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MYOCLONUS IN GUINEA PIGS IS INDUCED BY INDOLE-CONTAINING BUT NOT PIPERAZINE-CONTAINING 5HT AGONISTS

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Summary

L-5-Hydroxytryptophan (5HTP) induces in guinea pigs a myoclonic jerking which is dependent upon stimulation of brainstem 5-hydroxytryptamine (5HT) receptors. We have investigated the ability of 5HT precursors and a range of synthetic 5HT agonists to produce myoclonus. The 5HT precursors and 5HT agonists containing an indole nucleus induced dose-dependent jerking in guinea pigs. In contrast, 5HT agonists possessing a piperazine moiety induced occasional jerking only at toxic doses, but not at those doses normally associated with 5HT agonist activity. The difference in activity between the indole-containing compounds and piperazine-containing 5HT agonists suggests that myoclonus is due to activation of an indole-selective brainstem 5HT receptor and provides further evidence for multiple cerebral 5HT receptors.

Myoclonus induced by the peripheral administration of L-5-hydroxytryptophan (5HTP) to guinea pigs appears due to stimulation of brainstem 5HT receptors (1,2). This myoclonic syndrome in guinea pigs is particularly useful since 5HTP administration induces a single easily assessed behaviour, whereas in other rodent species 5HTP causes a range of behavioural changes (3,4). In addition, the syndrome is not induced by agonists of other central neurotransmitter systems, nor is it prevented by receptor antagonists other than those altering 5HT function (1,2). The model has been used to investigate the efficacy of a wide range of 5HT-active compounds, but a number of discrepancies in activity have been reported compared to the known 5HT properties of the drugs examined. Thus, the ability of cerebral 5HT antagonists to inhibit 5HTP-induced myoclonus, and the ability of potent and specific cerebral 5HT re-uptake blockers to potentiate the effects of a threshold dose of 5HTP does not correspond to their known potency on other cerebral 5HT systems (5,6). The most striking observation has been the suggestion from preliminary studies that established 5HT agonists containing a piperazine grouping (for example quipazine and MK 212) rarely produce jerking in guinea pigs, while 5HT agonists possessing an indole nucleus (for example d-LSD and 5-methoxy-N,N-dimethyltryptamine) induce dose-dependent myoclonus (5,6).

We have now investigated a large range of structural analogues of each of the groups of 5HT agonists and report that an indole nucleus appears to be a structural prerequisite for a 5HT agonist to induce myoclonus in guinea pigs.

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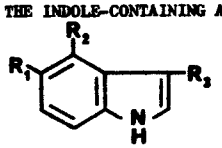
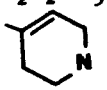
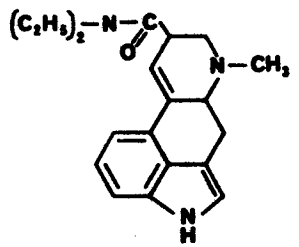
TABLE I

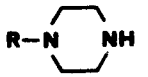
STRUCTURAL FORMULAE OF THE INDOLE-CONTAINING AND PIPERAZINE-CONTAINING COMPOUNDS EMPLOYED

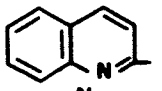
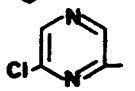
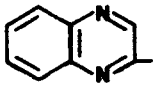
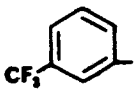
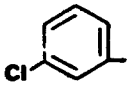
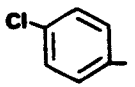


Name	R_1	R_2	R_3
L-tryptophan	H	H	$CH_2CH(NH_2)COOH$
L-5HTP	OH	H	$CH_2CH(NH_2)COOH$
5HT	OH	H	$CH_2CH_2NH_2$
Tryptamine	H	H	$CH_2CH_2NH_2$
N,N-dimethyltryptamine	H	H	$CH_2CH_2N(CH_3)_2$
5-methoxy-N,N-dimethyl-tryptamine	CH_3O	H	$CH_2CH_2N(CH_3)_2$
Bufotenine	OH	H	$CH_2CH_2N(CH_3)_2$
Psilocin	H	OH	$CH_2CH_2N(CH_3)_2$
Psilocybin	H	H_2PO_4	$CH_2CH_2N(CH_3)_2$
BU 24969	CH_3O	H	



d-LSD



Name	R
Quipazine	
MK 212	
2-(1-piperazinyl) quinoxaline	
1-(m-trifluoromethyl-phenyl)piperazine	
m-chlorophenyl-piperazine	
p-chlorophenyl-piperazine	
Phenylpiperazine	

Methods

Female Duncan Hartley guinea pigs (250-500 g; Little Lions Farm) housed under standard conditions of light, temperature and nutrition, were used in all experiments. Behavioural observation was carried out on individual animals in opaque plastic boxes (32 x 20 x 17 cm) at appropriate intervals following drug administration. A 0-4 observer rating system reflecting both the frequency and intensity of jerking was used to assess myoclonic behaviour.

The rating system used was as follows: 0 = no jerking; 1 = very occasional jerking; 2 = head jerking (frequency >very occasional); 3 = whole body jerking (frequency >very occasional <continuous); 3.5 = almost continuous whole body jerking; 4 = continuous rhythmic whole body jerking. In addition, animals were observed for the occurrence of seizure activity.

Each 5HT precursor or agonist (Table 1) was examined at 5 or more doses in at least 4 animals. All drugs and vehicles were given intraperitoneally, except L-tryptophan and L-5HTP which were administered subcutaneously. Drugs other than L-5HTP, L-tryptophan and tryptamine were administered to naive guinea pigs. The monoamine oxidase inhibitor pargyline hydrochloride (75 mg/kg ip, Sigma Chemical Co.) dissolved in saline was administered 1 h prior to L-tryptophan and tryptamine). The peripheral decarboxylase inhibitor carbidopa (25 mg/kg ip α-methyldopahydrazine; Merck, Sharpe & Dohme Ltd.) dissolved in 1% methyl cellulose, and was given 1 h before L-5HTP (5-hydroxytryptophan). L-tryptophan (Sigma Chemical Co.) and L-5HTP (Cambrian Chemicals or Sigma Chemical Co.) were dissolved in warmed saline minimally acidified with 12 N hydrochloric acid. 5-Hydroxytryptamine creatinine phosphate (5HT), tryptamine hydrochloride, bufotenine monooxalate hydrate (all Sigma Chemical Co.), psilocybin and d-lysergic acid diethylamide tartrate (d-LSD; both Sandoz Products Ltd.) and RU 24969 (5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole, succinate; Roussel-UCLAF) all were dissolved in saline. N,N-dimethyltryptamine, 5-methoxy N,N-dimethyltryptamine (both Sigma Chemical Co.) and psilocin (Sandoz Products Ltd.) were dissolved in acidified saline buffered to pH 6.5 with 2 N sodium hydroxide. At least 6 animals were tested at each of 6 or more doses of an indole-containing 5HT agonist. Quipazine maleate (2-(1-piperazinyl)quinoline maleate; Miles Laboratories), 2-(1'-piperazinyl)quinoxaline hydrochloride (Merck, Sharpe & Dohme Ltd.) were dissolved in saline, as were m-chlorophenylpiperazine dihydrochloride and p-chlorophenylpiperazine dihydrochloride (both Aldrich Chemical Co.) which were then neutralised with 2N sodium hydroxide. N-phenylpiperazine and 1-(m-trifluomethylphenyl)piperazine (both Aldrich Chemical Co.) were neutralised with dilute hydrochloride acid to produce an aqueous solution (pH 7.0). 6-chloro-2-(1-piperazinyl)pyrazine (MK 212: Merck, Sharpe and Dohme Ltd.) was dissolved in 1% methyl cellulose. At least 4 animals were tested at each of 5 or more doses of a piperazine-containing 5HT agonist.

Results

The results are summarised in Tables 2 and 3. The 5HT precursor L-tryptophan (25-200 mg/kg in pargyline 75 mg/kg pretreated animals) evoked dose-dependent myoclonus. Administration of 5HTP (20-200 mg/kg) to naive animals or 5HTP (5-120 mg/kg) to carbidopa-pretreated guinea pigs also evoked dose-dependent myoclonus. However, 5HT (1-36 mg/kg) evoked no myoclonus.

Tryptamine (6-160 mg/kg) induced dose-dependent myoclonus in pargyline pretreated animals. All other indole containing compounds (5-methoxy-N,N-dimethyltryptamine 2.5-20 mg/kg; N,N-dimethyltryptamine 20-160 mg/kg; bufotenine 80-160 mg/kg; psilocin 10-160 mg/kg; psilocybin 10-80 mg/kg; RU 24969 40-120 mg/kg and d-LSD 0.25-8.0 mg/kg) evoked dose-related myoclonus in naive guinea pigs.

TABLE 2

THE ABILITY OF 5HT PRECURSORS AND INDOLE-CONTAINING 5HT AGONISTS TO INDUCE MYOCLONIC JERKING IN GUINEA PIGS

Name	Doses tested (mg/kg)	Threshold myoclonic dose (mg/kg)	Maximum myoclonus score (± S.E.M. (dose, mg/kg))	Mean duration of myoclonus[1] (min)
L-tryptophan (plus pargyline)	25-200	25	2.9 ± 0.3 (150)	360
L-5HTP	5-200	20	3.8 ± 0.2 (200)	215
L-5HTP (plus carbidopa)	1-120	5	3.9 ± 0.1 (75)	360
5HT	1-36	>36	0	-
Tryptamine (plus pargyline)	1-160	6	3.6 ± 0.2 (80)	95
N,N-dimethyltryptamine	1-160	20	3.4 ± 0.2 (160)	110
5-methoxy-N,N-dimethyl-tryptamine	0.1-20	2.5	3.8 ± 0.2 (20)	30
Bufotenine	10-160	80	1.9 ± 0.6 (160)	25
Psilocin	2.5-160	10	3.0 ± 0.0 (160)	85
Psilocybin	1-80	10	2.5 ± 0.5 (80)	50
MU 24969	1-120	40	3.0 ± 0.0 (80)	270
d-LSD	0.1-8.0	0.25	3.9 ± 0.1 (8.0)	40

1 The mean duration of myoclonus was that observed for the dose of each compound causing the maximum myoclonus score shown

In marked contrast, the piperazine-containing 5HT agonists (quipazine 1.0-200 mg/kg; MK 212 1-80 mg/kg; 2-(1'-piperazinyl)quinoxaline 1-40 mg/kg; m- or p-chlorophenylpiperazine 1-160 mg/kg; N-phenylpiperazine 1-160 mg/kg; and 1-(m-trifluoromethylphenyl)piperazine 1-160 mg/kg) evoked only occasional myoclonus at toxic doses which often caused seizures sometimes resulting in death. Myoclonus was very rarely observed following doses effective in producing other behavioural and biochemical changes indicative of 5HT agonist properties (7-10). Pretreatment of animals with pargyline did not enhance the intensity of myoclonus or, indeed, induce myoclonus on subsequent administration of piperazine compounds (6) (and unpublished observations).

Discussion

The ability of 5HT precursors (in naive animals or in the presence of inhibition of peripheral decarboxylase activity or inhibition of monoamine oxidase activity), but not 5HT, to induce myoclonus confirms the central origin of this behaviour (2). The involvement of 5HT mechanisms,as previously demonstrated, is shown by the ability of a range of indole-containing 5HT agonists to induce the same behavioural response. None of the indole-containing compounds used can be considered as pure 5HT agonists. However, the only other common action with which they might be attributed is dopamine agonist activity. Previous study has demonstrated that dopamine agonists may inhibit 5HTP-induced myoclonus, but not induce the behaviour (11,12), so it would seem unlikely that such an action would explain the effects observed.

The ineffectiveness of the piperazine-containing 5HT agonists is surprising since all the compounds examined are known to be active on cerebral 5HT mechanisms. It might be argued that the piperazine-containing 5HT agonists did not penetrate into brain or that the doses used were inadequate to stimulate cerebral 5HT systems. However, the doses of quipazine, MK 212, 1-(m-trifluoromethylphenylpiperazine and m- or p-chlorophenylpiperazine employed are known to produce behavioural (for example head twitching in rodents (8-10) and biochemical effects (7-10) consistent with 5HT receptor stimulation. Cerebral penetration in guinea-pigs also is suggested by the capacity of quipazine, MK 212 and 1-(m-trifluoromethylphenyl)piperazine to potentiate jerking evoked by a threshold dose of 5HTP (6). This presumably reflects the 5HT releasing and re-uptake blocking properties also possessed by these compounds (13,14), but also indicates the piperazine-containing 5HT agonists administered to guinea pigs can exert central actions, as observed in other species. The direct *in vivo* interaction of the piperazine-containing compounds with cerebral 5HT receptors in the guinea-pig has not been studied. However, since *in vitro* these compounds can displace ^{3}H-5HT from rat brain membranes, there is little doubt they possess direct 5HT receptor activity (10,15). Therefore, as the piperazine-containing compounds appear to penetrate into guinea-pig brain, there is no reason why they should not interact with 5HT receptors sensitive to their actions if these were available.

Dopamine agonist actions of the piperazine-containing compounds might counteract the ability to induce myoclonus through 5HT agonist activity. Indeed, quipazine may possess direct (16) or indirect (17) agonist actions on cerebral dopamine systems. However, little evidence exists to support such properties in the other piperazine-containing compounds which were equally ineffective in inducing myoclonus. Thus, quipazine, MK 212 and 1-(m-trifluoromethylphenyl)piperazine show little ability *in vitro* to displace ^{3}H-N,n-propylnorapomorphine or ^{3}H-spiperone from their specific binding sites to rat striatal preparations (unpublished observations). The indole-containing 5HT agonists N,N-dimethyltryptamine and d-LSD also possess some dopamine agonist activity, but this did not prevent the induction of myoclonus. Indeed, if dopamine agonist actions were the reason for the failure of the piperazine-

TABLE 3

THE ABILITY OF PIPERAZINE-CONTAINING 5HT AGONISTS TO INDUCE MYOCLONIC JERKING IN GUINEA PIGS

Name	Doses tested (mg/kg)	Threshold myoclonic dose (mg/kg)	Maximum myoclonus score (± S.E.M. (dose, mg/kg))	Mean duration of myoclonus (min) [1]	Threshold seizure dose (mg/kg)
Quipazine	1-200	30	2.3 ± 0.5 (160)	10	80
MK 212	1-80	40	2.0 ± 0.0 (80)	1	20
2-(1-piperazinyl) quinoxaline	1-40	5	0.6 ± 0.3 (10)	10	20
1-(m-trifluoromethyl)-phenyl)piperazine	1-160	40	2.0 ± 1.0 (120)	55	80
m-chlorophenylpiperazine	1-160	80	1.7 ± 0.5 (160)	5	160
p-chlorophenylpiperazine	1-160	>160	0	-	160
Phenylpiperazine	1-160	>160	0	-	40

1. The mean duration of myoclonus was that observed for the dose of each compound causing the maximum myoclonus score shown.

containing compounds to induce myoclonus, they would have been expected to inhibit rather than enhance the effects of a threshold dose of 5HTP (6).

Much interest recently has centred on the possible existence of multiple 5HT receptors in brain. In guinea pig hippocampal slices (18) and in rat facial motor nucleus (19) microiontophoretic studies have indicated the existence of both excitatory and inhibitory 5HT receptors, with differing antagonist susceptibility. Ligand binding studies have shown the presence of two 5HT receptor populations in rat brain, one labelled selectively by ^{3}H-5HT ($5HT_1$ or S-1) and the other by ^{3}H-spiperone ($5HT_2$ or S-2), each with a differing regional distribution and with different agonist and antagonist affinities (20-23).

In addition to electrophysiological and biochemical evidence, other pharmacological data also would suggest the existence of multiple 5HT receptors. Thus, the rat acoustic startle reflex is increased by intrathecal 5HT and decreased by intraventricular 5HT (24). 5HT receptor antagonists also may differentially inhibit some other 5HT dependent responses, such as 5HT-induced hyperthermia in rabbits (25) or hyperactivity induced by L-tryptophan plus a monoamine oxidase inhibitor in rats (26).

Distinction also has been made in the rat between those 5HT receptors linked to adenylate cyclase and those measured by specific binding of ^{3}H-5HT. Piperazine-containing 5HT agonists did not affect 5HT-sensitive adenylate cyclase in collicular homogenates, but displaced ^{3}H-5HT from forebrain preparations (27). This absolute distinction might suggest that the 5HT receptors involved in 5HTP-induced myoclonus are of the type linked to adenylate cyclase. In contrast, others have suggested that head twitching in mice induced by 5HTP is related to the non-cyclase linked 5HT receptor preferentially labelled by ^{3}H-spiperone (28). However, in both situations a behaviour related to activation of 5HT receptors in rodent brain stem has been compared to ligand binding studies in forebrain preparations.

We conclude that the specificity of 5HT receptors in the guinea pig brain stem responsible for the induction of myoclonus represents a unique structure-activity relationship. The pharmacological properties of this particular brain stem 5HT receptor system may be due to a distinct physical conformation of this particular receptor protein, rendering it accessible to 5HT agonists with an indole nucleus, but not those with a piperazine group.

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