Acetylcholine and choline in cerebrospinal fluid of patients with Parkinson's disease and Huntington's chorea

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SYNOPSIS Lumbar cerebrospinal fluid (CSF) acetylcholine (ACh) and choline (Ch) levels were measured in patients with Huntington's chorea (N=11), Parkinson's disease (N=8), and subjects at risk for Huntington's chorea (N=4), and all three groups were found not to differ significantly from normal controls (N=10). The values found for lumbar CSF ACh and Ch levels in the normal subjects were comparable with previously reported values. The use of physostigmine, a cholinesterase inhibitor, in collecting the CSF samples did not appear to make a difference with regard to ACh and Ch concentrations. Evidence suggesting a ventricular-lumbar gradient, with lumbar CSF Ch concentration being less than ventricular CSF Ch concentration, was found. Finally, ACh levels in CSF did not correlate with corresponding Ch levels.

To date it has been very difficult to determine whether acetylcholine (ACh) is a normal constituent of cerebrospinal fluid (CSF) in man. Some investigators found that ACh could be measured in the CSF of patients with epilepsy, craniocerebral trauma, and those receiving electroshock treatment, but not in non-schizophrenic psychiatric patients who did not receive electroshock treatment, and not in other patients who may have been neurologically normal (Tower and McEachern, 1949a, b). Others, on the other hand, detected ACh in the CSF of normal subjects but not in certain neuropsychiatric patients such as schizophrenics (Poloni, 1951). An important problem in settling this issue is that the neurotransmitter does not seem to accumulate in CSF to such a degree as to be readily quantitated, and the fact that CSF contains both non-specific and specific cholinesterase activity (Tower and McEachern, 1949c; Jefferson, 1954) is probably important. When ACh or ACh-like activity has been measured in CSF using various bioassay methods, its con-

centration has been found to range from 0.0001 to 1.0 μM , with most values centring around 0.01 to 0.10 μM (Schain, 1960; Duvoisin and Dettbarn, 1967).

Because of the difficulty in measuring ACh in CSF, some investigators have looked at the major metabolite of ACh, choline (Ch). There is a certain amount of evidence to indicate that Ch in CSF may be used as an indicator of ACh turnover in the brain (Aquilonius *et al.*, 1970). The average concentrations of Ch that have been measured in human CSF range from 0.5 to 4.0 μM , depending on the investigator and the assay used (Aquilonius *et al.*, 1970; Aquilonius *et al.*, 1972). The assays include some type of bioassay such as was used by Bowers (1967), or an enzymatic-radioisotope method such as was used by Jonsson *et al.* (1969). Recently, a highly sensitive technique, integrated gas chromatography/mass spectrometry (GC/MS), has been applied by Jenden *et al.* (1973) to detect and accurately quantitate small amounts of biochemical compounds such as ACh and Ch in tissue samples.

One aim of the present study was to utilize the GC/MS technique to estimate not only Ch

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levels but also the very low ACh levels found in the CSF of human subjects, and to compare these results with previously obtained values. A second aim was to compare the ACh and Ch levels found in normal humans with those values found in patients with Huntington's chorea and patients with Parkinson's disease. Evidence has been accumulating suggesting that the cholinergic activity of the striatum (the caudate and the putamen) may be either relatively or possibly absolutely increased in Parkinson's disease (Calne, 1970; Hornykiewicz, 1971), and decreased in Huntington's chorea (Aquilonius and Sjostrom, 1971). In the case of Huntington's chorea, a cholinergic hypofunctioning could result from the drop-out of cholinergic striatal interneurons; with Parkinson's disease, the postulated hyperactivity might follow the release of cholinergic neurones in the striatum from dopaminergic inhibition. Further, Aquilonius *et al.* (1972) found a significant difference in Ch between patients with Huntington's chorea and controls, with the Huntington's chorea patients having lower values, but they found no difference between controls and patients with Parkinson's disease.

A third objective has been to determine if there exists a gradient in Ch concentration in the CSF from a higher ventricular value to a lower lumbar value as suggested by Bowers (1967). We have attempted to verify this in two different ways: (1) by comparing the average Ch levels in human ventricular CSF with the average Ch levels in lumbar CSF—that is, repeating what Bowers did—and (2) by comparing the Ch level in a sample of CSF taken soon after the initial lumbar puncture on a given patient with the level in a sample taken from 1 to 30 ml after the early sample. If a gradient truly exists, one might expect an early sample to contain less Ch than a later sample since a late sample represents CSF which, theoretically, more recently passed through the ventricles.

METHODS

PATIENTS Cerebrospinal fluid was obtained from six different patient populations:

1. *Lumbar CSF from 11 patients with Huntington's chorea* Ten were ambulatory outpatients, and one was bedridden in a nursing home. The duration of

illness ranged from two to nine years with a mean of 5.5 years. The patients' ages were from 43 to 64 years (mean of 51 years) and six were males. Dementia and choreoathetosis were graded as being absent, mild, moderate, or severe by a neurologist. One of the patients was on haloperidol (Haldol), 4 mg/day, and reserpine, 0.50 mg/day; another was on reserpine, 6 mg/day, but the drug was discontinued two days before removal of CSF.

2. *Lumbar CSF from four patients at-risk for Huntington's chorea* They were the teenage children of a single patient who had Huntington's chorea, and thus each had a 50% chance of ultimately manifesting the disease. Their ages were 16, 18, 19, and 20 years; three were males.

3. *Lumbar CSF from eight patients with Parkinson's disease* The patients' ages ranged from 43 to 77 years (mean of 62 years) and four were males. The duration of illness ranged from three to 21 years and averaged 7.5 years. Five patients had mild bilateral tremor and/or rigidity (stage II in the classification system of Hoehn and Yahr (1967)) and three had more severe tremor and rigidity and impaired righting reflexes (stage III). Five were on regular L-dopa medication ranging from 2.0 g/day to 7.5 g/day, and two were on Carbidopa, which is L-dopa plus a peripherally acting DOPA-decarboxylase inhibitor. One patient was taking 500 mg L-dopa and 50 mg inhibitor daily, and the other was taking 750 mg L-dopa and 75 mg inhibitor per day. Three patients were taking anticholinergic agents; one, trihexiphenidyl (Artane), 6 mg/day; another, procyclidine (Kemidrin), 10 mg/day; and the third, benzotropine (Cogentin), 4 mg/day. One patient had been off all anti-Parkinsonism drugs for four days at the time a CSF sample was taken.

4. *Lumbar CSF from 10 control subjects* These individuals were either spouses or friends of the patients with Huntington's chorea and were free of neurological disease. The ages of the control subjects were from 24 to 69 years (mean of 46 years) with five being males.

5. *Ventricular CSF from six patients undergoing ventriculograms* These individuals had the following diagnosis: neurofibromatosis, obstructive hydrocephalus (two patients), pseudotumor cerebri, myelomeningocele, and astrocytoma. Their ages ranged from 2 weeks to 45 years (mean of 20 years) with four being males.

6. *Lumbar CSF from 30 miscellaneous patients taken during course of pneumoencephalogram or myelo-*

gram Ten patients were ultimately considered neurologically normal, while 20 were found to have various neurological disorders including old poliomyelitis, presenile dementia, olivopontocerebellar atrophy, pituitary adenoma (two), cerebral palsy, seizure disorder (four), traumatic spinal cord injury (three), Guillain-Barré syndrome, cerebral atrophy (two), meningioma, headaches with elevated CSF protein, viral encephalitis, and posterior fossa teratoma. The 26 adults received ephedrine, droperidol, and/or meperidine as pretest medications; the four children were anaesthetized with cyclohexamine (Ketamine). The ages of the patients ranged from 3 to 68 years (mean of 42 years) with 15 being males.

SAMPLE COLLECTION Samples of CSF were collected in tubes containing 0.05 ml 6×10^{-3} molar physostigmine salicylate, a cholinesterase inhibitor which was added to protect the ACh from enzymatic breakdown. Further analysis, however, suggested that this did not make a significant difference in ACh or Ch values (see Results section), and thus was not done in the patients with Huntington's chorea, the normal controls, and in some patients with Parkinson's disease. After collection, all samples were immediately frozen and kept at 0°C until analysed.

In the 30 miscellaneous patients who underwent pneumoencephalograms or myelograms, sometimes up to 35 ml CSF was removed in small aliquots. It was therefore possible to obtain an early sample of

CSF taken soon after the initial lumbar puncture, and a late sample taken 1.0 to 30 ml later.

ACh AND Ch ANALYSIS The samples were thawed and any samples that were grossly bloody were discarded. As Aquilonius *et al.* (1972) reported for choline, and as was confirmed in this study for ACh and Ch, microscopic blood contamination (range of 1 to 145 cells/ml) did not appear to correlate with Ch or ACh values. The samples were then centrifuged in a clinical centrifuge and a 0.5 ml aliquot of CSF supernatant was drawn off for analysis. Each sample was run in duplicate. Levels of ACh and Ch were measured using the GC/MS method used by Freeman *et al.* (1975). Simply put, the method is one in which endogenous amounts of ACh and Ch in a tissue or fluid are determined by an isotope dilution assay using known amounts of deuterium-labelled variants of ACh and Ch as internal standards and determining the isotope ratio for each compound by GC/MS.

RESULTS

The mean values of two trials for ACh ranged from 0.02 to 0.07 μM and from 2.31 to 3.35 μM for Ch (Table 1). Choline was present, therefore, in a concentration about 50–60 times the concentration of ACh. The presence or absence of physostigmine, a cholinesterase inhibitor, in

TABLE 1
SUMMARY OF ACh AND Ch LEVELS IN VARIOUS PATIENT GROUPS STUDIED

Patient group	Site of CSF removal*	Mean (μM)	SEM† (μM)	N	Range
<i>Acetylcholine</i>					
Normal controls	L	0.07	0.02	10	0.00–0.13
Huntington's chorea	L	0.05	0.02	11	0.00–0.18
At-risk for Huntington's chorea	L	0.04	0.01	4	0.00–0.11
Parkinson's disease	L	0.07	0.04	8	0.00–0.32
Ventriculogram	V	0.04	0.01	6	0.00–0.10
Miscellaneous‡	L	0.06	0.01	30	0.00–0.18
<i>Choline</i>					
Normal controls	L	3.35	0.38	10	2.41–6.30
Huntington's chorea	L	2.92	0.24	11	1.79–4.03
At-risk for Huntington's chorea	L	3.07	0.13	4	2.67–3.27
Parkinson's disease	L	3.27	0.53	8	1.56–6.45
Ventriculogram	V	3.20	0.40	6	1.86–4.86
Miscellaneous‡	L	2.86	0.11	30	1.55–3.89

All groups not significantly different from normal controls using Student's *t* test with $P=0.05$ as level of significance.

* L = Lumbar. V = Ventricle.

† SEM = standard error of the mean.

‡ Since each patient in this group had both an early and late sample of lumbar CSF taken, it was necessary to average the ACh (Ch) levels of the early and late samples to obtain a single ACh (Ch) level for that patient. These individual averaged values were then used in calculating the mean for the group.

the CSF collecting tube did not seem to make a significant difference in the ACh and Ch levels measured. This followed from the finding that those groups of patients whose CSF was not collected using physostigmine—that is, the Huntington's chorea patients, the subjects at-risk for Huntington's chorea, and the normal control subjects—did not tend to have lower ACh or higher Ch levels in the CSF than those groups whose CSF was collected using the cholinesterase inhibitor—that is, the miscellaneous patients, the ventriculogram patients, and most of the patients with Parkinson's disease. Furthermore, there was no obvious relationship within the Parkinson's disease group between CSF ACh and Ch levels and the presence or absence of physostigmine in the collecting tubes.

The normal control group had a mean value of ACh in lumbar CSF of 0.07 μM with all values ranging from 0.00 to 0.13 μM . Two of the 10 normal control subjects had no detectable levels of ACh in their CSF. The mean level of Ch in this same group was 3.35 μM ; most of the values fell within the narrow range of 2.41 to 3.14 μM but two samples were found to have 4.61 and 6.30 μM , resulting in an overall mean of 3.35 μM .

As shown in Table 1, both mean ACh and Ch levels in lumbar CSF of patients with Huntington's chorea (0.05 and 2.92 μM , respectively) were found to be less than the corresponding levels found in the normal controls but these differences were not significant at the $P=0.05$ level. Duration of illness did not correlate significantly with individual CSF ACh or Ch levels in the patients with Huntington's chorea. Although the severity of the disease could not completely explain the individual differences in ACh and Ch levels in these patients, there was one patient with Huntington's chorea with the highest combined dementia-chorea score who also had the second lowest CSF Ch level and the highest ACh level. This same patient was the one on haloperidol (4 mg/day) and reserpine (0.50 mg/day). Finally, the four subjects at risk for Huntington's chorea all had ACh and Ch values that varied little from each other and as a group they did not have ACh or Ch concentrations significantly different from the normal controls (Table 1).

In the group with Parkinson's disease there was also no significant difference found between

TABLE 2
STAGE OF PARKINSON'S DISEASE
AND CSF ACh AND Ch LEVELS

Acetylcholine (μM)		Choline (μM)	
Stage II	Stage III	Stage II	Stage III
0.01	0.12	3.33	6.45
0.00	0.32	3.06	4.17
0.01	0.06	1.56	2.23
0.01		3.03	
0.01		2.35	
Mean			
0.01	0.17	2.66	4.28
$P < 0.05$ (by Student's <i>t</i> test)		$P > 0.05$ (by Student's <i>t</i> test)	

the ACh or Ch concentrations in lumbar CSF of patients with Parkinson's disease (0.07 and 3.27 μM respectively) when compared with normal controls (Table 1). The type of drug therapy—that is, the use of anticholinergics, the amount of L-dopa, the use of a decarboxylase inhibitor—did not significantly influence individual ACh and Ch levels. It should be noted, however, that the highest ACh value was found in the one patient who was on no L-dopa or anticholinergics at the time CSF was obtained. The duration of illness did not correlate with individual levels of CSF ACh and Ch. The severity of the disease, however, did seem to make a difference; two of the three patients with Parkinson's disease who were rated as being stage III in severity of their disease had ACh and Ch concentrations con-

TABLE 3
ACh AND Ch LEVELS
IN EARLY LUMBAR CSF* VS VENTRICULAR CSF†

	Mean (μM)	S.E.M. (μM)	N	Range (μM)
<i>Acetylcholine</i>				
Early lumbar CSF	0.05	0.01	30	0.00–0.22
Ventricular CSF	0.04	0.01	6	0.00–0.10
(P > 0.05 by Student's <i>t</i> test)				
<i>Choline</i>				
Early lumbar CSF	2.31	0.13	30	1.22–3.46
Ventricular CSF	3.20	0.40	6	1.86–4.86
(P < 0.05 by Student's <i>t</i> test)				

* As represented by CSF samples from miscellaneous patients (see definition in text) taken before 10 ml CSF had been drained off.

† As represented by CSF samples from patients undergoing ventriculograms.

FIGURE Relationship of CSF Ch concentration (μM) to CSF volume sequence (ml) ($N=30$).

siderably higher than the five patients who were rated stage II (Table 2).

The mean level of Ch in samples of ventricular CSF obtained from patients undergoing ventriculograms was not significantly different from the mean lumbar Ch levels in the normal controls (Table 1). However, the CSF from the latter group was taken in every instance after 10 ml of fluid had been drained off and discarded. If one considered the early samples of CSF—that is, samples taken before 10 ml of fluid had been drained off—taken from the miscellaneous patients more accurately to represent lumbar CSF, then the difference between ventricular CSF Ch, and lumbar CSF Ch was significant (3.20 vs 2.31 μM , respectively, which significantly differed with $P<0.05$) (Table 3). Unlike Ch, ACh levels in ventricular CSF were not significantly different from ACh levels in early lumbar CSF (Table 3).

Of the 30 miscellaneous patients from whom both an early sample and a late sample of CSF was obtained, 24 had the early sample containing a lower Ch concentration than the later sample. A chi squared test with a Yates correction showed this to be significant with $P<0.01$. In addition, the volume sequence of a CSF sample—that is, how many ml after the initial lumbar puncture the sample was taken—

correlated positively with the Ch concentration in that sample with a Pearson-product-moment correlation coefficient of 0.576 which was significant with $P<0.01$ (Figure). Such a difference between early and late samples was not found with ACh concentrations.

Ten of the 30 miscellaneous patients who ultimately were shown to have no demonstrable CNS disease did not have significantly different values for CSF Ch when compared with the remaining 20 patients who were known to have a definite CNS disorder (3.05 vs 2.76 μM ; t test showed $P>0.05$). Similarly, no appreciable difference was found between these two groups with regard to CSF ACh (0.06 vs 0.06 μM ; t test showed $P>0.05$).

Finally, the possibility that there existed some type of a relationship between ACh and Ch levels in CSF—for example, does ACh tend to be high when Ch is high?—was investigated. The results of the Pearson-product-moment correlation analysis indicated that for each group of patients, as well as generally for all patients as a whole, the Ch level in a given CSF sample tended to be quite independent of the corresponding ACh level in that sample ($P>0.05$).

DISCUSSION

It indeed appears that ACh is a normal con-

stituent of CSF in human subjects as evidenced by the definite although low level of ACh found in the normal control subjects. This level is in good agreement with that found by Duvoisin and Dettbarn (1967) using a much improved bioassay. Similarly, the mean Ch level in normal lumbar CSF found in the present study confirms previously reported values for CSF Ch (Bowers, 1967; Aquilonius *et al.*, 1970; Aquilonius *et al.*, 1972).

Because cholinergic mechanisms in the caudate nucleus and putamen appear to be involved in both Huntington's chorea and Parkinson's disease, it was initially suspected that CSF ACh and/or Ch in these two disorders might be different from levels in normal subjects. It is well established that ACh concentration in the striatum is significant (Campbell and Jenden, 1970; Butcher and Butcher, 1974) as is acetylcholinesterase and choline acetylase. Striatal ACh is concentrated in nerve endings, or at least in the subcellular fraction on ultracentrifugation which contains synaptic vesicles (Laverty *et al.*, 1963). It now appears that the cells of origin of these ACh-containing synaptic terminals belong entirely or almost entirely to one class of caudate interneurons, for damaging the areas which project to the striatum (the thalamus, cerebral cortex, and ventral midbrain tegmentum) does not materially alter striatal choline acetylase (McGeer *et al.*, 1971), an enzyme primarily confined to cholinergic neurones. In Huntington's chorea there is a major loss of striatal neurones (Bruyn, 1968), the brunt of the loss being to interneurons. This conclusion stems from Golgi and electron microscopic studies of the cat striatum which show that about 98% of striatal neurones are interneurons, and about 96% of the total cells are of one morphological cell type, the medium spiny neurone (Kemp and Powell, 1971a, b). In Huntington's chorea there is a reduction in choline acetylase of from 50–99% in the striatum in most cases (Bird *et al.*, 1973; McGeer *et al.*, 1973; Stahl and Swanson, 1974). A sizeable reduction in striatal ACh or Ch might therefore be reflected in the lumbar CSF; we did not find either, although Aquilonius *et al.* (1972) did find significantly lowered Ch as compared with controls. The discrepancy between their findings and those of the present study might be explained by the fact that their

patients may have had more advanced disease. Their patients were all inpatients unlike most of ours; the average duration of illness in their series was 9.2 years as compared with 5.2 years for ours; and more appeared to have severe dementia. However, the lack of correlation between Ch levels and duration and severity of illness in our own series makes this explanation unlikely.

Assuming that our results are correct, how can they be explained? There is little doubt that ACh metabolism is reduced in the striatum of patients with Huntington's chorea. If this is so, then lumbar CSF Ch and ACh do not reflect striatal ACh metabolism.

In Parkinson's disease, no direct observations on striatal ACh or its metabolites or enzymes have been shown to be abnormal. However, anticholinergic medication causes clear, mild to moderate benefit to symptoms of Parkinsonism. In human Parkinson's disease the dopamine content of the substantia nigra (Hornykiewicz, 1963) and the striatum (Bernheimer *et al.*, 1963) is greatly decreased, and the large pigmented nigral neurones are much reduced in number (Hassler, 1955). In animals it has been shown that nigral neurones containing dopamine project to the striatum and branch many times before terminating in irregular varicose endings. It is not known precisely on which cells these endings synapse, but the character of the endings, their widespread and dense distribution make it likely that they terminate on the medium sized spiny neurones which constitute some 96% of striatal cells. For reasons given above, some of these interneurons are almost surely cholinergic. A loss of dopamine, a neurotransmitter with possibly inhibitory transmitter function, could lead to overactivity of the post-synaptic cells, some of which contain ACh. If these several hypotheses be correct, then in Parkinson's disease there may be a relative or absolute overactivity of striatal cholinergic cells; and an increased ACh or Ch might possibly be expected in nearby CSF. However, we did not find a significant difference in CSF ACh and Ch levels between patients with Parkinson's disease as a group and the normal subjects, which is in agreement with Aquilonius *et al.* (1972) with regard to Ch. As with the inverse model of Huntington's chorea, this strongly suggests that

lumbar CSF ACh and Ch do not reflect striatal metabolism of these substances. A finding that is difficult to explain and should be noted is that although as a group the patients with Parkinson's disease did not differ from normal subjects, two of the three stage III patients with Parkinson's disease had Ch and ACh levels appreciably higher than both the normal subjects and the remaining Parkinson's disease patients who were stage II. Examination of the CSF from patients with stage IV and V of Parkinson's disease would seem indicated.

The evidence in favour of a major part of CSF Ch being derived from ACh metabolism in the brain has been summarized by Aquilonius *et al.* (1970). However, recent evidence has been found to question this theory (Schuberth and Jenden, 1975). It seems that other possibilities such as CSF Ch being derived from plasma, or CSF Ch representing a breakdown product of brain phospholipids can still not be excluded. The negative findings in this study support the view that lumbar CSF Ch is not representative of ACh metabolism in the brain.

There are some findings in the present study that fit with the theory that CSF Ch is derived primarily from the brain, although not necessarily the striatum or ACh metabolism. It was found that 80% of the time a small sample of CSF taken early after a lumbar puncture in a given patient had a lower Ch level than did a sample taken in the same patient anywhere from 1.0 to 30 ml after the early sample. In addition, the Ch levels in the early lumbar CSF samples from the miscellaneous patients were found to be significantly lower than the Ch concentrations in ventricular CSF samples of other patients. Both these findings suggest, as did the finding of Bowers (1967), that a ventricular-lumbar gradient exists in man with the ventricular CSF Ch being higher than lumbar CSF Ch. They also suggest that an important source of Ch in CSF is the brain surrounding the ventricles. The biochemical source within the brain, however, does not necessarily have to be ACh; it might be plasma Ch, brain phospholipids such as phosphatidyl choline, or some other unknown source. Regardless of the chemical or anatomical source, a gradient such as was found could also be due to the removal of Ch from CSF as it passed from the ventricles to the spinal sub-

arachnoid space. Such a transport process for Ch in CSF has already been described and studied in dogs and rabbits (Aquilonius and Windbladh, 1972).

In contrast with Ch, ventricular *vs* lumbar and early lumbar *vs* late lumbar differences were not found with CSF ACh. This finding agrees with that of Turner and Mauss (1959) who also found ACh levels in ventricular CSF to be essentially the same as those levels in lumbar CSF, each being about 0.04 μM . The most reasonable explanation for this is the fact that cholinesterase is present throughout the whole CSF system. Thus, ACh in ventricular fluid and in spinal fluid would be hydrolyzed to a universally low level, reducing any differences which may have existed initially. A second possibility may be that ACh levels in CSF are unrelated to Ch levels in CSF and just because Ch differences were found between lumbar and ventricular CSF does not mean that ACh differences should also be expected. Support for this notion is the finding that ACh levels in lumbar CSF bore no relationship with Ch levels.

As has been evident throughout this discussion, the question of whether or not lumbar CSF ACh and Ch truly reflect ACh turnover in the brain must be answered before the results of this study can be fully understood. Further studies in this area, however, should take into account an apparent ventricular-lumbar gradient for CSF Ch in man.

REFERENCES

- Aquilonius, S.-M., Nystrom, B., Schuberth, J., and Sundwall, A. (1972). Cerebrospinal fluid choline in extra-pyramidal disorders. *Journal of Neurology, Neurosurgery, and Psychiatry*, **35**, 720-725.
- Aquilonius, S.-M., Schuberth, J., and Sundwall, A. (1970). Choline in the cerebrospinal fluid as a marker for the release of acetylcholine. In *Drugs and Cholinergic Mechanisms in the CNS*, pp. 399-410. Edited by E. Heilbronn and A. Winter. Forsvarets Forskningsanstalt: Stockholm.
- Aquilonius, S.-M., and Sjostrom, R. (1971). Cholinergic and dopaminergic mechanisms in Huntington's chorea. *Life Sciences*, pt 1, **10**, 405-414.
- Aquilonius, S.-M., and Windbladh, B. (1972). Cerebrospinal fluid clearance of choline and some other amines. *Acta Physiologica Scandinavica*, **85**, 78-90.
- Bernheimer, H., Birkmayer, W., and Hornykiewicz, O. (1963). Zur biochemie des Parkinsonsyndroms des menschen. *Klinische Wochenschrift*, **41**, 465-469.
- Bird, E. D., Mackey, A. V. P., Rayner, C. N., and Iversen, L. L. (1973). Reduced glutamic acid decarboxylase activity of post-mortem brain in Huntington's chorea. *Lancet*, **1**, 1090-1092.

- Bowers, M. B. (1967). Choline in cerebrospinal fluid. *Life Sciences*, 6, 1927-1933.
- Bruyn, G. W. (1968). Huntington's chorea: historical, clinical and laboratory synopsis. In *Handbook of Clinical Neurology*, vol. 6, pp. 298-378. Edited by P. J. Vinken and G. W. Bruyn. American Elsevier: New York.
- Butcher, S. G., and Butcher, L. L. (1974). Acetylcholine and choline levels in the rat corpus striatum after microwave irradiation. *Proceedings of the Western Pharmacology Society*, 17, 37-40.
- Calne, D. B. (1970). *Parkinsonism: Physiology, Pharmacology and Treatment*. Arnold: London.
- Campbell, L. B., and Jenden, D. J. (1970). Gas chromatographic evaluation of the influence of oxotremorine upon the regional distribution of acetylcholine in the rat brain. *Journal of Neurochemistry*, 17, 1697-1699.
- Duvoisin, R. C., and Dettbarn, W.-D. (1967). Cerebrospinal fluid acetylcholine in man. *Neurology (Minneapolis)*, 17, 1077-1081.
- Freeman, J. J., Choi, R. L., and Jenden, D. J. (1975). Plasma choline: its turnover and exchange with brain choline. *Journal of Neurochemistry*, 24, 729-734.
- Hassler, R. (1955). The pathological and pathophysiological basis of tremors and Parkinsonism. *Proceedings of the Second International Congress of Neuropathology*, Excerpta Medica Foundation: Amsterdam, pp. 29-40.
- Hoehn, M. M., and Yahr, M. D. (1967). Parkinsonism: onset, progression and mortality. *Neurology (Minneapolis)*, 17, 427-442.
- Hornykiewicz, O. (1963). Die topische Lokalisation und das Verhalten von Noradrenalin und Dopamin (3-Hydroxytryptamine) in der Substantia nigra des normalen und Parkinsonkranken Menschen. *Wiener Klinische Wochenschrift*, 75, 309-312.
- Hornykiewicz, O. (1971). Neurochemical pathology and pharmacology of brain dopamine and acetylcholine: rational basis for the current treatment of Parkinsonism. In *Recent Advances in Parkinson's Disease*. Edited by C. H. Markham and F. H. McDonnell. Davis: Philadelphia.
- Jefferson, M. (1954). The cholinesterase activity of cerebrospinal fluid. *Clinical Science*, 13, 599-605.
- Jenden, D. J., Roch, M., and Booth, R. A. (1973). Simultaneous measurement of endogenous and deuterium-labelled tracer variants of choline and acetylcholine in sub-picogram quantities by gas chromatography/mass spectrometry. *Analytical Biochemistry*, 55, 438-448.
- Jonsson, L. E., Schuberth, J., and Sundwall, A. (1969). Amphetamine effect on the choline concentration of human cerebrospinal fluid. *Life Sciences*, pt 1, 8, 977-981.
- Kemp, J. M., and Powell, T. P. S. (1971a). The structure of the caudate nucleus of the cat: light and electron microscopy. *Philosophical Transactions of Royal Society of London, B*, 262, 383-401.
- Kemp, J. M., and Powell, T. P. S. (1971b). The synaptic organization of the caudate nucleus. *Philosophical Transactions of Royal Society of London, B*, 262, 403-412.
- Laverty, R., Michaelson, I. A., Sharman, D. F., and Whittaker, V. P. (1963). The subcellular localization of dopamine and acetylcholine in the dog caudate nucleus. *British Journal of Pharmacology*, 21, 482-490.
- McGeer, P. L., McGeer, E. G., and Fibiger, H. C. (1973). Choline acetylase and glutamic acid decarboxylase in Huntington's chorea. *Neurology (Minneapolis)*, 23, 912-917.
- McGeer, P. L., McGeer, E. G., Fibiger, H. C., and Wickson, V. (1971). Neostriatal choline acetylase and cholinesterase following selective brain lesions. *Brain Research*, 35, 308-314.
- Poloni, A. (1951). L'acetilcolinia nel liquor dei malati di mento. *Cervello*, 27, 81.
- Schain, R. J. (1960). Neurohumors and other pharmacologically active substances in CSF: a review of the literature. *Yale Journal of Biology and Medicine*, 33, 15-36.
- Schuberth, J., and Jenden, D. J. (1975). Transport of choline from plasma to cerebrospinal fluid in the rabbit with reference to the origin of choline and to acetylcholine metabolism in brain. *Brain Research*, 84, 245-256.
- Stahl, W. L., and Swanson, P. D. (1974). Biochemical abnormalities in Huntington's chorea brains. *Neurology (Minneapolis)*, 24, 813-819.
- Tower, D. B., and McEachern, D. (1949a). Acetylcholine and neuronal activity. 1. Cholinesterase patterns and acetylcholine in the cerebrospinal fluid of patients with cranio-cerebral trauma. *Canadian Journal of Research*, 27, 105-119.
- Tower, D. B., and McEachern, D. (1949b). Acetylcholine and neuronal activity. 2. Acetylcholine and cholinesterase activity in the cerebrospinal fluids of patients with epilepsy. *Canadian Journal of Research*, 27, 120-131.
- Tower, D. B., and McEachern, D. (1949c). The content and characterization of cholinesterases in human cerebrospinal fluids. *Canadian Journal of Research*, 27, 132-145.
- Turner, W., and Mauss, E. (1959). Serotonin (5-hydroxytryptamine) and acetylcholine in human ventricular and spinal fluids. *Archives of General Psychiatry*, 1, 646-650.