DISCUSSION
The past thirty years has seen our appreciation of the function of DA in brain elevated from that of a precursor for other catecholamines, principally norepinephrine, to a neurotransmitter in its own right. The association of disturbances of DAergic neurotransmission with neurological and psychiatric disorders has further emphasized the crucial role of this neurotransmitter in normal brain functioning and stimulated much basic research into DAergic neurotransmission. DAergic systems in various parts of the brain are involved in the control of motor activity, autonomic processes and emotional behaviour. DA agonists now have a firmly established role in the treatment of Parkinson's disease, while the DAergic antagonists have a longer history in the treatment of schizophrenia, Huntington's disease and Gilles de la Tourette syndrome. Although agents which alter the synthesis, release, reuptake or catabolism of DA are useful both therapeutically and experimentally, drugs which act directly on DA receptors, as agonists or antagonists, have proven most useful in delineating the biochemical, electrophysiological, and behavioural functioning of the DAergic system. This has geared considerable theoretical as well as therapeutical interest in defining specific characteristics of D_1 and D_2 DA receptor subtypes and their role in DA-mediated neuropsychiatric disorders. The possibility of a functional
interaction between these two receptor subtypes has also arisen from the use of highly selective D₁ and D₂ agonists in biochemical, physiological and behavioural studies.

Currently, the cellular localization of DA receptors is a matter of controversy, with some schools holding to the idea that D₁ and D₂ DA receptors occur on the same neuron (Seaman et al., 1989; Bertorello et al., 1990; Piomelli et al., 1991) while evidence from other laboratories has shown that D₁ and D₂ DA receptors co-exist on only a small fraction of striatal efferent neurons (Gerfen et al., 1990). The location and the roles of DA receptor subtypes have been further complicated by the discovery of three additional DA receptors: D₃, D₄ and D₅. While clearly the role of D₃, D₄ and D₅ receptors must eventually be determined, our current knowledge of these receptors and of selective drugs for manipulating them is still scant. Furthermore, the conventional therapy for schizophrenia and Parkinson’s disease is plagued by serious side effects. The antipsychotics currently in use cause various motor dysfunctions and hyperprolactinemia, while the drugs used to treat Parkinsonism can cause nausea and vomiting, choreiform movements, psychiatric disturbances and cardiovascular problems. Hence a clearer understanding of which subtypes mediate particular physiological effects, and how D₁ and D₂ receptors interact would promote the development of more specific drugs and improve the treatment of these disorders.
A thiazoloazepine derivative, B-HT 920 [2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d) azepine] has been characterized as a specific central and peripheral alpha₂- adrenoceptor agonist (Kobinger and Pichler, 1977, 1980). It causes hypotension and bradycardia like other alpha₂ agonists. B-HT 920 has been reported to possess several centrally mediated pharmacological actions similar to clonidine (Parale and Kulkarni, 1985; Kulkarni et al., 1986a,b; Kunchandy and Kulkarni, 1986). All of these responses had a central site of action sensitive to reversal by alpha₂- adrenoceptor blockers, yohimbine or idazoxan. B-HT 920 has also been shown to display its prejunctional alpha₂- adrenoceptor agonistic activity in various in vitro models (Mottram, 1983).

Subsequent studies have demonstrated that B-HT 920 also acts as an agonist at D₂ DA autoreceptors. For example, B-HT 920 decreases the rate of disappearance of brain DA after inhibition of tyrosine hydroxylase, decreases the accumulation of DOPA in the striatum and nucleus accumbens of rats treated with gamma-butyrolactone (Anden et al., 1982, 1983), and decreases the firing activity of DA neurons in the substantia nigra after both intravenous and iontophoretic administration (Eriksson et al., 1985; Bergstrom et al., 1986).
However, some data indicate that B-HT 920 has effects on postsynaptic DA receptors. In vitro, B-HT 920 inhibits the K⁺-stimulated release of both DA (autoreceptor action) and acetylcholine (postsynaptic action) at similar concentrations (Schmidt et al., 1986). In vivo, B-HT 920 increases the firing activity of globus pallidus neurons, an effect consistent with a postsynaptic DA receptor agonist action (Bergstrom et al., 1986). Hsu et al. (1986) reported that B-HT 920 induced emesis in dogs in a haloperidol-sensitive manner. Haloperidol was unable to block the sedative effect of B-HT 920 while it prevented the emetic effect. B-HT 920 produces weak stereotypy in normal animals (Verma and Kulkarni, 1991a) but intensification of postsynaptic D₂ DAergic actions of B-HT 920 is observed in animals depleted of brain DA by means of reserpine or destruction of forebrain DA pathways (Hinzen et al., 1986) or when the drug is given in combination with a D₁ agonist (Braun and Chase, 1986; Robertson and Robertson, 1987). The possibility that the lack of D₁ agonist activity of B-HT 920 might be accounting for its apparent lack of or weaker postsynaptic DA agonistic effects in behavioural tests has stirred considerable interest in the concept of D₁/D₂ interplay.

Recently much of the research work has been focused on D₁/D₂ receptor mechanisms and their interplay. The use of selective D₁ and D₂ receptor ligands has shown that
concomitant stimulation of D\textsubscript{1} receptors is required to reveal the postsynaptic effects of D\textsubscript{2} agonists (Braun and Chase, 1986; Hjorth and Carlsson, 1987; Meltzer et al., 1988; Verma and Kulkarni, 1991a,b,c,d). The classical signs of postsynaptic D\textsubscript{2} DA receptor stimulation such as hyperlocomotion and stereotypy manifest when a low dose of a D\textsubscript{2} agonist is administered concomitantly with a D\textsubscript{1} agonist (Barone et al., 1986; Jackson and Hashizume, 1986; Pifland Hornykiewicz, 1988). Electrophysiological studies also support the enabling effect of D\textsubscript{1} stimulation on the functional effects of D\textsubscript{2} agonists (White, 1987; White et al., 1988). The behavioural effects of psychomotor stimulants once thought to be mediated by direct or indirect actions on D\textsubscript{2} DA receptors are actually a function of a synergistic interaction of endogenous DA and/or exogenous receptor agonists acting on both D\textsubscript{1} and D\textsubscript{2} DA receptors (Clark and White, 1987; Waddington and O’Boyle, 1989). This is in contrast to the biochemical interaction of these receptor subtypes on adenylate cyclase, the D\textsubscript{1} receptor increasing and the D\textsubscript{2} receptor decreasing or having no effect on the activity of this second messenger system (Kebabian and Calne, 1979). Although the exact mechanism for D\textsubscript{1}/D\textsubscript{2} interplay is not clearly known at present, this may have important implications for the treatment of DA-mediated disorders. The concept of D\textsubscript{1}/D\textsubscript{2} interdependence
has thrown light on the significance of the role of D₁ receptors in Parkinson’s disease and has provided a means for potentiating the effects of D₂ agonists. This will enable reduction of the dose of the D₂ agonist and the associated side effects besides conferring antiparkinsonian efficacy to D₁ agonists, which by themselves appear of little therapeutic value in Parkinson’s disease. The blockade of the behavioural effects of D₂ agonists by the D₁ antagonist, SCH 23390 supports the contention that a D₁ antagonist may be useful for the tonic dampening of elevated DAergic tone in schizophrenia. The fact that D₁ receptor is highly abundant in limbic areas and that SCH 23390 is very efficacious in blocking A 10 mesolimbic-mediated behaviours (Nielsen and Scheel-Kruger, 1986) suggests that D₁ antagonism may provide a new avenue for the development of atypical neuroleptics.

In the recent years, a distinction has been made between the "classical" neuroleptics and some newer "atypical" neuroleptics (White and Wang, 1983; Lowe et al., 1988). Classical neuroleptics (e.g. chlorpromazine, haloperidol and trifluoperazine) induce a variety of common side effects like the extrapyramidal side effects, increased levels of plasma prolactin and orthostatic hypotension. Atypical neuroleptics (e.g. clozapine and sulpiride) (Fig.37) retain the antipsychotic effect in the relative absence of extrapyramidal side effects or effect on plasma
Fig. 37. Atypical neuroleptics
prolactin levels. The relationship between antipsychotic drug action and D₂ receptor antagonism created a situation where new antipsychotic drugs were being developed exclusively on the basis of their ability to block this particular DA receptor. Until recently, this was the case; however, in the last few years, several new principles for obtaining antipsychotic drugs have emerged. Most of these principles centre around the unique properties of the atypical neuroleptics in an attempt to develop novel neuroleptics with an atypical profile. Some of these approaches are based on the classical DA receptor antagonism principle, whereas others are based on indirect ways to modulate DAergic neurotransmission.

Cortical glutamatergic neurons project to presynaptic DAergic terminals and thereby exert a modulatory effect on the release of DA. This situation may allow for glutamate antagonism as a new antipsychotic treatment principle. Glutamate receptors exist in several subtypes: the NMDA, quisqualate and kainate receptor subtypes (Honore, 1989). Recent intracerebral microdialysis work has shown that antagonists of quisqualate receptors can block activated DA release induced by for example, amphetamine, while having no effect on basal DA release (Honore, 1989). There is also the intriguing possibility that glutamate antagonists may provide protection against a putative
ischemic process in schizophrenia. Very recent evidence has demonstrated abnormal levels of glutamatergic binding sites in the orbital frontal cortex of schizophrenics (Deakin et al., 1989). Since glutamatergic neurons project to DAergic neurons in the amygdala, it may provide the mechanism which underlies the increased levels of DA in that area.

Recently, examination of interaction between NMDA antagonists such as MK 801 and DA receptor subtype specific agonists has revealed that MK 801 acts synergistically with D₁ and antagonistically with D₂ agonists. The substrate of the interaction between D₁ and NMDA receptors is at the moment only a matter of conjecture. Freund et al. (1984) have reported that DA terminals synapse with the neck of dendritic spines of medium sized spiny neurons, whereas cortical glutamatergic neurons terminate on the spine heads. This arrangement might be the anatomical substrate for an interaction between the two types of inputs, with DA receptors (probably the D₁ type) (Calabresi et al., 1987) exerting an inhibitory influence on the ability of cortical glutamatergic input to depolarize neuronal spines in the striatum (Smith and Bolam, 1990). According to this mechanism, blockade of NMDA receptors would exert on striatal neurons an effect in the same direction of the stimulation of D₁ receptors. This would explain the synergism between NMDA blockade and D₁ stimulation.
A more direct interaction between DA and NMDA receptors is suggested by the studies of Halpin et al. (1990) who have proposed that in striatal slices, the activation of NMDA receptors exerts, through a calcineurin dependent mechanism, an inhibitory influence on the ability of D₁ receptors to promote the phosphorylation of DARPP-32. This effect, which is antagonized by MK 801, provides a biochemical mechanism for a positive interaction between D₁ receptor stimulation and NMDA receptor blockade. This is supported by c-fos expression, an early gene specifically linked with D₁ receptors, in striatum following treatment with MK 801 and SKF 38393 (Morelli et al., 1991,1992).

An explanation of the mechanism of the inhibitory effect of the blockade of NMDA receptors on D₂ receptor stimulation is also hypothetical. According to a currently held view which is, however, not supported by radioligand binding studies (Joyce and Marshall, 1987), stimulation of presynaptic D₂ receptors inhibits the release of glutamate from cortico-striatal terminals (Maura et al., 1988). Therefore, under reduced glutamatergic neurotransmission such as after D₂ stimulation, administration of an antagonist might add little to the action of D₂ stimulation.

Although this mechanism explains the lack of potentiation of D₂ responses by MK 801, it fails to explain why MK 801 actually inhibits these responses. One might
hypothesize that at some point of the neural pathway which mediates D₂ responses is intercalated a glutamatergic synapse, which facilitates or mediates the expression of D₂ receptor stimulation, but is not involved in the behavioural expression of D₁ responses. The localization of such pathways is still hypothetical. This differential interaction between D₁ and D₂ receptor subtypes with MK 801 may, therefore, serve useful in providing a means for potentiating the effect of D₁ receptor agonist in a condition of DA denervation such as Parkinson’s disease. The observations of the present investigation have been discussed on these backgrounds in the following pages.

DIFFERENTIAL ROLE OF DOPAMINE RECEPTOR SUBTYPES IN THERMOREGULATION AND STEREOTYPIC BEHAVIOUR IN NAIVE AND RESERPINIZED RATS

In contrast to norepinephrine (NE) and 5-hydroxytryptamine, a DA action on the thermoregulation has been relatively less investigated. The first evidence that DA itself could play a physiological role in thermoregulation was obtained after administration of drugs that stimulate DA receptors (Barnett et al., 1972; Fuxe and Sjoqvist, 1972). Intracerebroventricular injection of DA agonists suggested that central DA has a hypothermic role in thermoregulation (Kruk, 1972). Studies performed with microinjections of DA support the hypothesis that rostral incertohypothalamic neurons of the medial preoptic region could be the DA
system related to temperature control (Cox and Lee, 1980; Brown et al., 1982), but the mesolimbic and the nigrostriatal (Cox and Lee, 1977; Brown et al., 1982) pathways have also been implicated. A role of DA in the thermoregulation is also strengthened by the induction of a marked hypothermia in rodents by reserpine, which is thought to result from the depletion of intraneuronal vesicular stores of NE and DA in the CNS and periphery and their subsequent destruction by monoamine oxidase (Carlsson, 1975).

The present study suggests the involvement of D$_2$ DA receptors in the hypothermic action of DAergic drugs in naive as well as reserpinized rats. In naive rats, B-HT 920, a D$_2$ DA agonist (Hsu et al., 1986) produced significant hypothermia, an effect sensitive to blockade by mixed D$_1$/D$_2$ antagonist, haloperidol. The DAergic origin of B-HT 920-induced hypothermia is also suggested by the inability of alpha$_2$-adrenoceptor antagonist, idazoxan and 5-hydroxytryptamine antagonist, cyproheptadine to block the hypothermic action of B-HT 920 in naive rats. The hypothermic effect of the mixed D$_1$/D$_2$ DA agonist, apomorphine (Tsuruta et al., 1981; Iorio et al., 1983) can also be attributed to its ability to stimulate D$_2$ DA receptors subsequent to the blockade of apomorphine effect by the D$_2$ DA antagonist, sulpiride in naive as well as
acutely reserpinized rats. Stimulation of D₂ DA receptors by D₂ agonists has also been previously reported to be involved in the decrease of body temperature in naive animals (Lapin and Samsonova, 1968; Maj et al., 1974; Cox et al., 1978; Carruba et al., 1980; Colboc et al., 1983; Carboni et al., 1986).

D₁ DA receptors, however, do not seem to play a significant role in thermoregulation since D₁ agonist, SKF 38393 did not produce any significant change in body temperature in naive and acutely reserpinized rats. SKF 38393, however, enhanced the hypothermic effect of B-HT 920 in naive and acutely reserpinized rats when given concurrently with B-HT 920. Postsynaptic DA receptor sensitization might explain intensification of the thermogenic effect of the combination in acutely reserpinized rats. The blockade of the facilitatory effect of the combination of SKF 38393 and B-HT 920 by D₁ antagonist, SCH 23390 (Hyttel, 1983) is consistent with the recent suggestions that D₁ and D₂ receptor interplay is essential for the full expression of D₂ DA receptor-dependent behavioural patterns (Barone et al., 1986; Jackson and Hashizume, 1986; Arnt and Perregaard, 1987; Verma and Kulkarni, 1991a,b). The diminished effect of B-HT 920 following acute treatment with reserpine might be explained by the decreased D₁ receptor tone due to endogenous amine depletion by reserpine. The partial involvement of other
neurotransmitter systems in thermoregulation, following reserpinization, is also ruled out by the inability of the alpha$_2$-adrenoceptor antagonist, idazoxan and of the 5-hydroxytryptamine antagonist, cyproheptadine to modify the effect of B-HT 920.

In contrast, pharmacologically different DA receptor subtypes were found to mediate different effects on body temperature in rats chronically treated with reserpine. Chronic reserpine treatment produced a significant hypothermia, subsequent to the depletion of endogenous amines (Carlsson, 1975). Following chronic reserpinization, B-HT 920 produced a D$_2$ receptor-mediated hypothermia, similar to the effect in naive and acutely reserpinized rats. This is evidenced by the blockade of B-HT 920 hypothermia by haloperidol but not by cyproheptadine or idazoxan. An increase in D$_2$ receptor density, subsequent to a decrease in DA transmission following chronic treatment with reserpine, might explain an increase in sensitivity to the thermogenic effect of B-HT 920 and, therefore, an intensification of the hypothermic response. D$_1$ receptor stimulation by SKF 38393, on the other hand, produced a significant rise in body temperature, unlike the effect in naive and acutely reserpinized rats. The opposing effects of the selective DA receptor subtypes on body temperature are also evident by the inability of the combination of...
B-HT 920 and SKF 38393 (5 mg/kg) to produce any change in body temperature and a rise in body temperature following administration of the D₂ agonist, B-HT 920 with a higher dose of SKF 38393 (10 mg/kg). The D₁ agonist-induced rise in body temperature in chronically reserpine-treated rats might be explained (i) by the change in affinity for agonists of D₁ receptors, or (ii) by the hypothermia produced by chronic treatment with reserpine, so that the lower the basal temperature, the easier the increase in body temperature can be measured. Administration of reserpine for 5 days has been shown to increase the D₁ receptor-mediated adenylate cyclase activity without producing changes in D₁ receptor density (Missale et al., 1989). Administration of reserpine, for 2 weeks, however, has been shown to increase the D₁ receptor density in the striatum and nucleus accumbens (Schambron et al., 1987). So, acute treatment with reserpine might not be sufficient to produce a significant alteration in the affinity for D₁ agonists so as to exhibit a rise in body temperature, despite the reduction in basal temperature produced by acute reserpine treatment. The possibility that pharmacologically different DA receptor subtypes mediate different effects on temperature is also supported by the rise in body temperature produced by the mixed D₁/D₂ agonist, apomorphine in chronically reserpinized rats, unlike its effect in naive or acutely reserpinized rats. Furthermore, the hyperthermic effect of the combination of
B-HT 920 and SKF 38393 (10 mg/kg) was reduced or reversed to hypothermia by the D₁ antagonist, SCH 23390 and enhanced by the D₂ antagonist, sulpiride in chronically reserpinized rats. Similarly, the hyperthermic effect of apomorphine was significantly enhanced when given concurrently with SKF 38393 or reverted to a significant hypothermia when administered in combination with B-HT 920.

The thermogenic action of B-HT 920 in naive and reserpinized rats is mediated through postsynaptic D₂ DA receptors since, in the doses employed, B-HT 920 produced a stereotypic behaviour in rats, which is a classic effect of postsynaptic D₂ DA receptor activation. The intensification of the stereotypic response of B-HT 920 following acute as well as chronic reserpinization, might be attributed to a supersensitization of D₂ receptors. The postsynaptic stereotypic effect of B-HT 920 was facilitated by SKF 38393, when administered concurrently, in naive as well as acutely reserpinized rats, as described earlier (Braun and Chase, 1986; Pifl and Hornykiewicz, 1988; Verma and Kulkarni, 1991a). In contrast to the reciprocal interaction between DA receptor subtypes observed in chronically reserpine-pretreated rats, as regards thermoregulation, a facilitatory interaction between the D₁ and D₂ DA receptor agonists was observed as regards stereotypy. The stereotypic effect of the combination was extremely intense following chronic
reserpinization, which might be attributed to a significant upregulation of the D₁ as well as of the D₂ DA receptor density in the striatum (Neisewander et al., 1991).

The present study, therefore, demonstrates the enabling effect of D₁ DA receptors on the D₂-mediated stereotypic and hypothermic action in naive and acutely reserpinized rats. Though such an interaction was also observed for the stereotypic action in rats rendered supersensitive following chronic treatment with reserpine, a reciprocal interaction between D₁ and D₂ DA receptor subtypes was observed for the thermogenic action in chronically reserpine-treated rats.

ON THE D₁ AND D₂ DOPAMINE RECEPTOR PARTICIPATION IN LEARNING AND MEMORY IN MICE

In the present study haloperidol (0.05 and 0.1 mg/kg), a D₂ DA receptor antagonist (Hyttel, 1978; Billard et al., 1984; Andersen and Nielsen, 1986) impaired passive avoidance retrieval without affecting the acquisition phase of memory process. The higher doses of haloperidol (0.25 and 0.5 mg/kg) disrupted the acquisition as well as the retrieval of the passive avoidance response. Davidson and Weidley (1976) have put forth the hypothesis that the impairment of the avoidance response produced by treatment with neuroleptics during the acquisition phase is primarily due to the impairment of motor performance, rather than to the impairment of learning associated with stimuli and
response contingencies (Fibiger et al., 1975; Beninger et al., 1980). Our observation indicating that molindone and sulpiride, D$_2$ DA receptor antagonists, also impaired passive avoidance retrieval without disrupting the acquisition performance is also consistent with this theory. SCH 23390, a D$_1$ DA receptor antagonist (Iorio et al., 1983) disrupted the passive avoidance acquisition but not the retention of the learned task. In the dose used SCH 23390 neither produces catalepsy nor reduces the sensory intensity of the electric foot-shock (Ichihara et al., 1988a). Clozapine, an atypical neuroleptic with mixed D$_1$/D$_2$ antagonistic activity (Billard et al., 1984; Andersen and Nielsen, 1986), attenuated the ability of mice to acquire as well as retrieve the passive avoidance response.

To be considered as having memory - enhancing effect and to be eliminated as a "false positive" that merely affects locomotor activity, a compound must be active in more than one paradigm. In fact an ideal choice would be the use of tasks employing choice measures of performance. In general, the procedures that require an animal to make a choice are less likely to be affected by CNS stimulation or depression. In the present study TL in the retention test was significantly shorter than that on the 1st day in the vehicle-treated group. This suggests that TL can be one of the indicators for learning and memory. SCH 23390
(0.1 mg/kg) did not interfere with the retrieval phase of memory process but produced a significant disruption of the acquisition phase of the memory process as reflected by the prolongation of the TL on the 1st day. Haloperidol (0.1 mg/kg), sulpiride (100 mg/kg) or molindone (2.5 mg/kg) failed to change the TL on the 1st day although impaired the performance of mice in the retention test. Clozapine (10 mg/kg) on the other hand, prolonged the TL on the 1st as well as the 2nd day. The above data suggests the impairment of retention performance following the administration of sulpiride, molindone or low doses of haloperidol, attributable to the blockade of D2 DA receptors. Impairment of acquisition by SCH 23390 supports the role of D1 DA receptors in the acquisition phase of the memory process. The preferential role of D1 and D2 DA receptors in learning and memory, respectively is supported by the ability of clozapine, a mixed D1/D2 antagonist, to disrupt acquisition as well as retention of the learned tasks.

To investigate the functional differences between the stimulation of DA receptor subtypes in memory and learning, the effect of the selective D1 and D2 DA receptor agonists on short-term memory was also studied. B-HT 920 (0.1 and 0.25 mg/kg), an alpha2- adrenoceptor (Mottram, 1983) and non-selective D2 DA receptor agonist (Anden et al., 1983; Hsu et al., 1986), failed to influence the passive avoidance acquisition and retrieval. The higher dose
of B-HT 920 (0.5 mg/kg), however, produced significant impairment of passive avoidance acquisition but not the retention performance in mice. Similar results were obtained with bromocriptine, another D\textsubscript{2} DA agonist. Furthermore, the impairment of passive avoidance acquisition by B-HT 920 was more pronounced in mice pretreated with alpha\textsubscript{2}-adrenoceptor antagonist, yohimbine indicating an unmasking effect of alpha\textsubscript{2}-adrenoceptors on the preferential action of the drug on D\textsubscript{2} DA receptors. Unusual arousal might have contributed to the impaired performance of mice, since activation of central DA receptors is known to disrupt latent inhibition (Solomon et al., 1981), latent learning (Ahlenius et al., 1977) and passive avoidance tasks (Ichihara et al., 1988b). This might also explain the impairment of passive avoidance retrieval following D\textsubscript{1} DA receptor activation by the selective D\textsubscript{1} DA agonist, SKF 38393 (Setler et al., 1978). A facilitatory interaction between D\textsubscript{1} and D\textsubscript{2} DA receptors has been described for a variety of functional responses (Verma and Kulkarni, 1991a,b,c). In the present study, a facilitatory interaction between B-HT 920 and SKF 38393 was, however, not observed when both were administered in combination. The effect of the combination was similar to the effect of either drug alone on acquisition and retrieval, respectively. The reversal of clozapine-induced impairment of passive avoidance acquisition by SKF 38393 and of
retrieval by B-HT 920 suggests the selective involvement of D₁ and post-synaptic D₂ DA receptors in acquisition and retrieval phases of memory processes, respectively. The above proposition is also strengthened by the ability of B-HT 920 to reverse the disturbance of passive avoidance retrieval produced by molindone or haloperidol. SKF 38393, however, failed to reverse the effect of haloperidol or molindone on the passive avoidance retention deficits. Furthermore, B-HT 920 failed while SKF 38393 effectively reversed SCH 23390-induced disruption of passive avoidance acquisition in mice.

In accordance with the findings on the passive avoidance paradigm, B-HT 920 prolonged the TL on elevated plus-maze when measured on the 1st day without influencing the TL in the retention test. On elevated plus-maze, SKF 38393 impaired the retention performance of mice without significantly affecting the acquisition. A synergistic interaction could not be similarly observed following concomitant administration of B-HT 920 and SKF 38393. The effect of the combination on acquisition and retrieval performance was similar to the effect of each drug alone on the TL measured on the 1st and 2nd day, respectively. The reversal of haloperidol (0.1 mg/kg)-induced impairment of memory retrieval by B-HT 920 and the reversal of SCH 23390-induced acquisition deficit by SKF 38393 also provide parallel to the findings on the passive avoidance paradigm.
The above data suggests that the retrieval phase of the memory process is unaltered following $D_2$ DA receptor stimulation but is attenuated subsequent to the blockade of $D_2$ DA receptors. $D_1$ DA receptor stimulation, similarly, does not impair the acquisition phase of memory which is impaired following the administration of a $D_1$ receptor blocker.

Scopolamine, an anticholinergic, impaired passive avoidance acquisition in mice. On elevated plus-maze, scopolamine disrupted the acquisition as well as the retrieval performance. Scopolamine-induced amnesia was not reversed by B-HT 920 or SKF 38393 on both the paradigms. Recently, $D_1$ and $D_2$ DA receptor stimulation was shown to exert opposite effects on striatal acetylcholine release, with $D_1$ stimulation enhancing the activity of cholinergic neurons while $D_2$ receptor activation having an inhibitory function (Damsma et al., 1991). However, a similar interaction between $D_1$ or $D_2$ DA receptors and cholinergic neurons fails to account for their role in memory and learning.

In conclusion, two independent studies conducted on elevated plus-maze and passive avoidance paradigm, respectively have suggested a preferential role of $D_1$ DA receptors in acquisition and of $D_2$ DA receptors in the retrieval stage of memory process. No conclusive evidence could be drawn for the possible link of DA receptor subtypes.
in cholinergic modulation of learning and memory in the
present paradigms.

**EFFECT OF D₁ AND D₂ DOPAMINE AGONISTS ON NEOCORTICAL AND HIPPOCAMPAL EEG ACTIVITY OF RAT BRAIN**

The present results provide electrophysiological
evidence that the putative autoreceptor -selective DA
agonist, B-HT 920 (Anden and Grabowska-Anden, 1988) exerts
postsynaptic D₂ DA agonistic effects in normal rats.
Electrophysiologically, B-HT 920 inhibited the frequency as
well as the amplitude of hippocampal and cortical firing, an
effect which is probably associated with postsynaptic DA
receptor activation (Johansen et al., 1988). In low doses,
B-HT 920 (0.1 mg/kg) increased the amplitude as well as the
frequency of electrical impulses in the hippocampus. This
probably occurs because of the disinhibition due to
selective DA autoreceptor stimulation. The lack of an
identical effect in cortical neurons can be attributed to
the neurophysiological functional differences of both the
areas. A similar increase in the firing of CA1 pyramidal
cells in the hippocampal slice was reported following
administration of pergolide, a D₂ DA agonist (Smialowski and
Bijak, 1987). The role of the postsynaptic D₂ DA receptors
in the inhibitory effect of B-HT 920 was supported by the
blockade of the inhibitory effect of B-HT 920 on cortical or
hippocampal neurons by the DA antagonist, haloperidol but
not by the alpha₂- adrenoceptor antagonist, idazoxan.
Recent electrophysiological and behavioural studies have demonstrated that many functional consequences of D₂ DA receptor stimulation require concurrent stimulation of D₁ DA receptors (Clark and White, 1987; Johansen et al., 1988). The present experiments demonstrated an inhibitory effect of SKF 38393, a D₁ DA agonist (Setler et al., 1978) on cortical and hippocampal firing. The combination of B-HT 920 and SKF 38393 produced significantly greater inhibition of cortical and hippocampal neurons as compared with the effect of each drug alone. This suggests the necessity of D₁ DA receptor stimulation for the expression of postsynaptic D₂ DA receptor-mediated inhibition of frequency and amplitude of cortical or hippocampal firing. The inhibitory effect of the above combination on the frequency and the amplitude of cortical or hippocampal EEG was comparable to the effect of apomorphine and DA, respectively, which stimulate both D₁ and D₂ DA receptors. Similar interplay between DA receptor subtypes has been described for cortical neurons (Kropf et al., 1989; Kropf and Kuschinsky, 1991). The role of D₁ and D₂ DA receptor co-activation explains the fact that B-HT 920, in low doses, was much less effective in inhibiting cortical or hippocampal neuronal activity. This diminished effect might have been due to an autoreceptor-mediated decrease in DA release and consequent lack of sufficient activation of D₁ DA receptors.
Rimcazole, a competitive antagonist of sigma receptor binding, antagonizes apomorphine-induced fighting in rats (Ferris et al., 1986). Direct action of rimcazole involves facilitation of NMDA-mediated cationic conductance in a non-competitive allosteric manner, subsequent to the blockade of the sigma site. The fact that rimcazole could antagonize animal models of psychosis induced by the stimulation of postsynaptic D2 receptors in the mesolimbic area by an indirect effect on DA neuronal activity, together with the observations that the agonists of the sigma receptor can produce psychosis and also indirectly increase DAergic activity, led to the attempts to explore the interaction between rimcazole and DA agonists electrophysiologically. In the present study, rimcazole increased the firing of the cortical and hippocampal impulses. The observation that rimcazole blocked the inhibitory effect of SKF 38393 or apomorphine on cortical and hippocampal neurons, respectively, suggest the ability of rimcazole to block D1 DA receptor-mediated functional responses. The lack of the ability of rimcazole to block the inhibitory action of B-HT 920 could be attributed to the differential interaction of D1 and D2 DA agonists, respectively with rimcazole. A reciprocal interaction between D1 or D2 agonists and MK 801 has also been previously described (Morelli and Di Chiara, 1990a,b). D2 DA agonists, in contrast with the D1 agonists, reduce DA
release and firing, thus producing a functional effect similar to that of neuroleptics. Thus, interaction of rimcazole with D₁ DA receptors might explain its ability to block the response of apomorphine, a mixed D₁/D₂ DA agonist.

The present study, therefore, suggests a facilitatory interaction between D₁ and D₂ DA receptor subtypes in rat hippocampus and neocortex. A modulatory effect of D₁ and D₂ DA receptor activation on the effect of rimcazole is also evident.

MODULATION OF MK 801 RESPONSE BY DOPAMINERGIC AGENTS IN MICE

In the present study MK 801 (0.1-0.5 mg/kg) and ketamine (2.5-10 mg/kg) produced dose-dependent stereotypy in naive mice. The stereotypic action of MK 801 as well as ketamine was blocked by treatment with reserpine and AMPT. This supports the previous suggestions that the stimulatory effects of MK 801 are brought about by catecholamine release, via a mechanism similar to that of methylphenidate (Clineschmidt et al., 1982). Biochemical studies have reported an increase in DA turnover in the striatum following the administration of MK 801 (Hiramatsu et al., 1989; Liljequist et al., 1991). Thus, the stereotypic action of MK 801 can be attributed to the activation of DAergic system subsequent to the blockade of NMDA receptors. In keeping with this view is a study showing fluphenazine-
reversible locomotor activation as well as increased ipsilateral striatal 3,4-dihydroxyphenyl acetic acid and homovanillic acid levels following the administration of glutamatergic antagonists into the substantia nigra pars compacta and the ventral tegmental area (Dawbarn and Pycock, 1981). MK 801- or ketamine-induced activation of the DAergic system might be brought about by the blockade of NMDA receptors located on DAergic terminals in the striatum (Javitt, 1987).

The observation that the various DAergic blockers antagonized the MK 801-induced stereotypic behaviour in naive mice reinforces the conclusion that the blockade of NMDA receptors can produce stereotypic behaviour via the stimulation of endogenous DA transmission in mice. The stereotypic response of MK 801 was blocked by the neuroleptics, haloperidol (0.5 mg/kg) and molindone (2.5 mg/kg). Haloperidol and molindone (also other neuroleptics) act as antagonists at D$_2$ DA receptors (Seeman, 1980), thereby blocking the actions of endogenous DA. Alternatively, a haloperidol-sensitive D$_2$ DA receptor is also involved in regulating the release of glutamate from presynaptic glutamatergic terminals (Roberts et al., 1982; Javitt, 1987). The selective D$_1$ antagonist, SCH 23390 (Iorio et al., 1983), and the "atypical" neuroleptic, clozapine, also blocked MK 801 response in mice. D$_1$ DA receptor blockade (Ellenbroek et al., 1991) and/or non-DAergic
actions of clozapine (Walker et al., 1990) might also be responsible for its ability to block the stereotypic action of MK 801. The above data, therefore, suggest a modulatory role of D₁ and D₂ receptor tone in the stimulant effects of non-competitive NMDA antagonists in mice.

In the present study the D₁ DA agonist, SKF 38393 (Setler et al., 1978) increased while the D₂ DA agonist, B-HT 920 (Hsu et al., 1986) reduced the stereotypic response of MK 801 in naive mice. This reciprocal D₁/D₂ interaction was evident in the failure of the ability of apomorphine, a mixed D₁/D₂ DA agonist, to potentiate the stereotypic response of MK 801 or ketamine. This is supported by the ability of SCH 23390 in blocking the potentiating effect of SKF 38393 on MK 801 response in naive mice. One possible explanation for the differential effect of D₁ and D₂ DA agonists on MK 801 effect is that D₂ agonists, in contrast with the D₁ agonists, reduce DA release and DA firing, thus producing a functional effect similar to that of reserpine plus AMPT and of neuroleptics. Further, D₁ receptor stimulation might interact in a positive manner with the blockade of NMDA transmission. Different neural pathways have been reported to mediate the behavioural effects of D₁ as compared with D₂ DA receptor stimulation on the stimulant action of MK 801 (Morelli and Di Chiara, 1990a,b).
Contrary to the effect in naive mice, MK 801 potentiated apomorphine response in mice pretreated with reserpine and AMPT. In monoamine-depleted mice and rats, NMDA antagonists have been reported to disclose the activational potential of other neurotransmitter systems, as illustrated by the marked synergism observed with an adrenergic or DAergic agonist, or a muscarinic antagonist (Carlsson and Carlsson, 1989a,b). This leaves open the problem of DAergic specificity of MK 801 action in reserpinized mice. In sharp contrast to the findings of Carlsson and Carlsson (1989a,b) our results suggest that an intact DAergic activity greatly facilitates the stimulatory action of MK 801, based upon the observation that there is a ten-fold difference in the dosage between the present study and the former study. A role of central catecholaminergic system was previously implicated by Clineschmidt et al., (1982).

Based upon the present behavioural and previously reported biochemical data (Deutsch et al., 1987; Hiramatsu et al., 1989) the effect of NMDA agonists/antagonists on the DAergic system appears to be complex. Our findings are consistent with the previous reports of blockade of ipsilateral turning behaviour elicited in response to MK 801 by reserpine and the neuroleptics. The above observations suggest that MK 801 is an indirect DA-releasing agent, a notion supported by the fact that it increases DA turnover.
biochemical data suggest that MK 801 releases DA in the same manner as methylphenidate or PCP. Further, MK 801 has also been reported to inhibit DA re-uptake mechanism (Snell et al., 1988; Hiramatsu et al., 1989), as was suggested earlier for the effects of PCP on DAergic mechanisms (Deutsch et al., 1987). In the present study the stereotypic response of MK 801 to DA-depleting and DA-receptor blocking agents would also be consistent with such a mechanism. These observations indicate that activation of DAergic mechanisms may contribute to the behavioural stimulation caused by NMDA antagonists, through an as yet unknown mechanism. The complexity of this interaction is due to the fact that excitatory amino acids are widely distributed in various parts of the brain and different regulatory mechanisms, e.g. trans-synaptic feedback, may participate in the regulation of transmitter release and re-uptake in different parts of the brain. A further study by applying these substances into well-defined, discrete brain areas would give a better insight into the interaction between NMDA agonist/antagonist(s), DA agonists and their role in pathophysiology and drug treatment of DA-mediated CNS disorders.

The present study, therefore, provides evidence for an involvement of endogenous DA transmission in the stimulant effects of non-competitive NMDA antagonists in
mice. The results also suggest that D₁ and D₂ DA receptor stimulation causes positive and negative modulation, respectively, of MK 801 response in mice. This study therefore suggests that the blockade of NMDA transmission could possibly provide an efficient means for potentiating the therapeutic antiparkinsonian effects of DA agonists stimulating D₁ DA receptors.

D₁/D₂ DOPAMINE AND N-METHYL-D-ASPARTATE (NMDA) RECEPTOR PARTICIPATION IN EXPERIMENTAL CATALEPSY IN RATS

In the present study perphenazine (5 mg/kg) and haloperidol (2 mg/kg), mixed D₁/D₂ DA antagonists, produced catalepsy in rats. A selective D₁ antagonist, SCH 23390 (Iorio et al., 1983), also produced catalepsy at a dose of 1 mg/kg. SCH 23390 has only weak or no D₂ antagonistic activity, since SCH 23390 inhibits the activity of DA-stimulated adenylate cyclase and exhibits no or only a weak effect on the decline in the release of [³H] choline and [³H] DA from striatal slices induced by apomorphine (Hyttel, 1984). Thus, in addition to D₁ DA receptor blockade the cataleptogenic action of SCH 23390, at least in part, might be mediated by indirect inhibition of D₂ receptor function through its D₁ blocking action. The combined administration of perphenazine (0.5 mg/kg) and SCH 23390 (0.1 mg/kg) - at doses that were completely devoid of activity alone - produced a significant cataleptic response in rats. This extends support to the concept that the functional
interaction between DA receptor subtypes (Arnt et al., 1987; Meller et al., 1988; Verma and Kulkarni, 1991a,b,c,d) is observed not only for the behaviours mediated by DA agonists but also for the behaviours mediated by DA receptor subtype antagonists.

In the present study, B-HT 920, a D₂ DA agonist (Hsu et al., 1986), reversed the cataleptogenic effect of perphenazine, haloperidol and SCH 23390. The effects of SKF 38393 on the catalepsy induced by DA antagonists were more complicated. SKF 38393 (1-5 mg/kg) markedly reduced the cataleptic response of SCH 23390 but failed to antagonize the effects of perphenazine and haloperidol. SKF 38393 has partial D₁ agonistic but no D₂ agonistic activity (Setler et al., 1978; Nielsen and Andersen, 1985). D₁ DA receptor activation by SKF 38393 may therefore not be sufficient to reverse the cataleptogenic action of haloperidol and perphenazine, which have D₁ as well as D₂ DA receptor blocking properties. In the catalepsy induced by SCH 23390, however, the stimulatory effect of SKF 38393 on D₁ receptors may be sufficient since SCH 23390 lacks D₂ receptor blocking property. This also supports the potentiation of the reversal effect of B-HT 920 following its combined treatment with SKF 38393 (5 mg/kg) in animals treated with SCH 23390. The insufficient D₁ DA receptor stimulation by SKF 38393 may explain the failure of SKF 38393 (5 mg/kg) to potentiate...
B-HT 920 effect against perphenazine-induced catalepsy. Increasing the dose of SKF 38393 (10 mg/kg), however, not only reversed the cataleptic effect of perphenazine but also enhanced the anticataleptic action of B-HT 920 against perphenazine. The fact that D₁ and D₂ agonists reduced the cataleptogenic effects of D₂ and D₁ antagonists, respectively, suggest the existence of a facilitatory interaction between D₁ and D₂ DA receptors in catalepsy. A number of reports, using selective agonists, have previously suggested functional interaction between D₁ and D₂ agonists in stereotypy, locomotion and neuronal firing (Braun and Chase, 1986; Mashurano and Waddington, 1986; Robertson and Robertson, 1986; Arnt and Perregaard, 1987; Arnt et al., 1987; Waddington and O’Boyle, 1989).

MK 801 provided protection against perphenazine-, haloperidol- and SCH 23390-induced catalepsy. The observed anticataleptic effect of MK 801 is consistent with earlier reports (Schmidt and Bubser, 1989; Mehta and Ticku, 1990). This observation indicates the involvement of NMDA receptors in catalepsy and the potential role of glutamate-DAergic interaction (Kulkarni and Ticku, 1989) in the treatment/pathophysiology of catalepsy. The anticataleptic action of MK 801 supports the concept that the stimulatory effects of MK 801 are brought about by catecholamine release, the mechanism being similar to that of methylphenidate (Clineschmidt et al., 1982). Stimulation of
basal DA release from striatal slices by PCP has been reported (Snell et al., 1985). MK 801 also increases DA turnover in a manner identical to PCP (Rao et al., 1990). Thus, the anticataleptic action of MK 801 can be attributed to the activation of the DAergic system subsequent to the blockade of NMDA receptors (Carlsson and Carlsson, 1990). This suggests that DA glutamate interaction in the striatum is analogous to the presumed DA-cholinergic interaction within this structure. This is supported by the enhancement of the anticataleptic response following concomitant administration of MK 801 and scopolamine, an anticholinergic in perphenazine-treated animals. This calls for a therapeutic approach employing a NMDA antagonist with a muscarinic and/or DA agonist, in order to achieve optimal therapeutic effect and a minimum of side effects in the treatment of Parkinson’s disease. Clonidine, an alpha₂-adrenoceptor agonist, provided significant protection against perphenazine-induced catalepsy but failed to potentiate the effect of MK 801. In sharp contrast to our results, Carlsson and Carlsson (1989a,b) have suggested potentiation of MK 801 response by clonidine in monoamine-depleted mice. In fact, in monoamine depleted mice and rats NMDA antagonists have been reported to disclose the activational potential of other neurotransmitter systems, as illustrated by the marked synergism observed with an
adrenergic or DAergic agonist, or a muscarinic antagonist (Carlsson and Carlsson, 1989a,b). Further, considering the anticataleptic effect achieved with clonidine, the effect of B-HT 920 and bromocriptine may be mediated, in part, via alpha2- adrenoceptors. However, in combination with SKF 38393 the postsynaptic D2 DA receptor mediated behavioural effects of B-HT 920 have been reported to become manifest.

To further investigate the interaction between MK 801 and DA receptor subtypes, the effect of D1 and D2 receptor agonists on the anticataleptic effect of MK 801 was studied in animals treated with perphenazine or SCH 23390. SKF 38393 as well as B-HT 920 failed to enhance the anticataleptic effect of MK 801 in perphenazine-treated rats. A similar effect was obtained when B-HT 920 was administered in combination with MK 801 in animals treated with SCH 23390. SKF 38393 (5 mg/kg), however, potentiated the protective effect of MK 801 against cataleptogenic action of SCH 23390. A similar effect with B-HT 920 or SKF 38393 and MK 801 has been described in the previous study. D1 DA receptor stimulation might interact in a positive manner with the blockade of NMDA transmission, thereby explaining the facilitatory effect of D1 DA receptor stimulation by SKF 38393 on the anticataleptic action of MK 801 in SCH 23390-treated rats. Studies with a selective, full D1 agonist are necessary to examine the possibility of
the lack of a full stimulatory action of SKF 38393 in the presence of perphenazine. The lack of facilitatory action of B-HT 920 on the protective effect of MK 801 suggests that probably different neuronal pathways mediate the behavioural effects of D₁ as compared with D₂ DA receptor stimulation on the anticataleptic action of MK 801 (Morelli and Di Chiara, 1990a,b; Morelli et al., 1992). D₂ DA agonists, in contrast to the D₁ agonists, reduce DA release and DA firing, thus producing a functional effect similar to that of neuroleptics. This also explains the failure of the ability of bromocriptine, another D₂ DA agonist, to potentiate the anticataleptic effect of MK 801 in perphenazine-treated rats, although bromocriptine alone elicited a significant reversal of the cataleptic effect of perphenazine.

The present study provides evidence for the role of both D₁ and D₂ DA receptors in catalepsy and the existence of a functional dependence between D₁ and D₂ DA receptor blockade in catalepsy. The results also suggest possible involvement of endogenous DA in the anticataleptic action of MK 801. Further, D₁ DA receptor stimulation causes positive modulation of MK 801 response. This study, therefore, suggests that the blockade of NMDA transmission could possibly provide an efficient means for potentiating the antiparkinsonian effects of DA agonists acting via D₁ DA receptors, a strategy for therapeutic exploration.
MODULATORY ROLE OF D₁ AND D₂ DOPAMINE RECEPTOR SUBTYPES IN NOCICEPTION IN MICE

The present study demonstrates that acute administration of the D₂ DA agonists, B-HT 920 and bromocriptine produced antinociceptive response in mice. SKF 38393, a D₁ DA agonist (Setler et al., 1978), however, failed to elicit any significant antinociceptive response. The antinociceptive response of the mixed D₁/D₂ DA agonist, apomorphine can, therefore, be attributed to the stimulation of D₂ DA receptors. The above data is in agreement with the report of Ben-sreti et al., (1983a) who demonstrated the lack of antinociceptive activity of SKF 38393 in naive mice. Apomorphine has also been previously reported to exert antinociceptive effect (Paalzow and Paalzow, 1983; Jensen and Yaksh, 1984). In the doses used, B-HT 920 has been reported to produce classical effects of postsynaptic D₂ DA receptor stimulation such as stereotypy and hyperlocomotion (Verma and Kulkarni, 1991a). The postsynaptic D₂ DA receptor-mediated actions of B-HT 920 are, however, masked by its action on alpha₂-adrenoceptors and become unfolded only in presence of sufficient D₁ DA receptor stimulation. The potentiation of the antinociceptive action of B-HT 920 by SKF 38393 suggests a modulatory role of D₁ DA receptor system on B-HT 920-induced analgesia. The blockade of the potentiating response of the combination by haloperidol also lends credence to the above
proposal. The blockade of the antinociceptive activity of B-HT 920 by alpha$_2$-adrenoceptor antagonist, idazoxan but not by haloperidol and potentiation by SKF 38393 suggests the participation of both alpha$_2$-adrenoceptors as well as postsynaptic D$_2$ DA receptors in the analgesic action of B-HT 920.

Reserpine-induced hyperalgesia, accompanied by sedation, ptosis, and diarrhoea, could be attributed to its ability to deplete catecholamines and 5-hydroxytryptamine (Kulkarni and Robert, 1982). The hyperalgesic action of reserpine was reversed by B-HT 920 and bromocriptine but not by SKF 38393. Apomorphine also produced significant reversal of the hyperalgesic action of reserpine. The reversal action of B-HT 920 was blocked by haloperidol but not by idazoxan. The above data suggests the involvement of D$_2$ DA receptors in the reversal action of mixed D$_1$/D$_2$ agonist, apomorphine. A potentiating interaction was also observed following concomitant administration of B-HT 920 and SKF 38393 in reserpine-pretreated mice. A synergistic interaction between D$_1$ and D$_2$ DA receptors has also previously been described for a variety of functional responses under normosensitive and supersensitive conditions (Hjorth and Carlsson, 1987; Pifl and Hornykiewicz, 1988; Verma and Kulkarni, 1991a,c).

Morphine (5 and 10 mg/kg) produced a significant antinociceptive effect while at 1 mg/kg dose it failed to
alter the responsiveness to pain perception. Interaction between DAergic mechanisms and narcotic analgesics has been demonstrated in several studies. It is well known that morphine and other narcotic analgesics increase the turnover of DA in the brain and naloxone antagonizes these effects (Garcia-Sevilla et al., 1978). However, there is also evidence that the action of morphine in nigrostriatal neurons results from the blockade of striatal DA receptors (Wand et al., 1973; Celsen and Kuschinsky, 1974). In the present study, B-HT 920 potentiated morphine antinociception. This is in agreement with the observation of Zarrindast and Moghaddampour (1989) who showed potentiation of antinociceptive action of morphine by bromocriptine. The failure of the ability of idazoxan to block the effect of B-HT 920 in morphine-pretreated mice negates the possibility of involvement of alpha2-adrenoceptors in the facilitatory effect of B-HT 920 on morphine analgesia. The above finding highlights the participation of postsynaptic D2 DA receptors in the interaction of B-HT 920 with morphine. Unlike D2 DA receptor agonists, SKF 38393 reduced the antinociceptive action of morphine. The reciprocal D1/D2 DA receptor interaction is also evident in the failure of the ability of apomorphine to facilitate morphine antinociception. This also accounts for the lack of D1/D2 DA receptor interplay following concomitant administration of SKF 38393 and B-HT 920 in
morphine-pretreated mice. Further, the fact that reserpinization reduced the antinociceptive action of morphine suggests that central DAergic tone plays a role in opiate analgesia. Opiate receptors and their endogenous ligands have been reported to have a modulatory role on DA function (Nakamura et al., 1973; Garcia-Sevilla et al., 1978; Yonehara and Clouet, 1984). The observations, taken together, suggest not only a link in opioid and DAergic systems but also the opposing influences of the two distinct DA receptors on morphine-induced antinociception.

To further elucidate the interaction between DA receptor subtypes and opioid system, the effect of selective D₁ and D₂ DA receptor agonists on the nociceptive response of the opioid antagonist, naloxone was studied. In the present study naloxone produced hyperalgesia in mice. D₁ DA receptor activation, unlike D₂ DA receptor activation, did not reverse naloxone-induced hyperalgesia as evidenced by the failure of the ability of SKF 38393 to reverse the hyperalgesic effect of naloxone. Naloxone-induced hyperalgesia was, however, reversed by D₂ DA agonists, B-HT 920 and bromocriptine; the reversal effect of B-HT 920 being sensitive to blockade by D₁/D₂ antagonist, haloperidol and D₂ antagonist, sulpiride, respectively. It is, therefore, probable that only D₂ DA receptor stimulation by apomorphine might contribute to its ability to reverse the

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hyperalgesic action of naloxone. This is further substantiated by the lack of a facilitatory effect of SKF 38393 on the reversal action of B-HT 920 against naloxone.

The above study, therefore, supports (a) the role of DAergic system in antinociception and the possibility of existence of an interlink between opioid and DAergic system in the brain. (b) D₂ DA receptor activation plays a predominant role in the antinociceptive action of DAergic drugs (Phillips et al., 1992), the effect being facilitated by D₁ DA receptor activation in naive or reserpinized mice and (c) a reciprocal interaction of D₁ and D₂ DA receptor activation with morphine.

ROLE OF D₁/D₂ DOPAMINE AND N-METHYL-D-ASPARTATE (NMDA) RECEPTORS IN MORPHINE TOLERANCE AND DEPENDENCE IN MICE

The development of tolerance and dependence to the functional effects of morphine on chronic treatment is a characteristic feature in animals and man (Goudie and Emmett-Oglesby, 1989). Naloxone-precipitated withdrawal jumps in rodents constitute a classical test for the study of opioid dependence (Francis and Schneider, 1971). The role of the putative neurotransmitter(s) and their receptor system(s) in the action of morphine analgesia has long been envisaged. The functional consequences of DA receptor modulation on morphine analgesia is though known for a long time, the modulatory role of DA receptor subtypes is of
relatively recent origin (Nestler and Beitner-Johnson, 1992; Reddy et al., 1993; Shippenberg et al., 1993). In the present study, chronic administration of B-HT 920 or bromocriptine, D₂ DA receptor agonists, significantly attenuated the development of tolerance to morphine analgesia. Bromocriptine has been similarly reported to be useful in the treatment of cocaine abuse (Preston et al., 1992). Bromocriptine has been reported to acutely antagonize cocaine-induced behavioural arousal and depression at doses that are not behaviourally active when given alone (Cambell et al., 1989). Though in acute studies, B-HT 920 and bromocriptine significantly enhanced the analgesic effect of morphine yet an acute analgesic interaction between morphine and the respective D₂ agonists is unlikely to account for the lack of development of tolerance to the analgesic effect of morphine. This is substantiated by the observed analgesic response despite the lack of any pretreatment on day 10 and a significant reduction in the analgesic effect of B-HT 920 or bromocriptine in morphine-dependent mice as compared with their acute effects in naive mice. A decreased analgesic effect of B-HT 920 or bromocriptine in morphine dependent mice can be attributed to the possible sub-sensitization or down regulation of D₂ DA receptors in chronically morphine-treated mice. The failure of chronic D₁ receptor stimulation
to modify morphine tolerance is evident in the inability of repeated treatment with $D_1$ DA agonist, SKF 38393 (Setler et al., 1978) to influence morphine tolerance. An acute reciprocal interaction between SKF 38393 and morphine is, however, suggested by the reduction of morphine analgesia following acute injection of SKF 38393 or the decrease in tail-flick latency on day 1 and 3 in groups receiving repeated treatment with SKF 38393 followed by morphine as compared with the effect of morphine alone. This might also account for the failure of the ability of SKF 38393 to prevent the development of tolerance to the analgesic effect of morphine on day 9 and 10. The attenuation of morphine tolerance following repeated treatment with the mixed $D_1/D_2$ agonist, apomorphine may, therefore, be attributable to the repeated $D_2$ DA receptor stimulation. The lack of a $D_1/D_2$ interplay may explain the inability of $D_1$ stimulation by SKF 38393 to influence the analgesic effect of B-HT 920 in chronically morphine-treated mice. A down-regulation or sub-sensitization of $D_2$ DA receptors might similarly account for a weaker analgesic response of apomorphine or the combination of B-HT 920 and SKF 38393 on day 10 in morphine dependent mice in comparison with their acute interaction with morphine. The inability of the acute administration of the $D_2$ receptor agonists to prevent the development of morphine tolerance is, therefore, suggestive of a possible therapeutic benefit only following repeated exposure to $D_2$
agonists. However, the lack of any pretreatment on day 10 in groups receiving repeated administration of DA agonists followed by morphine on days 1 through 9 suggests that a D₂ agonist need not be present during testing to observe analgesia in chronically morphine-treated mice.

In addition to the effect on tolerance, the effect of DA subtype selective agonists on naloxone-precipitated morphine withdrawal was also investigated. Unlike the effect on morphine dependence, repeated administration of SKF 38393 or apomorphine suppressed morphine dependence as assessed by naloxone-precipitated jumps in mice receiving chronic treatment with morphine. An acute injection of SKF 38393 on day 10 in morphine-treated mice also produced significant attenuation of morphine withdrawal symptoms. However, the absence of SKF 38393 pretreatment on day 10, prior to the precipitated withdrawal signs rules out the possible involvement of an acute interaction between D₁ receptors and morphine in suppressing morphine withdrawal syndrome. On the other hand, chronic as well as acute administration of D₂ agonists, B-HT 920 and bromocriptine, respectively failed to attenuate the symptoms of morphine withdrawal. Alterations in the responsiveness of D₁ or D₂ agonists have been similarly described for other psychotropic drugs like cocaine, amphetamine and ethanol (Wise and Bozarth, 1987). Sensitization of nucleus accumbens neurons from cocaine -
treated rats to the inhibitory effects of SKF 38393 but not quinpirole has been reported (Henry and White, 1991). Various studies have also reported that D₁/D₂ receptors display enhanced responsiveness to agonists following certain schedules of repeated administration (White et al., 1988). The suppression of opiate withdrawal following D₁ receptor stimulation could, therefore, be due to competing alterations in DA receptor sensitivity in morphine dependent mice (D₂ subsensitivity vs D₁ supersensitivity). D₁ receptor stimulation could also account for the inhibitory effect of apomorphine or of the combination of B-HT 920 and SKF 38393 on naloxone-precipitated opiate withdrawal. The enabling effect of D₁ DA receptor stimulation, described earlier, for a variety of D₂ receptor-mediated functional responses (Braun and Chase, 1986; Verma and Kulkarni, 1991a,b,c) fails to account for the effectiveness of apomorphine or of the combination of B-HT 920 and SKF 38393 in suppressing naloxone-precipitated withdrawal jumps. It is possible that D₁ supersensitization, subsequent to the inhibition of DA neurotransmission following repeated morphine administration (Stinus et al., 1992) relieves the D₂ receptors of the necessity of concomitant D₁ stimulation. Such functional "uncoupling" of D₁ and D₂ receptors has been reported in various behavioural and electrophysiological studies in rats with supersensitive D₁ and D₂ receptors. The above data, therefore, suggests differential alterations in D₂ and D₁ DA
receptor responsiveness following repeated morphine administration (dependence) and withdrawal, respectively. The inhibition of morphine tolerance and dependence by D$_2$ and D$_1$ agonists, respectively suggest that selective DA receptor subtypes are involved in the behavioural changes and, therefore, presumably in the neural adaptations produced by repeated morphine administration.

The effectiveness of the non-competitive NMDA antagonist MK 801 following chronic administration, in attenuating morphine tolerance and dependence might suggest a possible similarity in the mechanism of action of MK 801 and DA agonists, respectively. Psychoactive drugs like morphine, amphetamine, cocaine and nicotine can increase the extracellular levels of DA at terminals of the mesolimbic DA system. MK 801 may, therefore, be affecting the functioning of these DA terminals. Support for the above contention comes from the recent studies showing that MK 801 can block the priming effect of apomorphine on turning behaviour produced by the DA agonist, SKF 38393 (Morelli and Di Chiara, 1990b) and long term sensitization of DA receptors with SKF 38393 (Griswell et al., 1990). In the present study, mice receiving chronic treatment with the combination of B-HT 920 or bromocripitine and MK 801 remained analgesic throughout the study. Similarly, the combination produced significantly greater inhibition of naloxone-
precipitated jumps as compared with the effect of each drug alone. Concomitant administration of SKF 38393 and MK 801, however, produced same effect on tolerance and dependence as the effect of MK 801 alone. The increase in the inhibitory effect of MK 801 against morphine withdrawal, when given in combination with apomorphine can, therefore, be attributed to the interaction of MK 801 with D_2 DA receptors. The interaction can, however, be additive since morphine effects are reported to be mediated through the DA-dependent as well as non-DA dependent mechanisms. Furthermore, since MK 801 has been reported to impair learning in the radial arm maze (Ward et al., 1990) it is conceivable that MK 801 may block learning processes that might be involved in sensitization to morphine. Thus, the adaptive changes seen with chronic morphine administration may involve DA as well as NMDA receptors. The (additive) interaction between D_2 agonists and NMDA antagonists may, therefore, provide a novel approach for modifying adaptive processes in morphine addiction.
The azepine derivative B-HT 920 was originally classified as a highly selective alpha2 adrenoceptor agonist (Mottram, 1983). Subsequent studies demonstrated that B-HT 920 acts as an agonist on DA autoreceptors (Anden et al., 1983; Eriksson et al., 1985). For example, B-HT 920 decreases the rate of disappearance of brain DA after inhibition of tyrosine hydroxylase, decreases the accumulation of DOPA in the striatum and nucleus accumbens of rats treated with gammabutyrolactone (Anden et al., 1982, 1983) and decreases the firing activity of DA neurons in the substantia nigra after both intravenous and iontophoretic administration (Eriksson et al., 1985; Bergstrom et al., 1986). Recent studies have demonstrated the postsynaptic D2 DA receptor- mediated pharmacological actions of B-HT 920 (Hsu et al., 1986; Hjorth and Carlsson, 1987; Pifl and Hornykiewicz, 1988). In the present study, in the doses used B-HT 920 increased the DA content of the whole rat brain, thereby supporting the above contention. This is in line with the reported behavioural and electrophysiological observations. In the doses used, B-HT 920 has been found to produce classical signs of postsynaptic D2 receptor stimulation such as hyperlocomotion and stereotypy (Braun and Chase, 1986; Barone et al., 1986; Verma and Kulkarni, 1991a) and inhibition of neuronal firing (Johansen et al.,
The lack of a significant effect of the lower dose of B-HT 920 (0.1 mg/kg) on the DA content can be attributed to the masking effect of alpha₂-adrenoceptor and D₂ DA autoreceptor agonistic actions of B-HT 920 on its stimulant action on postsynaptic D₂ DA receptors. However, the postsynaptic activity of B-HT 920 has been reported to be uncovered by concomitant D₁ DA receptor stimulation by SKF 38393. This has led to a general assumption that central D₁ and D₂ DA receptor interplay is essential for the full expression of the postsynaptic behavioural or electrophysiological effects of D₂ agonists (Barone et al., 1986; Clark and White, 1987; Robertson and Robertson, 1987).

In the present study, the D₁ agonist, SKF 38393 (Setler et al., 1978) failed to influence the DA content of the whole brain of rat. Contrary to the studies on animal behaviour, D₁ receptor stimulation by SKF 38393 failed to enhance B-HT 920-induced increase in DA content. This suggests that a facilitatory interaction between D₁ and D₂ DA agonists cannot be attributed to the enhancement of DAergic transmission in the brain. The only mechanism to explain D₁/D₂ synergy, to date, involves activation of D₁ receptors in the substantia nigra pars reticulata and D₂ receptors in the striatum (Robertson and Robertson, 1987).

The concept of DA-glutamate interaction has derived support from behavioural, biochemical as well as
electrophysiological observations (Freed et al., 1980; Clineschmidt et al., 1982; Javitt, 1987; Schmidt and Bury, 1988). Non-competitive NMDA antagonist, MK 801 has been reported to increase the rate of DA turnover (Hiramatsu et al., 1989; Liljequist et al., 1991) at doses which increased stereotypy, head weaving and ataxia. The present data indicating an increase in DA content of the whole brain following MK 801 administration supports the concept of the DAergic actions of MK 801. The reported stereotypic and anticataleptic actions of MK 801 can, therefore, be attributed to the enhanced DAergic transmission subsequent to MK 801 administration. The abolishment of stereotypic effect of MK 801 following reserpinization or haloperidol administration (previous studies) also supports the above proposal.

In the present study SKF 38393 increased while B-HT 920 decreased the DA content when administered in combination with MK 801. This is in line with the behavioural observations. D₁ receptor stimulation has similarly been found to increase while D₂ stimulation to decrease the stereotypic or anticataleptic action of MK 801. In the 6-OHDA model of turning, MK 801 reportedly potentiated the contralateral turning induced by the D₁ agonists, SKF 38393 and CY 208-243, while it reduced the contralateral turning induced by D₂ agonist LY 171555 (Morelli and Di Chiara, 1990a). These findings suggest that
an increase in the DAergic transmission is involved in the behavioural expression of NMDA receptor blockade. The present study, therefore, provides a biochemical correlate of the postsynaptic D₂ DAergic actions of B-HT 920 and of the modulatory influence of DA receptor subtype stimulation on the reported behavioural effects of MK 801 in experimental animals.