In summary, chiral syntheses have been develped for the four *erythro* and *threo* isomers related to *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA). As starting material, the synthetic routes employed a common synthon derived from the relatively inexpensive sugar, L-rhamnose. All steps were performed without need for resolution of racemic mixtures. These methods are readily adaptable to the preparation of additional related compounds.

The four chiral isomers have been examined with adenosine deaminase (ADA) from human erythrocytes and calf intestine. With both human and calf enzymes, (+)-(2S,3R)EHNA was the most potent isomer (K_i values, human ADA = 2 nM, calf ADA = 3.2 nM), whereas its enantiomer (−)-(2R,3S)EHNA was 200 to 250-fold less active (K_i values, human ADA = 500 nM, calf = 625 nM). With both enzymes the two *threo* isomers were about 40–75 times less potent than (+)-(2S,3R)EHNA. The K_i values for THNA were: (+)-(2R,3R)THNA, human ADA = 122 nM, calf = 120 nM; (−)-(2S,3S)THNA, human ADA = 80 nM, calf = 240 nM. Current studies include examination of these four isomers for cytotoxicity and antiviral properties.

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Calcium-stimulated glutamate decarboxylase activity in brain slices

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Calcium ions are established as critically important in excitation secretion coupling in neuronal synaptic function and in numerous other secretory cells in the mammal (for a review, see Ref. 1). Typical of the classical role of Ca^{2+} in synaptic function is the inhibition of stimulus-dependent γ-aminobutyric acid (GABA) release from synaptosomes when Ca^{2+} is omitted from the incubation medium [2]. At the molecular level, however, it is less clear how calcium couples neuronal stimulation to transmitter secretion and the extent to which the cation is important in other basic cellular functions. For example, there are voltage-sensitive Ca^{2+} channels in neuronal membranes and these channels participate in the generation of the action potential [3]. Data from studies with synaptosomes have shown that Ca^{2+} is sequestered within cells [4]. Ca^{2+}-dependent protein phosphorylation has been localized to neurons in brain [5], and calmodulin, as the intracellular receptor for calcium, is of key importance in cellular regulation [6].

There is some evidence that Ca^{2+} participates in the regulation of transmitter biosynthesis in serotonergic neurons [7], noradrenergic neurons [8], and in dopaminergic neurons [9]. The present study was carried out to define a role for Ca^{2+} in the regulation of GABA biosynthesis in brain GABAergic neurons.

The animals used were male Sprague–Dawley-derived rats from the Charles River Laboratories (Wilmington, MA). Labeled compounds and Aquassure were purchased from the New England Nuclear Corp. (Boston, MA), and ionophore A23187 was obtained from CalBiochem (La Jolla, CA). Amino acids and imidazole were purchased from the Sigma Chemical Co. (St. Louis, MO), $CoCl_2 \cdot 6H_2O$ was obtained from Fisher Scientific (Fair Lawn, NJ), and $LaCl_3 \cdot 7H_2O$ ("Gold Label") was supplied by the Aldrich Chemical Co. (Milwaukee, WI). Verapamil · HCl was a gift of the Knoll Pharmaceutical Co. (Whippany, NJ). All other chemicals and reagents were obtained

from standard commercial suppliers.

Slices of rat corpus striatum were prepared from freshly dissected brain, essentially as described previously [10]. Slices were nominally 225 μm thick and, in all experiments other than those with La^{3+}, they were suspended in 4.5 ml Krebs–Ringer–bicarbonate medium (KRB) with the following composition: NaCl, 124 mM; KCl, 5 mM; $MgSO_4$, 1.3 mM; KH_2PO_4, 1.25 mM; $NaHCO_3$, 26 mM; and glucose, 10.4 mM. $CaCl_2$ was present in concentrations varying from 0 to 2.6 mM. In experiments with La^{3+}, slices were suspended in a Krebs–Ringer–imidazole medium described previously [11]. [^{3}H]GABA synthesis was initiated by adding 0.5 ml L-[3,4-^{3}H]glutamate (0.2 μCi/μmole) to a final concentration of 0.5 mM. The slices were incubated at 37° for 10 min, and the reaction was terminated by adding 0.5 ml trichloroacetic acid (15%, w/v) which contained 3 mM carrier GABA. The acidified suspension was immediately homogenized with a Polytron (Brinkmann Instruments, Westbury, NY), and the protein was pelleted by centrifugation (47,000 *g*, 10 min). The supernatant fractions were aspirated and stored on ice for assay 2 hr later. Duplicate 1 ml fractions of each supernatant were applied to cation exchange columns made from Pasteur pipettes plugged with glass wool and containing as 1 ml bed volume of AG 50W X8 resin (Na^+ form, 200–400 mesh; Bio-Rad Laboratories, Richmond, CA) equilibrated with 0.2 M citric acid. The [^{3}H]GABA was separated by stepwise elution with 3 ml of citrate buffer (0.2 M, pH 3.5) followed by 3 ml of acetate buffer (0.2 M, pH 5.5) [12]. The final 2 ml fraction was collected into scintillation vials, 15 ml of Aquassure was added, and [^{3}H]GABA was estimated by liquid scintillation spectrometry. As verification of the identity of the labeled product, a column eluate was lyophilized and reconstituted in 0.5 ml of 50% (v/v) ethanol. A one hundred and twenty microliter sample was streaked on a thin-layer plate (0.1 mm cellulose, E. Merck, Darmstadt, FRG). Glutamate and GABA were spotted as standards, and the plate was developed in butanol–acetic acid–water (67:23:10). The standards were visualized with ninhydrin, and the glutamate migrated with an R_f of 0.18. GABA migrated with an R_f of 0.44, and 94% of the recovered radioactivity migrated with an R_f equal to that of the GABA standard. Representative recoveries were calculated each day by eluting a pair of columns, to which had been applied [^{14}C]GABA (3 mM, 0.1 μCi/μmole), in parallel with the experimental samples. Blank values were established by incubating and eluting a pair of samples to which slices were not added. Sample-to-blank ratios averaged 5:1 (6000–8000 dpm/sample), and daily recoveries were always greater than 90%. Data were evaluated using a Hewlitt–Packard 9815 desktop computer with software for one-way and two-way analysis of variance. Where necessary, *posteriori* multiple comparisons were made by the Newmann–Keuls multiple comparison test.

In an earlier study of glutamate decarboxylase (GAD) activity in striatal slices, it was reported that the omission of Ca^{2+} from the incubation medium decreased GABA synthesis and abolished the increase produced by high K^+ [10]. There was no further effect when Ca^{2+} was deleted and ethyleneglycol bis (amino-ethylether)tetra-acetate (EGTA) included in the medium. Data presented in Fig. 1 confirm and extend those preliminary findings. [^{3}H]GABA synthesis by the slices was decreased in a Ca^{2+}-deficient medium and the addition of Ca^{2+} produced a concentration-related increase in activity which was maximal at 2.6 mM Ca^{2+}. Results in Fig. 2 show that the response to 1.3 mM Ca^{2+} could be completely reversed by 1.0 mM La^{3+} but not by 1.0 mM Co^{2+}. This may be explained by the different activities of La^{3+} and Co^{2+} as inhibitors of Ca^{2+} binding [13], or it may reflect the ability of Co^{2+} to cross neuronal membranes [14] and the lack of ability of La^{3+} to cross the same membranes [15]. Data in Fig. 3 show that the response to 1.3 mM Ca^{2+} was not

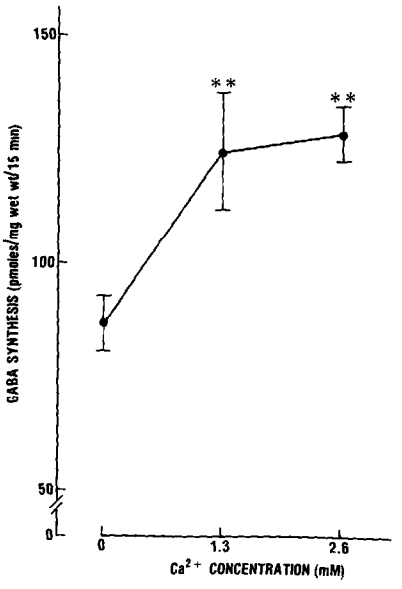

Fig. 1. Effect of Ca^{2+} on GABA synthesis in striatal slices. Each point is the mean ± S.E.M. of eight samples, pooled from two experiments. Key: (**) significantly different from control ($P \leq 0.01$) by analysis of variance.

affected by the divalent cationic ionophore A23187 (6 μM), nor was it blocked by 100 μM verapamil. It may be that the amplification of the slice system was too low to see a change in the presence of the ionophore, or that the ionophore concentration was too low to see an effect. A third possibility is consistent with the other data presented here, that Ca^{2+} need not cross the neuronal membrane to stimulate GABA synthesis. Of the four treatments reported here, only La^{3+} acts solely in the extracellular compartment to prevent Ca^{2+} from binding [15]. Co^{2+} crosses neuronal membranes as discussed above, and blocks both intracellular and extracellular Ca^{2+} flux [3, 14]. Ionophore A23187 stimulates Ca^{2+} flux [16], and verapamil blocks Ca^{2+} flux [17].

In summary, we have reported that Ca^{2+} in the incubation medium is necessary for maximal rates of GAD activity in a brain slice preparation. The Ca^{2+} stimulation was

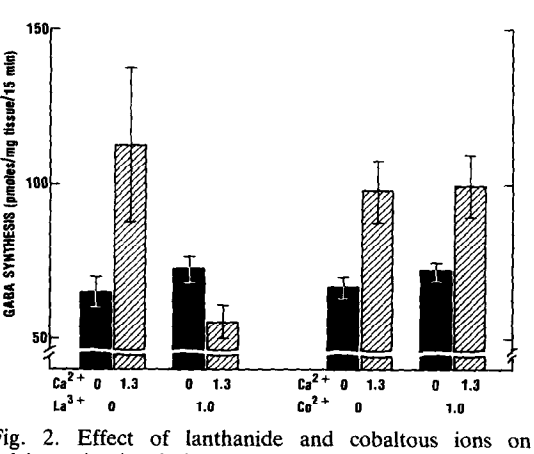

Fig. 2. Effect of lanthanide and cobaltous ions on calcium-stimulated GAD activity in striatal slices. Each value is the mean ± S.E.M. of four samples. Incubations in the La^{3+} studies were in Krebs–Ringer–imidazole; those in the Co^{2+} studies were in KRB. Cation concentrations are all mM.

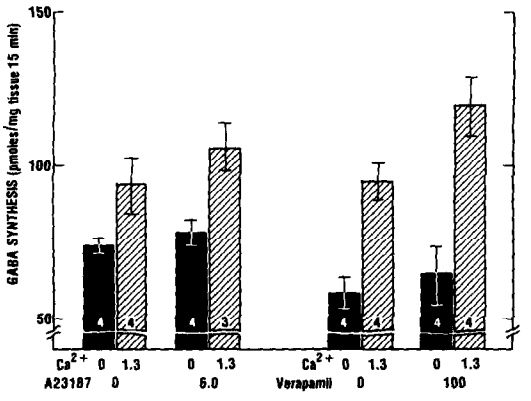

Fig. 3. Effect of A23187 and verapamil on calcium-stimulated GAD activity in striatal slices. Each value is the mean ± S.E.M. of the number of samples indicated in the figure. A23187 was dissolved in absolute ethanol and then added to the incubation. Ethanol was included in the control incubations. Ca^{2+} concentrations are mM; A23187 and verapamil concentrations are μM.

concentration-related and could be blocked by La^{3+}, but not by Co^{2+} or by verapamil at the concentrations tested. Submaximal stimulation by Ca^{2+} was not potentiated by the ionophore A23187. These data are consistent with the hypothesis that extracellular Ca^{2+} plays a role in the regulation of GABA biosynthesis by interaction with binding sites on the exterior surface of neuronal membranes. The mechanism by which Ca^{2+} is transduced to stimulate GAD activity cannot be explained by these data.

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Isolation and separation of heme *a* and heme *b* from cardiac tissue by thin-layer chromatography

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Hemoproteins found in mammalian cells are composed of a protein moiety coupled with either heme *a*, *b*, or *c*. Hemes *a* and *b* are loosely associated with the protein moiety of hemoproteins and are extractable under acid conditions into certain organic solvents [1]. Heme *c* is covalently linked to the protein moiety of hemoproteins and special procedures are required to break the covalent bond prior to heme *c* isolation [2].

The isolation of heme *b* from tissues is facilitated by the isolation of subcellular fractions (i.e. microsomal fractions) which are essentially free of heme *a*. The isolation of heme *a* from tissue is complicated by the fact that heme *a* is primarily located in the mitochondria, which also contains heme *b*.

Most published procedures for the isolation of heme *a* are relatively complex [3–7] and utilize a large amount of tissue for heme *a* isolation. This paper will describe a simple method for the isolation of heme *a* and heme *b* from small quantities of cardiac tissue.

Materials and methods

Hemin (Type III) was purchased from the Sigma Chemical Co. (St. Louis, MO). Silica gel 7 was obtained from the J. T. Baker Co. (Phillipsburg, NJ). Polyvinyl alcohol (100% hydrolyzed, average molecular weight of 14,000) was obtained from the Aldrich Chemical Co. (Milwaukee, WI). Aquasol-2 and [4-^{14}C]-δ-aminolevulinic acid (25.4 mCi/mmole) were purchased from the New England