ARE ANANDAMIDE AND CANNABINOID RECEPTORS INVOLVED IN ETHANOL TOLERANCE? A REVIEW OF THE EVIDENCE

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(Received 9 June 1999; in revised form 23 August 1999; accepted 7 October 1999)

Abstract—There have been significant developments towards the elucidation of molecular and cellular changes in neuronal second messenger pathways involved in the development of tolerance to and dependence on ethanol (EtOH). The long-term exposure to EtOH has been shown to affect several aspects of neuronal signal transduction as well as ligand-gated ion channels and receptor systems, including the receptors that are coupled to the superfamily of GTP binding regulatory proteins (G-proteins). The recent identification of a G-protein coupled receptor that was activated by \textit{\delta}-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, led to the discovery of endogenous agonists. One such agonist found to exist in mammalian brain was characterized to be an arachidonic acid (AA) metabolite and was named anandamide (AnNH). AnNH has been shown to bind specifically to the cannabinoid receptor (CB\textsubscript{1}) and mimics many of the pharmacological and behavioural effects of THC including tolerance development. The role of endocannabinoids and the CB\textsubscript{1} receptor signal transduction system in tolerance development to drugs of abuse has not been explored until recently. The findings presented in this review provide evidence for the first time that some of the pharmacological actions of EtOH including tolerance development may be mediated through participation of the endocannabinoid–CB\textsubscript{1} receptor signal transduction system. Recent studies have shown that chronic EtOH exposure produces downregulation of CB\textsubscript{1} receptors and an inhibition of CB\textsubscript{1} receptor agonist-stimulated GTP\textsubscript{S} binding in mouse brain synaptic plasma membranes (SPM). The observed receptor downregulation results from the persistent stimulation of the receptors by the endogenous CB\textsubscript{1} receptor agonist AnNH, the synthesis of which is increased by chronic EtOH exposure. Further, the CB\textsubscript{1} receptor antagonist SR-141716A has been shown to block voluntary EtOH intake in rats and mice. Based on these studies, a hypothesis is presented to explain the possible involvement of the endocannabinoid system in the pharmacological and behavioural effects of EtOH.

INTRODUCTION

Alcohol is one of the most widely used and abused drugs contributing to a variety of medical and socio-economic problems (US Public Health Service, 1990). Chronic excessive intake of ethanol (EtOH) for long periods causes development of tolerance to and dependence on EtOH. Furthermore, abusive drinking results in organ and tissue damage (Hunt, 1985). The mechanism by which EtOH exerts its pharmacological effects is not clearly understood. EtOH does not appear to have a specific receptor site for its action. However, it has been shown to modulate a variety of receptors which are coupled to neurotransmission and second messenger systems (Nevo and Hamon, 1995; Fadda and Rossetti, 1998). It has recently been reported that chronic EtOH exposure increases the levels of an endogenous cannabinimimetic substance, anandamide (AnNH), an arachidonic acid (AA) metabolite, in neuroblastoma cells (Basavarajappa and Hungund, 1999a). It was also demonstrated that chronic exposure of mice to EtOH (EtOH-tolerant) resulted in downregulation of cannabinoid (CB\textsubscript{1}) receptors and CB\textsubscript{1} receptor agonist-stimulated guanyl-5'-O-(thio)triphosphate (GTP\textsubscript{S}) binding in synaptic plasma membranes (SPM) (Basavarajappa et al., 1998a; Basavarajappa and Hungund, 1999b). These results, taken together, point to participation of yet another receptor system in the pharmacological and behavioural actions of EtOH. Based on the evidence presented here, we hypothesize that: chronic EtOH intake increases synthesis and release of AnNH leading to reduction in the number and function of CB\textsubscript{1} receptors, and thus the cannabinoidergic system may play a role in many aspects of EtOH action including development of tolerance to EtOH. This article will also provide a review of the historical connection between tetrahydrocannabinol (THC) and EtOH and recent findings on the similarities in their mechanism of action.

CROSS-TOLERANCE BETWEEN THC AND ETOH

There are several studies reported in the literature describing cross-tolerance between THC and EtOH. Only THC, of all the cannabinoids, has a significant pharmacodynamic interaction with EtOH (Hollister and Gillespie, 1970). Both drugs have many similarities in their actions. At low doses, they tend to produce euphoria and stimulation and at high doses produce sedation (Hollister and Gillespie, 1970). A number of behavioural and pharmacological effects of EtOH, such as hypothermia, euphoria, analgesia, and motor dysfunction (ataxia, sleep time), in addition to development of tolerance and dependence, have been shown to be similar to the effects produced by THC (Kalant and LeBlanc, 1974). The rats made tolerant to the depressant effects of THC were also tolerant to the behavioural depressant effects of EtOH (Newman et al., 1972). Rats made tolerant to either EtOH or THC exhibited symmetrical cross-tolerance to challenge doses of the opposite compounds in an avoidance learning task and rotordor performance test (Newman et al., 1972; Sprague and Craigmill, 1976; Siemens...
and Doyle, 1979). Sprague and Craigmill (1978) have also studied the effects of THC upon abstinence signs (handling-induced convulsions) and responsiveness to electric foot shock in EtOH-dependent mice. It was found that the responsiveness to electric foot shock, but not the severity of handling-induced convulsions, was alleviated by EtOH or high doses of THC (10−40 mg/kg) (Sprague and Craigmill, 1978). In a related study, it was shown that low doses of THC reduced, and high doses increased, the severity of handling-induced convulsions in EtOH-dependent mice (Blum et al., 1975). McMillan and Snodgrass (1991) have demonstrated that EtOH intake through lever pressing increased in rats during acute and chronic THC administration and withdrawal. These data strongly suggest possible cross-tolerance between the two drugs and similarities in their mechanism of action.

WHAT ARE ETHANOLAMIDES?

In 1992, Devane and co-workers made the observation that a compound isolated from porcine brain and identified as AnNH, an ethanolamide derivative of arachidonic acid (AA), competed with the binding of the cannabinoid receptor ligand [3H]HU-210 to brain membranes (Devane et al., 1992). The N-acyl ethanolamides (NAEs) are the lipid products containing long-chain fatty acids ranging from palmitic (C:16:0) to arachidonic (C20:4) acid, conjugated with ethanolamine (NH2CH2CH2OH), both of which are essential components of membrane phospholipids (PL). The chemical structure of N-arachidonyl-ethanolamid (AnNH) is shown in Fig. 1. AnNH, one of the many species of NAEs found in the brain, has been shown to mimic many of the pharmacological and behavioural properties of THC, a psychoactive component of marijuana (Devane et al., 1992). Like THC, AnNH has been shown specifically to bind with high affinity to brain CB1 receptors, modulate both adenylate cyclase (Vogel et al., 1993), and mobilize Ca2+ (Mackie et al., 1993). AnNH has also been shown to display other pharmacological actions such as activation of phospholipase A2 (PLA2) (Hunter et al., 1986; Audette et al., 1991; Burststein et al., 1994; Wartmann et al., 1995). Furthermore, in vivo, AnNH produced a series of behavioural responses typical of cannabinoid drug administration, which included hypothermia, analgesia, catalepsy, mobility, and tolerance development (Fride and Mechoulam, 1993).

CROSS-TOLERANCE BETWEEN THC AND AnNH

Chronic THC treatment has been shown to lead to behavioural tolerance in both humans and animals (Carlini, 1968; Kalant et al., 1971; Jones et al., 1981; Dewey, 1986; Deadwyler et al., 1990, 1995b). Chronic treatment with AnNH for 2 weeks has been shown to produce tolerance to a challenge dose of AnNH and cross-tolerance to THC in mice similar to that seen after chronic THC exposure (Fride and Mechoulam, 1993). It was suggested that the high dose, but not the low dose, of AnNH produced tolerance to AnNH and cross-tolerance to THC for motor activity in open field, catalepsy, hypothermia, and analgesia on a hot plate (Fride and Mechoulam, 1993). Since EtOH also produces similar behavioural effects, the studies reported here further suggest that these drugs (THC, EtOH, and AnNH) may share a common site to induce tolerance and may have a common mechanism for their actions.

ALCOHOL AND NEURORECEPTORS

Comprehensive reviews on this subject have recently been published (Nevo and Hamon, 1995; Fadda and Rossetti, 1998). Both acute and chronic EtOH administration have been shown to affect many of the known neurotransmitter systems in the central nervous system. The brain’s major amino acid transmitter systems, inhibitory γ-aminobutyric acid (GABA) and excitatory glutamate, have been widely studied over the past decade. The consensus has been that acute EtOH facilitates GABAergic transmission (by enhancing chloride conductance through the GABA_A receptor) (Morrow et al., 1988; Grobin et al., 1998) and initially inhibits the actions of glutamate on the N-methyl-D-aspartate (NMDA) receptors (by decreasing cationic conductance through NMDA receptors). This adaptation involves upregulation in the number and/or function of the respective receptors (Foliesa and Ticku, 1995, 1996; Hu et al., 1996; Kalluri et al., 1998). The development of tolerance associated with chronic EtOH consumption is attributed to reduced GABAergic (McQuilkin and Harris, 1990; Calkin and Barnes, 1994) and increased glutamatergic (Hoffman, 1995; Hu and Ticku, 1995; Hu et al., 1996) function. Dopaminergic (Fadda et al., 1985; Samson and Harris, 1992) and noradrenergic (Davis et al., 1978; Amit and Brown, 1982; Docherty et al., 1993) mechanisms along with opioid systems (Davis and Walsh, 1970; Reid et al., 1991; Ulm et al., 1995) have been suggested to play a role in the rewarding effects of EtOH, while the serotoninergic system mediates negative reinforcement (Myers et al., 1972; Gill and Amit, 1989; Lu et al., 1993). The central cholinergic system has been suggested to participate in the EtOH effect on learning and memory function in alcohol-dependent patients (Arendt et al., 1988a; Hodges et al., 1991; Melis et al., 1996).

ROLE OF THE ENDOCANNABINOID AnNH AND CB1 RECEPTOR-MEDIATED SIGNALLING MECHANISM IN THE ACTIONS OF ETOH: CURRENT STATUS

Increased accumulation of AnNH in chronic EtoH exposed SK-N-SH cells

Earlier studies have demonstrated that chronic EtOH exposure leads to accumulation of fatty acid ethyl esters (FAEes) in various mammalian organs including the brain (Laposata and Lange, 1986; Hungund et al., 1988). Although AA is a major component of membrane phospholipids (PL), none of the studies have identified free AA or ethyl ester of AA or...
other metabolites of AA including eicosanoids in the EtOH-exposed tissues and organs. The recent discovery of AnNH prompted an investigation to examine whether the missing AA in EtOH-exposed tissue may be diverted to AnNH synthesis. Indeed, it was found that the exposure of SK-N-SH cells to chronic EtOH resulted in increased accumulation of AnNH (Basavarajappa and Hungund, 1999a) (Table 1). In this study, it was demonstrated that AnNH synthesis increased with increasing period of EtOH exposure, peaking at 72 h with 100 mM EtOH, and was further enhanced by Ca\(^{2+}\)-ionophore, and membrane-depolarizing agents. Co-exposure of cells to pertussis toxin (PTX) (100 ng/ml), which selectively inactivates G-protein, and EtOH (100 mM) for 72 h, inhibited the EtOH-induced formation of \(^{[3]}\)H-AnNH (by 100%). Interestingly, cotreatment of SK-N-SH cells with the CB\(_1\) receptor antagonist, SR-141716A (1.0 \(\mu\)M) and EtOH (100 mM) for 72 h, blocked (by 100%) the EtOH-induced formation of \(^{[3]}\)H-AnNH. The results with SR-141716A and PTX suggest that the level of AnNH in chronic EtOH-treated SK-N-SH cells may be regulated by CB\(_1\) receptors and G\(_i/o\)-proteins (Basavarajappa and Hungund, 1999a) (Table 1). Several investigators have used similar in vitro models to explain different aspects of cellular tolerance and dependence of EtOH (Gordon et al., 1986; Coe et al., 1996; Diamond and Gordon, 1997; Bhave et al., 1999). A similar increase in the levels of AnNH was observed in brains of mice which were made EtOH tolerant by continuous exposure to EtOH vapours (Goldstein and Pal, 1971; Blum et al., 1975; Sprague and Craigmill, 1978; Littleton and Little, 1994). The results of this study indicate that the chronic EtOH exposure not only decreased the \(B_{max}\) and CB\(_1\) receptors (without any changes in the receptor affinities) (Basavarajappa et al., 1998a) but also reduced the net CP-55940-stimulated ([\(^{125}\)I]GTP\(\gamma\)S) binding in mouse SPM (Basavarajappa and Hungund, 1999b) Table 2. A similar decrease in \(B_{max}\) of CB\(_1\) receptors (downregulation) after chronic THC or CP-55940, a CB\(_1\) receptor agonist or AnNH treatment has also been reported (Oviedo et al., 1993; Rodriguez de Fonseca et al., 1994; Rubio et al., 1994; Romero et al., 1995, 1997, 1998a,b,c; Fan et al., 1996). It has also been shown that besides the CB\(_1\) receptor modification by CB\(_1\) receptor agonists, downregulation of G-protein expression and CB\(_1\) receptor agonist-stimulated GTP\(\gamma\)S binding was also observed in the rat central nervous system (Sim et al., 1996; Rubio et al., 1997; Zhuang et al., 1998). In cultured neuroblastoma cells, chronic THC exposure desensitized the cannabinoïd-induced inhibition of adenylate cyclase, indicating that cannabinoid tolerance may involve alterations in the CB\(_1\) receptor signal transduction (Dill and Howlett, 1988). These studies strongly support the hypothesis that the pharmacological and behavioural effects of EtOH may be mediated through the AnNH–CB\(_1\) receptor signal transduction mechanism and EtOH, AnNH, and THC may have a common mechanism for their actions.

### Table 1. Chronic ethanol (EtOH) enhances the formation of the CB\(_1\) receptor agonist anandamide and its precursor N-ArPE in SK-N-SH cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>EtOH (%) of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>AnNH</td>
<td>100.00 ± 5.78</td>
<td>138.30 ± 5.77*</td>
</tr>
<tr>
<td>Basal</td>
<td>91.90 ± 10.90</td>
<td>92.45 ± 6.20*</td>
</tr>
<tr>
<td>SR-141716 (1 (\mu)M)</td>
<td>106.80 ± 8.40</td>
<td>115.70 ± 18.10*</td>
</tr>
<tr>
<td>PTX (100 ng/ml)</td>
<td>108.00 ± 4.04</td>
<td>180.70 ± 8.39*</td>
</tr>
<tr>
<td>N-ArPE</td>
<td>100.00 ± 5.80</td>
<td>169.70 ± 5.48*</td>
</tr>
<tr>
<td>Basal</td>
<td>102.30 ± 2.85</td>
<td>174.70 ± 7.35*</td>
</tr>
<tr>
<td>SR-141716 (1 (\mu)M)</td>
<td>108.00 ± 4.04</td>
<td>180.70 ± 8.39*</td>
</tr>
</tbody>
</table>

*Basavarajappa and Hungund (1999a). Effect of the CB\(_1\) receptor antagonist SR-141716A and pertussis toxin (PTX) on the chronic EtOH induced formation of \(^{[3]}\)H-AnNH and \(^{[3]}\)H-N-ArPE in SK-N-SH cells showing exposure to 100 mM EtOH for 72 h. Cells were labelled with \(^{[3]}\)H AA (1 \(\mu\)Ci/ml) in 0.1% FBS: DMEM for 5 h and then co-treated with SR-141716A (1 \(\mu\)M) or PTX (100 ng/ml) and with or without EtOH. The \(^{[3]}\)H-AnNH and \(^{[3]}\)H-N-ArPE were extracted from media and cells and separated on thin-layer chromatograms using solvent system A (chloroform/methanol/acetic acid (90:6:6, v/v/v)). Data are expressed as percentage of control. Control values were 5700 ± 400 for \(^{[3]}\)H-AnNH and 2500 ± 300 dpm/mg cellular protein for \(^{[3]}\)H-N-ArPE. Each value represents the means ± SEM (\(n = 9\)). *\(P < 0.05\) (ANOVA).

### Mechanism of CB\(_1\) receptor downregulation by chronic EtOH treatment; signal transduction pathway

It has been suggested that desensitization of cannabinoïd–activated signal transduction may be responsible for tolerance development to THC (Oviedo et al., 1993; Rodriguez de Fonseca et al., 1994; Rubio et al., 1994; Romero et al., 1995, 1997, 1998a,b,c; Fan et al., 1996). Similarly, the observed downregulation of CB\(_1\) receptors by chronic EtOH (EtOH-tolerant mouse SPM) may also result from over-stimulation of receptors through increased synthesis of the endogenous CB\(_1\) receptor agonist (AnNH) (Basavarajappa et al., 1998a; Basavarajappa and Hungund, 1999a). Another plausible mechanism by which chronic EtOH might downregulate CB\(_1\) receptors is by a direct effect on membrane lipids. Such an effect may change the physical nature of the selective portions
of the neuronal membrane and thereby alter the receptor distribution and its function (Hunt, 1985; Salem and Ward, 1993; Macdonald, 1995; Zheng et al., 1996; Basavarajappa et al., 1997, 1998a,b; Zhang et al., 1997). Further, this may disturb the functional interactions of the ligand at the receptor–lipid interface.

The primary actions of cannabinoids are mediated through G-protein coupled receptors and an intracellular signaling mechanism that initiates cellular response by cannabinoid activated G-proteins (Matsuda et al., 1990; Howlett, 1995). Cannabinoid inhibition of adenyl cyclase plays an important role in several aspects of cannabinoid functions, such as modulation of conductance at a voltage-dependent K⁺-channel (‘A’ current) (Deadwyler et al., 1995a; Mackie et al., 1995) and inhibition of Ca²⁺ current through a voltage-gated N-channel (Caulfield and Brown, 1992; Mackie and Hille, 1992), thus providing an effective rationale for behavioural effects of cannabinoids (Howlett et al., 1986). Further studies to examine if the chronic EtOH-mediated downregulation of CB₁ receptors has any functional effect on CB₁ receptor-activated G-proteins revealed that the net CB₁ receptor agonist (CP-55940) stimulated [³⁵S]GTPγS binding was reduced significantly in SPM from chronic EtOH-exposed mice without any significant changes in the G-protein affinity (Basavarajappa and Hungund, 1999b). The CP-55940 stimulated [³⁵S]GTPγS binding was blocked by the CB₁ receptor antagonist, SR-141716A (Basavarajappa and Hungund, 1999b) (Table 2). These results suggest that the observed downregulation of CB₁ receptors by chronic EtOH has a profound effect on desensitization of cannabinoid-activated signal transduction similar to the one observed for THC.

Table 2. Effect of chronic ethanol (EtOH) exposure on CB₁ receptors and CB₁ receptor agonist stimulated GTPγS binding in synaptic plasma membrane (SPM) from mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB₁ receptors&lt;br&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bₘₐₓ (pmol/mg protein)</td>
<td>12.00 ± 0.30</td>
<td>7.00 ± 0.25*</td>
</tr>
<tr>
<td>Kᵦ (nM)</td>
<td>2.30 ± 0.20</td>
<td>3.00 ± 0.30</td>
</tr>
<tr>
<td>Kᵦ (μM) (AnNH with PMSF)</td>
<td>1.61 ± 0.02</td>
<td>0.13 ± 0.01*</td>
</tr>
<tr>
<td>G-protein&lt;br&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bₘₐₓ (pmol/mg protein)</td>
<td>7.58 ± 0.22</td>
<td>6.42 ± 0.20*</td>
</tr>
<tr>
<td>Kᵦ (nM)</td>
<td>2.68 ± 0.24</td>
<td>3.42 ± 0.31</td>
</tr>
<tr>
<td>Eₘₐₓ (% basal binding) (CP-55940)</td>
<td>175 ± 5.25</td>
<td>150 ± 8.14*</td>
</tr>
<tr>
<td>Eₑₘₓ,ₑₕₐₜ (μM) (CP-55940)</td>
<td>0.20 ± 0.08</td>
<td>0.54 ± 0.10*</td>
</tr>
<tr>
<td>Iₐₒₜ (μM) (SR-141716A)</td>
<td>0.04 ± 0.003</td>
<td>0.021 ± 0.005*</td>
</tr>
</tbody>
</table>

*Basavarajappa et al. (1998a); Basavarajappa and Hungund (1999b).

Changes in G-protein activity and G-protein levels after chronic drug treatment have been reported for various receptor systems (Suzdak et al., 1986; Werling et al., 1988; Wand et al., 1993; Williams et al., 1993; Tabakoff et al., 1995; Traynor and Nahorski, 1995). A number of in vitro and in vivo studies have demonstrated that chronic EtOH treatment leads to reduced sensitivity of adenyl cyclase (Gordon et al., 1986; Charness et al., 1988). It was reported that a variety of agonists acting at various receptors coupled through G-proteins to adenyl cyclase have been shown to be reduced by EtOH (Rabin, 1990). Such a modification was suggested to alter the ability of the enzymes to interact with G-proteins and G-protein coupled receptors (Tabakoff et al., 1995). Regulation of either G-protein or G-protein mRNA level by chronic EtOH treatment is also a possibility. There have also been studies showing a decrease in adenyl cyclase activity (Deitrich et al., 1989; Tabakoff et al., 1995) and a two- to fourfold increase in Gi levels, but no changes in Gαi in brains of mice treated chronically with EtOH (Wand et al., 1993). Further studies along these lines will reveal the function of AnNH and CB₁ receptors and the role played by the CB₁ receptor signal transduction pathway in mediation of the pharmacological and behavioural effects of EtOH. If EtOH affects particular regions of the brain selectively, the findings will have a greater significance in terms of explaining the behavioural effects of the drug.

Studies with SR-141716A, a potent and selective antagonist of the brain CB₁ receptors

Little is known about the interaction of EtOH and CB₁ receptor function, other than for recent reports (Basavarajappa et al., 1998a; Basavarajappa and Hungund, 1999a,b) and other reports describing a linkage between CB₁ receptor function and alcohol consumption (Arnone et al., 1997; Colombo et al., 1998). In these studies, it was shown that the administration of the CB₁ receptor antagonist SR-141716A, reduced consumption of EtOH in two different EtOH-prefering rodent models (C57BL/6 mice and Sardinian sp rats). Thus, it is suggested that the cannabinoid system may mediate the reward mechanism associated with alcohol consumption. It has also been demonstrated that the CB₁ receptor antagonist, SR-141716A, when administered orally to animals, antagonized hypothermia, ring immobility, and antinociception produced by the CB₁ receptor agonists CP-55940 and WIN-55212-1. It antagonized the inhibitory effect of the CB₁ agonist on both mouse vas deferens contraction and adenyl cyclase activity in rat brain membranes (Rinaldi-Carmona et al., 1994, 1995; Cook et al., 1998; Welch et al., 1998). Many of these behavioural effects are similar to those produced by EtOH (Kalant and LeBlanc, 1974). Thus, these behavioural effects may be related to the antagonist’s effect produced by its action on agonist-induced inhibition of the adenyl cyclase (AC)–cAMP system in the signal transduction pathway. Recently, SR-141716 has also been shown to inhibit the motivation to consume alcoholic beverages in rats (Gallate and McGregor, 1999). These findings provide further support for the hypothesis that some of the pharmacological actions of EtOH may be mediated through CB₁ receptors.

Neuromodulatory role of AnNH and CB₁ receptors; physiological implications

AnNH has been suggested to meet the criteria of a neurotransmitter. AnNH activates the brain CB₁ receptor and has
putative mechanisms for biosynthesis and inactivation via re-uptake and intracellular degradation (Self, 1999). Unlike other known neurotransmitters, it is stored in membrane lipids, rather than in the vesicles, and is released by phospholipase-mediated cleavage, followed by passive diffusion across the plasma membrane.

Neuropharmacological studies have established an important role for the dopaminergic system in the reinforcing effects of drugs of abuse including EtOH (Koob et al., 1998 and references therein). Neurochemical evidence indicates that at pharmacologically relevant doses, EtOH activates the mesolimbic dopaminergic system (Brodie et al., 1990; Weiss et al., 1996; Fadda and Rossetti, 1998). Withdrawal from chronic EtOH treatment is associated with a profound decrease in dopamine (DA) release (Darden and Hunt, 1977; Rossetti et al., 1996; Fadda and Rossetti, 1998). Although the actions of AnNH on the dopaminergic system are not clear at this time, AnNH has been shown to inhibit synthesis and release of DA in the striatum and to potentiate in dopaminergic neurons of the tuberoinfundibular system (Di Marzo et al., 1992). Modulation of DA and GABA-mediated neurotransmission by AnNH in the basal ganglia were related to the inhibition of motor activity (Mechoulam et al., 1995; Mechoulam and Fride, 1995). These modulations include a direct inhibition of striatal DA release, inhibition of DA D2 or D3 receptor-mediated neurotransmission through reversal of adenyllyl cyclase inhibition or activation, respectively (Di Marzo et al., 1998). Whether such a mechanism through participation of AnNH can explain the development of tolerance to EtOH remains to be established.

The CB1 agonist WIN-55211-2 blocks acetylcholine (ACh) (but not GABA) release from hippocampal slices (Gifford and Ashby, 1996). This suggests that endogenous cannabinoids tonically inhibit the release of ACh. Chronic EtOH treatment has also been shown to decrease ACh content and the activities of both choline acetyltransferase (ChAT) (an enzyme of ACh synthesis) and acetylcholinesterase (AChE) (an enzyme which degrades ACh) (Arendt et al., 1988b). These results could provide an important molecular link for the CB1 receptor-mediated impairment of working memory by AnNH as well as for EtOH (Gifford and Ashby, 1996; Di Marzo et al., 1998). Chronically, EtOH and AnNH have been shown to inhibit the release of glutamate, long-term potentiation (LTP) and NMDA-mediated synaptic neurotransmission (Castellano et al., 1997; Fadda and Rossetti, 1998).

These studies suggest a possible interaction between CB1 receptors in specific brain regions and other neurotransmitters and in modulation of neurotransmitter systems involved in control of neuronal functions.

GENERAL CONCLUSIONS AND COMMENTS

In summary, endocannabinoid–CB1 receptor research as such is in its early infancy and the literature relating to interaction between EtOH and endocannabinoids and CB1 receptors is sparse. The evidence presented here for the first time suggests that: (a) chronic EtOH treatment downregulates CB1 receptors in brain SPM; (b) chronic EtOH treatment leads to increased accumulation of the endocannabinoid AnNH in both in vitro and in vivo models; (c) treatment with the CB1 receptor antagonist SR-141716A leads to reduced consumption of EtOH in EtOH-prefering strains of rodents, strongly supporting the participation of an AnNH–CB1 receptor system in the pharmacological and behavioural actions of EtOH including tolerance development. Furthermore, the reported study on downregulation of CB1-receptor agonist stimulated [35S]GTPγS in EtOH-tolerant mouse SPM suggests that the endocannabinoid–CB1 receptor signal transduction pathway may be involved in EtOH actions. However, further studies are necessary to elucidate the exact mechanism by which the endocannabinoid–CB1 receptor system modulates the pharmacological and behavioural actions of EtOH.

This review of the current literature provides many opportunities to explore the participation of the endocannabinoid–CB1 receptor signal transduction mechanism, not only in tolerance development to EtOH, but also in many aspects of alcohol misuse and alcoholism, including the fetal alcohol syndrome. Furthermore, establishing the brain regional levels and distribution of both endocannabinoids and CB1 receptors will be of great utility in understanding their physiological and functional roles in several neurological disorders (Consroe, 1998) including behavioural neuroadaptation to EtOH. Such studies may also lead to the development of medications for treatment of problems associated with abusive drinking and alcoholism.

REFERENCES


Abbreviations used: AA, arachidonic acid; AAPC, arachidonoyl-PC; AnNH, anandamide; CB, cannabinoid receptors; DA, dopamine; EtOH, ethanol; FAEE, fatty acid ethyl esters; LTP, long-term potentiation; NAA, N-acetyl ethanolamide; NPE, N-acetyl ethanolamine; N-ArPE, N-arachidonylethanolamine; PC, phosphatidylcholine; PL, phospholipid; PLA, phospholipase A; PLD, phospholipase D; PMSF, phenylmethylsulphonyl fluoride; PTX, pertussis toxin; SPM, synaptic plasma membranes; THC, tetrahydrocannabinol.