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THE RELEASE OF ACETYLCHOLINE AND OF 3-HYDROXYTYRAMINE FROM THE CAUDATE NUCLEUS

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The criteria by which certain chemical entities may be ascribed roles as transmitters of synaptic action at peripheral sites are well recognized (Florey, 1960). They include demonstrations of the presence of the substance in presynaptic nerve terminals, its release therefrom upon stimulation and the mimicry of the effects of activation by exogenous application of the substance to the synaptic region.

Clearly these criteria are much more difficult to satisfy at synapses within the central nervous system (C.N.S.). Perfusion of a known local area through its blood supply, with collection of the venous effluent for assay of liberated substances, is impossible; and stimulations are hard to conduct in such a way that the details of the neuronal pathway are fully known. Certain valuable data have been amassed through the application of materials to single neurones by iontophoresis (e.g. Curtis, Phillis & Watkins, 1961), but even here it may be difficult to distinguish pharmacological effects from those which mimic synaptic action. Thus, for example, Curtis & Davis (1963) have concluded that, although acetylcholine is able to excite neurones of the lateral geniculate body, from other pharmacological evidence these cells are unlikely to be cholinoceptive.

The basal ganglia are peculiarly rich in their content of many of the materials which are felt to be likely candidates for transmitter function, and of certain enzyme systems which are implicated in both anabolism and catabolism of these materials. The caudate nucleus contains a strikingly high concentration of 3-hydroxytyramine, and amounts almost as high as are found anywhere else in the C.N.S. of 5-hydroxytryptamine, acetylcholine, DOPA decarboxylase, monoamine oxidase, choline acetylase, and cholinesterase (for references see McLennan, 1963). If the biogenic amines mentioned act as synaptic transmitters within the caudate nucleus, it should be possible to detect their release upon stimulation of appropriate structures. 3-Hydroxytyramine is not normally regarded as a

likely transmitter substance; however, its high concentration in some nuclei of the basal ganglia, where the amounts of noradrenaline are not correspondingly high, has led Holtz (1960) to conclude that 'dopamine itself is the end-product of biosynthesis', and that it is of importance to the function of those areas. Changes in its amount in certain basal ganglia have been described in some extrapyramidal disorders (Ehringer & Hornykiewicz, 1960, *et seq.*). Neurones containing 3-hydroxytyramine are known from other situations (Häggendal & Malmfors, 1963). The possibility that 3-hydroxytyramine may mimic synaptic action in the reticulospinal inhibitory pathway has been raised (McLennan, 1961, 1962; Curtis, 1962).

In the present experiments a small volume of the caudate nucleus of cats has been irrigated with a saline medium, and the nucleus was activated from different sources. A selective output of 3-hydroxytyramine and of acetylcholine has been demonstrated.

METHODS

The experiments have been performed on adult cats of both sexes, anaesthetized with pentobarbital (35–40 mg/kg). The animals were mounted in a stereotaxic instrument, and a push–pull cannula (Gaddum, 1961), fashioned from a 20-gauge (S.W.G.) hypodermic needle, was directed through a small hole drilled in the skull into the centre of the head of one caudate nucleus, at frontal planes 15·5–17 in the atlas of Jasper & Ajmone-Marsan (1954). Fluid was forced into the cannula from a motor-driven syringe at a rate of 0·12 ml./min, and only those experiments in which the effluent was recovered at the same rate were accepted. Passage of a coloured solution through the cannula inserted into a 1% agar gel gave an estimate of 0·5–1 mm^3 for the volume of tissue bathed by the fluid. The effluents were collected for 15 min periods, and intervening periods of 5 min rest with the irrigation running were allowed between each collection period.

The irrigation fluid was Locke's solution modified by the omission of bicarbonate. When acetylcholine was to be estimated in the effluent fluids, the medium usually contained 50 mg/l. eserine sulphate; for 3-hydroxytyramine the effluents were collected into tubes containing one drop of 0·5 N-acetic acid, but no inhibitor of monoamine oxidase was added to the medium.

Monopolar square wave stimuli of 0·1 msec duration were delivered to cortical or subcortical structures through a 26-gauge (S.W.G.) steel electrode insulated to within 0·3 mm of its tip. Records of the electrical activity of the caudate nucleus were obtained from a fine nichrome wire similarly insulated, which was either inserted within or attached with lacquer to the outer tube of the cannula. Such records were amplified, displayed on an oscilloscope, and photographed in the usual way. At the end of each experiment, the brain was removed and fixed in Tellyesniczky's fluid, and paraffin sections stained with cresyl violet were examined to determine electrode placements.

Acetylcholine assays were performed upon the eserinized dorsal muscle of the leech, and the following tests were carried out in some experiments to support the assertion that this substance was actually being measured. The assayed activity could be completely destroyed by heating at 100° C for 5 min at pH 12. Fluids not containing eserine collected from the caudate nucleus occasionally showed some slight activity on the leech, but never more than one-tenth of that recovered with eserine. 3-Hydroxytyramine assays were kindly carried out by Dr E. G. McGeer, using the spectrophotofluorometric method described by

McGeer & McGeer (1962), but simplified by the omission of the perchlorate precipitation step and with all volumes scaled down for the detection of the small quantities encountered. Values are expressed in terms of acetylcholine chloride or of 3-hydroxytyramine hydrochloride.

RESULTS

Release of acetylcholine from the caudate nucleus. Mitchell & Szerb (1962) briefly reported that the resting release of acetylcholine from the caudate nucleus, under experimental conditions apparently similar to the present, amounted to 15–60 ng/ml., which is the equivalent of an average of 1600 pg/min. In the present experiments, the spontaneous output was 290 pg/min (15 determinations, range 95–625 pg). Mitchell & Szerb further reported that the quantities released could be increased up to 10 times by low-frequency stimulation of a portion of the anterior sigmoid gyrus. In the present experiments it has not been possible to reproduce this effect (see below), the most likely explanation for which appears to be that different areas of the gyrus were stimulated (J. F. Mitchell, personal communication).

TABLE 1. Liberation of acetylcholine from the irrigated caudate nucleus (pg/min of irrigation)

	Nucleus ventralis anterior (VA) stim.		Anterior sigmoid gyrus stim.		Sigmoid + VA stim.	Nucleus centro-medianus stim.	
Resting	3–5/sec	7–10/sec	3–5/sec	7–10/sec	5–8/sec	3–5/sec	7–10/sec
240	355	—	—	—	—	—	—
320	—	—	345	—	160	—	—
625	—	805	—	—	—	—	—
285	—	415	380	350	325	—	205
215	340	—	220	—	220	—	—
105	—	195	—	—	—	—	—
215	340	—	315	—	225	—	—
325	420	375	—	—	—	315	—
95	—	—	—	—	—	75	—
185	—	275	120	—	140	—	—
100	175	—	165	—	—	155	—
115	—	—	55	55	—	—	—
350	—	—	180	—	—	—	—
295	—	—	—	—	—	—	280
350	—	—	—	—	—	—	320

Low frequency stimulation of the nucleus ventralis anterior (VA) in the thalamus invariably led to an increase in the amount of acetylcholine liberated into the irrigation fluid (Table 1). The effect could be noted at frequencies of stimulation as low as 3/sec and appeared maximal at 5–7/sec, although frequencies > 12/sec were not tested. At 5 pulses/sec and above typical recruiting responses (Fig. 1) were fully evident. The

increase for all frequencies of stimulation was on the average 29·6 ± 6·6 % (S.E., 10 determinations).

Stimulation of points in the anterior sigmoid gyrus of the cortex which provoked prolonged electrical discharges in the caudate nucleus (Fig. 2) had a rather variable effect upon the acetylcholine output from the nucleus (Table 1); but on the average this was not different from the controls. However, simultaneous stimulation of this cortical region and of VA invariably resulted in the abolition of the increased output of

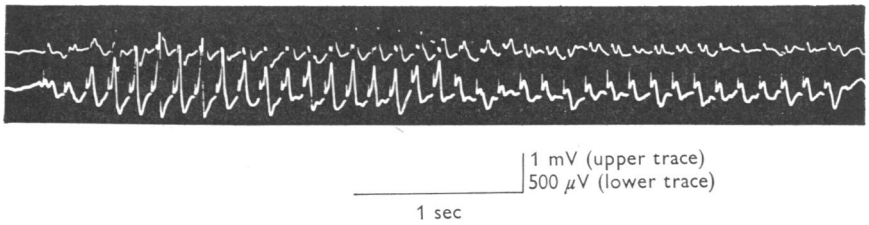

Fig. 1. Recruiting responses recorded from the anterior sigmoid gyrus (above) and the caudate nucleus (below) following activation of the nucleus ventralis anterior. Stimulus 5 V, 0·1 msec, 7/sec.

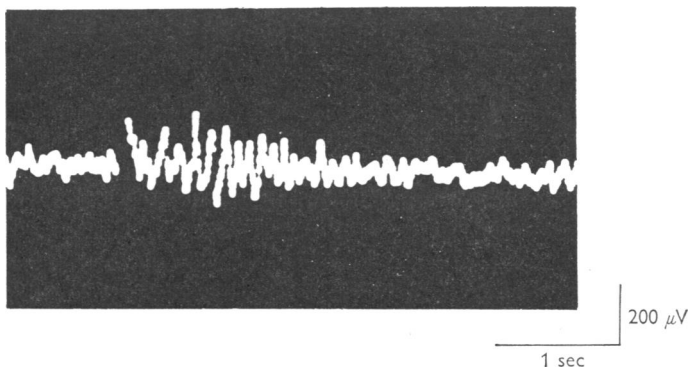

Fig. 2. Electrical activity recorded from the caudate nucleus following a single shock to the sigmoid gyrus. Stimulus 7 V, 0·1 msec.

acetylcholine from the caudate which followed VA excitation alone. Excitation of the thalamic nucleus centromedianus (CM) failed to influence the output of acetylcholine from the caudate.

Release of 3-hydroxytyramine. A 'resting' release of 3-hydroxytyramine from the caudate nucleus has been found, amounting to *ca.* 1000 pg/min (12 determinations, range 350–1950 pg). This output, in contrast to that of acetylcholine, was not significantly altered following activation of VA, whereas excitation of CM using similar parameters of stimulation consistently produced an increased output averaging 80·3 ± 23·6 % (S.E., 10

determinations) (Table 2). The electrical response of the caudate nucleus to CM stimulation differed from that found following activation of VA (Fig. 1) in that the short latency response which followed a single shock (Fig. 3) was not altered by repetitive stimulation.

TABLE 2. Liberation of 3-hydroxytyramine from the irrigated caudate nucleus (pg/min of irrigation)

	Nucleus centromedianus (cm) stim.		Anterior sigmoid gyrus stim.	Sigmoid + CM stim.	Nucleus ventralis anterior stim.	
Resting	3–5/sec	7–10/sec	3–5/sec	3–5/sec	3–5/sec	7–10/sec
1950	2500	—	—	—	1350	—
1150	1800	—	850	—	1200	—
1900	—	2650	—	—	—	1650
1100	—	—	—	—	—	850
750	1000	1300	—	—	950	1000
850	1350	—	—	—	—	—
650	—	—	850	—	—	—
750	—	—	1100	—	—	—
350	1350	—	—	350	—	—
350	650	—	1000	350	—	—
700	1200	—	800	600	—	—
1150	1950	—	1750	1300	950	—

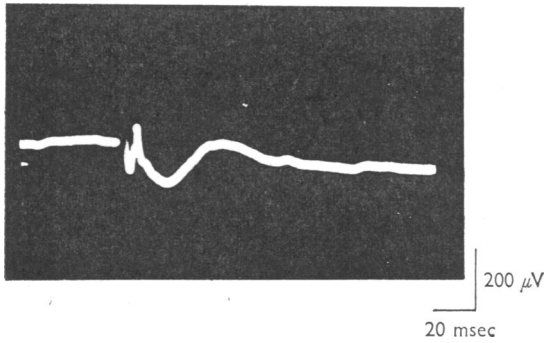

Fig. 3. Response evoked in the caudate nucleus by a single shock to the nucleus centromedianus. Stimulus 5 V, 0·1 msec.

In five of six experiments in which the anterior sigmoid gyrus was stimulated an increased output of 3-hydroxytyramine from the caudate nucleus was observed. However, when this stimulation was delivered together with that to CM a complete abolition of the increased release of 3-hydroxytyramine was produced (Table 2).

Stimulation of other structures. In a number of experiments the thalamic stimulating electrode was misplaced, so that certain other nuclei were inadvertently stimulated; and in other animals the stimulating electrodes were directed towards different subcortical structures which are believed to project on to the caudate nucleus. These experiments were uniformly

negative, whether the irrigation fluid was analysed for acetylcholine or for 3-hydroxytyramine. The structures stimulated were as follows: (1) assay for acetylcholine: nucleus commissurae posterioris, nucleus ventralis lateralis, nucleus supragenicularis, nucleus caudatus; (2) assay for 3-hydroxytyramine: nucleus ventralis lateralis, globus pallidus medialis, nucleus caudatus, substantia nigra, nucleus medialis dorsalis, putamen, nucleus ventralis posteromedialis.

DISCUSSION

Anatomical relationships. There seems to be general agreement that a major efferent pathway of the nucleus centromedianus (CM) leads to the corpus striatum (caudate nucleus and putamen) (Vogt & Vogt, 1941 *et seq.*); there is, however, uncertainty about whether both of these structures receive the projection fibres. Some authors (e.g. Powell & Cowan, 1956) have failed to find evidence for connexions from CM to caudate; while others (Hassler, 1948) have reported that the large-celled portion of the nucleus projects only to the caudate. The electrical records obtained in the present study would favour the latter view, and the fact that a specific liberation of 3-hydroxytyramine was obtained from the caudate nucleus when CM was stimulated would also indicate the interconnexion.

The relation between the nucleus ventralis anterior (VA) and the caudate is even less clear. Von Monakow (1895) described a connexion between VA and the corpus striatum, but apparently regarded its fibres as striofugal. However, later workers have felt that thalamostriate fibres arising from this nucleus may be present as well. The evidence strongly suggests that there is a considerable cortical projection from VA particularly to the frontal regions (Freeman & Watts, 1947) and it is therefore possible that the effects which have been observed here could be attributable to an indirect action through the cortex rather than to a direct one from the nucleus itself projecting to the caudate. That there is a clear relation between cortical and caudate activity following stimulation of VA is clear from Fig. 1, in which the recruiting type of response described earlier as appearing in the caudate nucleus is seen to be present also in the sigmoid gyrus.

This consideration raises the question of the anatomical evidence for a cortical projection to the caudate nucleus. Although there has been considerable controversy upon this subject in the past, the existence of corticocaudate fibres especially from the frontal regions now seems accepted (Walker, Andy & Poggio, 1955). The evocation of a complex electrical response in the caudate nucleus upon stimulation of the sigmoid gyrus (Fig. 2) would confirm the existence of a functional pathway. The

resemblance of this response to the well known 'spindles' evoked in the cortex following stimulation of the caudate nucleus (Buchwald, Wyers, Okuma & Heuser, 1961) may be noted as suggestive of a close two-way connexion between the structures involved.

Liberation of possible transmitters. It is axiomatic that a transmitter of synaptic activity must be liberated upon stimulation, and the detection of this event is necessary to the definition of any synapse as mediated by a given transmitter substance. In the present experiments it has been

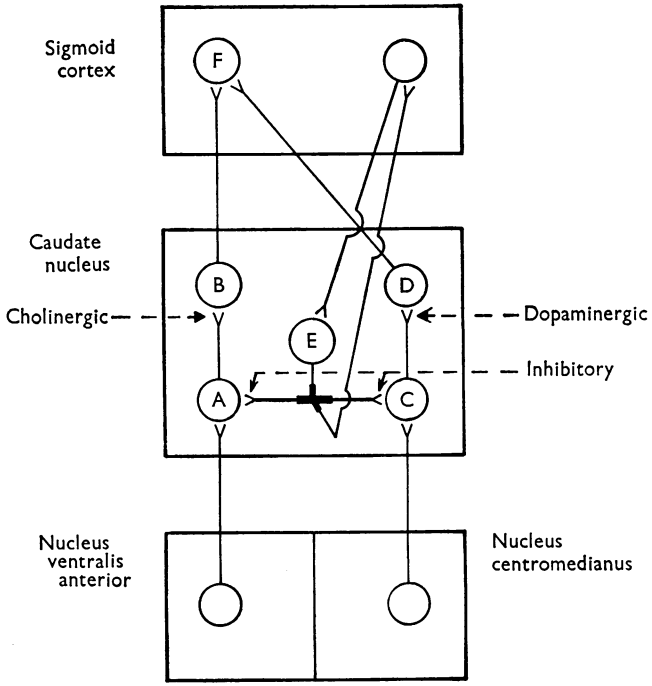

Fig. 4. Diagram representing possible neuronal relations of and within the caudate. See text for further details.

demonstrated that both acetylcholine and 3-hydroxytyramine are released into a fluid bathing a portion of the caudate nucleus in the absence of deliberately applied stimulation. This accords with the statement of Beleslin, Carmichael & Feldberg (1963) that most of the acetylcholine liberated in the anterior horns of the ventricular system originates from the caudate nucleus. The amounts of both materials liberated are specifically enhanced through the activation of structures which can be shown to influence the electrical activity of the caudate nucleus. Thus stimulation of VA increased the liberation of acetylcholine but not of 3-hydroxytyramine, while the reverse was true for excitation of CM.

These findings suggest, but certainly do not prove, that the final synapses in the VA-caudate and CM-caudate pathways, however complex these may be, are respectively cholinergic and 'dopaminergic'. It is noteworthy that the concentration both of acetylcholine and of 3-hydroxytyramine in the caudate nucleus is conspicuously high in comparison with other areas of the central nervous system (McLennan, 1963). In these experiments stimulation of the anterior sigmoid gyrus has not resulted in the unequivocal liberation of either of these materials, but the enhanced outputs due to thalamic stimulation could be completely suppressed by this cortical activation.

A possible mechanism to explain these results is shown diagrammatically in Fig. 4. This scheme would postulate the existence within the head of the caudate nucleus of five different types of neurones concerned with the processes under discussion here. The cells A are those directly excited by stimulation of VA, and their endings release acetylcholine upon cells B. These latter show a marked recruiting response and project to the cortex, there to impinge upon neurones F which also show recruiting. An analogous pathway from CM involves cells C, which are directly excited by CM and whose endings release 3-hydroxytyramine upon cells D. These do not show the recruiting phenomenon, but it is suggested that their projections may be to the same group of cortical cells F showing recruiting since cortical recruiting responses following CM stimulation are well recognized (e.g. Dempsey & Morison, 1942). Stimulation of the caudate nucleus itself is also known to produce cortical recruiting responses (Stoupel & Terzuolo, 1954). Stimulation of the cortex results in the activation of cells E which respond by a long-lasting 'spindle burst' (Fig. 2), and the endings of E act through the release of an unknown transmitter upon cell types A and C respectively to inhibit the release of acetylcholine and of 3-hydroxytyramine, when these cells are activated from the thalamus. In addition it is tentatively suggested that collateral fibres from cells E project back to the cortex, there to initiate the typical caudate-induced 'spindles'. The close connexion between the 'spindles' induced in the cortex by stimulation of the caudate, and in the caudate from the cortex, seems well established (Stoupel & Terzuolo, 1954; Wieck, Kuhn, Kohlmann, Huffmann & Stammler, 1961). In addition to providing an explanation for the observations made in the course of this study the scheme also accords with the findings of Traczyk & Sadowski (1962) that: (*a*) cortical recruiting following stimulation of the caudate nucleus involves a cholinergic step (stimulation of cell-type A in Fig. 4), and (*b*) in spite of many similarities between the cortical responses of recruiting and of 'spindle bursts', they depend in fact upon distinct neuronal processes. It must be mentioned, however, that Stoupel & Terzuolo (1954) do not accept the concept of the

intervention of the caudate nucleus in the development of cortical recruiting responses following thalamic stimulation. Nevertheless, their published records do show a shorter latency for activity in the caudate nucleus than in the cortex following excitation of VA.

SUMMARY

1. A resting release of acetylcholine and of 3-hydroxytyramine from the caudate nuclei of anaesthetized cats has been observed.

2. The output of acetylcholine could be increased by low frequency stimulation of the thalamic nucleus ventralis anterior, and that of 3-hydroxytyramine by excitation of the nucleus centromedianus. These enhanced outputs were prevented by excitation of points in the anterior sigmoid gyrus of the cortex.

3. The possible function of acetylcholine and of 3-hydroxytyramine as mediators of synaptic transmission in the caudate nucleus is discussed.

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