Biochemical Pharmacology, Vol. 32, No. 12, pp. 1857–1864, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + .00

ALTERATIONS IN BRAIN 5HT AND TRYPTAMINE CONTENT DURING INDOLEAMINE-INDUCED MYOCLONUS IN GUINEA PIGS

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(*Received* 15 *September* 1982; *accepted* 21 *January* 1983)

Abstract—L-5-Hydroxytryptophan (5HTP) (with or without carbidopa pretreatment), L-tryptophan (plus pargyline pretreatment), or tryptamine (plus pargyline pretreatment) all induced dose-dependent myoclonus in guinea pigs. At the time of maximal behavioural response animals were killed for determination of brain indoleamine content. Administration of 5HTP (50–200 mg/kg) to naive guinea pigs, or of 5HTP (20–80 mg/kg) to carbidopa- (25 mg/kg 1 hr previously) pretreated animals, markedly elevated brain 5-hydroxytryptamine (5HT) concentrations but depressed whole brain tryptamine content. L-Tryptophan (50–200 mg/kg) administration to pargyline- (75 mg/kg 30 min previously) pretreated animals also increased cerebral 5HT levels. L-Tryptophan (200 mg/kg plus pargyline), elevated whole brain tryptamine content. Administration of tryptamine (40 mg/kg) to pargyline-pretreated guinea pigs caused a small increase in brain 5HT levels, but markedly elevated cerebral tryptamine content. 5HT appears to be the indoleamine mainly responsible for 5HTP-induced myoclonus but tryptamine predominates in tryptamine-induced myoclonus. Both 5HT and tryptamine may contribute to myoclonus induced by L-tryptophan.

Myoclonic jerking can be evoked in guinea pigs following the administration of 5-hydroxytryptamine (5HT) precursors and by indole-containing 5HT agonists [1–3]. In the presence of a monoamine oxidase inhibitor both L-tryptophan and tryptamine induce dose-dependent myoclonus. 5-Hydroxytryptophan (5HTP) evokes dose-related jerking in naive guinea pigs and in animals pretreated with a peripheral decarboxylase inhibitor [3]. Although a similar behavioural response was observed following each of these treatments, the syndromes can be differentiated by pharmacological manipulation [4]. The differential inhibition of 5HT-dependent and tryptamine-induced responses by indoleamine receptor antagonists may indicate pharmacologically distinct 5HT and tryptamine receptors [4, 5–7].

The suggestion of a central action for tryptamine was supported by the observation that the indoleamine may play an important role in the behavioural effects of L-tryptophan in rats pretreated with a monoamine oxidase inhibitor [8]. This treatment produces a proportionately greater increase in brain tryptamine than in cerebral 5HT [9]. The identical behaviours induced in rats pretreated with a monoamine oxidase inhibitor either by L-tryptophan or by tryptamine both appeared to correlate to increased brain tryptamine content. However, administration of 5HTP (plus tranylcypromine) produced a similar behavioural response but this was associated with large increases in cerebral 5HT levels but little change in tryptamine content [8].

So in rats, differential involvement of 5HT or tryptamine apparently underlies the similar behaviours induced by 5HTP, L-tryptophan or tryptamine.

We have investigated, therefore, the biochemistry of myoclonus induced in guinea pigs by L-tryptophan, L-5HTP and tryptamine. We find that both tryptamine and 5HT may play some part in the production of myoclonic behaviour dependent on the indoleamine precursor or indoleamine administered.

MATERIALS AND METHODS

Drug administration. Female Dunkin Hartley guinea pigs (250–500 g; Little Lions Farm) were used in all studies. Animals were housed under standard conditions of lighting (12 hr light/dark cycle) and temperature (22–24°) and were allowed free access to food and water.

L-Tryptophan (25–500 mg/kg i.p.; Sigma Chemical Co.) dissolved in warmed normal saline (0.9% sodium chloride solution) acidified with a minimum volume of 6 N hydrochloric acid was administered to naive guinea pigs. L-Tryptophan (50–200 mg/kg i.p.) was given to animals pretreated 30 min previously with pargyline hydrochloride (75 mg/kg i.p.; Abbott Laboratories) dissolved in saline. L-5-Hydroxytryptophan (50–200 mg/kg s.c.; Cambrian Chemicals Ltd.) dissolved in warmed acidified saline was administered to naive animals. 5HTP (20–80 mg/kg s.c.) was administered to guinea pigs pretreated with the peripheral decarboxylase inhibitor carbidopa (α-methyldopahydrazine; 25 mg/kg i.p. 1 hr previously; Merck, Sharpe & Dohme Ltd.) dissolved in 1% methylcellulose (Fisons Ltd.).

Tryptamine hydrochloride (40 mg/kg i.p.; Sigma

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Chemical Co.) dissolved in saline was administered to naive guinea pigs. Animals previously treated with pargyline hydrochloride (75 mg/kg i.p.; 1 hr previously) subsequently received tryptamine hydrochloride (10 or 40 mg/kg i.p.).

Behavioural observations. Behavioural observations were carried out on individual animals housed in opaque plastic boxes (32 × 20 × 17 cm) using the following observer rating system: 0 = no jerking; 1 = very occasional jerking; 2 = head jerking (frequency > very occasional); 3 = whole body jerking (frequency > very occasional < continuous); 4 = continuous rhythmic whole body jerking. Myoclonus was assessed at the time of maximal drug effect immediately prior to sacrifice for biochemical analysis, that is 30 min following tryptamine administration, 60 min following 5HTP administration, 90 min following L-tryptophan adminstration, 120 min following carbidopa administration and 90 or 120 min following administration of pargyline.

Biochemical studies. Regional cerebral concentrations of tryptophan, 5HT and 5-hydroxyindoleacetic acid (5HIAA), and whole brain tryptamine levels were measured following each of a series of pharmacological treatments designed to manipulate cerebral indoleamine function.

Following drug or vehicle administration, guinea pigs were killed by cervical dislocation and decapitation immediately after behavioural observation at the times stated above. The whole brain was rapidly removed and placed on ice. For the determination of regional tryptophan, 5HT and 5HIAA content, a series of brain areas were immediately dissected out. The brain was placed on its dorsal surface and the tuberculum olfactorium and nucleus accumbens (mesolimbic area) and the hypothalamus were removed. The brain was then placed on its ventral surface and a portion of frontal cortex removed. The hemispheres were split and the striatum and hippocampus removed. The cerebellum was removed and the midbrain and pons were obtained by a series of coronal and sagittal cuts through the brainstem.

Regional brain tryptophan concentrations following administration of L-tryptophan to naive or pargyline-pretreated guinea pigs. Regional brain tryptophan levels were determined using a modification [10] of the fluorimetric technique of Denckla and Dewey [11].

Regional brain 5HT and 5HIAA concentrations following administration of indoleamines. Guinea pigs were treated as previously stated and regional 5HT and 5HIAA concentrations were determined by the fluorimetric technique of Curzon and Green [12].

Whole brain tryptamine concentrations following administration of indoleamines. Naive guinea pigs received either L-tryptophan (200 mg/kg i.p.) or 5HTP (200 mg/kg s.c.) or pargyline (75 mg/kg i.p.) or carbidopa (25 mg/kg i.p.) or saline. Some animals pretreated with pargyline (75 mg/kg i.p.) received L-tryptophan (200 mg/kg i.p. 30 min later) or tryptamine (10 or 40 mg/kg i.p. 60 min later). Some guinea pigs previously treated with carbidopa (25 mg/kg i.p. 1 hr previously) subsequently received 5HTP (80 mg/kg s.c.). Following death the whole brain was rapidly removed and placed on ice. The brain was divided in half rostro-caudally and each hemisphere was homogenised in 2.5 ml 0.1 N hydrochloric acid. Tryptamine was extracted according to a method based on those of Martin *et al.* [13] and Marsden and Curzon [14]. The homogenates were washed with 10 ml redistilled diethyl ether and the ether phase discarded. The homogenate was made alkaline by the addition of 0.3 ml 10 N sodium hydroxide and extracted with 10 ml benzene. Following phase separation by centrifugation, 7 ml of the benzene phase was removed and washed twice with 2.5 ml 0.1 N sodium hydroxide. The benzene phase was then back-extracted into 1 ml 0.1 N sulphuric acid. Tryptamine was determined fluorimetrically in 0.6 ml of this extract using the procedure of Hess and Udenfriend [15] as modified by Martin *et al.* [13]. Using this procedure an absolute recovery of 54.5 ± 2.4% was obtained for synthetic tryptamine added to brain tissue. Since all samples were compared with synthetic tryptamine added to brain tissue and run through the above procedure on the same occasion, no allowance for recovery was made. Some experiments involved the administration of L-tryptophan or L-5-hydroxytryptophan, the specificity of the method therefore was routinely assessed by incorporation of 100 μg of each compound into brain homogenates. Neither compound produced appreciable fluorescence in the final extracts or interfered with the fluorescence of either endogenous or added synthetic tryptamine.

The excitation maxima at 367 nm and the emission maxima at 430 nm for both endogenous tryptamine in guinea pig brain and for synthetic tryptamine agreed with previous reports. However, it was impossible to estimate the tryptamine content of samples accurately using a fixed emission wavelength. The inflection point between the excitation wavelength maxima and the emission maxima for tryptamine occurred at varying fluorescence intensities, causing an apparent wide variation in tryptamine values at a fixed emission wavelength. The emission spectra for each sample therefore was obtained, and the difference between the fluorescence intensity and the maxima for tryptamine (430 nm) and the minima (380–393 nm) occurring between this peak and the excitation wavelength maxima (367 nm) was used to calculate the tryptamine content of the samples. Using this approach a linear increase in fluorescence intensity was obtained in the range 0.02–2.0 μg tryptamine.

Statistical analysis. Biochemical data were analysed by a two-tailed Student's *t*-test. Control levels of 5HT and 5HIAA were derived from three individual experiments (cumulative $n = 12$). Regional tryptophan and whole brain tryptamine levels were each determined in four saline-treated guinea pigs. The behavioural and biochemical effects of all drug treatments were each assessed in four individual guinea pigs.

RESULTS

The effect of drug treatments on cerebellar and pontine tryptophan, 5HT and 5HIAA is shown, unless otherwise indicated in the text, as representative of their effect on all brain regions. Basal

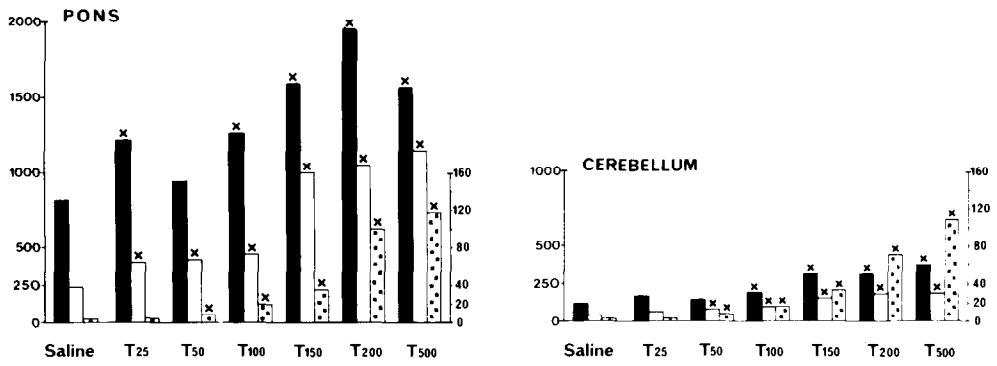

Fig. 1. Effect of L-tryptophan (25–500 mg/kg i.p.; 90 min previously) on levels of tryptophan, 5HT and 5HIAA in the pons and cerebellum. Left-hand axes—5HT (■) and 5HIAA (□) (ng/g); right-hand axes—tryptophan (⊡) (μg/g). T25—L-tryptophan (25 mg/kg), etc. X: $P < 0.05$ vs saline (Student's *t*-test—two-tailed). $n = 4$ guinea pigs for all determinations. S.E.M.s did not exceed 16% (5HT), 35% (5HIAA) or 48% (tryptophan).

regional 5HT and 5HIAA levels are displayed in Fig. 5.

Regional brain tryptophan concentrations following administration of L-tryptophan to naive or pargyline-pretreated guinea pigs (Figs. 1 and 2)

L-Tryptophan (25–500 mg/kg i.p. 90 min previously) did not induce myoclonus in naive guinea pigs (Fig. 3). L-Tryptophan (25 mg/kg) did not alter brain tryptophan concentrations but larger doses (50–500 mg/kg) produced a dose-related elevation in cerebral tryptophan concentrations (Fig. 1). The effect was similar in most brain regions although the increase was less pronounced in the striatum and mesolimbic area, and tryptophan concentrations in the hypothalamus were elevated only after administration of the highest doses of L-tryptophan (200 and 500 mg/kg). The maximal elevation of brain tryptophan concentrations caused by L-tryptophan (500 mg/kg) increased tryptophan levels approximately 30-fold in all areas except the striatum (24-fold), mesolimbic area (14-fold) and hypothalamus (11-fold).

Administration of L-tryptophan (50–200 mg/kg i.p.) to pargyline- (75 mg/kg i.p. 30 min previously) pretreated guinea pigs induced dose-dependent myoclonus (Fig. 3). Pargyline (75 mg/kg 2 hr previously) did not modify cerebral tryptophan concentrations, and administration of L-tryptophan (50 mg/kg) to animals pretreated with pargyline (75 mg/kg 30 min previously) also was without effect (Fig. 2). Administration of larger doses of L-tryptophan (100–200 mg/kg) to pargyline- (75 mg/kg 30 min previously) pretreated animals, however, produced a dose-dependent increase in tryptophan levels in all brain areas compared to animals receiving pargyline (75 mg/kg) alone (Fig. 2). The elevation was uniform in all brain regions except the mesolimbic area and hypothalamus where the increases were again less pronounced. Thus, compared to the effect of pargyline (75 mg/kg) alone, L-tryptophan (200 mg/kg plus pargyline 75 mg/kg 30 min pre-

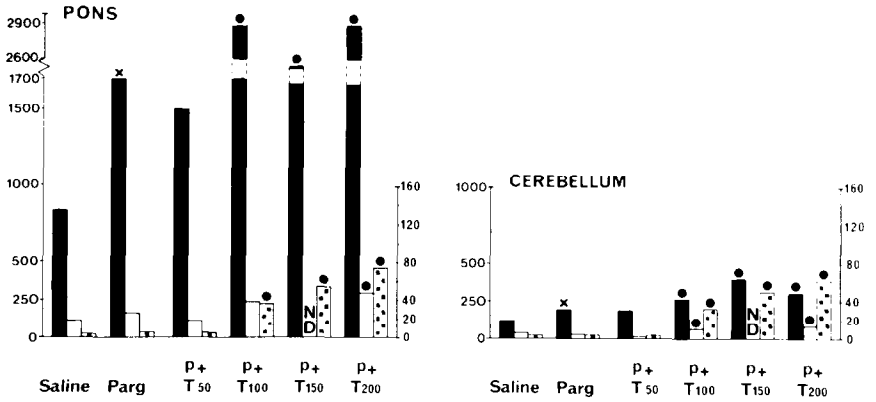

Fig. 2. Effect of L-tryptophan (50–200 mg/kg i.p.; 90 min prior to death) plus pargyline (75 mg/kg i.p.; 30 min previously), and pargyline (75 mg/kg; 120 min prior to death) alone on levels of tryptophan, 5HT and 5HIAA in the pons and cerebellum. Left-hand axes—5HT (■) and 5HIAA (□) (ng/g); right-hand axes—tryptophan (⊡) (μg/g). Parg—pargyline (p); T50—L-tryptophan (50 mg/kg), etc; ND—not determined. X: $P < 0.05$ vs saline. ●: $P < 0.05$ vs pargyline (Student's *t*-test—two-tailed). $n = 4$ guinea pigs for all determinations. S.E.M.s did not exceed 26% (5HT), 56% (5HIAA) or 31% (tryptophan).

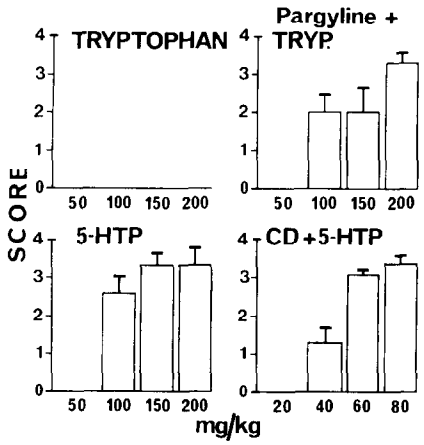

Fig. 3. Effect of L-tryptophan (50–200 mg/kg i.p.), L-tryptophan (50–200 mg/kg) plus pargyline (75 mg/kg i.p. 30 min previously), L-5HTP (50–200 mg/kg s.c.) and L-5HTP (20–80 mg/kg) plus carbidopa (25 mg/kg i.p. 60 min previously) on guinea pig behaviour. CD—carbidopa; 5-HTP = L-5-hydroxytryptophan. Myoclonus was assessed by a 0–4 observer rating system (see Materials and Methods) immediately prior to sacrifice for biochemical assay at the times stated in Materials and Methods. $n = 4$ guinea pigs for all treatments.

viously) elevated regional tryptophan levels by approximately 16-fold in all brain areas except the mesolimbic area (6-fold) and hypothalamus (7-fold).

Regional brain 5HT and 5HIAA concentrations following administration of indoleamine precursors or tryptamine (Figs. 1, 2, 4 and 5)

L-Tryptophan (25–500 mg/kg i.p. 90 min previously) administration to naive guinea pigs did not induce myoclonus (Fig. 3) but produced a dose-related elevation of 5HT and 5HIAA in all brain regions (Fig. 1). A maximum 2-fold increase in 5HT levels was observed in most brain areas although the elevation was greater in the cerebellum and less marked in the frontal cortex.

Pargyline (75 mg/kg i.p. 2 hr previously) did not evoke myoclonic jerking but increased regional 5HT levels by an average of approximately 60% and decreased 5HIAA concentrations in naive animals.

Pargyline (75 mg/kg i.p. 30 min previously) plus L-tryptophan (50–200 mg/kg i.p. 90 min prior to death) evoked dose dependent myoclonus (Fig. 3). Administration of L-tryptophan (50 mg/kg) to pargyline- (75 mg/kg 30 min previously) pretreated guinea pigs did not change cerebral 5HT concentrations compared to pargyline (75 mg/kg) alone but lowered 5HIAA levels, notably in the hippocampus and hypothalamus. The elevation of 5HT levels by L-tryptophan (100, 150 and 200 mg/kg plus pargyline 75 mg/kg 30 min previously) was similar at each dose (Fig. 2). The increase in regional 5HT concentrations usually was less than 2-fold compared to the effect of pargyline (75 mg/kg) alone and was less than 50% in mesolimbic and cortical tissue. In pargyline- (75 mg/kg 30 min previously) pretreated animals L-tryptophan (100 mg/kg) markedly elevated 5HIAA levels only in the striatum and cerebellum compared to pargyline (75 mg/kg) alone, but an increase in metabolite content of all regions was observed following L-tryptophan (200 mg/kg) administration. Thus the increase in intensity of myoclonus due to elevating the dose of L-tryptophan from 100 to 200 mg/kg in pargyline- (75 mg/kg 30 min previously) pretreated guinea pigs was associated with increased brain tryptophan and 5HIAA levels and unchanged cerebral 5HT concentrations (Figs. 2 and 3).

5HTP (50–200 mg/kg s.c. 60 min previously) evoked dose-dependent myoclonus in naive guinea pigs (Fig. 3). These treatments also induced a dose-dependent increase of 5HT and 5HIAA levels in all brain regions compared to administration of saline ('control'; Fig. 4). A uniform elevation of 5HT content was observed in most brain areas, typically 5 to 10-fold following 5HTP (200 mg/kg) but 15-fold in the striatum and 30-fold in the cerebellum.

Carbidopa (25 mg/kg i.p. 2 hr previously) uniformly reduced the 5HT content of cerebral tissues by approximately 45%; 5HIAA levels were elevated only in the cerebellum (Fig. 4).

5HTP (20–80 mg/kg s.c. 60 min prior to death) administration to carbidopa- (25 mg/kg i.p. 1 hr previously) pretreated animals induced dose-related myoclonus (Fig. 3) and a dose-dependent elevation of 5HT and 5HIAA concentrations in all brain areas (Fig. 4). The largest doses of 5HTP (60 and 80 mg/kg) had a similar effect on behaviour (Fig. 3) and

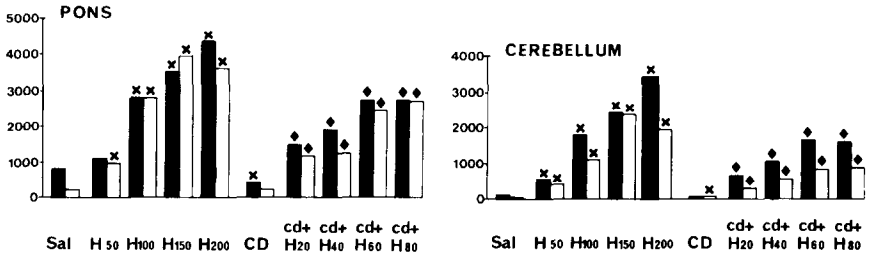

Fig. 4. Effect of L-5HTP (50–200 mg/kg s.c. 60 min prior to death), carbidopa (25 mg/kg i.p. 120 min prior to death) and L-5HTP (20–80 mg/kg s.c. 60 min prior to death) plus carbidopa (25 mg/kg 60 min previously) on levels of 5HT and 5HIAA in the pons and cerebellum. Left-hand axis—5HT (■) and 5HIAA (□) (ng/g). Sal—saline; CD—carbidopa (cd); H—L-5-hydroxytryptophan (5HTP). X: $P < 0.05$ vs saline. ◆: $P < 0.05$ vs carbidopa (Student's t-test—two-tailed). $n = 4$ guinea pigs for all determinations. 5HTP (50–200 mg/kg): S.E.M.s did not exceed 14% (5HT) or 21% (5HIAA). 5HTP (20–80 mg/kg) plus carbidopa: S.E.M.s did not exceed 13% (5HT) or 24% (5HIAA).

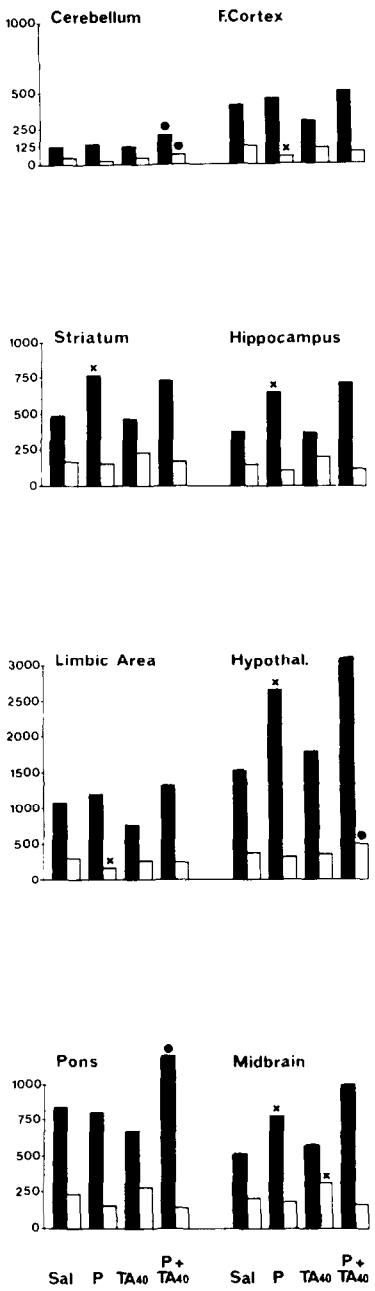

Fig. 5. Effect of tryptamine (40 mg/kg i.p. 30 min prior to death), pargyline (75 mg/kg i.p. 90 min prior to death) and tryptamine (40 mg/kg 30 min prior to death) plus pargyline (75 mg/kg 60 min previously). Left-hand axis—5HT (■) and 5HIAA (□) (ng/g). N.B. Histograms for the hypothalamus and limbic area are displayed at half-scale. Sal—saline; P—pargyline; TA—tryptamine; F. cortex—frontal cortex; Hypothal.—hypothalamus. X: $P < 0.05$ vs saline. ●: $P < 0.05$ vs pargyline (Student's t-test—two-tailed). $n = 4$ guinea pigs for all determinations. S.E.M.s did not exceed 14% (5HT) or 31% (5HIAA).

cerebral amine and metabolite levels (Fig. 4), increasing 5HT levels by 6 to 12-fold in all areas except the striatum and cerebellum where a 20-fold elevation was observed compared to animals receiving carbidopa alone.

Tryptamine (40 mg/kg i.p. 30 min previously) administration to naive guinea pigs did not induce myoclonus or change cerebral 5HT levels, and 5HIAA levels were increased only in mid-brain (Fig. 5).

Pargyline (75 mg/kg i.p. 90 min previously) elevated 5HT levels in midbrain, hippocampus, striatum and hypothalamus and decreased the 5HIAA content of mesolimbic and cortical tissue (Fig. 5). This effect of pargyline (75 mg/kg) on amine and metabolite levels was less pronounced than reported above (Fig. 2); probably this was due to the different times of death—90 min after drug administration in this experiment, but 120 min following pargyline administration in the previous experiment.

Tryptamine (40 mg/kg i.p. 30 min prior to death) administration to pargyline- (75 mg/kg i.p. 1 hr previously) pretreated guinea pigs produced marked myoclonus (mean score 2.3 ± 0.8). This treatment elevated 5HT levels markedly (50%) only in the pons and cerebellum, and increased the 5HIAA content only of cerebellar and hypothalamic tissue compared to pargyline (75 mg/kg) alone (Fig. 5).

Whole brain tryptamine levels following administration of indoleamine precursors or tryptamine (Table 1)

The tryptamine content of whole brain was elevated by administration of pargyline (75 mg/kg i.p.) or L-tryptophan (200 mg/kg i.p.) to naive guinea pigs, but myoclonus was not evoked. The jerking induced by administration of L-tryptophan (200 mg/kg) to pargyline- (75 mg/kg 30 min previously) pretreated guinea pigs, however, was accompanied by an elevation in whole brain tryptamine content which was less than that produced by the amino acid alone (Table 1).

Tryptamine (10 or 40 mg/kg i.p.) caused a dose-dependent elevation of whole brain tryptamine levels in guinea pigs previously treated with pargyline (75 mg/kg 1 hr previously) but myoclonus was observed only following the larger dose of tryptamine (Table 1).

5HTP (200 mg/kg s.c.) administration to naive guinea pigs induced marked myoclonus but decreased whole brain tryptamine levels, with the amine being undetectable in one animal. Administration of 5HTP (80 mg/kg) to carbidopa- (25 mg/kg 1 hr previously) pretreated animals also induced marked myoclonus and reduced the tryptamine content of whole brain compared to the effect of carbidopa (25 mg/kg) alone (Table 1).

DISCUSSION

The adminstration of either L-tryptophan or tryptamine to pargyline-pretreated guinea pigs, or of 5HTP to naive or carbidopa-pretreated animals, induced a similar behavioural response, but different biochemical changes (see Table 2).

Myoclonus induced in guinea pigs by 5HTP was

Table 1. Effect of indoleamines (L-tryptophan, 5HTP and tryptamine), pargyline and carbidopa on whole brain tryptamine content and on guinea pig behaviour.

Treatment	Whole brain tryptamine [ng/g mean ± S.E.M.]	Myoclonus score (mean ± S.E.M.)
Saline	29 ± 6	0 ± 0
L-Tryptophan (200 mg/kg)	279 ± 14*	0 ± 0
Pargyline (75 mg/kg)	72 ± 4*	0 ± 0
L-Tryptophan (200 mg/kg) + pargyline (75 mg/kg)	175 ± 29**	2.7 ± 0.3
Tryptamine (10 mg/kg) + pargyline (75 mg/kg)	354 ± 35**	0 ± 0
Tryptamine (40 mg/kg) + pargyline (75 mg/kg)	2798 ± 345**	3.3 ± 0.5
5HTP (200 mg/kg)	9 ± 4*	3.3 ± 0.5
Carbidopa (25 mg/kg)	47 ± 8	0 ± 0
5HTP (80 mg/kg)	21 ± 3***	3.5 ± 0.3

* $P < 0.05$ vs saline.
** $P < 0.05$ vs pargyline.
*** $P < 0.05$ vs carbidopa.
Biochemical data analysed by Student's t-test—two-tailed. $n = 4$ guinea pigs for all determinations. Animals killed at times indicated in Materials and Methods.

related to an increase in brain 5HT levels with no change in cerebral tryptamine content (Table 2). Dose-related jerking evoked by 5HTP (50–200 mg/kg) administration to naive guinea pigs was associated with a dose-dependent increase in brain 5HT concentrations, but 5HTP (200 mg/kg) caused no change or even a reduction in brain tryptamine content. Similarly, 5HTP (20–80 mg/kg) in conjunction with carbidopa induced dose-dependent myoclonus and caused a dose-related elevation of cerebral 5HT levels, but no change in tryptamine content.

However, there was no absolute relationship between cerebral 5HT content and the degree of myoclonus induced by 5HTP. The additional rise in brain 5HT content caused by increasing the dose of 5HTP from 100 to 200 mg/kg was accompanied by only a small increment in the intensity of myoclonus. This may indicate that a plateau had been reached such that extra availability of 5HT was unable to enhance jerking further. Administration of 5HTP (200 mg/kg) to naive guinea pigs, or of 5HTP (80 mg/kg) to carbidopa-pretreated animals, evoked a similar intensity of myoclonus although brain 5HT levels were considerably greater following the former treatment.

Klawans *et al.* [1] reported that the myoclonus induced in guinea pigs by 5HTP correlated with the time course of elevation of whole brain 5HT levels. There also was a relationship between brain 5HT content and observed behaviour following 5HTP

Table 2. Summary of the effect of L-tryptophan, 5HTP and tryptamine on guinea pig behaviour and on cerebral levels of 5HT and tryptamine

Drug treatment	Effect on brain 5HT levels	Effect on brain tryptamine content	Effect on behaviour
L-tryptophan (200 mg/kg)	+/++	++	0
Pargyline (75 mg/kg)	+	+	0
Pargyline (75 mg/kg) + L-tryptophan (200 mg/kg)	++	+/++	++/+++
5HTP (200 mg/kg)	+++	—	+++
Carbidopa (25 mg/kg)	—	+	0
Carbidopa (25 mg/kg) + 5HTP (80 mg/kg)	+++	0	+++
Tryptamine (40 mg/kg)	0	N.D.	0
Pargyline (75 mg/kg) + tryptamine (10 mg/kg)	N.D.	++	0
Pargyline (75 mg/kg) + tryptamine (40 mg/kg)	+	+++	++/+++

—, Decrease; 0, no effect; +, weak increase; ++, moderate increase; +++, marked increase; N.D., not determined.
Behaviour was assessed at the times stated (see Materials and Methods) immediately prior to sacrifice for biochemical measurements.

administration in tranylcypromine-pretreated rats [8]. The 5HTP-induced head twitch in the mouse parallels changes in brainstem 5HT levels but not alterations in whole brain 5HT concentrations [16]. Brain tryptamine levels were not determined in these experiments.

5-HTP treatment induces 'wet-dog' shakes in rats [17], a behavioural response possibly analogous to 5HTP-induced myoclonus in guinea pigs. Transection studies indicate that both myoclonus and 'wet-dog' shakes originate in the brainstem [2, 17]. However, investigation of regional biochemical changes induced by 5HTP has not demonstrated any pronounced local elevation of cerebral 5HT levels ([17]; this study).

The general elevation of regional 5HT concentrations by 5HTP administration reflects the widespread distribution of aromatic L-amino acid decarboxylase in rodent brain. Tryptophan hydroxylase, however, is associated only with 5HT neurones. Treatment with L-tryptophan might have caused a more revealing picture of changes in regional 5HT biochemistry. Administration of L-tryptophan to naive guinea pigs or to pargyline-pretreated animals, however, caused a uniform elevation of 5HT levels in all areas except the frontal cortex where the increase was less marked.

L-Tryptophan (100, 150 and 200 mg/kg) administration to pargyline-pretreated guinea pigs produced a dose dependent increase in brain tryptophan content but cerebral 5HT levels were equally elevated by all three doses. Myoclonus, however, was more marked following L-tryptophan (200 mg/kg) than after lower doses (100 and 150 mg/kg), despite brain 5HT levels following pargyline plus L-tryptophan (200 mg/kg) being lower than after carbidopa plus 5HTP (40 mg/kg), a treatment which evoked myoclonus only very occasionally. This may suggest involvement of another active substance in the production of myoclonus but, although pargyline plus L-tryptophan (200 mg/kg) elevated brain tryptamine 7-fold, this was less than L-tryptophan (200 mg/kg) alone which was behaviourally inactive. The mechanism of pargyline plus L-tryptophan-induced myoclonus thus is difficult to explain, although Hess *et al.* [18] noted that pretreatment with iproniazid also attenuated the L-tryptophan-induced increase in tryptamine content of guinea pig brain.

Increasing the dose of L-tryptophan administered to tranylcypromine-pretreated rats from 50 to 100 mg/kg caused a striking increase in behaviour associated with a considerable elevation of brain tryptamine content, but only a small rise in cerebral 5HT levels [14]. This suggestion of an association between the increase in cerebral tryptamine content and the enhanced behavioural score, however, seems incompatible with the present data unless the modest increase in guinea pig brain tryptamine content caused by pargyline plus L-tryptophan (200 mg/kg) acts to release 5HT, whose levels also are elevated, or to block 5HT re-uptake.

The behavioural effect in pargyline-pretreated guinea pigs of tryptamine (10 or 40 mg/kg) administration appears related to cerebral tryptamine levels. Thus myoclonus induced by pargyline plus tryptamine (40 mg/kg) was associated with a 100-fold elevation in cerebral tryptamine content; the brain 5HT levels of these animals were less than those measured after two behaviourally inactive treatments, 5HTP (50 mg/kg) and carbidopa plus 5HTP (20 mg/kg). Even so, the role of tryptamine in indoleamine-induced myoclonus is still unclear because pargyline plus tryptamine (10 mg/kg), which does not evoke myoclonus, induced a far larger increase in brain tryptamine levels than the behaviourally active combinations of pargyline plus L-tryptophan (200 mg/kg) and carbidopa plus 5HTP (80 mg/kg). Tryptamine (1–5 mg/kg) plus tranylcypromine produced a dose-related behavioural syndrome in rats which also has been associated with a dose-dependent increase in brain tryptamine content while cerebral 5HT levels are unchanged [14].

In summary, 5HTP-induced myoclonus in naive and carbidopa-pretreated guinea pigs appears to be due to stimulation of 5HT systems. Myoclonus evoked by low doses of L-tryptophan (plus pargyline) apparently is related to direct activation of 5HT mechanisms. At higher L-tryptophan doses, however, tryptamine may be directly or indirectly involved by, for example, acting itself on a postsynaptic indoleamine receptor, or facilitating 5HT release and/or blocking 5HT re-uptake. The production of myoclonus by pargyline plus tryptamine (40 mg/kg) appears predominantly due to an action of tryptamine, possibly at a specific tryptamine receptor. The differential inhibition of 5HTP- and tryptamine-induced myoclonus in guinea pigs by 5HT antagonists may indicate pharmacologically distinct 5HT and tryptamine receptors [4].

Acknowledgements—The study was supported by the Medical Research Council and the Research Funds of King's College Hospital and the Bethlem Royal and Maudsley Hospitals. G.L. is a SRC CASE award student, held in conjunction with Organon Laboratories Ltd.

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