ABSTRACTS

AMINO ACIDS IN HEALTH AND DISEASE: NEW PERSPECTIVES
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Lewis Stegink and Savio Woo, Organizers
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Amino Acid Transport, Barriers and Compartmentation

R1 MEMBRANE TRANSPORT IN INTERORGAN AMINO ACID FLOWS: WHERE DO DEPLETED PLASMA AMINO ACIDS GO IN PKU? H.N.Christensen, Dept.of Biol.Chem.,Univ.of Mich.,Ann Arbor 48109

Virtually every cell in the body is nourished in part by nutrient flows from various other tissues. The direction, intensity, and regulation of uphill amino acid transport by several catalytic systems are important factors in determining these flows. The various tissues compete in determining to what extent each will be a source and which will be a target for this traffic. By treating the alimentary canal as the source of the flows, traditional global consideration of nutrition has led to an overly sharp division of amino acids between the indispensable and those that appear dispensable. A key question of nutrition at the cellular level is this one: Is the concentration of each amino acid in or its flow sustained to a pertinent cellular compartment functionally sufficient[1]? For example, in infants with a variety of inborn errors of amino acid metabolism requiring restricted protein intake,dietary alanine supplements exert an important protein-sparing action, presumably by assisting the interorgan flows of the alanine-glucose cycle[2]. I proposed in 1953[3] that the phenylalanine accumulations of PKU inhibit the transport of analogous amino acids into brain, an action since rendered understandable by their common movement by System L across the blood-brain barrier. Unless membrane transport is perturbed, spontaneous variations of the levels of a given amino acid tend to occur together in plasma and tissues[3]. We now show with old data[4] that amino acids supplied singly in excess fall into 2 classes, those whose transport competition lowers and those whose action instead elevates various other amino acids in liver and muscle. Those of the first group inhibit as a net the influx of endogenous amino acids, especially by sodium-dependent System A, whereas those of the second group inhibit net efflux by Na^+-independent System L. This dichotomy arises from a cooperation by which System L is forced into service for exodus by the stronger amino acid gradients generated by the more uphill systems. I propose that pathological accumulations of an efflux-inhibiting amino acid (e.g. phenylalanine in PKU, leucine in maple syrup urine disease) cause sequestering of several endogenous amino acids into various tissues, and hence their accelerated catabolism. This action may well account for the previously puzzling fall of the plasma levels of a number of amino acids in PKU (e.g. threonine, glutamine, asparagine, proline, alanine, etc.[5]). Because System L operates as a net inward across the blood-brain barrier, the sequestering action by other tissues will not be balanced for the brain. The aggregate effect of a) inhibited inward transport at the blood-brain barrier and b) stimulated amino acid sequestration in other tissues complicates prediction of the full list of brain amino acids depleted in PKU. Ackn. support, HD01233,NIH.

1) H.N. Christensen, Physiol. Rev. 62: 1193, 1982. 2) J.A. Wolff et al., J. Neurogenet. 2: 41, 1985. 3) H.N. Christensen, Ann. Rev. Biochem. 22: 235, 1913. 4) H.N. Christensen et al. J. Biol. Chem. 172: 515, 1948. 5) M.L. Efron et al., J. Pediatrics 74: 539, 1969.

R2 THE ROLE OF DIET IN ADAPTATION OF RENAL TUBULE TRANSPORT, Russell W. Chesney, M.D., Department of Pediatrics, University of California-Davis, CA 95616.

The sulfur-containing B-amino acid, taurine, is a putative neuromodulating compound and may be important in osmoregulation and cell volume regulation. The whole body homeostasis of this compound appears to be regulated by the kidney.

The renal tubular epithelium is able to conserve ions and amino acids during periods of decreased dietary intake and to excrete them following dietary excess. In rats or mice fed a diet containing a reduced content of sulfur amino acids, the renal reabsorption and isolated tubule uptake of taurine, a sulfur-containing B-amino acid, is greatly enhanced. This has been termed "the renal adaptive response to altered dietary intake of sulfur amino acids". This response is expressed at the apical surface of the proximal tubule, since this is the site of active amino acid accumulation and since brush border membrane vesicles (BBMV) prepared from animals fed the low sulfur amino acid diet (LTD) take up taurine to a greater degree than BBMV from animals fed normal amounts of sulfur amino acids. A diet high in taurine (HTD) results in massive (17-fold higher) taurinuria and decreased tubule and BBMV uptake. These changes in tubule and BBMV accumulation are associated with a change in the initial (15 sec) rate of taurine uptake (Vmax) rather than a change in the affinity of the uptake system for taurine (Km) connected with dietary manipulation. Moreover, at equilibrium (45 min), taurine accumulated within vesicles is the same regardless of diet indicating that the adaptive response occurs only in the early, Na^+ gradient-energized portion of taurine accumulation across the apical surface. This adaptive response also is expressed in the immature kidney and in collagenase-isolated tubule segments.

The signal for and the membrane-related events that underlie this adaptive response are unclear. From a nutritional point of view it is appropriate to conserve glucose and amino acids within the renal tubule following periods of dietary deprivation. The problem of the signal underlying taurine conservation within the proximal tubule is particularly germain since whole body taurine content is likely regulated by the kidney. The plasma concentration of taurine or the concentrations of taurine found in the intracellular water of the renal cortex cell may govern this renal adaptive response. Maneuvers directed toward further reducing renal cortex taurine content, including simultaneous feeding with B-alanine, fasting and feeding the LTD, enhance initial rate Vmax of taurine uptake without significantly altering plasma taurine values. In addition, feeding 3% methionine, which raises plasma taurine, does not alter either renal cortex or BBMV uptake. Thus, the latter change (renal cortex content) is more likely to govern the adaptive response.

R3 PHENYLALANINE TRANSPORT AT THE HUMAN BLOOD-BRAIN BARRIER, William M. Pardridge, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Nutritional regulation of brain amino acid metabolism, including neurotransmitter production, arises from two physical properties of brain amino acid transport and metabolism. First, the rate-limiting enzymes within most pathways of brain neutral amino acid metabolism are characterized by Km values > the existing precursor amino acid concentration in brain; thus, fluxes through these rate-limiting steps rise or fall in proportion to the ambient amino acid concentration. Second, amino acid transport through the brain capillary endothelial wall, i.e., the blood-brain barrier (BBB), the rate-limiting step in amino acid movement from blood to brain intracellular space, is characterized by Km values that are uniquely low (compared to cell membrane transport in nonbrain organs) and these BBB Km values approximate the normal plasma concentrations of neutral amino acids. Since all of the essential neutral amino acids traverse the BBB via a common transport system, the availability of a given amino acid in brain, e.g., tryptophan, is a function, not only of blood tryptophan concentration, but also of the concentration of the other neutral amino acids in blood that compete with tryptophan for BBB transport sites. The affinity of the various neutral amino acids for the BBB transport system differs over a log order of magnitude and phenylalanine has the highest affinity for the BBB transport system of any of the neutral amino acids in blood. Knowledge of the kinetics of amino acid transport through the BBB provides insight into the pathophysiologic significance of the hyperaminoacidemias, in particular hyperphenylalaninemia, e.g., severe sustained hyperphenylalaninemia associated with phenylketonuria (PKU) or mild intermittent hyperphenylalaninemia associated with aspartame ingestion. A caveat in explaining the pathophysiology of hyperaminoacidemias in humans, on the basis of the BBB Km paradigm, is that the Km values were determined in rats. Conceivably, BBB Km values for amino acid transport at the human BBB may be high (e.g., relative to the existing plasma amino acid concentrations) and, if this were so, the human brain would not be subject to amino acid competition effects. A new model system of the human BBB is the isolated human brain capillary (Pardridge, et. al., 1985). Recent studies of the transport of ^{3}H-phenylalanine into human brain capillaries indicate the Km of phenylalanine transport at the human BBB is approximately 20 μM, which is nearly identical to the Km value found for the rat BBB using either the carotid injection technique or isolated rat brain capillaries. Phenylalanine uptake into the isolated human brain capillary is inhibited by physiologic concentration of large neutral amino acids, modestly inhibited by small neutral amino acids, and not inhibited by basic, acidic, or immunoglycine amino acids. These studies indicate that the human brain, like the rat brain, is subject to exquisite amino acid transport competition effects at the BBB within the physiologic range of plasma amino acid concentrations.

R4 KINETICS OF NEUTRAL AMINO ACID TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER STUDIED USING AN IN SITU BRAIN PERFUSION TECHNIQUE. Quentin R. Smith, Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Neutral amino acids are transported across the blood-brain barrier by a single facilitated transport system. To characterize the transport kinetics of this system, we determined the concentration dependence of cerebrovascular influx for 11 amino acids in pentobarbital-anesthetized rats. Influx was measured with the brain perfusion technique of Takasato et al. (1), which allows accurate measurements of cerebrovascular transport from saline or blood. Then, for each amino acid we calculated the four parameters that describe influx into the brain; the Vmax and Km of the saturable component, the apparent Km for saturable transport from plasma (Km(app)), and the constant of nonsaturable diffusion (Kd).

Cerebrovascular Vmax and Km values differed significantly among the 11 amino acids. Vmax ranged from 3.1×10^{-4} umol/s/g for Thr to 13×10^{-4} umol/s/g for Tyr. The average Vmax for the 11 amino acids equaled 8.6×10^{-4} umol/s/g. For each amino acid regional Vmax was 30% greater in the cortical lobes than in other brain regions, consistent with the greater vascularity of the cortex. Km values ranged from 0.01 umol/ml for Phe to 0.94 umol/ml for Gln, and were inversely related to amino acid size and lipohilicity. There were no regional differences in Km. Kd values were small and ranged from 0 to 3×10^{-4} s^{-1}.

For each amino acid the apparent Km for transport from plasma was 10-30 fold greater than the true Km, and ranged from 0.22 umol/ml for Phe to >5 umol/ml for Gln and Thr. The Km(app) for transport from plasma was markedly greater than the true Km because 11 or more amino acids compete for the same system and because the plasma concentration of each amino acid approximates the Km of the transport system. Competitive inhibition increased the Km(app) for each amino acid so that Km(app) >> normal plasma concentration.

In conclusion, this study presents a new kinetic analysis of neutral amino acid transport across the blood-brain barrier. The results indicate that both transport capacity and affinity are greater than previously thought, and that at normal plasma concentrations the transport system is effectively saturated (96%) with amino acids. As a result, influx of each neurtral amino acid will remain approximately constant when plasma concentrations of all amino acids increase or decrease by the same fraction. However, influx into the brain will be sensitive to imbalances in plasma amino acid concentrations because an increase in the influx of one amino acid can occur only at the expense of the influxes of other amino acids.

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Neurotransmitter Regulation

R5 UNCONVENTIONAL CONTROL MECHANISMS IN MONOAMINERGIC NEURONES. John W. Commissiong, Department of Physiology, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6

Examples of assumptions that guide our thinking about regulatory mechanisms in monoaminergic neurons will be illustrated with results from the spinal cord and striatum. 1) In the dorsal horn of the cervical spinal cord of rat, the concentration of NE (40 ± 3 pmol/mg protein) is 5 times that of DA (7.1 ± 0.9). However, the rate constant (Hr^{-1}) of DA is 2.7 and of NE only 0.32, with the result that the apparent rate of utilization of DA is actually higher than that of NE. 2) At 1 hr after the injection of morphine (10 mg/kg s.c.), the concentration of 5-HT is unchanged, but 5-HIIA is increased equally in the three main functional regions (sensory, autonomic and somatic motor) of the rat spinal cord. There is no evidence of a more significant increase in the dorsal horn. 3) At 100 days after a complete mid-thoracic cordotomy, the isolated caudal region of the cord, is devoid of descending monoaminergic nerve terminals, but is able to metabolize DA (synthesized from injected L-DOPA) to its two major metabolites DOPAC and HVA, as efficiently as the intact spinal cord. 4) The increases in DOPAC and HVA normally seen in the striatum after electrical stimulation of the nigrostriatal tract, are not an invariant result. When stress is minimized, the increases are small, and are not statistically significant. 5) After a complete lesion of the left nigrostriatal tract, the isolated nerve terminals in the left striatum retain the ability to regulate the synthesis and metabolism of DA for up to 24 hours. 6) After chemical blockade (300 μM TTX), or an acute knife cut of the left nigrostriatal tract, haloperidol (HAL), 1.0 mg/kg i.p., is still able to induce a significant increase in DOPAC and HVA in the left striatum. 7) Under some conditions, at 4 hrs after transection of the left nigrostriatal tract, there is a supranormal increase in the synthesis and metabolism of DA in the left striatum. DA is unchanged, and DOPAC and HVA are increased by more than 3 and 6 times respectively. The results detailed above lead to the following questions: 1) In a physiological context, what information is gained from a knowledge of the steady-state concentration of monoamine neurotransmitters? 2) What is the functional significance of monoamine metabolites (5-HIAA, DOPAC, HVA, MHPG, DHPG etc)? 3) Do these metabolites provide any useful information about the incidence of orthodromic action potential activity in monoaminergic neurons? 4) Do monoamine metabolites always reflect monoamine release? 5) Is there a significant role for the direct presynaptic action of neuroleptic drugs on dopaminergic nerve terminals. 6) Is the negative feedback hypothesis a correct explanation for regulation of dopaminergic cell firing? A critical re-examination of some of the accepted explanations in this field could prove to be valuable in guiding future research activity in this area.

R6 EFFECTS OF NUTRIENTS ON BRAIN FUNCTION AND BEHAVIOR -- EXAMINATION OF SOME ASSUMPTIONS, Francis V. DeFeudis, Department of Biological Chemistry, Faculty of Medicine, University of Strasbourg, 67084 Strasbourg Cedex, France

Administration of high doses of precursors for serotonin, dopamine, norepinephrine or acetylcholine to experimental animals, either systemically or by dietary supplementation, can, in some cases, lead to changes in brain levels of the precursor and its associated neurotransmitter(s). Competition among large ("bulky") neutral amino acids (e.g., L-DOPA, tryptophan, tyrosine, phenylalanine) for transport sites of the cerebrovascular endothelium ("blood-brain barrier") can be useful in explaining changes that might occur in the cerebral accumulation of a given large neutral amino acid. However, the proportions of precursor uptake and neurotransmitter synthesis that might occur in the cerebrovascular endothelium, as opposed to the brain parenchyma (neurones and glia), remain largely unknown. In this regard, recent studies have revealed that both serotonin (1) and acetylcholine (2) can be synthesized in cerebrovascular endothelial cells. Therefore, it seems necessary to conduct further studies on the uptake, synthetic and metabolic activities of the brain's vascular endothelial compartment before one can expect to understand the mechanisms underlying the changes in CNS neuronal activity, or the behavioral or neurologic effects, that might follow "loading" with monoamine- or acetylcholine-precursors (3).

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(2) Parnavelas, J.G., Kelly, W. and Burnstock, G. (1985) Nature (Lond.) 316, 724-725.
(3) DeFeudis, F.V. (1986) Trends Pharmac. Sci. In press.

R7 Regulatory Control of Midbrain Dopamine Neurons

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Biochemical and electrophysiological studies have demonstrated that, in contrast to the intensively studied nigrostriatal(NS) and mesolimbic(ML) dopamine(DA) neurons, certain subpopulations of midbrain(MB) DA neurons appear to lack impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors. The absence or insensitivity of this important class of receptors on the mesoprefrontal(MP) and mesocingulate(MC) DA neurons may, in part, explain some of the unique biochemical properties of these two subpopulations of MB DA neurons. For example, the MP and MC DA neurons appear to have a faster firing rate, exhibit more bursting and have a more rapid turnover of transmitter than the NS, ML and mesopiriform(MPY) DA neurons. The MP and MC DA neurons also show a diminished biochemical responsiveness to DA agonists and antagonists. Administration of low doses of apomorphine or autoreceptor selective agonists, in contrast to their inhibitory effects on other MB DA neurons are ineffective in inhibiting the physiological activity or lowering DA metabolite levels in these two cortical DA projections. DA receptor blocking drugs produce large increases in synthesis and the accumulation of DA metabolites in the NS, ML and MPY DA neurons, but have only a modest effect in the MP and MC DA neurons. Following chronic administration of antipsychotic drugs, tolerance develops to the metabolite elevating effects of these agents in the MB DA systems possessing autoreceptors, but not in the systems lacking autoreceptors. Transmitter synthesis is more readily influenced by altered availability of precursor tyrosine in MB DA neurons lacking autoreceptors(MP and MC) than in those midbrain DA neurons possessing autoreceptors, perhaps related to the enhanced rate of physiological activity of this subpopulation of MB DA neurons.

The MP DA neurons have additional unique properties which do not appear completely related to the absence of autoreceptors. For example, the MP DA neurons are very sensitive to activation by stress, conditioned fear and other environmental perturbations. The activation produced can be prevented by pretreatment with anxiolytic benzodiazepines and appears related to the activation of habenulo-VTA substance P neurons. Recent studies have demonstrated that MP DA neurons are also selectively activated by systemic administration of anxiogenic beta carbolines. This activation is reversed by pretreatment with central benzodiazepine receptor agonists and antagonists suggesting that a benzodiazepine-GABA recognition site exerts a selective and powerful modulatory control on this subset of VTA DA neurons. MH-14092.

R8 BRAIN TRYPTOPHAN AVAILABILITY AND SEROTONIN SYNTHESIS, John D. Fernstrom, Department of Psychiatry and Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh PA 15213.

The rate of serotonin (5HT) synthesis in the mammalian brain is sensitive to variations in tryptophan supply. This relationship occurs because tryptophan hydroxylase, which catalyzes the rate limiting step in 5HT synthesis, is normally not saturated with its substrate, L-tryptophan (TRP). In fact, brain TRP levels normally approximate the enzyme's K_m for this substrate. As a consequence, raising or lowering brain TRP levels rapidly increases or decreases, respectively, the rate of 5HT synthesis (see [1]). The ability of TRP availability to influence 5HT synthesis appears to be the dominant regulatory feature of this biochemical pathway. Even the chronic elevation or depression of brain TRP levels continues for an extended period of time to be associated with increased or decreased 5HT formation, respectively, suggesting that no biochemical compensatory mechanisms are activated to neutralize such precursor-induced alterations in transmitter synthesis. Indeed, this extraordinary vulnerability of the 5HT synthetic pathway to variations in precursor supply sets it apart from other transmitter synthetic pathways in brain. This direct relationship of TRP level in brain to the rate of 5HT synthesis evokes two general questions: (a) does normal (and abnormal) body functioning lead to changes in brain TRP uptake, and as a consequence, in 5HT synthesis ? And (b) do TRP-induced changes in 5HT synthesis influence the amount of transmitter released into the synaptic cleft, thereby altering brain function ? The answer to both questions appears to be yes, though at present with some qualifications. First, a large body of literature now demonstrates that variations in brain tryptophan level, induced by both physiologic (e.g., meal consumption) and pathophysiologic (e.g., diabetes) phenomenon, produce expected changes in brain 5HT synthesis [1]. And second, a variety of studies demonstrate clear effects of TRP administration on brain functions thought to be mediated in part by 5HT neurons in brain, suggesting that TRP-induced increases in 5HT synthesis do enhance transmitter release. Important qualifications are that (a) though the diet under some well-defined circumstances will predictably alter brain TRP level and 5HT synthesis, it does not do so under all circumstances (e.g.[2]), and (b) though TRP administration can influence particular brain functions, to date no changes in brain functions mediated by 5HT neurons have been produced by changes in brain TRP and 5HT induced by physiologic phenomenon. These qualifications spotlight important areas for contemporary and future investigation. Such studies should elucidate the extent to which the 5HT neuron, and ultimately particular brain functions, are physiologically tied to variations in brain TRP.

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Hydroxylases

R9 THE ENZYMOLOGY OF THE AROMATIC AMINO ACID HYDROXYLASES, Seymour Kaufman, Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, MD 20892

The conversions of phenylalanine to tyrosine, tyrosine to 3,4-dihydroxyphenylalanine and tryptophan to 5-hydroxytryptophan are catalyzed by three distinct enzymes, phenylalanine, tyrosine and tryptophan hydroxylases. These hydroxylases function in vivo as part of a more complex hydroxylating system; the other essential components of the system are the pterin coenzyme, tetrahydrobiopterin and a second enzyme, dihydropteridine reductase.(1)

The reactions involved in the hydroxylation of the aromatic amino acids by these enzyme systems are shown in equations 1 and 2, where RH and ROH stand for the aromatic amino acid and the hydroxylated product, respectively, and BH_4 and q-BH_2 stand for tetrahydrobiopterin and its oxidation product, quinonoid dihydrobiopterin, respectively. Reaction 1 is catalyzed by the hydroxylase and reaction 2 by dihydropteridine reductase.

$$RH + O_2 + BH_4 \rightarrow ROH + q\text{-}BH_2 + H_2O \qquad 1$$

$$q\text{-}BH_2 + NADH + H^+ \rightarrow BH_4 + NAD^+ \qquad 2$$

Although all three hydroxylases share many regulatory properties, there are also distinctive features of their regulatory behavior that appear to be related to the separate roles that these enzymes play in vivo.

There is evidence that the activity of all three hydroxylases can be regulated by phosphorylation-dephosphorylation. The phosphorylation-mediated activation, however, is not expressed in the same way for all of the enzymes. In addition, activation of phenylalanine hydroxylase by phosphorylation appears to be closely integrated with activation by its substrate, phenylalanine. By contrast, neither tyrosine nor tryptophan hydroxylases are activated by their respective substrates.

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R10 DEVELOPMENT OF TYROSINE HYDROXYLASE IN THE BRAIN NUCLEUS LOCUS COERULEUS IN CULTURE, Cheryl F. Dreyfus, Wilma J. Friedman, Keith A. Markey and Ira B. Black, Division of Developmental Neurology, Cornell University Medical College., New York, New York 10021

To define mechanisms governing brain development and function we have been studying ontogeny of noradrenergic (NA) traits in the mouse brain nucleus locus coeruleus in vivo and in culture. In particular, the catecholamine (CA) biosynthetic enzyme, tyrosine hydroxylase (TH), has been used to monitor development.

Initial in vivo studies defined a developmental profile. TH catalytic activity was first detectable at 13 days of gestation (E13) and increased approximately 20-fold by E18, immediately prior to birth. From birth to adulthood TH activity increased an additional 10-fold. Immunotitration studies indicated that the increase in TH activity represented an increase in enzyme molecules. Moreover, immunocytochemical analysis of the tissue revealed that TH is localized in neurons.

To determine whether comparable development can occur in vitro, explants of embryonic brainstem have been placed in organotypic tissue culture. Tyrosine hydroxylase (as well as the CA enzyme dopamine-β-hydroxylase) increased markedly during development of the embryonic catecholaminergic nucleus, reflecting ontogeny in vivo. Moreover, the increase reflected a specific increase in TH enzyme per cell.

To begin to examine regulatory mechanisms we have studied the influence of membrane depolarization on TH. Initially the effects of the depolarizing agents veratridine and elevated K^+ on the locus cultures were evaluated. Exposure to the depolarizing agents veratridine or elevated K^+ significantly increased TH catalytic activity. The effects of veratridine were prevented by tetrodotoxin, suggesting that transmembrane Na^+ influx was necessary for the rise in TH. Morphometric analysis indicated that the rise in TH activity was not accompanied by altered TH - positive cell number or cell diameter. Instead, TH fluorescence increased in intensity in each neuron, suggesting that depolarization increased TH per cell. Immunoblot and densitometric analysis indicated that depolarization did, indeed, increase TH immunoreactive protein.

In conclusion, our experiments have defined a tissue culture system valuable for evaluation of regulatory events associated with TH development in central neurons. More specifically, these studies suggest that membrane depolarization and/or Na^+ influx regulates a critical transmitter macromolecule in developing brain neurons, as in the periphery, by altering enzyme molecule number. We are now in a position to define underlying molecular mechanisms governing these phenomena. (Supported by NIH Grants NS 10259, HD 12108, and NS 20788 and NSF Grant 8024081. CFD is the recipient of a Teacher Scientist Award from the Andrew W. Mellon Foundation.)

R11 REGULATION OF TYROSINE HYDROXYLASE ACTIVITY BY ITS ENDPRODUCTS AND CYCLIC AMP-DEPENDENT PROTEIN KINASE, Hitoshi Fujisawa, Sachiko Okuno, and Takashi Yamauchi, Department of Biochemistry, Asahikawa Medical College, Asahikawa 078-11, Japan

Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of catecholamines in the nervous system and therefore the regulatory mechanism of the activity of this enzyme is very important. A variety of conditions, including enzymatic phosphorylation by cyclic AMP-dependent protein kinase, calmodulin-dependent protein kinase II, Ca^{2+}-, phospholipid-dependent protein kinase, partial proteolytic digestion, and the presence of polyanions, anionic phospholipids, salts, and catecholamines, have so far been reported to affect the enzymatic activity.

Tyrosine hydroxylase activity of the crude extract from rat striatum, where the enzyme exists most abundantly, has a sharp pH optimum at around pH 5.5 and shows almost no activity at pH 7, which is the intracellular pH of rat brain. However, when the crude extract is adjusted to pH 5, the resulting precipitate shows a high activity of the enzyme at the physiological pH (pH 7). When the acid-precipitated fraction is incubated with the acid-soluble fraction at 30°C, the enzyme activity at pH 7 again decreases remarkably, indicating that there is an endogenous factor inactivating tyrosine hydroxylase in rat brain. When the acid-precipitated enzyme is incubated with the endproducts of the enzyme, catecholamines such as dopamine, norepinephrine, and epinephrine, a similar decrease in the enzyme activity is observed. This inactivation of the enzyme by catecholamines differs from the well-known inhibition which is competitive with respect to the pterin cofactor: the inactivation occurs at a very much lower concentration of catecholamines (10^{-8} M) even in the presence of a near-saturating concentration of a pterin cofactor and it is a time-dependent reaction. Thus, the very low concentrations of the endproducts of the enzyme are capable of converting the enzyme to an inactive form and it would mean that tyrosine hydroxylase usually exists as an inactive form in the unstimulated neurons.

The inactive enzyme which has been incubated with its endproduct is dramatically activated by the action of cyclic AMP-dependent protein kinase. The specific activity of the enzyme activated by cyclic AMP-dependent protein kinase is about 2,800 units/mg protein, which is very much higher than the highest so far reported.

Among a variety of conditions affecting the enzyme activity so far reported, the inactivation by the endproducts and the activation by cyclic AMP-dependent protein kinase are particularly striking, when the enzyme is assayed at the physiological pH. Thus, the Biosynthesis of catecholamines may be mainly regulated by the mechanism that the inactive tyrosine hydroxylase induced by the action of the endproduct is converted to a highly active form by the action of cyclic AMP-dependent protein kinase.

R12 MOLECULAR STRUCTURE OF MAMMALIAN AROMATIC AMINO ACID HYDROXYLASES AND DIHYDROPTERIDINE REDUCTASE, Fred D. Ledley, M.D., Baylor College of Medicine, Houston, Texas, 77030

The cloning of the pterin dependent hydroxylases phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TYH), and tryptophan hydroxylase (TRH) provides insight into their genetic and molecular structure, structure-activity relationships, and evolution. PAH is a protein of 451 amino acids encoded by a cDNA of 2400 bases. The genomic locus contains 13 exons over 90 Kb of DNA. TYH is a protein of 498 amino acids coded by a cDNA of 1900 bases. The nucleic acid and predicted protein sequences of these proteins are highly homologous reflecting evolutionary conservation of determinants for their common activities. These proteins are comprised of two distinct domains. The first domain comprising approximately 35,000 Daltons at the carboxy end, contains sequences which are homologus between PAH and TYH, and functions as the catalytic core. The second domain at the amino terminal end, contains no homologus sequences, and apparently contributes regulatory elements and determinants of substrate specificity. The predicted secondary structure of the amino terminal domain is highly helical while the carboxy terminal domain is largely indeterminate. The carboxyl terminal domain of PAH is encoded by 8 exons clustered within 15 kb at the 3' end of the gene. The amino terminal domain is encoded by 5 exons spread over greater than 80 kb and may have a different evolutionary origin.

Human dihydropteridine reductase (DHPR) has been cloned and a large portion of the amino acid sequence of sheep DHPR determined by protein sequencing revealing the primary structure of this gene and protein.

R13 MOLECULAR BIOLOGICAL STUDIES ON THE REGULATION OF TYROSINE HYDROXYLASE GENE ACTIVITY. B.B.Kaplan, M.K.Stachowiak, A.E.Gioio, E.M.Stricker and M.J.Zigmond, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213

At present, we are using the cold stress induction of rat adrenomedullary TH as a model system to investigate trans-synaptic regulation of TH gene activity. Previously, we demonstrated that the induction of TH activity during chronic cold stress (5-7 days cold exposure) was accompanied by a parallel increase (4- to 5-fold) in the relative abundance and in vitro translation activity of TH mRNA (1,2). Increases in TH mRNA levels (approx. 90%) also were observed in the brain stem of cold-stressed animals. The change in TH mRNA appears specific, as no significant differences were observed in the content of adrenomedullary total RNA or poly(A)$^+$RNA. Importantly, the relative abundance of PNMT mRNA changed by only 20-40% under the identical conditions. In recent studies, we examined the extracellular mechanisms underlying stress-induced alterations in adrenomedullary TH mRNA levels. Adrenal glands of male Sprague-Dawley rats were unilaterally denervated, and 2 wk later, animals were exposed to cold (5^{o}C) for 5 days. Estimates of TH mRNA levels were obtained by RNA dot-blot hybridization and northern analysis using a cloned TH cDNA as probe. No detectable differences were observed in basal TH mRNA levels in the innervated compared to the denervated adrenal gland. In the innervated glands, cold stress increased TH mRNA levels 3- to 4-fold. Denervation abolished this effect, indicating that stress-induced alterations of TH mRNA levels require an intact splanchnic nerve. Interestingly, short-term exposure to cold (12 hr) resulted in a 5- to 8-fold increase in TH RNA levels. This change could be reduced 80-90% by treating stressed animals with the ganglionic blocker, chlorisondamine 1 hr before and 5 hr after the onset of cold exposure (2 times for 6 hr each; 10 mg/kg,ip). The administration of chlorisondamine had little effect on the basal levels of TH mRNA (-20%; drug vs vehicle-treated controls). In contrast to these findings, the administration of the cholinergic agonists carbachol (8.2 umoles/kg,ip) or nicotine (5.0 mg/kg, sc), injected every 12 hr for 3 days resulted in only a small increase (approx. 50%) in the relative abundance of adrenomedullary TH mRNA. This finding suggests that activation of cholinergic receptors are necessary, but not sufficient, to mediate the effects of cold stress on adrenomedullary TH mRNA. Taken together, these data indicate that cold stress-induced alterations in TH RNA levels (1) are neurally mediated, i.e. require an intact sympathetic input, (2) are mediated, in part, by nicotinic cholinergic receptors, and (3) precede by 24-36 hr comparable alterations in TH enzyme activity. Additionally, it appears that TH and PNMT mRNA levels are regulated differentially during cold stress. (Work supported by MH00518, MH29670 and NS19608).

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Behavior and Function: Appetite

R14 ASSOCIATIONS AMONG DIET, COMPETITION FOR LYSINE TRANSPORT INTO BRAIN, BRAIN AND PLASMA AMINO ACIDS, AND FEEDING BEHAVIOR, Jean K. Tews & Alfred E. Harper, Departments of Biochemistry and Nutritional Sciences, University of Wisconsin, Madison, WI 53706.

Depressed food intake often occurs when rats are fed a low protein diet limiting in one indispensable amino acid (AA) and containing disproportionate amounts of other AA. Concentration in brain of the limiting AA also usually decreases, presumably because increased plasma concentrations of those AA added to the diet to induce the disproportion increase competition for transport of the limiting AA across the blood-brain barrier (BBB) (1). Our studies with lysine illustrate these points. Transport of 14C-lysine across the BBB, as determined by carotid artery injection, (2), was almost completely inhibited by other basic AA, e.g., the analog, homoarginine (HA) (3). In another study rats were trained to eat a control, lysine-limiting diet (8% AA) during a daily 8 hr period. On the day of the experiment they ate single meals of this diet containing adequate lysine ± added HA (1.9%); 4-7 hr later the rats were briefly infused intravenously (4) with tracer amounts of 14C-lysine. The rate of lysine influx in the control group was 8.7 ± 0.5 nmoles/g brain/min: influx was reduced to 2.0 ± 0.5 nmoles/g brain/min in the rats fed HA. Corresponding lysine concentrations were 0.33 ± 0.03 and 0.17 ± 0.02 μmoles/g brain, respectively. Plasma lysine concentration was 0.72 mM in the control and 0.45 mM in the HA group; mean plasma HA concentration was 0.6 mM in the latter group. Other rats learned to eat daily 3-hr meals of a control, AA diet limiting in lysine before receiving a single meal of the experimental diets. A 3-fold increase in dietary lysine caused 10 and 4-fold increases in plasma and brain lysine; dietary HA invariably lowered brain lysine, ornithine and arginine but did not affect concentrations of large neutral AA. Total 10 day food intake was decreased by about 20% in rats fed a lysine-limiting, 8% AA diet containing HA; increased dietary lysine content lessened these effects. When rats selected between diets (computer-monitored system), they chose control (9.0 ± 0.3 g/day) rather than HA diet (1.7 ± 0.3 g/day). Avoidance of the HA diet occurred early on day 1 in 4/5 rats. When choice was between the HA and HA+lysine diets 3/10 rats preferred the former, 6/10 the latter, and 1/10 neither diet. Our results support the concept that effects of dietary disproportions of AA on feeding behavior involve competition for AA entry into the brain from the blood.

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R15 AMINO ACIDS IN FOOD INTAKE AND SELECTION, Edmund T. S. Li and G. Harvey Anderson, Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada.

Complex physiological processes are involved in the control of ingestive behaviour. Recent use of the self-selecting feeding paradigm has provided evidence that both quantitative and qualitative aspects of food intake are regulated. We have proposed that protein and amino acids play key roles in regulating food intake and selection (1). In rats, a protein meal reduces total energy intake and enhances preference for carbohydrate during the subsequent eating episode (2). Rats with subdiaphragmatic vagotomy behave similarly, suggesting that the mechanisms are vagal independent (3). Therefore, the signals may be based on changes in plasma and brain amino acid profile after food consumption. A single carbohydrate meal increases the concentration of TRP, TYR and PHE in the brain but decreases that of VAL, LEU and ILE. Conversely, protein consumption increases brain concentration of TYR and the branched-chain amino acids but decreases that of TRP and PHE. We have, therefore, systematically examined the effects of each of these amino acids, administered i.p., on food intake and selection.

		LEU^{+}	ILE^{+}	VAL	VAL	TRP^{+}	PHE^{+}	TYR^{+}
	Dose	96^{a}(50)^{b}	96(87)	75(55)	300(221)	75(414)	60(59)	60(51)
Time								
0-1	h	↓TF	↓TF	-	↓TF	↓TF	↓TF	↓TF
1-2	h	↓TF	-	-	-	↑PRO	↓TF	↓TF
2-12	h	↑CHO	↓PRO	-	↑CHO	-	-	-
0-12	h	-	↓PRO	-	↓PRO	↓CHO	-	↓TF

($^{+}$Lowest effective dose, ^{a}mg/kg, ^{b}% meal intake, based on a 20% casein diet, 7 meals a day, ↓= Reduced, ↑= Increased, -= No change, TF=Total food, CHO=Carbohydrate, PRO=Protein). The results indicate that (a) all amino acids tested, when given independently at a sufficiently large amount, will suppress total food intake in the next hour of feeding, (b) the lowest effective doses are within a narrow range when expressed as mg/kg, but vary widely when expressed as % of meal intake, (c) TRP and the branched-chain amino acids influenced food selection, in general in opposite directions, consistent with the effects of feeding a carbohydrate meal or a protein meal, respectively. We conclude that both the aromatic and branched-chain amino acids are involved in regulating food intake and selection.

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(Supported by NSERC of Canada)

R16 PLASMA AMINO ACID RESPONSES TO CARBOHYDRATE INGESTION IN OBESITY. Benjamin Caballero, Nicholas Finer and Richard J. Wurtman, Dept. of Applied Biological Sciences and the Clinical Research Center, Massachusetts Institute of Technology, Cambridge, MA 02139.

Carbohydrate ingestion normally produces an insulin-stimulated fall in plasma brached chain amino acid levels, and therefore rises the ratio of tryptophan to the other large neutral amino acids (Trp/LNAA ratio) in blood (1). Protein ingestion, by providing proportionally larger amounts of branched chain amino acids (BCAA) than of Trp, has the opposite effect (2). Since the Trp/LNAA ratio determines the availability of Trp to the brain, and consequently, brain serotonin synthesis (3), changes of the Trp/LNAA plasma ratio after food intake may allow the brain to sense the macronutrient composition of meals and regulate food intake accordingly. We determined the plasma Trp/LNAA ratio in obese subjects (who often crave and consume large amounts of carbohydrate) in the fasting state and after ingestion of a 30g carbohydrate snack at mid-afternoon. Basal plasma BCAA were similar in lean controls and obese, but Trp was lower in obese ($p<0.001$). One hour after carbohydrate ingestion, lean subjects exhibited a significant rise in the Trp/LNAA ratio ($p<0.02$), whereas obese showed no change. This difference was accounted for by a blunted decrease of BCAA in obese subjects, in spite of their significantly higher insulin response. The glycemic response was similar in both groups. In subsequent tests using different doses of carbohydrate, it was determined that obese subjects had to ingest at least 75g of carbohydrate to exhibit an increase in their plasma Trp/LNAA ratio similar to that attained by lean controls after 30g. The blunted Trp/LNAA response of obese may have neurochemical consequences, as obese persons would have to consume more carbohydrate than lean subjects to exert a comparable effect on brain serotonin output.

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R17

NEUROCHEMICAL APPROACHES TO THE STUDY OF DIET SELECTION, Robin B. Kanarek, Department of Psychology, Tufts University, Medford, MA 02155

During the past decade, renewed interest has been expressed in the mechanisms controlling the intake of specific dietary components. In particular, research interest has focused on the role of neurotransmitters and neuromodulators within the central nervous system in regulating intakes of the three macronutrients, protein, fat and carbohydrate. Within this research framework, investigators have examined the effects of 1) the destruction of particular areas or neurotransmitter systems on nutrient selection and 2) pharmacological manipulations, such as the administration of neurotransmitter precursors and endogenous neuropeptides, on diet selection. Several hypotheses relating specific neurotransmitter systems to macronutrient selection have been developed from this research. However, conflicting data have arisen from this research, making the acceptance of any one hypothesis about the mechanisms controlling nutrient selection untenable at this time. As an attempt to reconcile these conflicting data, a variety of factors which can influence patterns of nutrient choice, including diet composition, the experimental environment and the animal's genetic and nutritional background, will be delineated. Additionally, research suggesting that an animal's intake of specific nutrients may influence its patterns of drug self-administration will be discussed.

R18 INTERACTIVE EFFECT OF TRYPTOPHAN AND MACRONUTRIENTS ON HUNGER MOTIVATION AND DIETARY PREFERENCES, John E. Blundell, Vijay Mavjee, Christopher J. Williams and Andrew J. Hill, BioPsychology Group, Psychology Department, University of Leeds, Leeds, LS2 9JT, U.K.

A series of experiments have been conducted to examine the action of tryptophan (TRYP) on various measures of hunger motivation, calorie intake, ingestion of macronutrients and nutrient preferences, in addition to psychomotor performance, cognitive performance, sleep, mood and bodily sensations. Different techniques have been used to deliver the TRYP including administering the amino acid in capsules, mixed in fruit drinks and bound into a small (10g) bar of chocolate. Various experimental strategies have been employed including the administration of TRYP without food, in advance of eating or as part of a lunch-time meal. Doses ranging from 0.5 to 2.0g have been given. We have previously reported that certain effects on food intake are apparent at the upper end of this range (1). When administered without food in mid-morning (10.00am) and tracked for 2.5 hours, 0.5g TRYP (embedded in chocolate) produced minimal changes in hunger motivation, food preferences, mood or cognitive/psychomotor performance. During the experimental period subjects became more hungry and displayed a typically enhanced preference for protein (2), but TRYP failed to modify this profile. However, TRYP did prevent the increase in feelings of stomach emptiness which occurred as lunch-time approached. A further study using a large dose of TRYP (2.0g) given in orange juice before a mainly protein or CHO lunch (586 kcal) gave rise to a number of effects. Subjects were significantly more sedated, showed a slower motor response component in a choice reaction time task, and reported themselves as more lethargic, tired and listless. This high dose showed clear interactive effects with meal type (nutrient composition) on hunger and food preferences. On the basis of these findings a further study was carried out incorporating 1.0g TRYP (embedded in chocolate) as the dessert component of a 580 kcal high protein or high CHO meal. In a free-selection test meal 3 hours later there was a significant suppression of total food intake (kcals; $F(1,8)=8.58$, $p<.05$) and protein intake ($F(1,8)=7.78$, $p<.05$) by the prior protein meal. For carbohydrate intake there was a significant TRYP x nutrient interaction ($F(1,8)=7.16$, $p<.05$); TRYP taken with the protein meal selectively decreased the amount of carbohydrate eaten. This appears to be the first time that a dose of TRYP as low as 1.0g has been shown to affect consumption. These studies indicate that TRYP exerts notable effects on aspects of eating and food selection which are markedly modulated when the compound is administered in conjunction with nutrient specific foods.

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R19 SUGAR AND BEHAVIOR, Mark Wolraich, M.D., University of Iowa.
Studies examining the effects of sugar ingestion on the behavior of children, both normal and hyperactive, will be reviewed. Although the results of correlational studies suggested that high levels of sugar consumption may be associated with increased rates of inappropriate behavior, the results of dietary challenge studies have been inconsistent and inconclusive. Most studies have failed to find any effects associated with sugar ingestion, and the few studies that have found effects have been as likely to find sugar improving behavior as making it worse. Design parameters unique to undertaking sugar challenge studies will be identified, and suggestions for future research will be offered.

Behavior and Function: Blood Pressure

R20 EFFECTS OF TYROSINE AND TRYPTOPHAN ON BLOOD PRESSURE IN THE RAT, Timothy J. Maher, Department of Pharmacology, Massachusetts College of Pharmacy, Boston, MA 02115
One of the most potent homeostatic mechanisms operating in the mammalian organism involves the regulation of arterial blood pressure (BP). Many of these mechanisms involve neurons which utilize the monoamine neurotransmitters, norepinephrine and epinephrine, derived from the amino acid tyrosine, while others utilize serotonin, derived from the amino acid tryptophan. For example, when BP is elevated, neurons within the brainstem release norepinephrine which act to inhibit sympathetic outflow and reduce BP. Conversely, when BP is acutely decreased, sympathoadrenal neurons are activated and release epinephrine and norepinephrine to restore BP. When a catecholamine-containing neuron fires rapidly (i.e., in an attempt to maintain BP), tyrosine hydroxylase is activated via phosphorylation and the levels of substrate, tyrosine, now become the limiting factor for synthesis rather than the normally limiting tetrahydrobiopterin cofactor. Thus the administration of tyrosine, or tyrosine-progenitors, such as aspartame, lower BP in a dose-dependent fashion in spontaneously hypertensive rats. These decreases in BP are associated with increases in the levels of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol in the brain, and can be attenuated by the coadministration of a large neutral amino acid, such as valine, which competes for uptake at the blood-brain barrier. Further, the administration of tyrosine increases BP in rats made hypotensive via hemorrhage, a response blocked by bilateral adrenalectomy. As expected, the administration of tyrosine does not lead to dramatic BP changes in normotensive animals since tyrosine hydroxylase would not be expected to be activated. Serotonin-containing neurons within the central nervous system are also involved in the regulation of BP, and can lead to increases or decreases, depending on its locus within the central nervous system. While having no effect in normotensive rats, acute tryptophan administration decreases BP. Levels of serotonin are increased by such treatments, while the hypotensive activity is blocked by coadministration of valine or pretreatment with a serotonin receptor blocker. There is evidence from some investigators that a metabolite of tryptophan other than serotonin might be contributing to the observed hypotensive actions. Tryptophan administration also increases BP in hypotensive rats, but this effect is much less potent than that seen with tyrosine. Thus, the amino acids tyrosine and tryptophan can alter BP in rats when administered acutely. The direction of the BP change depends upon the animal's starting blood pressure.

R21 AMINO ACID-INDUCED CARDIOVASCULAR CHANGES IN UNANESTHETIZED, UNRESTRAINED RATS. Debra L. Yourick and Richard E. Tessel, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS. 66045

Recent studies suggest that some amino acids can alter blood pressure in restrained rats[1,2]. However, inspite of its increased ingestion in the form of aspartame, little is known concerning the cardiovascular actions of phenylalanine (Phe). Phe, in vivo, can be converted to tyrosine (Tyr) by Phe hydroxylase or to the indirectly acting sympathomimetic amine, phenethylamine (PEA), by l-aromatic amino acid decarboxylase. Based on previous studies in which Tyr has been found to lower blood pressure in both normotensive and hypertensive rats, one might expect that Phe administration to rats would be similarly hypotensive. Alternatively, Phe might elevate pressure due to Phe-derived PEA. To investigate these possibilities, it was necessary to eliminate restraint and other stressful stimuli from the cardiovascular measurement technique due to recent evidence that stress alters PEA excretion[3].

After one or more days of recovery from femoral artery and vein catheterization, adult normotensive Sprague-Dawley rats were placed in an opaque cage with a light permeable cover and connected to a pressure transducer and grass polygraph. Continuously monitored mean arterial pressure (MAP) and heart rate (HR) were allowed to reach stable baseline values (approximately 2 hours). At this time, rats were injected intraperitoneally with saline or one of several amino acids and MAP and HR were measured for at least 5 hours post-injection.

Phe (287 mg/kg) elevated MAP about 20 mmHg. Lower doses (69-216 mg/kg) produced qualitatively similar although quantitatively smaller effects. Saline and an equimolar dose of valine had little effect on MAP revealing that responses to Phe were not attributable to nonspecific consequences of injection or to neutral amino acid administration. The Phe-induced increase in MAP was antagonized by the peripheral decarboxylase inhibitor, carbidopa, and by the alpha$_1$-adrenergic receptor antagonist, prazosin. Cardiovascular responses to Phe cannot be attributed to PEA since Phe-induced increases in PEA were not temporally correlated with the pressure response and a dose of the norepinephrine uptake inhibitor desipramine, that antagonized pressure increases produced by exogenous PEA administration, did not antagonize Phe-induced pressure changes. Moreover, Tyr administration increased MAP, and DOPA, which is not converted to indirectly acting sympathomimetics, markedly elevated MAP. We conclude that catecholamine precursors exert their cardiovascular effects in unrestrained rats by increasing the synthesis and release of peripheral catecholamines.

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R22 SEROTONIN AND BLOOD PRESSURE CONTROL, Walter Lovenberg, Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.

Serotonergic neurons in the brain participate in numerous physiologic regulatory systems. Evidence is accumulating that serotonin containing nerves are active participants in the control of blood pressure in rats. Electrical stimulation of specific serotonin cell groups in the dorsal and median raphe areas of the brain of anesthetized rats results in a rapid and marked (up to 80 mm Hg) increase in blood pressure. A variety of pharmacologic and anatomical studies provide strong evidence that synaptic release of serotonin in the brain was responsible for this pressor response. In contrast to this observation, modification of serotonin synthesis or release has minimal and variable effects on blood pressure in resting animals. Peripheral administration of L-tryptophan which is known to enhance serotonin synthesis in brain was reported to have a hypotensive effect. Further evaluation of this effect by Wolf and Kuhn (Brain Res. 295:356, 1984) led to the conclusion that hypotensive effect of peripherally administered L-tryptophan was unrelated to the effect of this amino acid on brain serotonin synthesis, and probably resulted from some metabolite of the tryptophan pyrrolase pathway.

A review of the evidence for a central role for serotonin and recent studies on the mechanism of the effects of peripheral tryptophan on blood pressure will be presented.

Disease States: The PKU Paradigm

R23

CLASSICAL PHENYLKETONURIA AND ITS VARIANTS CAUSED BY DEFECTS IN BIOPTERIN METABOLISM, Seymour Kaufman, Laboratory of Neurochemistry, National Institute of Mental Health, Bethesda, MD 20892

The hepatic phenylalanine hydroxylating system consists of three essential components, phenylalanine hydroxylase, dihydropteridine reductase and the non-protein coenzyme, tetrahydrobiopterin. The reductase and the pterin coenzyme are also essential components of the tyrosine and tryptophan hydroxylating systems. During the hydroxylation reaction, tetrahydrobiopterin is stoichiometrically converted to quinonoid dihydrobiopterin. The reduction of this latter compound back to tetrahydrobiopterin, a reaction catalyzed by the reductase in the presence of NADH, permits the pterin coenzyme to function catalytically.(1)

Recent studies have shown that there are three distinct forms of phenylketonuria or hyperphenylalaninemia, each caused by the lack of one of these essential components. In addition to the classic form of the disease, which is caused by a lack of phenylalanine hydroxylase, a variant form has been described that is caused by a lack of dihydropteridine reductase and another form that is caused by a deficiency of tetrahydrobiopterin secondary to a defect in one of the enzymes involved it its de novo biosynthesis.(2) Besides hyperphenylalaninemia, these variant forms are characterized by severe neurological deterioration, and impaired functioning of phenylalanine, tyrosine, and tryptophan hydroxylases. The impaired functioning of the latter two hydroxylating systems leads to a deficiency of tyrosine- and tryptophan-derived monoamine neurotransmitters in the brain, deficiencies that, in turn, cause at least some of the neuropathology that is seen in these diseases. Current therapy for hyperphenylalaninemia due to a deficiency of dihydropteridine reductase involves administration of the precursors of the deficient neurotransmitters, 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan, in addition to supplementation with a tetrahydrofolate derivative, such as 5-formyl tetrahydrofolate. Therapy for hyperphenylalaninemia due to a deficiency of an enzyme in the biosynthetic pathway for tetrahydrobiopterin involves administration of tetrahydrobiopterin, or the just mentioned neurotransmitter precursors, or both.

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R24

THE PKU PARADIGM: THE MIXED RESULTS FROM EARLY DIETARY TREATMENT, Harvey L. Levy, Department of Neurology, Harvard Medical School, Boston, MA 02114

For 20 years newborn screening has identified PKU early in infancy. As a result treatment with the phenylalanine restricted diet has been initiated in infancy and continued at least through early childhood in thousands of children. This diet is successful in controlling the overt biochemical abnormalities of PKU and it has prevented mental retardation in affected children. Other neurologic complications of PKU, such as seizures and autism, as well as systemic complications such as eczema are also prevented by early dietary therapy.

Despite this notable, even monumental success of newborn screening and early treatment of PKU, a number of distressing problems remain. The mean IQ of early treated children is several points lower than that of their nonphenylketonuric siblings. Even the most carefully treated children often have learning disabilities, short attention spans and hyperactive behavior. Discontinuance of the diet in childhood is often followed by reduction in the IQ, sometimes by as many as 20 or 30 points. Adolescents who were early treated but no longer on diet often experience depression, poor concentration and a lack of well-being. Psychotic-like characteristics have also been observed in some of these adolescents and young adults. The adverse effects of maternal PKU on the fetus threaten to result in birth defects and mental retardation in the generation of offspring from young women with PKU.

New and ancillary therapeutic modalities are required to control and prevent these complications in early treated PKU.

R25 MOLECULAR GENETICS OF PHENYLKETONURIA, Savio L.C. Woo, Ph.D., Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas, 77030

Phenylketonuria (PKU) is a recessive human genetic disorder caused by a deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH). The metabolic disorder causes severe mental retardation in untreated children and has a prevalence of about 1 in 10,000 births among Caucasians. Using a full-length human PAH cDNA clone as the hybridization probe, ten polymorphic restriction sites have been identified in the corresponding chromosomal locus. By comparison of the restriction fragment profiles in most PKU families, the PAH genes in the parents can be distinguished and the ones transmitted to the affected child identified. Thus, the genomic polymorphism has led to the development of an analytical procedure for prenatal diagnosis of the genetic disorder. This method of analysis however, is limited to families that have had a previously affected child. In order to develop methodologies for detection of mutant alleles in the population without previous PKU history, we attempted to characterize the mutations by molecular cloning. PKU is a genetic disorder that displays a variety of phenotypes at the clinical level. Using the full-length cDNA clone as the hybridization probe, a number of liver biopsy samples from PKU patients were analyzed for the presence of PAH mRNA. Results demonstrated that some patients are mRNA+ while others are mRNA-. Thus, the disorder is heterogenious at the mRNA level and suggests the presence of multiple mutations in the PAH gene that can cause PKU. In order to characterize the mutations underlying PKU, the human chromosomal PAH gene was cloned in 4 overlapping cosmids. Structural characterization has demonstrated that it contains 13 exons and is 90 Kb in length to code for a mature mRNA of 2.4 Kb. The polymorphic restriction sites defined specific haplotypes of individual PAH genes and their exact positions in the gene had been determined. Detailed RFLP haplotype analysis of the Northern European population has demonstrated that over 90% of PKU genes are represented by four distinct RFLP haplotypes. Mutant PAH alleles of 2 of the 4 RFLP haplotypes seem to be associated with a more severe clinical phenotype in patients, suggesting that the corresponding mutations might be more deleterious. The observation represents the first correlation between various genotypes of the PAH gene and different phenotypes of PKU. In order to determine whether each of the RFLP haplotype of the PAH gene is associated with a specific mutation due to linkage disequilibrium, all four mutation genes have been isolated by cosmid cloning from patients who are haplotype homozygotes. Sequencing analysis followed by expression studies will define the molecular basis of the hereditary disorder, which may lead to development of specific probes to detect the corresponding mutation chromosomes in individuals. This will permit development of specific procedures for carrier detection in the population without previous PKU history.

R26 PROSPECTS FOR GENETIC THERAPY OF PHENYLKETONURIA, Fred D. Ledley, Department of Cell Biology, Bayor College of Medicine, Houston, TX 77030.

The cloning of a full length human phenylalanine hydroxylase (PAH) cDNA clone has enabled investigation of methods for alternative therapy of PKU involving gene transfer. Two approaches are being investigated. The first involves genetic transfer of the recombinant human PAH into bacteria to produce large amounts of the human PAH enzyme for use in enzyme replacement therapy, and the second involves construction of recombinant viruses carrying human PAH for use in gene replacement therapy.

The human PAH cDNA has been recombined with a prokaryotic promoter and introduced into E. coli. The transformed E. coli overproduce human PAH accounting for 1% of total protein and 10-20 times more activity (/mg protein) than human liver. The physical properties and kinetic properties of the recombinant enzyme are indistinguishable from native human liver PAH. Thus, the recombinant E. coli represent a readily accessible source of unlimited quantities of authentic, and apparently normal human PAH for consideration of enzyme replacement.

The human PAH cDNA has also been incorporated into retroviral vectors to produce recombinant retroviruses capable of transducing the human PAH gene into target cells via infection. The scheme for production of recombinant retroviruses involves cloning the PAH gene into a retroviral vector that contains only the retroviral long terminal repeats and a Ψ "packaging sequence" and introducing this clone into cells that contain a packaging defective "Ψ^-" retroviral clone that can synthesize the complete retroviral capsule but cannot package the Ψ^- mRNA. The recombinant virus consists of the capsule from the Ψ^- provirus but carries the Ψ^+ proviral genome containing PAH.

These viruses which contain the human PAH sequence have been used to infect a variety of cells including NIH3T3 (fibroblast-like) cells and the mouse hepatoma cell line hepal a. Infected cells express human PAH mRNA, protein, and enzymatic activity. The hepatoma cells, which are capable of the synthesis and reduction of the tetrahydrobiopterin cofactor, are transformed to grow in tyrosine free media. NIH3T3 cells, which cannot synthesize the cofactor, will not grow in tyrosine free media unless the cofactor is supplemented by the administration of exogenous tetrahydrobiopterin or 6-methyltetrahydrobiopterin. These experiments demonstrate that the PAH holoenzyme can be reconstituted in various types of PAH deficient cells by retroviral mediated transfer of human PAH. Future experiments will be directed at the introduction of the recombinant PAH gene into primary bone marrow or hepatocyte cultures. One model for somatic gene therapy of PKU involves transplantation of the PAH deficient host with transformed primary cells, providing a source of PAH activity to prevent the accumulation of unmetabolized phenylalanine.

Poster Abstracts

R27 PHENYLETHANOLAMINE N-METHYLTRANSFERASE IN THE BRAIN OF DIABETIC RATS, Jennifer K. Stewart and Krista J. Fischer, Virginia Commonwealth University, Richmond, VA 23284

We investigated the effects of Streptozotocin-induced diabetes on the activity of phenylethanolamine N-methyltransferase (PNMT) in the brainstem and hypothalamus of the rat. Diabetes of one month duration was associated with a 2-fold increase in brainstem PNMT activity ($P < 0.0001$), and chronic insulin treatment partially reversed the effects of the diabetes ($P < 0.01$). Brainstem PNMT activity exhibited a positive ($P < 0.001$) correlation with plasma glucose concentrations ($r = 0.51$). Diabetes had no apparent effect on enzyme activity in the hypothalamus. In contrast to the effects of other types of stress on central adrenergic neurons, diabetes had no effect on epinephrine concentrations in the brainstem and hypothalamus and caused a decrease in epinephrine turnover in the hypothalamus. The increased PNMT activity maintained steady state levels of epinephrine in diabetic animals and may have compensated for limitations on the synthesis of catecholamines, e.g., reduced availability of precursor. This hypothesis is consistent with evidence that high levels of branched-chain amino acids in diabetic animals compete with tyrosine for transport into the brain and thereby limit the availability of this precursor of catecholamine synthesis.

R28 PRECURSOR CONTROL AND INFLUENCE OF ASPARTAME ON MIDBRAIN DOPAMINE See-Ying Tam, Nobufumi Ono and Robert H. Roth, Yale University School of Medicine, Department of Pharmacology, New Haven, CT 06510

The mesocortical dopamine (DA) neurons projecting to the prefrontal and cingulate cortices exhibit a higher rate of basal physiological activity than other midbrain DA neurons, and DA synthesis in these neurons is more sensitive to control by precursor tyrosine. Tyrosine administration in physiologically relevant doses (25 mg/kg) causes significant increases in in vivo tyrosine hydroxylation in the prefrontal and cingulate cortices but not in other DA projection fields examined. Since it has been speculated that the dipeptide, aspartame, may under some special circumstances, influence brain catecholamine metabolism, we examined the effects of this agent on midbrain DA systems, including those cortical systems which we have shown to be sensitive to precursor regulation. The influence of carbohydrate loading on the ability of aspartame to alter dopamine metabolism in discrete midbrain DA systems was also examined. Aspartame administration in doses of 50-200 mg/kg i.p. did not significantly alter DA metabolism in the striatum or mesolimbic DA projections. When aspartame was administered in high dosages (100-400 mg/kg, p.o.) to glucose-preloaded fasting rats, no inhibition of DA synthesis was observed in any of the brain regions examined including the prefrontal cortex. In fact, at the higher dose range we observed an apparent increase in DA synthesis and metabolite accumulation in the precursor sensitive DA neurons in the prefrontal cortex. Supported in part by a grant MH-14092 and a gift from G.D. Searle Co.

R29 DEPOLARIZATION-INDUCED STIMULATION OF DA SYNTHESIS IN STRIATAL SLICES INVOLVES BOTH CA++-CALMODULIN AND CA++--PHOSPHOLIPID-DEPENDENT EVENTS Marina E. Wolf, Diane L. Rosin, Amy M. Knorr, and Robert H. Roth
Yale University, Dept. of Pharmacology, New Haven, CT 06510

K^+-depolarization of striatal slices results in increased tyrosine hydroxylation, measured as DOPA formation after decarboxylase inhibition. This is accompanied by a kinetic activation of tyrosine hydroxylase (TH) characterized by a decrease in the apparent Km for cofactor ($6\text{-}MPH_4$) and an increase in the Vmax. Considerable evidence suggests that Ca++-calmodulin and Ca++-phospholipid-dependent mechanisms may play a role in the activation of TH produced by neuronal depolarization. We have therefore examined the effects of TPA and sn-1,2-dioctanoylglycerol, as well as the calmodulin antagonist W7, on both the kinetic state of TH and the rate of in situ tyrosine hydroxylation. Incubation of striatal slices with TPA or sn-1, 2-dioctanoylglycerol results in a decrease in the Km of TH for cofactor similar to that produced by K^+-depolarization, but fails to elicit increased DOPA formation. Incubation with W7 blocks the increase in DOPA formation elicited by K^+-depolarization, but fails to prevent K^+-induced kinetic activation of TH. These findings suggest the involvement of a two-step mechanism in K^+-induced stimulation of DA synthesis in striatal slices. The Km of TH for cofactor appears to be decreased via Ca++-phospholipid-dependent events. However this kinetic change in itself does not appear to be sufficient to elicit increased DOPA formation unless accompanied by a calmodulin-dependent activational process. Supported by MH 14092 and the State of Connecticut.

R30 EVIDENCE FOR N-METHYL-D-ASPARTIC ACID (NMDA) RECEPTORS MEDIATING BARORECEPTOR REFLEXES IN CAUDAL VENTROLATERAL MEDULLA (CVM). Frank J. Gordon, Dept. of Pharmacology, Emory Univ. School of Medicine, Atlanta, GA 30322.

The baroreceptor reflex is one of the fundamental mechanisms by which the CNS regulates cardiovascular function. However, little is known of the CNS pathways, neurotransmitters or synaptic receptors which mediate this reflex. Microinjection of L-glutamate (1 nmol, 50 nl) into the CVM elicits a fall in arterial pressure analogous to a baroreceptor reflex. To determine whether excitatory amino acid receptors in the CVM might play a role in baroreflex-mediated reductions in arterial pressure, the aortic nerve of urethane anesthetized rats was electrically stimulated before and after microinjection (50 nl) into the CVM of the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (D-AP5). Before D-AP5 injection, graded electrical stimulation of the aortic nerve produced reflex reductions in mean arterial pressure of 10-40 mmHg. Bilateral administration of D-AP5 (2 nmole) into the CVM completely abolished synaptically-mediated baroreflex responses as well as depressor responses to subsequent microinjection of NMDA. Depressor responses elicited by L-glutamate were not altered by D-AP5, an observation consistent with the mixed agonist properties of this excitatory amino acid. These observations suggest that neural transmission of baroreceptor information in the CVM is mediated by activation of synaptic NMDA receptors and that the neurotransmitter released at these synapses may be an excitatory amino acid.

R31 ASPARTAME; LACK OF EFFECT ON CONVULSANT THRESHOLDS IN MICE, H.J. Haigler, M.E. Nevins and S.M. Arnolde, Searle Research and Development, Skokie, IL 60077

In order to determine if aspartame (NutraSweet®, Equal®) had an effect on seizure threshold, the effects that aspartame had on pentylenetetrazol (PTZ)-induced and electroconvulsive shock (ECS)-induced convulsant thresholds was determined. Male CD-1 mice received either 0, 50 or 500 mg/kg of aspartame by gavage 30 min prior to one of four doses of PTZ (25,30, 35 and 40 mg/kg i.v.) or one of four ECS currents (5,8,10 and 12 mA, 60 pps for 0.2 sec applied transcorneally). The presence or absence of clonus (PTZ) or tonus (ECS) was recorded and the percent of mice convulsing in each group was calculated. The effects of ECS appear to be an analog of generalized tonic-clonic convulsions and the effects of PTZ appear to be an analog of absence seizures (Goodman and Gilman, Pharmacological Basis of Therapeutics; 7th Edition, 1985). Convulsant thresholds for PTZ (clonic dose$_{50}$, CD_{50}) and for ECS (tonic current$_{50}$, TC_{50}) were calculated for all treatment. Convulsant thresholds for mice treated with aspartame were statistically compared to those for mice treated with vehicle. Aspartame treatment had no effect on either PTZ CD_{50} (31.8, 31.3 and 32.7 mg/kg for vehicle, 50 and 500 mg/kg of aspartame respectively) or on ECS TC_{50} (8.3, 9.0, and 8.6 mA respectively). These data demonstrate that aspartame treatment does not affect convulsant thresholds in mice even at very high doses.

R32 BEHAVIORAL ASSESSMENT OF THE TOXICTY OF ASPARTAME, Mark D. Holder, Psychology Department, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9 and Raz Yirmiya, Neuroscience Program, Brain Research Institute, University of California, Los Angeles, CA 90024.

In 1981 FDA approved the use of L-asparty1-L-phenylalanine methyl ester (aspartame) as a sugar substitute in foods ranging from beverages to cold cereal to chewing gum. After entering the stomach, aspartame is immediately broken down into its constituent amino acids and methyl group: phenylalanine, aspartate and methanol. Though these substances are normally in the body, aspartame may have unwanted side-effects because it raises the levels of these substances relative to other substances. Possible toxic effects of aspartame were assessed with two behavioral tasks: conditioned taste aversions (CTA) and spontaneous wheel running. CTA refers to the learned aversion to a taste that was previously followed by a toxin. CTA were observed when 353, or 706 mg/kg aspartame (.3 M) was injected i.p. after thirsty rats drank tasty water. However, CTA were not observed when the identical amounts of aspartame were intragastrically intubated or when the rats freely consumed aspartame. The second task measured the voluntary running of rats. Rats were given 30 min access to running wheels each day. After the rats' running rates had stabilzed, they were injected or intragastrically intubated with aspartame (353 ml/kg of 0.3M solution). Relative to the control groups given isotonic saline, injections, but not intubations, of aspartame resulted in a precipitous drop in running. The 24 hr running rates of rats that lived in the running wheels did not change when the rats' water was replaced with solutions of aspartame (0.8% w/v). Overall, the results suggest that aspartame has toxic effects when intraperitoneally injected but that these effects are eliminated when the route of administration is oral.

R33 ASPARTAME: REDUCED ISOLATION CRIES AND ANALGESIA IN 10-DAY-OLD RAT PUPS, PRISCILLA KEHOE AND SHARIN SAKURAI, TRINITY COLLEGE, HARTFORD, CT. 06106

Previous data indicate that the sweet taste of sucrose produces an opioid response in neonatal rats (Blass & Kehoe, 1986). In an effort to determine a correlated response to the taste of aspartame, two opioid-mediated behaviors were assayed. In the first experiment, Day 10 pups were isolated for 10 min from siblings and their dam while receiving aspartame (.034%), quinine (.05%), or distilled water through a tongue cannula. Each pup was pretreated with either an opioid antagonist or saline given intraperitoneally. Ultrasonic vocalizations were monitored during the isolation period. Pups receiving aspartame emitted significantly fewer cries than the pups in the other conditions. Naltrexone pretreatment reversed the quieting effects of aspartame. In the second study pups were tested for paw-withdrawal from heat after the oral infusions were completed. The aspartame-infused pups demonstrated significantly longer latencies than any other pups. This analgesic effect was naltrexone-reversible. It appears that the sweet taste of aspartame elicits an opioid response similar to that of sucrose perhaps reflecting recruitment of a positive affective system.

R34 TYROSINE ALONE MAY BE ANALGESIC IF APPROPRIATELY TRANSPORTED TO THE RECEPTOR SITE(S): M. Abu Khaled[1], G.M. Anatharamaiah[1], John M. Beaton[1] and I.K. Ho[2]. [1]University of Alabama, Birmingham, AL 35294 and [2]University of Mississippi, Jackson, MS 39216.

Previously it was demonstrated by using NMR methods (Khaled, et al., BBRC, 76, 224, 1977) that the amino acid residue tyrosine in enkaphalins adopts a particular conformation for its side-chain. Such side-chain orientation places the N atom of amino and the O atom of hydroxyl groups of tyrosine approximately 7Å apart. The distance between the tertiary amino and the hydroxyl moieties in many narcotics has also been found to be around 7Å. Based on these findings, it was hypothesized that tyrosine alone may mimic the opioid activity if appropriately transported to the receptor site(s). In order to prove this hypothesis some small molecules were synthesized and Tyr-$NH(CH_2)_{12}$-NH_2(T_{12}) is one such compound. In the behavioral studies, using the flinch jump method, the average jump thresholds, in mA, at 4 mg/kg, indicated that T_{12} has analgesic properties analogous to morphine. A similar dose of T_{12} significantly decreased the number and the duration of phenyl-p-quinone induced writhes. A dose of 4 mg/kg of naloxone completely reversed the analgesia induced by this compound. The effect of T_{12} on naloxone binding in rat brain synaptic membrane was also performed and found that T_{12} is about 60% as potent as morphine in terms of IC_{50} for replacing [^{3}H] naloxone used as ligand. Although the binding studies of T_{12} to other subtypes of opioid receptors, e.g., δ and κ-receptor, are in progress, the overall results strongly support our hypothesis.

R35 A SIMPLE METHOD FOR ASSESSMENT OF NEUTRAL AMINO ACID TRANSPORT INTO THE HUMAN BRAIN, Lorcan A. O'Tuama, Tomas R. Guilarte, Robert F. Dannals, Henry N. Wagner, Jr., The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Transport of large neutral amino acids (LNAA) is of major importance in brain function, and may influence the pathogenesis of neurometabolic disorders such as phenylketonuria. We have quantified LNAA uptake by the human brain using a simple dual probe radiation detector. Normal, fasted, adult male volunteers received 360-410 μCi (1.5-4.2 μg) C11-L-methionine (MET) iv. All studies showed a rapid initial accumulation of brain 11C-L-MET which plateaued after 5 min. A second study was acquired 1 hour after the oral administration of L-phenylalanine (PHE) 100 mg/kg and showed a decreased accumulation of radio MET (mean ± SD = -32.5% ± 14.7%). Analysis of total LNAA levels (except L-tryptophan) showed: (a) a 1.8- to 12-fold elevation of serum PHE after loading; (b) no change in other LNAA levels. Data fitting to an integral (Patlak) plot indicated that initial blood-to-brain transfer of MET was undirectional and also decreased after PHE challenge (e.g., y = .009012 + .0005 theta baseline vs. y = .00093 + .00002 theta post PHE). Thus, the dual probe system allows quantification and detailed analysis by modeling of LNAA transport. Such information has previously been obtainable only with PET imaging using approximately 40 times the present radiation dose. The probe detection system provides a unique method for repeated, non-invasive studies of the LNAA transport system in the living brain. This novel method makes it possible to monitor brain amino acid transport and other mental disorders.

R36 ABSENCE OF BLOOD PRESSURE LOWERING EFFECTS OF HIGH ORAL DOSES OF ASPARTAME IN SPONTANEOUSLY HYPERTENSIVE RATS, Gerald M. Walsh, Stephen E. Bittner, Anita K. Mooney, and Leonard F. Rozek. G. D. Searle & Co., Skokie, Ill. 60077.

The effects of oral and intraperitoneal doses of aspartame on arterial blood pressure of conscious spontaneously hypertensive rats (SHR) were measured using both direct catheter and indirect tail cuff techniques. We tested the possibility that high doses of aspartame would increase tissue levels of tyrosine which might decrease arterial pressure via the central nervous system. In some studies administration of aspartame was accompanied by the oral administration of glucose. Mean arterial pressure (MAP, mmHg) measured directly in 13-week male SHR (n=10) was 171 $\pm$ 5.2 before aspartame and at 1, 2, 4, and 6 hrs after aspartame (200 mg/kg ig) was 168, 166, 164, and 160, respectively. The post treatment values were not different from the pretreatment value nor from vehicle values. Heart rate was unaltered. This was also the case when glucose was coadministered 3 g/kg ig or when the studies were repeated using the tailcuff technique. When aspartame or aspartame plus glucose was administered by the intraperitoneal route, again there were no significant changes in arterial pressure. The results of these studies demonstrate that large oral or ip doses of aspartame or aspartame plus glucose do not decrease MAP in SHR.

R37 HYPERSERINEMIA IN PSYCHOTIC ILLNESS, Rafiq Waziri
University of Iowa, Iowa City, IA 52242

In our studies on close to 300 psychiatric patients and normal control subjects, we have found that patients with psychosis (delusions and hallucinations) have significantly higher plasma serine levels (PSL) when compared to nonpsychotic patients and normal controls. We have also found that the specific activity of the enzyme serine hydroxymethyltransferase (SHMT), which catalyzes the major degradative route of serine to glycine and 1-carbon units, is significantly lower in psychotic subjects than in nonpsychotic subjects. The activity of SHMT in plasma is significantly and negatively correlated to PSL in these subjects. This would suggest that the hyperserinemia in psychotic subjects is largely due to diminished SHMT activity. Kinetic studies show a decreased V_{max} and K_i and an increased K_m for SHMT from psychotics, suggesting a diminished affinity for serine due to configurational differences in the enzyme protein. We have found no significant influence from age, sex, dietary and drug intake in these subjects on SHMT activity. Since abnormally low SHMT activity is observed in recovered psychotics and in some 10-15% of nonpsychotic subjects, it is likely that abnormal serine metabolism is a trait and a vulnerability marker for psychosis. An etiological role for this abnormality in serine metabolism may be postulated on the basis of decreased glycine and 1-carbon units in the brain of psychotic patients with diminished SHMT activity.

R38 PERINATAL EXPOSURE TO ASPARTAME AFFECTS RATS MAZE PERFORMANCE BUT NOT REFLEX OR MORPHOLOGICAL DEVELOPMENT. Raz Yirmiya[1], Mark D. Holder[2], Edward D. Levine[1], and Clinton D. Chapman[1]. [1]Department of Psychology, University of California, Los Angeles, CA 90024 and [2]Department of Psychology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9.

After ingestion, the widely used nonnutritive sweetener L-aspartyl-L-phenylalanine methyl ester (aspartame) is immediately broken down into its constituent amino acids and methyl group: phenylalanine, aspartate and methanol. Though these substances are normally in the body, aspartame may have unwanted side-effects because it raises the levels of these substances in the body relative to other substances. To evaluate the effects of aspartame, aspartame-containing water (.8% w/v) was given to female rats for 30 days before pregnancy and for the entire preweaning period. Compared to rat pups with mothers who drank plain water, no effects on morphological (i.e. pinnae detatchment, eye opening, incisor eruption and body weight) and reflex (i.e. surface righting and negative geotaxis) development were observed. Additionally, no difference was found in the time taken by mothers to retrieve their litters spread out around their home cages. However, when the rats were later tested on a radial-arm maze, the performance of aspartame-exposed rats differed from rats not exposed to aspartame. The radial maze consisted of 8 arms arranged like spokes on a wheel without the rim. The number of arms chosen until an arm was reentered, was higher for the aspartame-exposed rats. Though perinatal exposure to aspartame did not change preweaning reflex and morphological development, post weaning behavior was altered.

R39 A MUTANT MOUSE WITH HYPERPHENYLALANINEMIA DUE TO TETRAHYDROBIOPTERIN DEFICIENCY
Vernon C. Bode & James D. McDonald, Kansas State University, Manhattan, KS 66506

Genetically based mouse models for phenylketonuria (PKU) and related defects in phenylalanine (PHE) metabolism would be valuable tools in the study of these diseases. We are using ethylnitrosourea to efficiently mutagenize spermatogonial stem cells in the mouse and thereby generate the desired mutants. Our breeding and screening protocol identifies mutations which cause high blood PHE so long as they permit the animal to survive for one to two weeks.

The first mutant isolated in this way, hph-1, exhibits neonatal hyperphenylalaninemia (20 mg/dl). Its phenylalanine hydroxylase (PH) activity is reduced 30-50% but this appears to be due to less normal enzyme. Genetic analysis indicates that hph-1 behaves as a single Mendelian gene and does not cosegregate with the PH structural gene, as defined by a full length rat-PH cDNA probe. Mutant adults maintain normal serum PHE levels when fed laboratory chow but they still are defective in PHE metabolism. Normal or heterozygous mice clear an intraperitoneal challenge (1 mg PHE/gm body weight) in 90 min. In mutant animals, PHE levels rapidly rise to 30-60 mg/dl and decline slowly with a half-life of 5-6 hours. If the animals are treated with tetrahydrobiopterin (BH_4), or certain BH_4 precursors, they rapidly metabolize the PHE-load. We are trying to define the site of the hph-1 biochemical defect more precisely and to evaluate the mutant's usefulness for studies of cofactor synthesis or as a model for certain types of atypical hyperphenylalaninemia.

R40 Chromosomal Location of Human Aldolase Isozymes: Mapping a Nonspherocytic Hemolytic Anemia Disorder to Chromosome 16 by Spot Blot Analysis. Dean R. Tolan, Joshua Niclas, Roger Lebo*, and Edward E. Penhoet
Biochemistry Department University of California, Berkeley CA 94720 and the *Howard Hughes Medical Institute University of California, San Francisco CA 94143.

One hereditary disorder which is among the nonspherocytic hemolytic class of anemias has been ascribed to a defect in the glycolytic enzyme, fructose diphosphate aldolase. The aldolase isozyme found in reticulocytes and erythrocytes is aldolase A, the most common form in muscle tissue. The other isozymes aldolase B and aldolase C are found predominantly in the liver and brain, respectively. A clone was isolated from a human endothelial cell cDNA library using a cDNA probe encoding aldolase A from rabbit muscle (Tolan *et al.*, 1983). The sequence of the cDNA was determined and the largest open reading frame coded for a protein which was 100% homologous to the protein sequence of the previously determined human aldolase A enzyme (Freemont *et al.*, 1984). The clone encoded amino acids 220 to 363, the carboxyl-terminus, and the entire 3'-untranslated region. This clone provided a probe for analysis of the genes for the human aldolase isozymes. Five *EcoR* I genomic fragments were found in the human genome; 20, 13.3, 9.6, 8.1 & 5.7 kilobases (kb) in size. Under more stringent conditions, and using other isozyme probes specific for aldolases B and C, each of the fragments could be ascribed to one of the aldolase genes. The aldolase B probe hybridized to two predicted fragments, 9.6 & 5.7 kb (Tolan and Penhoet, 1986). The aldolase C probe hybridized to the single 20 kb fragment. The aldolase A probe hybridized to a single 13.3 kb fragment. The aldolase pseudogene probe hybridized to the other 8.1 kb fragment. These probes accounted for all of the hybridizable genomic fragments revealing four aldolase genes in humans; A, B, C, and an A pseudogene.

Studies of the chomosomal location of all the human aldolase genes were done using spot blot analysis of sorted chromosomes. Under very stringent conditions for hybridization we report the location for aldolase A on chromosome 16. This therefore maps the class of hereditary nonspherocytic hemolytic anemic disorders involving aldolase A to chromosome 16.

R41 NUTRIENT SELF-SELECTION BY CORN EARWORM LARVAE: THE ROLE OF SEROTONIN, R.W. Conen, Department of Entomology, University of Illinois, Urbana, IL 61801.
The neurotransmitter 5-hydroxytryptamine (serotonin) has been implicated in the regulation of carbohydrate appetite in rats. It is well known that insects produce serotonin, and research has shown that its functions generally parallel those in mammals. Thus, it may be asked whether serotonin is involved in determining the level of carbohydrate intake by insects. One possible candidate is the corn earworm, Heliothis zea (Lepidoptera: Noctuidae), a major field crop pest in the Western Hemisphere. A previous study has shown that final instar corn earworm larvae, like laboratory rats, have the ability to self-select a balanced diet from a choice of two diet cubes which are identical except one lacks only protein and the other lacks only carbohydrate. These caterpillars consume a ratio by weight of about 80% protein and 20% carbohydrate. To assess the impact of serotonin concentration on carbohydrate intake, I performed two pharmacological experiments involving the incorporation of p-chlorophenylalanine (PCPA), a known serotonin inhibitor, and tryptophan (serotonin precursor) into diet cubes. Larvae feeding on PCPA-treated diets significantly increased carbohydrate consumption, while those feeding on tryptophan-treated diets significantly decreased carbohydrate intake. Direct measurements of brain serotonin, using an HPLC assay, show that an increase in serotonin concentration is correlated with increased carbohydrate consumption. This correlation suggests that the phenomenon of diet-mixing is mediated by internal physiological feedback mechanisms monitoring nutrient intake.

R42 WHERE DO DEPLETED PLASMA AMINO ACIDS GO IN PKU? Halvor N. Christensen, Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109

I proposed in 1953 that the phenylalanine accumulations of PKU inhibit the transport of analogous amino acids into brain, an action rendered more understandable by their common movement by System L across the blood-brain barrier. Unless membrane transport is perturbed, spontaneous variations of the levels of a given amino acid tend to occur together in plasma and tissues. We now show with old data that amino acids supplied singly in excess include two classes, those whose transport competition lowers and those whose action instead elevates various other amino acids in liver and muscle. Those of the first group inhibit as a net the influx of endogenous amino acids, especially by sodium-dependent System A, whereas those of the second group inhibit net efflux via Na^+-independent System L. This dichotomy arises from a cooperation by which System L is forced into service for exodus by the stronger amino acid gradients generated by the more uphill systems. I propose that pathological accumulations of an efflux-inhibiting amino acid (e.g. phenylalanine in PKU, leucine in maple syrup urine disease) cause sequestering of several endogenous amino acids into various tissues, and hence their accelerated catabolism. This action may well account for the previously puzzling fall of the plasma levels of a number of amino acids in PKU (e.g. threonine, glutamine, asparagine, proline, alanine, etc.). Because System L operates as a net inward across the blood-brain barrier, the sequestering action by other tissues will not be balanced for the brain. The aggregate effect of a) inhibited inward transport at the blood-brain barrier and b) stimulated amino acid sequestration in other tissues complicates prediction of the full list of brain amino acids depleted in PKU. I ackn. support, HD01233, Natl. Inst.Health.

R43 IN VIVO INHIBITION OF TYROSINE UPTAKE INTO RAT RETINA BY LARGE NEUTRAL, BUT NOT ACIDIC AMINO ACIDS. John D. Fernstrom, Madelyn H. Fernstrom, and Etta A. Volk. University of Pittsburgh, Pittsburgh PA, 15213.

The uptake of tyrosine into rat retina and brain was studied in vivo following its peripheral injection alone or with other amino acids. Both retinal and brain tyrosine levels rose for at least 60 minutes following tyrosine administration. The levels of tyrosine in retina and brain 60 minutes after injection were also found to be dose-related (the tyrosine doses studied were 75, 200, and 500 mg/kg, or 414, 1194, and 2760 micromoles/kg ip). When tyrosine at each dose was coadministered with a mixture of branched-chain amino acids (comined dose of 270 mg/kg, or 2143 micromoles/kg), the increments in retinal and brain tyrosine levels were significantly attenuated. When tyrosine was coadministered at each dose with aspartate and glutamate (combined dose of 300 mg/kg or 2148 micromoles/kg), no attenuation of the rise in retinal or brain tyrosine level was noted. Blood tyrosine levels were always comparable between groups receiving tyrosine alone and those receiving tyrosine plus either acidic or large neutral amino acids. The post-injection tyrosine level in serum was therefore not predictive of the tyrosine level in retina or brain. Instead, retinal and brain tyrosine levels were found to parallel the serum ratio of tyrosine to the sum of the other large neutral amino acids (which include the branched-chain amino acids), a quantity previously shown in rats to be predictive of the competitive transport into brain of the large neutral amino acids (Fernstrom JD, Faller DV. J Neurochem 30: 1531, 1978). These results therefore support the notion that tyrosine uptake into rat retina, like that into brain, is mediated by a competitive transport system shared among the large neutral amino acids.

R44 AMINO ACID TRANSPORT IN THE DEVELOPING FETAL ERYTHROID CELL, Jaydutt V. Vadgama and Halvor N. Christensen, University of Michigan, Ann Arbor, MI 48109.

We have examined the changes in amino acid transport in erythroid cells isolated from rat fetal livers at different days in gestation. Our results confirm the presence of Na^+-dependent MeAIB transport by System A at days 13 and 14 in gestation. Soon after, this activity begins to decline and disappears on maturation of the red cell. In contrast, the Na^+-dependent System ASC as measured with threonine uptake, increases in activity after day 14 and peaks at day 16. Although this activity decreases on further maturation, its net uptake remains conspicuosly saturable.The newly discovered Na^+-independent System asc is also present as an independent biological entity in the nucleated erythroid cells. The Na^+-independent System L transport is expressed as a high affinity low Km component at the early stages in differentiation. It's afffinty as measured with leucine transport, decreases on maturation however, with a small change in transport capacity. In addition, the Na^+-independent transport of arginine presumably by System y^+, is expressed at the very early stages and virtually remains unaltered during the developmental stages. The changes in amino acid transport may constitute an important signal for erythroid cell differentiation, in addition to the nutritional requirements for the developing fetus. (Ackn. support, HD01233, NIH).