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The histamine H_3 receptor antagonist clobenpropit enhances GABA release to protect against NMDA-induced excitotoxicity through the cAMP/protein kinase A pathway in cultured cortical neurons

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Abstract

Using the histamine H_3 receptor antagonist clobenpropit, the roles of histamine H_3 receptors in NMDA-induced necrosis were investigated in rat cultured cortical neurons. Clobenpropit reversed the neurotoxicity in a concentration-dependent manner, and showed peak protection at a concentration of 10^{-7} M. This protection was antagonized by the histamine H_3 receptor agonist (R)-α-methylhistamine, but not by the histamine H_1 receptor antagonist pyrilamine or the histamine H_2 receptor antagonist cimetidine. In addition, the protection by clobenpropit was inhibited by the $GABA_A$ receptor antagonists picrotoxin and bicuculline. Further study demonstrated that the protection by clobenpropit was due to increased GABA release. The inducible GABA release was also inhibited by (R)-α-methylhistamine, but not by pyrilamine or cimetidine. Furthermore, both the adenylyl cyclase inhibitor SQ-22536 and the protein kinase A (PKA) inhibitor H-89 reversed the protection and the GABA release by clobenpropit. In addition, clobenpropit reversed the NMDA-induced increase in intracellular calcium level, which was antagonized by (R)-α-methylhistamine. These results indicate that clobenpropit enhanced GABA release to protect against NMDA-induced excitotoxicity, which was induced through the cAMP/PKA pathway, and reduction of intracellular calcium level may also be involved.

Keywords: Histamine H_3 receptor; Clobenpropit; NMDA; Excitotoxicity; GABA; Intracellular calcium level

1. Introduction

Histamine is regarded as a neurotransmitter and neuromodulator within the central nervous system (CNS) (Haas and Panula, 2003; Wada et al., 1991). Cell bodies of histaminergic neurons are exclusively located in the tuberomamillary nuclei of the hypothalamus (Panula et al., 1984; Watanabe et al., 1984; Wouterlood et al., 1986) and give rise to widespread projections extending through the basal forebrain to the whole CNS (Inagaki et al., 1988; Panula et al., 1989; Watanabe et al., 1984). This morphology implicates histamine in the regulation of numerous physiological functions and behaviors such as thermoregulation, circadian rhythms, neuroendocrine and cardiovascular functions, catalepsy, locomotion, drinking and feeding, as well as learning and memory (Haas and Panula, 2003).

Evidence shows that histamine is involved in the responses to ischemia. For example, middle cerebral artery occlusion in rat induces a long-lasting increase in neuronal histamine release in the striatum (Adachi et al., 1992). Moreover, histamine depletion with α-fluoromethylhistidine, an inhibitor of histidine decarboxylase (the synthesizing enzyme for histamine), significantly increases the number of necrotic pyramidal cells in the hippocampal CA1 region in rats subjected to cerebral ischemia (Adachi et al., 1993). In contrast, post-ischemic loading with

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histidine, a precursor of histamine, decreases the amount of neuronal damage in the rat striatum (Adachi et al., 2004). In cultured cortical neurons, histamine protects against NMDA-induced necrosis *via* the histamine H_2 receptor (Dai et al., 2006).

The histamine H_3 receptor is a member of the large superfamily of G protein-coupled receptors that are characterized by the presence of seven putative transmembrane spanning domains. The relative expression of histamine H_3 receptors is very high in the CNS, where they participate in the modulation of arousal, learning and memory and food intake by their autoreceptors and heteroreceptors. So far, the histamine H_3 receptor has been postulated to be a good target for drug discovery for a variety of indications (Leurs et al., 2005; Witkin and Nelson, 2004). In addition, in various cell lines, histamine H_3 receptor activation leads to an inhibition of forskolin-stimulated cAMP formation (Lovenberg et al., 1999) and decreases extracellular calcium inflow (Blandizzi et al., 2001; Seyedi et al., 2005; Takeshita et al., 1998) but its physiological action *in situ* remains to be demonstrated.

The general aim of the present study was to explore whether clobenpropit, a representative histamine H_3 antagonist, could protect against NMDA-induced excitotoxicity, and to explore possible underlying mechanisms.

2. Materials and methods

2.1. Materials

Cell culture plates were obtained from Corning Inc. (NY, USA). NMDA, pyrilamine, diphenhydramine, cimetidine, (R)-α-methylhistamine, clobenpropit, 8-Bromo-cAMP (8-Br-cAMP), 9-(tetrahydro-2-furanyl)-9H-purine-6-amine (SQ-22536), *N*-[2-(*p*-bromocinnamylamino) ethyl]-5-isoquinolinesulfonamide (H-89), picrotoxin and bicuculline were purchased from Sigma (St. Louis, MO, USA). Zolantidine was from Tocris Cookson Ltd. (Bristol, UK). L-glutamine, B-27 supplement, penicillin, trypsin, streptomycin, poly-L-lysine, and Dulbecco's modified Eagle's medium (DMEM) were from GIBCO-BRL (Grand Island, NY, USA). Clobenpropit was kindly supplied by Prof. Timmerman (Amsterdam, The Netherlands).

2.2. Cell culture

All experiments were carried out in accordance with the National Institutes of Health guide for Care and Use of Laboratory Animal. Primary cultures of cerebrocortical neurons were obtained from rat pups (Sprague–Dawley) on the first postnatal day using previously described procedures (Dai et al., 2006). In brief, cortices were dissected from the brains under sterile conditions and were digested in 0.25% trypsin in Hank's balanced salt solution for 15 min at 37 °C, then mechanically dissociated. The cells were spun down for 5 min at 1000 ×*g* and resuspended in DMEM containing 100 IU/ml penicillin, 100 mg/ml streptomycin, 0.5 mM L-glutamine, 33 mM (final concentration) glucose, 10% horse serum and 10% foetal calf serum. Cells were seeded to a density of 1.5×10^5 cells/cm^2 in 96-well plates previously coated with poly-L-lysine (0.1 mg/ml).

After 24 h *in vitro*, the culture medium was replaced with Neurobasal medium (Life Technologies, Grand Island, NY, USA) supplemented with 100 IU/ml penicillin, 100 mg/ml streptomycin, 0.5 mM L-glutamine, 33 mM (final concentration) glucose and 2% B-27 supplement. The cultures were maintained in a humidified CO_2 incubator (5% CO_2, 95% air and 37 °C). Cytosine arabinoside (5 μM) was added 96 h after culture to inhibit the replication of non-neuronal cells. Cells were used for experiments after 12–14 days *in vitro*.

2.3. Drug exposure

As a general rule, drugs were dissolved in sterile purified water prior to dilution into Neurobasal medium. On days 12–14, *in vitro* cell cultures were exposed for 3 h to NMDA (100 μM). Glycine (10 μM) was present in all external solutions used to elicit NMDA responses. Clobenpropit and 8-Br-cAMP were added 15 min before NMDA. The histamine agonist or antagonists and GABA antagonists were added 10 min before clobenpropit.

2.4. Viability studies

Neurons were cultured on 96-well plates, with 3 wells in each group. The cells were incubated with 1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT, final concentration 0.5 mg/ml) for 3 h at 37 °C. Then, the supernatant layer was removed, and 100 μl of dimethyl sulfoxide was added to each well. MTT metabolism was quantified spectrophotometrically at 490 nm in a Biorad microplate reader. Results were expressed as the percentage MTT reduction, assuming the absorbance of control cells was 100%.

2.5. Neurochemical analysis of GABA

After stimulation, the supernatant layer was removed and cells were broken by the addition of 0.5 ml of distilled water. The extracellular accumulation was stored at − 80 °C until assayed, when samples were homogenized in 3% perchloric acid containing 5 mM disodium EDTA and 5-hydro-N^{ω}-methyltryptamine in a Polytron homogenizer (Kinematica, Lucern, Switzerland) at the maximum setting for 20 s in an ice bath. The homogenate was centrifuged at 15,000 ×*g* for 20 min at 4 °C, then the supernatant was removed and filtered through a 0.22 μm polyvinylidene difluoride membrane. GABA concentrations were determined by a HPLC-ECD system consisting of a solvent delivery module (Model 582, ESA, Chelmsford, MA, USA), a 3 mm reversed-phase column (3.0 mm × 50 mm, CAPCELL PAK C18 MG, Shiseido, Japan), and an HPLC autosampler (Model 542, ESA, Chelmsford, MA, USA); a 4-channel CoulArray electrochemical detector (E1 = 250 mV, E2 = 550 mV) was used for the analysis of GABA. The mobile phase (0.1 M Na_2HPO_4 in 22% methanol and 13% acetonitrile, pH 6.8 with H_3PO_4) was filtered through a 0.22 μm filter (Millipore, Bedford, MA, USA) and degassed before pumping at a flow rate of 0.75 ml/min. The samples were derivatized according to the method of Donzanti and Yamamoto (1988) with minor modifications. The derivatization stock

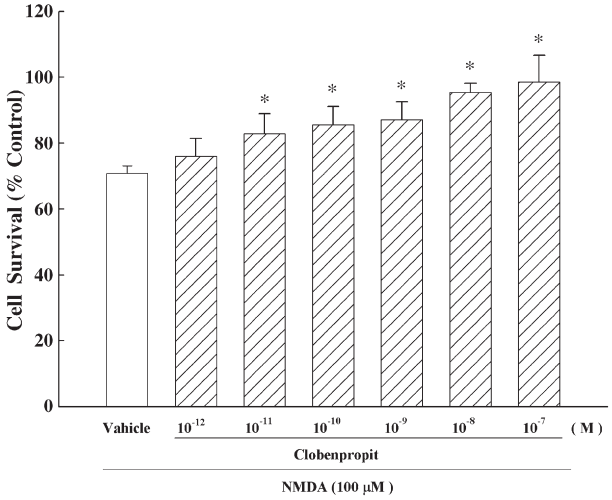

Fig. 1. Effects of clobenpropit on NMDA-induced neurotoxicity. Cortical neurons, after 12–14 days in primary culture, were exposed to vehicle or the indicated drugs. Clobenpropit was added 15 min before NMDA (100 μM). Neuronal viability was determined by MTT assay 3 h after application of NMDA. Values are expressed as percentage of control values and are from 4 to 6 independent experiments with 3 replicates for each condition. *$P<0.05$, compared with NMDA group (ANOVA followed by Student–Newman–Keuls test).

reagent consisted of 27 mg of *o*-phthalaldehyde (OPA, Pickering, Mountain View, CA, USA) dissolved in 1 ml of ethylhydroxide with 10 mg thioflour (Pickering, Mountain View, CA, USA) and 9 ml 0.1 M sodium tetraborate (pH 9.3). The working solution was prepared by diluting 1 ml OPA-thioflour stock solution with 4 ml 0.1 M sodium tetraborate, pH 9.3. Pre-column amino acid derivatization was performed by mixing 15 μl volumes of the standard GABA or sample and 20 μl OPA-thioflour working solution in the autosampler before injection onto the analytical column. Data were collected and analyzed by CoulArray for Windows software (ESA, Chelmsford, MA, USA).

2.6. Measurement of intracellular Ca^{2+}

Intracellular free Ca^{2+}-levels were assessed semiquantitatively according to Kao et al. (1989) by loading cells with 5 μM of the cell-permeant fluorescent calcium indicator Fluo-3/AM for 45 min at 37 °C, then rinsing cells once with phosphate-buffered saline and subjecting them to fresh medium before the experiment. The fluorescent signal was measured at indicated time points using a Fluoview FV1000 confocal microscope (Olympus, Tokyo, Japan). Concentrations of intracellular calcium were calculated from the fluorescence intensity averaged for 20–30 neurons per field. Data are presented as difference in percentage fluorescence relative to controls.

2.7. Statistics

The data are given as mean ± S.D. of four to six experiments. Drug-induced percentage inhibition of protection was calculated from the values for percentage MTT metabolism and determined by the formula:

$$\frac{(\text{protective drug}+\text{NMDA})-(\text{inhibitory drug}+\text{protective drug}+\text{NMDA})}{(\text{protective drug}+\text{NMDA})-(\text{NMDA alone})}\times 100\%^{*}$$

(*In this case, "protective drug" means clobenpropit and "inhibitory drug" means histamine receptor agonist or antagonist, or GABA antagonist.)

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Newman–Keuls test for multiple comparison tests (performed with SPSS software). ED_{50} values were calculated according to the graded concentration–response curve (performed with GraphPad Prism software). *P* values lower than 0.05 were considered to be statistically significant.

3. Results

The histamine H_3 receptor antagonist clobenpropit alone had no effect on cell survival at the concentrations used (data not shown); however, it reversed NMDA-induced neurototoxicity in a concentration-dependent manner (Fig. 1). At concentrations of 10^{-11}–10^{-7} M, clobenpropit significantly prevented NMDA-induced neurototoxicity in cortical neurons. The maximal effect was observed at 10^{-7} M (viability reversed to 98.6±8.1%).

Exposure of neurons to the histamine H_3 receptor agonist (R)-α-methylhistamine (10^{-8}–10^{-4} M) alone had no effect on cell survival (data not shown). Pretreatment with (R)-α-methylhistamine inhibited the protection induced by 10^{-7} M clobenpropit in a concentration-dependent manner, reaching significance at 10^{-6}, 10^{-5} and 10^{-4} M (Fig. 2). However, neither the histamine H_1 receptor antagonist pyrilamine nor the histamine H_2 receptor antagonist cimetidine blocked the

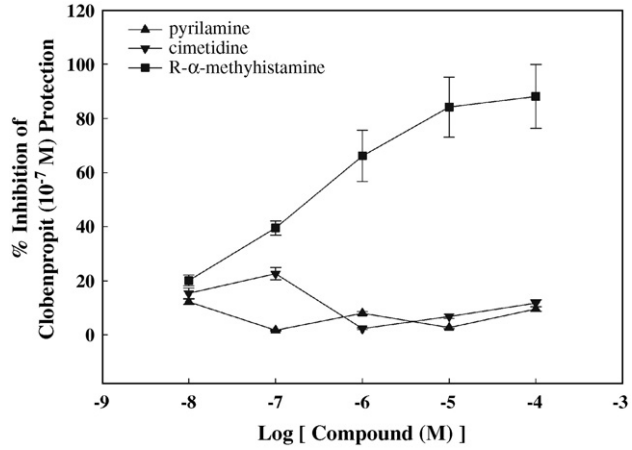

Fig. 2. Effects of histamine receptor ligands on the protection by clobenpropit. Cortical neurons, after 12–14 days in primary culture, were exposed to vehicle or the indicated drugs. Pyrilamine, cimetidine or (R)-α-methylhistamine was added 10 min before clobenpropit. Clobenpropit (10^{-7} M) was added 15 min before NMDA (100 μM). Neuronal viability was determined by MTT assay 3 h after application of NMDA. Values are expressed as percentage inhibition of the protection by clobenpropit and are from 4 to 6 independent experiments with 3 replicates for each condition.

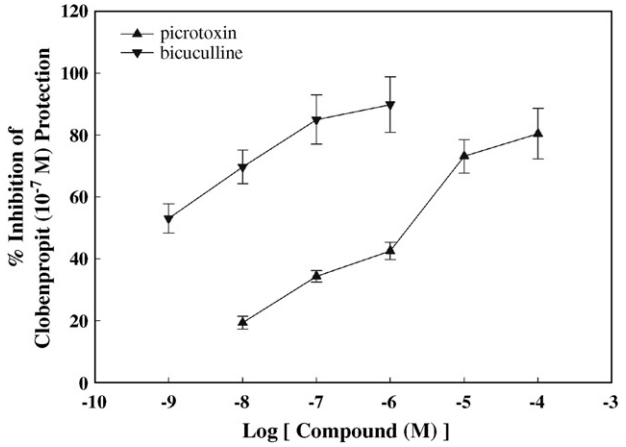

Fig. 3. Effects of $GABA_A$ receptor antagonists on the protection by clobenpropit. Cortical neurons, after 12–14 days in primary culture, were exposed to vehicle or the indicated drugs. Picrotoxin or bicuculline was added 10 min before clobenpropit. Clobenpropit (10^{-7} M) was added 15 min before NMDA (100 μM). Neuronal viability was determined by MTT assay 3 h after application of NMDA. Values are expressed as percentage inhibition of the protection by clobenpropit and are from 4 to 6 independent experiments with 3 replicates for each condition.

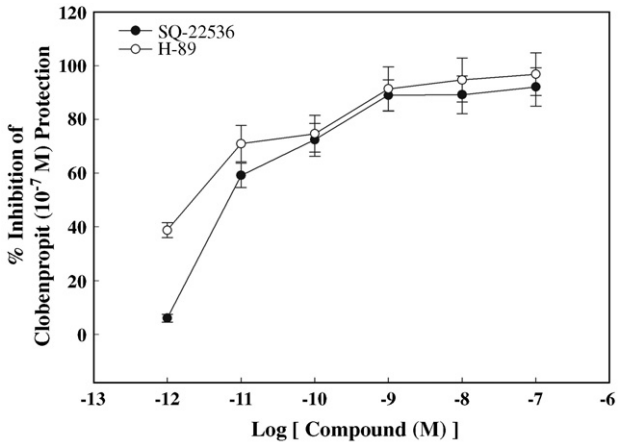

Fig. 5. Effects of the AC inhibitor SQ-22536 and the PKA inhibitor H-89 on the protection conferred by clobenpropit. Cortical neurons, after 12–14 days in primary culture, were exposed to vehicle or the indicated drugs. SQ-22536 or H-89 was added 10 min before clobenpropit. Clobenpropit (10^{-7} M) was added 15 min before NMDA (100 μM). Neuronal viability was determined by MTT assay 3 h after application of NMDA. Values are expressed as percentage inhibition of the protection by histamine and are from 4 to 6 independent experiments with 3 replicates for each condition.

clobenpropit-induced protection, even at a concentration of 10^{-4} M (Fig. 2).

Clobenpropit is also reported to have heteroreceptor action and enhances the release of other neurotransmitters, such as GABA. Bicuculline is a competitive antagonist at the $GABA_A$ receptor, while picrotoxin blocks $GABA_A$ responses by acting at a different, non-receptor site, such as the chloride channel. As shown in Fig. 3, pretreatment with picrotoxin or bicuculline resulted in a concentration-related reduction of the protection induced by 10^{-7} M clobenpropit, while these antagonists alone had no appreciable effects on cortical neurons at the concentrations used (data not shown).

In addition, in our culture system, a time-dependent increase in GABA release was observed after clobenpropit application (10^{-7} M; Fig. 4). After clobenpropit application for 5 min or longer, the concentrations of GABA in the buffer were markedly elevated. The maximum GABA release was about 268% at 150 min, compared with vehicle-treated samples which was only about 89.6±7.1 nmol/mg protein (Fig. 4). But pretreatment with (R)-α-methylhistamine (10^{-4} M) blocked the clobenpropit-induced GABA increase (Fig. 4).

In rat cortical primary cultures, KCl-induced GABA release is modulated by protein kinase A (PKA) (Schaffhauser et al., 1998). In addition, the histamine H_3 receptor is a G-protein-coupled receptor linked to the inhibition of adenylyl cyclase (Lovenberg et al., 1999). So we further tested whether blockade of clobenpropit-stimulated cAMP formation would affect the GABA release. SQ-22536 (10^{-7} M), a compound that inhibits adenylyl cyclase (AC), reversed the GABA release by clobenpropit (Fig. 4). Having established a role for cAMP, we tested whether PKA was also involved. H-89 (10^{-7} M), a PKA inhibitor, reversed the GABA release by clobenpropit (Fig. 4). While SQ-22536 and H-89 alone had no appreciable effects on GABA release in cortical neurons at the concentrations used (data not shown). Thus, PKA appeared to be necessary for this GABA release.

We also tested whether blockade of agonist-stimulated cAMP formation would affect the protection by 10^{-7} M clobenpropit. Both SQ-22536 and H-89 concentration-relatedly reversed this protective effect (Fig. 5). The AC and PKA inhibitor alone had no appreciable effect on cortical neurons at the concentrations used (data not shown). Thus, PKA appeared to be necessary for the protection by clobenpropit in NMDA-induced excitotoxicity.

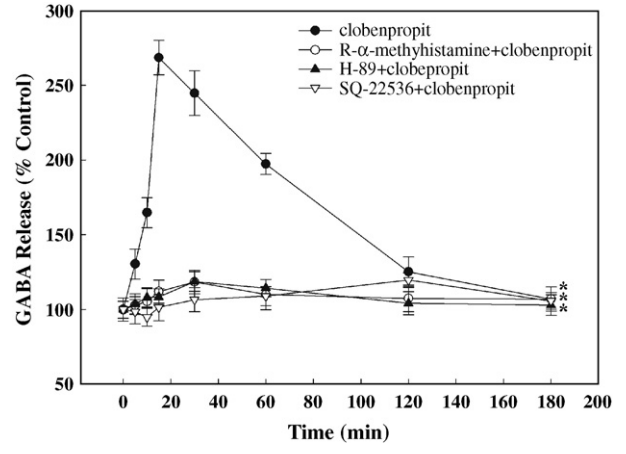

Fig. 4. Time course analysis of GABA release evoked by clobenpropit. Cortical neurons, after 12–14 days in primary culture, were exposed to vehicle or the indicated drugs. (R)-α-methylhistamine (10^{-4} M), SQ-22536 (10^{-7} M) or H-89 (10^{-7} M) was added 10 min before clobenpropit. Clobenpropit (10^{-7} M) was added 15 min before NMDA (100 μM). The amount of GABA was quantified by HPLC at the indicated times after exposure to clobenpropit. Values are from 4 to 6 independent experiments with 3 replicates for each condition. A two-way ANOVA showed a significant difference between groups (*$p<0.05$ compared with clobenpropit).

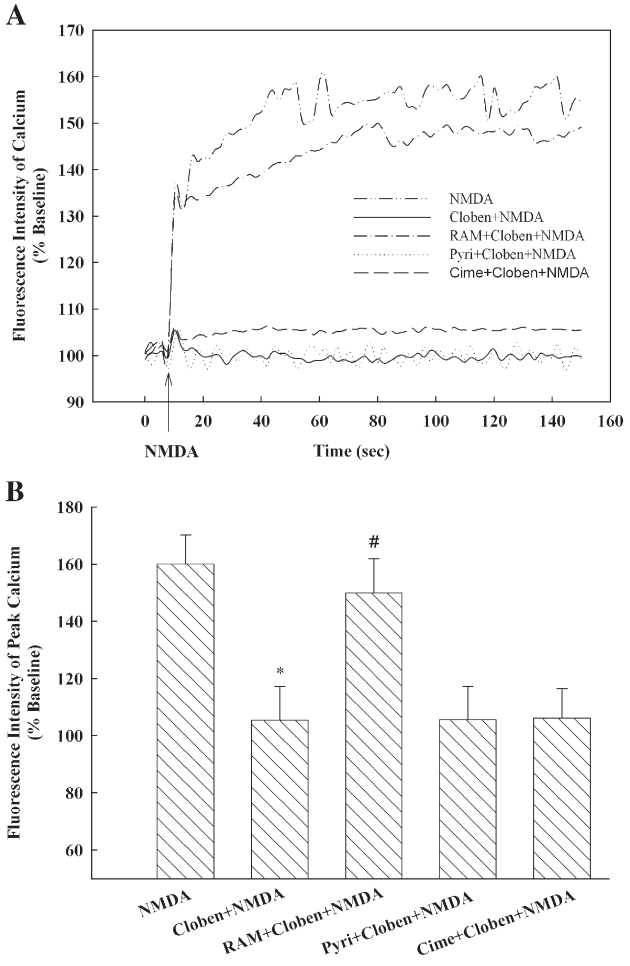

Fig. 6. Clobenpropit pre-treatment protects from NMDA-induced $[Ca^{2+}]_i$ increase. Neuronal cultures were loaded for 45 min with the intracellular calcium-indicator Fluo-3/AM ester (5 μM) and then examined under a laser confocal microscope. Primary cortical rat cultures loaded with Fluo-3/AM were pre-treated with 10^{-7} M clobenpropit or vehicle for 15 min and then exposed to 100 μM NMDA. Pyrilamine (pyri, 10^{-4} M), cimetidine (cime, 10^{-4} M) or (R)-α-methylhistamine (RAM,10^{-4} M) was added 10 min before clobenpropit. $[Ca^{2+}]_i$ levels measured for 140 sec after NMDA exposure are visualized in an exemplary experiment with calcium measurements every two seconds (A), and were significantly lower in clobenpropit pre-treated neurons (B). Concentrations of intracellular calcium were calculated from the fluorescence intensity averaged for 20–30 neurons per field. $^{*}P<0.05$, compared with NMDA group; $^{\#}P<0.05$, compared with clobenpropit+NMDA group (ANOVA followed by Student–Newman–Keuls test).

It is known that intracellular calcium overload is a prominent feature of excitotoxicity (Choi, 1985). The histamine H_3 receptor signaling pathway, which is different from those of histamine H_1 and histamine H_2 receptors, involves pertussis toxin-sensitive Gi/Go protein and a decrease in calcium influx through N-type calcium channels (Endou et al., 1994). To further investigate the potential underlying mechanism of clobenpropit's anti-excitotoxic properties, we measured intracellular calcium levels ($[Ca^{2+}]_i$) after exposure to 100 μM NMDA. Clobenpropit itself did not influence the intracellular calcium (data not shown). However, it significantly inhibited NMDA-induced elevation of $[Ca^{2+}]_i$ (Fig. 6). In addition, (R)-α-methylhistamine attenuated its effect, while neither cimetidine nor pyrilamine blocked the clobenpropit-induced reduction of $[Ca^{2+}]_i$ overload (Fig. 6). So, the neuroprotective effects of clobenpropit may correlate with a reduction of the increase in $[Ca^{2+}]_i$.

4. Discussion

Neuronal degeneration upon NMDA exposure has been reported to occur either by necrosis (Rothman, 1985) or apoptosis (Lesort et al., 1997). Our previous studies showed that within 3 h of intense NMDA insult, most neurons die by necrosis, with swollen nuclei stained by propidium iodide. Six hours later, however, the cells progressively and mainly display the morphological signs of apoptosis (Dai et al., 2006). In the present study, we found that clobenpropit induced a marked protection against the NMDA-induced necrosis in cultured cortical neurons, and showed an ED_{50} estimated at 3.5 nM. A similar potency estimate was obtained for thioperamide (ED_{50}=23 nM) (Dai et al., 2006). These data indicate that, like thioperamide, clobenpropit is a more potent and selective antagonist for histamine H_3 receptors *in vitro*.

The protective effect of clobenpropit was abolished by concomitant treatment with the histamine H_3 receptor agonist (R)-α-methylhistamine, suggesting that histamine H_3 receptors play a role in regulating the NMDA-induced necrosis. The histamine H_3 receptors are located on histaminergic and other cell somata, dendrites and axons (varicosities), where they provide negative feedback to restrict histamine synthesis and release (Haas and Panula, 2003). A previous report also showed that histamine ameliorates the delayed ischemic damage produced in hippocampal CA1 pyramidal cells by transient forebrain ischemia (Fujitani et al., 1996). However, both pyrilamine and cimetidine failed to block clobenpropit-induced protection. Thus, the observed effect of clobenpropit does not appear to be due to an interaction of released histamine with histamine H_1 and histamine H_2 receptors. It is likely that the amount of histamine released by clobenpropit is insufficient to generate a postsynaptic response.

On the other hand, we were interested to find that the protective effect of clobenpropit was significantly reversed not only by the $GABA_A$ receptor competitive antagonist bicuculline but also by the non-competitive antagonist picrotoxin. These results suggest that the protection of clobenpropit against NMDA-induced effects may at least in part be mediated *via* GABAergic systems. Activation of histamine H_3 receptor leads to the selective inhibition of the component of depolarization-induced GABA release in substantia nigra pars reticulata slices (Garcia et al., 1997). The present data showed that when the cultured neurons were exposed to 10^{-7} M clobenpropit, GABA release was enhanced to a maximum of 268% compared with vehicle-treated neurons. Pretreatment with (R)-α-methylhistamine (10^{-4} M) completely blocked this protective effect. Thus, the GABA increase induced by clobenpropit may be mediated by the histamine H_3 receptor. In addition, it has been reported that increasing GABA function by activating the $GABA_A$ receptor complex results in increased chloride flux across the post-synaptic membrane (de Groat, 1970). GABA can block

NMDA-stimulated calcium influx in the rat cortical slice (Riveros and Orrego, 1986), and the $GABA_A$ receptor agonist muscimol inhibits NMDA-induced neurotoxicity in primary cell cultures, an effect abolished by bicuculline (Ohkuma et al., 1994). Therefore, the massive GABA release induced by clobenpropit may constitute an important protective mechanism against NMDA-induced necrosis.

In auditory brain stem nuclei, dibutyryl-cyclic adenosine monophosphate, a PKA activator, elevates GABA release, which is blocked by H-89. These findings demonstrate that PKA can positively regulate GABA release (Zhang et al., 2004). In various cell lines, histamine H_3 receptor activation induces an inhibition of forskolin-stimulated cAMP formation (Lovenberg et al., 1999), but the physiological consequences of this effect *in situ* remain to be shown. In the present study, we also found that SQ-22536, an AC inhibitor, and H-89, a PKA inhibitor, inhibited the GABA release evoked by clobenpropit. SQ-22536 and H-89 alone had no appreciable effect on GABA release at the concentrations used (data not shown). In addition, it has also been reported that agents that can elevate cAMP levels, such as forskolin, protect hippocampal or cortical neurons against glutamate-induced toxicity *in vitro* (Bruno et al., 1995; Mattson et al., 1988). Pretreatment with dibutyryl cAMP, the permeant cAMP analogue, significantly attenuates the neuronal loss induced by NMDA (Nakao, 1998). In addition, SQ-22536 and H-89 reversed the protective effect of clobenpropit. So, our results further indicate that this effect may be mediated *via* the histamine H_3 receptor/cAMP/PKA pathway. This raises the possibility that cAMP-related signals enhancing GABA release operate against the toxic cascade of events that follows overstimulation of the NMDA receptor.

Excitotoxicity by stimulation of NMDA receptors contributes to neuronal death in brain injuries, including stroke (Choi, 1985). Several lines of evidence suggest that influx and accumulation of Ca^{2+} are required for NMDA receptor-mediated neuronal death (Choi, 1985). In the present study, clobenpropit reversed the NMDA-induced increase in $[Ca^{2+}]_i$, which was antagonized by (R)-α-methylhistamine. It is also reported that in cells expressing human H_3 (445), clobenpropit fully antagonizes the (R)-α-methylhistamine-mediated increases in intracellular calcium levels (Krueger et al., 2005). Thus, the neuroprotective effect of clobenpropit may be attributable to prevention of NMDA-induced $[Ca^{2+}]_i$ accumulation. On the other hand, it was also reported that a histamine H_3 receptor-mediated inhibition of cAMP-dependent phosphorylation of Ca^{2+} channels causes a sequential attenuation of Ca^{2+} influx and $[Ca^{2+}]_i$ (Seyedi et al., 2005). One possible explanation for the differences in efficacy and the potency reversals observed in the different histamine H_3-mediated signaling systems by clobenpropit may be the presence of different ligand-bound active state conformations that couple differentially to the signaling systems.

Therefore, functional histamine H_3 receptor in NMDA-induced excitotoxicity may play an important role as an inhibitory modulator of GABAergic neurotransmission, acting through the cAMP/PKA pathway, and that the inhibitory action probably leads to attenuating the NMDA-induced neurotoxicity. However, the consequences of reduction of $[Ca^{2+}]_i$ by clobenpropit cannot be excluded.

Acknowledgements

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