Cerebrospinal fluid choline in extrapyramidal disorders¹

S-M. AQUILONIUS², B. NYSTRÖM, J. SCHUBERTH, AND A. SUNDWALL

From the Research Institute of National Defence, Department 1, Sundbyberg; Department of Neurosurgery, University of Uppsala; Psychiatric Research Center, Ulleråker Hospital, University of Uppsala, and Department of Pharmacology, University of Uppsala, Sweden

SUMMARY Cerebrospinal fluid from patients with Parkinson's disease and Huntington's chorea has been investigated with regard to the concentration of choline. In Parkinson's disease the choline concentration of lumbar spinal fluid was not different from that of a control group, nor was it related to medication, duration of illness, or severity of symptoms. A comparison between choline in ventricular cerebrospinal fluid from patients with Parkinson's disease and with intention tremor showed no significant differences. Patients with Huntington's chorea had a lower concentration of choline in lumbar spinal fluid as compared with a control group. The results are discussed in relation to the possible sources of cerebrospinal fluid choline.

The possibilities of studying the function of neurotransmitter systems in the human brain are limited and our knowledge is to a great extent based on analogies from animal experiments. In the last years, however, biochemical analyses of the monoamine transmitters noradrenaline, dopamine, and 5-hydroxytryptamine in brain material obtained at necropsy (Ehringer and Hornykiewicz, 1960) and of their metabolites in the cerebrospinal fluid (CSF) (Guldberg, Turner, Hanieh, Ashcroft, Crawford, Perry, and Gillingham, 1967; Johansson and Roos 1967) have given new approaches to the study of these transmitters in man.

The cholinergic transmitter acetylcholine (ACh), however, cannot be investigated in brain material from necropsy due to a rapid post-mortem change of the ACh concentration. Further, investigations on ACh in the CSF have so far been rather inconclusive and its occurrence as a normal constituent of CSF has been a matter of controversy (Schain, 1960; Duvoisin and Dettbarn, 1967). The cholinergic precursor and end-product choline (Ch) is, on the other hand, detectable in the CSF in a fairly low con-

centration. We thus raised the question whether a change in the concentration of Ch in human CSF could reflect an altered cholinergic activity in the central nervous system.

In Parkinson's disease, evidence points to cholinergic dominance in the striatum due to decreased dopaminergic inhibition on cholinergic neurones in this region (Calne, 1970). In Huntington's chorea, on the other hand, it was recently suggested that a cholinergic hypofunction might exist in striatal mechanisms regulating motor activity (Aquilonius and Sjöström, 1971). As Parkinson's disease and Huntington's chorea thus could be in contrasting relationship regarding central cholinergic activity, we found it interesting to study the concentration of Ch in CSF of patients suffering from these two disorders.

METHODS

SELECTION OF PATIENTS AND CONTROLS The diagnoses of the patients suffering from Parkinson's disease were considered unmistakable in the departments of neurology or neurosurgery. In one group of 10 patients with Parkinson's disease, lumbar puncture (2-3 ml.) were performed with the patients on their usual anticholinergic medication (orphenadrine hydrochloride and/or benzhexol chloride). In 10

¹ A preliminary report of this work was presented at the XXVth Scandinavian Pharmacological Meeting, Copenhagen 1971.

² Present address: Department of Neurology, University of Uppsala, Sweden.

patients lumbar punctures were also undertaken, either before treatment had started or after withdrawal of drugs for five days. The patients exhibited more pronounced symptoms without anticholinergic therapy, which could also be documented by objective measurement of rigidity and tremor by a method earlier described (Aquilonius and Tiselius, 1969). Another group of five patients with Parkinson's disease who were on anticholinergic medication had tremor as the dominating symptom. In these patients lumbar and ventricular CSF was taken at ventriculography¹ before stereotaxic surgery. A third group of patients were on stationary L-dopa treatment when lumbar punctures were performed.

Lumbar CSF from 17 patients suffering from Huntington's chorea was analysed. These people were inpatients at different clinics and mental hospitals in Sweden. They had typical heredity and were all examined by one of the authors who considered the diagnosis to be well established. It was not feasible to withdraw drug therapy (mainly phenothiazines and/or haloperidol) in the majority of patients with Huntington's chorea. In three cases at our own clinic, drug therapy was withdrawn for five days before lumbar puncture.

Duration of illness has been estimated from the date of appearance of clinical symptoms as given in the case history. The patients with Huntington's chorea were crudely classified as one group with mild to moderate and one group with severe dementia. Patients unable to comprehend easy instructions and permitting no verbal contact were referred to the severe group.

The control group consisted of 19 persons with no sign or history of neurological disease; three of the controls were healthy volunteers, while the rest were patients submitted to the Department of General Surgery for the diagnoses shown in Table 1. The age distribution of the controls corresponded satisfactorily with that of the patients (Fig. 5). Ventricular CSF concentration of Ch was further determined in five patients with intention tremor of idiopathic origin undergoing ventriculography and in 16 patients with different diagnoses (Table 2) undergoing operation in the Department of Neurosurgery. The general anaesthetics used in the latter patients are shown in Table 2. Ventricular size was rated as normal or increased, based on the encephalographic findings, and CSF pressure was graded as normal or increased at ventricular puncture.

DETERMINATION OF CH IN CSF Choline was determined by an enzymatic method (Jönsson, Schuberth,

¹ Patients were awake at ventriculography, having been given a premedication of triethylperazine (8.6 mg) as suppository and an analgesic containing caffeine (0.2 g), phenacetin (0.6 g) orally 30 min before the examination.

TABLE 1
CSF CHOLINE CONCENTRATION IN EXTRAPYRAMIDAL DISEASE

	No.	Site of sampling	CSF-Ch (n-mole/ml. $\pm$ SEM)
Parkinson's disease			
on anticholinergic therapy	21	Lumbar	2.6 $\pm$ 0.15
tremor dominating	5	Lumbar	2.6 $\pm$ 0.19
tremor dominating	5	Ventricular	3.2 $\pm$ 0.23
no medication	10	Lumbar	2.6 $\pm$ 0.14
L-dopa therapy	4	Lumbar	2.2 $\pm$ 0.29
Huntington's chorea	17	Lumbar	1.9 $\pm$ 0.10*
on drug therapy (mainly phenothiazines)	14	Lumbar	1.9 $\pm$ 0.12*
no medication	3	Lumbar	1.7 $\pm$ 0.13*
Controls (prostatectomy, cholecystectomy, breast tumour, minor surgery, lumbago)	19	Lumbar	2.5 $\pm$ 0.09
Intention tremor	5	Ventricular	3.7 $\pm$ 0.45

* Significantly different from controls ($P < 0.01$).

and Sundwall, 1969; Schuberth and Sundwall, 1971), based on the choline acetyltransferase catalysed formation of labelled ACh from labelled acetyl-CoA and the Ch in the sample. The CSF samples were stored frozen until analysed. All samples showing macroscopic blood contamination were discarded. A microscopic blood contamination was not related to the Ch value in the CSF (Fig. 1). All Ch analyses were performed as at least duplicates on different days and Ch concentrations are given as means of those determinations.

FIG. 1. Ch concentration (n-mole/ml.) and microscopic blood contamination in 24 samples of lumbar CSF. (RBC = red blood count per mm³.)

TABLE 2
CHOLINE CONCENTRATION OF VENTRICULAR CSF IN DIFFERENT BRAIN DISORDERS

Patient	Age (yr)	Diagnosis	CSF pressure	Ventricular size	General anaesthetic	Ch (n-mole/ml.)
M.P.	2	Medulloblastoma	Increased	Slightly increased	O ₂ + N ₂ O + halothane	1.9
C.G.	4	Medulloblastoma	Increased	Increased	O ₂ + N ₂ O + halothane	5.3
E.A.	6	Medulloblastoma	Increased	Increased	O ₂ + N ₂ O + halothane	2.1
L.P.	19	Medulloblastoma	Increased	Increased	O ₂ + N ₂ O + halothane	4.0
Å.M.	1	Dandy Walker cyst	Increased	Increased	O ₂ + N ₂ O + halothane	6.5
K.K.	24	Dandy Walker cyst	Increased	Increased	O ₂ + N ₂ O + halothane	5.0
R.W.	18	Cerebellar tumour	Increased	Slightly increased	O ₂ + N ₂ O + halothane	3.4
S.N.	35	Cerebellar tumour	Increased	Abnormal	O ₂ + N ₂ O + methoxyflurane	4.8
A.N.	61	Brain-stem tumour	Increased	Slightly increased	O ₂ + N ₂ O + methoxyflurane	4.6
B.N.	42	Craniopharyngioma	Increased	Slightly increased	O ₂ + N ₂ O + halothane	5.5
I.H.	57	Inflammatory expansive process	Increased	Normal	O ₂ + N ₂ O	7.0
E.Å.	53	Cerebral glioma	Increased	Normal	O ₂ + N ₂ O	3.0
E.K.	54	Cerebral glioma	Increased	Increased	O ₂ + N ₂ O	2.4
S.J.	61	Cerebral meningioma	Increased	Increased	O ₂ + N ₂ O + halothane	3.0
G.D.	60	Hydrocephalus	Normal	Slightly increased	O ₂ + N ₂ O + halothane	4.3
A.B.	66	Cerebral atrophy	Normal	Normal	O ₂ + N ₂ O + methoxyflurane	3.5

RESULTS

The mean values of CSF Ch concentration of the different groups are summarized in Table 1. As seen from this Table there is no difference in the Ch concentration of lumbar CSF between the control group and the patients suffering from Parkinson's disease—neither with nor without anticholinergic therapy. There seemed to be no relationship between individual CSF Ch values

FIG. 2. Lumbar CSF Ch concentration (n-mole/ml.) and duration of illness in 10 patients with Parkinson's disease. No drug therapy.

FIG. 3. Lumbar CSF Ch concentration (n-mole/ml.) and rigidity objectively measured. Rigidity in ponds measured as described by Aquilonius and Tiselius (1969). Rigidity values of right and left side are added. ○ = no medication, ■ = anticholinergic therapy.

in the Parkinsonism group and duration of illness (Fig. 2) or rigidity objectively measured in some of the patients (Fig. 3). The five patients with tremor dominating showed the same mean value as the rest of the material. A somewhat lower mean value than in the controls was found in the group on L-dopa treatment (Table 1).

In the patients with Huntington's chorea, on the other hand, the mean Ch concentration of

FIG. 4. Lumbar CSF Ch concentration (n-mole/ml.) and duration of illness in 17 patients suffering from Huntington's chorea.

lumbar CSF was significantly lower than that in the controls and in the patients with Parkinson's disease. This is true for patients with and without drug therapy (Table 1). No correlation existed between lumbar CSF Ch concentration and duration of illness (Fig. 4). The same Ch concentration was found in patients with severe ($n=6$) and moderate ($n=11$) dementia (1.8 ± 0.23 and 1.9 ± 0.10 n-mole/ml respectively). Although a highly significant difference was found between the mean lumbar CFS Ch con-

FIG. 6. Frequency of lumbar CSF Ch values (n-mole/ml.) in controls, Parkinson's disease, and Huntington's chorea.

centration of controls and patients with Huntington's chorea, a considerable overlapping between the materials exists (Fig. 5). This is also seen from the frequency distribution of values (Fig. 6).

As seen from Table 1, the Ch concentration in ventricular CSF from patients with Parkinson's disease and intention tremor is somewhat higher than the lumbar CSF Ch concentration. Furthermore, in most of the ventricular CSF samples from the patients in Table 2, a higher Ch concentration than the mean lumbar concentration of the controls (Table 1) was found. The highest ventricular CSF Ch concentrations were found

FIG. 5. Lumbar CSF Ch concentration (n-mole/ml.) in controls and patients with Parkinson's disease (without medication) and patients with Huntington's chorea. Age distribution.

in cases with increased ventricular size and CSF pressure and in the case with an inflammatory process.

DISCUSSION

In the present investigation it has been shown that the lumbar CSF concentration of Ch is rather constant in man. Earlier reported values for human CSF, based on a small number of observations, range from 12–60 n-mole/ml. (Guggenheim and Löffler, 1916), to values of the same order as ours, 0.5–2.2 n-mole/ml. (Page and Schmidt, 1931) and 0.8–1.6 n-mole/ml. (Bowers, 1967). In these studies, bioassay determination of the ACh derived from the Ch in the sample, after non-enzymatic acetylation, was used. This involves treatment of the sample with acetic anhydride. Since CSF contains more than 7 n-mole/ml. choline phospholipids (Sastry and Stancer, 1968), this acetylation procedure may cause an *in vitro* increase of choline to be acetylated. In fact, Bowers (1967) reported different choline concentrations when acetylation was carried out with acetic anhydride before and after removal of proteins and phospholipids from the CSF. Using the enzymatic acetylation, no such difference in the choline concentration of the CSF was obtained (Schuberth and Sundwall, 1971). Furthermore, by enzymatic acetylation of CSF Ch with labelled acetyl-CoA, bioassay of ACh is avoided, which is advantageous, since bioassay procedures are not completely specific and the presence of drugs and modifying substances in the samples may interfere with the determination.

Several authors using bioassay have reported ACh-like activity in human lumbar CSF (Schain, 1960). Whether ACh is a normal constituent of human CSF or not, has, however, been a subject of controversy. Duvoisin and Dettbarn (1967), who added an acetylcholinesterase inhibitor to the sample immediately after lumbar puncture and performed appropriate controls to identify ACh, found ACh in all samples examined. No conclusive difference in CSF ACh concentration was found between controls and patients suffering from different diseases, including Parkinsonism and chorea. They reported a mean ACh concentration in lumbar CSF from controls of 17 ng/ml., which is about 4% of the mean lumbar CSF Ch con-

centration found by us. It was noted in their study that CSF samples not treated by an acetylcholinesterase inhibitor showed only a very slow decline in ACh upon storage in room temperature. This suggests that ACh entering CSF had been hydrolysed to values below an effective substrate concentration for the acetylcholinesterase present in the CSF. In view of these findings, it seemed more fruitful to investigate the CSF concentration of the ACh metabolite Ch in an attempt biochemically to evaluate cholinergic activity.

Our finding of lower lumbar CSF Ch in Huntington's chorea is interesting in view of the suggested cholinergic hypoactivity in the striatum and stresses the need for further studies on Ch metabolism in this disorder (Aquilonius and Sjöström, 1971). It is by no means certain, however, that a low lumbar CSF Ch concentration reflects altered ACh metabolism in the central nervous system. Choline in CSF can be derived from plasma Ch, phospholipid metabolism and ACh being released from nervous tissue adjacent to the lumbar subarachnoid space. The relative proportions of these sources are not known. That the Ch concentration in CSF is not merely a reflection of plasma Ch concentration has earlier been demonstrated (Gardiner and Domer, 1968; Jönsson *et al.*, 1969). If CSF Ch concentration reflects, to a major part, phospholipid metabolism, the low CSF Ch found in Huntington's chorea might parallel the shrinkage of nervous tissue as well as the reduction of phospholipids in particular reported in the disease (Borri, Opden Velde, Hooghwinkel, and Bruyn, 1967).

Although it is not known to what extent the CSF Ch is derived from the brain ACh, there are some data in favour of CSF Ch as a marker of central cholinergic activity. Oxotremorine, which decreases acetylcholine turnover in the brain (Schuberth, Sparf, and Sundwall, 1969) and which exerts its main effects on the structures around the lateral ventricles (Bartolini, Bartolini, and Pepeu, 1970; Campbell and Jenden, 1970), caused a decrease of Ch in the CSF from the lateral ventricle of the dog, while the Ch concentration in the cisternal CSF remained unaltered (Aquilonius, Schuberth, and Sundwall, 1970). Also, from the results of the amphetamine effect on CSF Ch in man, there is

some evidence for a correlation between the Ch concentration in CSF and ACh turnover in the central nervous system. It has been shown that amphetamine causes an increased release of ACh from exposed cortex of experimental animals (Beani, Bianchi, Santinoceto, and Marchetti, 1968). In accordance with this, it was found that the Ch concentration of lumbar spinal fluid from amphetamine addicts was significantly higher during intoxication than after detoxication (Jönsson *et al.*, 1969).

If a major part of CSF Ch is derived from ACh hydrolysis in brain regions adjacent to CSF, an increased Ch concentration should be suspected in Parkinson's disease, where a cholinergic overactivity in striatum is likely. The failure to find an increased Ch concentration in lumbar CSF could however be due to the efficient removal of Ch from CSF during the ventriculocisternal passage (Aquilonius and Winblad, 1971). For this reason, we studied ventricular and lumbar CSF Ch in five patients with Parkinson's disease undergoing ventriculography. The mean value of ventricular CSF Ch concentration (3.2 ± 0.23 n-mol/ml.) was higher than in lumbar CSF (2.6 ± 0.19 n-mole/ml.). In five patients with intention tremor, however, the ventricular CSF Ch concentration was even somewhat higher (3.7 ± 0.45 n-mole/ml.) than in the Parkinsonism group. Conclusions are, however, hard to draw from this comparison as nothing is known about central cholinergic mechanism in intention tremor. For obvious reasons, no satisfactory control material of ventricular CSF could be obtained and the wide variation of ventricular CSF Ch concentration in the group consisting mainly of brain tumour cases with increased CSF pressure makes comparison with this material useless.

REFERENCES

- Aquilonius, S.-M., and Tiselius, P. (1969). Measurement of rigidity and tremor in Parkinson's disease. *Acta Neurologica Scandinavica*, **45**, 327-334.
- Aquilonius, S.-M., Schuberth, J., and Sundwall, A. (1970). Studies on choline in cerebrospinal fluid. *Acta Pharmacologica et Toxicologica*, **28**, Suppl. 1, 35.
- Aquilonius, S.-M., and Sjöström, R. (1971). Cholinergic and dopaminergic mechanisms in Huntington's chorea. *Life Sciences*, Part 1, **10**, 405-414.
- Aquilonius, S.-M., and Winblad, B. (1971). Cerebrospinal fluid clearance of choline. *Acta Pharmacologica et Toxicologica*, **29**, Suppl. 4, 64.
- Aquilonius, S.-M., Nyström, B., Schuberth, J., and Sundwall, A. (1971). Cerebrospinal fluid choline in extrapyramidal diseases. *Acta Pharmacologica et Toxicologica*, **29**, Suppl. 4, 3.
- Bartolini, A., Bartolini, R., and Pepeu, G. C. (1970). The effect of oxotremorine on the acetylcholine content of different parts of cat brain. *Journal of Pharmacy and Pharmacology*, **22**, 59-60.
- Beani, L., Bianchi, C., Santinoceto, L., and Marchetti, P. (1968). The cerebral acetylcholine release in conscious rabbits with semi-permanently implanted epidural cups. *International Journal of Neuropharmacology*, **7**, 469-481.
- Borri, P. F., Opden Velde, W. M., Hooghwinkel, G. J. M., and Bruyn, G. W. (1967). Biochemical studies in Huntington's chorea. VI. Composition of striatal neutral lipids, phospholipids, glycolipids, fatty acids, and amino acids. *Neurology (Minneapolis)*, **17**, 172-178.
- Bowers, M. B. Jr., (1967). Choline in cerebrospinal fluid. *Life Sciences*, Part 2, **6**, 1927-1933.
- 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 rat brain. *Journal of Neurochemistry*, **17**, 1697-1699.
- Dvoisin, R. C., and Dettbarn, W.-D. (1967). Cerebrospinal fluid acetylcholine in man. *Neurology (Minneapolis)*, **17**, 1077-1081.
- Ehringer, H., and Hornykiewicz, O. (1960). Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. *Klinische Wochenschrift*, **38**, 1236-1239.
- Gardiner, J. E., and Domer, F. R. (1968). Movement of choline between the blood and cerebrospinal fluid in the cat. *Archives Internationales de Pharmacodynamie et de Thérapie*, **175**, 482-496.
- Guggenheim, M., and Löffler, W. (1916). Über das Vorkommen und Schicksal des Cholins im Tierkörper. Eine Methode zum Nachweis kleiner Cholinmengen. *Biochemische Zeitschrift*, **74**, 208-218.
- Guldberg, H. C., Turner, J. W., Hanieh, A., Ashcroft, G. W., Crawford, T. B. B., Perry, W. L. M., and Gillingham, F. J. (1967). On the occurrence of homovanillic acid and 5-hydroxyindol-3-ylacetic acid in the ventricular C.S.F. from patients suffering from Parkinsonism. *Confinia Neurologica*, **29**, 73-77.
- Johansson, B., and Roos, B.-E. (1967). 5-hydroxyindoleacetic and homovanillic acid levels in the cerebrospinal fluid of healthy volunteers and patients with Parkinson's syndrome. *Life Sciences*, Part 1, **6**, 1449-1454.
- Jönsson, L.-E., Schuberth, J., and Sundwall, A. (1969). Amphetamine effect on the choline concentration of human cerebrospinal fluid. *Life Sciences*, Part 1, **8**, 977-981.
- Page, I. H., and Schmidt, E. (1931). Über die Abspaltbarkeit des Cholins aus Lecithin und den Cholingehalt der Cerebrospinalflüssigkeit. *Hoppe-Seylers Zeitschrift für physiologische Chemie*, **199**, 1-9.
- Sastry, P. S., and Stancer, H. C. (1968). Quantitative analysis and fatty acid composition of phospholipid constituents in cerebrospinal fluid of various age groups. *Clinica Chimica Acta*, **22**, 301-307.
- Schain, R. J. (1960). Neurohumors and other pharmacologically active substances in cerebrospinal fluid: A review of the literature. *Yale Journal of Biology and Medicine*, **33**, 15-36.
- Schuberth, J., Sparf, B., and Sundwall, A. (1969). A technique for the study of acetylcholine turnover in mouse brain *in vivo*. *Journal of Neurochemistry*, **16**, 695-700.
- Schuberth, J., and Sundwall, A. (1972). A method for the determination of choline in body fluids. (To be published.)