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STIMULATORY EFFECT OF TAURINE ON Ca-UPTAKE BY DISC MEMBRANES FROM PHOTORECEPTOR CELL OUTER SEGMENTS

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SUMMARY : Effects of taurine and related compounds on Ca-uptake by the disc membranes prepared from dark-adapted frog retina were studied. Taurine stimulated ATP-dependent Ca-uptake and the turnover of 45Ca in the disc membranes without affecting basal activity, but it was not observed with the synaptic plasma membranes from rat brain. The stimulatory effect appears to be specific to taurine, since cysteine sulfinic acid, hypotaurine, isethionic acid, β-alanine and γ-aminobutyric acid (GABA) did not stimulate Ca-uptake. The maximal activation, observed at about 30 mM taurine, was about 3 fold, and the Km value for taurine was 10 mM. These results might suggest that taurine modifies translocation of Ca ion in the rod outer segment.

INTRODUCTION : The vital role of taurine, an ubiquitous sulphur-containing amino acid, in the retinal function is discussed in accordance with the following evidence ; 1) Accumulation of taurine in the retina not only occurs in the Na-dependent taurine-uptake system (1), but taurine is also synthesized by cysteine sulfinate decarboxylase from cysteine sulfinic acid via hypotaurine (2). 2) Taurine is highly concentrated in the retina, especially in photoreceptor cell (about 75 % of the amino acids pool is occupied by taurine) and the physiological concentration is estimated to be about 2.5 to 25 mM (3-5). 3) Destruction of photoreceptor cells is observed in the taurine-deficient cat with a concomitant decrease in the taurine content of retina (6). 4) Taurine has depressant effects on the spinal and cortical neurones and on b-wave of the electroretinogram (7). 5) Release of taurine from the retina and rod outer segment is significantly enhanced by illumination (8,9). 6) Taurine significantly inhibits Ca-uptake by rod outer segment (5).

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Considerable evidence also supports the belief that one of the actions of taurine in the heart and central nervous system involves its effect on the Ca-transport system (10, 11).

In the present paper, we describe the effects of the amino acids related to taurine on Ca-uptake by the disc membranes prepared from frog rod photoreceptor cells in comparison with those of synaptic plasma membranes from the rat brain.

MATERIALS AND METHODS : Disc membranes were prepared as reported previously (12) and all procedures were carried out under dim red light. Retinas were removed from a dark-adapted bull frog (Rana Catesbiana) and were shaken for 10 sec in 42 % (W/W) sucrose. After centrifugation at 80,000 x g for 40 min, the material (rod outer segment) in the air-sucrose interface was collected and suspended in 20 mM bicarbonate buffer (pH 7.5) to disrupt the organella. Then, the material was precipitated by centrifugation at 12,000 x g for 5 min. The pellet was homogenized with 20 mM bicarbonate buffer (pH 7.5) and used as a disc membrane preparation for the Ca-uptake experiment. Synaptic plasma membranes from rat brain were prepared by a discontinuous sucrose gradient method as reported previously (13). Ca-uptake assay medium in a total volume of 1.0 ml, consisted of 50 mM bicarbonate buffer ($NaHCO_3$/ KH_2PO_4, pH 7.5), 50 mM KCl, 50 mM NaCl, 50 μM ouabain, 2 mM $MgCl_2$, 10^{-5}M $CaCl_2$ 0.25 μCi/tube) and 1 mM ATP-Tris with or without 30 mM taurine. The reaction mixture was incubated for 10 min at 37°C and the reaction was terminated by adding 5 ml of the same cold Ca-uptake assay medium without ATP and ^{45}Ca. The mixture was filtrated on a Millipore filter and the amount of ^{45}Ca was counted in a liquid scintillation counter as described previously (12). Protein was determined by the method of Lowry et al. (14). Millipore filters (TM-2, 0.45 μm pore size) were obtained from Toyo Roshi., Ltd . Taurine, hypotaurine, isethionic acid, cysteine sulfinic acid, β-alanine and GABA were purchased from Sigma Chemical Co.

RESULTS AND DISCUSSIONS : The time course of ATP-dependent Ca-uptake and the turnover of Ca^{++} by the disc membranes in the presence of 30 mM taurine are shown in Fig. 1. Ca-uptake was stimulated about 10 fold by 1 mM ATP and reached a steady level within 5 min at 37°C. On the other hand, it took about 10 min to obtain a maximal Ca-uptake level in the presence of taurine. This effect of taurine seems to differ from that of oxalate on Ca-uptake by mitochondria and synaptic plasma membranes, since these membranes accumulated ^{45}Ca linearly up to 20 min (data not shown). ATP-dependent Ca-uptake was enhanced approximately 3 fold by 30 mM taurine, increasing from the initial velocity. If small amounts of ^{45}Ca were added at 5 min (as indicated with arrow) to

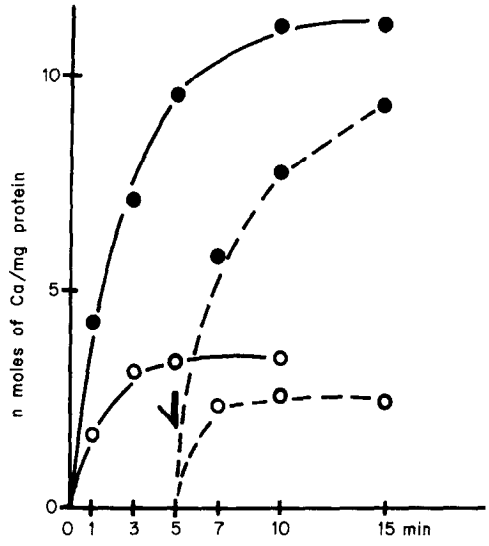

Fig. 1. ATP-dependent Ca-uptake and turnover of Ca by disc membranes in the presence or absence of taurine. Ca-uptake ; Reaction was started by the addition of disc membranes in the presence of 1 mM ATP with (●—●) or without (o—o) 30 mM taurine. Turnover ; Small amounts of ^{45}Ca were added to the Ca-uptake assay medium (after time 5 minutes as indicated with arrow) which had been incubated with 1 mM ATP in the presence (●---●) or absence (o---o) of 30 mM taurine without labelled Ca at 37°C. The amount of ^{45}Ca in the disc membranes was determined as described in MATERIALS AND METHODS.

Ca-uptake assay medium in which disc membranes had been incubated in the presence or absence of 30 mM taurine without labelled Ca, the time courses and levels of ^{45}Ca-uptake by the disc membranes were similar to those of Ca-uptake observed without preincubation. The result might suggest that taurine modifies a Ca-pool in the disc membranes evoked by ATP. We emphasize the fact that taurine failed to stimulate Ca-uptake in Tris-HCl buffer as described by Pasantes-Morales et al who also use bicarbonate buffer and demonstrate an inhibitory effect on Ca-uptake by rod outer segments in the chick retina (5). It is not clear why taurine stimulates Ca-uptake only in bicarbonate buffer.

The effect of various concentrations of taurine on Ca-uptake in the presence or absence of 1 mM ATP are illustrated in Fig. 2. The stimulatory effect of taurine was observed only in the presence of ATP, and its effect was dose-dependent. The Km value for taurine on ATP-dependent Ca-uptake was estimated to be about 10 mM, well within the physiological concentration range in the retina.

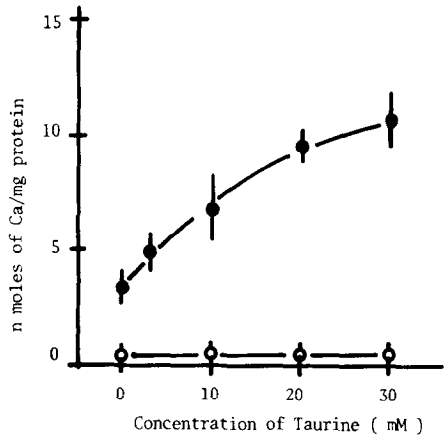

Fig. 2. Effect of taurine on Ca-uptake by the disc membranes. The reaction mixture was incubated for 10 min at 37°C in the presence of various concentrations of taurine with (●—●) or without (○—○) ATP. The vertical lines indicate standard deviation from the mean for 4 to 6 experiments.

The specificity of the stimulatory effect of taurine was studied by examining cysteine sulfinic acid, hypotaurine, isethionic acid, β-alanine and GABA on ATP-dependent Ca-uptake by the disc membranes. All compounds were tested at a concentration of 30 mM. None of the substances activated ATP-dependent Ca-uptake as markedly as taurine (Fig. 3). Isethionic acid caused a slight inhibition (about 35 %) rather than stimulation. Under the same experimental conditions, the effect of taurine in the Ca-uptake system

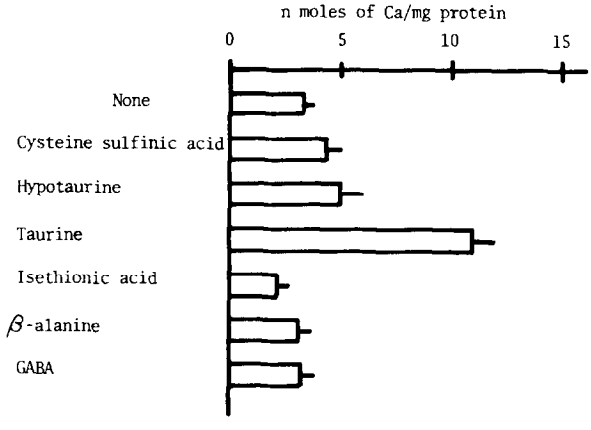

Fig. 3. Effects of taurine and the related amino acids on ATP-dependent Ca-uptake by the disc membranes. The disc membranes were incubated for 10 min at 37°C in the presence of taurine or its analogues at each concentration of 30 mM. The results are mean ± S.D. of 4-6 separate experiments.

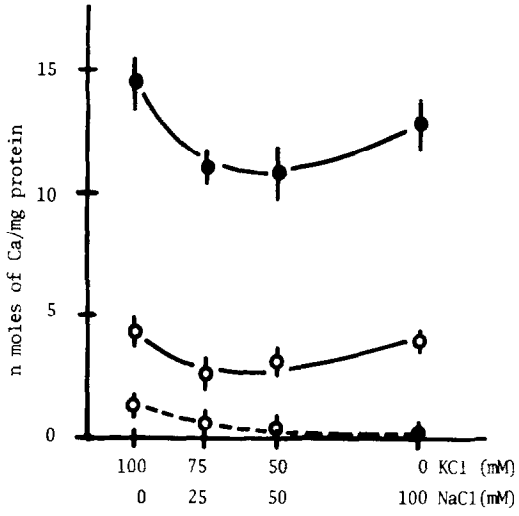

Fig. 4. Effect of taurine on ATP-dependent Ca-uptake by disc membranes in the medium containing various concentrations of potassium and sodium ion. Ca-uptake by the disc membranes was measured as in Fig. 2 and 3 except that $KHCO_3$ was substituted for $NaHCO_3$ in the bicarbonate buffer and the ratio of K^+ and Na^+ in the Ca-uptake assay medium was varied, with the total concentration of both ions kept at 100 mM. Ca-uptake was assayed in the presence (o—o) or absence (o···o) of 1 mM ATP or with 1 mM and 30 mM taurine (●—●). The vertical lines indicate standard deviation from the mean for 3 to 5 experiments.

was also studied with the isolated synaptic plasma membranes from rat brain. The Ca-uptake by the synaptic plasma membranes was enhanced by 30 mM taurine (9.2 ± 0.6 to 11.8 ± 0.8 n moles of Ca/mg protein), but the stimulatory activity is less than one-tenth compared with that of the disc membranes. Fig. 4 illustrated Ca-uptake by the disc membranes with or without 30 mM taurine in the Ca-uptake assay medium containing various concentrations of K^+ and Na^+, keeping a total concentration of both ions at 100 mM. ATP-dependent Ca-uptake was decreased by increasing concentrations of Na^+ up to 50 mM. The maximal inhibition (about 30 %) was observed at about 25 mM Na^+ and 75 mM K^+. Amounts of ATP-dependent Ca-uptake were increased about 3 fold by 30 mM taurine as shown in Fig. 4.

It is widely accepted that a part of the pharmacological action of taurine is elicited by an effect on the Ca^{++} flux (15). Hagins and Yoshikami (16) have shown that hyperpolarization of the retina by light is caused by the translocation of Ca ion in the rod outer segment. In

conjunction with the Ca-hypothesis, it is reported that the release of taurine from rod outer segment is markedly enhanced by illumination (8,9). Our result may suggest the posibility that translocation of Ca ion within the rod outer segment is regulated by taurine. Further experiments will be required to elucidate this.

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