CHAPTER V

DISCUSSION

In the present study, adopting GBL as a practical substitute for GHB is based on specific reasons. Firstly, GHB by itself is in a list of Schedule I controlled substance in Thailand. Secondly, National Toxicology Program (NTP) reviewed that GBL is converted to GHB very rapidly. Because of the more rapid absorption of less polar lactone form in GBL compared with that of free acid form in GHB, bioavailability of GHB as a metabolite of GBL is greater than that observed after administration of Na-GHB. Therefore, the evaluation of GBL was in fact an evaluation of GHB (Irwin, 2006).

Previous animal experiments indicated similarities in the neuropharmacological action of GHB, GBL, and THF. Marcus et al. (1976) reported that THF (21 mmol/kg i.p.) induced high amplitude EEG, slow wave activity, loss of righting reflex, myoclonic jerks and vibrissae movements to tactile stimulation, in rats. In addition, THF induced progression of EEG and behavioral changes characteristic of generalized non-convulsive epilepsy similar to that produced by 4-hydroxybutyric acid (GHB) and butyrolactone (GBL). Acute toxicity of THF could lead to narcosis, muscular hypotonia, and disappearance of corneal reflexs, followed by coma and death (Hazardous Substances Databank, 2002). However, detailed comparative studies of THF and GBL had not been published so far.

Locomotion is a complex behavior affected by many different brain systems, including the telencephalic dopaminergic system and the cerebellum, as well as by peripheral abnormalities (i.e. muscle weakness). Because locomotor activity is required for many behavioral tasks, increases or decreases in locomotor activity nonspecifically affect performance in many behavioral tests and should be measured before any other behavioral characterization is performed (Karl, Pabst, and von Horsten, 2003). In our study, locomotor activity was analyzed and considered as a possible confounder in all behavioral tests.

In addition to locomotor activity test, the rotarod test is the widely used analysis of neuromotor performance in which motor coordination, balance, and ataxia can be tested. This behavioral task measures the ability of the mouse to maintain balance on a rotating rod, therefore the fore- and hind limb motor coordination and

balance can be analyzed. Rotarod performance requires an intact cerebellar function and motor coordination. Mice with severe motor coordination problems will have difficulties to remain on the rotating rod (Karl et al., 2003). Therefore, we used rotatrod test that was more sensitive and reproducible than loss of righting reflex test to study various pharmacological aspects of THF and GBL in details.

Our study compared effects of THF and GBL on behavioral models in ICR mice when administered in equivalent doses on a mmol/kg basis (i.e., 1 mmol/kg for THF equates to 72.11 mg/kg and 86.09 mg/kg for GBL). We compared the TD_{50} of GBL and THF generated by the dose-response curves from the righting reflex and the rotarod test. The TD_{50} values of THF and GBL for the loss of righting reflex were 15.18 mmol/kg and 4.60 mmol/kg, respectively. The TD_{50} values of THF and GBL for the failure in rotarod test were 7.00 mmol/kg and 0.85 mmol/kg, respectively. Therefore, the present study clearly indicated that GBL is more potent than THF in producing the loss of righting reflex and the failure to perform rotarod test. Guidotti and Ballotti (1970) and Arena and Fung (1980) provided evidence that GBL was lipophilic and could easily traverse the cell membrane mainly because of its cyclic structure. Further, the lactone could also be taken up from the blood into the lean body mass rapidly and efficiently, which then served as a depot pool for the drug that would be metabolized more slowly, as it would not be readily accessible to plasma and liver lactonases.

Moody (1991) summarized the literature concerning the lethality of THF in which most of the studies employed rats and mice, with only slight species differences. In the comparative studies, rats were consistently slightly less susceptible to the lethal effects of THF, and in one of these studies, guinea pigs were equivalent to mice. These findings provided estimates for acute LD_{50} of 2-3 g/kg and 20-70 mg/L for oral and inhalational routes of exposure, respectively. Bamford et al. (1970) examined the intravenous anesthetic activity of THF and reported the intravenous dose of THF which caused anesthesia in 50% of mice injected (AD_{50}), and dose that of killed 50% of mice injected (LD_{50}) were 607 and 759 mg/kg, respectively. Induction of anesthesia was usually accompanied by convulsive side-effects and animals which received lethal doses died of respiratory failure. Data pertaining to the toxicity of THF in humans is quite limited. The probable oral lethal dose in human is 50-500 mg/kg.

In our study, we found that mice received THF via intracerebroventricular injection showed different pattern of neurobehavioral effects from those induced by intraperitoneal injection. Intracerebroventricular administration of THF induced seizure-like behaviors and death instead of reduced locomotion, impaired motor coordination, and loss of righting reflex. The LD_{50} of THF for intracerebroventricular injection was 79.28 μmol/mouse. It is conceivable that THF vapors cause irritation of the mucous membranes, respiratory system, and skin. When we administered THF by intracerebroventricular injection, due to its lipophilic nature and high local concentrations, THF might cause direct neurocytotoxicity that consequently led to neuronal damages, seizures-like behaviors, and death.

In our study, we used THF that was preserved with <0.025% butylated hydroxytoluene (BHT) as a test compound. BHT is a substituted toluene used as an antioxidant in cosmetic product formulations. Phenolic antioxidants such as BHT form stable free radicals that interrupt the propagation step of oxidation processes. The intraperitoneal LD_{50} of BHT in rats was 8.0 g/kg and no clinical signs or pathologic changes of the organs were observed. Lanigan, and Yamarik (2002) reported that Wistar rats received one or two doses of 800 mg/kg BHT (route of administration not specified), 24 hours apart, the liver weights increased 20% within the first 24 hours and up to 33% by 48 hours. In the present study, the effect of BHT on locomotor activity was observed by preparing BHT in corn oil. BHT at a dose 2 mg/kg, i.p. did not cause any differences in locomotor activity as compared to those of saline or corn oil. We also found that BHT had no effect on the righting reflex test and rotarod test (data not shown). Therefore, effects of THF observed in behavioral studies were presumably not confounded by BHT.

de Fiebre et al. (2004) measured locomotor activity in Swiss-Webster mice for 2 hours following a single injection of GBL and 1,4-butanediol. They observed that GBL (0-100 mg/kg) dose-dependently decreased locomotor activity. At 25 mg/kg, GBL did not differ from saline. At 50 mg/kg, GBL produced an initial depression of locomotor activity which was followed by stimulation of locomotor activity. At 100 and 150 mg/kg, GBL produced primarily a dose-dependent decrease in locomotor activity that returned to baseline within 50 min.

In our study, we observed that GBL had depressant effects on locomotor activity. Locomotion was reduced and was significantly different from that

of control group for the first 60 min of treatment with GBL at doses of 1 and 3 mmol/kg and for the first 80 min of treatment with GBL at a dose of 5 mmol/kg. Thereafter, locomotor activity was gradually returned to baseline. At GBL dose of 10 mmol/kg, locomotor activity was totally suppressed for the entire 150-min test period. In essence, our study supports more evidence for the depressive effects of GBL on locomotor activity.

Malley et al. (2001) performed acute and subchronic neurotoxicological evaluations of THF by inhalation in rats. Evaluations included clinical observations, motor activity assessments, and a battery of functional test designed to reveal nervous system dysfunction. Acute exposure concentrations were 0, 500, 2500, or 5,000 ppm for 6 hours. During exposure to 2,500 and 5,000 ppm, rats had a diminished or absent startle response to a punctuate auditory alerting stimulus. Following exposure to 5,000 ppm, male and female rats were lethargic, exhibited abnormal gait or mobility, and splayed rear feet. Lethargy and splayed rear feet were also observed in females exposed to 2,500 ppm. Males exposed to 5,000 ppm had a lower incidence of palpebral closure, higher incidences of slow or absent righting reflex, and a biphasic pattern of reduced motor activity followed by increased motor activity. Females exposed to 5,000 ppm had increased incidences of palpebral closure in the open-field, increased incidences of slow or absent righting reflex, and decreased motor activity.

Our study demonstrated that THF dose-dependently decreased locomotor activity without a biphasic pattern on locomotor activity of mice. At THF doses of 3, 5, and 10 mmol/kg, i.p., locomotion was reduced and significantly different from that of control group throughout the entire 150-min test period. These inconsistent findings suggested that the differences in species and route of administration of THF might account for the different observable results on locomotor activity.

Itzhak and Ali (2002) demonstrated that GHB (100-300 mg/kg) induced dose-dependent inhibition of locomotor activity. The effect of GHB on motor activity can be explained by its action on the dopaminergic system (Fattore et al, 2000). The GHBergic system seems to participate in the control of dopaminergic neurotransmission, mainly by reducing impulse flow in the nigrostriatal and in the meso-corticolimbic pathways (Roth, Doherty and Walters, 1980). The attenuation of

dopamine neurotransmission may underlie the effects of GHB on motor activity (Nicholson and Balster, 2001).

Our data demonstrated that the time course for effects of THF and GBL on locomotor activity at a dose range of 1 to 5 mmol/kg were not identical, presumably due to differences in GHB formation and distribution and/or effects on the mouse neurochemistry. As considered from the combination of behavioral tests, THF possessed a longer duration with a less potency than those of GBL. It is possible that THF might be transformed to GBL, and/or GHB via enzymatic reactions in the body and complicated the pharmacological action of THF. However, the current study did not assess the biotransformation of THF, we could not conclude that THF mediated direct effects on the locomotor activity via itself or indirectly via its metabolites.

The open-field is an additional and important tool for analyzing the locomotion of rodents. However, open-field behavior of mice and rats is affected by two behavioral dimensions, activity, and emotionality/anxiety. The interpretation of results is confounded by these two underlying constructs. Therefore, this test usually should not be used as a single measure of activity or anxiety (Karl et al., 2003). In the present study, we investigated effects of THF and GBL in open-field test to measure their anxiolytic activities in conjunction with another model, the elevated plus maze test.

An anxiolytic action of GHB has been observed in rats using the elevated plus maze (Schmidt-Mutter et al., 1998). The number of entries and the time spent in the open arms of the maze were increased by GHB (50, 150, 250 mg/kg, i.p.). There was no sedative effect at these doses as measured by the spontaneous locomotor activity or the total number of arm entries. The anti-anxiety effect of GHB was antagonized by the benzodiazepine receptor antagonist, flumazenil (10 mg/kg, i.p.). In our study, two doses of THF or GBL which did not show sedation (0.1 and 0.3 mmol/kg) were tested for anxiolytic activity in mice. In the open-field test, the parameters were thigmotactic ratio, inner ambulation, outer ambulation, number of rearing, number of grooming, open-field defecation and open-field urination. One of the most prominent fear related open-field responses is thigmotactic or wall-seeking behavior, which refers to the propensity of a rodent to stay in close contact with the walls of the field because of the underlying tendency to avoid open, and thus potentially dangerous places. The thigmotactic ratio was calculated by dividing the

number of inner units the mouse entered by the total sum of units it visited. The smaller the ratio, the more prone the mouse was to keeping close to the open-field walls. In addition to thigmotaxis, many other parameters have been used to measure emotionality in rodents. The most traditional index being defecation. A high level of defecation has been interpreted as an indicator of a high level of emotionality. In our study, there were no significant differences in the mean of all parameters in all treated groups in comparison with the control group except for the inner ambulation. Administration with THF at a dose of 0.1 mmol/kg and GBL at doses of 0.1 and 0.3 mmol/kg decreased the inner ambulation in the open-field during 10-min test period ($p<0.05$). Although the inner unit ambulation showed significant difference, the thigmotactic ratios showed no difference. From limited data from the open-field test, it appeared that THF and GBL possessed no anxiolytic activity. On the other hand, they might have a certain degree of anxiogenic property.

The elevated plus maze is an ethologically-based approach-avoidance conflict test, which is sensitive to anxiolytic drug treatment. Mice prefer a dark, enclosed, small, place over a brightly lit, open large space. Anxiety can be measured by the time spent on open arms as well as the percentage of open-arm entries. These parameters are inversely related to anxiety. The number of total arms entries reflects also the general motor activity. In our elevated plus maze test, the percentage of open-arm entries and the time spent in open arms was not different among saline-treated, THF (0.1 and 0.3 mmol/kg)-treated, and GBL (0.1 and 0.3 mmol/kg)-treated groups. However, diazepam, a reference anxiolytic drug at a dose of 2 mg/kg, i.p, significantly increased the percentage of open-arm entries as well as the time spent in open arms ($p<0.05$).

Unlike the study of Schmidt-Mutter et al. (1998), our open-field and elevated plus maze tests of THF (0.1 and 0.3 mmol/kg, i.p.), and GBL (0.1 and 0.3 mmol/kg, i.p.) did not reveal anxiolytic property. This discrepancy might be due to possible differences in many factors including animal species, behavioral protocols, doses used, and experimental settings.

Learning is a complex phenomenon subserved by the activity of many brain regions. Some aspects of learning that can be measured in rodents include attention, working memory (the short-term memory used while a task is being performed), memory consolidation, and reference memory (the long-term memory, which lasts from 24 hours to the lifetime of the animal) (Karl et al., 2003). In adult

rodents, behavioral effects of GHB were dose-dependent, ranging from amnesia to sedation to catalepsy, absence seizures, coma and death (Wong et al., 2004). In humans, GHB is known to have amnesic effects (Wong et al., 2004). In our study, we determined the effects of THF and GBL on different aspects of learning and memory by using the Y-maze and Morris water maze. The Y-maze, in which rodents are trained to visit a pattern of arms in the maze, is particularly geared toward measuring short-term memory. The spontaneous alternation may have a component of spatial working memory since the animals should recall the previous memory of explored arm in order to explore another arm in consecutive choices. The current study demonstrated that THF (0.1 and 0.3 mmol/kg, i.p.) and GBL (0.1 and 0.3 mmol/kg, i.p.) had no effect on the percentage of alternation behavior and total arm entries. Although, THF (3 mmol/kg, i.p.) significantly reduced total arm entries, the percentage of alternation behavior was not significant different from that of control mice. These findings implied that THF did not impair short-term working memory in spite of suppressing locomotor activity at a high dose. With GBL at doses of 1 and 3 mmol/kg, i.p., mice were nearly immobile so the alternation behavior could not be analyzed. These results suggested that GBL at test doses markedly affected motor activity and thus intervened learning and memory task.

The Morris water maze, in which an animal uses three-dimensional cues in the testing room to learn to find a hidden platform in a swimming pool, measures spatial learning and long-term reference memory which is critically dependent on hippocampal function. The test involves repeated trials in which the animal is placed in different parts of the pool, and the time taken to find the hidden platform is measured. Plotting the time to find the platform on successive trials generates a learning curve that can be used to compare the acquisition of the spatial learning task between animals. Long-term memory can also be measured 24 hours or more after the final training trial in a task called the transfer test. In the task, the hidden platform is removed, and the time that the animal swims in the area where the platform used to be is recorded.

Sircar and Basak (2004) reported that adolescent rats that received GHB (10-100 mg, i.p.) exhibited decrease in cortical N-methyl-D-aspartate receptor level and impaired spatial learning. GHB-treated rats took longer and swam greater distances to find the hidden platform than control rats in the Morris water maze. Memory impairments in adolescent GHB exposed rats were not seen until the fourth

day of exposure, indicating that repeated GHB treatment may cause some degree of sensitization. In the probe trial, adolescent rats exposed to GHB spent less time in the target quadrant than control rats.

In the present study, we modified the training protocol to measure memory retrieval ability of animals once already learnt the task. Mice were daily trained to find the hidden platform in a Morris water maze for 5 consecutive days after which they were subjected to drug treatment and another course of spatial learning task. In this particular protocol, mice retrieved their consolidated long term memory and used it in relearning the same memory task. The daily escape latencies of the THF- and GBL-treated mice (1 and 3 mmol/kg, i.p.) were longer than that of control mice and significantly different from control mice on different days of testing. The escape latencies of the THF-treated mice (1 mmol/kg, i.p.) were significantly longer than that of control mice on day 2 of testing. The escape latencies of the THF-treated mice (3 mmol/kg, i.p.) were significantly longer than that of control mice on day 3 and 5 of testing. The escape latencies of the GBL-treated mice (1 mmol/kg, i.p.) were significantly longer than that of control mice on day 2, day 4 and day 5 of testing. The escape latencies of the GBL-treated mice (3 mmol/kg, i.p.) were significantly longer than that of control mice on day 2, day 3, day 4 and day 5 of testing. In the probe trial, the percentage of time spent in the platform quadrant of THF-treated mice (1 and 3 mmol/kg), and GBL-treated mice (1 and 3 mmol/kg) was significantly shorter than that of control mice. With GBL at a dose of 3 mmol/kg, i.p., mice were nearly immobile and the time spent in the target quadrant was not accessible. These experimental findings suggested that THF and GBL interrupted the memory retrieval process once the long term memory had been formed. In addition, they might interfere with spatial learning and memory consolidation in the relearning process. The exact mode of inhibitory action of THF and GBL on learning and memory has been undetermined. It was notable that high doses of GBL produced marked suppression on motor activity which interfered with the memory task. Therefore, non-sedative and low doses of GBL should be chosen to investigate effects on learning and memory.

In conclusion, a deficit in spatial learning ability was observed in 1 and 3 mmol/kg THF, and 1 mmol/kg GBL groups with repeated administration. The mode and mechanism for inducing impaired spatial memory are needed to be further elucidated whether they involve glutamate levels or NMDA receptors. It is quite

notable that there were no significant effects of THF and GBL at 0.1 and 0.3 mmol/kg on working memory in Y-maze test.

Studies looking at mechanisms by which GHB affect neural functioning indicated that GHB interacts with the GHB receptor as well as the $GABA_B$ receptor in the brain. Since neither GHB nor $GABA_B$ receptors can fully explain the effects of GHB in the brain, it is not surprising that other mechanisms may be involved. Intrahippocampal infusion of low doses (in nanomolar concentrations) of GHB and GHB analogues with agonist properties at the GHB receptor such as t-HCA (*trans*-γ-hydroxycrotonic acid) and NCS-435 (γ-(*p*-methoxybenzil)-γ-hydroxycrotonic acid) have been shown to increase extracellular glutamate (Castelli et al., 2003); at micromolar concentrations GHB decreases glutamate levels (Ferraro et al., 2001). GHB-induced glutamate release is blocked by GHB antagonists but $GABA_B$ antagonists fail to do so (Castelli et al., 2003). Thus some of the behavioral effects of GHB appear to be mediated by alterations in glutamate neurotransmission.

Dean (2003) mentioned that GHB had antidepressive effects. Among the hottest of new pharmacological agents are the serotonin-reuptake inhibitors like Prozac, Paxil, and Zoloft, all of which act to increase levels of serotonin in the brain by blocking the uptake of serotonin by receptor sites in brain neurons. It also may be this effect of GHB which is the reason for its persecution. It is not clear whether the endogenous GHB system influences the serotonergic activity in brain directly or indirectly via the interaction with another system (dopaminergic or GABAergic). However pharmacological doses of GHB (400-500 mg/kg) in rats induce an increase in serotonin turnover in the striatum and mesolimbic areas (Maitre, 1997). It is postulated that this increase in serotonin turnover is most probably due to an increase in tissue concentration and bioavailability of tryptophan, the precursor of serotonin, which has been shown to increase after administration of GHB *in vivo*. The transport of tryptophan through the blood-brain barrier and/or through the neuronal membrane could possibly be affected by GHB. Also baclofen, a $GABA_B$ agonist mimics some aspects of GHB on serotonergic system. Thus it is tempting to suggest that GHB induced GABAergic stimulation of serotonin synthesis and degradation via either a presynaptic control of GABA release by GHB or the synthesis of GABA using GHB as precursor (Maitre, 1997).

Our study has also determined the effects of THF and GBL in an animal model of depression, an open-space swimming test. Mice that received saline showed a gradual reduction in the mobility time over successive trials. The mobility time included all the time that mice moved during the entire 15 min, as caused by active swimming. Active swimming was defined as when a mouse is making active swimming motions as to move around in the pool. Imipramine was used in this study as the positive control to determine whether depressive behavior that induced by the open-space swimming test was sensitive to antidepressant treatment. Imipramine administration attenuated the reduction in mobility time over trials, compared with that of the control group. The imipramine-treated mice showed more and long-lasting periods of active swimming measured as the mobility time. Statistical analysis revealed significant improvement from the 3^{rd} trial to the 4^{th} trial ($p<0.05$) which indicated a persistent increase in motivational behavior of mice that received the antidepressant treatment. THF- and GBL-treated groups (0.1 mmol/kg and 0.3 mmol/kg, × 3 injections per day, i.p.) showed no differences in percentage of mobility time as compared to saline treatment. THF and GBL were not effective in attenuating the reduction in mobility time of mice in open-space swimming test as compared to saline group. The results suggested that THF- and GBL administration, at the doses of 0.1 and 0.3 mmol/kg, i.p. did not possess antidepressant effects in this animal model.

Following a 4-week exposure to GBL in drinking water, Nowycky and Roth (1979) showed tolerance development in rats to the sedative effects and increased DA synthesis produced by acute GBL administration. These results were confirmed by Giorgi and Rubio (1981), who showed that the anesthetic effects of acute GBL were greatly attenuated after 3 week-chronic administration, and the brain levels of GHB at time of recovery were 50% greater than those in control rats.

In our study, mice receiving saline, THF 5 mmol/kg, i.p., and 10 mmol/kg, i.p., once daily for a total period of 14 consecutive days were challenged with THF 15 mmol/kg, i.p. and the righting reflexes were evaluated until recovery. The percentage of mice that lost the righting reflex in a group receiving repeated treatment with 10 mmol/kg THF and challenged with 15 mmol/kg THF were decreased significantly until 165 min as compared to that of THF naïve group. The percentage of mice that lost the righting reflex in a group receiving repeated treatment

with 5 mmol/kg THF challenged with 15 mmol/kg THF were inconsistently decreased up to 150 min. These results demonstrated that chronic treatment with THF induced tolerance on its sedative-hypnotic effect in accordance with previous studies.

In other experimental rodent models for studying reinforcing properties, it has been shown that GHB induced conditioned placed preference in rats (Martellota et al., 1997) and maintained intravenous self-administration in mice (Fattore et al., 2000), which was antagonized by NCS-382 (Martellotta et al., 1998) and baclofen (Fattore et al., 2001). In contrast, i.v. self-administration studies in monkey have yielded negative results (Beardsley, Balster, and Harris, 1996; Woolverton et al., 1999). Thus, because of these conflicting reports, it is difficult to unambiguously conclude that GHB possesses reinforcing properties. Our study indicated that mice treated with THF (3 and 5 mmol/kg, i.p.) and GBL (0.5 and 1 mmol/kg, i.p.) showed no differences in place preference score and time spent in white compartment parameters as compared to saline treatment group. Therefore, it was suggestive that THF (3 and 5 mmol/kg, i.p.) and GBL (0.5 and 1 mmol/kg, i.p.) might not have reinforcing properties, and hence might not induce dependence.

Although GHB does not bind to $GABA_A$ receptor (Serra et al., 1991). GHB is metabolically converted to GABA (Doherty, Snead, and Roth, 1975), and activation of $GABA_B$ receptors by GHB can stimulate the synthesis of neurosteroids that positively modulate the actions of GABA at the $GABA_A$ receptor complex (Barbaccia et al, 2002). The antagonist pretreatment tests may clarify the mechanisms of action of THF. Specific antagonists for $GABA_A$, $GABA_B$, and GHB receptors, were administered 15 min prior to THF and the percentage of animals that failed in the rotarod test were determined. Mice that received $GABA_A$ antagonists, picrotoxin (2 mg/kg, i.p.) or flumazenil (10 mg/kg, i.p.), 15 min prior to THF (15 mmol/kg, i.p.) treatment showed no differences in motor impairment as compared to the THF-treatment group. Mice that received a GHB receptor antagonist, NCS-382 (250 mg/kg, i.p.), 15 min before THF (15 mmol/kg, i.p.) treatment did not recover the rotarod performance within 360-min test session as compared to control group. While mice that received a $GABA_B$ receptor antagonist, CGP-35348 (200 mg/kg, i.p.), 15 min prior to THF (15 mmol/kg, i.p.) treatment clearly passed the rotarod test with gradually increased failures up to 120-150 min and did not recover within 360 min. In conclusion, our experimental results suggested that a $GABA_B$ receptor antagonist could antagonize the deteriorated effects of THF on motor function in the rotarod test

with relatively short duration. It may be due to the short half-life of CGP-35348. Therefore, the mechanism of THF on the impairment of motor function may be mediated, at least partly, through $GABA_B$ receptors.

To our knowledge, this is the first study to demonstrate that the GHB receptor antagonist, NCS-382, can have deleterious effects when combined with THF. In our animal model of acute overdose with THF, NCS-382 and CGP-35348 prolonged the duration of rotarod failure in pretreated mice versus control mice. There are two possible explanations for the unexpected observation. Firstly, T-HCA, the metabolite of GHB via β-oxidation, has demonstrated a greater affinity for the GHB receptor than has GHB itself. Perhaps this T-HCA affinity for the GHB receptor might also be greater than the receptor antagonist, NCS-382. Alternatively, perhaps NCS-382 antagonism of GHB receptors results in a metabolic shunt of GHB back to succinic semialdehyde (SSA) by NADP-dependent GHB dehydrogenase. While a proportion of SSA would then be oxidized in the Kreb's cycle, some SSA could alternatively be converted to GABA by GABA transaminase. In overdose situations, this could potentially result in a significant increase in the total brain GABA pool, leading to toxicity (Quang et al., 2002). This theory is supported by the experimental results in which pretreatment with NCS-382 prior to THF overdose postponed the recovery phase in the rotarod test as compared to control. With CGP-35348 pretreatment, the recovery phase in the rotarod test was masked (at least up to 6 h). This phenomenon was presumably derived from pharmacokinetic interactions between THF and CGP-35348. Perhaps CGP-35348 had some inhibitory effects on the metabolizing enzyme of THF and prolonged the metabolism and/or excretion of THF.