Colchicine Is a Competitive Antagonist at Human Recombinant γ-Aminobutyric Acid A Receptors1

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ABSTRACT

Colchicine is an alkaloid that is used clinically in the treatment of arthritic gout. This potent microtubule disrupting agent has also been used extensively as an experimental tool in studies characterizing the role of the cytoskeleton in a variety of cellular processes. Colchicine has also been used as a selective neurotoxin and in animal models of Alzheimer’s disease and epilepsy. Although the mechanism(s) mediating the neurotoxic actions of colchicine have not been established, most studies have attributed these effects to its microtubule depolymerizing actions. Here we report another central nervous system action of colchicine, competitive antagonism of γ-aminobutyric acid (GABA)A receptor function. By use of a rapid drug perfusion system, colchicine (10–1000 μM) significantly inhibited GABA currents recorded from L(tk-1) cells stably transfected with human α1β2γ2L GABA A receptor subunits. The inhibition was rapid and reversible, with 100 μM colchicine shifting the GABA EC50 from 2.5 to 5.1 μM with no effect on currents evoked by saturating concentrations of GABA. Colchicine also significantly inhibited binding of the competitive GABA A receptor antagonist [3H]SR-95531. Other microtubule disrupting agents (10 μM vinblastine, 10 μg/ml nocodazole, 1 μM taxol) had no acute effects on GABA currents, nor did the inactive analog γ-lumicolchicine (100 μM). Moreover, pretreating cells with colchicine, vinblastine, nocodazole or taxol for 1 to 4 hr did not occlude the acute inhibitory action of colchicine. We conclude that, in addition to its well characterized effects on microtubule assembly, colchicine can also inhibit GABA A receptor function through a direct interaction with the receptor/ion channel complex.

Colchicine is a plant-derived alkaloid that binds to tubulin and depolymerizes microtubules (Osborn and Weber, 1976; Walker and Whitfield, 1985), disrupts axonal transport (Karlsson and Sjostrand, 1969; Fink et al., 1973; Wooten et al., 1975) and inhibits mitosis (Wilson and Friedkin, 1966, 1967; Wang et al., 1975). This compound has been used clinically in the treatment of gout (Hastie, 1991) and has also been used extensively as an experimental tool to characterize cellular processes that involve microtubule structure and axoplasmic transport (Chiaia et al., 1996; Schmalz et al., 1996; Tandon et al., 1996; Saib et al., 1997). Numerous studies have also used colchicine as a neurotoxin to cause lesions in discrete brain regions, such as the dentate gyrus (Brady et al., 1992; Newell et al., 1993; Tandon et al., 1994; Gobbi et al., 1996) and the septum (Peterson and McGinty, 1988; Gilbert and Peterson, 1991), and several recent studies have suggested that colchicine neurotoxicity may model some of the neuropathological aspects of Alzheimer’s dementia (Nakagawa et al., 1987; Mattson, 1992). In all of these cases, the mechanism of colchicine action has been attributed to its inhibition of tubulin binding and subsequent disruption of processes that depend on the integrity of cytoskeletal architecture. However, the mechanism(s) underlying these diverse actions of colchicine have not been determined experimentally.

Several studies have also demonstrated that colchicine can significantly inhibit the function of several ion channels, including voltage-gated sodium (Matsumoto et al., 1984) and calcium (Johnson and Byerly, 1993) channels and ligand-gated nicotinic (Hardwick and Parsons, 1995) and GABA A receptor-gated ion channels (Whatley et al., 1994; Whately and Harris, 1996). The mechanism(s) through which colchicine exerts these effects are unclear, but have been hypothesized to involve the depolymerizing actions of this drug.

We have characterized the interaction of colchicine and other microtubule disrupting agents with recombinant GABA A receptors in a stable transfection system to further delineate the mechanism(s) through which microtubule depolymerization might inhibit GABA A receptor function. Here we report that colchicine appears to have an additional direct action on the GABA A receptor that may play a role in the neurotoxic effects of this drug.
inhibitory effect on GABA<sub>A</sub> receptor function, one that is independent of the microtubule disrupting actions of this drug. Unlike the indirect inhibition of GABA<sub>A</sub> receptor function that appears to be related to effects on microtubules (Whatley et al., 1994; Whatley and Harris, 1996), this direct effect is rapid and competitive, and it is not mimicked or occluded by other microtubule disrupting agents.

Materials and Methods

Cell culture conditions. Stable transfection of mouse L (tk<sup>−</sup>) cells with human GABA<sub>A</sub> receptor subunits was carried out as described previously (Hadingham et al., 1992). Expression was controlled by a dexamethasone-sensitive promoter. Cells were grown to confluence in 75-ml flasks and then plated onto 12-mm-diameter glass coverslips coated with poly-L-lysine with Dulbecco's Modified Eagle's Medium (DME/high glucose, Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Gemini Bio-Product, Calabasas, CA), 100 U/ml penicillin, 0.1 mg/ml streptomycin (Sigma Chemicals, St. Louis, MO) and 2 mM l-glutamine (Dexter CO/Gibco Labs Division, Grand Island, NY). Cells were grown on coverslips for 24 to 48 hr at 37°C/5% CO<sub>2</sub>, treated with 1 µM dexamethasone (Sigma Chemical, St. Louis, MO) and then grown for an additional 3 to 6 days.

Electrophysiology. Coverslips were transferred to a recording chamber perfused with a HEPES-buffered external solution (in mM): NaCl, 130; KCl, 5; CaCl<sub>2</sub>, 2; MgCl<sub>2</sub>, 1; HEPES, 10; d-glucose, 11; pH adjusted to 7.4 with NaOH; 300–310 mMTris. Whole-cell patch recordings were made from individual cells with borosilicate glass electrodes (1.5 mm outer diameter, 0.86 mm internal diameter, Sutter Instruments, Novato, CA). The intracellular recording solution contained (in mM): KCl, 130; CaCl<sub>2</sub>, 0.1; EGTA, 1.0; Mg-ATP, 2; Tris-GTP, 0.2; HEPES, 10; pH adjusted with KOH; 275–285 mMTris. Currents were recorded with an Axopatch 200 amplifier (Axon Instruments, Foster City, CA), low pass filtered with a 4 pole Bessel Filter (2 kHz) and analyzed on- and off-line with software developed in this laboratory. All external solutions were gravity fed from glass 12-cc syringe reservoirs through a two-barrel flow tube array (450 µm internal diameter, Polymicro Tech. Inc., Phoenix, AZ). The flow tube array was affixed to a piezoelectric double ceramic plate (Bimorph) (Morgan Matroc, Inc., Bedford, OH). By applying a voltage across the Bimorph, the solution interface across the surface of the cell being recorded could be rapidly exchanged (approximately 100 msec half-time). Cells were voltage-clamped at –40 to –60 mV under our recording conditions, were completely antagonized by 20 µM bicuculline (data not shown) and were therefore mediated by the activation of GABA<sub>A</sub> receptors.

The effect of colchicine was initially determined on currents evoked by a half-maximal concentration of GABA (2 µM). Colchicine was applied continuously for a 10-min period, during which GABA currents were evoked every 30 sec. With this protocol, 100 µM colchicine caused a rapid inhibition of GABA-evoked currents (fig. 1). The mean inhibition was 63 ± 5% (n = 6; P < .01, paired t test) and both the onset and washout of the inhibition exhibited a latency of less than 1 to 2 min, which was similar to the time required to introduce a new drug through the drug delivery system. To determine whether the continuous colchicine application was necessary to observe the inhibition, a protocol was used in which 100 µM colchicine was applied concurrently with the GABA applications (fig. 1). The inhibition observed with this protocol (61 ± 2%, n = 13) was not statistically different from that observed with continuous application of colchicine (P > .5, unpaired t test).

To further resolve the time course of colchicine inhibition, we next used a protocol in which the solution bathing the cell was stepped from a low concentration of GABA (200–500 nM) to one containing the same concentration of GABA plus 100 µM colchicine. The latency of the resulting change in current amplitude was therefore limited only by the kinetics associated with colchicine inhibition and the time required to exchange the solution interface across the cell being recorded. With this protocol, colchicine inhibited the GABA-evoked currents with onset and offset half-times of approximately 140 msec (fig. 2A). Although the rapid perfusion system used in this study could effectively exchange the solution at the tip of the electrode with a half-time of <10 msec, the time required to equilibrate a solution exchange across a whole cell was considerably slower (50–100 msec). We therefore compared the kinetics of colchicine inhibition with that induced by an equieffective concentration of bicuculline methiodide, a well-characterized competitive antagonist of GABA<sub>A</sub> receptors (fig. 2B). No difference was observed between the onset
Colchicine inhibition of GABA-evoked currents is dose-dependent and competitive. A separate series of experiments were carried out to characterize the pharmacological characteristics of the colchicine antagonism of GABA receptor-mediated responses. With use of the concurrent application protocol, the colchicine inhibition of currents evoked by 2 μM GABA was dose-dependent, with an EC50 of 56 μM and a Hill coefficient of 1.2 (fig. 3). The lowest concentration of colchicine that significantly inhibited currents evoked by 2 μM GABA was 10 μM (14 ± 2%, P < .01, paired t test, n = 7), and a concentration of 1 mM colchicine almost completely antagonized these currents.

The acute effect of 100 μM colchicine was next tested across a full range of GABA concentrations. Colchicine produced greater than 80% inhibition of currents evoked by an EC10 concentration of GABA but had almost no effect at saturating GABA concentrations (fig. 4A). Colchicine significantly shifted the GABA dose-response curve to the right, and offset kinetics of bicuculline inhibition and that of colchicine.
increasing the GABA EC_{50} from $2.5 \pm 0.3 \mu M$ to $5.1 \pm 0.5 \mu M$ (paired t test, $P < .001$, $n = 6$) with no significant change in the Hill coefficient (control $= 1.2 \pm 0.1$; colchicine $= 1.3 \pm 0.2$, $P > .3$, paired t test, $n = 6$). The estimated pA_{2} value for colchicine was 4.02, corresponding to an IC_{50} of 96 \mu M.

To confirm that colchicine was a competitive antagonist at the GABA binding site, the effect of colchicine on [3H]SR 95531 binding to membranes of cells expressing \textalpha 1\gamma 1\delta 2L \textbeta GABA_A receptor subunits was determined (table 1). Colchicine (100 \mu M) significantly increased the $K_{i}$ of [3H]SR 95531 binding (control $= 37 \pm 3$ nM; colchicine $= 101 \pm 9$ nM; $P < .001$) with no effect on the $B_{\text{max}}$ (control $= 0.4 \pm 0.1$; colchicine $= 0.6 \pm 0.1$) or Hill coefficient (0.9 $\pm$ 0.1; colchicine $= 0.9 \pm 0.1$; table 1). The estimated $K_{i}$ value for colchicine calculated from the binding assay was 58 \mu M.

The colchicine inhibition does not involve the benzodiazepine binding site. \textbeta-Lumicolchicine is a structural analog of colchicine that has no effect on microtubules (Wilson and Friedkin,
GABAA receptor function observed with colchicine was mediated by et al., 1994). We therefore determined whether the antagonism of GABA-evoked currents (fig. 6). We also tested another inactive an-
GABA. None of these compounds had any significant effect on

\[ P, 1 \text{m} \] significantly potentiated currents evoked by 1 \text{m} 

used for colchicine. We tested the acute actions of 10 \text{microtubule disrupting agents were also tested by the same protocol 
exclude the acute actions of colchicine.

The acute effects of other 
experiments, however flumazenil had no effect on the colchicine 
interaction with the benzodiazepine site on GABAA receptors (Mihic
enhancement was completely blocked by flumazenil. Colchicine (100 
M) significantly inhibited GABA currents, as observed in the initial 
mean of 147 \pm 4\% (P < .001, paired t test) (fig. 7).

In a second set of experiments, we tested whether prolonged treatment with other microtubule disrupting agents could occlude the acute effects of colchicine. In preliminary experiments, continu-
ous application of 10 \mu g/ml nocodazole or 1 \mu M taxol for 20 to 30 min had no effect on GABA-evoked currents. However, a 20-min application of 100 \mu M vinblastine did significantly inhibit these responses (Whatley et al., submitted). These disparate effects may reflect diff-
differences in the time required for these compounds to depolymerize microtubules under our recording conditions. Because the microtubu-
le depolymerizing actions of these drugs require at least 60 min (Whatley and Harris, 1996; Whatley et al., submitted) and we cannot 
routinely maintain stable recordings for that long, cells were prein-
cubated with 10 \mu g/ml nocodazole, 1 \mu M taxol or 100 \mu M vinblastine 
for 1 to 4 hr before electrophysiological recordings to ensure that 
these compounds had sufficient time to maximally depolymerize microtubules (Whatley and Harris, 1996; Whatley et al., submitted).

After the drug pretreatments, the effect of concurrent application of 100 \mu M colchicine and 2 \mu M GABA was assayed in the continuous 
presence of these compounds. None of these pretreatments had an 
effect on the inhibitory action of 100 \mu M colchicine on GABA-evoked 
currents (fig. 7).

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( K_D )</th>
<th>( B_{max} )</th>
<th>Hill Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.0 ± 2.6</td>
<td>0.4 ± 0.1</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td>Colchicine (100 \mu M)</td>
<td>101.0 ± 9.4</td>
<td>0.6 ± 0.1</td>
<td>0.91 ± 0.04</td>
</tr>
</tbody>
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\( ^a \) Values are mean ± S.E.M. of 3 to 12 experiments, each carried out in triplicate. 
\( ^b \) P < .001.

1967; Walker and Whitfield, 1985). A previous report demonstrated that this analog potentiated GABAA receptor function by a direct interaction with the benzodiazepine site on GABAA receptors (Mihic et al., 1994). We therefore determined whether the antagonism of GABAA receptor function observed with colchicine was mediated by an interaction with this same site. Under the recording conditions used in this study, acute application of 100 \mu M \beta-lumicolchicine significantly potentiated currents evoked by 1 \mu M GABA in cells expressing \( \alpha 1\beta 2\gamma 2 \) GABAA receptor subunits (fig. 5). The mean potentiation was 147 \pm 4\% \( (P < .001, \text{paired } t \text{ test}, n = 5) \) and this enhancement was completely blocked by flumazenil. Colchicine (100 \mu M) significantly inhibited GABA currents, as observed in the initial experiments, however flumazenil had no effect on the colchicine inhibition (fig. 5).

**Other microtubule disrupting agents do not mimic or occlude the acute actions of colchicine.** The acute effects of other microtubule disrupting agents were also tested by the same protocol used for colchicine. We tested the acute actions of 10 \mu M vinblastine, 1 \mu M taxol and 10 \mu g/ml nocodazole on currents evoked by 2 \mu M GABA. None of these compounds had any significant effect on GABA-evoked currents (fig. 6). We also tested another inactive an-
olog of colchicine, \gamma-lumicolchicine, which was also without effect on GABA-evoked currents.

We next tested whether microtubule depolymerization could occlude the acute inhibitory effect of colchicine on GABAA receptor 
function. In an initial series of experiments, cells were preincubated 
with 100 \mu M colchicine for 1 to 4 hr, a protocol that has previously 
been shown to maximally depolymerize microtubules in these cells (Whatley and Harris, 1996; Whatley et al., submitted). Cells were then patch-clamped with the standard intracellular recording solu-
tion in the continuous presence of colchicine. After obtaining a stable base-line current evoked by 2 \mu M GABA, the colchicine was then washed off. This resulted in a rapid potentiation of the current presumably caused by a washout of the acute inhibitory effect of colchicine. Once a new base line was obtained (2–4 min), the acute effect of 100 \mu M colchicine was assessed. No difference was observed between the acute effect of 100 \mu M colchicine on control cells and 
cells pretreated with colchicine. (The inhibition of the GABA re-

tion in the continuous presence of colchicine. After obtaining a stable base-line current evoked by 2 \mu M GABA, the colchicine was then washed off. This resulted in a rapid potentiation of the current presumably caused by a washout of the acute inhibitory effect of colchicine. Once a new base line was obtained (2–4 min), the acute effect of 100 \mu M colchicine was assessed. No difference was observed between the acute effect of 100 \mu M colchicine on control cells and 
cells pretreated with colchicine. (The inhibition of the GABA re-

\[ 100 \mu M \text{ GABA}, \text{the colchicine was then } 6, P < .001 \text{, unpaired } t \text{ test.} \] (fig. 7).

Fig. 5. Colchicine inhibition does not in-
volve the benzodiazepine binding site. (A) 
Representative traces illustrating the ef-
fect of 1 \mu M flumazenil on the enhance-
ment of GABA responses induced by 100 
\mu M \beta-lumicolchicine and on the antago-
nism of GABA responses induced by 100 
\mu M colchicine. (B) Bar graph summarizing 
the effect of 100 \mu M \beta-lumicolchicine and 100 
\mu M colchicine on GABA-evoked cur-
rents in the presence and absence of 1 \mu M 
flumazenil. \( n = 3–7 \) cells/group. Note that 
flumazenil completely antagonized the 
\beta-lumicolchicine-induced potentiation 
of GABA-evoked currents but had no effect 
on the colchicine-mediated inhibition.
Discussion

The results of this study demonstrate that colchicine can act as a competitive antagonist at GABAA receptors through a mechanism that appears to be distinct from the effects of this drug on microtubule depolymerization. Two studies have previously demonstrated that colchicine and other microtubule depolymerizing agents can inhibit GABAA receptor function in cerebral cortical microsacs, in *Xenopus* oocytes transiently transfected with recombinant GABAA receptor subunits (Whatley et al., 1994) and in cells stably transfected with GABAA receptor subunits (Whatley et al., submitted). In both of these studies, this inhibition was attributed to the depolymerizing actions of these compounds on microtubule structure. However, the time course and characteristics of the inhibition were significantly different. In microsacs and oocytes, 100 μM colchicine inhibited GABAA receptor function in as little as 3 to 20 sec and the inhibition appeared to be competitive. Other microtubule depolymerizing drugs also inhibited GABAA receptor-mediated chloride flux, although their actions were only characterized at 30 to 60 min. In contrast, in chloride flux studies of the stably transfected cell line characterized in the present study, 100 μM colchicine required 60 min to significantly inhibit GABAA receptor function, and this inhibition was noncompetitive, having no effect on GABAA receptor affinity. Moreover, using immunolabeling of tubulin, it was shown that colchicine did not appreciably depolymerize microtubule structure for at least 30 min. Based on the results of these studies, it is clear that colchicine (but not the other depolymerizing agents) has a direct, competitive inhibitory interaction with the GABAA receptor.
that is apparent within 100 msec of colchicine application, and does not involve the microtubule depolymerizing actions of this drug. In contrast, the noncompetitive inhibitory effects of colchicine and other similar agents that are observed at longer treatment intervals (>60 minutes) are likely to involve a mechanism related to the depolymerizing actions of these drugs.

Several lines of evidence indicate that the inhibition of GABA<sub>أ</sub> receptor function observed in this study did not involve the microtubule depolymerizing actions of colchicine. First, the time course of the colchicine inhibition of GABA-evoked currents is inconsistent with the microtubule depolymerizing actions of this drug (Whatley and Harris, 1996; Whatley et al., submitted). With concurrent application of colchicine and GABA, the inhibition developed with a half-time of approximately 140 msec, whereas colchicine-mediated microtubule depolymerization in these cells required at least 30 min (Whatley et al., submitted). In addition, within the temporal resolution of the drug delivery system used in this study, no difference was observed between colchicine antagonism of GABA-evoked currents and that produced by equieffective concentrations of bicusculine methiodide, a competitive GABA<sub>أ</sub> receptor antagonist. Second, the effects of colchicine in the present study were characterized by a competitive interaction with GABA, whereas the inhibition attributed to depolymerization produces a noncompetitive decrease in GABA efficacy with no change in receptor affinity for GABA (Whatley et al., submitted). Third, the rapid inhibitory effect of colchicine on GABA-evoked currents was not observed with other microtubule disrupting agents like nocodazole, vinblastine and taxol. In contrast, all three of these compounds have been shown to significantly inhibit GABA<sub>أ</sub> receptor function when applied for durations that are sufficient to depolymerize microtubule assembly (Whatley et al., 1994; Whatley and Harris, 1996). Finally, prolonged incubation of cells in colchicine or other microtubule depolymerizing drugs for durations that were long enough to fully depolymerize microtubules in these cells (Whatley and Harris, 1996; Whatley et al., submitted) had no effect on the rapid colchicine inhibition of GABA<sub>أ</sub> receptor function, which again suggests the independence of these two actions.

The results of this study suggest that colchicine is a competitive antagonist at GABA<sub>أ</sub> receptors, an action that is independent from the noncompetitive colchicine inhibition associated with the microtubule depolymerizing actions of this drug. Presently, the mechanism by which colchicine exerts this effect is not known. Colchicine may inhibit GABA<sub>أ</sub> receptor function by direct interaction with the GABA binding site or alternatively it may modulate GABA affinity via an allosteric interaction with another site on the GABA<sub>أ</sub> receptor or even with some protein closely associated with it. This latter possibility, that colchicine is acting as an allosteric, apparent competitive antagonist, is supported by the observation that colchicine had no effect on GABA<sub>أ</sub> receptors reconstituted into proteoliposomes (Whatley et al., 1994) where any proteins that might normally associate with these receptors in neurons would not be present. There is evidence that a structural analog of colchicine, β-lumicolchicine, can interact directly with the benzodiazepine binding site on GABA<sub>أ</sub> receptors (Mihic et al., 1994). This analog does not bind tubulin and is therefore often used as a control for the microtubule disrupting actions of colchicine. However, β-lumicolchicine was shown to compete with flunitrazepam binding in cerebral cortical microsacs and potentiate muscimol-stimulated chloride flux in microsacs and GABA-evoked currents in Xenopus oocytes stably transfected with recombinant GABA<sub>أ</sub> receptor subunits (Mihic et al., 1994). In addition, the potentiation of GABA-evoked currents was completely antagonized by flumazenil, a competitive benzodiazepine antagonist. In the present experiments, similar effects of β-lumicolchicine on GABA-evoked currents were observed, but the acute inhibitory effect of colchicine was not antagonized by flumazenil. This suggests that colchicine and β-lumicolchicine, despite being structural analogs, do not interact with the same site on the GABA<sub>أ</sub> receptor complex.

In preliminary experiments, continuous application of microtubule disrupting drugs such as nocodazole and taxol for up to 30 min did not significantly inhibit GABA-evoked currents. Similar treatments with colchicine did inhibit GABA<sub>أ</sub> receptor function; however, this inhibition could all be attributed to the acute, competitive inhibition that appears to be independent of colchicine's microtubule depolymerizing effects. In contrast, a 20-min continuous application of vinblastine did significantly inhibit GABA responses (Whatley et al., submitted). These disparate findings may reflect differences in the depolymerization kinetics of these compounds under our experimental conditions; however, incubating cells for up to 4 hr with these compounds, an interval sufficient to ensure maximal microtubule depolymerization (Whatley and Harris, 1996; Whatley et al., submitted), had no effect on the acute inhibitory action of colchicine.

Colchicine has been used as an experimental tool in numerous studies to characterize the role of microtubule assembly in various aspects of cellular function (Chiaia et al., 1996; Schmalz et al., 1996; Tandon et al., 1996; Saib et al., 1997) and as a selective neurotoxin in studies of epilepsy (Lee and Hong, 1990; Barnes and Mitchell, 1993; Gobbi et al., 1996; and Alzheimer's disease (Nakagawa et al., 1987; Mattson, 1992). The mechanism underlying the neurotoxic effects of colchicine is not known, but has usually been attributed to its ability to disrupt microtubule assembly, because structural analogs of colchicine, such as β- and γ-lumicolchicine, that do not bind tubulin, do not mimic the neurotoxic effects of colchicine.

Our findings suggest that at least some of the neurotoxic actions of colchicine may be a result of direct inhibition of GABA<sub>أ</sub> receptor function. This is particularly likely in those studies in which colchicine was injected directly into specific brain regions to induce seizures (Lee and Hong, 1990; Barnes and Mitchell, 1993; Gobbi et al., 1996). Other competitive GABA<sub>أ</sub> receptor antagonists can elicit seizures in vivo and frequently have been used in models of epileptogenesis (Uemura and Kimura, 1988; Catalpe et al., 1995; Reigel and Bourn, 1995). In addition, some of the neuropathological actions of colchicine on the phosphorylation state of the microtubule-associated protein, tau, are also observed with glutamate administration (Nakagawa et al., 1987; Mattson, 1992) and are not mimicked by other microtubule disrupting agents (Sygowski et al., 1993). These effects may stem from a generalized increase in neuronal excitability resulting, in the case of glutamate, from direct activation of (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and/or N-methyl-d-aspartate receptors and, in the case of colchicine, from...
acute inhibition of GABA<sub>A</sub> receptor function. Preliminary experiments from our laboratory have shown that colchicine, at concentrations used in this study, can significantly depress GABA<sub>A</sub> receptor-mediated synaptic currents in rat hippocampal CA1 neurons. These findings further support the hypothesis that direct antagonism of neuronal GABA<sub>A</sub> receptor function may underlie some of colchicine's neurotoxic actions.

Our results also suggest that the use of structural analogs of colchicine that do not bind tubulin may not be sufficient to demonstrate that effects of colchicine are mediated by microtubule depolymerization. Because these analogs either potentiate (γ-lumicolchicine) or have no acute effect (α-lumicolchicine) on GABA<sub>A</sub> receptor function, they would not mimic the effects of colchicine that reflect its acute inhibitory action on GABA<sub>A</sub> receptor function. Because other microtubule disrupting agents, such as nocodazole, taxol and vinblastine, do not share this acute inhibitory effect on GABA<sub>A</sub> receptor function, these agents may prove more useful in identifying the actions of colchicine that are mediated by depolymerization of microtubules. A detailed understanding of the cellular substrates that underlie colchicine-mediated neurotoxicity is needed to evaluate and interpret the results of studies that use this compound in animal models of complex disease states such as epilepsy and Alzheimer's disease. Further studies will be needed to determine the extent to which competitive antagonism of GABA<sub>A</sub> receptors contributes to the neuropathological actions of colchicine.

References


