Analysis of Tremorgenic Effects of Intracaudate Serotonin[1] (37476)

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Normal integrative functioning of the caudate neuroregulatory system appears to depend on critically balanced local neurotransmitter levels (1, 2). For example, caudate instability as reflected in the development of tremors has been ascribed to disturbances in a neurotransmitter balance, involving either augmented cholinergic excitation (3) or diminished dopaminergic inhibition (4). However, despite the presence of appreciable quantities of serotonin (5-HT) in the caudate nucleus (5, 6), its potential role in the regulation of caudate neuronal excitability is undefined and its relationship to the major neurotransmitters acetylcholine (ACh) and dopamine (DA) remains relatively undetermined.

A reciprocal relationship between the levels of DA and 5-HT in the caudate has been proposed (7, 8), and it has been shown that inhibition of monoamine oxidase can upset the caudate biogenic amine balance by selectively elevating 5-HT levels (9, 10). Furthermore, certain monoamine oxidase inhibitors are capable of inducing involuntary movements in experimental animals (10, 11). On this basis, it seemed reasonable to test the hypothesis that 5-HT might participate in an intracaudate neuroregulatory system to influence local neuronal excitability and play a significant role in modifying caudate function. Since hindlimb tremor has been utilized by our group as an endpoint and sensitive index of disturbances in neurotransmitter functioning within the caudate, the present studies were structured to determine the direct effects of 5-HT on local caudate activity and to explore its relationship to the local effects of ACh and DA.

Materials and Methods. Details of the neurosurgical procedure followed in preparing the chronic cats utilized in this study may be found in a previous report (3). In short, the technique involved stereotaxic implantation of combined recording electrode-injection guides (injectrodes) into various brain areas (12) including the caudate nucleus (A_{15}, R_5 or L_5, D+5), hippocampus (A_2, R_8 or L_8, D+5) and substantia nigra (A_5, R_5, or L_5, D—5). Bipolar twisted recording electrodes were positioned on the left and right frontal cortex, while a screw in the occipital portion of the skull served as the indifferent electrode. Insulated recording wires from each electrode were terminated in an Amphenol plug (series 223-1217) permanently anchored to the skull.

After a 2 wk recovery period, each cat was tested at intervals of 7–10 days. The animals were suspended in a canvas sling which permitted free movement of the left hind leg and a sensitive phonocartridge assembly (ASTATIC 13TB) was positioned on the dorsal surface of the left hindfoot. Tremor and local electrographic activity were simultaneously recorded on an electroencephalograph (Grass Model 6). Tremor parameters (frequency, amplitude, percentage tremor time, etc.) were semiquantitated and analyzed as previously described (3). Solutions of drugs were microinjected locally in volumes not exceeding 10 μl; when indicated appropriate controls for volume, pH and tonicity were carried out. Precise volumes were administered by means of delivery tubing coupled to a calibrated micrometer-drive which when inserted into the injectrode projected 1 mm be-

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TABLE I. Characteristics of Tremors Produced by Intracaudate Injection of 5-Hydroxytryptamine.

Tremor parameters	Mean values[a] (range)
Dose (μg)[b]	30[c]
Latency (min)	2.5 (1–5)
Activity ratio[d]	23/25
Maximal tremor characteristics	
Onset of peak effect (min)	8 (3–18)
Duration of maximal tremor (min)[e]	11 (7–16)
Amplitude (μV)	469 (217–1150)
Amplitude (graded intensity)[f]	+++
Tremor bursts/min	21 (16–25)
Tremor frequency (cycles/sec)	15 (12–19)
Tremor time (%)	42 (33–54)
Total duration of tremor (min)[e]	23 (15–35)

[a] Average of 25 experiments.
[b] Dose expressed as free base.
[c] Fixed dose of 30 μg administered to each animal.
[d] Activity ratio = tremorgenic responses/total no. of experiments.
[e] Values obtained from 10 cats.
[f] Graded against physostigmine (150 μg) tremor (+++) as standard.

low the tip of the permanently implanted cannula guide.

The following drugs were utilized in this investigation: 5-hydroxytryptamine creatinine sulfate (5-HT), methysergide maleate, dopamine hydrochloride (DA), scopolamine hydrochloride, hemicholinium hydrobromide, pargyline hydrochloride and physostigmine salicylate (PHYSO). All drugs were dissolved in physiological saline except methysergide, which was dissolved in a solution of 10% *N,N*-dimethyl-formamide in saline. At these levels, formamide was found to be devoid of any demonstrable caudate action. Doses of all drugs are expressed in terms of the free base. Electrode placements were confirmed in formalin-fixed brains previously lesioned at each injection site.

Results. Characteristics of 5-HT tremor. Intracaudate 5-HT elicited hindlimb tremors in chronic cats throughout a wide range of doses. No tremor activity was observed below a dose of 5 μg (6 animals); slight but inconsistent tremor responses of short duration were evident at 10 μg (4 animals); however, at 30 μg, 5-HT produced strong, reproducible tremors in 23 of the 25 animals tested. The duration but not the intensity of the tremors was proportional to the dose, in that higher doses (50–120 μg) of 5-HT did not appreciably increase the amplitude of the tremors, but substantially prolonged the duration ($> 2\times$). Significant tremor activity was not observed, however, when 5-HT (30 μg) was injected into the hippocampus or substantia nigra. A summary of the maximal tremor characteristics resulting from intracaudate 5-HT (30 μg) is shown in Table I. Maximal tremor represents the period of peak amplitude response which generally plateaued within 5–10 min after injection, was sustained for 7–16 min and then slowly declined over the next 10 min. At 30 μg, tremor responses were consistent, reproducible and comparable to those produced in previous studies by 150 μg of PHYSO (13). Significantly, tachyphylaxis was not apparent in that repetitive challenging doses (30 μg) continued to result in tremor responses of comparable intensity. On this basis, 30 μg of 5-HT was selected as the test dose for subsequent analysis of local drug mechanisms. Rather consistent behavioral and electrographic changes paralleled the tremor activity following intracaudate 5-HT injection. The cat appeared much less alert, usually ceased all vocalization and frequently closed his eyelids. During this 15–35 min interval, the electrographic pattern became synchronized with high amplitude, low frequency slow waves which periodically appeared bilaterally in the caudate nuclei and occasionally projected to the frontal cortex and hippocampus.

Pharmacologic analysis of 5-HT tremor. In an effort to characterize 5-HT tremor pharmacologically, several agents which might

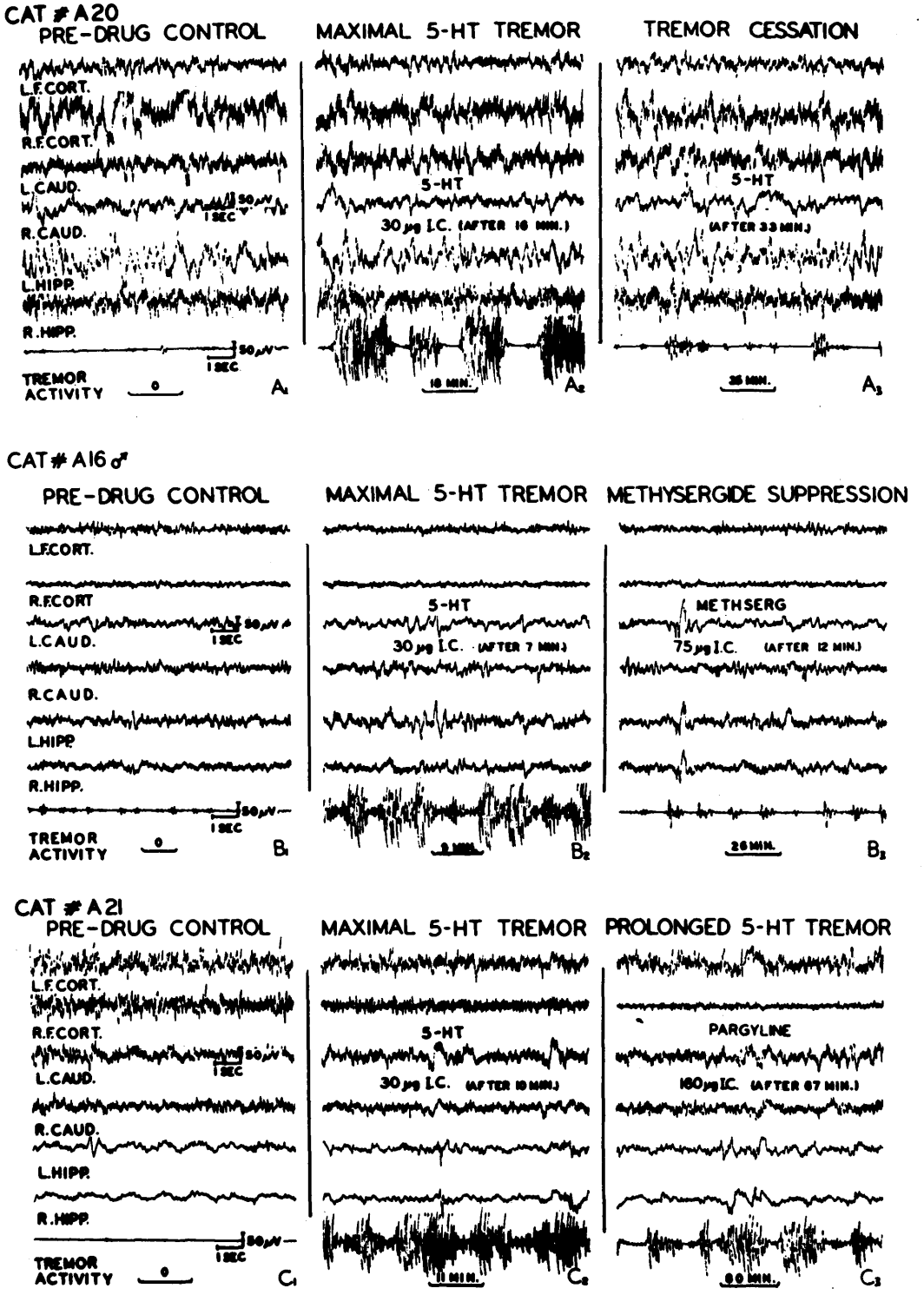
CAT # A20
PRE-DRUG CONTROL
MAXIMAL 5-HT TREMOR
TREMOR CESSATION
L.F.CORT.
R.F.CORT.
L.CAUD.
R.CAUD.
L.HIPP.
R.HIPP.
50 μV
1 SEC
TREMOR ACTIVITY
0
A1
5-HT
30 μg I.C. (AFTER 16 MIN.)
10 MIN.
A2
5-HT
(AFTER 33 MIN.)
25 MIN.
A3
CAT # A16 ♂
PRE-DRUG CONTROL
MAXIMAL 5-HT TREMOR
METHYSERGIDE SUPPRESSION
L.F.CORT.
R.F.CORT
L.CAUD.
R.CAUD.
L.HIPP
R.HIPP.
50 μV
1 SEC
TREMOR ACTIVITY
0
B1
5-HT
30 μg I.C. (AFTER 7 MIN.)
B2
METHSERG
75 μg I.C. (AFTER 12 MIN.)
26 MIN.
B3
CAT # A21
PRE-DRUG CONTROL
MAXIMAL 5-HT TREMOR
PROLONGED 5-HT TREMOR
L.F.CORT.
R.F.CORT.
L.CAUD.
R.CAUD.
L.HIPP.
R.HIPP.
50 μV
1 SEC
TREMOR ACTIVITY
0
C1
5-HT
30 μg I.C. (AFTER 10 MIN.)
11 MIN.
C2
PARGYLINE
160 μg I.C. (AFTER 87 MIN.)
90 MIN.
C3

TABLE II. Effects of Locally Administered Neuropharmacologic Agents on Serotonergic (5-HT) Tremors.

Agent[a]	5-HT tremor			
	Effect on tremor[b]	Dose[c] (μg)	Latency (min)	Activity ratio[d]
Methysergide	(↓)	84 (75–112)	9 (4–14)	10/10
Dopamine	(↓)	80	3 (1–7)	7/7
Pargyline	(+)	160	—	6/6
Scopolamine	(0)	64 (45–90)	—	6/6
Hemicholinium	(0)	108	—	6/6

[a] Drugs evaluated against 5-HT tremor produced by standard dose of 30 μg of base.
[b] (↓) Tremor suppressed; (+) tremor prolonged; (0) no effect on tremor.
[c] Mean dose of base (range in parentheses); if no range is indicated, a fixed dose was given to each animal.
[d] Activity ratio = no. of responses/total no. of experiments.

influence local neurotransmitter function were used as chemical tools and their effects evaluated on the maximal 5-HT tremor responses produced by the standardized optimal dose of 30 μg (Table II). As shown in Fig. 1B, methysergide (75–112 μg), a competitive 5-HT receptor antagonist readily blocked 5-HT tremor within 4–14 min. Local pretreatment with methysergide also effectively interfered with the development of 5-HT tremors; however, a substantially lower mean dose (38 μg) was required. Dopamine was likewise effective in suppressing 5-HT tremors at a dose (80 μg) previously found to be effective in suppressing equipotent cholinergic (PHYSO) tremors (13); only 1–7 min, however, were required for total suppression by DA. Although tremor could be reestablished after both methysergide and DA suppression, considerably higher doses (2–3× test dose) of 5-HT were required to reestablish control activity. At a dose (160 μg) found to be effective in abolishing endogenous PHYSO tremors (11), pargyline, an inhibitor of monoamine oxidase, exerted little effect on the intensity (amplitude) of 5-HT tremor, but more than doubled the average duration from 23 to 65 min (Fig. 1C).

To determine whether ACh participates in 5-HT tremorgenic responses, the effects of agents which interfere with local ACh mechanisms were examined for their actions on 5-HT tremor activity (Table II). In contrast to the inhibitory actions of dopamine and methysergide, scopolamine, a specific ACh receptor antagonist, failed to alter 5-HT tremor throughout a range of doses (45–90 μg) demonstrated to be effective against cholinergic (PHYSO) tremors. Hemicholinium (108 μg), an agent which interferes with ACh synthesis was likewise ineffective in either diminishing maximal 5-HT tremor activity or preventing the subsequent redevelopment of tremor following a supplemental injection of 5-HT. Conversely, local administration (30 μg) of 5-HT during periods of maximal cholinergic (PHYSO, 150 μg) tremor intensified ongoing tremor activity for 10–30 min following a short latency (2–4 min). The overall duration of PHYSO tremor, however, remained essentially unchanged. This additive and synergistic action of 5-HT on ongo-

FIG. 1. Analysis of tremor and local brain electrographic responses following intracaudate serotonin. (A_1–A_3) Development of serotonin (5-HT) tremors subsequent to intracaudate (ic) microinjection (30 μg). (B_1–B_3) Antagonism of 5-HT tremors by ic methysergide (75 μg). (C_1–C_3) Prolongation of 5-HT tremor activity from usual 30 to 80 min by ic pargyline (160 μg). Bracketed values represent continuous time sequence (min) following 5-HT injections.

ing PHYSO tremor was observed consistently in an experimental series of six animals.

Discussion. Our findings demonstrate that relatively small doses of 5-HT alter the functioning of the caudate to produce tremors. The rapidity with which tremor activity developed and the marked effectiveness of the specific 5-HT antagonist methysergide in abolishing these tremors strongly suggest that 5-HT acts directly and specifically on intracaudate serotonergic receptors which are readily accessible. The duration of these tremors seems to be determined largely by the action of local monoamine oxidase, evidenced by the ability of pargyline to substantially prolong 5-HT tremor activity presumably by interfering with the metabolism of 5-HT. This observation is in keeping with out findings that higher doses of 5-HT also greatly prolong tremor duration without any further increase in amplitude. In contrast to the previously described cholinergic (PHYSO, 150 μg) tremor profile (13), 5-HT tremors developed more rapidly, exhibited a shorter duration and although of comparable intensity at the test dose, differed from ACh (PHYSO) tremors in that the range of responses with increasing dosage was considerably more limited. The tremorgenic action of 5-HT apparently develops independently of ACh activity as indicated by the inability of the cholinergic antagonists scopolamine and hemicholinium to interfere with either development or maintenance of 5-HT tremor. Conversely, serotonergic tremor activity can be superimposed upon and is additive to that of ongoing PHYSO tremor within the investigated range of tremorgenic doses. Thus, although 5-HT and PHYSO are both tremorgenic, it is evident from their patterns of action and their differential susceptibility to pharmacologic (scopolamine, hemicholinium) antagonists that they are operating substantially independent of one another.

In contrast to ACh, 5-HT and DA functionally oppose one another at a local level: 5-HT is tremorgenic, whereas DA is inhibitory and stabilizes the caudate. Furthermore, following DA inhibition, the tremor threshold for 5-HT is raised 2–3×, suggesting the occurrence *in situ* of a mutual antagonism between the two. Along these lines, Hall *et al.* (14) proposed that the balance between central DA and 5-HT plays a critical role in movement disorders; and although an increased ratio of 5-HT relative to DA is postulated to produce a hyperactive state, there is no direct evidence for an excitatory function for 5-HT in the caudate (15). Yet, the reversal of local DA inhibition by 5-HT and the additive tremorgenic effect of 5-HT on ongoing PHYSO tremor can be advanced as arguments that 5-HT tremorgenesis may be related to alterations in the local neuro-excitatory state.

If we take into account its local pattern of responses, 5-HT might reasonably be assigned a modulator role in the caudate. The following lines of evidence developed in our study can be marshalled in support of such a modulatory function for intracaudate 5-HT: (a) rapid onset; (b) short duration; (c) limited range of responses; (d) absence of tachyphylaxis; (e) antagonistic action to that of DA and (f) synergism with ACh (potentiation of PHYSO tremor). It might thus be appropriate to include 5-HT as part of the local homeostatic neuroregulatory system in the caudate previously attributed entirely to the interactions between ACh and DA (13). It is interesting to note that Kety (16) envisioned central 5-HT levels as regulating the volatility or instability of central synapses, a role generally considered to be of a modulatory nature. Increasing evidence for a broader participation of the caudate nucleus in motor, autonomic and behavioral brain activities (17, 18) suggest that the potential role of 5-HT as a modulator in the caudate may acquire added significance.

Summary. In chronic cats, 5-HT injected into the caudate elicited tremors which were ascribed to a local excitatory effect. The 5-HT tremors developed rapidly, were of short duration and exhibited no tachyphylaxis. Tremor responses were readily antagonized by methysergide, reversibly suppressed by DA and prolonged by pargyline without a corresponding increase in amplitude. Development and maintenance of 5-HT tremor activity is substantially independent of cholinergic intervention. Based on its intracaudate

profile of action and its ability to modify the neuroregulatory actions of local neurotransmitters (ACh, DA), 5-HT has been assigned a modulator role in the caudate.

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