Running head: Boron distribution in the normal rat brain after BPA-f IV.

Title: Boron distribution in the normal rat brain after intravenous injection of boronophenylalanine-fructose

Author: Yasushi Shibata

Affiliations: Department of Neurosurgery, University of Tsukuba, Tsukuba, Ibaraki, Japan

Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

Department of Nuclear Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Corresponding author:

Yasushi Shibata, M.D., Ph.D.

Department of Neurosurgery, University of Tsukuba, Tsukuba, Ibaraki, 305-8575, Japan

Phone: +81-29853-3220

Fax: +81-29853-3214

E-mail: yshibata@md.tsukuba.ac.jp

ABSTRACT

Boron neutron capture therapy (BNCT) is an experimental form of radiation therapy for malignant brain tumors and peripheral melanoma. The micro-distribution of the boron compound is critical to determine the radiation effects for both tumors and normal tissue. In the current dose calculation of BNCT, normal brain tissue is considered to have a homogeneous boron concentration. The purpose of this study was to examine the structure-specific boron concentration in normal rat neural tissue. At 10, 30 and 60 minutes after intravenous injection of 300 mg/kg boronophenylalanine-fructose to 10-week-old CD Fisher rats, neural tissue and blood were collected. Various neural structures were anatomically and histologically identified and specific boron concentrations were analyzed using high-resolution quantitative autoradiography. At 60 minutes after the injection, only the pituitary gland showed a higher boron concentration than that in blood, with the former being three-fold higher. All other neural structures showed lower boron concentrations than that in blood. The present study thus demonstrated an extremely high boron concentration in the pituitary gland following intravenous injection of boronophenylalanine-fructose. In clinical trials of BNCT using an epithermal neutron beam, the radiation dose to the pituitary gland should be carefully evaluated.

Key words: boron, BPA, brain, neutron capture therapy, rat

INTRODUCTION

Boron neutron capture therapy (BNCT) is undergoing clinical trial for the treatment of malignant brain tumors and peripheral melanoma [1,2,3]. In this therapy, selective accumulation of boron in the tumor is critical for success. Precise measurement of the boron concentrations in the tumor and venous blood is essential to calculate the dose delivered to the tumor and the normal tissue. Most important dose-limiting factor is normal tissue tolerance. It is thus crucial to determine the exact boron uptake into normal tissue and the radiation dose of normal tissue. In dose calculation in BNCT, normal brain tissues have generally been treated as a single tissue having homogeneous boron distribution and homogeneous radiosensitivity. Reports describing the boron concentration in the normal brain after intravenous infusion of boron compound are scarce [4,5]. To our knowledge, there has been no study of the boron distribution in normal neural structures. High Resolution Quantitative Autoradiography (HRQAR) is a sensitive method for analyzing boron micro-distribution in tissue [6,7,8,9]. Using HRQAR, boron distributions are observed on histological structure and boron concentrations in specific anatomical structure are calculated. The aim of this study was to investigate the structure-specific distribution of boron in the normal rat brain using HRQAR. We considered that this knowledge would be helpful both for calculating the normal tissue dose and determining the critical normal structure in BNCT.

MATERIALS AND METHODS

Boronophenylalanine-fructose (BPA-f)

Boronophenylalanine (BPA) was purchased from Ryscor Science Inc. (North Raleigh, NC, USA) and converted to BPA-fructose (BPA-f) in our laboratory using previously reported methods [10,11]. Briefly, BPA and fructose were combined in water, the pH was adjusted to 10.0 with NaOH, the mixture was stirred until all solids dissolved, and the pH was

readjusted to 7.4 with HCL.

Animals

Male 10-week-old CD Fisher rats weighing 200 g were purchased from Charles River Laboratories, Inc. (Wilmington, MA, USA). Rats were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (5 mg/kg). Once the rats were sufficiently anesthetized, they were injected with 300 mg/kg of BPA-f through the tail vein using a 27G needle. The rats were sacrificed by pentobarbital overdose (100 mg/kg intraperitoneally) at 3 different time points (10, 30, and 60 min after the injection of BPA-f). Blood was collected by cardiac puncture and kept in EDTA-filled test tubes. Blood boron concentrations were measured using prompt gamma ray neutron activation analysis in the Massachusetts Institute of Technology Research Reactor II (MITR-II) [12]. The brain, optic nerve, trigeminal nerve, pituitary gland and cervical spinal cord were removed immediately and put into test tubes. These tubes were stored in a freezer and kept frozen for histological and HRQAR study. Pituitary gland is composed of large adenohypophysis and small neurohypophysis. We anatomically and histologically identified the adenohypophysis, but did not analyze the neurohypophysis in this study. These studies were conducted in accordance with accepted ethical and humane practices and were approved by the Harvard Medical Area Standing Committee on Animals and The Beth Israel Deaconess Medical Center Institutional Animal Care & Use Committee.

High Resolution Quantitative Autoradiography (HRQAR)

HRQAR is a special technique developed in our laboratory. Using this technique we can investigate the subcellular distribution of boron in tumors and the normal brain. The detailed procedures of this technique have been described in other publications [6]. In brief, a quartz glass microslide was coated with a film of Lexan and a thin film of Ixan. Lexan was used as an α and Li- particle detector, and Ixan as a protective layer over the tissue. The 2

films were laminated together. A boron containing frozen tissue were cut to a thickness of 4 microns using a cryostat microtome, then immediately placed on a coated and laminated quartz glass microslide. The quartz glass microslides were packed in dry ice and irradiated with thermal neutrons at MITR-II. The tissue on the quartz glass microslide was stained with hematoxylin and eosin (HE). Another glass slide is mounted on the tissue using glycerol/gelatin mountant , and the slides were refrigerated for 5 days at a temperature of 4°C. The quartz glass slides were separated and the outermost Lexan films were etched in KOH solution. The track etchings in the Lexan produced by α and Li-particles and the HE staining of tissue were simultaneously observed under a light microscope. Microscopic images were captured using OpenLab software (Improvision, Coventry, UK) on a Power Macintosh G3 computer connected to a microscope mounted with a CCD camera. The acquired images were analyzed using Trackanalysis software developed in our laboratory on a Power Macintosh G3 computer. The boron concentration in each anatomical and histological structure was measured by comparing with standard samples of known boron concentration. Boron concentrations in the following normal neural tissues were examined: the olfactory bulb, frontal cortex, optic nerve, caudate nucleus, thalamus, hypothalamus, pituitary gland, trigeminal nerve, hippocampus, occipital cortex, cerebellar cortex (molecular layer, granular layer), cerebellar white matter, ventral and dorsal midbrain, pons, medulla, cervical spinal cord, and posterior cerebral artery (PCA). Ten images for each neural structure were analyzed and the mean boron concentrations were calculated.

RESULTS

Figure 1 shows typical HRQAR images of the frontal lobe, thalamus and pituitary gland. All samples were taken from the same mouse at 60 min after i.v. administration of 300 mg/kg BPA-f. Figure 2 shows the mean boron concentrations in various nervous

system structures. All boron concentrations in neural structures and blood decreased with time. All neural structures showed lower boron concentration than that in blood, except for that in pituitary gland. Boron concentrations in the pituitary gland at 30 and 60 min after intravenous injection of BPA-f were higher than those in blood. The trigeminal nerve showed higher boron concentrations than other neural structures at 10 and 30 min after the injection. At 60 min after the injection, only the pituitary gland showed a higher boron concentration than that in blood, with the former being threefold greater.

DISCUSSION

Analysis of the micro-distribution of boron

Several methods are available to investigate the micro-distribution of boron in tissue. Traditional neutron-induced autoradiography has high sensitivity for measuring low concentrations of boron in tissue, but its low spatial resolution does not permit investigation of the micro-distribution of boron at the cellular or sub-cellular level [13,14,15,16]. Secondary ion mass spectrometry ion microscopy is another of the methods used to measure the micro-distribution and concentration of boron [17,18,19]. In this method, the distribution of sodium, potassium and calcium can be investigated at the same time. However, it is difficult to correlate the boron micro-distribution with the histological findings using this method, because the cell and nucleus boundaries are not seen directly. Using a resonance ionization microprobe, on the other hand, the boron micro-distribution can be investigated with a spatial resolution at the μm level [20]. In this method, the detection limit of boron is relatively high and accurate quantification of boron concentration in tissue was not reported. HRQAR has both high sensitivity and high resolution. HRQAR is the only method that can observe both the boron micro-distribution and the histological findings simultaneously without any superimposing.

Mechanism of BPA uptake into neural tissue

There have been many studies on BPA uptake into tumor cells and tumor tissue. An *in vitro* tumor study showed that transport of BPA across the cell membrane was dependent both on the time of exposure and the concentration of BPA in the culture medium [21]. A rat brain tumor study has shown that the accumulation of BPA in rapidly growing animal tumors could be due to the metabolic demand for the amino acids needed for protein synthesis [22]. A fluorine-18-labeled L-fluoro-BPA (18F-FBPA) PET study demonstrated that 18F-FBPA was accumulated in the amino acid pool of the tumor tissue by a retention mechanism via the amino acid anabolic pathway rather than by incorporation into proteins [23]. On the other hand, the uptake mechanisms of BPA into normal neural tissue are not completely understood. Chuang examined the boron concentration in normal neural tissues of rats after intravenous injection of BPA-f [24]. The boron concentrations in the cerebellum and brain stem were slightly higher and that in the cranial nerve was 1.5 times higher than that in the cerebrum. The boron concentration was measured with inductively coupled plasma atomic emission spectroscopy (ICP-AES), so more detailed anatomical structure-specific data were not available.

Regional cerebral blood flow

Because blood flow delivers intravenously injected BPA-f into neural structures, we reviewed the correlation between regional cerebral blood flow (rCBF) and boron concentration in neural tissue. Various authors have reported on rCBF in rats [25,26,27,28,29,30,31]. All these rCBF data were measured using autoradiography with 14C-iodoantipyrine. This method is well established and is used as a standard reference when examining the efficacy of new methods [32,33,34]. Generally, rCBF values are closely coupled to the respective metabolic rates [26,28]. And rCBF depends on age, anesthetic condition and species of animals [27,30,31]. Generally, the cerebral cortex, basal ganglia

(caudate, putamen, thalamus), cerebellar cortex, midbrain and pons show high mean rCBF values of between 1.0 and 1.5 ml/g/min; the cerebral white matter, corpus callosum, optic nerve, pituitary gland, cerebellar white matter and spinal cord show low mean rCBF values of about 0.5 ml/g/min; and the hypothalamus, hippocampus and medulla show intermediate mean rCBF values of between 0.5 and 1.0 ml/g/min. The cerebral cortex, caudate, thalamus, hypothalamus, hippocampus and cerebellar cortex showed high boron concentrations at 10 min after i.v. injection. Most of these high boron concentrations can be attributed to the high rCBF levels. However, the extremely high boron concentrations in the pituitary gland and trigeminal nerve can not be explained by rCBF.

Phenylalanine influx into neural tissue

Hawkins et al. examined radioactive phenylalanine influx into various cerebral structures in rats after intravenous injection using quantitative autoradiography [35]. They demonstrated that the phenylalanine influx varied considerably among structures, with the highest rate of influx occurring in the inferior colliculus in the dorsal midbrain and the lowest in the globus pallidus. Moguilevsky examined phenylalanine incorporation into neural tissue after intravenous injection [36]. The anterior pituitary gland showed high phenylalanine incorporation, more than double those of the hypothalamus and cerebral cortex. They concluded that the anterior pituitary gland has a higher protein synthetic activity than the hypothalamus or the cortex. Movement of large neutral amino acids across the blood brain barrier (BBB) is mediated by a common high affinity, low capacity L amino acid transport system. A large excess of one of the large neutral amino acids will saturate this carrier system, thereby excluding other amino acids in the group from entry into the brain [37]. Organs outside the BBB, such as the pituitary gland, have high capacity amino acid transport systems, and competitive effects are not seen [38,39].

Generally, metabolic demand, amino acid supply, glucose utilization and blood flow

are well correlated [26,28]. In the present study, the cerebral cortex, cerebellar cortex, thalamus, midbrain and pons showed high rCBF and high phenylalanine influx. The dorsal midbrain showed particularly high values for both these parameters. The cerebral white matter, cerebellar white matter and hypothalamus showed low rCBF and low phenylalanine influx. The pituitary gland had a low rCBF value, but the finding of a high boron concentration in the pituitary gland was reasonable because the pituitary gland has a high protein synthetic activity and high capacity amino acid transport system.

Boron accumulation in the dopaminergic neurons

Dopamine synthesis originates from the amino acid precursors phenylalanine and tyrosine. Setiawan et al. investigated the accumulation of BPA in the catecholamine-containing neurons, because BPA may be a precursor for the catecholamine neurotransmitters [40]. However, it was not possible to measure boron localization in the mouse brain using the neutron capture autoradiography employed in their study. Their immunohistochemical study showed a transient decrease of tyrosine hydroxylase activity in the dopaminergic fibers and cell bodies 4 hours after BNCT with BPA. Although this effect recovered by 48 hours, it should be dependent on the dose of injected BPA and neutron fluence. These results demonstrated that BPA accumulates in the dopaminergic neurons and transiently affects catecholamine-synthesizing pathways.

We did not investigate dopamine neuron-specific BPA uptake. The central nervous dopaminergic system includes the olfactory, tuberohypophysial, incentohypothalamic, and medullary neurons, and the ventral tegmental, substantia nigra, caudate-putamen and limbic systems [41]. Previous studies have demonstrated the accumulation of dopamine and L-dopa in the pituitary gland [42,43,44]. In the present study, we observed an extremely high BPA uptake only in the pituitary gland. This high boron concentration in the pituitary gland might be partially attributed to BPA uptake into the pituitary gland as a precursor of

dopamine. However, we did not observe a high boron concentration in other neural structures containing dopaminergic neurons. The purpose of the present study was to quantify the boron localization in gross anatomical structures. The boron uptake to specific types of neurons should be investigated in a future study. The combination of HRQAR and immunohistochemical staining might provide valuable findings. The current treatment planning system, however, cannot avoid the irradiation of particular types of neurons, although it can avoid irradiation to critical structures by means of fine treatment planning.

Normal tissue tolerance dose

Because BNCT is given as high dose radiation therapy with or without some fractionations, the normal tissue tolerance in radiosurgery becomes the reference value. Recent developments and clinical experiences of radiosurgery have revealed the normal tissue tolerance dose of high dose radiation therapy. The optic nerve, brain stem and hypothalamo-pituitary axis are critical normal structures in radiosurgery. The reported tolerance doses of the optic nerve to radiosurgery range from 8 Gy to 10 Gy [45,46]. Other cranial nerves are relatively resistant to single large doses of radiation, except for the chochlear nerve [46,47,48]. The brain stem is radiosensitive, and brain stem doses greater than 16 Gy are associated with a high risk of cranial neuropathy [49]. The hypothalamo-pituitary axis is also radiosensitive, and the effect of irradiation of this axis is time- and dose-dependent [50]. The pituitary complications occurred at doses substantially less than those commonly used for the tumor treatment and the minimum reported total dose required to induce pituitary dysfunction was 30 Gy with 20 fractionations [51,52]. The clinical manifestations of hypopituitarism depend on which hormones are lost and the extent of the hormone deficiency [53]. Provocative tests may be required to assess pituitary reserve. These endocrine consequences of radiation are believed to reflect hypothalamic damage rather than anterior pituitary damage [50,51]. In BNCT, the boron concentration in

tissue is one of the factors used to determine the radiation dose. Based on our present findings, the pituitary gland and trigeminal nerve should be treated as critical structures that have high boron concentrations. There have been no reported cases of pituitary dysfunction or cranial nerve palsy directly as a consequence of BNCT for intracranial tumor. Epithermal neutrons are able to deliver a larger amount of neutron fluence into the deep intracranial structures than thermal neutrons. The BNCT clinician should be aware of the possibility of pituitary dysfunction and cranial nerve palsy after BNCT. To avoid these complications, precise knowledge of the boron concentration in normal neural tissue and fine treatment planning that includes consideration of the pituitary gland and cranial nerve are required. For numerous reasons, the present results in rats cannot be directly translated to humans. A boron distribution study using large primates or other mammals would be helpful for estimating the boron distribution in human neural tissue.

The compound biological effectiveness (CBE) factor has been used to describe the effects of the dose component due to B(n,α)Li reaction [54,55]. The CBE factor is a combination of the relative biological effectiveness of α particles and the biodistribution of the boron compound at the cellular and subcellular levels. The CBE factor for normal brain was derived in the rat spinal cord [56]. At 60 min after the intravenous injection of BPA, the boron concentration in the spinal cord was almost the same or higher than the boron concentration in most normal neural tissues, except for the pituitary gland. Thus the CBE factor derived from the rat spinal cord is reasonable as the representative CBE factor for normal neural tissue, except in the case of the pituitary gland. More detailed, structure-specific measurements of CBE should be made in a future study.

ACKNOWLEDGMENTS

This study was supported by an Innovations in Nuclear Infrastructure and Education

(INIE) grant from the Department of Energy to the Massachusetts Institute of Technology (No. DE-FG07-02ID14420). I am grateful to Dr. Stead Kiger, Dr. Hemant Patel and Dr. Robert Zamenhof for their help with the sample preparation and analysis. I would also like to thank the operational and radiation safety staff members at MITR-II for their assistance with the sample irradiation.

FIGURE LEGENDS

FIG. 1. High resolution quantitative autoradiograms of the frontal cortex (left), thalamus (middle) and pituitary gland (right). The tissue was taken 60 min after i.v. administration of 300 mg/kg of BPA-f. Hematoxylin and eosin staining. Original magnification is 400X.

FIG. 2. Boron concentration in various neural tissues and blood. PCA: posterior cerebral artery; molecular and granular layer: molecular and granular layer of the cerebellar cortex

REFERENCES

1. Miyatake S, Kawabata S, Kajimoto Y, et al: Modified boron neutron capture therapy for malignant gliomas performed using epithermal neutron and two boron compounds with different accumulation mechanisms: an efficacy study based on findings on neuroimages. J Neurosurg.103: 1000-1009, 2005
2. Matsumura A, Yamamoto T, Shibata Y, et al: "Intraoperative Boron Neutron Capture Therapy using Thermal/Epithermal Mixed," *Proc 10th Int Symp on Neutron Capture Therapy for Cancer*, Monduzzi Editore, Bologna, 2002, pp.1073-1078
3. Barth, RF, Coderre JA, Vicente MG, et al: Boron neutron capture therapy of cancer: current status and future prospects. Clin Cancer Res 11: 3987-4002, 2005
4. Elowitz EH, Bergland RM, Coderre JA, et al: Biodistribution of p-boronophenylalanine in patients with glioblastoma multiforme for use in boron neutron capture therapy. Neurosurgery 42: 463-468, 1998
5. Mallesch JL, Moore DE, Allen BJ, et al: The pharmacokinetics of p-Boronophenylalanine.fructose in human patients with glioma and metastatic melanoma. Int J Radiat Oncol Biol Phys 28: 1183-1188, 1994
6. Kiger III WS: Developments in Micro- and Macrodosimetry of Boron Neutron Capture Therapy, Nuclear Engineering. Cambridge, Massachusetts Institute of Technology, 2000
7. Solares GR, Zamenhof RG: A novel approach to the microdosimetry of neutron capture therapy. Part I. High-resolution quantitative autoradiography applied to microdosimetry in neutron capture therapy. Radiat Res 144: 50-58, 1995
8. Zamenhof RG: Microdosimetry for boron neutron capture therapy: a review. J Neurooncol 33: 81-92, 1997
9. Zamenhof RG, Clement S, Lin K, et al: Monte Carlo treatment planning and high-resolution alpha- track autoradiography for neutron capture therapy. Strahlenther

10. Yoshino K, Suzuki A, Mori Y, et al: Improvement of solubility of p-boronophenylalanine by complex formation with monosaccharides. Strahlenther Onkol 165: 127-129, 1989
11. Coderre JA, Chanana AD, Joel DD, et al: Biodistribution of boronophenylalanine in patients with glioblastoma multiforme: boron concentration correlates with tumor cellularity. Radiat Res 149: 163-170, 1998
12. Riley KJ, Harling OK: An improved prompt gamma neutron activation analysis facility using a focused diffracted neutron beam. Nucl Instrum Methods Phys Res B 143: 414-421, 1998
13. Abe M, Amano K, Kitamura K, et al: Boron distribution analysis by alpha-autoradiography. J Nucl Med 27: 677-684, 1986
14. Gabel D, Holstein H, Larsson B, et al: Quantitative neutron capture radiography for studying the biodistribution of tumor-seeking boron-containing compounds. Cancer Res 47: 5451-5454, 1987
15. Kubota R, Yamada S, Ishiwata K, et al: Cellular accumulation of 18F-labelled boronophenylalanine depending on DNA synthesis and melanin incorporation: a double-tracer microautoradiographic study of B16 melanomas in vivo. Br J Cancer 67: 701-705, 1993
16. Pettersson OA, Grusell E, Larsson B, et al: Quantitative neutron capture radiography for boron in biological specimens. Phys Med Biol 38: 1089-1097, 1993
17. Bennett B, J MZ, JA C, et al: Subcellular localization of p-boronophenylalanine-delivered boron-10 in the rat 9L gliosarcoma: cryogenic preparation in vitro and in vivo. Radiat Res 140: 72-78, 1994
18. Morris GM, Smith DR, Patel H, et al: Boron microlocalization in oral mucosal tissue: implications for boron neutron capture therapy. Br J Cancer 82: 1764-1771, 2000

19. Smith DR, Chandra S, Coderre JA, et al: Ion microscopy imaging of 10B from p-boronophenylalanine in a brain tumor model for boron neutron capture therapy. Cancer Res 56: 4302-4306, 1996
20. Arlinghaus HF, Spaar MT, Switzer RC, et al: Imaging of boron in tissue at the cellular level for boron neutron capture therapy. Anal Chem 69: 3169-3176, 1997
21. Wittig A, Sauerwein WA, Coderre JA: Mechanisms of transport of p-borono-phenylalanine through the cell membrane in vitro. Radiat Res 153: 173-180, 2000
22. Coderre JA, Glass JD, Fairchild RG, et al: Selective delivery of boron by the melanin precursor analogue p-boronophenylalanine to tumors other than melanoma. Cancer Res 50: 138-141, 1990
23. Imahori Y, Ueda S, Ohmori Y, et al: Fluorine-18-labeled fluoroboronophenylalanine PET in patients with glioma. J Nucl Med 39: 325-333, 1998
24. Chuang CF: Experimental evaluation and mathematical modeling of the pharmacokinetics of boronophenylalanine-fructose(BPA-f) in murine tumor models, Nuclear Engineering. Cambridge, Massachusetts Institute of Technology, 1999
25. Bryan RM, Jr., Cherian L, Robertson C: Regional cerebral blood flow after controlled cortical impact injury in rats. Anesth Analg 80: 687-695, 1995
26. Ginsberg MD, Busto R, Boothe TE, et al: A radioisotopic method for the simultaneous quantitation of regional cerebral blood flow and glucose utilization in small dissected samples: validation studies and values in the nitrous oxide-anesthetized rat. Brain Res 230: 165-179, 1981
27. Ohata M, Sundaram U, Fredericks WR, et al: Regional cerebral blood flow during development and ageing of the rat brain. Brain 104: 319-332, 1981
28. Pulsinelli WA, Levy DE, Duffy TE: Regional cerebral blood flow and glucose

29. Rosenorn J, Diemer NH: Reduction of regional cerebral blood flow during brain retraction pressure in the rat. J Neurosurg 56: 826-829, 1982
30. Sakurada O, Kennedy C, Jehle J, et al: Measurement of local cerebral blood flow with iodo [14C] antipyrine. Am J Physiol 234: H59-66, 1978
31. Salter JM, Cassone VM, Wilkerson MK, et al: Ocular and regional cerebral blood flow in aging Fischer-344 rats. J Appl Physiol 85: 1024-1029, 1998
32. Prunte C, Flammer J, Markstein R, et al: Quantification of optic nerve blood flow changes using magnetic resonance imaging. Invest Ophthalmol Vis Sci 36: 247-251, 1995
33. Rudin M, Sauter A: Noninvasive determination of regional cerebral blood flow in rats using dynamic imaging with Gd(DTPA). Magn Reson Med 22: 32-46, 1991
34. Wittlich F, Kohno K, Mies G, et al: Quantitative measurement of regional blood flow with gadolinium diethylenetriaminepentaacetate bolus track NMR imaging in cerebral infarcts in rats: validation with the iodo[14C]antipyrine technique. Proc Natl Acad Sci U S A 92: 1846-1850, 1995
35. Hawkins RA, Mans AM, Biebuyck JF: Amino acid supply to individual cerebral structures in awake and anesthetized rats. Am J Physiol 242: E1-11, 1982
36. Moguilevsky JA, Kalbermann LE, Libertun C, et al: Effect of orchiectomy on the amino acid incorporation into proteins of anterior pituitary and hypothalamus of rats. Proc Soc Exp Biol Med 136: 1115-1118, 1971
37. Carlson HE, Hyman DB, Blitzer MG: Evidence for an intracerebral action of phenylalanine in stimulation of prolactin secretion: interaction of large neutral amino acids. J Clin Endocrinol Metab 70: 814-816, 1990
38. Ablett RF, MacMillan M, Sole MJ, et al: Free tyrosine levels of rat brain and tissues with sympathetic innervation following administration of L-tyrosine in the presence and

absence of large neutral amino acids. J Nutr 114: 835-839, 1984

39. Hargreaves KM, Pardridge WM: Neutral amino acid transport at the human blood-brain barrier. J Biol Chem 263: 19392-19397, 1988
40. Setiawan Y, Halliday GM, Harding AJ, et al: Effect of L-10B-p-boronophenylalanine-fructose and the boron neutron capture reaction on mouse brain dopaminergic neurons. Cancer Res 55: 874-877, 1995
41. Friedman JH: Mood, Emotion, and Thought, in Goetz CG, Pappert EJ (eds): Textbook of Clinical Neurology (ed 1st). Philadelphia, W. B. Saunders, 1999, pp 2003
42. Demarest KT, Alper RH, Moore KE: Dopa accumulation is a measure of dopamine synthesis in the median eminence and posterior pituitary. J Neural Transm 46: 183-193, 1979
43. Partanen S: Fluorescence histochemical observations on the uptake and metabolism of tryptophan in the endocrine cells of the hypophysis. Med Biol 53: 114-120, 1975
44. Partanen S, Rechardt L, Back N: Histochemical observations on uptake of L-dopa into endocrine cells of the rat pituitary gland during the postnatal development. Cell Tissue Res 156: 451-461, 1975
45. Leber KA, Bergloff J, Pendl G: Dose-response tolerance of the visual pathways and cranial nerves of the cavernous sinus to stereotactic radiosurgery. J Neurosurg 88: 43-50, 1998
46. Tishler RB, Loeffler JS, Lunsford LD, et al: Tolerance of cranial nerves of the cavernous sinus to radiosurgery. Int J Radiat Oncol Biol Phys 27: 215-221, 1993
47. Leber KA, Bergloff J, Langmann G, et al: Radiation sensitivity of visual and oculomotor pathways. Stereotact Funct Neurosurg 64 Suppl 1: 233-238, 1995
48. Urie MM, Fullerton B, Tatsuzaki H, et al: A dose response analysis of injury to cranial nerves and/or nuclei following proton beam radiation therapy. Int J Radiat Oncol Biol

Phys 23: 27-39, 1992

49. Meeks SL, Buatti JM, Foote KD, et al: Calculation of cranial nerve complication probability for acoustic neuroma radiosurgery. Int J Radiat Oncol Biol Phys 47: 597-602, 2000
50. Robinson IC, Fairhall KM, Hendry JH, et al: Differential radiosensitivity of hypothalamo-pituitary function in the young adult rat. J Endocrinol 169: 519-526, 2001
51. Chrousos GP, Poplack D, Brown T, et al: Effects of cranial radiation on hypothalamic-adenohypophyseal function: abnormal growth hormone secretory dynamics. J Clin Endocrinol Metab 54: 1135-1139, 1982
52. Wigg DR, Murray RM, Koschel K: Tolerance of the central nervous system to photon irradiation: endocrine complications. Acta Radiol Oncol 21: 49-60, 1982
53. Melmed S: Disorders of the Anterior Pituitary and Hypothalamus, in Eugene Braunwald ASF, Kurt J. Isselbacher, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, and J. Larry Jameson (ed): Harrison's Online (ed 15th edition). New York, McGraw-Hill, 2003
54. Coderre JA, Morris GM: The radiation biology of boron neutron capture therapy. Radiat Res 151: 1-18, 1999
55. Gabel D, Philipp KH, Wheeler FJ, et al: The compound factor of the 10B(n,alpha)7Li reaction from borocaptate sodium and the relative biological effectiveness of recoil protons for induction of brain damage in boron neutron capture therapy. Radiat Res 149: 378-386, 1998
56. Morris GM, Coderre JA, Hopewell JW, et al: Response of the central nervous system to boron neutron capture irradiation: evaluation using rat spinal cord model. Radiother Oncol 32: 249-255, 1994