglossus activity (Garpestad, Basner, Ringler, Lilly, Schwartzstein, Weinberger & Weiss, 1992). The genioglossus depression and the resulting obstructive apnoea reported here may therefore have involved a 5-HT-induced hypertension (the arterial pressure was not monitored for technical reasons). Secondly, 5-HT has been reported to affect gastric motility (McCann, Herman & Rogers, 1989; Gamse & Buchheit, 1992) and the occurrence of gastro-oesophageal reflux might result in obstructive apnoea (Herbst, Book & Bray, 1978; Herbst *et al.*

1979; Bartlett, 1989). Thirdly, 5-HT is a vigilance-suppressing agent which is known to induce slow-wave sleep and to have an anti-waking effect (Wauquier & Dugovic, 1990). Sleep deepening might facilitate the obstructive apnoea which was reported to occur during sleep with elevated 5-HIAA levels (Cramer *et al.* 1981). Furthermore, our experiments were performed under barbiturate anaesthesia and interactions may have occurred between 5-HT and barbiturate. Besides the central respiratory effects of 5-HT in the observed obstructive apnoea, the possibility cannot therefore be ruled out that other mechanisms may have been involved.

*Obstructive apnoea, enhanced 5-HT biosynthesis and sudden infant death syndrome*

Sudden infant death syndrome (SIDS, for reviews, see Valdes-Dapena, 1982; Kinney, Filiano & Harper, 1992) is the main cause of death in infants up to the age of 18 months in industrialized countries. A very complex aetiology has been reported with suspected disorders in several fields (respiratory, cardiovascular, digestive, vigilance, thermoregulatory, etc.) and this has resulted in the formulation of numerous, so far unconfirmed, hypotheses as to the cause(s) of SIDS. Necropsy, epidemiological studies, and monitoring of newborn infants have met with little success and predicting and preventing SIDS remains impossible.

A broad hypothesis is that SIDS is due to abnormality in cardiopulmonary control mechanisms (Valdes-Dapena, 1982; Kinney *et al.* 1992). Central respiratory disorders might be due to immaturity of the central respiratory network leading to either central or obstructive apnoeas. There have been numerous SIDS studies in the respiratory field, but the findings have often been controversial. For example, in subjects who died from SIDS frequent sleep apnoeas were described (Schechtman, Harper, Wilson & Southall, 1991) but so were normal pneumograms (Rahilly, 1989). Predicting SIDS on the basis of easily monitored respiratory parameters will obviously remain impossible until we can establish exactly what the 'abnormality' consists of, but even criteria as to 'normality' are lacking.

The finding that an activation of 5-HT biosynthesis in the newborn rat elicits drastic, even lethal, obstructive apnoea could be of significance as regards SIDS, since SIDS victims have been reported to show (i) signs of hypoxaemia and intrathoracic petechiae which might result from obstructive apnoeas (Valdes-Dapena, 1982; Kinney *et al.* 1992), (ii) histologically abnormal profiles on central respiratory neurons (Quattrochi, McBride & Yates, 1985; Takashima & Becker, 1985) as well as on hypoglossal motoneurons (Quattrochi *et al.* 1985), and (iii) abnormally high levels of the 5-HT metabolite, 5-HIAA, which suggests that an increase in the 5-HT biosynthesis levels occurs prior to death (Caroff *et al.* 1992). The 5-HT mechanisms are not yet fully mature at birth, and considerable changes in the 5-HT, 5-HIAA (Hamon & Bourgoin, 1982), and $5\text{-HT}_2$ receptor mRNA levels (Roth, Hamblin & Ciaranello, 1991) occur postnatally. The molecular and biochemical processes regulating these developmental changes are still unknown but it could be speculated that any disturbance which might affect 5-HT mechanism maturation at a particular developmental stage, might result in respiratory and other disorders.

To conclude, it is possible that dysfunction of the 5-HT mechanism (i) may occur just before SIDS as suggested by the high 5-HIAA levels observed in SIDS victims (Caroff *et al.* 1992), (ii) may affect the numerous processes modulated by 5-HT

(Osborne, 1982) which almost overlap with the multiple functions thought to be involved in SIDS (Kinney *et al.* 1992) and (iii) may induce drastic respiratory disorders such as those described above. In the future, defining why L-tryptophan elicits obstructive apnoea in some newborn rats but not in others should constitute a major step, but whether the results obtained on newborn rats can be extrapolated to newborn infants remains an open question.

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