Volume 247, number 2, 438–444 FEB 07074 April 1989

Neuroactive metabolites of L-tryptophan, serotonin and quinolinic acid, in striatal extracellular fluid

Effect of tryptophan loading

Matthew J. During*+, Andrew Freese+, Melvyn P. Heyes, Kenton J. Swartz+, Sanford P. Markey, Robert H. Roth* and Joseph B. Martin+

*Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510, +Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114 and Section of Analytical Biochemistry, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892, USA

Received 7 March 1989

Extracellular fluid levels of the neurotoxin quinolinic acid in the corpus striatum of rats, measured by in vivo microdialysis, were increased in a dose-dependent manner following the intraperitoneal administration of tryptophan. The lowest dose of tryptophan (12.5 mg/kg), equivalent to about 5% of the normal daily intake, increased peak quinolinic acid levels nearly 3-fold. At higher doses of tryptophan (up to 250 mg/kg), concentrations of quinolinic acid increased over 200-fold and exceeded potentially neurotoxic levels (10 μM). In contrast, the increase in extracellular serotonin following even the highest tryptophan dose was small (less than 2-fold). These data indicate that quinolinic acid is present in the extracellular fluid where it may function as a neuromodulator and that it is very responsive to physiological changes in precursor availability.

Tryptophan; Quinolinic acid; Hydroxytryptamine, 5-; Microdialysis

1. INTRODUCTION

The precursor relationship of the essential aromatic amino acid, L-tryptophan to the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) is well established [1–8]. Indeed, therapeutic interventions for several neuropsychiatric and other disorders are based on this relationship. These include mania and depression, insomnia, obesity, hypertension, pain and aggression [9–16]. The rationale behind this therapy is the recognition that after crossing the blood-brain barrier, tryptophan is hydroxylated by tryptophan 5-monoxygenase, to 5-hydroxytryptophan and then decarboxylated to 5-HT. In the central nervous system (CNS), tryptophan 5-monoxygenase is restricted to serotoninergic neurons and hence oral administration of tryptophan is believed to increase selectively 5-HT synthesis and release from serotoninergic neurons.

However, the metabolism of tryptophan is complex and 5-HT is neither the major nor the only neuroactive tryptophan metabolite. Following a systemic load, the amount of tryptophan available to the brain for 5-HT synthesis is estimated to be only 3% of that administered [19,20]. In the periphery the vast majority of tryptophan not used for protein synthesis is metabolized in the liver through the kynurenine pathway via the hepatic enzyme tryptophan 2,3-dioxygenase. Although this

Correspondence address: M.J. During, B254 SHM, Yale University School of Medicine, 333 Cedar St, New Haven, CT 06510, USA

Abbreviations: CNS, central nervous system; 5-HT, 5-hydroxytryptamine, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; N-Me-D-Asp, *N*-methyl-D-aspartate; QUIN, quinolinic acid; KYNA, kynurenic acid; ECF, extracellular fluid

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

enzyme is found exclusively in the liver, indole 2,3-dioxygenase has been shown to be present in other tissues including the brain [21–23]. However, both the activity and function of the kynurenine pathway in the brain are poorly characterized [24]. Of the several neuroactive intermediates of kynurenine metabolism, including kynurenine itself, perhaps the most relevant is quinolinic acid (QUIN). As an agonist of the *N*-methyl-D-aspartate (N-Me-D-Asp)-type excitatory amino acid receptor [25], this dicarboxylic acid is an excitotoxin [26–28], and has been implicated in the pathogenesis of a number of neurological and metabolic disorders, including Huntington's Disease, temporal lobe epilepsy, hepatic encephalopathy and glutaric aciduria [29–34].

We were interested in determining whether a physiological coupling of substrate control, similar to the relationship observed between brain tryptophan availability and serotoninergic neurotransmission, also existed between tryptophan and QUIN. Specifically, we wished to demonstrate a metabolic relationship using systemic doses of tryptophan that are believed to elevate plasma and brain tryptophan concentrations to levels within the physiological range. The concentrations of tryptophan in both plasma and brain exhibit a diurnal variation with nocturnal peak levels typically 50 to 150% above the daytime nadir [35]. A tryptophan dose of 12.5 mg/kg intraperitoneally is less than 5% of the normal daily intake and increases plasma and brain tryptophan to levels which do not exceed their normal daily peaks [36]. A precursor relationship of QUIN in the CNS to systemic levels of tryptophan would have several major implications: (i) The generation of a mechanism whereby plasma fluctuations in tryptophan may influence brain levels of a potent neurotoxin. (ii) QUIN might mediate some of the CNS effects attributed to alterations in brain tryptophan levels. (iii) At high doses systemic administration of tryptophan might elicit selective neuronal cell loss comparable to that found in Huntington's Disease.

The site of action of 5-HT, and potentially QUIN, is the synaptic cleft. This space is hidden from current neurochemical techniques. However, recent developments in in vivo perfusion, in particular, microdialysis, have enabled sampling of brain extracellular fluid (ECF), a space believed to be in equilibrium with the synaptic fluid. Measurement using this technique reveals the spillover of released neurotransmitters and metabolites and provides a dynamic record of synaptic events averaged over several minutes [37]. We have used this technique to follow the extracellular concentrations of tryptophan and some of its metabolites in the striatum following systemic tryptophan administration.

We have chosen the corpus striatum for two reasons. First, the N-Me-D-Asp receptor, which is unevenly distributed in mammalian brain, is present in high concentrations in the striatum [38]. This may account for the regional variation in neurotoxic sensitivity observed with a low threshold for toxicity found in the corpus striatum [39]. Second, the neostriatum is also the brain region primarily affected in Huntington's Disease.

2. EXPERIMENTAL

Male Sprague-Dawley rats (250–350 g) were housed in a animal facility with ad libitum access to Agway 3000 (Syracuse, NY) rat chow. On the morning (08:00–09:00 h) of the day of experimentation, rats were anesthetized with chloralose/urethane (50/500 mg · kg^{-1}) intraperitoneally with small, additional doses administered as necessary to maintain stable levels of anesthesia. Rats were placed in a Kopf stereotaxic frame and kept at 37°C using a homeostatically controlled heating pad. Dialysis probes were implanted into left and right striata at coordinates AP: +0.5, L: 2.5, V: −7.0 [40]. Correct probe placement was verified by post-mortem sectioning and light microscope visualization.

The probes were of cannula design and had a 4 mm length of exposed membrane; the diffusion surface spanned the entire dorsoventral coordinates of the striatum. Probes were constructed using the design of Sandberg et al. [41] with minor modifications. Hollow dialysis fibers (5 kDa cut-off; Cuprophan, Hospal, Edison, NJ) were sealed at one end with epoxy resin (Devcon, Danvers, MA). A length of hollow vitreous silica fiber (0.17 mm outer diameter, Anspec, MI) was inserted into the dialysis tube flush to the sealed end. The dialysis tubing with the vitreous silica fiber in situ was then inserted through a length of 23 gauge stainless-steel tubing into which another length of vitreous silica fiber (the outlet) had been placed. Both ends of the 23 gauge tubing were sealed with epoxy resin, with the dialysis membrane protruding from one end of the probe. The length of exposed membrane surface was controlled by coating the dialysis membrane with epoxy resin. 23 gauge tubing was glued with epoxy to the inlet vitreous tube enabling the probe to be perfused using a Harvard microperfusion pump (Harvard Instruments, Natick, MA) via PE-50 tubing. The probes were perfused with an artificial ECF (125 mM Na^+, 2.8 mM K^+, 1.0 mM Mg^{2+}, 1.2 mM Ca^{2+}, 100 μM ascorbate, 2 mM phosphate, pH 7.4) at 0.6 μl/min. Probes were calibrated in vitro by placing them in standard solutions (10^{-6} and 10^{-7} M) and determining the relative concentration recovered.

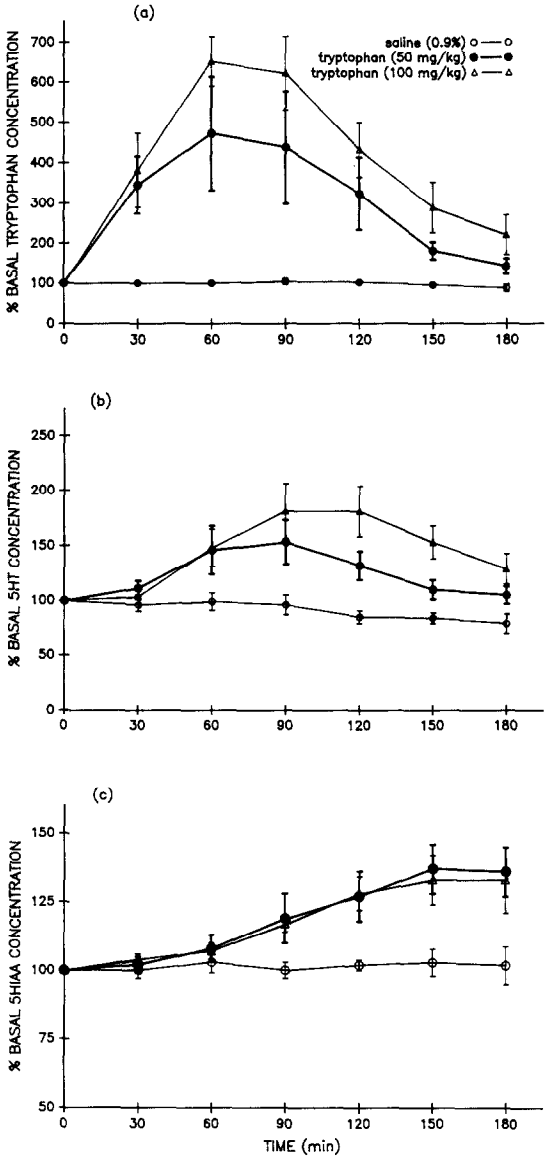
The left striatal dialysates were assayed for QUIN using negative ionization gas chromatography mass spectrometry as described [42,43] and the right dialysates were analyzed for tryptophan, 5-HT and its acidic metabolite, 5-hydroxyindoleacetic acid (5-HIAA) using HPLC with electrochemical detection. The HPLC system used a 15 cm by 2.1 mm 3 μm C18 narrow-bore column with a low-volume (<0.1 μl) electrochemical cell with an applied voltage of 0.95 V using a BAS LC3 potentiostat (Bioanalytical Systems, West Lafayette, IN). The mobile phase used to achieve separation was 0.06 M sodium phosphate buffer, 0.2 mM EDTA, 0.5 mM heptanesulfonic acid and 25% methanol (v/v), pH 2.1. Dialysate samples were injected directly onto the HPLC column without any need for an internal standard or sample preparation. Chromatograms were completed within 25 min. Detection limits with a signal-to-noise ratio exceeding 2:1 were 5 fmol for 5-HT and 5-HIAA, and 200 fmol per sample for tryptophan. Additional characterization of the 5-HT signal included a greater than 60% reduction in peak height following *p*-chlorophenylalanine (150 mg/kg slow intravenous infusion) and verification by Dr G.M. Anderson of selected samples using HPLC with fluorometric detection [44].

Groups of rats received saline, or tryptophan in doses of 12.5, 50, and 100 mg/kg intraperitoneally. L-Tryptophan (Sigma, St. Louis, MO) was administered as a saline solution in a volume of 5 ml/kg. The tryptophan was dissolved in 1 M NaOH, diluted close to final volume with 0.9% saline, then adjusted to pH 7.4 using 6 M HCl, with saline added to final volume. Treatments were administered following three 30-min baseline samples, collected at approx. 120–210 min following probe implantation. Perfusion continued for 3 h after treatments, with dialysates taken at 30-min intervals. Samples for QUIN assay were immediately frozen on dry ice and stored at -70°C until assay. The dialysates for tryptophan, 5-HIAA and 5-HT measurements were directly injected onto the HPLC system.

As an additional control to ensure that dialysate QUIN represented de novo brain synthesis and ECF spillover, and not disruption of the blood-brain barrier, a group of rats were cannulated with intrafemoral catheters prior to dialysis probe implantation. These rats underwent simultaneous dialysis and plasma measurements following the intravenous administration of QUIN (2.5 mmol/kg). Plasma QUIN levels increased by 100-fold, whereas dialysate QUIN concentrations remained unchanged (not shown).

3. RESULTS

Dialysate tryptophan levels remained stable in the saline-treated animals but increased by 4.7 ± 1.4-fold in the 50 mg/kg group and by 6.5 ± 0.6-fold in the 100 mg/kg treated animals (fig.1). QUIN increased markedly by 2.9 ± 0.5-fold (mean $\pm$ SE) in the 12.5 mg/kg tryptophan-treated animals, 41 ± 15-fold in the 50 mg/kg group and by 81 ± 15-fold in the 100 mg/kg group (fig.2). An additional group of animals received tryptophan at 250 mg/kg. In these rats, dialysate tryptophan levels increased by 17 ± 3-fold and QUIN concentrations increased by 230 ± 70-fold (not shown).



The levels of QUIN peaked at 150 min in the 100 mg/kg animals and at 30 min in the 12.5 mg/kg animals. The rate of increase in QUIN levels over the first 30 min however appears identical, suggesting saturation of the enzyme even at the lowest dose of tryptophan and obtainment of maximal enzymatic rate (V_{max}). Tryptophan levels peaked between 30 and 60 min, 5-HT peaked at 90 min and 5-HIAA levels had a delayed increase reaching a plateau at 150–180 min (fig.1).

Fig.1. Rats were anaesthetized and microdialysis probes implanted into left and right striata. 120 min following probe implantation, 3 consecutive 30-min samples were collected to obtain a baseline measurement. Rats then received either 0.9% saline ($n=5$) or tryptophan in a saline solution in doses of 50 ($n=5$) or 100 mg/kg i.p. ($n=5$). Dialysates were collected for the following three hours, with the right striatal dialysates assayed for tryptophan, 5-HT and 5-HIAA. Mean basal levels of tryptophan, 5-HIAA and 5-HT did not differ between groups, with concentrations of 0.45 ± 0.12 μM, 210 ± 28 nM and 0.73 ± 0.1 nM, respectively, uncorrected for recovery (means $\pm$ SE). Data represent (a) tryptophan, (b) 5-HT, and (c) 5-HIAA concentrations as a percentage of baseline values; vertical bars represent the SE. Tryptophan values in both 50 and 100 mg/kg treated animals at all time points are significantly elevated compared to saline treated controls. 5-HT levels are significantly elevated above control values at all time points between 60 and 150 min in the animals receiving 100 mg/kg, and at 60, 90 and 120 min in the 50 mg/kg group. 5-HIAA levels are significantly elevated from 90 to 180 min and from 60 to 180 min in the 50 and 100 mg/kg groups, respectively. Values significant at $p<0.05$, Student's t-test.

The basal QUIN levels obtained in dialysates prior to tryptophan or saline administration were 45 ± 12 nM. The in vitro recovery efficiency of the probes at the 0.6 μl/min perfusion rate was between 30 and 40%. Therefore, assuming identical diffusion and recovery kinetics in vivo, the basal extracellular concentration of QUIN would be approx. 120 nM. However, microdialysis is believed to underestimate true ECF levels of compounds using in vitro recovery and it is likely that actual ECF concentrations may be 5- or even 10-times higher [45].

4. DISCUSSION

These data suggest that changes in brain tryptophan availability have a marked influence on

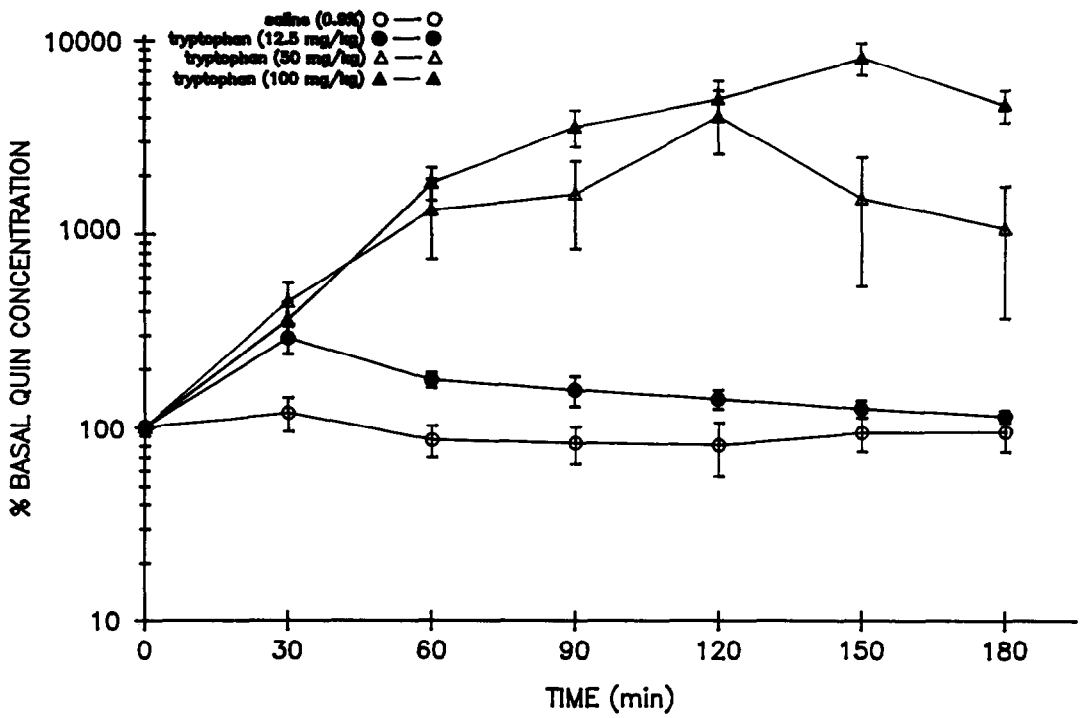


Fig.2. Rats were anaesthetized and microdialysis probes were implanted in left and right striata. 120 min following probe implantation, 3 consecutive 30-min samples were collected to obtain a baseline measurement. Tryptophan (12.5, 50 or 100 mg/kg, all groups ($n=5$)), or 0.9% saline ($n=5$) was then administered i.p. and dialysis continued for a further 180 min. The left striatal dialysates were immediately frozen and subsequently assayed for QUIN using GC/MS. Basal QUIN levels were similar in all groups, 45 ± 9 nM, mean $\pm$ SE, uncorrected for in vitro recovery. Data represent dialysate QUIN concentrations as a percentage of baseline with vertical bars representing the SE. Levels of quinolinic acid are significantly elevated above saline-treated control values in the 30 and 60 min sample for the 12.5 mg/kg tryptophan treated group; and at 30 min and all time points thereafter in the 50 and 100 mg/kg groups. $p<0.05$, Student's t-test, unpaired.

levels of QUIN within striatal ECF. In addition, this relationship exists even with doses of tryptophan which may result in alterations in plasma levels well within the physiological range. This coupling between acute tryptophan loading and striatal ECF QUIN is of much greater magnitude than the relationship which exists between brain tryptophan availability and ECF 5-HT. However, the ECF levels of 5-HT obtained by microdialysis may not accurately reflect synaptic release, and are perhaps more appropriately considered as an indicator of synaptic spillover, as high-affinity uptake of the transmitter may attenuate the changes in release as monitored by microdialysis.

Tryptophan inhibits raphe neuron firing within minutes of its administration [46] and although the 5-HT content within striatal serotoninergic neuronal terminals is likely to be increased before 60 min, the delayed, small increase in ECF levels we observed may in part be mediated by partial return of raphe neuron firing as seen in slice preparations (Sprouse, J. and Aghajanian, G.K., personal communication). The small and delayed release of 5-HT in vivo following tryptophan administration is also in agreement with previous studies using ventricular fluid withdrawal [47], ependymal caudate surface fluid collections [48], and in vivo voltammetry [49].

Levels of QUIN of 10 μM have been shown to be toxic in neuronal cell cultures [50] and injection of as little as 12 nmol directly into the striatum causes neuronal death at the site of infusion [28,51]. Peak estimated ECF levels of QUIN after both the 100 and 250 mg/kg doses exceed 10 μM; these levels may prove to be neurotoxic. The functional significance of the QUIN elevations observed following the administration of lower doses of tryptophan is unknown. However, as a specific agonist of the N-Me-D-Asp receptor, small alterations in ECF QUIN levels may modulate N-Me-D-Asp receptor number and/or activity and thereby influence excitatory amino acid neurotransmission. Thus, it appears possible that QUIN, a compound predominantly synthesized and catabolized by glia [52] may act as a neuromodulatory substance, although not meeting classical criteria for neurotransmitters [53].

In Hungtinton's Disease, the activity of the QUIN-synthesizing enzyme, 3-hydroxyanthranilic oxygenase is increased by almost 4-fold in the striatum [54], whereas that of the QUIN-degrading enzyme, quinolinate phosphoribosyltransferase, is relatively unaffected [55]. Combined with evidence from our laboratory that QUIN injected into the striatum may produce selective neuronal degeneration similar to the pattern observed in Huntington's Disease [30], these data indicate that this disease may be caused by a disorder of tryptophan metabolism. Indeed, isolated case reports have shown rapid clinical deterioration in juvenile Huntington's Disease patients following the repeated ingestion of pharmacological doses of tryptophan [56], and one study has demonstrated correlation of free plasma tryptophan levels with the severity of chorea [57]. The potential excitatory effects of the tryptophan-induced increase in ECF QUIN may be antagonized however by concomitant increases in kynurenic acid (KYNA). KYNA, another tryptophan metabolite of the kynurenine pathway, acts as a N-Me-D-Asp antagonist [58] and when coinjected with QUIN can protect against both the neurotoxicity and convulsant activity of QUIN [59]. Tissue levels of KYNA have been shown to increase following tryptophan loading (100 mg/kg tryptophan increasing levels less than 2-fold) [60]. Although this is a trivial increase compared to that of 100-fold we observed in ECF QUIN, ECF KYNA was not measured in the present study (see below) and it is possible that levels within this compartment increase much more than tissue levels. Elevated ECF concentrations of KYNA might therefore protect against the putative QUIN toxicity under tryptophan loading conditions.

The sensitivity of current methods for assay of KYNA is in the low picomole range [60] (cf. low femtomole sensitivity for QUIN) and such methods will require major improvement before KYNA will be able to be measured in dialysis perfusates.

In conclusion, QUIN may be added to the list of endogenous neuroactive substances whose brain levels are influenced by physiological changes in plasma precursor concentrations. The functional significance of the observed changes in brain extracellular levels of QUIN remains to be elucidated. Because of the well established neurotoxicity of QUIN and the large increases seen in ECF QUIN levels after tryptophan administration we recommend that the indiscriminate sale and casual self-administration of tryptophan from health food stores should be curtailed [61], as cer-

tain subsets of the population may be particularly vulnerable to QUIN-mediated toxic effects.

Acknowledgements: We thank George Anderson for carrying out confirming analysis of ECF serotonin. We thank Marian DiFiglia for expert guidance and support. This work was supported in part by NIH grants NS 16367 and MH-14092. M.J.D. is a recipient of a USPHS Fogarty International Research Fellowship; A.F. is a recipient of a Harvard/MIT Health Sciences and Technology Predoctoral Fellowship. K.J.S. is a recipient of a Boston University predoctoral fellowship.

REFERENCES

[1] Wang, H.L., Harwalker, V.H. and Waisman, H.A. (1962) Arch. Biochem. Biophys. 97, 181–184.
[2] Green, H., Greenburg, S.M., Erickson, R.W., Sawyer, J.L. and Ellizon, T. (1962) J. Pharmacol. Exp. Ther. 136, 174–178.
[3] Ashcroft, G.W. and Crawford, T.B.B. (1965) J. Neurochem. 12, 483–492.
[4] Eccleston, D., Ashcroft, G.W. and Crawford, T.B.B. (1965) J. Neurochem. 12, 493–503.
[5] Moir, A.T.B. and Eccleston, D. (1968) J. Neurochem. 15, 1093–1108.
[6] Fernstrom, J.D. and Wurtman, R.J. (1971) Science 173, 149–151.
[7] Fernstrom, J.D. and Wurtman, R.J. (1971) Science 174, 1023–1025.
[8] Fernstrom, J.D. and Wurtman, R.J. (1972) Science 178, 414–416.
[9] Hartmann, E. and Spinweber, C.L. (1979) J. Nerv. Ment. Dis. 167, 497–499.
[10] Nicholson, A.N. and Stone, B.M. (1979) EEG Clin. Neurophysiol. 47, 539–545.
[11] Moldofsky, H. and Luc, F.A. (1980) EEG Clin. Neurophysiol. 50, 71–80.
[12] Moller, S.E., Kirk, C. and Honore, P. (1980) J. Affective Dis. 2, 47–59.
[13] Sved, A.F., van Itallie, C.M. and Fernstrom, J.D. (1982) J. Pharmacol. Exp. Ther. 221, 329–332.
[14] Hedaya, R.J. (1984) J. Clin. Psychopharmacol. 46, 347–348.
[15] Hrboticky, N., Leiter, L.A. and Anderson, G.H. (1985) Nutrition Res. 5, 595–607.
[16] Wilcock, G.F., Stevens, J. and Perkins, A. (1987) Lancet i, 929–930.
[17] Carlsson, A. and Lindquist, M. (1972) J. Neural. Transm. 33, 23–43.
[18] Carlsson, A. and Lindquist, M. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 303, 157–165.
[19] Sjoerdsma, A., Weissbach, H. and Udenfriend, S. (1956) Am. J. Med. 20, 520–532.
[20] Young, S.N., St.-Arnaud-McKenzie, D. and Sourkes, T.L. (1978) Biochem. Pharmacol. 27, 763–767.
[21] Gal, E.M., Armstrong, J.C. and Ginsberg, B. (1966) J. Neurochem. 13, 643–654.
[22] Tsuda, H., Noguchi, T. and Kido, R. (1972) J. Neurochem. 19, 887–890.
[23] Hirata, F., Hayaishi, O., Tokuyama, T. and Senoh, S. (1974) J. Biol. Chem. 249, 1311–1313.
[24] Gal, E.M. and Sherman, A.D. (1980) Neurochem. Res. 5, 223–239.
[25] Perkins, M.N. and Stone, T.W. (1983) J. Pharmacol. Exp. Ther. 226, 551–557.
[26] Lapin, I.P. (1978) J. Neural Transm. 42, 37–43.
[27] Stone, T.W. and Perkins, M.N. (1981) Eur. J. Pharmacol. 72, 411–412.
[28] Schwarcz, R., Whetsell, W.O. jr and Mangano, R.M. (1983) Science 219, 316–318.
[29] Curzon, G., Katameneni, B.D., Winch, J., Rojas-Bueno, A., Murray-Lyon, I.M. and Williams, R. (1973) J. Neurochem. 21, 137–145.
[30] Beal, M.F., Kowall, N.W., Ellison, D.W., Mazurek, M.F., Swartz, K.J. and Martin, J.B. (1986) Nature 321, 168–171.
[31] Moroni, F., Lombardi, G., Carla, V., Pellegrini, D., Carassale, G.L. and Cortesini, C. (1986) J. Neurochem. 46, 869–874.
[32] Moroni, F., Lombardi, G., Carla, V., Cal, S., Etienne, P. and Nair, N.P.V. (1986) J. Neurochem. 47, 1667–1671.
[33] Heyes, M.P. (1987) Can. J. Neurol. Sci. 14, 441–443.
[34] Schwarcz, R., Okuno, E., Speciale, C., Kohler, C. and Whetsell, W.O. jr (1987) in: Neurotoxins and Their Pharmacological Implications (Jenner, P. ed.) pp. 19–32, Raven, New York.
[35] Wurtman, R.J., Rose, C.M., Chou, C. and Larin, F. (1968) N. Engl. J. Med. 279, 171–175.
[36] Fernstrom, J.D. and Wurtman, R.J. (1971) Science 173, 149–152.
[37] Ungerstedt, U. (1984) in: Measurement of Neurotransmitter Release In Vivo (Marsden, C.A. ed.) pp. 81–105, Wiley, New York.
[38] Monaghan, D.T. and Cotman, C.W. (1985) J. Neurosci. 5, 2909–2919.
[39] Perkins, M.N. and Stone, T.W. (1983) J. Pharmacol. Exp. Ther. 226, 551–554.
[40] Paxinos, G. and Watson, C. (1982) The Rat Brain in Stereotaxic Coordinates, Academic Press, New York.
[41] Sandberg, M., Butcher, S.P. and Hagberg, H. (1986) J. Neurochem. 47, 178–184.
[42] Heyes, M.P. and Markey, S.P. (1988) Biomed. Environ. Mass Spectrom. 15, 291–293.
[43] Heye, M.P. and Markey, S.P. (1988) Anal. Biochem. 174, 349–359.
[44] Anderson, G.M., Teff, K.L. and Young, S.N. (1987) Life Sci. 40, 2253–2260.
[45] Gallagher, D.W. and Aghajanian, G.K. (1976) Neuropharmacology 15, 149–156.
[46] Ternaux, J.P., Boireau, A., Bourgoin, S., Hamon, M., Hery, F. and Glowinski, J. (1976) Brain Res. 101, 533–548.
[47] Ternau, J.P., Hery, F., Hamon, M., Bourgoin, S. and Glowinski, J. (1977) Brain Res. 132, 575–579.
[48] Kennett, G.A. and Joseph, M.H. (1982) Brain Res. 236, 305–316.
[49] Adams, R.N. (1986) Ann. NY Acad. Sci. 473, 42–49.
[50] Whetsell, W.O. jr (1984) Clin. Neuropharmacol. 7, 248–250.
[51] Schwarcz, R., Whetsell, W.O. jr and Foster, A.C. (1983) in: Excitotoxins (Fuxe, K. et al. eds) pp. 122–137, Macmillan, London.

[52] Okuno, E. and Schwarcz, R. (1985) Biochim. Biophys. Acta 841, 112–119.
[53] Cooper, J., Bloom, F. and Roth, R.H. (1986) The Biochemical Basis of Neuropharmacology, Oxford University Press, Oxford.
[54] Schwarcz, R., Okuno, E., White, R.J., Bird, E.D. and Whetsell, W.O. (1988) Proc. Natl. Acad. Sci. USA 85, 4079–4081.
[55] Foster, A.C. and Schwarcz, R. (1985) J. Neurochem. 45, 199–205.
[56] Barbeau, A. (1969) Lancet ii, 1066.
[57] Belendiuk, K., Belendiuk, G.W. and Freedman, D.X. (1980) Arch. Gen. Psychol. 37, 325–332.
[58] Perkins, M.N. and Stone, T.W. (1982) Brain Res. 247, 184–187.
[59] Foster, A.C., Vezzani, A.M., French, E.D. and Schwarcz, R. (1984) Neurosci. Lett. 48, 273–278.
[60] Moroni, F., Russi, P., Lombardi, G., Beni, M. and Carla, V. (1988) J. Neurochem. 51, 177–180.
[61] Freese, A., Swartz, K.J. and During, M.J. (1988) Ann. Int. Med. 108, 312–313.