Neurochemical Research, Vol. 15, No. 4, 1990, pp. 419–423

Clinical Relevance of Measuring GABA Concentrations in Cerebrospinal Fluid*

Paul J. Schechter[1,3] and Albert Sjoerdsma[2]

(Accepted August 18, 1989)

Determination of GABA concentrations in human cerebrospinal fluid can be used to assess GABA-ergic activity in the central nervous system. As CSF free GABA concentrations may vary with age, sex, CSF fraction, and collection and storage conditions, careful attention to these factors are necessary to allow interpretation of results. Longitudinal studies to investigate the influence of pharmacological agents on CSF GABA have proven especially useful to define clinical biochemical activity and have been utilized to attribute the anti-epileptic action of vigabatrin, a selective inhibitor of GABA-transaminase, to its effects on brain GABA metabolism.

KEY WORDS: GABA; cerebrospinal fluid; homocarnosine; vigabatrin.

Although gamma-aminobutyric acid (GABA) was known to exist in plants, bacteria, yeast and various animal tissues previously, the hallmark event in establishing the importance of this amino acid in biological function occurred with the publication of two companion papers in Volume 187 of the *Journal of Biological Chemistry* in 1950. In the first paper, Roberts and Frankel reported the discovery of GABA in mammalian brain and demonstrated its formation via the decarboxylation of glutamic acid (1). In the following communication, Udenfriend, using his new isotope derivative technique which permitted chemical identification at the microgram level, verified conclusively the presence of GABA in extracts of mouse brain (2). These nascent findings eventually led to the appreciation that GABA functions as the major inhibitory neurotransmitter in the central nervous system (CNS).

Recognition of the role of GABA in neuronal function has prompted numerous studies to measure its concentration in the CNS following various pharmacologic interventions as well as the influence of a spectrum of disease states. Whereas post-mortem examination of brain to assess GABA concentrations is possible in humans, a more convenient method to monitor CNS GABA is to determine GABA concentrations in the cerebrospinal fluid (CSF). CSF is readily accessible during life and can be repeatedly sampled in the same individual.

Measurement of GABA in Human CSF. Three analytical methods are currently being used in various laboratories to determine GABA concentrations in human CSF, a radioreceptor assay (3), ion-exchange-fluorometry (4,5), and gas chromatography-mass spectrometry (6). An enzymatic assay has been described (7), but has been criticized as to specificity (8) and is seldom used.

The problems that have arisen in measuring GABA concentrations in human CSF have been less related to the methodology used (with the exception of the enzymatic method, as noted above) than with certain biological factors. This is illustrated in Table I in which lumbar CSF GABA values from selected publications are presented. In all cases values were considered to be from control or normal adult subjects. Variations do not ap-

[1]Merrell Dow Research Institute, 2 rue de Stockholm, 67000 Strasbourg, France.
[2]Merrell Dow Research Institute, 2110 East Galbraith Road, Cincinnati, Ohio 45215.
[3]To whom to address reprint request, at Ohio address.
*Special issue dedicated to Dr. Sidney Udenfriend.

0364-3190/90/0400-0419$06.00/0

Table I. Values of GABA Concentrations in Human Lumbar Cerebrospinal Fluid in "Control" or "Normal" Adult Subjects From Selected Publications

Analytical Method*	Subjects	n	GABA (pmol/ml ± SD)	Reference
IE/F	Neurological	9	534 ± 266	9
IE/F	Healthy Volunteers	40	233 ± 75	10
IE/F	Intervertebral disc disease	38	220 ± 81	4
IE/F	Healthy volunteers	10	97 ± 34	11
IE/F	Neurological	18	89 ± 49	12
IE/F	Neurologically normal	35	87 ± 41	13
RR	Neurologically normal	41	273 ± 122	14
RR	Neurological & psychiatric	26	230 ± 122	15
RR	Neurological	27	203 ± 29	16
RR	Not stated	25	127 ± 26	17
GC/MS	Not stated	4	1080 ± 130	18
GC/MS	Neurological	25	330 ± 214	6

* IE/F = ion-exchange-fluorometry
RR = radioreceptor
GC/MS = gas chromatography-mass spectrometry

pear to be related to assay methodology used, but rather can be attributed to collection and storage conditions.

Our laboratory first reported that when untreated human CSF was kept at room temperature, GABA concentrations increased progressively with the rate of increase differing in different individuals (4). This rate of increase could be retarded by cooling (4, 19) and accelerated by deproteinization with acid (19). The origin of this "artifactual" increase in CSF GABA has been attributed to the stoichiometric hydrolysis of homocarnosine, the histidine-GABA dipeptide, with the rate of GABA increase correlating with the CSF homocarnosine concentration (20). Thus, it is clear that extreme care and uniformity of collection and storage procedures must be used to allow interpretation of results of CSF GABA determinations.

During our investigations on human CSF GABA concentrations we discovered that, after strong acid hydrolysis, the GABA concentration in CSF increases 40-100 times. We assigned the term "total GABA" to this GABA fraction and "free GABA" to the concentration found prior to hydrolysis (21). As the concentration of homocarnosine could only account for about 30% of total GABA, we initially attributed the origin of the remainder to "unidentified conjugates" (12) since, in addition to homocarnosine, other GABA conjugates have been identified in human CSF, e.g., GABA-cystathionine (22), GABA-lysine (23), N-carboxyethyl-GABA (24). This designation was never satisfying since the total molar concentrations of all known GABA-conjugates in CSF represent only a small fraction of the GABA generated upon hydrolysis. We recently reported that essentially all the GABA previously unaccounted for in the total GABA fraction of human CSF derives not from a GABA-conjugate but from the cyclized lactam of GABA, 2-pyrrolidinone (25). The biological activity of 2-pyrrolidinone has not been investigated and its clinical relevance is unknown.

Does CSF GABA Bear any Relationship to Brain GABA. As CSF bathes the brain and spinal cord, it is assumed that it reflects variations in the state of activity of these organs. In the case of GABA, there is evidence to support this assumption. In rats treated with various doses of vigabatrin (gamma-vinyl GABA), a selective, enzyme-activated, irreversible inhibitor of GABA-transaminase, a linear correlation was observed between the increase in free and total GABA in whole brain and the increases in cisternal CSF (26). Hence, increases (but not necessarily decreases) in brain GABA concentrations are mirrored by increases in CSF. In addition, in man a rostrocaudal gradient for free and total GABA has been demonstrated, both when successive fractions of lumbar CSF were examined as well as when suboccipital and lumbar CSF from the same individuals were compared (27). Such a gradient suggests a brain origin of the GABA found in lumbar CSF. This gradient, estimated at about 0.35 and 33 pmol/ml for free GABA and total GABA, respectively, also indicates that comparisons of lumbar CSF concentrations are only valid if similar CSF fractions are obtained.

Influence of Disease States on CSF GABA Concentrations. The concentrations of free GABA in lumbar CSF have been assessed in a wide variety of neurological and psychiatric conditions. There is seldom agreement as to whether a disease state is associated with a CSF GABA concentration different from a control group. For example, decreases in CSF GABA concentrations of patients with Huntington's disease claimed by some authors (15, 28, 29) have not been confirmed by others (13, 30); decreases associated with Parkinson disease (30, 31) were not found by others (15, 32). Similar discrepancies can be found with epilepsy, schizophrenia, spasticity, Alzheimer's disease and other clinical states where multiple research groups have investigated this relationship. These differences in results are not surprising in view of the considerations mentioned above with respect to problems associated with sampling, handling and storage. In addition, decreases in CSF free and conjugated GABA (total minus free fraction) concentrations

have been reported with age in adults (33, 34), females showing a greater change with age than males (35). Thus, inattention to age and sex of control populations may also contribute to these disagreements.

Effect of Drug Treatment on CSF GABA Concentrations. It is obvious from the above discussions that the most sensitive design to assess changes in CSF GABA would be a longitudinal-type of study. Such a design is most applicable to assess the effects of pharmacological agents on CNS GABA metabolism, with values for each individual prior to drug administration serving as the control for subsequent results. CSF GABA concentrations in the same individual appear to be stable over time with repeated lumbar punctures and do not vary as a function of time of day (11).

Vigabatrin was specifically designed as an irreversible inhibitor of GABA-transaminase (GABA-T), the enzyme which catalyzes the inactivation of GABA (36, 37). Following the demonstration of its biochemical effects in animals (38, 39), a longitudinal study was carried out in patients to determine if vigabatrin was biochemically active in man. Oral administration of this GABA-T inhibitor to patients with various neurological diseases produces dose-dependent increases in CSF concentrations of free and total GABA as well as increases in homocarnosine and beta-alanine, an alternate substrate for the enzyme (21, 40). The biochemical effects of vigabatrin in CSF outlast the presence of inhibitor in this compartment by several days, consistent with its action as an irreversible enzyme inhibitor (41). An example of changes in CSF concentrations with vigabatrin is illustrated in Figure 1. Drug-refractory epileptic patients had oral vigabatrin treatment added to their anti-epileptic regimen, initially 1 g/day for 14 days and then 2 g/day for 14 days (40). Prior to vigabatrin administration and after 14 days at each dose, CSF obtained via suboccipital puncture was analyzed. Dose-dependent increases in free GABA, total GABA, homocarnosine and vigabatrin concentrations are evident.

Knowledge of the biochemical efficacy of vigabatrin in man and the doses at which these effects occurred, could be exploited to demonstrate therapeutic efficacy of this agent at these same doses. Vigabatrin has proved to be extremely useful in the treatment of refractory epilepsy (42). Its biochemical specificity, as shown by CSF studies (40, 43), allows the attribution of its anti-epileptic activity to its effect on CNS GABA metabolism.

Other pharmacological agents have also been evaluated in longitudinal studies to assess effects on human CSF GABA concentrations. Thus, gamma-acetylenic GABA and isoniazid, two other GABA-T inhibitors, increased CSF GABA concentrations (44, 45). Valproic acid, believed by some to act as an anti-epileptic agent via its effect on brain GABA metabolism, increased CSF GABA in some studies (46) but not in others (47, 48). Diazepam has also been shown to increase CSF GABA in one study (49), whereas haloperidol had no effect (50).

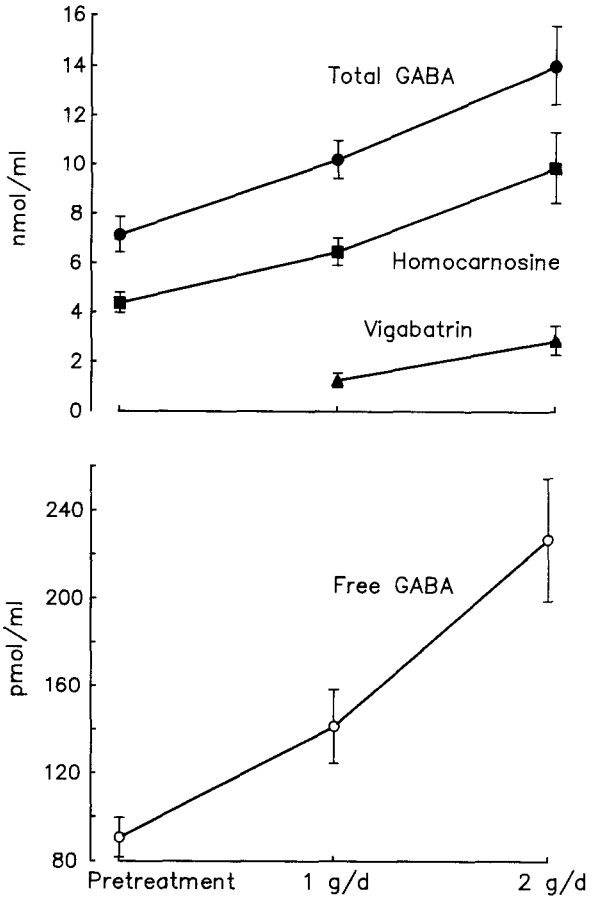

Fig. 1. Suboccipital CSF concentrations of total GABA, homocarnosine, vigabatrin and free GABA of epileptic patients prior to treatment and after sequential treatment with 1 g/d and 2 g/d oral vigabatrin, 14 days each. Values represent means ± SE (n = 7-9/point). Data from reference 40.

CONCLUSIONS

The determination of GABA concentrations in human CSF has clinical utility only in those cases where scrupulous attention is paid to sampling, collection and storage conditions of the fluid obtained. When populations are compared, properly age-matched and sex-matched individuals are required to allow interpretation of results. An argument has been advanced that the mea-

surement of total GABA, rather than free GABA, in CSF may have major advantages (12). Assuming the necessary precautions are taken in sample handling, the most useful application of CSF GABA studies is in assessing the influence of pharmacological agents on CNS GABA metabolism, with individual pre-treatment values serving as control. Studies with vigabatrin, a selective irreversible inhibitor of GABA-transaminase, illustrate this utility. Based on these studies, it can be asserted that vigabatrin represents the first effective anti-epileptic agent whose action can be unequivocally attributed in man to its effects on the CNS GABA-ergic system. These findings focus on the potential to target this neuronal system to discover effective new therapeutic agents.

REFERENCES

1. Roberts, E., and Frankel, S. 1950. Gama-aminobutyric acid in brain: Its formation from glutamic acid. J. Biol. Chem. 187:55–63.
2. Udenfriend, S. 1950. Identification of gamma-aminobutyric acid in brain by the isotope derivative method. J. Biol. Chem. 187:65–69.
3. Enna, S.J., Wood, J.H., and Snyder, S.H. 1977. Gamma-aminobutyric acid (GABA) in human cerebrospinal fluid: Radioreceptor assay. J. Neurochem. 28:1121–1124.
4. Bohlen, P., Schechter, P.J., van Damme, W., Coquillat G., Dosch, J.-C., and Koch-Weser, J. 1978. Automated assay of gamma-aminobutyric acid in human cerebrospinal fluid. Clin. Chem. 24:256–260.
5. Hare, T.A., and Manyam, N.V.B. 1980. Rapid and sensitive ion-exchange fluorometric measurement of gamma-aminobutyric acid in physiological fluids. Anal. Biochem. 101:349–355.
6. Huizinga, J.D., Teelken, A.W., Muskiet, F.A.J., Jeuring, H.J., and Wolthers, B.G. 1978. Gamma-aminobutyric acid determination in human cerebrospinal fluid by mass-fragmentography. J. Neurochem. 30:911–913.
7. Achar, V.S., Welch, K.M.A., Chabi, E., Bartosh, K., and Meyer, J.S. 1976. Cerebrospinal fluid gamma-aminobutyric acid in neurological disease. Neurology 26:777–780.
8. Hare, T.A., Bala Manyam, N.V., and Glaeser, B.S. 1980. Evaluation of cerebrospinal fluid gamma-aminobutyric acid content in neurological and psychiatric disorders. Pages 171–187, *in* Wood, J.H. (ed.), Neurobiology of Cerebrospinal Fluid, Vol. 1, Plenum Press, New York.
9. Glaeser, B.S., Vogel, W.H., Oleweiler, D.B., and Hare, T.A. 1975. GABA levels in cerebrospinal fluid of patients with Huntington's chorea: A preliminary report. Biochem. Med. 12:380–385.
10. Hare, T.A., Wood, J.H., Ballenger, J.C., and Post, R.M. 1979. Gamma-aminobutyric acid in human cerebrospinal fluid: Normal values. The Lancet II:534–535.
11. Ben-Menachem, E., Persson, L., Schechter, P.J., Haegele, K.D., Huebert, N., and Hardenberg, J. 1989. Cerebrospinal fluid parameters in healthy volunteers during serial lumbar punctures. J. Neurochem. 52:632–635.
12. Grove, J., Palfreyman, M.G., and Schechter, P.J. 1983. Cerebrospinal fluid GABA as an index of brain GABA activity. Clin. Neuropharmacol. 6:223–229.
13. Perry, T.L., Hansen, S., Wall, R.A., and Gauthier, S.G. 1982. Human CSF GABA concentrations: Revised downward for controls, but not decreased in Huntington's chorea. J. Neurochem. 38:766–773.
14. McCarthy, B.W., Gomes, U.R., Neethling, A.C., Shanley, B.C., Taljaard, J.J.F., Potgieter, L., and Roux, J.T. 1981. Gamma-aminobutyric acid concentration in cerebrospinal fluid in schizophrenia. J. Neurochem. 36:1406–1408.
15. Enna, S.J., Stern, L.Z., Wastek, G.J., and Yamamura, H.I. 1977. Cerebrospinal fluid gamma-aminobutyric acid variations in neurological disorders. Arch. Neurol. 34:683–685.
16. Airaksinen, E.M., and Leino, E. 1982. Decrease of GABA in the cerebrospinal fluid of patients with progressive myoclonus epilepsy and its correlation with the decrease of 5HIAA and HVA. Acta. Neurol. Scand. 66:666–672.
17. Kuroda, H. 1983. Gamma-aminobutyric acid (GABA) in cerebrospinal fluid. Acta. Med. Okayama 37:167–177.
18. Norman, E.J., Wee, E.L., Berry, H.K., and Zimmerman, E.F. 1985. Rapid gas chromatographic-mass spectrometric quantitation of gamma-aminobutyric acid in biological specimens. J. Chromatog. 337:21–27.
19. Grossman, M.H., Hare, T.A., Manyam, N.V.B., Glaeser, B.S., and Wood, J.H. 1980. Stability of GABA levels in CSF under various conditions of storage. Brain Res. 182:99–106.
20. Grove, J., Schechter, P.J., Tell, G., Rumbach, L., Marescaux, C., Warter, J.-M., and Koch-Weser, J. 1982. Artifactual increases in the concentration of free GABA in samples of human cerebrospinal fluid are due to degradation of homocarnosine. J. Neurochem. 39:1061–1065.
21. Grove, J., Schechter, P.J., Tell, G., Koch-Weser, J., Sjoerdsma, A., Warter, J.-M., Marescaux, C., and Rumbach, L. 1981. Increased gamma-aminobutyric acid (GABA), homocarnosine and beta-alanine in cerebrospinal fluid of patients treated with gamma-vinyl GABA (4-amino-hex-5-enoic acid). Life Sci. 28:2431–2439.
22. Perry, T.L., Hansen, S., Schier, G.M., and Halpern, B. 1977. Isolation and identification of gamma-aminobutyryl-cystathionine from human brain and CSF. J. Neurochem. 29:791–795.
23. Perry, T.L., Hansen, S., and Kennedy, J. 1975. CSF amino acids and plasma-CSF amino acid ratios in adults. J. Neurochem. 24:587–589.
24. Fussi, F., Savoldi, F., and Curti, M. 1987. Identification of N-carboxyethyl gamma-aminobutyric acid in bovine brain and human cerebrospinal fluid. Neurosci. Lett. 77:308–310.
25. Haegele, K.D., Schwartz, J.-J., Schoun, J., Schmitt, A.H., and Schechter, P.J. 1987. 2-Pyrrolidinone in human cerebrospinal fluid: A major constituent of total gamma-aminobutyric acid. J. Neurochem. 49:1402–1406.
26. Bohlen, P., Huot, S., and Palfreyman, M.G. 1979. The relationship between GABA concentrations in brain and cerebrospinal fluid. Brain Res. 167:297–305.
27. Grove, J., Schechter, P.J., Hanke, N.F.J., de Smet, Y., Agid, Y., Tell, G., and Koch-Weser, J. 1982. Concentration gradients of free and total gamma-aminobutyric acid and homocarnosine in human CSF: Comparison of suboccipital and lumbar sampling. J. Neurochem. 39:1618–1622.
28. Manyam, N.V.B., Hare, T.A., Katz, L., and Glaeser, B.S. 1978. Huntington's disease. Cerebrospinal fluid GABA levels in at-risk individuals. Arch. Neurol. 35:728–730.
29. Uhlhaas, S., Lange, H., Wappenschmidt, J., and Olek, K. 1986. Free and conjugated CSF and plasma GABA in Huntington's chorea. Acta. Neurol. Scand. 74:261–265.
30. Kuroda, H., Ogawa, N., Yamawaki, Y., Nukina, I., Ofuji, T., Yamamoto, M., and Otsuki, S. 1982. Cerebrospinal fluid GABA levels in various neurological and psychiatric diseases. J. Neurol. Neurosurg. Psychiat. 45:257–260.
31. Manyam, B.V. 1982. Low CSF gamma-aminobutyric acid levels in Parkinson's disease. Effect of levodopa and carbidopa. Arch. Neurol. 39:391–392.
32. Teychenne, P.F., Lake, C.R., and Ziegler, M.G. 1980. Cerebrospinal fluid studies in Parkinson's Disease. Pages 197–206, Vol.

1, *in* Wood, J.H. (ed.), Neurobiology of Cerebrospinal Fluid, Plenum Press, New York.
33. Bareggi, S.R., Franceschi, M., Bonini, L., Zecca, L., and Smirne, S. 1982. Decreased CSF concentrations of homovanillic acid and gamma-aminobutyric acid in Alzheimer's disease. Age- or disease-related modifications? Arch. Neurol. 39:709–712.
34. Ferraro, T.N., and Hare, T.A. 1985. Free and conjugated amino acids in human CSF: Influence of age and sex. Brain Res. 338:53–60.
35. Hare, T.A., Wood, J.H., Manyam, B.V., Gerner, R.H., Ballenger, J.C., and Post, R.M. 1982. Central nervous system gamma-aminobutyric acid activity in man. Relationship to age and sex as reflected in CSF. Arch. Neurol. 39:247–249.
36. Lippert, B., Metcalf, B.W., Jung, M.J., and Casara, P. 1977. 4-Amino-hex-5-enoic acid, a selective catalytic inhibitor of 4-aminobutyric-acid aminotransferase in mammalian brain. Eur. J. Biochem. 74:441–445.
37. Metcalf, B.W., Lippert, B., and Casara, P. 1978. Enzyme-activated irreversible inhibition of transaminases. Pages 123–133, *in* Seiler, N., Jung, M.J. and Koch-Weser, J. (eds.), Enzyme-activated Irreversible Inhibitors, Elsevier/North Holland Press, Amsterdam.
38. Jung, M.J., Lippert, B., Metcalf, B.W., Bohlen, P., and Schechter, P.J. 1977. Gamma-vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: Effects on brain GABA metabolism in mice. J. Neurochem. 29:797–802.
39. Schechter, P.J., Trainer, Y., Jung, M.J., and Bohlen, P. 1977. Audiogenic seizure protection by elevated brain GABA concentration in mice: Effects of gamma-acetylenic GABA and gamma-vinyl GABA, two irreversible GABA-T inhibitors. Eur. J. Pharmacol. 45:319–328.
40. Schechter, P.J., Hanke, N.F.J., Grove, J., Huebert, N., and Sjoerdsma, A. 1984. Biochemical and clinical effects of gamma-vinyl GABA in patients with epilepsy. Neurology 34:182–186.
41. Ben-Menachem, E., Persson, L.I., Schechter, P.J., Haegele, K.D., Huebert, N., Hardenberg, J., Dahlgren, L., and Mumford, J.P. 1988. Effects of single doses of vigabatrin on CSF concentrations of GABA, homovanillic acid and 5-hydroxyindoleacetic acid in patients with complex partial epilepsy. Epil. Res. 2:96–101.
42. Schechter, P.J. 1986. Vigabatrin. Pages 265–275, *in* Meldrum, B.S. and Porter, R.J. (eds.), Current problems in epilepsy. IV. New Anticonvulsant Drugs, John Libbey and Co., London.
43. Ben-Menachem, E., Persson, L.I., Schechter, P.J., Haegele, K.D., Huebert, N., Hardenberg, J., Dahlgren, L., and Mumford, J.P. 1989. The effect of different vigabatrin treatment regimens on CSF biochemistry and seizure control in epileptic patients. Br. J. Clin. Pharm. 27:79S–85S.
44. Tell, G., Bohlen, P., Schechter, P.J., Koch-Weser, J., Agid, Y., Bonnet, A.M., Coquillat, G., Chazot, G., and Fischer, C. 1981. Treatment of Huntington's disease with gamma-acetylenic GABA, an irreversible inhibitor of GABA-transaminase: Increased CSF GABA and homocarnosine without clinical amelioration. Neurology 31:207–211.
45. Manyam, B.V., Katz, L., Hare, T., Kaniefsky, K., and Trembley, R.D. 1981. Isoniazid-induced elevation of GABA levels and effects on chorea in Huntington's chorea. Ann. Neurol. 10:35–37.
46. Zimmer, R., Teelken, A.W., Cramer, H., Ruther, E., and Gundurewa, M. 1980. Biochemical investigations into the mode of action of Na-valproate and valproinic acid, respectively. Arzneimittelforschung 30:1213.
47. Neophytides, A.N., Suria, A., and Chase, T.N. 1978. Cerebrospinal fluid GABA in neurologic disease. Neurology 28:359.
48. Snoek, J.W., Van Weerden, T.W., Teelken, A.W., Van den Burg, W., and Lakke, J.P.W.F. 1987. Meige syndrome: Double-blind crossover study of sodium valproate. J. Neurol. Neurosurg. Psychiat. 50:1522–1525.
49. Loscher, W., and Schmidt, D. 1987. Diazepam increases gamma-aminobutyric acid in human cerebrospinal fluid. J. Neurochem. 49:152–157.
50. Gattaz, W.F., Roberts, E., and Beckmann, H. 1986. Cerebrospinal fluid concentrations of free GABA in schizophrenia: No changes after haloperidol treatment. J. Neural. Transm. 66:69–73.