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5-HYDROXYTRYPTOPHAN-INDUCED MYOCLONUS: INCREASED SENSITIVITY TO SEROTONIN AFTER INTRACRANIAL 5,7-DIHYDROXYTRYPTAMINE IN THE ADULT RAT*

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Summary—Systemic administration of the serotonin precursor, 5-hydroxytryptophan, produced tremor and myoclonus in rats previously treated with intracisternal injections of 5,7-dihydroxytryptamine and systemic desmethylimipramine, but not in their controls. The behavioural syndrome developed to a maximum at 10–14 days, and persisted at least 16 weeks following intracisternal 5,7-dihydroxytryptamine. The myoclonic syndrome was potentiated by pargyline, but was blocked by D-lysergic acid diethylamide, 2-bromolysergic acid diethylamide, methysergide, and centrally active high doses of the decarboxylase inhibitor, Ro4-4602. Drugs that increase or decrease the effects of other proposed central nervous system neurotransmitters (dopamine, norepinephrine, acetylcholine, and γ-aminobutyric acid) neither reproduced the syndrome nor altered the myoclonus following 5-hydroxytryptophan. Thus, myoclonus induced by 5-hydroxytryptophan following treatment with 5,7-dihydroxytryptamine + desmethylimipramine appears to be specifically related to central indoleamine neurones and may reflect pre- or postsynaptically mediated supersensitivity to serotonin. This animal behavioural syndrome may be relevant to some forms of clinical myoclonus, and should be useful in testing agents that act as agonists or antagonists at serotonin receptors in the central nervous system.

Dihydroxylated tryptamines, including 5,7-dihydroxytryptamine (5,7-DHT) and 5,6-dihydroxytryptamine (5,6-DHT) are powerful neurotoxic chemicals that destroy monoamine-containing cells, including neurones containing serotonin in the central nervous system (CNS). These agents previously have been utilized extensively to characterize the neuroanatomy, biochemistry, and pharmacology of serotonin in the mammalian CNS (BAUMGARTEN and LACHENMAYER, 1972; BAUMGARTEN, LACHENMAYER and SCHLOSSBERGER, 1972; BALDESSARINI and GERSON, 1973; BAUMGARTEN, BJÖRKLUND, LACHENMAYER and NOBIN, 1973a; BAUMGARTEN, VICTOR and LOVENBERG, 1973b; GERSON, BALDESSARINI and WHEELER, 1974). Relatively few studies have focussed on the behavioural correlates of these neurochemical lesions (DALY, FUXE and JONSSON, 1974; LONGO, SCOTTI DE CAROLIS, LIUZZI and MASSOTTI, 1974; BALDESSARINI, AMATRUDA, GRIFFITH and GERSON, 1975). Further, the usefulness of the dihydroxylated tryptamines has been limited to some extent by their non-selective toxicity for both indoleamine and catecholamine neurones. A recently devised technique of administering desmethylimipramine (DMI) parenterally prior to intracisternal 5,7-DHT, appears to provide increased selectivity to lesion indoleamine neurones and spare catecholamine neurones (GERSON and BALDESSARINI, 1975; BJÖRKLUND, BAUMGARTEN and RENSCH, 1975). We have examined behavioural consequences of using this improved method to prepare a relatively selective neurochemical lesion of central serotonergic neurones and now describe and evaluate a characteristic myoclonic syndrome provoked by L-5-hydroxytryptophan (5-HTP).

MATERIALS AND METHODS

Male Sprague–Dawley rats (Charles River) initially weighing 190–210 g were housed three per cage and maintained in a quiet room with controlled lighting (7:00 to 19:00 hr) and constant temperature (21–23°C). They had free access to water and Purina laboratory chow. In some experiments, newborn rats of the same strain, with their mothers, were used.

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Freshly prepared drug solutions were used for all intraperitoneal and intracisternal injections. The rats were neurochemically lesioned with 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT, Regis) as previously described (GERSON *et al.*, 1974; GERSON and BALDESSARINI, 1975). Briefly, desmethylimipramine HCl (DMI, Norpramin,® donated by Lakeside Labs, 25 mg/kg i.p.) was injected 4 hr and again 1 hr prior to the intracisternal injection of 5,7-DHT (200 μg, calculated as the free base). The 5,7-DHT was administered in a volume of 25 μl of artificial cerebrospinal fluid gassed with nitrogen, and with ascorbic acid added (0.5% w/v) to retard oxidation. This vehicle was also used as a placebo in control animals. The intracisternal injection was performed under ether anaesthesia, and sodium pentobarbital (Diabutal,® Diamond Labs, 15 mg/kg s.c.) was given immediately after the 5,7-DHT to prevent generalized seizures. The animals were allowed to recover for at least 12 hr before behavioural testing was undertaken.

Rats were tested in individual wire cages. They were observed and evaluated for 20 sec periods at 5 and 15 min following the injection of L-5-hydroxytryptophan (5-HTP, Calbiochem, up to 600 mg/kg i.p.), and at 15 min intervals thereafter until tremor, ataxia, and myoclonic activity stopped. The raters were kept unaware of the prior or present treatment given. Behaviours were rated according to the following scale, established by observing responses to a wide range of doses of 5-HTP in rats previously treated with 5,7-DHT and DMI: 0 = no change in behaviour, normal appearing rat; 1 = unsteady gait, head tremor; 2 = occasional and intermittent myoclonus; 3 = nearly continuous myoclonus; 4 = generalized seizures, death.

The mean total behaviour score for each rat was computed as the mean of all raters' total scores, each of which was obtained by summing a rater's scores at all time points. Alternatively, mean scores at each time point were computed. Variance is always expressed as ± S.E.M. The inter-rater reliability was evaluated by comparison of paired behavioural ratings made by "blind" observers and was found to be very high (correlation coefficient, $r \geq 0.95$).

Freshly prepared drug solutions were administered by injection either alone or 15 min to 2 hr prior to a dose of 5-HTP (usually 65 mg/kg i.p.). Dosages (as salts) were calculated according to body weight determined on the day of testing. Drugs administered included (±)-α-methyl-*p*-tyrosine methyl ester HCl (Calbiochem, 50 mg/kg); apomorphine HCl (Merck, 2 mg/kg); atropine sulphate (Matheson, Coleman and Bell, 1 mg/kg); bicuculline (K & K Laboratories, 3 mg/kg); 2-bromolysergic acid diethylamide tartrate (donated by Sandoz through the NIMH, 0.5 mg/kg); chlorimipramine (Anafranil, donated by CIBA-Geigy, 15 mg/kg); haloperidol (Haldol,® for injection, McNeil, 10 mg/kg); imipramine HCl (Tofranil,® donated by CIBA-Geigy, 35 mg/kg); L-dihydroxyphenylalanine (L-DOPA, Sigma, 25–500 mg/kg); L-tryptophan (Sigma, 100–300 mg/kg); D-lysergic acid diethylamide (LSD, donated by Sandoz through the NIMH, 0.1 mg/kg); methysergide maleate (Sansert,® donated by Sandoz, 1 mg/kg); pargyline HCl (Eutonyl,® donated by Abbott Laboratories, 100 mg/kg); (±)-*p*-chlorophenylalanine methyl ester HCl (*p*-CPA, Calbiochem, 300 mg/kg, 2 hr previously); *p*-chlorophenyl-γ-aminobutyric acid (Lioresal,® donated by CIBA-Geigy, 5 mg/kg); phenoxybenzamine HCl (Dibenzyline,® donated by Smith, Kline and French, 10 mg/kg); phentolamine (Regitine,® parenteral solution, CIBA-Geigy, 5 mg/kg); physostigmine sulphate (eserine sulphate, Calbiochem, 1 mg/kg); propranolol (Inderal,® parenteral solution, Ayerst, 0.2 mg/kg); Ro4-4602 (N^1-(DL-seryl)-N^2(2,3,4-trihydroxybenzyl)-hydrazine, donated by Hoffman-LaRoche, 10, 50, 200 mg/kg); serotonin creatinine sulphate complex (5-HT, Calbiochem, 65 mg/kg).

RESULTS

Behaviour induced by 5-HTP after pretreatment with 5,7-DHT plus DMI

Although seizures commonly occurred immediately after the administration of 5,7-DHT + DMI, no spontaneous tremor, ataxia, myoclonus or seizures were noted after the first 12 hr in any animal, several of which were observed for as long as 16 weeks. Occasionally when two or more rats were in the same cage, they displayed increased irritability, aggressiveness, rearing and biting compared with controls.

When the pretreated animals were challenged by a dose of 5-HTP, they developed a reproducible and characteristic myoclonic syndrome. Within 3–5 min after the intraperitoneal injection of an approximately half-maximally effective dose of 5-HTP (65 mg/kg), the rats stopped moving about in their cages and crouched in an immobile fashion. They resisted movement and had difficulty regaining their posture when pushed off balance. They developed a rapid small-amplitude termor of the head and heightened startle response to noise. Autonomic signs were prominent, including salivation, piloerection, loose stools and occasionally ejaculation. By 5–15 min, multifocal intermittent muscle twitches became prominent in the forelimbs, and progressed to a more continuous state of myoclonic activity which occasionally involved the entire body. The myoclonus was accentuated by activity, but after 30 min it gradually became limited to isolated irregular contractions. During this time in many instances, the rat's tail was stiffened, elevated, and curled back on itself in a "U" shape (Straub's tail phenomenon). Usually by 45–60 min the myoclonus had ceased, although occasional contractions, tremor and irritability continued. The total syndrome lasted for about 60–90 min, and by the following day the treated rats had returned to their baseline behaviour and level of activity.

The behavioural response to 5-HTP was evaluated at various times after the administration of

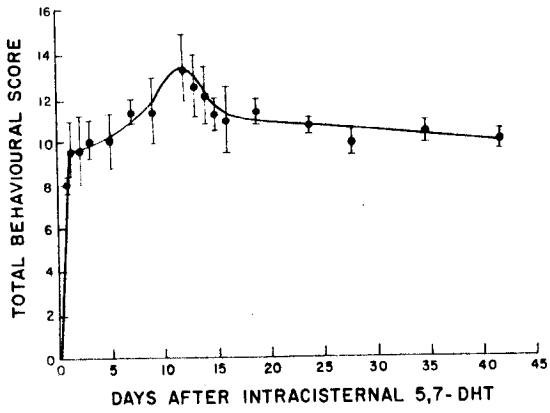

Fig. 1. Time course for development of the 5-HTP-induced myoclonic syndrome expressed as a total behavioural score (see Methods) at times following the intracisternal injection of 5,7-DHT after intraperitoneal DMI. Data are mean scores ± S.E.M. (n = 4–8). The response was discernible at 24 hr, increased to a maximum at 10–14 days, then stabilized at about 3 weeks and persisted at least 16 weeks.

5,7-DHT + DMI by giving a single dose of 5-HTP (65 mg/kg). Some myoclonic response was evident by 20–24 hr after the intracranial injection 5,7-DHT; it increased to a maximum at about 10–14 days and then declined slightly thereafter to remain at a stable level after 3 weeks (Fig. 1). The behavioural response did not seem to be appreciably diminished up to 4 months after the initial treatment with 5,7-DHT + DMI.

The behavioural response evaluated at 3 to 6 weeks after 5,7-DHT + DMI (on the stable portion of the time course shown in Fig. 1) increased in strength and duration with increasing doses of 5-HTP (Figs. 2 and 3). Thus, a 'dose–response' curve for myoclonus

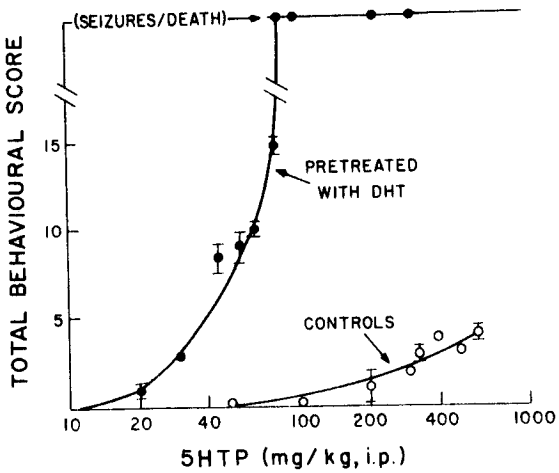

Fig. 2. Log dose vs. response curve for the 5-hydroxytryptophan (5-HTP)-induced myoclonic syndrome expressed as the total behavioural score (calculated as in Methods) in response to 5-HTP in controls (○), or in rats 3 to 6 weeks following intracisternal injection of 5,7-DHT (●). Data are mean behavioural scores ± S.E.M. (n = 4–6). There is a marked shift in the dose–response curve upward and to the left for 5,7-DHT-pretreated animals.

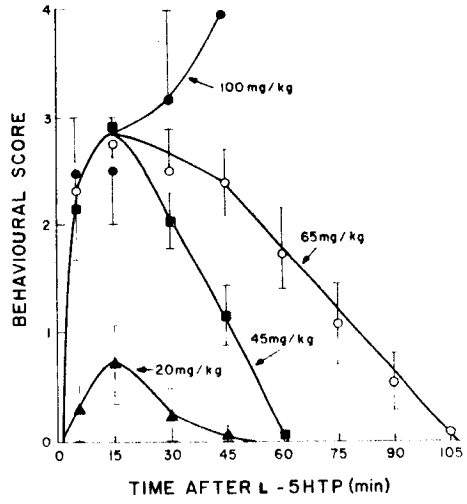

Fig. 3. Time course of response to four doses of 5-HTP: 20 (▲), 45 (■), 65 (○), and 100 (●) mg/kg, i.p., a month after an intracisternal injection of 5,7-DHT (and i.p. DMI). Data are mean behavioural scores ± S.E.M. evaluated as in Methods. There is an increased and more prolonged behavioural response to increasing doses of 5-HTP. After 45 min at 100 mg/kg, all rats died.

induced by 5-HTP was constructed using the total behavioural score obtained after challenge with various doses of 5-HTP (Fig. 2). In rats lesioned 3 to 6 weeks previously with 5,7-DHT + DMI, the response increased with increasing doses of 5-HTP and demonstrated a marked shift upward and to the left compared with normal controls (Fig. 2). In 5,7-DHT pretreated animals, the threshold dose for a perceptible change in behaviour (a score of 1) was approximately 20 mg/kg) (Figs. 2 and 3). The behavioural response was not only stronger, but also more prolonged (Fig. 3) with increasing doses of 5-HTP. Brief generalized tonic-clonic seizures and death (a score of 4) usually occurred at doses of 5-HTP ≥ 80 mg/kg (Figs. 2 and 3). All lesioned animals which were given 5-HTP 3 or more weeks after 5,7-DHT in doses of 100 mg/kg or greater, died from seizures within 30 min so that a precise total behavioural score could not be determined. These animals are shown, however, along the top of Figure 2 to indicate the lethality of 5-HTP at the higher doses of 5-HTP. On the other hand, rats lesioned less than 1 week previously (not shown) tolerated 5-HTP in intraperitoneal doses up to approximately 180 mg/kg, before generalized seizures and death occurred.

In contrast, placebo-control animals given intraperitoneal doses of 5-HTP up to 600 mg/kg, developed increased muscular tone in the extremities, and mild head tremor at the higher doses but none had myoclonus or seizures (Fig. 2). Furthermore, normal newborn rats developed generalized seizures but not tremor or myoclonus following 5-HTP (50–200 mg/kg, i.p.). When the 5,7-DHT (200 μg) was given intraperitoneally 10 days before a dose of 5-HTP (65 mg/kg i.p.), no myoclonic response occurred. However, in

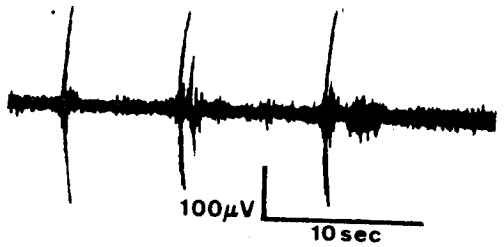

Fig. 4. Representative record of myoclonus induced by 5-HTP in a rat recorded from the posterior cervical region with intramuscular electrodes. The animal had received 200 μg of 5,7-DHT following DMI 7 days previously, and 65 mg of 5-HTP 20 min previously. Note the brief bursts of high amplitude muscle activity. During peak activity (10–30 min after injection of 65 mg/kg of 5-HTP), 8–13 contractions/min were recorded.

normal adult rats pretreated with the monoamine oxidase (MAO) inhibitor, pargyline (100 mg/kg, i.p.), 5-HTP (65 mg/kg, i.p.) did produce a typical myoclonic syndrome which was prolonged (> 150 min) and was about as intense as in 5,7-DHT-pretreated rats. Pargyline also prolonged the response to 5-HTP in rats pretreated with 5,7-DHT + DMI (Table 1). Tryptophan given to normal animals pretreated with pargyline also provoked myoclonus which, however, was delayed in onset (20 min) and was of much lesser magnitude and duration than that produced by pargyline and 5-HTP.

In several animals, the myoclonus was recorded by copper wire electrodes implanted in a forelimb or the posterior cervical musculature. This muscle activity was amplified and displayed with either a cathode ray oscilloscope or an ink writer polygraph. Such recordings confirmed the presence of myoclonus and permitted a more accurate estimate of the frequency of the myoclonic bursts, which ranged from 8–13 contractions/min during peak activity for a dose of 65 mg/kg of 5-HTP (Fig. 4).

Specificity of the myoclonic response

5-Hydroxytryptophan appeared to be the only agent of several given alone that induced the myoclonic response in adult rats previously lesioned with 5,7-DHT + DMI (Table 1). Tryptophan in doses up to 200 mg/kg appeared to sedate both pretreated rats and normal animals but produced no other behavioural changes. Serotonin (65 mg/kg) likewise did not produce myoclonus. Administration of L-DOPA (20–500 mg/kg) did not induce myoclonus in either lesioned adult, or normal newborn rats. Moreover, apomorphine (2–5 mg/kg) did not produce myoclonus, although it did induce stereotyped sniffing, licking and chewing, which were qualitatively and

Table 1. The effect of acute parenteral drug treatment on the myoclonic syndrome in rats previously lesioned with intracisternal 5,7-DHT after parenteral DMI

Agent	Dose (mg/kg)	n	Score ($\bar{x}$ ± S.E.M.)	% of Control
DHT + DMI pretreatment followed by:				
L-5-Hydroxytryptophan	65	(18)	9.88 ± 0.32	100
L-Tryptophan	up to 300	(8)	0	0
5-Hydroxytryptamine (serotonin)	65	(4)	0	0
L-Dihydroxyphenylalanine	up to 500	(6)	0	0
Apomorphine	up to 5	(5)	0	0
DHT + DMI pretreatment followed by 5-HTP (65 mg/kg i.p.) after:				
(±)-*p*-Chlorophenylalanine	300	(4)	11.56 ± 0.74	117*
Ro4-4602	10	(4)	11.01 ± 0.70	111
	50	(6)	4.10 ± 1.83	41**
D-Lysergic acid diethylamide	200	(4)	1.75 ± 1.59	17**
	0.10	(5)	9.92 ± 0.85	101
	0.15	(3)	7.92 ± 0.82	80*
2-Bromo-LSD (BOL)	0.20	(3)	4.67 ± 0.33	47**
Methysergide	0.5	(3)	5.42 ± 1.83	42**
Atropine	1.0	(5)	6.07 ± 0.62	61**
Physostigmine	1.0	(5)	10.76 ± 0.79	108
(±)-α-Methyl-*p*-tyrosine	0.5	(5)	10.01 ± 0.88	101
Haloperidol	50	(4)	9.76 ± 0.67	99
Imipramine	1.0	(4)	9.39 ± 0.72	95
Desmethylimipramine	35	(4)	8.79 ± 0.92	89
Chlorimipramine	25	(4)	11.99 ± 0.72	121*
Pargyline	15	(3)	11.77 ± 2.49	119*
Propranolol	100	(3)	30.02 ± 0.98†	304**
Phenoxybenzamine	0.2	(3)	9.48 ± 0.37	96
Phentolamine	10.0	(4)	10.39 ± 0.87	105
p-Chlorophenyl-GABA	5.0	(9)	8.87 ± 0.54	90
Bicuculline	5.0	(3)	9.66 ± 0.51	98
	3.0	(4)	10.58 ± 0.76	107

* $P < 0.05$ by *t*-test; ** $P < 0.01$, in comparison to 5-HTP alone (100%, or control value).
† Behaviour rated for 180 min, but persisted more than 3 hr.

quantitatively similar in the rats pretreated with 5,7-DHT + DMI and their controls. Finally, some fine tremor was noted following systemic physostigmine in both lesioned and control rats, but neither group showed myoclonus.

Effect of acute drug treatments on 5-HTP-induced myoclonus

The responses of 5-HTP-induced myoclonus to drugs that are believed to influence various neurotransmitters in the CNS, including serotonin, dopamine, norepinephrine, acetylcholine, and γ-aminobutyric acid (GABA) were studied on the stable portion of the response curve (Fig. 1) three or more weeks after 5,7-DHT + DMI. Following pretreatment with one of the drugs listed in Table 1, the rats were challenged with an approximately half-maximally effective dose of 5-HTP (65 mg/kg).

Agents that decreased the 5-HTP-myoclonus included methysergide, D-lysergic acid diethylamide (LSD), 2-bromolysergic acid diethylamide, and high centrally-active doses of Ro4-4602, an inhibitor of L-aromatic amino acid decarboxylase. Pretreatment with methysergide (1 mg/kg) or LSD (0.2 mg/kg) decreased the myoclonus but did not completely abolish it. The partial blocking effect of methysergide (1 mg/kg) was more apparent when the 5,7-DHT-lesioned rats were given high doses of 5-HTP (200 mg/kg), as in this instance the expected seizures and deaths did not occur. The responses to a range of doses of 5-HTP when displayed as a log dose–response curve (not shown) appeared to be shifted to the right when methysergide (1 mg/kg) was given. 2-Bromolysergic acid diethylamide (0.5 mg/kg), the bromine derivative of LSD, also decreased the myoclonic response, but otherwise did not alter the rat's behaviour. At high doses (200 mg/kg), Ro4-4602 blocked the myoclonus, whereas the syndrome may have been slightly potentiated by a low dose (10 mg/kg), possibly by a selective blockade of the peripheral decarboxylation of 5-HTP. Only high doses of Ro4-4602 (as well as bromolysergic acid diethylamide) blocked the autonomic manifestations of the syndrome as well as the myoclonus, suggesting that all of the effects of 5-HTP were centrally mediated.

Atropine (1 mg/kg) partially blocked the salivation and diarrhea, but had no effect on the motor manifestations of the syndrome. Physostigmine (0.5 mg/kg) by itself produced a fine tremor in both normal and 5,7-DHT-lesioned rats, but neither group developed myoclonus. The typical 5-HTP-induced myoclonus was also unaffected by physostigmine, although the tremor was more evident.

Other agents had little or no effect on the 5-HTP-induced myoclonus. The administration of α-methyl-*p*-tyrosine (50 mg/kg) did not block the myoclonus elicited by 5-HTP. Haloperidol, at a dose (1 mg/kg) that increased muscular tone, did not affect the behavioural response to 5-HTP. The amine uptake-blocking agents imipramine, desmethylimipramine and chlorimipramine (up to 35 mg/kg) had little effect. Adrenergic blocking agents, both alpha- (phenoxybenzamine, 10 mg/kg; phentolamine, 5 mg/kg) and beta-antagonists (propranolol, 0.2 mg/kg) did not significantly alter the behaviour induced by 5-HTP. Pretreatment with *p*-chlorophenyl-GABA (5 mg/kg) did not alter the myoclonus induced by 5-HTP, but did decrease tremor and autonomic manifestations. Bicuculline (3 mg/kg), an antagonist of GABA, increased the tremor but did not appreciably increase the myoclonus except in response to startle.

DISCUSSION

Evidently, myoclonus can be provoked by mechanisms within the CNS of the rat associated with increased sensitivity to serotonin. Intracranially injected 5,7-DHT exerts its toxicity chiefly at axon terminals of monoamine neurones (BAUMGARTEN and LACHENMAYER, 1972; BAUMGARTEN *et al.*, 1973a; GERSON and BALDESSARINI, 1974). Dihydroxylated tryptamines can affect serotonin-containing nerve terminals as indicated by histofluorescence and electron microscopic observations (BAUMGARTEN *et al.*, 1972) and biochemical evidence (BAUMGARTEN *et al.*, 1973b; GERSON *et al.*, 1974; GERSON and BALDESSARINI, 1975) of prolonged damage. To produce a more specific lesion of serotonergic neurones, DMI was given to protect catecholamine neurones, evidently by preventing their taking up the 5,7-DHT (GERSON and BALDESSARINI, 1975; BJÖRKLUND *et al.*, 1975). This selective chemical lesion of central serotonin neurones leads to a state of increased behavioural sensitivity to the subsequent administration of L-5-HTP, indicated by an acute behavioural syndrome which includes myoclonus in adult rats lesioned with 5,7-DHT + DMI. The myoclonus seemed to be uniquely associated with indoleamine metabolism, since agents that interact with other classes of neurotransmitter substances failed to produce myoclonus or to block its production by 5-HTP (Table 1).

Similar reports of increased sensitivity to 5-HTP include induction of tremor by 5-HTP after 5,6-DHT (LONGO *et al.*, 1974), and an increased inhibitory effect of 5-HTP on the extensor hind limb reflex in spinal rats after 5,7-DHT (DALY *et al.*, 1974); in both cases, 5-HTP was administered with an inhibitor of MAO. A "hyperactivity syndrome" occurs in normal animals given tryptophan and an MAO inhibitor (GRAHAME-SMITH, 1971), and we found that 5-HTP or tryptophan produced prolonged myoclonus in normal adult rats pretreated with pargyline (100 mg/kg), and that this MAO inhibitor potentiated the effects of 5-HTP in 5,7-DHT-lesioned rats. 5-Hydroxytryptophan alone has previously been reported to produce myoclonus in immature, but not in adult normal guinea pigs (KLAWANS, GOETZ and WEINER, 1973), although we failed to find a similar effect in the immature rat.

The myoclonic syndrome that we observed is probably related to abnormalities in neuronal function

within the CNS itself. Thus, 5,7-DHT led to this response to 5-HTP when administered intracranially, but not intraperitoneally. Furthermore, intraperitoneal serotonin crosses the blood-brain barrier poorly (DOUGLAS, 1970) and did not produce myoclonus after intracranial 5,7-DHT. Moreover, inhibition of peripheral decarboxylation of 5-HTP with low doses of Ro4-4602 did not block (and may even have potentiated) the effects of 5-HTP (Table 1), while high centrally-active (PORTER, 1971) doses did so. After DHT, the 5-HTP must undergo decarboxylation, either in the remaining serotonergic neurones or in other sites that have aromatic amino acid decarboxylase activity, such as capillaries or catecholamine-containing neurones. This effect of Ro4-4602 and the potentiating effect of pargyline together suggest that the active metabolite of 5-HTP responsible for inducing myoclonus is an amine, presumably serotonin.

The time course for development of the myoclonus response to 5-HTP following 5,7-DHT was complex (Fig. 1), suggesting that several processes may occur. There was an early increase in sensitivity to 5-HTP that gradually increased to a maximum at 12 days, and then declined somewhat to become stable from 3 weeks to at least 16 weeks after 5,7-DHT. The onset of 5-HTP-induced myoclonus after 5,7-DHT + DMI could not be timed accurately due to initial toxicity of 5,7-DHT that often leads to generalized seizures and the need for a barbiturate for a few hours after intracranial administration of the DHT. Nevertheless, it appeared that myoclonus could be provoked within 24 hr after the pretreatment. This early onset of 5-HTP-myoclonus differs somewhat from the slower development of other examples of apparent supersensitivity believed to reflect postsynaptic changes, including changes in response to agonists that may involve CNS catecholamine receptors and that usually evolved over at least several days (UNGERSTEDT, 1971; PERKINS and MOORE, 1973; MISHRA, GARDNER, KATZMAN and MAKMAN, 1974; TARSY and BALDESSARINI, 1974; THORNBURG and MOORE, 1975). On the other hand, there is some biochemical and behavioural evidence that the sensitivity of intracranial catecholamine receptors can be altered in a matter of hours (DEGUCHI and AXELROD, 1973; COSTENTIN, PROTAIS and SCHWARTZ, 1975). Alternatively, it may be that treatment with 5,7-DHT leads to the early emergence of myoclonus due to changes unrelated to postsynaptic mechanisms: presynaptic effects might decrease the inactivation of newly formed serotonin by enzymatic or uptake processes, allowing the increased serotonin which follows adminstration of 5-HTP to be more effective at postsynaptic receptors. A similar presynaptic mechanism is believed to be an important aspect of post-denervation supersensitivity in peripheral (TRENDELENBERG, 1966) and central (PERKINS and MOORE, 1973) noradrenergic neurones. Furthermore, the rapid production of myoclonus in intact rats by pretreatment with an MAO inhibitor, followed by either tryptophan or 5-HTP suggests that increased availability of serotonin at postsynaptic sites may contribute to myoclonus. On the other hand, myoclonus was not produced in intact rats by treatment with chlorimipramine (a blocker of serotonin uptake) and 5-HTP (STEWART, GERSON and BALDESSARINI, unpublished observations).

The subsequent gradual increase in sensitivity to 5-HTP to a maximum at 12 days after DHT (Fig. 1) could represent postsynaptic adaptations to a decreased availability of serotonin. Thus, the postsynaptic indoleamine receptor or other aspects of the postsynaptic apparatus might be altered as a result of disuse or denervation induced by DHT so as to become more sensitive to serotonin, thereby producing enhanced behavioural effects of 5-HTP. If postsynaptic receptor changes are involved, actual denervation by DHT rather than decreased availability of serotonin ("disuse") may be required, since myoclonus was not produced by 5-HTP after a week of treatment with reserpine, *p*-chlorophenylalanine or even *p*-chloroamphetamine (which may also have some limited lesioning actions) to deplete serotonin levels (STEWART, GERSON and BALDESSARINI, unpublished observations). It is also possible that 5,7-DHT + DMI has some as yet unknown toxic effects that contribute to the production of myoclonus with 5-HTP. The final partial decline in response to 5-HTP to a plateau after 3 weeks (Fig. 1) may represent partial recovery of damaged serotonin-containing neurones or a re-equilibration of activities in pathways mediated by neurotransmitters other than serotonin.

The experimentally induced myoclonus that we have described may have potential relevance to certain kinds of clinical myoclonus, some of which appear to be associated with abnormalities of serotonin metabolism or to be modified by agonists or antagonists of serotonin (GUILLEMINAULT, THORP and COUSIN, 1973; KLAWANS *et al.*, 1973; VAN WOERT and SETHY, 1975). In addition, the present behavioural model might provide an approach for testing new agents that act at central receptors as serotonin agonists or antagonists and for evaluating potential new therapies for clinical myoclonus.

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