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Effect of melatonin on the retinal glutamate/glutamine cycle in the golden hamster retina

Daniel A. Sáenz, Andrea P. Goldin, Luciana Minces, Mónica Chianelli, María I. Keller Sarmiento, and Ruth E. Rosenstein

Laboratorio de Neuroquímica Retiniana y Oftalmología Experimental, Departamento de Bioquímica Humana, Facultad de Medicina, Universidad de Buenos Aires, Conicet, Buenos Aires, Argentina

Corresponding author: Dr. Ruth E. Rosenstein, Departamento de Bioquímica Humana, Facultad de Medicina, UBA. Paraguay 2155, 5°P, (1121), Buenos Aires, Argentina. E-mail: ruthr@fmed.uba.ar

ABSTRACT

Glutamate is the main excitatory neurotransmitter in the retina, but it is neurotoxic when present in excessive amounts. The metabolic dependence of glutamatergic neurons upon glia via the glutamate/glutamine cycle to provide the precursor for neurotransmitter glutamate is well established. Since melatonin has been shown to be neuroprotective in several systems, in the present report, its effect on the glutamate/glutamine cycle activity was examined in the golden hamster retina. Melatonin (0.1–10 nM) significantly increased retinal glutamine synthetase activity but it did not affect L-glutamine release. A characterization of the hamster retinal L-glutamine uptake mechanism was performed. This mechanism was partly Na^+-dependent, and it was significantly inhibited by 2-aminobicyclo (2, 2, 1) heptane 2-carboxylic acid (BCH, a selective antagonists for the L-type system) and by α-(methylamino)-isobutyric acid (MeAIB, substrate characteristic for the A -type transporter) suggesting the coexistence of these transport systems in the hamster retina. Melatonin (0.1–10 nM) significantly increased total glutamine uptake as well as the BCH and the MeAIB-insensitive transporters activity. On the other hand, melatonin significantly decreased retinal glutaminase activity. On the basis of these results, it might be presumed that hamster retinal glutamate/glutamine cycle activity is regulated by physiological concentrations of melatonin. Furthermore, these findings suggest that a treatment with melatonin could be considered as a new approach to handling glutamate-mediated neuronal degeneration.

Key words: glutamine synthetase • L-glutamine influx • glutaminase

Melatonin (5-methoxy-N-acetyltryptamine) is a putative endogenous neuromodulator in the retina of various vertebrate species (1, 2, 3). Melatonin levels in both the pineal gland (4) and the retina of several species (3) dramatically change during the day–night cycle. We have demonstrated that melatonin levels in the golden hamster retina significantly vary during the light–dark cycle, peaking during the night (5), and that mean retinal

melatonin levels significantly increase after pinealectomy, supporting the proposal that in the golden hamster, retinal melatonin is generated within the tissue itself (6). In contrast to pineal melatonin which is secreted to the body fluids, retinal melatonin is thought to act locally within the eye (7). Melatonin selectively inhibits the calcium-dependent release of dopamine (8), and it has been implicated in the regulation of photoreceptor disc shedding and phagocytosis (1), melanosome aggregation in pigment epithelium, and cone photoreceptor retinomotor movements (9). In addition, we have demonstrated that melatonin decreases cAMP and increases cGMP accumulation in the hamster retina (5, 10). These functions of melatonin are believed to be mediated directly through activation of specific melatonin receptors (11, 12).

The vertebrate retina can be parceled into two kinds of signal processing pathways: 1) a vertical pathway, composed of photoreceptor → bipolar cells→ ganglion cell chains; 2) a lateral pathway, composed of a variety of horizontal cell/amacrine cell networks. Glutamate is the main retinal excitatory transmitter (13–15). In the outer retina, glutamate is released from photoreceptors; in the inner plexiform layer (IPL), it is released from ON- and OFF-bipolar cells (14, 16–17), and it is also the neurotransmitter released by ganglion cells (18). Besides its physiological role, it has been clearly demonstrated that pathological levels of glutamate may be toxic to retinal cells. Retinal tissue is in fact, an established paradigm for glutamate neurotoxicity for several reasons: 1) insult leads to accumulation of relatively high levels of glutamate in the extracellular fluid (19); 2) administration of glutamate leads to neuronal cell death (20); and 3) glutamate receptor antagonists can protect against neuronal degeneration (21). Consequently, an appropriate clearance of synaptic glutamate is required for the normal function of retinal excitatory synapses and for prevention of neurotoxicity. Glial cells, mainly astrocytes and Müller glia, surround glutamatergic synapses, and express glutamate transporters and the glutamate-metabolizing enzyme, glutamine synthetase (GS) (22, 23). Glutamate is transported into glial cells and amidated by GS to the nontoxic amino acid glutamine. Glutamine is then released by the glial cells and taken up by neurons, where it is hydrolyzed by glutaminase to form glutamate again, completing the retinal glutamate/glutamine cycle (24, 25). In this way, the neurotransmitter pool is replenished and glutamate neurotoxicity is prevented. Nitric oxide synthase (NOS) inhibition provides partial but significant protection against the lethal effects of glutamate on retinal cells, suggesting the involvement of nitric oxide (NO) in glutamate-induced retinal neurotoxicity (26). In previous reports, we showed that melatonin modulates retinal glutamatergic neurotransmission (27), and that it is a potent inhibitor of the retinal nitridergic pathway in the golden hamster (28). Despite the highly significant role of glutamate in retinal physiology and pathology, the biochemical mechanisms that regulate the transmitter clearance and recycling are far from being completely understood. Because it has been demonstrated that melatonin exerts a neuroprotective effect against glutamate-induced excitotoxicity in several tissues, including chick retinal neurons (29, 30), the aim of the present report was to study the effect of melatonin on the glutamate/glutamine cycle activity in the hamster retina.

MATERIALS AND METHODS

Reagents and drugs

L-[^{3}H]-glutamine was obtained from New England Nuclear Corp. (Boston, MA, USA). Melatonin, 2-aminobicyclo (2, 2, 1) heptane 2-carboxylic acid (BCH), α-(methylamino)-isobutyric acid (MeAIB), L-serine, L-cysteine, L-histidine, L-asparagine, and L-arginine were

obtained from Sigma Chemical Co. (St. Louis, MO, USA), and Dowex resin was purchased from Bio-Rad Laboratories (Richmond, CA, USA)

Animals and tissues

Male golden hamsters (average weight 120 ± 20 g), derived from a stock supplied by Charles River Breeding Laboratories (Wilmington, MA, USA), were purchased from a local dealer. The animals were kept under a photoperiod of 14 h of light and 10 h of darkness (lights on at 0600 h), with free access to food and water. Since the maximal response to melatonin in the hamster retina occurs at 2400 h (5, 6, 12), animals were killed at midnight by decapitation. Eyeballs were quickly enucleated after death and corneas were removed; the lens and vitreous were dissected under a surgical microscope and the retinas were detached by blunt dissection. Retinas were examined to eliminate possible choroidal tissues. Immediately after dissecting, retinas were incubated or processed as described below for each protocol. Killing of animals, extraction of retinas, and in vitro incubations were carried out under a 10-W red light.

Glutamine synthetase assessment

Retinas were incubated in 500 μl of buffer HEPES-Tris, containing 140 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, (adjusted to pH 7.4 with Tris base) for 30 min at 37°C, with or without melatonin. At the end of incubation, each retina was homogenized in 200 μl of 10 mM potassium phosphate, pH 7.2. GS activity was assessed as described (31). Reaction mixtures contained 150 μl of retinal homogenates and 150 μl of a stock solution (100 mM imidazole-HCl buffer, 40 mM $MgCl_2$, 50 mM β-mercaptoethanol, 20 mM ATP, 100 mM glutamate and 200 mM hydroxylamine, adjusted to pH 7.2). Tubes were incubated at 37°C for 15 min. The reaction was stopped by adding 0.6 ml of ferric chloride reagent (0.37 M $FeCl_3$, 0.67 M HCl, and 0.20 M trichloroacetic acid). Samples were placed for 5 min on ice. Precipitated proteins were removed by centrifugation, and the absorbance of the supernatants was read at 535 nm against a reagent blank. Under these conditions, 1 μmol of γ-glutamylhydroxamic acids gives an absorbance of 0.340. GS specific activity was expressed as μmoles of γ-glutamylhydroxamate per hour per milligram of protein.

L-[^{3}H]-glutamine uptake assessment

L-glutamine uptake was examined in a crude synaptosomal fraction of hamster retinas. Retinas were homogenized (1:9 w/v) in 0.32 M sucrose containing 1 mM $MgCl_2$, and centrifuged at 900 × *g* for 10 min at 4°C. Nuclei-free homogenates were further centrifuged at 30,000 × *g* for 20 min. The pellet was immediately resuspended in buffer HEPES-Tris and aliquots of 100–300 μg protein/100 μl were used to assess glutamine uptake. The effect of the absence of sodium was examined by the iso-osmotic substitution of sodium chloride by choline chloride. The effect of amino acids (L-serine, L-cysteine, L-arginine, L-histidine, L-asparagine, BCH, and MeAIB) was examined by adding them together with the radioligand. For experiments in which the effect of melatonin was examined, aliquots of synaptosomal fractions were preincubated for 15 min at 37°C in the absence or presence of melatonin and with or without BCH and/or MeAIB, before the addition of L-[^{3}H]-glutamine. At the end of the preincubation period, 100 μl of L-[^{3}H]-glutamine (500,000–800,000 dpm/tube, specific activity 51 Ci/mmol) were added. After 4 min, L-[^{3}H]-glutamine uptake was terminated by adding 4 ml of ice-cold HEPES-Tris buffer. The

mixture was immediately poured onto Whatman GF/B filters under vacuum. The filters were washed twice with 4-ml aliquots of ice-cold buffer, and the radioactivity on the filters was counted in a liquid scintillation counter. Nonspecific uptake of L-[^{3}H]-glutamine into synaptosomes was assessed by adding an excess of L-glutamine (10 mM).

L-[^{3}H]-glutamine release

For glutamine release studies, retinal synaptosomal fractions were incubated for 30 min at 37°C with [^{3}H]-glutamine (500,000-800,000 dpm/tube) in 500 µl of HEPES-Tris buffer. According to Deitmer et al. (32), this preloading period is a compromise between equilibration of intracellular glutamine pools and avoiding metabolism of glutamine inside the tissue. The synaptosomal fractions were washed several times in fresh buffer in order to remove the excess of [^{3}H]-glutamine, and incubated for 20 min with gentle shaking in the presence or absence of melatonin (0.1 nM–10 nM), with or without 10 mM BCH, 20 mM MeAIB or a high K^+ (50 mM) buffer in which osmolarity was conserved by equimolar reduction of Na^+ concentration. The tissues were digested with hyamine hydroxide and radioactivity in the medium and that incorporated into the tissue were determined in a scintillation counter. Fractional release was calculated as the ratio of radioactivity released/total radioactivity taken up by the tissue. Greater than 80% of the released radioactivity was identified as authentic glutamine by paper chromatography.

Glutaminase activity assessment

Retinas were incubated in 500 µl of buffer HEPES-Tris for 30 min at 37°C, with or without melatonin. Glutaminase activity was assessed as described (33). Each retina was homogenized in 40 µl of 0.1% Triton X-100 in 7.5 mM Tris HCl, pH 8.8. The assay mixture contained 20 µl of retinal homogenate (200–400 µg of proteins), 50 mM glutamine, 0.2–0.5 µCi L-[^{3}H]-glutamine, and 63 mM potassium phosphate, pH 8.2, in a total volume of 100 µl. Tubes were incubated for 1 h at 30°C, with gentle agitation. The reaction was stopped by adding 1 ml of cold 20 mM imidazole, pH 7.0. Samples were briefly centrifuged, and the supernatants were applied to 0.6 × 3.5 cm beds of anion exchange resin (Dowex, AG1-X2, 200–400 mesh hydroxide form, Bio-Rad Laboratories) previously charged with 1M HCl and washed with water. The reaction substrate was removed with 6 ml of imidazole buffer, which was discarded and the reaction product was eluted with 3 ml of 0.1 M HCl. Aliquots of this fraction were mixed with a scintillation cocktail to measure radioactivity. Blanks were determined from samples lacking retinal homogenates. Glutaminase-specific activity was expressed as µmoles of glutamate per milligram protein per hour.

Protein content was determined by the method of Lowry et al. (34), using bovine serum albumin as the standard. Statistical analysis of results was made by a Student's *t* test or by a two-way ANOVA followed by a Dunnett's or Tukey's test, as stated. All animal use procedures were in strict accordance with the NIH Guide for Care and Use of Laboratory Animals.

RESULTS

Figure 1 depicts the effect of melatonin on GS activity in the hamster retina. Melatonin (0.1, 1, and 10 nM) significantly increased the conversion of glutamate to glutamine. The effect of melatonin on glutamine efflux was assessed as shown in Fig. 2. In control conditions, ~75% of

the preloaded radioactivity was released from retinal synaptosomal fractions during the efflux period. In the presence of 50 mM K^+ or 10 mM BCH, a significant increase of L-[^{3}H]-glutamine efflux was observed, whereas 20 mM MeAIB was ineffective. Melatonin alone or in the presence of high K^+, BCH, or MeAIB did not modify glutamine efflux (Fig. 2).

The characterization of L-glutamine uptake in the golden hamster retina is summarized in Fig. 3. The influx of L-glutamine was partly Na^+-dependent since it was diminished by the iso-osmotic substitution of Na^+ with choline. Both BCH and MeAIB significantly inhibited L-glutamine uptake, and when added together, a strong inhibition (≅85% inhibition) of glutamine influx was observed. In addition, the Na^+-independent glutamine uptake (assessed in the presence of choline instead of Na^+) was almost completely inhibited by BCH (≅95% inhibition). L-histidine and L-asparagine weakly (but significantly) inhibited L-glutamine influx, whereas L-serine, L-cysteine, and L-arginine were ineffective. As shown in Fig. 4, nanomolar concentrations of melatonin significantly increased total L-[^{3}H]-glutamine uptake in retinal crude synaptosomal fractions. Figure 5 shows the effect of 10 nM melatonin on glutamine influx in the presence of BCH and/or MeAIB. Melatonin significantly increased both the BCH- and the MeaIB-insensitive L-glutamine transport. When glutamine influx was assessed in the presence of BCH plus MeAIB, no effect of melatonin was observed. The effect of melatonin on retinal glutaminase activity is shown in Fig. 6. The conversion of glutamine to glutamate was significantly decreased by melatonin at all of the concentrations examined.

DISCUSSION

The foregoing results indicate that nanomolar concentrations of melatonin significantly modulate the glutamate/glutamine cycle activity in the golden hamster retina. Glutamate is the primary excitatory neurotransmitter in the retina, but it is neurotoxic when present in excessive amounts. Thus, tight control of glutamate concentrations in the synaptic cleft is crucial for the prevention of neurotoxicity. Since no enzymes exist extracellularly that degrade glutamate, glutamate transporters are responsible for maintaining low extracellular glutamate concentrations. The synaptically released glutamate is taken up into glial cells, where it is converted into glutamine by GS activity. Thus, the regulated coordination between glutamate release, its uptake, and its degradation (e.g., an effective glutamate/glutamine cycle), is critical in regulating the balance between physiological signaling and pathological overactivation. In fact, although glutamate uptake is controlled by the expression and post-translational modification of specific transporters (35), physiological measurements suggest that glutamate uptake may also depend on its metabolism. In this sense, it was shown that an increase in internal glutamate concentrations significantly slows down the net transport of glutamate (36), and it was suggested that instantaneous intracellular glutamate metabolism may be needed for efficient glutamate clearance of the extracellular milieu. In a previous report, we showed that nanomolar concentrations of melatonin significantly increase retinal glutamate release and uptake in hamster retinas excised at midnight (27). Present results indicate that by increasing the activity of GS, melatonin also increased the metabolism of this neurotransmitter. Because the assay of GS activity involves the incubation of retinal homogenates in the presence of controlled concentrations of L-glutamate, one can assume that the higher GS activity observed in the presence of melatonin is not merely a consequence of an increase in L-glutamate uptake. Although it was demonstrated a common transcriptional regulation of the most abundant retinal glutamate transporter (GLAST1) and GS in cultured retinal Müller cells (37), the fact that the

effect of melatonin on both parameters was evident after a short period of incubation (e.g., 30 min), makes highly unlikely the involvement of transcriptional and/or translational mechanisms. Thus, it seems possible that not only the expression but also the activity of the proteins mediating retinal uptake and degradation of synaptically released glutamate may be coordinately regulated. In agreement is a recent study that demonstrated that GS activity influences the retinal clearance of extracellular glutamate and that the inhibition of GS causes a decline in glutamate uptake (38).

Five amino acid uptake systems named A, ASC, N, L and y^+ are the major carriers for glutamine uptake in mammalian cells (39–41). The L- and y^+- type systems are Na^+-independent, whereas the A, N, and ASC are Na^+-dependent transport systems. Only the L-system is specifically inhibited by BCH (41), and the A-like transporters are distinguished by their ability to transport N-methylated substrates such as MeAIB (41). BCH and MeAIB significantly inhibited hamster retinal L-[^{3}H]-glutamine influx, indicating the presence of the L- and the A - type systems. Furthermore, since BCH and MeAIB added together strongly inhibited L-glutamine influx (≅85%), the L and A systems seem to be the primary uptake routes for L-glutamine in this tissue. In fact, the Na^+-independent uptake of this amino acid was almost totally abolished by BCH (≅95%). Since L-arginine did not modify the uptake of L-glutamine, it is unlikely that the y^+ system is involved in the hamster retinal glutamine influx. Moreover, while BCH did not impair retinal L-arginine influx (42), it significantly reduced L-glutamine uptake. Other transporters may coexist in this tissue, but their contribution must be less than 15% of the total transport activity. L-histidine and L-asparagine slightly (but significantly) decreased the influx of L-glutamine, suggesting that system N (43) may also be active in the hamster retina. Since there is no specific compound available for further discrimination between systems N and ASC, we did not pursue further this characterization. In contrast to these results, the N-system was found to be the predominant one in the mouse retina (43). There is no ready explanation for this discrepancy, although it appears that species differences may account for it.

Present results suggest that melatonin increased the activity of both L- and A-transport systems. The A-system is restricted to neurons and is absent in astrocytes (44), whereas system L has been localized in astrocytes and cultured neurons (45, 46). Assuming a similar distribution in the hamster retina, it seems likely that melatonin could increase both the glial and the neuronal influx of L-glutamine.

L-glutamine efflux involves specific carriers mostly belonging to the N, ASC, and L transport systems (32, 46–48). Since the N-system is highly dependent on the electrical potential (47), the increase in glutamine efflux induced by high K^+ concentration, could suggest the involvement of this transporter in retinal glutamine release. (48). The fact that melatonin did not affect L-glutamine efflux in basal conditions or in the presence of high K^+ supports that if N and ASC systems operate in the retina, their activity is not influenced by melatonin. Although the L-system may also participate in retinal glutamine release, melatonin did not affect the efflux-induced by BCH. The asymmetrical recognition of substrate by the L- system may influence the preferred direction of transport (49), as well as the differential effect of melatonin on glutamine uptake and its efflux mediated by this system.

As shown here, melatonin not only increased the synthesis of glutamine catalyzed by GS but also decreased its conversion to glutamate by inhibiting glutaminase activity. In this way, melatonin

may contribute to the conversion of glutamate to glutamine through a possibly redundant mechanism.

Although there remain to be established the intracellular events triggered by melatonin that could explain the effects described here, the increase in GS activity as well as in glutamine uptake, taken together with the decrease in glutaminase activity induced by melatonin, may contribute to controlling glutamate synaptic concentrations.

Aside from its role as a precursor and degradation product of glutamate, glutamine is also a precursor for nucleotides, GABA, glucose, glutathione, protein synthesis, an oxidative energy source, and it is also the major interorgan ammonia shuttle. Thus, the effects of melatonin described herein may also have other metabolic consequences. Experiments are now planned to address this question.

The full range of physiological actions of melatonin is not completely known. Melatonin is known to possess widespread free radical scavenging and antioxidant activities (50, 51). In the retina, melatonin protects retinal cells from lipid peroxidation (52), and it prevents retinal oxidative damage from ischemia-reperfusion injury (53). Although physiological levels of the methoxyindole have been shown to be beneficial in preventing toxicity in some systems (54), most studies have used pharmacological concentrations of melatonin to protect against free radical damage. The effective melatonin concentrations for induction of the effects described here are compatible with the affinity of melatonin receptors ($K_d \cong 0.1$ nM) (12), as well as with melatonin levels in the hamster retina (5), suggesting that physiological concentrations of melatonin modulate retinal glutamate/glutamine cycle activity through receptor-mediated mechanisms. We have previously demonstrated that 2-[^{125}I]-melatonin specific binding varies significantly throughout the 24 h period, with maximal density at 2400 h (12). Thus, the high sensitivity of the effects of melatonin described here could also be related to the fact that these effects were assessed in animals killed at midnight.

Besides its antioxidant potential, several other mechanisms are considered to be involved in the neuroprotection mediated by melatonin, like its interaction with calmodulin (55) and microtubular components (56), the blockade of increases in intracellular Ca^{2+} levels (57), inhibition of activation of NF-κB by cytokines (58), and inhibition of the nitridergic pathway (28), among others. The physiological consequences of a modulation by melatonin of the retinal glutamate/glutamine cycle are yet to be determined, although this effect could provide new insight into the neuroprotective potential of melatonin. In that respect, it was demonstrated that an increase in GS provides neuroprotection in experimental models of neurodegeneration (59). Induction of GS in vivo or in vitro by glucocorticoids was clearly demonstrated in different tissues, including the retina (60, 61). Physiological levels of glucocorticoids regulate GS expression by stimulating transcription of the gene. This effect of glucocorticoids has been associated to their ability to protect against neuronal degeneration (59) as shown in animal models brain damage (62), as well as after retinal photic injury (63). However, since induction of GS expression by glucocorticoids takes ~24 h, there are some potential weaknesses in glucocorticoid treatment. This might explain why glucocorticoids must be supplied early to achieve a beneficial effect and suggests that only in programmed neurological interventions, prophylactic administration of glucocorticoids might be advisable. In contrast, since the effect of melatonin was much faster, a treatment with the methoxyindole may circumvent this obstacle.

Furthermore, this beneficial effect of melatonin may be further improved by its effect on glutamate uptake, on glutaminase activity, and on the nitridergic pathway (28). In summary, these findings suggest that a treatment with melatonin could be considered as a new approach to handling glutamate-mediated neuronal degeneration.

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Fig. 1

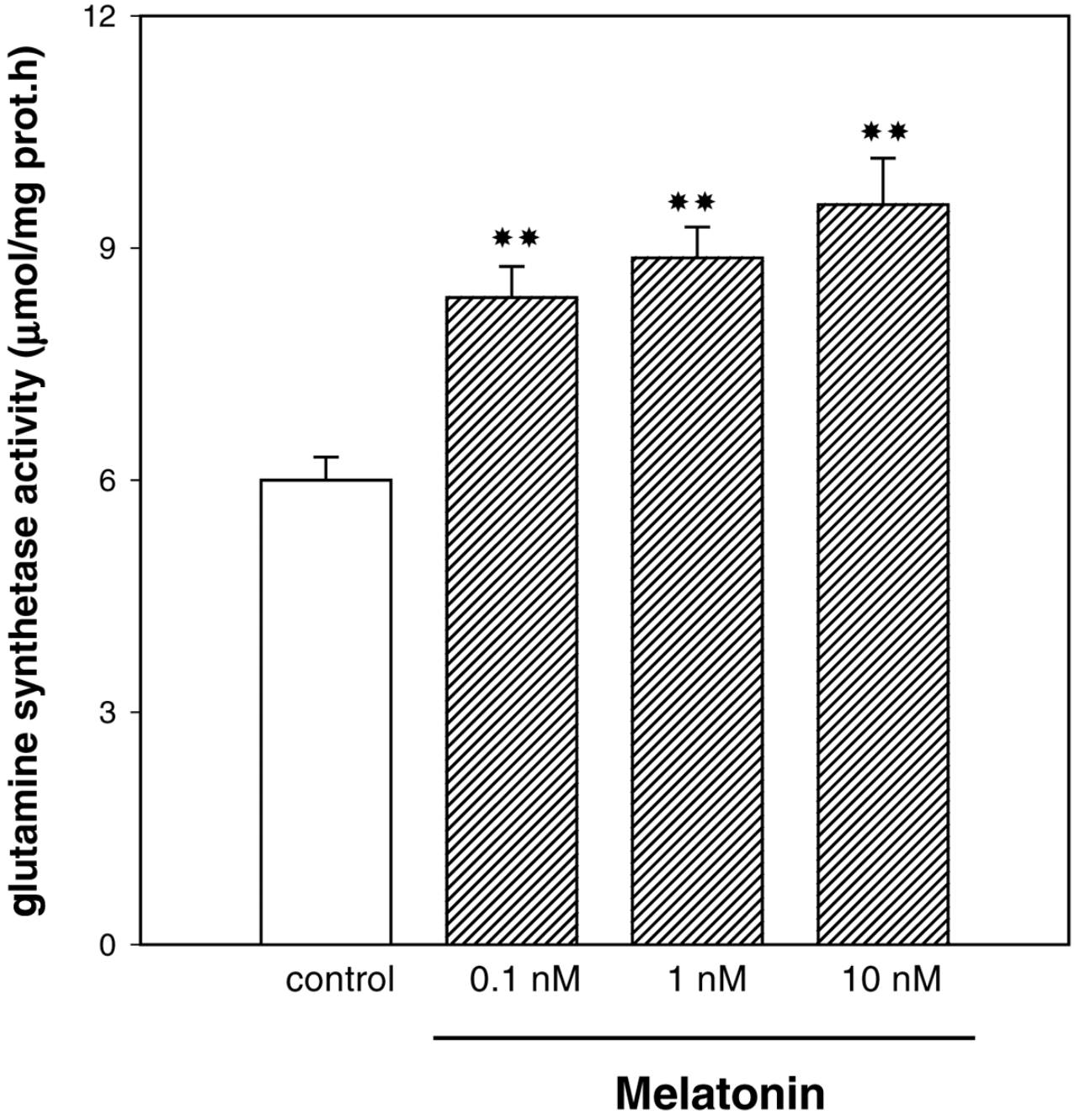

Figure 1. Effect of melatonin on retinal GS activity. Retinas were preincubated for 30 min in the presence or absence of melatonin (0.1–10 nM). Then, tissues were homogenized and GS activity was assessed as described in Materials and Methods. Melatonin significantly increased retinal GS activity at any concentration tested. Data are mean ± SEM (n = 8–10 animals per group). $^{**}P < 0.01$, by Dunnett's test.

Fig. 2

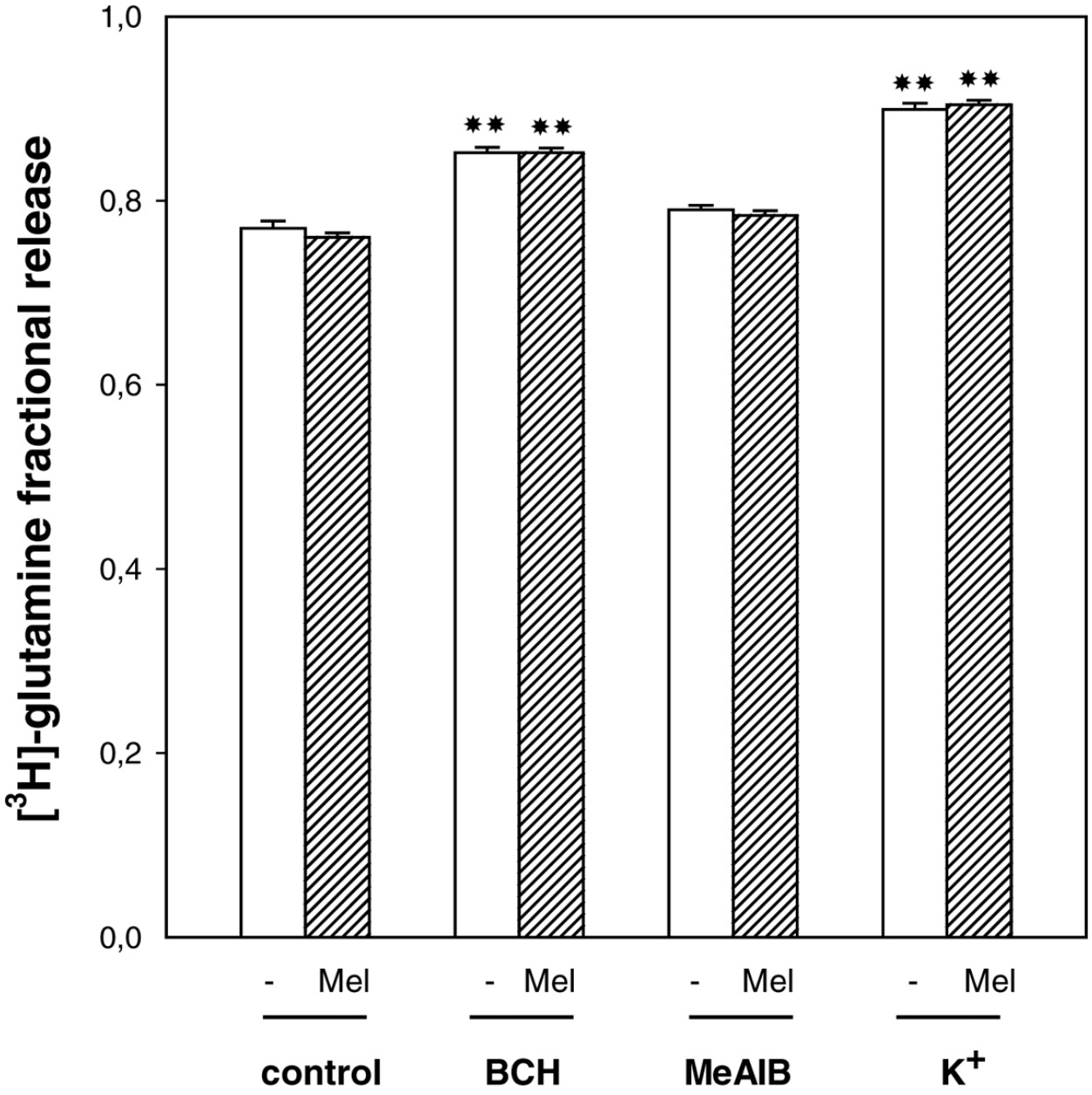

Figure 2. L-[^{3}H]-glutamine release in the golden hamster retina. The efflux of L-glutamine was assessed as described in Materials and Methods. High K^+ (50 mM), and 10 mM BCH but not 20 mM MeAIB induced a significant increase in [^{3}H]-glutamine release. Melatonin alone or in the presence of high K^+, BCH, or MeAIB did not modify glutamine efflux. Shown are means ± SEM (n=12 animals/group), $^{**}P < 0.01$, by Dunnett's test).

Fig. 3

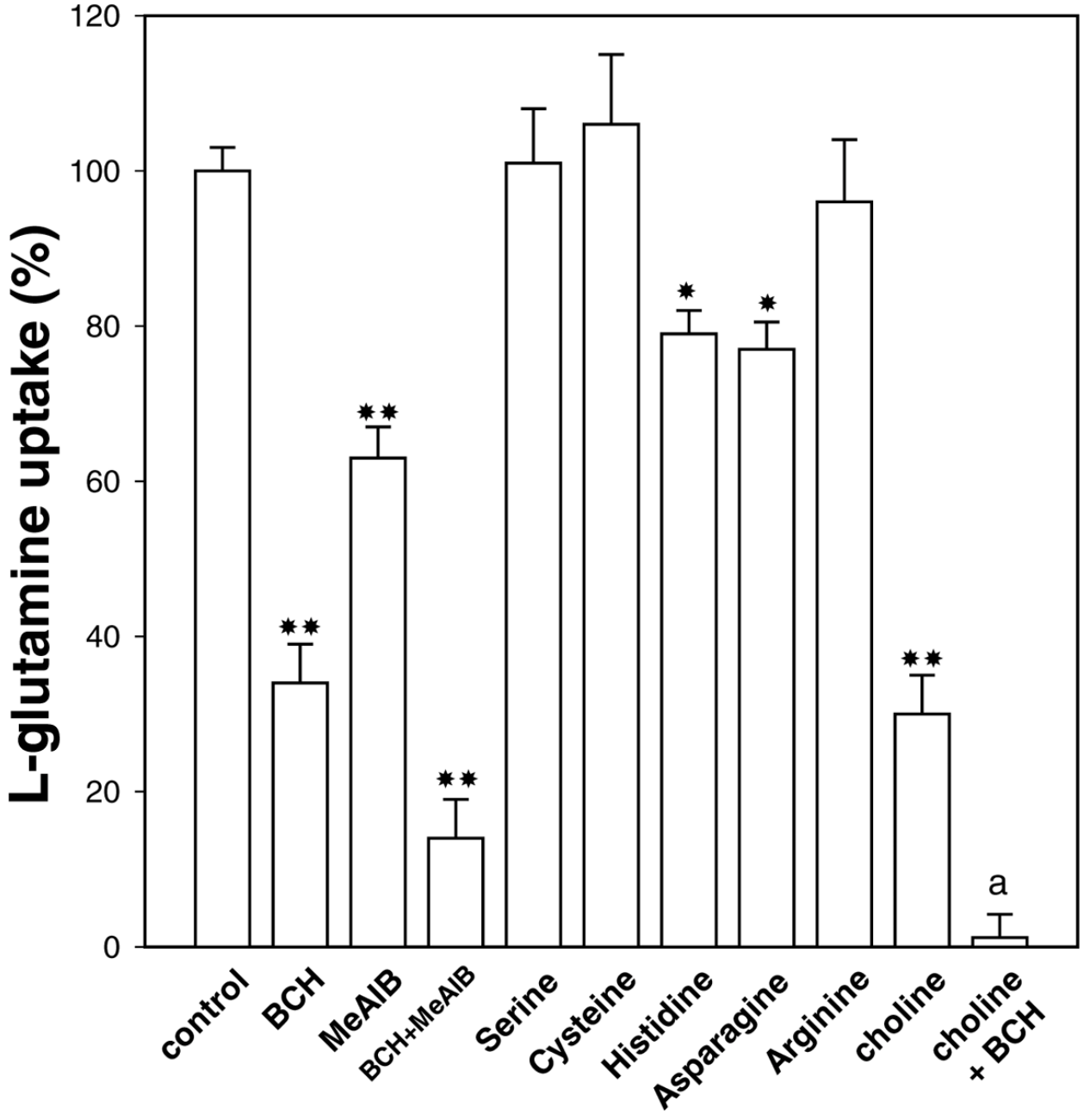

Figure 3. Characterization of L-[^{3}H]-glutamine uptake in the hamster retina. The retinal synaptosomal fraction was incubated with 10 μM L-[^{3}H]-glutamine in the presence of various amino acids (10 mM), BCH (10 mM), or MeAIB (20 mM). The effect of the absence of sodium was examined by the iso-osmotic substitution of sodium chloride by choline chloride. Histidine and Asparagine, BCH, MeAIB, BCH plus MeAIB, and choline significantly decreased the influx of L-glutamine, whereas serine, cysteine, and arginine showed no effect. The 100% of L-[^{3}H]-glutamine uptake was 5.5 ± 0.1 pmol/mg prot.min. Data are the mean ± SEM of four independent experiments, performed in triplicates. *$P <$ 0.05, **$P < 0.01$ by Dunnett's test; $^a P < 0.01$ vs. choline.

Fig. 4

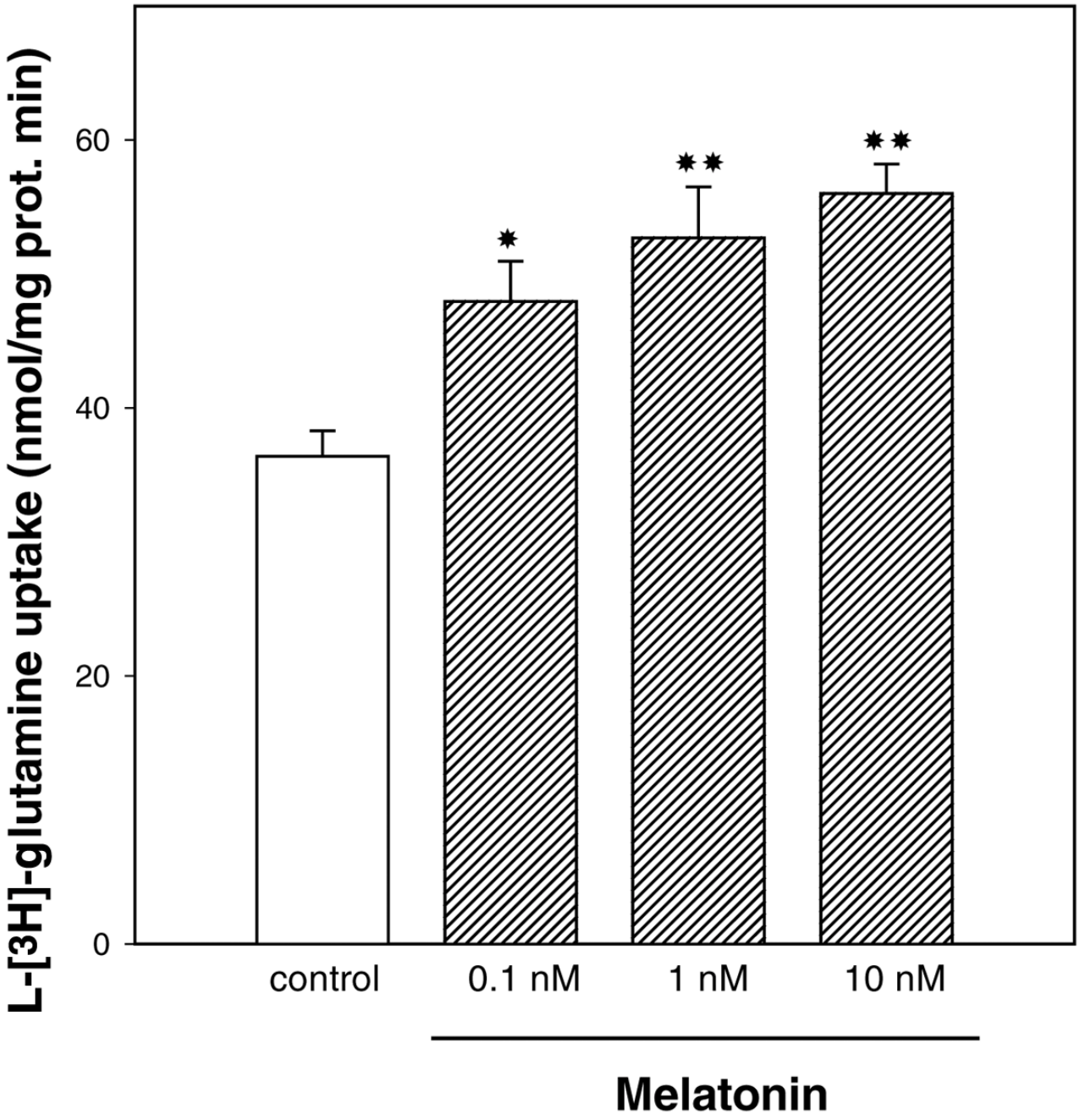

Figure 4. Effect of melatonin on retinal L-glutamine uptake. Retinas were preincubated for 15 min in the presence of melatonin (0.1 nM–10 nM). Then, the tissues were homogenized, and glutamine influx was assessed as described in Materials and Methods. Melatonin significantly increased retinal glutamine influx at any concentration tested. Data are mean ± SEM (n = 8–10 animals per group). *$P < 0.05$, **$P < 0.01$ by Dunnett's test.

Fig. 5

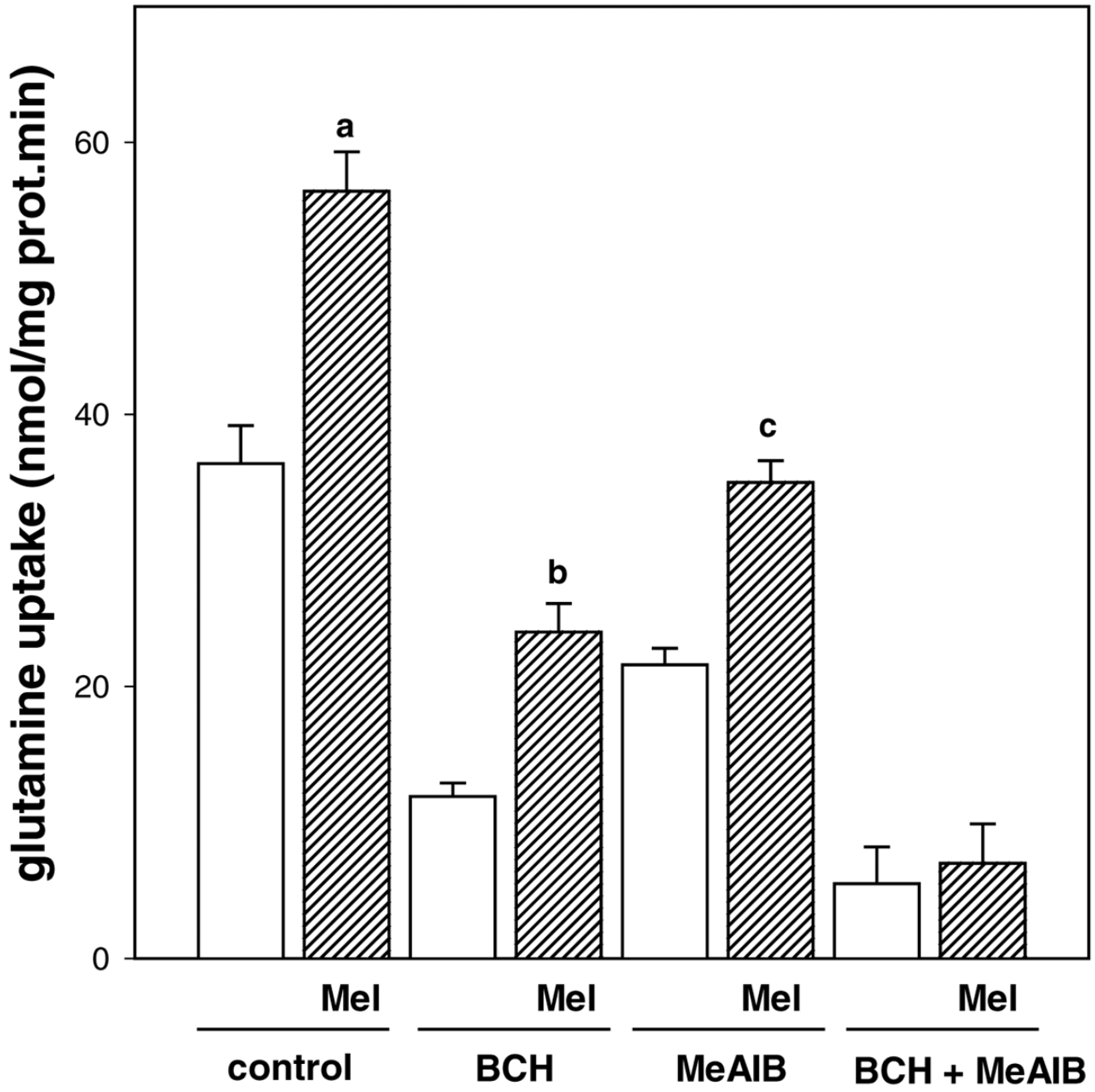

Figure 5. Effect of melatonin on hamster retinal glutamine transporters. Melatonin (10 nM) significantly increased both the BCH- and MeAIB-insensitive influx of L-glutamine. When synaptosomal fractions were incubated in the presence of BCH plus MeAIB, melatonin showed no effect. Data are mean ± SEM (n = 8–10 animals per group). a: $P <$ 0.01 vs. control; b: $P < 0.05$ vs. BCH; c: $P < 0.01$ vs. MeAIB, by Tukey's test.

Fig. 6

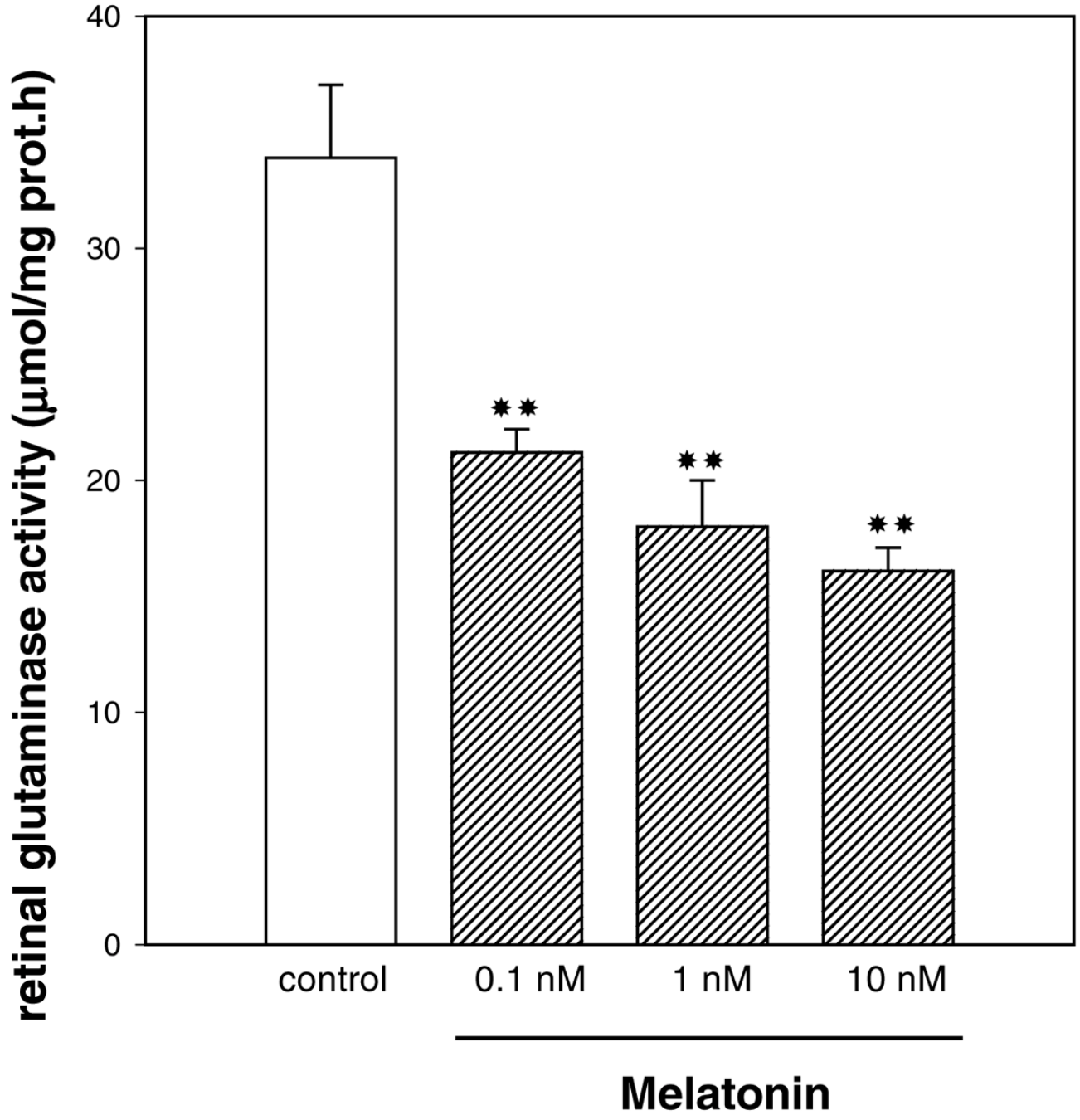

Figure 6. Effect of melatonin on glutaminase activity in golden hamster retina. The retinas were excised and incubated as described in Materials and Methods, with (0.1 nM–10 nM) or without melatonin. Melatonin significantly decreased this enzymatic activity at any concentration tested. Data are mean ± SEM values (*n*: 10 animals per group), $^{**}P < 0.01$ by Dunnett's test.