Uptake of D-glucose anomers by rat retina

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Summary. Uptake of D-glucose anomers by isolated rat retina was studied. After 3 min incubation at 37 °C in the presence of α or β anomer (750 μg/ml), a significantly greater uptake (1.32 mg/g wet tissue) of β-anomer was observed compared with that of α-D-glucose (1.11 mg/g wet tissue). This result and other data suggest that the carrier for D-glucose transport in the retina prefers the β-anomer stereospecifically.

The retina is a kind of neuron-rich tissue characterized by extremely rapid metabolism of D-glucose as well as brain cortex[3-5].

Recently we showed that the α anomer of D-glucose is more effective than the β anomer in triggering insulin secretion from isolated rat pancreatic islets[6], and the β anomer of D-glucose is more easily transported into the islets than the α anomer[7].

From the points of physiological function of D-glucose anomers, it is interesting to study whether or not anomeric stereospecificity is present in the D-glucose uptake by the retina of rat.

Materials and methods. The two anomers of D-glucose were prepared by applying our method[7] for preparation of α- and β-D-glucose-1-^{3}H. Each anomeric purity of the two anomers was determined to be more than 98% by our method using β-D-glucose oxidase [EC 1.1.3.4] (Nagase & Co., Osaka), hog kidney mutarotase [EC 5.1.3.3], and a Beckman oxygen electrode (model 777)[8,9]. Total amount of D-glucose in the reaction mixture before and after incubation was determined by a reagent kit of Boehringer (β-D-glucose oxidase-peroxidase-ABTS system)[10]. The mutarotase activity of the retina was assayed by our method using β-D-glucose oxidase and an oxygen electrode[11]. The eyes were removed from male Wistar strain rats weighing 150–200 g under anesthesia with ether and then hemisected just behind the corneoscleral junction. The lens and vitreous body were carefully discharged and the retina was gently peeled off with a blunt spatula from the sclera. All incubations were performed at 37 °C in gassed (95% O_2 and 5% CO_2) Krebs-Ringer bicarbonate solution. After a preliminary incubation period of 5 min, batches of 2 retinas (about 20 mg) were incubated for 3 and 5 min in 300 μl of the media with either the pure α or β anomer, or with the mixture of both anomers at the equilibrated ratio (α:β, 36:64) of D-glucose. These anomers were rapidly dissolved in the gassed Krebs-Ringer bicarbonate solution, which had been warmed to 37 °C, by vigorous shaking just before use. The final concentration of D-glucose was 750 μg/ml. The major portion of each anomer in the incubation media just before the incubation was more than 98%, and during the 5 min incubation, the α and β anomers were converted to the other anomers by 42.4% and

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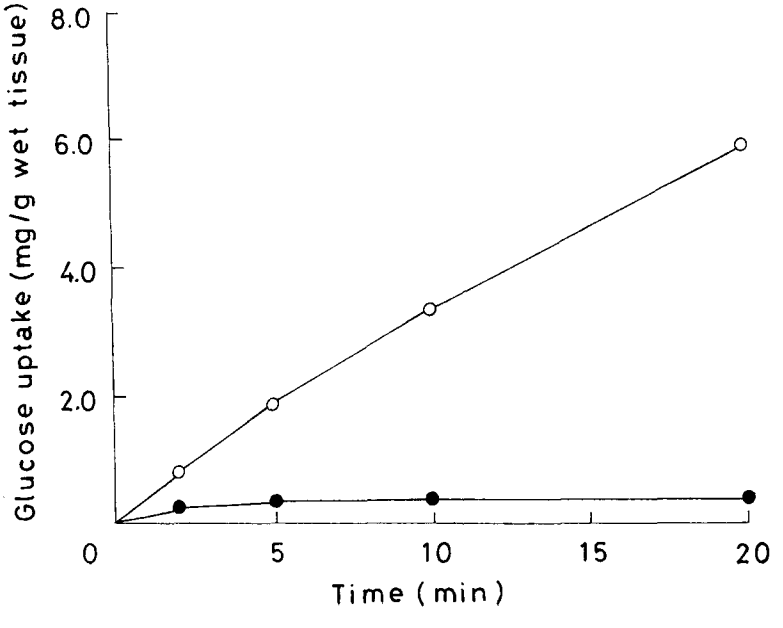

Influence of temperature on D-glucose uptake by the retina. The retinas were incubated in the media containing 750 μg/ml equilibrated D-glucose at 4 °C (●) or 37 °C (○). Preincubations of 5 min were performed at the respective temperatures. See the text for the detailed conditions.

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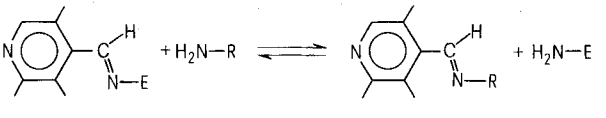
Summary. N-(5′-Phosphopyridoxyl)-4-aminobutyric acid, a stable adduct of pyridoxal phosphate and 4-aminobutyric acid, has been shown to be a potent inhibitor of rat brain 4-aminobutyric acid aminotransferase (GABA-T) with a K_i of 1.4 μM.

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