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Anandamide and noladin ether prevent neurotoxicity of the human amyloid-β peptide

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Abstract

Cannabinoid receptor agonists including anandamide and noladin ether have recently been suggested to exhibit neuroprotective properties. The amyloid-β (Aβ) peptide is thought to be responsible for the neurodegenerative changes associated with Alzheimer's disease pathology. This study characterizes the effects of anandamide and noladin ether on the neurotoxicity of Aβ in differentiated human teratocarcinoma cell line, Ntera 2/cl-D1 neurons. Anandamide and noladin ether, at nanomolar concentrations, showed concentration dependent inhibition of Aβ toxicity. A CB_1 cannabinoid receptor antagonist, AM251, prevented the protective effects of anandamide and noladin ether. The mitogen activated protein kinase (MAPK) pathway inhibitor PD98059 also prevented the protective effects of cannabinoids and corticotrophin-releasing hormone. These results suggest that activation of the MAPK pathway by either cannabinoids or corticotrophin-releasing hormone could be used to prevent Aβ peptide induced neurodegeneration.

Keywords: Amyloid-β; Noladin ether; Anandamide; Cannabinoid; Mitogen activated protein kinase; Neurons; Alzheimer's; Corticotrophin-releasing hormone

The amyloid-β (Aβ) peptide which accumulates in the senile plaques characteristic of Alzheimer's disease (AD) is thought to be responsible for the neurodegenerative changes [18] seen in the disease. The precise mechanisms responsible for the neurotoxicity of Aβ however remain unclear. Oxidative stress, apoptosis, cell cycle re-entry, G-protein activation, lipids and peptide aggregation are all suggested to play causative roles in Aβ neurotoxicity based on the pharmacological properties of Aβ toxicity inhibitors [10]. Elevated levels of Aβ found in AD brains are associated with accumulation of aggregated peptide in the extracellular space. The toxicity of Aβ is thought to be mediated via intracellular actions [19] and to follow endocytosis of soluble peptide aggregates. Prevention of Aβ toxicity by corticotrophin-releasing hormone (CRH) receptor ligands is mediated via activation of mitogen activated protein kinase (MAPK) pathways [17] raising the possibility that compounds which activate MAPK may be potential agents to prevent Aβ induced neurodegeneration.

Cannabinoids including anandamide, 2-arachidonoyl glycerol and noladin ether have been identified in brain extracts [4,7] providing a potential endogenous signaling system. These endogenous cannabinoids have also been suggested as neuroprotective agents [6,16]. Potential therapeutic use of cannabinoids for the treatment of neurodegenerative disorders including Multiple Sclerosis, Parkinson's and ischaemia has also been suggested [6,11,16]. The actions of cannabinoids are mediated principally via specific cannabinoid receptors [2,7,9]. However, some cannabinoids including anandamide can also act via vanilloid receptors [9] and cellular uptake of endogenous cannabinoids may play a role in some of their actions [5]. Both the CB_1 and CB_2 cannabinoid receptors have been shown to activate the p42/p44 MAPK [8] and antagonists of p42/p44 MAPK can prevent some actions of cannabinoids [2,8]. This raises the possibility that cannabinoids could act in a similar manner to CRH and prevent Aβ toxicity.

This study set out to determine the effects of the endogenous cannabinoids anandamide and noladin ether on Aβ toxicity. Synthetic Aβ peptides were obtained from Insight Biotechnology Ltd., Wembley, UK and prepared freshly from trifluoracetic acid treated stock solutions prior to use. For all cytotoxicity experiments Aβ peptides were resus-

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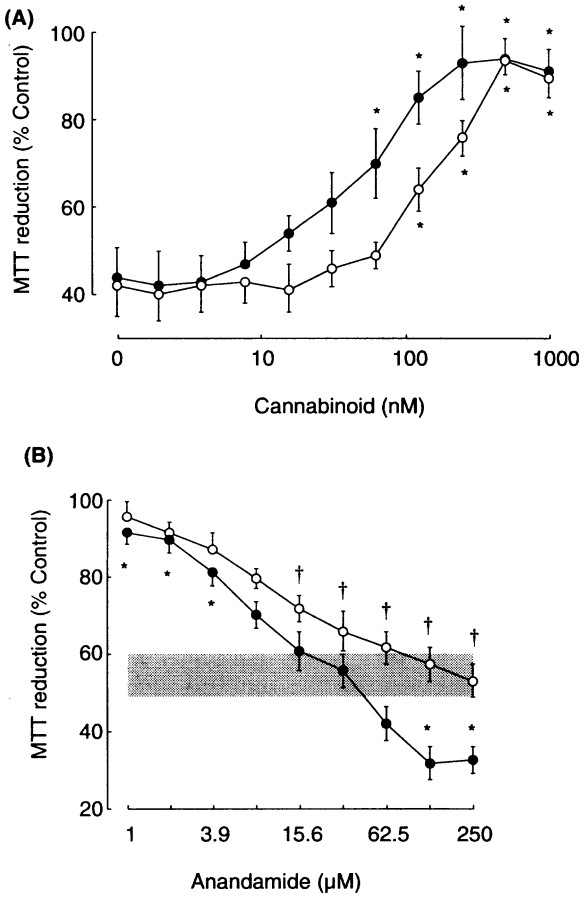

Fig. 1. (A) Effect of Aβ 1–40 (5 μM) plus anandamide (open circles) and Aβ 1–40 (5 μM) plus noladin ether (closed circles) on MTT reduction in human NT-2 neurons. (B) Effects of anandamide alone (open circles) or in the presence of 5 μM Aβ 1–40 (closed circles) on MTT reduction in human NT-2 neurons. Shaded box indicates the MTT reduction (mean ± SEM) for 5 μM Aβ 1–40 alone. All results are mean ± SEM, $n = 6$ for each group, * = $P < 0.05$ versus Aβ alone, † = $P < 0.05$ versus control.

pended in phosphate buffered saline (PBS) and incubated for 24 h at 37°C prior to addition to cells. The effects of anandamide and noladin ether on cytotoxicity were determined using differentiated human teratocarcinoma cell line, Ntera 2/cl-D1 (NT-2) neurons. The NT-2 neurons were propagated as previously described and incubated with test peptides (20 μM) for 24 h prior to determination of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye exclusion [15]. Anandamide, noladin ether, AM251, AM404 and AM630 were obtained from Tocris Cookson Ltd., Bristol, UK and stock solutions prepared in ethanol for anandamide, noladin ether and AM404 or dimethylsulfoxide (DMSO) for AM251 and AM630. The ethanol and DMSO vehicles were tested in control experiments and found to have no effect on NT-2 neuron reduction of MTT in the presence or absence of Aβ.

Anandamide and noladin ether (2–1000 nM) caused a concentration dependent inhibition of Aβ 1–40 toxicity in NT-2 neurons (Fig. 1a). Anandamide and noladin ether are both arachidonic acid derivatives, however, arachidonic acid had no effect on Aβ toxicity. Dose response curves showed that noladin ether was the more effective agent against Aβ toxicity. At concentrations greater than 7.8 μM anandamide was toxic to the neuronal cells in agreement with previous studies [2,6,7,9]. The toxicity of the Aβ peptide was enhanced by anandamide concentrations between 62.5 and 250 μM (Fig. 1b). Similar in vivo neuroprotective effects have recently been demonstrated for BAY 38-7271, a cannabinoid agonist [11]. The dose responses of the BAY 38-7271 suggest that low concentration neuroprotection is lost at higher concentrations in a similar fashion to the effects observed here with anandamide. The neuroprotective actions of anandamide and noladin ether occur at concentrations that are likely to activate CB_1 cannabinoid receptors [8] and may therefore also produce typical cannabinoid side effects. The selectivity of noladin ether for CB_1 cannabinoid receptors at these concentrations [8] implicates this receptor type in the observed responses.

The protective effects of cannabinoids were compared with those of CRH [17]. The CRH and α-helical CRH 9–41 peptides were resuspended in PBS prior to addition to cells. The cytotoxicity of Aβ 1–42, 1–40, 17–35 and 25–35 peptides could be inhibited by noladin ether or CRH (Fig. 2). However, the noladin ether and CRH had no effect on the toxicity of the Aβ 31–35 fragment (Fig. 2). Previous studies have characterized the ability of Aβ 31–35 to cause apoptosis [20], activate the cyclin-dependent kinase-1 enzyme [12] and inhibit the catalase enzyme [13]. It has been assumed that Aβ 31–35 represented the toxic domain of Aβ based on these and other studies [12,13,20]. The different protective ability of cannabinoids and CRH against Aβ 25–35 and 31–35 would suggest that the Aβ 31–35 region is not solely responsible for the toxicity of longer the Aβ

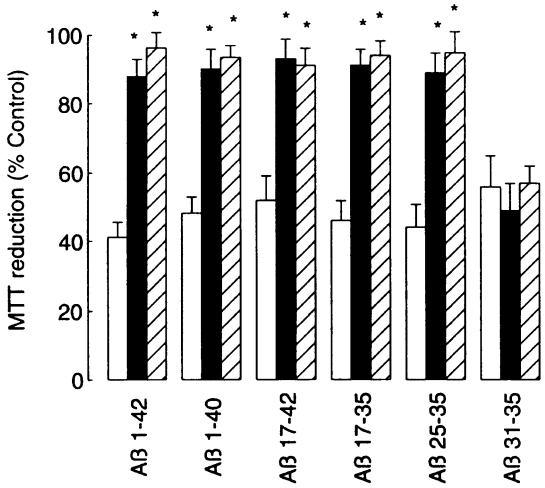

Fig. 2. Effect of Aβ peptides (25 μM) alone (open columns) or plus noladin ether (1 μM: closed columns) or plus CRH (1 nM: hatched columns) on MTT reduction in human NT-2 neurons. All results are mean ± SEM, $n = 6$ for each group, * = $P < 0.05$ versus Aβ.

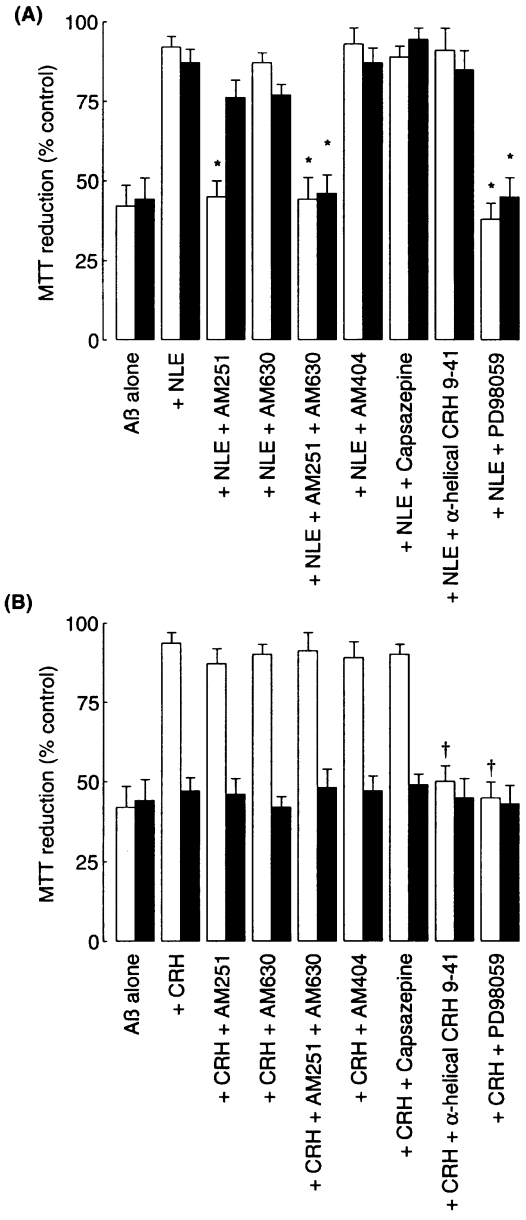

Fig. 3. (A) Effects of the CB_1 receptor antagonist AM251 (10 μM), the CB_2 receptor antagonist AM 630 (10 μM), AM251 (10 μM) plus AM630 (10 μM), the anandamide uptake inhibitor AM404 (10 μM), the vanilloid receptor antagonist capsazepine (10 μM), the CRH antagonist α-helical CRH 9–41 (1 μM) and the MAPK kinase inhibitor PD98059 (50 μM) on noladin ether (NLE: 1 μM) protection against Aβ 1–40 (5 μM) in human NT-2 neurons (open columns) or mouse SP2 myeloma cells (closed columns). (B) Effects of the CB_1 receptor antagonist AM251 (10 μM), the CB_2 receptor antagonist AM 630 (10 μM), AM251 (10 μM) plus AM630 (10 μM), the anandamide uptake inhibitor AM404 (10 μM), the vanilloid receptor antagonist capsazepine (10 μM), the CRH antagonist α-helical CRH 9–41 and the MAPK kinase inhibitor PD98059 (50 μM) on CRH (1 nM) protection against Aβ 1–40 (5 μM) in human NT-2 neurons (open columns) or mouse SP2 myeloma cells (closed columns). All results are mean ± SEM, $n = 6$ for each group, * = $P < 0.05$ versus Aβ 1–40 plus noladin ether, † = $P < 0.05$ versus Aβ 1–40 plus CRH.

peptide fragments. All the toxic forms of Aβ have been shown to aggregate [3,18] and this is thought to be essential for Aβ toxicity. However, aggregated peptides in general can exert toxic actions at similar concentrations [3] and it is possible that this mechanism is responsible for the toxicity of Aβ 31–35.

Previous studies have shown that the Aβ peptides are toxic to both neuronal and non-neuronal cells [12,13]. Cannabinoids [11] and CRH [14] can also exert effects outside the CNS via actions on peripheral receptors. Specific antagonists were used to determine the mechanisms underlying cannabinoid and CRH protection against Aβ toxicity in both NT-2 neurons and SP2/0-Ag-14 mouse myeloma cells as previously described [12]. The protective actions of noladin ether against Aβ 1–40 could be prevented in neuronal cells by the CB_1 cannabinoid receptor antagonist AM251 whilst the CB_2 cannabinoid receptor antagonist AM630 had no affect. The concentrations of AM251 and AM630 used (10 μM) are similar to previously published studies [9], however they are significantly greater than the published K_i values for these compounds and as such the compounds could be acting via a different mechanism [8]. At lower concentrations (0.1–1 μM) AM251 was effective at reducing noladin ether neuroprotection, however, it was unable to completely abolish the neuroprotective actions. The anandamide uptake inhibitor AM404, the vanilloid receptor antagonist capsazepine and the CRH antagonist α-helical CRH 9–41 [14] had no affect on the protective actions of noladin ether. The MAP kinase pathway inhibitor PD98059 [1] prevented the protective action of the noladin ether (Fig. 3a). In myeloma cells the protective effects of noladin ether was blocked by a combination of the CB_1 and CB_2 antagonists but not the individual antagonists (Fig. 3a). The myeloma cells are derived from B-cells and this immune cell type has been shown to express primarily CB_2 cannabinoid receptors with low levels of CB_1 receptors [8], suggesting that CB_2 receptors may be the main type in the myeloma cells. Noladin ether shows a marked CB_1 receptor selectivity and is unlikely to be a CB_2 ligand at submicromolar concentrations. The actions of the CB_1 and CB_2 receptor antagonists in this cell type do suggest a contribution from CB_2 cannabinoid receptors in myeloma cells. It cannot be excluded that this contribution is mediated via an endogenous cannabinoid produced by the myeloma cells during the experimental manipulations.

In neuronal cells protection against Aβ toxicity by CRH was unaffected by AM251, AM630, AM404 and capsazepine, but was blocked by PD98059 and the CRH antagonist α-helical CRH 9–41 (Fig. 3b). In myeloma cells the CRH had no effect on Aβ 1–40 toxicity, suggesting that this cell line lacks an appropriate CRH receptor [17]. The toxicity of Aβ was unaffected by AM251, AM630, AM404, capsazepine, α-helical CRH 9–41 and PD98059. The failure of cannabinoid receptor antagonists and uptake inhibitors to directly affect Aβ toxicity suggests that endogenous cannabinoids produced by the cells used in these studies are not providing protection.

The cannabinoids act through CB_1 receptors in neurons and it is likely that activation of a protective signaling pathway may be responsible for the observed prevention of Aβ toxicity. Cannabinoid CB_1 receptors are located within the brain suggesting that these compounds could act to prevent neurodegeneration [2]. Peripherally administered cannabinoids can act on brain receptors, suggesting that such compounds may be useful therapeutic agents for CNS disorders. This is supported by the beneficial actions of cannabinoid receptor agonists in neurodegenerative disorders including Multiple Sclerosis, Parkinson's disease and vascular dementia [6,11,16]. The protective mechanism of cannabinoids against Aβ toxicity, like CRH [17], involves activation of a MAP kinase cascade.

In conclusion the endogenous cannabinoids anandamide and noladin ether were effective neuroprotective agents against Aβ toxicity, via activation of MAP kinase pathways. Compounds such as cannabinoids and CRH that specifically activate such a MAP kinase cascade in neurons may provide an effective way of preventing Aβ induced neurodegeneration and may therefore be of use in the treatment of AD.

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