Brief communication

A pyrazole derivative of curcumin enhances memory

Pamela Maher,a Tatsuhiro Akaishi,b David Schuberta,⁎, Kazuho Abeb

a The Salk Institute, Laboratories for Cellular Neurobiology, 10010 North Torrey Pines Road, La Jolla, CA 92037-1099, USA
b Laboratory of Pharmacology, Research Institute of Pharmaceutical Sciences, Musashino University, Nishitokyo-shi, Tokyo 202-8585, Japan

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Abstract

Reduced cognitive function is associated with Alzheimer’s and Parkinson’s diseases as well as brain trauma and ischemia. However, there are few compounds that enhance memory and are also orally active. We recently synthesized a pyrazole derivative of curcumin called CNB-001 that enhances the activity of Ca2+/calmodulin dependent protein kinase II (CaMKII). Since CaMKII plays a central role in long-term potentiation (LTP) and memory, it was asked if CNB-001 can facilitate the induction of LTP in rat hippocampal slices and enhance memory in a rat object recognition test. It is shown that CNB-001 enhances both LTP and memory.

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1. Introduction

Impaired cognition and memory are associated with a large number of clinical disorders including neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) as well as head trauma, stroke, and a variety of drug-associated toxicities. A decrease in cognition also occurs with age. Therefore, there is a critical need for drugs that improve cognitive performance. Cyclic AMP (cAMP) has a major role in synaptic transmission, and phosphodiesterase inhibitors have been viable drug candidates (Blokland et al., 2006). However, cAMP is involved in a large number of other processes, making phosphodiesterases less than ideal targets for memory enhancement. It is therefore necessary to identify other mechanisms to improve memory and related aspects of cognitive performance.

We recently synthesized a pyrazole derivative of curcumin called CNB-001 (Fig. 1A) that is neuroprotective in a variety of nerve cell toxicity assays, including excitotoxicity, oxidative stress, amyloid toxicity, and glucose starvation (Liu et al., 2008). During these studies, it was observed that CNB-001 activates CaMKII in nerve cells. Since CaMKII plays a role in synaptic facilitation, it was asked if CNB-001 has the ability to enhance long-term potentiation (LTP) and memory. It is shown that CNB-001 activates CaMKII by a cAMP-independent mechanism, facilitates the induction of hippocampal LTP, and is orally active in a rat object recognition test for memory. Since CNB-001 has both neuroprotective and memory enhancing properties, it may be a unique lead drug for treating clinical disorders associated with the loss of nerve cells and cognitive abilities.

2. Materials and methods

2.1. Chemicals

Rolipram was from Calbiochem. All other chemicals were from Sigma.
Fig. 1. (A) Structure of CNB-001. (B) CNB-001 increases CaMKII activity. Primary rat cortical neurons were treated with 1 \( \mu \)M 001 for the indicated times and analyzed for CaMKII activity and phosphorylation. The amount of activity was normalized to protein and phospho CaMKII to total CaMKII, and are presented relative to the activity in vehicle treated cultures. The results are means of three independent experiments. *Indicates significantly different from control \((p<0.05)\). (C) Effect of compounds on object recognition task in Wistar rats. Data represents mean ± S.E.M. *\( p < 0.05 \) compared with the vehicle control. V = vehicle; G = galantamine, 3 mg/kg IP; 001A, CNB-001 5 mg/kg gavage; 001B, CNB-001 10 mg/kg gavage; C, inactive CNB-001-2OMe, 75 mg/kg, gavage. (D) CNB-001 does not increase cAMP levels in hippocampal slices. Hippocampal slices were treated with 1 \( \mu \)M CNB-001 or 3 \( \mu \)M rolipram for 30 min and then either immediately frozen or treated with 5 \( \mu \)M forskolin for an additional 15 min (rolipram + forskolin; 001 + forskolin) prior to freezing. Additional slices were treated only with 5 \( \mu \)M forskolin for 15 min (forskolin). The levels of cAMP in the slices were measured using a Scintillation Proximity Assay and are presented as pmole/mg protein ± S.D. *Indicates significantly different from control \( n = 3 \) \((p<0.05)\).

2.2. LTP

Slice preparations and field potential recordings were made as previously using hippocampal slices from male Wistar rats (Maher et al., 2006). Data are presented as the mean ± S.E.M. of 5–13 slices per condition. Data were analyzed by one-way ANOVA followed by Dunnett’s test.

2.3. Object recognition

Adult male Wistar rats (Jackson Laboratories) were used and the testing was done blind by PsychoGenics (Tarrytown, NY) exactly as described (Ennaceur and Delacour, 1988) using 10 rats in each treatment group. For each dose tested, a 10× solution of compound was prepared in 95% ethanol and then diluted with 4 volumes polyethylene glycol 660 hydroxystearate (Solut HS15 from BASF) and 5 volumes phosphate buffered saline. The vehicle contained the same ratios of ethanol, Solut HS15 and phosphate buffered saline. All were administered orally 60 min prior to training at a volume of 1 ml/kg body weight. Galantamine was administered intraperitoneally at 3 mg/kg 1 h prior to training. Object recognition was computed using the formula: time spent with novel object \( \times 100 \)/total time spent exploring both objects. Data are presented as the mean ± S.E.M. Data were analyzed by a one-way ANOVA followed by the Fisher’s test. An effect was considered significant if \( p < 0.05 \).

2.4. cAMP and CaMKII assays

cAMP was assayed in hippocampal slices as described (Maher et al., 2006). CaMKII activity was measured using the SignaTECT calcium/calmodulin-dependent protein kinase assay system from Promega according to the manufacturer’s instructions and normalized to protein. Western blotting was done as described (Maher et al., 2006) using anti-phospho thr286 (Novus) and anti-total (Zymed) CaMKII. The data were analyzed using ANOVA followed by Tukey’s test.
3. Results

During an investigation of the role of steroids in amyloid toxicity (Liu and Schubert, 1998), we identified a pseudosteroid, cyclohexyl-bisphenol A, with neurotrophic activity. The neurotrophic activity of this compound was greatly improved by creating a hybrid molecule, CNB-001, with the neuroprotective compound, curcumin (Fig. 1A) (Liu et al., 2008). To determine the mode of action of CNB-001, it was tested in a variety of kinase assays using primary cultures of nerve cells and found to increase the activity of CaMKII using both phosphorylation (Yamauchi, 2005) and enzyme activity assays (Fig. 1B). Because of the role of CaMKII in memory (Yamauchi, 2005), we then asked if CNB-001 can affect LTP in the CA1 area of rat hippocampal slices. When the slices were perfused with 1 or 10 μM CNB-001 for 60 min, there was no change in basal synaptic transmission (not shown, n = 4). The magnitude of LTP induced by the standard 100-pulse, 100-Hz tetanic stimulation was not significantly different between control slices (the average of the fEPSP slopes 30–60 min after tetanus, 151.8 ± 9.4% of baseline, n = 5) and slices perfused with 10 μM CNB-001 (164.7 ± 31.4%, n = 6). However, while a weak tetanic stimulation of 15 pulses at 100 Hz alone failed to induce LTP in control slices, the same stimulation produced LTP in slices perfused with CNB-001 (Fig. 2A). The facilitation of LTP induction by CNB-001 was concentration dependent in the range of 0.1–10 μM (Fig. 2B). To ask if these effects are specific for CNB-001, we synthesized a derivative of CNB-001 in which the ring hydroxyl groups are replaced with methoxy groups (CNB-001-2OMe) and is inactive in neurotrophic and kinase assays (Liu et al., 2008, and not shown). CNB-001-2OMe had no effect on the induction of LTP (Fig. 2C and D).

To determine if the effect of CNB-001 seen on LTP translates into alterations in memory enhancement in animals, it was tested in rats using the object recognition test (Ennaceur and Delacour, 1988). This model is based upon normal rodent behavior in which there is greater spontaneous exploration of a novel object compared to a familiar object. During the training period rats are presented with two identical objects, which they explore for a fixed time period. To test for memory, they are presented 1 day later with two different objects, one of which was present during the training and is thus familiar; the other that is new. The better they remember the familiar object, the more time they will spend exploring the novel object. CNB-001 was administered orally before the start of the training period. Galantamine, a phosphodiesterase inhibitor that potentiates memory in this assay (Musial et al., 2007), requires injection and was used as a positive control. Fig. 1C shows that CNB-001 has a significant memory-enhancing effect, while...
the inactive analogue, CNB-001-2OMe has no effect at a maximal dose. An open field test 5 min immediately prior to the test period showed that there is no effect of CNB-001 on locomotion (not shown).

Phosphodiesterase 4 (PDE4) inhibitors such as rolipram and galantamine enhance memory through cAMP and CREB phosphorylation (Musial et al., 2007). To determine whether CNB-001 works through a similar mechanism, hippocampal slices were treated with CNB-001 using the conditions where maximal facilitation of LTP were seen, and the slices assayed for cAMP. Forskolin, an activator of adenyl cyclase and rolipram were used as positive controls. Rolipram alone modestly increased cAMP levels in the slices and significantly potentiated the effect of forskolin on cAMP levels. In contrast, CNB-001 had no effect on cAMP levels by itself, nor did it potentiate the effect of forskolin (Fig. 1D).

4. Discussion

The above data show that CNB-001 is able to facilitate LTP in rat hippocampal slices, and memory in a rat object recognition assay. Unlike drugs that stimulate memory through the inhibition of phosphodiesterase, CNB-001 does not inhibit this enzyme but rather activates CaMKII. Although we have not formally shown the CNB-001 stimulates LTP and memory through the activation of CaMKII, low levels of CaMKII activation are required for the induction of LTP (Bramham and Messaoudi, 2005) and memory in various animal models (Yamauchi, 2005). CNB-001 and fisetin (Maher et al., 2006) are among the few orally active compounds that enhance memory independently of cAMP. Fisetin activates CREB, but CNB-001 does not (Maher et al., 2006) and not shown).

Multiple pathways can lead to enhanced synaptic transmission. Because many drugs that are highly potent and selective for synaptic transmission may not be safe because the status quo of normal cognitive function must be maintained, perhaps drugs with lower target affinity and toxicity are required. CNB-001 is a simple one-step synthetic derivative of curcumin, an FDA-approved natural product for the treatment of cancer that also has great promise for the treatment of Alzheimer’s disease (Cole et al., 2007). Since CNB-001 has an EC50 of around 0.5 μM in both LTP (Fig. 2B) and cell culture assays (Liu et al., 2008) and is active in animals at 10 mg/kg (Fig. 1C), it is not a highly potent compound, but it is within the range of some established drugs and thus has potential as a lead compound for the treatment of disorders affecting memory and cognition.

Conflicts of Interest statement

There are no actual or potential conflicts of interest.

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