Chapter 7

DISCUSSION
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1. UNDERSTANDING AND MANAGEMENT OF EPILEPSY: A NOVEL HISTAMINERGIC APPROACH

1.1 Convulsions

At the present state of knowledge, the understanding of underlying mechanisms in the pathogenesis of epilepsy (and therefore its management) is grossly inadequate. There is an urgent need for more scientific effort in this area using novel approaches. A survey of literature revealed that while varied neurotransmitters and neuropeptide systems have been extensively investigated for their involvement in seizures and epilepsy (Dragunow, 1986; Meldrum, 1995; Dichter, 1997; Rogawski, 1998), little is known about the role of histaminergic mechanisms in this disease. This approach was chosen for the present study because though the histaminergic neuronal system has now gained the status of a regulatory centre for whole brain activity (Wada et al., 1991), it has not received the requisite scientific attention to probe problems related to epilepsy. There are indicators from recent studies by some Japanese researchers (Onodera et al., 1992; Inuma et al., 1993; Yokoyama et al., 1992, 1993a,b, 1994a,b; Kakinoki et al., 1998; Kamei et al., 1998) that effort in this direction might well be rewarding. We found a significant increase in whole brain and brain stem histamine concentrations following MES-induced convulsions. It is noteworthy that brain stem (and a number of brainstem structures) has been implicated in seizure arrest (Dragunow, 1986). Elevation of brain histamine following MES may be due to maximal neuronal activity with enhanced neurotransmitter synthesis/release. Seizure arrest and postictal refractory period (PIRP) are associated with corresponding activity or release of endogenous substances in the brain (Dragunow, 1986). The observed increase in histamine, in this situation, may reflect protective physiological mechanisms supporting the concept of histamine as an
endogenous anticonvulsant (Leurs et al., 1998). Contrary to the effects in the MES model, a tendency towards decrease in brain histamine levels was observed following PTZ-induced clonic convulsions. The reduction was seen in the cerebral cortex, hypothalamus, brain stem and cerebellum. Consistent with our findings, a decrease in the histamine content of amygdala and hypothalamus has been reported following kindling (Kamei et al., 1998). Similar decrease in histamine content has been reported by other workers (Onodera et al., 1992) in many brain regions (striatum, hippocampus, amygdala, midbrain, thalamus & hypothalamus) of genetically epilepsy-prone rats (GEPRs) vs those in epilepsy-resistant Wistar rats. This suggests an inverse relationship between brain histamine levels and epileptogenic activity.

1.2 Antiepileptic Drugs (AEDs)

Generally the AEDs, under study, did not affect the histamine levels in the whole brain at their ED<sub>100</sub> doses. An exception to this was GBP which caused a significant rise in the neurotransmitter. An increase was observed in the brain regions such as the cerebral cortex and brain stem following treatment with PHT and GBP. It is interesting to note that the brain regions showing elevation in histamine concentrations are considered relevant for epilepsy (Gale and Browning, 1988). Among other AEDs, CBZ did not affect brain histamine while SVP caused a significant reduction in the hypothalamic histamine content. The pharmacological significance of such differences observed with SVP is difficult to explain at this stage. These findings, however, preclude a role for histaminergic neuronal system in mediating the anticonvulsant effects of SVP and CBZ. Tuomisto and Tacke (1986) reported a linear correlation between the rise in brain histamine levels and protection against MES seizures in rats. Besides, drugs
which enhance brain histamine (e.g. L-histidine, metoprine and histamine itself) are known to act as anticonvulsants in various experimental models (Tuomisto and Tacke, 1986; Scherkl et al., 1991; Yokoyama et al., 1994a). These reports, coupled with the present PHT and GBP effects on brain histamine concentrations, point to the involvement of the histaminergic neuronal system in the anticonvulsant effects of these drugs.

Methionine-sulfoximine (MSO), a convulsant agent, was reported to deplete brain histamine levels by enhancement of its catabolism through a stimulatory action on histamine-N-methyl transferase activity (Schayer and Reilly, 1970; Schatz and Sellinger, 1975). SVP and GBP significantly protected mice against MSO-induced convulsions. While SVP is known to have such effect, we report protective action of GBP in the MSO model for the first time. Several studies indicate that GABA system plays a role in the convulsive mechanisms of MSO (Stone and Javid, 1978; Blizard and Balkoski, 1982). Results of the present study for effects of GBP alone provides additional evidence for attributing it to GABAergic mechanisms. We did not find any modulation of MSO-induced convulsions by THP and RAMH alone or by the combination of THP with AEDs. Therefore the convulsant action of MSO, which is attributed to its modulatory effect on GABA synthesis (Stone and Javid, 1978), does not appear to be mediated via histaminergic mechanisms.

1.3 Histamine \( H_3 \)-Receptor Ligands and Experimental Convulsions

Thioperamide (THP: an \( H_3 \)-receptor antagonist) caused a marked and dose-dependent increase in the latencies to and reduced the incidence of myoclonic jerks and clonic generalized seizures in the PTZ model. This study presents the first experimental
evidence for any H3-receptor antagonist against PTZ-induced clonic convulsions. Though THP exhibited a protective action against MES-induced convulsions also, the effects were far less pronounced as compared to those in the PTZ model. Thus, while a dose as low as 3.75 mg/kg of THP elicited moderate protection against PTZ, no effect was observed against MES seizures even at a dose of 7.5 mg/kg. When the dose was further increased to 15 mg/kg, it could only partly reduce, but not abolish, the various phases of MES-induced convulsions. Our findings are similar to those of Yokoyama and co-workers (1993a) who demonstrated a protective action of THP in the MES model with appreciable effect against clonic convulsions and only a marginal effect on tonic convulsions. This report and results of the present study suggest a preferential action of THP for clonic type of convulsions with potential for therapeutic applications in petitmal epilepsy.

Administration of R(α)-methylhistamine (RAMH: an H3-receptor agonist) enhanced MES-induced convulsions and a tendency towards similar effect (non-significant) in the PTZ model. A pro-convulsive action for RAMH could not be shown in this model because the dose of PTZ used was already producing convulsions in 100% animals leaving no scope for further enhancement. However, its administration 15 min before THP (15 mg/kg, ip) significantly antagonised the protective effect of THP against both MES and PTZ-induced convulsions. This suggests that the observed effects of THP were elicited through H3-receptors. Targeting these receptors, thus, embody exciting possibilities with immense therapeutic potential.

Potentiation of PTZ-induced seizures by H3-antagonists has been reported (Fairbairn and Sturman, 1989). Scherkl and associates (1991) showed that a rise in PTZ seizure threshold parallels a concomitant elevation in the brain histamine concentrations.
Thus, it is likely that THP exerts its protective action by enhancing neuronal histamine release and thus normalizing the observed PTZ-induced reduction in the brain histamine content. However, further biochemical studies are required to correlate an enhanced histamine release with the observed anticonvulsant effects of THP.

It is well known that H$_3$-receptors are autoreceptors on presynaptic histaminergic terminals with an inhibitory action on the synthesis and release of histamine (Arrang et al., 1983). Blockade of these receptors by selective H$_3$ antagonists e.g. THP would lead to an enhanced neuronal histamine release in the brain resulting in anticonvulsant effects (Arrang et al., 1987; Mochizuki et al., 1991; Yokoyama et al., 1993a, 1994b; Kakinoki et al., 1998). Enhanced histamine release \textit{in vivo} corresponds to decreased tissue levels and vice versa (Yokoyama et al., 1994b; Kakinoki et al., 1998). We found a significant reduction in the histamine content of the whole brain, hypothalamus and midbrain following THP administration. Conversely, RAMH exhibited a rise in histamine concentrations of the whole brain and select brain regions. Our findings are in consistent with those of other workers demonstrating an enhanced neuronal release following THP with a concomitant reduction in the tissue levels and vice versa following RAMH (Oishi et al., 1989, 1990). The anticonvulsant effects of H$_3$-receptor antagonists are reported to be reversed either by H$_3$-receptor agonists or by H$_1$-receptor antagonists but not by H$_2$-receptor antagonists suggesting an interaction of the THP-induced release of histamine with histamine H$_1$-receptors on the postsynaptic neurons (Yokoyama et al., 1993a; Kakinoki et al., 1998; Vohora et al., 2000a).

However, H$_3$-receptors are now considered as heteroreceptors on both central and peripheral nervous systems (Schlicker et al., 1996). They regulate not only the release of histamine but also of other neurotransmitters e.g. 5HT (Schlicker et al., 1988), NE
(Schlicker et al., 1989), DA (Schlicker et al., 1993), ACh (Blandina et al., 1996) and GABA (Kishino et al., 1989; Yamamoto et al., 1997). Whether the observed action of THP, in the present study, was elicited through influence on other neurotransmitters? While the precise answer to this question is not available, it has to be viewed in the light of the following facts:

a) Heteroreceptor function of \( H_{2} \)-receptor ligands in the regulation of monoaminergic activity is suggested to be minor in contrast with their function in modulating histaminergic activity (Oishi et al., 1990).

b) The dose of THP, used in this study, is reported to produce no effects on the brain levels of DA, 3,4-dihydroxyphenyl acetic acid, 5HT and 5-hydroxyindole acetic acid (Yokoyama et al., 1993a), ruling out the possibility of the involvement of these neurotransmitters in causing the observed effects.

c) A role for GABAergic mechanisms appears to be more likely. Recently, THP was shown to increase the release of GABA from the rat hypothalamus (Yamamoto et al., 1997). A disinhibition of GABA is known to be involved in the initiation and generalization of PTZ-induced seizures (MacDonald and Barker, 1977). It may, therefore, be speculated that the protective effect of THP on PTZ was mediated through an indirect action on GABAergic mechanisms.

d) An interplay of NE sites may not be overlooked as \( H_{2} \)-receptors regulating the release of NE are located on the catecholaminergic nerve terminals (Schlicker et al., 1989). These aspects have not been looked into.
1.4 Combination Studies of H3-receptor Ligands with AEDs

Combination of THP with PHT and GBP, in their respective subeffective doses, revealed protection (additive effect) against MES-induced convulsions. We have also shown that the combination of subeffective doses of THP and GBP elicited a protection (synergistic action) in the PTZ model. Further, the protective action was completely countered by RAMH in both PTZ and MES models. These findings opens up exciting newer approaches to the management of epilepsy. Thus, an H3-receptor antagonist can serve as a useful adjunct in epileptic patients not responding to a conventional AED regimen. The added advantages include: a) possible reduction of sedative action and cognitive deficits associated with AED therapy by reduction in AEDs dose, and b) reported beneficial effects of H3-receptor antagonists in maintaining wakefulness (Lin et al., 1990; Monti et al., 1991) and in improving cognitive function (Meguro et al., 1995; Blandina et al., 1996; Miyazaki et al., 1997; Onodera et al., 1998). Our findings clearly indicate the involvement of H3-receptor mechanisms in epilepsy. The present PHT-GBP-THP effects on seizures (and brain histamine concentrations) further point to the involvement of the histaminergic neuronal system in the anticonvulsant effects of these drugs.

2. COGNITIVE DEFICITS: MAJOR CLINICAL PROBLEM IN EPILEPSY

2.1 Convulsions

Are convulsions essential correlates of cognitive dysfunction? To find an answer to this question, we studied the effects of convulsive and subconvulsive electric stimuli of MES and doses of PTZ on experimental models of learning and memory. The study revealed an impairment of cognitive performance in both passive avoidance paradigm
(PAP) and spatial alternation (SAP) task following convulsions with MES and PTZ. At sub-convulsive doses, differences were discernible; impairment in PAP following electroshock (ES) and no such effect following PTZ. Thus, ES produced amnesia even at a current intensity lower than that necessary for producing convulsions. Consistent with our observations, Becker et al. (1995) demonstrated that motor convulsion suppression did not prevent kindling-induced learning deficits. The latter involves a more complex interaction of the pathological process and the acute syndrome. The findings in PTZ model, on the contrary, revealed that convulsions are critical for producing amnesia.

To explore the possibility of motor stimulation by sub-convulsive ES following PAP, we probed the effect of locomotor activity (LA) on this test (see Section 2.5). Further, sub-convulsive ES did not show an impairment in the alternation task. Apparently, different brain mechanisms are involved in PAP and SAP (Meguro et al., 1995; Blandina et al., 1996).

It has been reported that ECS follows an inverted U pattern on memory i.e. enhancement at moderate and impairment at high intensities. Footshock, used for training, has been shown to enhance retention possibly by releasing moderate amounts of epinephrine/norepinephrine (Stenberg et al., 1983). This study showed no enhancement of spatial memory by low ES. Increasing current intensities (2 mA, 9mA and 48mA), however, resulted in an inverted U pattern response in SAP.

PTZ produced a dual effect on memory, enhancement at lower dose and impairment at higher (convulsive) dose; the latter affecting both acquisition and short-term and long-term memory retention (STM and LTM) in the PA and SA tasks. Becker et al. (1995) reported similar learning impairment in PTZ-kindled rats in the shuttle box.
paradigm. Sub-convulsive dose of PTZ exhibited improved retention of STM in the PAP and a similar tendency for SAP.

There is now extensive experimental evidence indicating a role for GABA in learning and memory. Retention of recently acquired information was shown to be impaired by post-training systemic injections of a GABA agonist (muscimol) or a GABA transaminase inhibitor (amino oxyacetic acid) (Brioni et al., 1990; Singh and Dhawan, 1992). A disinhibition of GABA is known to be involved in the actions of PTZ (MacDonald and Barker, 1977). Possibly, the observed enhancement of memory retention was due to its effect on GABA. This hypothesis gets support by several experimental studies showing enhanced maze learning and retention in the active avoidance tasks following treatment with picrotoxin, PTZ, bemeegrade and bicuculline (Buskirk and McGaugh, 1974; Castellano and McGaugh, 1989; Singh and Dhawan, 1992; Gold, 1995).

2.2. Antiepileptic Drugs

Almost all AEDs can cause some adverse effect on cognitive function. This statement is based on a vast body of experimental and clinical literature available on the subject (Mondadori and Classen, 1984; Meador et al., 1990; Smith, 1991; Thompson, 1992; Aldencamp et al., 1994; Sudha et al., 1995; Vermeulen and Aldencamp, 1995). While the disease \textit{per se} and a variety of other factors are also responsible for cognitive impairments (Lennox, 1942; Halgren et al., 1991), the iatrogenic effect of AEDs is of primary concern to the clinician. The very drugs meant to control seizures and comfort the patients are paradoxically adding to their problems by inducing cognitive deficits. It is very unfortunate that no true ‘drug of choice’ exists from the stand point of such
side effects. Vermeulen and Aldencamp (1995) reviewed over 90 clinical investigations
during the last 25 years and found no satisfactory answer to the problem.

Varied effects were observed on cognitive function in this study with different AEDs: impairment with PHT and SVP and enhancement with CBZ and GBP in two experimental models viz PAP and SAP in mice. Our results reconfirm, the well documented memory impairing effects of PHT and SVP (Meador et al., 1990; Smith, 1991; Aldencamp et al., 1994; Aldencamp and Vermeulen, 1995). In the present study, while PHT could elicit amnestic action when administered at its ED_{100} dose for two weeks, SVP showed such an effect with a single ED_{100} dose. Sudha et al. (1995) demonstrated effects, similar to our findings for PHT, in experimental models even when the antiepileptic blood levels were within the required therapeutic range. The dose of PHT employed in this study (given for two weeks) is reported to result in a drug concentration well with in the required therapeutic plasma concentration range (10-20 μg/ml) (Sudha et al., 1995). Mondadori and Classen (1984) also reported a dose-dependent amnesic effects of PHT and SVP in ES-induced amnesia following convulsions. Locomotor activity/motor stimulation might influence PAP (see Section 2.5). The mild stimulation observed with PHT, in the present study, was not found to be statistically significant. Although LA was increased following single treatment with SVP, it is, however, unlikely that this contributed to SVP-induced cognitive deficits as we have recently shown that only SDE is influenced by LA, other parameters (SDL and TSZ) remained unaffected (Vohora et al., 2000c). Further, one week treatment with SVP did not alter LA but resulted in amnesic action. An increase number of arm entries in the SAP test following single dose SVP could be responsible for its lack of effect in single dose administration.
In contrast to PHT and SVP, we found significant enhancement of cognitive function in both PAP and SAP following one week treatment with CBZ and GBP; the latter showing improvement even after two weeks treatment. These findings indicate pro-cognitive (rather than amnesic) effects for CBZ and GBP. Similar effects were reported by other workers (Mondadori and Classen, 1984; Rostock and Siegemund, 1993) for CBZ with improved retention in ES-induced amnesia and in active avoidance task. Additional evidence for the beneficial effects on cognitive function is provided by some clinical reports (Thompson and Trimble, 1981, 1982) where substitution of CBZ for one or all of the AEDs in patients on polymedication resulted in a significant improvement of cognitive performance. Meador (1998), on the contrary, found adverse cognitive effects with CBZ comparable to those of PHT and SVP.

Observed differences in the effects of PHT and SVP on one hand and those of CBZ and GBP on the other hand are baffling but hold promise in choosing a drug with less/or hopefully no ill effects on cognition. This is a challenge for researchers in designing new AEDs with ideal features: maximum seizure control with minimum discomfort to the patient. While the reasons for cognitive facilitating effects of CBZ and GBP are not precisely known, these may possibly be attributed to their reported psychotropic properties e.g. antidepressants, antimania, improvement in alertness etc (Ballenger and Post, 1980; Post et al., 1985; Dimond et al., 1996; Magnus, 1999). Optimum improvement in the quality of life in epileptic patients can not be achieved if we loose sight of the major clinical problem. The cognitive profile of newer AEDs is limited. If newer drugs are to attain a useful place in the therapeutic armamentarium, they must be at least as well tolerated as the older agents. While several newer AEDs are considered well tolerated and demonstrate fewer adverse effect (Perucca, 1996), the
available information on cognitive functions is insufficient warranting more investigations in this area. Some useful information is available on vigabatrin, lamotrigine, tiagabine, topiramate and GBP (Martin et al., 1991; Meador, 1998). The latter drug was shown to have minimal ill effects on cognitive function in an add-on, double-blind, placebo-controlled study on refractory epilepsy (Leach et al., 1997) and a study on healthy volunteers (Meador et al., 1999). Our experimental findings on GBP agree well with these reports. We report a pro-cognitive effect for this drug for the first time. This lead should be probed further to explore its clinical potential. The observed increase in brain histamine concentrations following GBP is quite interesting in view of some recent reports on low histamine levels in Alzheimer’s disease (Marurkiewicz-Kwilecki and Noswah, 1989) and possibility of the use of histamine receptor ligands in this disease (Onodera et al., 1994). Thus, it may be speculated that the pro-cognitive effects of GBP were elicited through its effects on brain histamine. Further biochemical studies are needed to establish a correlation between cognitive performance and brain histamine levels following treatment with GBP.

2.3 Histamine H₃-Receptor Ligands and Tacrine

THP significantly improved the acquisition and short-term memory (STM) retention measured at 2h post-training in a PAP with a tendency to improve long term memory (LTM). The other drug TAC could only facilitate acquisition with no effect on either STM or LTM in the PAP. Both THP and TAC improved SAP without affecting the number of arm entries suggesting a positive effect on spatial memory. Further, subeffective doses of these two drugs, when given together, resulted in a significant enhancement of percent alternation (FED index : 0.6) indicating a synergistic action on cognitive function. RAMH was without any effect on the PA task but significantly enhanced SAP with a concomitant increase in the number of arm entries.
Central cholinergic system is known to play an important role in learning and memory (L&M) processes (Bartus et al., 1985; Decker and McGaugh, 1991; Giacobini, 1993). Recently, involvement of histaminergic mechanisms has also been suggested in cognitive function (Kamei and Tasaka, 1991, 1992, 1993, Meguro et al., 1995b,c; Onodera et al., 1998). THP and other H3-receptor antagonists have been shown to improve cognitive function in a variety of tasks including elevated plus maze (Miyazaki et al., 1995; Onodera et al., 1998), scopolamine-induced learning deficits in senescence accelerated mice (Meguro et al., 1995), object recognition task and footshock avoidance in a T-maze (Flood et al., 1998) etc. This is the first study reporting improvement of spontaneous alternation by an H3-receptor antagonist. While the form of memory tested in PAP depends on intact frontal cortex (Blandina et al., 1996), SAP measures spatial memory requiring an intact hippocampus (Douglass, 1989). Autoradiographic studies have demonstrated a widespread distribution of H3 receptors in many regions of the brain (Pollard et al., 1993). Their presence in areas such as the cerebral cortex, striatum, hippocampus and amygdala is consistent with a functional role for these receptors in arousal and cognition (Monti et al., 1991; Smith et al., 1994; Miyazaki et al., 1997).

A close relationship exists between the cholinergic and histaminergic systems in L&M. This is evident from the fact that ACh reverses mepyramine-induced inhibition of avoidance response (Smith et al., 1994), the memory facilitating effect of 2-methyl histamine is attenuated by a muscarinic antagonist, pirenzepine (Bhattacharya, 1990) and that the muscarinic receptor stimulation is associated with decreased release and turnover of histamine in the rat brain (Smith et al., 1994). Both PAR and SAP tasks are highly correlated with the integrity of the cholinergic system (Blandina et al., 1996) and are
blocked by cholinergic antagonists (Bammer, 1982; Doughlas, 1989). Activation of the central histaminergic system (e.g. by H3-R antagonists) is known to antagonise scopolamine-induced learning deficits (Miyazaki et al., 1995a,b). Thus, it is possible that THP improved cognition through its ability to alter other neurotransmitters particularly ACh. Smith and co-workers (1994) reported an improvement of spatial learning by RAMH. This is surprising since RAMH is reported to decrease and THP increase ACh release from the hippocampus (Clapham and Kilpatrick, 1992; Blandina et al., 1996). We found facilitation of SAP with an increased number of arm entries. Thus, it is reasonable to attribute improved performance by RAMH to an increase in the locomotor activity.

Tacrine, which alleviates symptoms of Alzheimer's disease in some patients, is believed to act mainly through the inhibition of acetylcholinesterase (AChE) (Freeman and Dawson, 1991). Recently, tacrine was shown to inhibit histamine-N-methyltransferase and to augment cerebral histamine levels both in vitro and in vivo (Nishibori et al., 1991). The nootropic action of tacrine can not be exclusively attributed to AChE inhibition in view of its muscarinic antagonistic activity and because of the failure of other AChE inhibitors to improve cognitive function (Freeman and Dawson, 1991; Morisset et al., 1996). Our results suggests the possible involvement of the histaminergic system (in addition to the cholinergic system) in producing the observed SAP synergism. The fact that tacrine inhibits histamine-N-methyl transferase more potently than AChE (Morisset et al., 1996) further supports this hypothesis. But as H3-receptor antagonists are known to increase ACh release (Blandina et al., 1996), an interplay of both cholinergic and histaminergic systems appears to be more likely.
2.4 Protection of Cognitive Deficits: Use of Novel Adjuncts with Antiepileptic Drugs

a) Thioperamide

Both THP and TAC could reverse AEDs (PHT and SVP)-induced cognitive deficits in the PAP and CM tests; the effect of TAC was observed only on acquisition, its retention was not influenced in the PAP. PHT has been reported to reduce brain ACh concentrations (Agarwal and Bhargava, 1964; Domino and Olds, 1974), decrease EAA-evoked release of ACh from rat striatal slices (Sethy and Sage, 1992) and the response of g. pig ileum to this neurotransmitter (Sanyal 1996). Its impairing effects on L&M are attributed to such alterations (Sudha et al., 1995). H₃-receptor antagonists including THP have been demonstrated both in vitro and in vivo to enhance ACh release in the brain mediated by H₃-heteroreceptors (Blandina et al., 1996). Recent evidence, however, casts doubt on the cholinergic mechanism of action of TAC (Morisset et al., 1996). While the reasons for different responses to TAC on acquisition and retention are not clear, the above facts point to the involvement of other (may be multiple) mechanisms in its action on cognitive function. Both the drugs proved effective against SVP-induced amnesia. This AED has no direct effect on the cholinergic system but may affect it indirectly through GABAergic system. This is evidenced by the following reports. Intraseptal administration of muscimol, a GABA agonist, is reported decrease hippocampal ACh turnover. There are indicators that GABA regulates the release of ACh via its presynaptic actions. Further, the amnesic effects of diazepam and scopolamine bear some notable similarities on the kind of memory affected by these drugs (Decker and McGaugh, 1991). Considerable evidence, thus, suggests that interactions with ACh underlie some of the GABA's actions on memory.
AEDs may also interfere with the NMDA system directly or indirectly and thus influence the expression of LTP. While PHT is known to suppress NMDA-stimulated NE efflux in the hippocampus and cerebral cortex, SVP exerts such effect only from the latter region (Lee et al., 1993; Brown et al., 1994). Recently, both PHT and SVP were shown to directly affect LTP (Lee et al., 1996). H$_3$-receptors modulate not only ACh release, but also other neurotransmitters including NE (Schlicker et al., 1989) and NMDA (Kishino et al., 1989) in the central nervous system. The observed effects of THP on AEDs-induced amnesia may, therefore, be due to its modulatory action on NE and NMDA via H$_3$-heteroreceptors. It has, however, been suggested that the contribution of these receptors in modulating monoaminergic system is minor in contrast to their effects on histaminergic system (Oishi et al., 1990).

Considerable experimental and clinical evidence points to an important role for histaminergic neuronal system in learning and memory (DeAlmeida and Izquiedo, 1986, 1988; Kamei and Tasaka 1991, 1992, 1993; Flood et al., 1998). In the present study, memory impairing doses of SVP caused a significant decrease of the histamine content in the hypothalamus (and also in whole brain after one week). This is consistent with the reported correlation between reduction in histamine levels in the hypothalamus and impaired cognitive performance (Kamei et al., 1993).

From the foregoing discussion, no clear picture emerges regarding the precise role for any neurotransmitter(s) in the mechanism of protective action of THP on AEDs-induced cognitive deficit. Further investigations on rational polytherapy in epilepsy to exploit the cognitive-deficit corrective potential of H$_3$-receptor antagonists as adjuncts to AED therapy are warranted. While the precise mechanisms are yet to be elucidated, the evidence clearly indicates that H$_3$-receptor antagonists can serve as useful adjuncts.
to conventional AED regimen in patients not responding to AEDs alone (Section 1.4) with the added advantage of their favourable action on cognitive function _per se_ and also on AEDs-induced amnesia.

b) _Bacopa monniera_

The antiepileptic treatment may last a lifetime in many patients (Vermeulen and Aldencamp, 1995). This implies that the nootropic agents may also need to be given for long periods of time. Unfortunately, antiepileptic drug therapy is associated with a variety of adverse effects (Mattson, 1995). Thus, the adjunct chosen for reducing such adverse effects should be free of additional risk of side effects. Natural products e.g. medicinal plants appear to be the ideal choice. Extract of _Bacopa monniera_ (BM), a reputed Indian nootropic plant, when administered for one week along with PHT in the second week of the two-week regimen, significantly reversed PHT-induced cognitive impairment both on acquisition and retention in the PAP in mice (Vohora et al. 2000b). A protection was observed with all the parameters tested except the initial latency to step-down (SDL) but BM alone too did not affect this parameter. This indicates that BM is probably ineffective in consolidating an early retention of memory (immediate recall) but improves it subsequently. BM is known to have nootropic effects on acquisition, consolidation and retention of memory in varied experimental models such as shock-motivated brightness discrimination reaction, conditioned-avoidance response (CAR) with taste aversion, electroconvulsive shock-induced amnesia, Y-maze etc (Singh and Dhawan, 1997). Our results on passive-avoidance task are in agreement with these reports.

Some recent studies report anticonvulsant effects of BM against MES and PTZ-induced seizures (Singh et al., 1996). These effects were observed at slightly higher
doses than those required for nootropic action. Pilot studies in our laboratory revealed no anticonvulsant effect in BM against MES-seizures both at memory enhancing and at higher doses (data not shown for higher doses). We did not observe any inhibition or potentiation of anticonvulsant activity following a combined treatment with BM and PHT. These results suggest that BM has a lot of promise and should be systematically investigated as an add-on therapy for improving the cognitive functions of patients being treated with PHT. Many commercial formulations are available in Indian markets, including one developed at the Central Drug Research Institute, Lucknow (Memory Plus) and used by people for memory enhancing effects. This fact, as also availability of a lot a pre-clinical data (Singh and Dhawan, 1997) should pave way for the proposed clinical trials. It is clear from the present study that addition of BM to PHT regimen will in no way influence the anticonvulsant effect of PHT.

It is unlikely that the observed reduction in LA, following 1 week treatment with BM, contributed to its improved performance in the PA task as we have recently shown that only SDE is influenced by the locomotor activity; other parameters viz SDL and TSZ remained unaffected (Vohora et al., 2000c). Moreover, BM is known to elicit nootropic effects in several experimental models including those with no LA component (Singh and Dhawan, 1997).

The precise mechanism by which BM elicits its nootropic effects is not known. The impairing effects of PHT on L&M are attributed to alterations in the cholinergic system (Sudha et al., 1995). It has been reported that PHT lowers brain ACh levels (Agarwal and Bhargava, 1964; Domino and Olds, 1972). Biochemical as well as electrophysiological evidence exists for interactions between cholinergic, noradrenergic and serotonergic systems (Decker and McGaugh, 1991). Sara (1989) suggested that the
behavioural effects of cholinergic degeneration can be alleviated by a reduction in the noradrenergic function. BM has been shown to have no direct effects on ACh or NMDA receptors. However, it is known to lower NE and increase 5HT levels in the hippocampus, hypothalamus and cerebral cortex (Singh and Dhawan, 1997). BM may thus modify ACh concentrations indirectly, through its influence on other NT systems. Removal of the negative influence (i.e. decreased NE concentrations) possibly helped in the manifestation of cholinergic effects resulting in improved cognitive function. Further, BM has been shown to enhance protein kinase activity in the hippocampus which could also contribute to its nootropic action (Singh and Dhawan, 1997). Future studies could aim at understanding the neurochemical basis for the nootropic effects of BM. Further investigations using a combination of BM and other AEDs are warranted to explore the full potential of BM in correcting the AEDs-induced cognitive impairment.

2.6 Locomotor Activity and Passive Avoidance Response

Passive-avoidance paradigm (PAP) is extensively used for the screening of drugs affecting L&M (Calhoun and Smith, 1968; Bamber, 1982; Papazova et al., 1994; Miyamoto et al., 1996). As the test involves training of rodents to avoid punishment (normally an electric shock) by curbing a normal behaviour (e.g. exploratory behaviour), it is likely to be influenced by locomotor activity. If this is so, its authenticity for true measurement of cognitive function becomes suspect. To probe this question, PAP and LA were studied in mice with known amnesic (scopolamine, diazepam) and nootropic (piracetam, BM) agents. The results revealed a direct relationship between LA and SDE. Thus, a drug which increased LA, augmented SDE and vice versa. While scopolamine
(amnesic agent) increased LA and SDE, BM (nootropic drug) decreased them. It is not clear whether these drugs affected SDE due to their true effects on cognitive function or due to their action on LA. In case of diazepam, a mixed response was observed on different parameters, both amnesic (TSZ, SDL) and nootropic (SDE). The latter effect may be due to the decreased LA observed in the diazepam-treated mice. Thus, the LA effect may lead to the detection of false positive or false negative nootropic drugs in the PAP. Piracetam, a reputed nootropic drug, did not show the expected reduction in SDE in the PAP, possibly because of absence of a significant effect on LA.

No correlation was observed between LA and other two parameters of PAP viz. SDL and TSZ. The former was not always affected by drugs having known effects on L&M, TSZ, on the other hand, revealed consistent results i.e., increase with amnesic and decrease with nootropic drugs. The introduction of TSZ (in addition to SDL and SDE) by us should, therefore, measure the cognitive function with better precision (Vohora et al., 2000c).

3. Intracellular calcium

The role for intracellular Ca$^{2+}$ concentrations in the generation of epileptic activity is well documented (Heinemann and Hamon, 1986; Baethmann, 1990; Lucke et al., 1990; Sugaya and Sugaya, 1991; Wiemann et al., 1996). The levels increase prior to or during seizure onset/epileptic discharges (Perkin and DeLorenzo, 1992). Many studies have demonstrated either a block or a reduction of Ca$^{2+}$ entry into cells by both conventional (PHT, CBZ, SVP, BDZs, Phenobarbitone) and newer (felbamate, lamotrigine) AEDs (Sohn and Ferrendelli, 1973; Ferrendelli and Kinochy, 1977; DeLorenzo, 1988; Stefani et al., 1997). During PTZ-induced bursting activity also,
intracellularly stored Ca\(^{2+}\) is released and moved towards the inner surface of the cell membrane (Sugaya and Onozuka, 1978). We found a significant and concentration-dependent reduction of intracellular Ca\(^{2+}\) concentrations in the brain synaptosomes following treatment with THP and GBP. This is interesting because preventing elevation of intracellular Ca\(^{2+}\) levels is generally associated with reduction in neuronal damage produced by epilepsy (Mody and MacDonald, 1995). Further, raised intracellular calcium levels are currently considered to be the major cause of functional degeneration in aged neurons with impaired cognition (Ouanounou et al., 1999). Recently, a modest increase in Ca\(^{2+}\) influx through L-channels was shown to raise the threshold for induction of LTP (Norris et al., 1998). Thus, it is possible that the reduced Ca\(^{2+}\) concentrations in the brain were related to the observed pro-cognitive effects of THP and GBP. This gets support by the reports exhibiting facilitation of LTP induction (Norris et al., 1998) and nootropic effects (Deyo et al., 1989) in experimental models of L&M by calcium channel blockers. All this is apparently paradoxical because induction of synaptic plasticity and LTP is known to parallel a rise in Ca\(^{2+}\) concentrations (Foster, 1999). Nevertheless, agents that stabilize Ca\(^{2+}\) within the cells afford protection against the kinds of insults believed to underlie neuronal injury in Alzheimer’s disease (Jimenez-Jimenez et al., 1996; Schousboe et al., 1997).

The antiepileptic mechanism of action of GBP is not fully understood (Kelly, 1998). Rock et al. (1993) suggested that its antiepileptic action is not due to direct effects on receptors for inhibitory or excitatory amino acids. Very recently, it has been shown that GBP interacts with a subunit of voltage-dependent Ca\(^{2+}\) channels (McLean, 1999). Stefani et al. (1998) demonstrated a significant inhibition of Ca\(^{2+}\) currents by GBP in isolated rat brain neurons. Our observations on mouse brain synaptosomes are
in agreement with these reports and suggests the possibility of reduced brain Ca$^{2+}$ concentrations by GBP as one of its antiepileptic mechanisms.

Very little is known about the intracellular signal transduction pathways initiated by histamine H$_3$-receptor activation (Schwartz et al., 1991; Hill et al., 1997). We found a significant reduction of brain intrasynaptosomal Ca$^{2+}$ concentrations by THP. It is interesting to note that THP, GBP (our study) and PHT (Sohn and Ferendelli, 1973; DeLorenzo, 1988), all reduces Ca$^{2+}$ concentrations in the brain. The latter thus, may represent a common mechanism for their anticonvulsant effects \textit{per se} and/or in the observed additive/synergistic effects in combination. While THP significantly altered brain Ca$^{2+}$, RAMH was without any effect. The reason for such differential effects observed with H$_3$-receptor agonist and antagonist is not clear. However, two possibilities may be considered. Firstly, the effect of THP on intracellular Ca$^{2+}$ may be independent of its action on H$_3$-receptors. Secondly, two different receptor sites for H$_3$-receptors may be responsible. West et al. (1990) identified an H$_3$A site with high affinity and an H$_3$B site with low affinity for THP. Further, ample evidence exists for functional distinctions between the H$_3$-receptor subtypes (Arang et al., 1987; West et al., 1990). Our observations, thus, provide support for the possible role of distinct H$_3$-receptor subtypes in mediating such differential effects with agonist and antagonist. However, this needs to be investigated further to exclude alternative explanations.

Our findings suggests a neuroprotective role for GBP and THP. Further, intracellular Ca$^{2+}$ in the brain appears to be the direct target for their anticonvulsant and/or pro-cognitive effects. This lead is quite interesting as neuroprotective action is now considered a pre-requisite for an ideal AED (Wiard et al., 1995; Stefani et al., 1997). Besides, potential usage of these drugs might also be speculated in varied clinical
conditions in which intracellular Ca\textsuperscript{2+} accumulation plays a central role in neuronal excitability and development of cellular damage such as Epilepsy, Parkinson's disease, Alzheimer's disease and Ischemic brain injury (DeCoster, 1995; Jimenez-Jimenez et al., 1996; Schousboe et al., 1997). Since newer AEDs have been designed on this premise (Wiard et al., 1995; Stefani et al., 1997), such optimistic speculation does not appear to be irrational.

4. OVERVIEW

A perusal of the results show that histaminergic mechanisms play a significant role in seizure generation and its control. Histamine appears to be an anticonvulsant neurotransmitter with an inverse relationship with epileptogenic activity. Most of the findings, in this work, are first reports e.g. protective action of THP against PTZ-induced convulsions and that of GBP against MSO seizures, effect of AEDs on brain histamine levels, measurement of Ca\textsuperscript{2+} in brain synaptosomes following THP and GBP, interactions of H\textsubscript{3}-receptor ligands with AEDs on both seizures and cognitive function and with anticholinesterase TAC and BM extract on cognitive function, pro-cognitive effects of GBP and that of THP on spatial alternation, introduction of TSZ as an additional and more reliable parameter in the PAP test etc. Results of special practical importance/clinical significance include protective action of H\textsubscript{3}-receptor antagonist THP and nootropic plant drug BM against AEDs-induced cognitive deficit without compromising with their anticonvulsant efficacy. The study gives indicators that adverse effects on cognitive function are not unavoidable. The present results hold great promise for choosing AED (e.g. CBZ and GBP) and for rational polytherapy (AEDs + adjuncts) in epilepsy management. The latter represents a significant advance towards the cherished aim of maximum seizure control with minimum adverse effects and discomfort to the patient.

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