26.8% respectively, as reported before[6]. Although the mutarotase activity of 0.66 unit/g wet tissue was detected in the retina, the mutarotase in the retina was considered scarcely to affect the mutarotation rate of D-glucose in the media. Uptake of D-glucose by the retina during the incubation of 3 and 5 min was measured by determining the glucose contents in the media before and after the incubation.

Results. After 3 min incubation, 1.11 mg of α-D-glucose was incorporated in the retina per g wet tissue, while 1.32 mg of β-D-glucose was incorporated (table). The incorporated amount of the equilibrated D-glucose was between those of α and β anomers. The ratio β/α was calculated to be 1.19. This value was statistically significant ($p < 0.05$) according to the *t*-test. Considering the rapid mutarotation during incubation, the real ratio should be greater than this. After 5 min incubation, the incorporation clearly increased in every case. However, the ratio β/α became smaller than that after 3 min incubation. This decrease of the ratio will be attributable to the progress of equilibration.

Uptake of two anomers of D-glucose and equilibrated D-glucose by rat retina

Incubation time	α anomer	β anomer	equilibrated	β/α
3 min	1.11±0.19	1.32±0.18	1.15±0.24	1.19[a]
5 min	1.88±0.31	2.15±0.22	1.99±0.36	1.14[b]

Values are mean ± S.D. of 9 experiments and are expressed as mg of D-glucose/g wet issue. [a] $p<0.05$; [b] insignificant.

Discussion. In a preliminary experiment, we found that the glucose uptake by the retina is dependent on the temperature as shown in the figure, by incubating the retina at different temperatures (4 °C and 37 °C). DOLLERY et al.[12] compared the assimilation of D-glucose-1-^{14}C with that of L-glucose-1-^{14}C in the retina of rat. They found that 5 min after an intravenous injection of the radioactive D- and L-glucose the ratio of D/L radioactivity in the retina was 23.7. KEEN and CHLOUVERAKIS[13] reported that the D-glucose uptake by the rat retina showed the saturation for D-glucose concentration. These 3 data indicate the presence of a stereospecific carrier for D-glucose in the retina. From our result, it should be mentioned that the carrier for D-glucose in the retina prefers the β anomer of D-glucose stereospecifically.

The preference for β-D-glucose in D-glucose uptake by the retina was similar to those of rat pancreatic islets[7], human red blood cells[14], Ehrlich ascites tumor cells[15]. Therefore, it should be notified that any cells of higher animals generally utilize the β anomer of D-glucose more predominantly than the α anomer as an energy source.

On the other hand, it seems likely that the receptor site of D-glucose-recognizing cells accepts the α anomer of D-glucose preferentially, since it is known that the preference for α-D-glucose is found in triggering insulin secretion[6], suppressing glucagon secretion[16] and sensing the sweetness of D-glucose[2,17].

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N-(5′-Phosphopyridoxyl)-4-aminobutyric acid: A stable bisubstrate adduct inhibitor of rat brain 4-aminobutyric acid aminotransferase[1]

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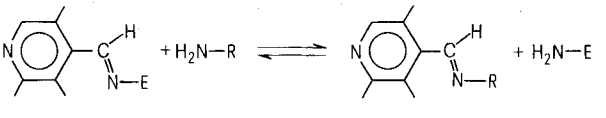
Summary. N-(5′-Phosphopyridoxyl)-4-aminobutyric acid, a stable adduct of pyridoxal phosphate and 4-aminobutyric acid, has been shown to be a potent inhibitor of rat brain 4-aminobutyric acid aminotransferase (GABA-T) with a K_i of 1.4 μM.

Introduction. The growing acceptance of 4-aminobutyric acid (GABA) as an inhibitory neurotransmitter[3,4] and the proposal that some anticonvulsant drugs exert their action by elevating brain GABA levels as a result of inhibition 4-aminobutyric acid aminotransferase (GABA-T)[5,6] have given impetus to the search for potent GABA-T inhibitors in anticipation that these compounds will be potential antiepileptic drugs.

GABA-T belongs to a class of enzymes called transaminases. The mechanism of action of these enzymes is well studied[7–9]. The catalytic activity of transaminases depends on the presence of pyridoxal phosphate. The enzyme-bound pyridoxal phosphate exists as the Schiff's base rather than as the free aldehyde. The initial step in the transaminase-catalyzed reaction is a transimination that involves the conversion of the pyridoxal phosphate-enzyme Schiff's base into a pyridoxal phosphate-amino acid Schiff's base:



With the participation of the appropriate acidic and basic groups of the enzyme in a hydrolytic reaction, a pyridoxamine phosphate derivative of the enzyme and a keto acid are formed. On the basis of this reaction mechanism it was anticipated that N-(5′-phosphopyridoxyl)-amino acids would be good inhibitors of pyridoxal phosphate dependent enzymes[10] since these compounds structurally resemble the intermediates in the enzymatic reaction pathway.

Severin et al.[10] reported that N-(5′-phosphopyridoxyl)-4-aminobutyric acid at 1 mM concentration inhibited rat brain GABA-T by 30%. However, the mechanism of

action of this compound on the enzyme was not investigated.

Our interest in GABA metabolism in brain has prompted us to synthesize N-(5′-phosphopyridoxyl)-4-aminobutyric acid (PLP-GABA) and investigate its mode of inhibition on rat brain GABA-T.

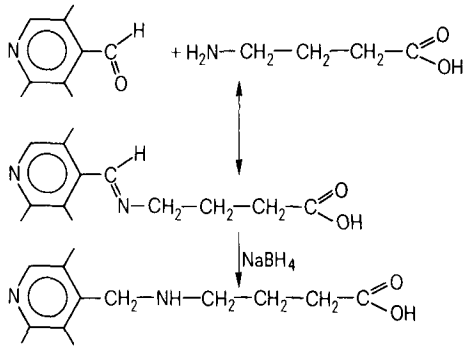
Materials and methods. Animals. Male Charles River rats weighing about 200 g were used for this study. The animals were maintained in our animal quarters for at least 3 days prior to use and during this time had unlimited access to food and water.

Chemicals. All chemicals used in this study were purchased from Sigma Chemical Co., St. Louis, Mo., USA.

Enzyme preparations. The enzyme used in this investigation was prepared by the method of Maître et al.[11] up to the stage of ammonium sulfate precipitation. Proteins precipitated between 42.5% and 67.5% ammonium sulfate were dissolved in 0.1 mM potassium phosphate buffer (pH 7.5) containing 10 μM pyridoxal phosphate and 1 mM 2-aminoethyl-isothiouronium bromide hydrobromide. The enzyme was dialized against 2 changes of 3 litres of the dissolving buffer. The dialysate was used as the enzyme source.

Determination of GABA-T activity. The activity of GABA-T was measured by coupling with succinic semialdehyde dehydrogenase and changes in absorbance at 340 nm were monitored continuously in a Beckmann Acta M VI recording spectrophotometer[12,13]. Enzymic reactions were carried out at 30 °C.

Preparation of PLP-GABA. PLP-GABA was synthesized by the procedure of Severin et al.[10] and Khomutov et al.[14] according to the following scheme:



1 mmole of pyridoxal-5′-phosphate was dissolved in 5 ml of anhydrous methanol containing 2 mmoles of KOH. To the resulting yellow solution was added 8 ml of methanol containing 1 mmole KOH and 1 mmole of GABA. The solution was cooled in an ice bath for 10 min. Then 40 mg of sodium borohydride was slowly added with stirring to the solution over 10 min period and the mixture was continued to be stirred for an additional 10 min, after which it was neutralized with acetic acid to pH 6. The solvent was removed in vacuo at 50 °C using a rotary evaporator. The solid was dissolved in 25 ml of a solution of 0.3 M acetic acid and 0.3 M-pyridine and chromatographed on a column of DEAE-cellulose (30 × 3 cm) which had been equilibrated with the same buffer. The column was eluted with this buffer. Fractions of 15 ml were collected and those with an extinction at 327 nm were pooled and evaporated to dryness using a rotary evaporator. Ammonia-saturated ethanol was added to the dry solid and evaporation repeated until crystallization occurred.

Results and discussion. N-(5′-phosphopyridoxyl)-4-aminobutyric acid has been synthesized according to the procedure of Severin et al.[10] and Khomutov et al.[14]. It shows characteristic absorption bands at ca. 295, 310 and 325 nm in acidic (0.1 M HCl) basic (0.1 M NaOH) and neutral (pH 7.0) solution respectively.

A number of N-(5′-phosphopyridoxyl)-amino acid adducts have been synthesized and shown to be inhibitors of pyridoxal phosphate dependent enzymes[10,14,15].

The effects of PLP-GABA of the activity of rat brain GABA-T are shown in the figure. It is clear that PLP-GABA inhibits GABA-T competitively with respect to GABA. In view of the results of Severin et al.[10] the inhibitory effects of PLP-GABA were anticipated. However, its potency as a GABA-T inhibitor was unexpected. From the data presented in the figure and from the following equation (1) which describes the competitive in-

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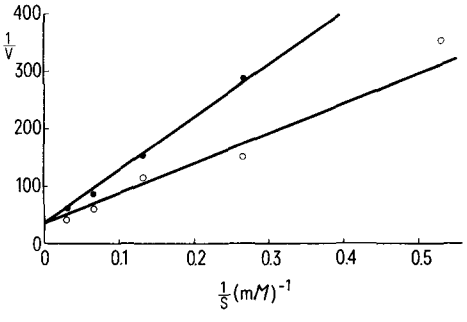

Lineweaver-Burk plots of GABA-T activity against GABA concentration in the absence (○) and in the presence of inhibitor (●). The inhibitor (PLP-GABA) concentration was 1 μM. [NAD]: 4 mM, [Enzyme]: 0.5 mg/ml, temperature: 30 °C. 50 mM potassium phosphate buffer pH 7.5, pyridoxal phosphate: 10 μM, [GABA]: 1.8–30 mM. v: Δ0.D at 340 nm per min; s: GABA concentration. Each point represents the mean of 4 determinations.

hibitor of the enzyme by PLP-GABA, the inhibitor (PLP-GABA)-enzyme dissociation constant (K_i) was calculated as 1.4 μM,

$$\frac{1}{v} = \frac{K_m}{V_m}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_m} \quad (1)$$

where v: initial rate of the enzymic reaction, Vm: maximum rate of the enzymic reaction, Km: Michael's constant, [I]: concentration of PLP-GABA, K_i: PLP-GABA and enzyme dissociation constant, [S]: concentration of GABA.

The magnitude of the K_i indicates that PLP-GABA is a powerful GABA-T inhibitor. Such an inhibitor might be a useful tool in the in vivo study of brain GABA metabolism if it transpires that it can cross the blood-brain barrier. As well, it could serve as a potential ligand in affinity chromatography for the purification of GABA-T.

Effect of chronic alloxan diabetes and insulin treatment on adipose tissue lipid composition of rats

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Summary. Decreased content of the adipose tissue lipids was observed in chronic alloxan diabetic rats and was restored to normal with insulin treatment. Prolonged insulin treatment in normal rats also resulted in increase of the lipid content of the adipose tissue.

Adipose tissue is characterized by its high content of fat which comprizes mostly triglycerides. The composition of yellow and brown adipose tissue lipids and its variations with different periods of starvation have been reported[1]. BENJAMIN et al.[2] and PECKHOLM et al.[3] studied the effects of age and diet on adipose tissue lipids of rats. The adipose tissue lipids are also under the influence of the hormonal status of the animal[4]. However, information regarding the effect of diabetes on the adipose tissue lipids and on their changes with insulin treatment are scanty. Therefore a study was undertaken to examine the detailed lipid composition of the adipose tissue of chronic alloxan diabetic rats and the effect of insulin treatment on the composition.

Materials and methods. Male rats of Institute colony (Wistar strain) weighing 160 ± 25 g were kept on commercial rat pellets (Hind Lever, Bombay) and water ad libitum. Diabetes was produced in 36 h fasted rats by i.p. injections of freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg body wt.). After the production of diabetes, half of the animals were treated with s.c. injections of 3 units of protamine zinc insulin (Boots) daily, and the other half were given the same amount of 0.15 *M* saline. In non-diabetic animals, the dose of insulin was started from 0.3 units which was increased every 3rd day by 0.2 units depending on the tolerance of the animals, till a final level of 3 units was reached, and this dose was maintained throughout the rest of the experimental period. The animals were observed for 120 days. Then they were sacrificed after overnight fast under light ether anaesthesia. Insulin injections were stopped 24 h before sacrifice.

Epididymal fat pads were quickly removed and the lipids were extracted[5]. Neutral lipids were separated by thin layer chromatography on silica gel G plates using solvent system petroleum ether-ether-acetic acid, 90:10:1 (V/V). Neutral lipids from silica gel G plates were eluted successively with chloroform and chloroform-methanol 2:1 (V/V). Cholesterol[6], triglycerides[7], free fatty acids[8] and phospholipid phosphorus[9] were measured in the lipid extracts. Blood sugar[10] estimations were also done in the animals before sacrifice.

Results and discussion. The initial and final body weights and the blood sugar levels of rats at the time of sacrifice have been presented in Table I. There was increase of body weights in all the groups except in the saline-treated diabetic group. Blood sugar levels of the diabetic groups (Groups C and D) were higher as compared to those of the non-diabetic groups (groups A and B). However, insulin-treated diabetic group (group D) showed much lower values as compared to that of the saline-treated diabetic group (group D).

Table I. Body weight and blood sugar values of rats in different groups

Groups	Body weight (g)		Blood sugar at the time of sacrifice (mg/100 ml)
	Initial	Final	
A) Saline treated control	156±8	222±11	80±7
B) Insulin treated control	171±7	266±5	68±6
C) Saline treated diabetic	152±3	144±4	393±12
D) Insulin treated diabetic	174±9	231±18	276±6

Values were Mean ± SE of 5 animals in each group

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