Yawning Behavior for Preclinical Drug Evaluation

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Introduction

The use of psychotropic agents is widespread, as is evident from the fact that many patients have been treated with them since their introduction. Concepts of the relationship between the actions of psychotropic drugs and the functions of specific brain systems have particularly progressed through the relatively brief history of psychopharmacology. Furthermore, efforts to provide explanations for drug-induced neurological changes will continue to focus on synaptic transmitters and their mechanisms. New approaches in drug development have been advanced by studies which have permitted the identification of receptor subtypes, undetected by traditional pharmacological approaches, with receptor heterogeneity providing an opportunity for greater pharmacological selectivity. All of these factors must be kept in mind when attempting to develop comprehensive explanations of the effects of drugs.

The association between specific clinical syndromes and predictable responses to psychotropic drugs has supported the impressive recent progress in this area. Testable hypotheses about possible biological bases of severe psychiatric illnesses have been stimulated by knowledge of the mechanisrns of action of psychotropic agents. In recent years, emphasis has been focused on biogenic amines and their receptors in the brain, their probable mediation of many effects of psychotropic drugs and their possible causal involvement in mental illness. Antipsychotic or neuroleptic drugs, which have been used to treat psychoses, have beneficial effects on mood and thought and antagonize the neurotransmitter actions of dopamine in the forebrain. It has, therefore, been proposed that there may be a state of functional overactivity of dopamine in the limbic system or cerebral cortex in schizophrenia or mania. However, these drugs carry the risk of producing characteristic side effects that mimic neurological diseases. Although the antipsychotic drugs have had a revolutionary, beneficial impact on medical and psychiatric practice, much attention has been given to the disadvantages of treatment with psychotherapeutic drugs, especially to their limited efficacy in severe or chronic mental illnesses, their frequent association with extrapyramidal neurological effects and their risk of occasional serious toxic effects.

Antipsychotic drugs which block postsynaptic dopamine receptors cause extrapyramidal symptoms that resemble parkinsonism, especially in older patients. In addition, there is a marked deficiency in dopaminergic innervation of the basal ganglia due to degeneration of neurons in the substantia nigra. The loss of this catecholamine from the basal ganglia has been shown to underlie all of the major motor manifestations of parkinsonism. Restoration of dopaminergic transmission restores motor function in parkinsonism and forms the central strategy in virtually all current drugs regimens for treatment of the disease. Many antipsychotic drugs interfere with the neurotransmitter actions of dopamine, and their antidopaminergic effects may well account for the diverse extrapyramidal effects of neuroleptic drugs. The antidopamine receptor effects of neuroleptic drugs also influence hypothalamic regulatory hormones and result in profound changes in the endocrine system, such as increased secretion of prolactin.

Although evaluating the efficacy of any drug is problematic, with psychoactive drugs it is particularly difficult because of the limitations of screening and testing methods used to develop new agents, most of which offer few advantages over drugs already available for treatment. The essential characteristics of human mental disorders cannot be reproduced in animals. Cognition.. communication, and social relationships in animals are difficult to compare with those in humans, and thus, screening procedures in animals are of limited utility for the discovery of unique therapeutic agents. In addition, clinical evaluation of new drugs is hampered by nonhomogeneity of diagnostic groups and difficulty in applying valid, sensitive measurements of the effects of therapy.

The present review has been prepared on the basis of our experimental results obtained on yawning and prolactin with respect to drug evaluation of new potential antipsychotic agents.

Behavioral drug evaluation of agents acting on dopaminergic function

A major goal of basic science research in the design of animal models has been to elucidate the neurochemical basis of psychotropic disorders in order to define pathogenesis and to improve treatment. As the biochemical bases of several abnormal symptoms are understood, the role of neurotransmitter systems in the pathogenesis of the disease might evolve. Because of the difficulty in defining one animal model that precisely matches all of the components of human psychosis, this goal may never be achieved. However, it is feasible to design an animal model for one facet of a complex clinical syndrome and thereby analyze the neurochemical mechanism responsible for the behavioral abnormality. For analysis of dopaminergic rnechanisms, behavioral animal models such as hyperaclivity, stereotypy and rotation have been used.

Hyperactivity

Results of the considerable work performed in animals suggest that increased motor activity (locomotion) induced by stimulants is related to stimulation of brain dopaminergic activity. In addition, biochemical and behavioral findings indicate the participation of activation at the brain norepinephrine level as well. Other experiments have implicated an inhibitory function of serotonergic systems in controlling motor activity. Thus, locomotor activity seems to involve alterations in several brain neurotransmitter systems and, therefore, may not be necessarily selective for dopaminergic mechanisms (1, 2).

Stereotypy

Agents that facilitate dopaminergic activity, such as apomorphine and d-amphetamine, can produce stereotypy (repetitive movements such as sniffing, licking, biting and gnawing), and neuroleptics, which block dopaminergic receptors, contrarily reduce the intensity of this behavior. Pergolide, a dopamine Dl and D2 receptor agonist, also evokes stereotyped behavior (3-5). It is also suggested that amphetamine-induced locomotor activity is at least partly mediated by dopamine released from mesolimbic dopamine neurons, whereas the stereotyped behavior is more closely related to the activity of nigrostriatal dopamine neurons (6). Hence, a predominant view at this time is that excessive dopaminergic activities in the brain are involved in causing stereotyped behavior (1).

Rotation (circling, turning) behavior

In animals with ascending nigrostriatal dopamine neurons unilaterally destroyed by local injections of 6-OHDA, d-amphetamine causes rotation toward the side of the lesion, whereas

apomorphine causes rotation away from it; thus animals rotate away from the side of the greatest dopaminergic activity in the striatum. This appears to be due to the development of supersensitive dopamine receptors in the lesioned striatum. Furthermore, bilateral destruction of mesolimbic dopaminergic nerve terminals by injection of 6-OHDA into the nucleus accumbens alters drug-induced rotation behavior of rats and results in a reduced rate of rotation in response to d-amphetamine and an enhanced rate in response to apomorphine (6). Pergolide, a dopamine receptor agonist, elicits turning behavior (5, 7).

Yawning

Dopamine

It has been reported that apomorphine exerts biphasic effects on behavior, that is, a decrease of motor activity at low doses, and stereotypy and hypermotility at high doses. We administered apomorphine to rats in order to find a certain modification of behavior, especially stereotypy, which has been proposed to be caused by dopamine receptor stimulation under several experimental conditions. However, the rats unexpectedly showed recurrent episodes of yawning with or without penile erections after injection of low doses of apomorphine (8). Each yawn was preceded by grooming or chewing or sometimes by sudden stretching of the forelimbs. Yawning began within 5 min after injection and was marked after 10-20 min. These responses to apomorphine were most pronounced at a dose of 0.25 mg/kg; the incidence of yawning at this dose was 87.5%, with a mean number of 4 yawns in 60 min. At higher doses, apomorphine induced dose-dependent stereotypy, characterized by slight sniffing at 0.5 mg/kg and by continuous licking and biting at 2.0 mg/kg, as previously reported. These responses were greatest 20 min after injection.

Yawning and stereotypy did not appear simultaneously (8). It was reported that intraventricular administration of the cholinergic agents ACTH, MSH or P-LPH, including their synthetic peptides, elicited yawning accompanied by sexual excitement, such as penile erections, in rats (9) and rabbits (l 0, 11). These agents were also reported to cause yawning in infant rats (12). The dose-response curve of yawning to apomorphine showed two peaks at doses of 0.05 and 0.5 mg/kg (8) i.p., suggesting that the apomorphine used in this experiment may have been decomposed since it had been stored for a fairly long period of time. We then performed a dose-response run with a new batch of the drug and obtained a dose-response curve with one peak at a lower dose of 0.25 mg/kg (Fig. 1) (13). The dopamine receptor agonists bromocriptine (1-32 mg/kg), piribedil (0.2-5 mg/kg), and 3-PPP (5-20 mg/kg) also produced dose-dependent yawning behavior with one peak, dernonstrating that the incidence of yawning decreases at higher doses of dopaminergic agents with optimal peak doses (14), as occurs with apomorphine. The dopamine receptor agonists thus produce yawning at lower doses and stereotypy at higher doses (13-16).

It has also been reported that low doses of apomorphine preferentially activate presynaptic dopamine autoreceptors, which results in an inhibition of dopamine release and consequent decrease in its synthesis, whereas higher doses stimulate postsynaptic receptors (17. 18). Accordingly, we first proposed that yawning elicited by low doses of apomorphine may be due to activation of presynaptic dopamine autoreceptors, while stereotypy induced by higher doses may be attributed to stimulation of postsynaptic dopamine receptors. However, after development of the microdialysis method, Stahle et al. determined apomorphine-induced extracellular dopamine levels in the rat corpus striatum and proposed that yawning and suppression of exploration induced by dopamine agonists are not related to changes in extracellular dopamine levels. On the basis of such findings, they proposed that autoreceptors are not mediators of behavioral effects of dopamine receptor agonists and that postsynaptic receptors mediate agonist-induced yawning (19, 20).

Apomorphine-induced yawning was completely antagonized by fluphenazine, a dopamine receptor antagonist (8). In addition, yawning produced by apomorphine, piribedil and 3-PPP, dopamine receptor agonists, was also strongly antagonized after sulpiride (14) and haloperidol (13), dopamine D2 receptor antagonists (Fig. 2).

Talipexole (B-HT 920), a selective dopamine D2 receptor agonist, dose-dependently evoked yawning but did not cause or caused only slight stereotyped behavior even at larger doses (21, 22). Yawning caused by talipexole was strongly inhibited by spiperone and YM-0915 1, D2 receptor antagonists, but was unaffected by SCH 23390, a Dl receptor antagonist. On the other hand, SK&F 38393, a Dl receptor agonist, did not elicit yawning behavior (21).

There as been substantial evidence that functional responsiveness of central dopamine receptors can be altered in response to synaptic situation. The supersensitivity of post-synaptic dopamine receptors occured after long-term impairment of dopamine neural transmission by 6-hydoxydopamine or reserpine (23, 24). In this respect, reserpine is known to cause supersensitivity of dopamine D2 receptors 18 h or more, but not 5 h, after treatment in rats (25). Twenty-four hours after treatment with reserpine, yawning induced by apomorphine, piribedil and talipexole was markedly potentiated (8, 26) and was antagonized by spiperone, a D2 receptor antagonist. Under even such supersensitive conditions with dopamine receptors, SCH 23390, a D1 selective agonist, was not able to cause yawning. These results suggest that dopamine D2 receptors, but not Dl receptors, participate in provoking yawning.

Experiments were also performed to determine the different properties of the dopamine D2 receptors related to yawning and stereotypy (22). The incidence of yawning produced by low doses of talipexole and SND 919, dopamine D2 receptor agonists, was decreased dose-dependently by SK&F 38393, a dopamine D1 receptor agonist (Fig. 3). On the other hand, talipexole and SND 919 at a high dose did not evoke or evoked only slight stercotypy, but the incidence of stereotypy by these agents was increased dose-dependently by SK&F 38393 (Fig. 4). Accordingly, the D2 receptors related to yawning are more sensitive to dopamine receptor agonists than those related to stereotypy. Moreover, concurrent stimulation of postsynaptic dopamine D1 receptors with D2 receptors reduces the incidence of yawning but enhances that of stereotypy (22).

Recent gene cloning studies have demonstrated the existence of different families of D1 -like (D A, D1B, D5) and D2-like (D2 long/short, D3, D4) receptors (27). Currently, considerable interest is focused on dopamine D3 receptors (28). Many antipsychotics display very high affinity for D3 receptors expressed in Chinese hamster ovary cells (29). For example, quinpirole was believed to be a selective dopamine D2 receptor agonist until recently, when it was demonstrated to have a 113-fold greater affinity for D3 receptors than for D2 receptors following discovery of dopamine receptor subtypes (28). Recently, 7-OH-DPAT was also identified as a dopamine receptor agonist having a higher affinity for D3 than for D2, D4 and D1 receptors (30). In addition, the signal transduction mechanism involved in D3 receptor responses seems to differ from that of its closest homologue, the D2 receptor (31).

Both 7-OH-DPAT and quinpirole evoked similar yawning behavior; the dose-response was bellshaped wilh a maximal effect at 25 and 100 μg/kg, respectively. These responses were strongly inhibited by spiperone, a D2 receptor antagonist (32). In the study on rat serum prolactin levels, 7-OH-DPAT and quinpirole also decreased levels dose-dependently (33) at a dose range similar to those of talipexole and SND 919, D2 receptor agonists (22). The decrease in prolactin levels induced by both drugs was antagonized by spiperone (33). Regarding these results, spiperone has been used as a dopamine D2 receptor antagonist but may not necessarily be selective for D2 receptors since Ki values were 0.069 and 0.61 nM, respectively, for D2 and D3 receptors (28). It bas also been proposed that the anterior pituitary is rich in D2 receptors but lacks D3 receptors (28). Consequently, dopamine D2 receptors seem to be involved in evoking yawning and decreasing

prolactin release, although proof of the possible involvement of D3 receptors must await the results of future experiments.

Acetylcholine

Physostigmine, an anticholinesterase agent, and pilocarpine, a direct acetylcholine agonist, elicited yawning at low doses and chattering at high doses in rats (Fig. 1) (8). Yawning behavior was unaffected by mecamylamine, a nicotinic receptor antagonist, implying that nicotinic receptors may not be involved (13, 16). In addition, since the behavior was abolished by scopolamine but not by methylscopolamine, a peripheral muscarinic receptor antagonist, it appears that yawning is mediated by muscarinic receptor activation in the brain (8).

Furthermore, apomorphine-induced yawning was strongly inhibited by dopamine receptor antagonists and scopolamine, but was not affected by methylscopolamine (8) and mecamylamine (13). After treatment with haloperidol, the yawning was practically eliminated, while with pilocarpine and physostigmine it was interestingly unchanged (13, 16). Yawning elicited by talipexole and SND 919 was strongly reduced not only by spiperone and YM- 09151-2, D2 receptor antagonists, but also by scopolamine. These results indicate that a dopaminergic mechanism precedes the cholinergic one, and doparninergic-cholinergic activation seems to he Closely involved in causing yawning behavior (Fig. 2).

M1, M2 and M3 receptors have been proposed as subtypes of the muscarinic receptor. M1 receptors exist in the brain, in such areas as the hippocampus, cerebral cortex and striatum. Several M 1 receptor agonists have been developed as potential antidementia agents. (-)-YM 796, a new muscarinic M1 receptor agonist, induced yawning which was potentiated by beta-receptor blocking agents. Yawning produced by YM 796 in combination with pindolol was inhibited by scopolamine, pirenzepine, and EEDQ, a M1 receptor antagonist, but not by spiperone and 4-DAP, a muscarinic M3 receptor antagonist (34). RS-86, a putative muscarinic M1 receptor agonist, administered intracerebroventricularly at low doses and subcutaneously at high doses, also produced yawning which was antagonized by scopolamine (35). Accordingly, the muscarinic M1 receptor seems to participate in evoking yawning.

Noradrenaline, adrenaline

Apomorphine-induced yawning was increased by pindolol, propranolol, indenolol, alprenolol and bucumolol which block the central beta-adrenoceptors, but not by the peripheral beta-adrenoceptor antagonists carteolol and atenolol (Fig. 5) (36). These beta-adrenoceptor antagonists given alone did not elicit yawning. Conversely, yawning was inhibited by salbutamol, a beta-adrenoceptor agonist, without being affected by prazosin, an alpha-adrenoceptor antagonist. The combined administration of SK&F 38393, a dopamine D1 receptor agonist, and the beta-adrenoceptor antagonists did not induce yawning (36). Yawning produced by talipexole and SND 919 was also potentiated by pindolol, without causing stereotypy (37). Yawning elicited by either apomorphine or piribedil in combination with pindoloi was suppressed by spiperone and YM-09151, dopamine D2-receptor antagonists, and scopolamine, a muscarinic receptor antagonist, but not by SCH 23390, a dopamine D1-receptor antagonist. Physostigmine or pilocarpine-induced yawning was also enhanced by pindolol and propranolol. This enhanced yawning was inhibited by scopolamine, but not by spiperone, YM-09151-2 and SCH 23390. Since the beta-adrenoceptor blockade facilitates the occurrence of yawning induced by dopaminergic and cholinergic agonists, the central adrenergic neuron systems may take part in the regulation of yawning responses (36).

There is evidence that adrenergic neurons, possessing high phenylethanolamine-N-methyltransferase activity which converts noradrenaline to adrenaline, exist in the brain (.38, 39). Intraperitoneal injection of tacrine or NIK-247, cholinesterase inhibitors, induced yawning which was markedly increased by pretreatment with a beta-adrenoceptor antagonist, pindolol. Yawning evoked by tacrine or NIK-247 given alone or in combination with pindolol was inhibited by pretreatment with scopolamine, but not by spiperone. Treatment with tacrine or NIK-247 increased acetylcholine content in the striatum, but this effect was not enhanced by pindolol which per se did not affect basal acetylcholine content (40). Pretreatment with the central adrenaline synthesis inhibitors LY-78335 and UK- 1187A also increased tacrine-induced yawning (40). Subcutaneous injection of talipexole evoked yawning, which was also increased by pindolol, LY-78335 and UK-1187A (37). These receptor antagonists and synthesis inhibitors per se did not cause yawning responses.

Since beta-adrenoceptor blockade and inhibition of adrenaline synthesis similarly facilitate yawning induced by cholinergic and dopaminergic agonists, the central adrenergic neuronal systems seem to be implicated in the regulation of yawning responses (40). Our results indicate that adrenergic neuronal activity inhibits cholinergic but not dopaminergic activation which is involved in causing yawning behavior . It is also suggested that adrenergic neurons interact with cholinergic neurons, and yawning caused by cholinergic activation is increased via adrenergic beta-receptor blockade. In addition, it has been confirmed that the stimulation of D1 receptors is not involved in the occurrence of yawning since dopamine D1 receptor stimulants are not able to evoke yawning even after treatment with beta-receptor blockers.

Yawning behavior elicited by talipexole was increased not only by pindolol, a beta-adrenoceptor antagonist, but also by prazosin and bunazosin, alpha-adrenoceptor antagonists. However, the yawning induced by physostigmine, an anticholinesterase agent, and pilocarpine, a direct muscarinic receptor agonist, was increased by pindolol but was unaffected by prazosin and bunazosin. In addition, yawning induced by the dopaminergic agonists, but not by the cholinergic agonists, was markedly suppressed by ST587, an alpha1-adrenoceptor agonist. All the yawning responses to dopaminergic and cholinergic agents were reduced not only by scopolamine, a muscannic receptor antagonist, but also by idazoxan, rauwolscine and yohimbine, alpha2-adrenoceptor antagonists (41).

Alpha-adrenoceptors have been subclassified into alpha1 and alpha2 subtypes. It has also been proposed that alpha2-adrenoceptors are located on noradrenergic and adrenergic neuronal pathways, and alpha2-adrenoceptor antagonists increase both noradrenaline and adrenaline release via blockade Of alpha2-receptor at central catecholaminergic nerve terminals (42, 43). Consequently, the noradrenergic neuronal mechanism appears to interact with dopaminergic mechanisms and participates via alpha1-receptor in decreasing the incidence of yawning caused by dopaminergic agonists without influencing the behavior induced by cholinergic agonists. It is also suggested that the stimulation of noradrenergic and adrenergic mechanisms induced by an increase in noradrenaline and adrenaline release resulting from presynaptic alpha2-receptor blockade might result in inhibition of yawning evoked by both dopaminergic and cholinergic activation (Fig. 2) (41).

Serotonin

The yawning responses to apomorphine, piribedil and talipexole, dopamine receptor agonists, were markedly increased by pretreatment with reserpine without eliciting stereotypy (26).

Piribedil-induced yawning was markedly inhibited after treatment with fluphenazine, scopolamine and methysergide (44). Yawning induced by apomorphine and talipexole was also increased by the serotonin synthesis inhibitor, p-chlorophenylalanine (PCPA), but was not affected by a-methyl-p-tyrosine (alpha-MT), implying that depletion of serotonin plays an important role in potentiation of yawning (26). It was later reported that apomorphine-elicited yawning was enhanced by pretreatment with PCPA or the serotonergic neurotoxin, 5,7-dihydroxytryptamine, and was contrarily reduced by the serotonin precursor, 5-hydroxytryptophan (45). In fact, serotonin is found in relatively high concentrations in the rat striatum (46, 47), one of the sites of action of dopamine receptor agonists in yawning (45, 48). Various lines of evidence have shown that the origin of serotonergic neurons in the striatum is the dorsal raphe (49), and the inhibitory serotonin receptors are located on terminals of dopaminergic neurons in the striatum (150-52). Lesioning of the raphe nucleus which reduces serotonin levels in the forebrain has been reported to cause an increase in dopamine release (53). The yawning evoked by combined administration of talipexole and PCPA was completely inhibited following spiperone or scopolamine (26). Therefore, treatment with PCPA may evoke an increased release of dopamine which plays a facilitatory role in the occurrence of yawning. Thus, it is assumed that the potentiation by reserpine or PCPA of yawning induced by dopamine receptor agonists involves decreases in serotonergic neuronal activity.

Intraventricular injection of a-melanocyte-stimulating hormone (alpha-MSH) elicited not only yawning-stretching syndrome but also 'wet dog' body shaking. Yawning was synchronized with stretching in almost all cases. The alpha-MSH-induced yawningstretching syndrome was blocked by scopolamine, apomorphine, fluphenazine and methysergide. Therefore, reciprocal balance of serotonergic activation, dopaminergic inhibition and cholinergic activation is involved in yawning produced by (alpha-MSH (44).

Neuropeptides

Central administration of adrenocorticotropic hormone (ACTH) was reported to cause yawning behavior (54, 55). Wood et al. (56) have proposed that the septal-hippocampal cholinergic neurons are necessary to elicit a specific stretching-yawning syndrome following ACTH or alpha MSH.

The peptide oxytocin also elicited yawning. The yawning response to oxytocin was markedly increased by pretreatment with an beta-adrenoceptor antagonist, pindolol (20 mg/kg), which per se did not elicit yawning. The yawning induced by oxytocin (50 ng/rat, i.c.v.) plus pindolol, but not that by alpha-MSH (20 µg/rat, i.c.v.) plus pindolol, was inhibited by [d(CH2)5,Tyr(Me)2,Om8l-vasotocin (100 ng/rat, i.c.v.), an oxytocin receptor antagonist. Yawning induced by oxytocin or alpha-MSH administered in combination with pindolol was inhibited by scopolamine (0.5 µng/kg, s.c.), a muscarinic receptor antagonist, without being affected by spiperone (0.5 µng/kg, s.c.). a dopamine D2 receptor antagonist. Thus, yawning produced by the neuropeptides, oxytocin and alpha-MSH, is modulated by beta-adrenoceptor activity in an inhibitory manner similar to that of muscarinic M1 receptor agonists, and involves cholinergic, but not doparninergic, activation (35). These results are in agreement with the previous proposal that the expression of yawning induced by dopaminergic agonists involves dopamine-oxytocin, but not oxytocin- dopamine, neuronal linkage (57). Yawning evoked by an alpha-MSH-related peptide, ACTH, which was unaffected by oxytocin receptor antagonists, was also reported to be prevented by cholinergic receptor antagonists (58).

Our previous results have indicated that none of the behavioral responses to alpha-MSH, such as yawning, stretching and body shaking, are associated with changes in the activities of the nigrostriatal, mesolimbic, tuberoinfundibular, or tuberohypophyseal dopaminergic neurons (44, 59), and that alpha-MSH-induced yawning is decreased by administration of cholinergic receptor antagonists (44). Yawning evoked by alpha-MSH administered after pindolol was antagonized by scopolamine but not by spiperone. From such findings, the oxytocin- and alpha-MSH-induced yawning responses appear to involve cholinergic but not dopaminergic activation. According to our proposal that dopaminergic cholinergic activation is involved as a common principal mechanism in causing yawning, the peptidergic mechanisms appear to be positioned between the dopaminergic and cholinergic neuronal system. Moreover, the present results also indicate that beta-adrenoceptors link to cholinergic neurons in the yawn-inducing neuronal mechanism and thereby play an inhibitory role in the modulation of such behavior.Thus, the neuropeptides, oxytocin and alpha-MSH, produce yawning via activation of cholinergic mechanisms, and beta-adrenoceptors are involved in the regulation of this yawning (Fig. 2).

Sites in the brain

We have investigated possible areas in the brain where a doparninergic-cholinergic neuron link may be involved with the incidence of yawning. Various lines of evidence suggest that the nigrostriatal dopaminergic neurons interact with the striatal cholinergic neurons, while a dopaminergic-cholinergic link is lacking in the mesolimbic area such as the nucleus accumbens and olfactory tubercle. Other evidence suggests that the mesoseptal dopaminergic neurons play a role in the control of the septal-hippocampal cholinergic neurons. The septal-hippocampal cholinergic neurons have been proposed to be necessary to elicit a specific stretching-yawning syndrome following a-MSH, since intraventricular injection of alpha-MSH caused yawning and also increased acetylcholine turnover rate in the rat hippocampus (60).

When dopamine receptor agonists such as apomorphine, piribedil and 3-PPP are bilaterally injected into the striatum and septum at smaller doses, yawning is markedly evoked (14). Consequently, the striatal and septal dopaminergic system may be related to the occurrence of yawning behavior, although other possible sites in the brain are still unclear.

PROLACTIN

Dopamine in the brain is involved in the regulation of hormone secretion. The tuberoinfundibular dopamine neuron, which originates in the arcuate and periventricular nuclei of the hypothalamus and projects to the external layer of the median eminence, is especially known to mainly regulate prolactin secretion from the anterior pituitary. Dopamine released from the median eminence into the hypophyseal portal vessels reaches the anterior pituitary and tonically suppresses prolactin secretion by acting on D2 receptor at prolactin-secreting cells which are endowed with inhibitory D2 receptor (6 1). In additiion, D2 receptors in lactotroph cells are expressed by the same DNA found in areas of the brain such as the striatum, cerebral cortex and nucleus accumbens (62). There is little evidence that tuberoinfundibular dopamine neuron of the hypothalamus is endowed with autoreceptors regulating activity of neurons and release of dopamine from the nerve terminals in the median eminence (63). Consequently, with regard to regulation of prolactin release, dopaminergic drugs administered in vivo appear to act preferentially on D2 receptors of the pituitary lactotroph cells rather than autoreceptors in the hypothalamus.

Consequently, in addition to observing yawning behavior, we also determined prolactin release from isolated rat pituitary slices and serum prolactin levels in male rats.

Pergolide, a dopamine D1 and D2 receptor agonist, decreased plasma prolactin levels (5), and perphenazine, a dopamine receptor antagonist, elevated them (64). 3-PPP, talipexole and SND 919, dopamine D2 receptor agonists, at respective yawninducing doses also caused a reduction in both the basal prolactin levels and ct-methyl-p-tyrosineinduced hyperprolactinemia (21). The high serum prolactin levels produced by daily treatment with estradiol were also reduced by talipexole and SND 919 in a dose-dependent fashion. These inhibitory effects were blocked by concornitant administration of YM09151-2, a dopamine D2 receptor antagonist (65). As described above, 7-OH-DPAT and quinpirole, dopamine D3 receptor agonists, also dose-dependently reduced prolactin levels and the reductions were antagonized by spiperone, a dopamine D2 receptor antagonist, presumably because the anterior pituitary is rich in D2 receptors but lacks D3 receptors (33).

YAWNING FOR PRECLINICAL DRUG EVALUATION

Talipexole (B-HT 920)

Talipexole was developed in Europe. On motor activity, the agent decreases locomotion but increases the activity when postsynaptic DA receptors become supersensitive 12-48 h after reserpine administration in mice and rats. Talipexole, at a wide range of doses, does not cause stereotyped behavior. The drug does not evoke rotation behavior but does cause the behavior only when supersensitive DA receptors exist after treatment with 6-OHdopamine. On the basis of such behavioral, electrophysiological and biochemical studies, it was proposed that talipexole, because it does not cause stereotypy and rotation, is a presynaptic dopamine D2-autoreceptor agonist with or without minor actions on postsynaptic dopamine D2 receptors. Therefore, it was proposed that talipexole may be therapeutically valuable in diseases presumed to be accompanied by a predominance of brain dopamine activity, such as Huntington's disease, mania and schizophrenia (66-68.).

In our studies, talipexole markedly induced yawning with bell-shaped dose responses at smaller doses but did not cause or caused only slight stereotyped behavior even at higher doses (21, 22). SND 919, having a similar chemical structure as talipexole, also caused yawning with bell-shaped dose responses at smaller doses and caused slight stereotypy (21, 22). The yawning caused by talipexole and SND 919 was inhibited by spiperone and YM-09151-2. dopamine D2 receptor antagonists, and scopolamine but was unaffected by SCH 23390, a dopamine D1 receptor antagonist (21), showing that talipexole and SND 919 are postsynaptic dopamine D2 receptor agonists.

As lactotroph dopamine receptors are more similar to dopamine autoreceptors than to postsynaptic dopamine receptors in the brain (68), we also studied effects on prolactin release. Talipexole and SND 919 dose-dependently decreased basal prolactin levels in rats. The decreasing effect of both drugs was marked in (i-methyl-p-tyrosine-induced hyperprolactinemia (21). We suggest that talipexole and SND 919 exert selective agonistic activities for specific dopamine D2 receptors which are related to causing yawning, but not to stereotypy and rotation, and that both drugs have a high affinity for dopamine receptor agonists similar to that of the pituitary lactotroph

dopamine D2 receptors. Finally, talipexole was recognized as a full agonist at both pre- and postsynaptic D2 dopamine receptors (69-71).

Clinical evaluation oftalipexole in Europe and a pilotstudy in Japan for the treatment of schizophrenia has already begun on the basis of its possible presynaptic dopamine autoreceptor agonist action without exerting postsynaptic action. These clinical studies have shown that the drug is effective at smaller doses, presumably because of presynaptic agonistic action, but with increased doses efficacy disappears and patients' symptoms are often aggravated, probably because of postsynaptic dopamine D2 receptor stimulation. Based on our experimental results on yawning and prolactin release showing talipexole to be a postsynaptic dopamine D2 receptor agonist, clinical trials were focused on the drug as an antiparkinsonian. Talipexole exhibits good efficacy with less gastrointestinal side effects, such as nausea and vomiting, and was recently approved in Japan for the treatment of Parkinson's.

Aripiprazole (OPC-14597)

Aripiprazole inhibited reserpine- and gamma-butyrolactone-induced increase in tyrosine hydroxylase activity in the mouse and rat brain and the effects were completely antagonized by haloperidol. Aripiprazole, unlike apomorphine, did not evoke postsynaptic DA receptor-stimulating behavioral signs such as hyperlocomotion in reserpinized mice and contralateral rotation in rats with unilateral striatal 6-hydroxydopamine lesions. The agent inhibited apomorphine-induced postsynaptic behavioral changes such as stereotypy and hyperlocomotion in mice and rats and rotation in rats with unilateral striatal lesions by kainic acid. From these results, aripiprazole was proposed to be a unique antipsychotic drug candidate with DA autoreceptor agonistic and postsynaptic D2 receptor antagonistic activity (72).

In our studies (73), aripiprazole did not cause hyperlocomotion or stereotypy in rats and did not evoke rotation behavior in 6-hydroxydopaminepretreated rats. In addition, apomorphine-induced stereotypy was antagonized by haloperidol and aripiprazole. showing that aripiprazole exhibits the profile of a postsynaptic D2 receptor antagonist. However, the experiments on yawning (73) demonstrated that aripiprazole dose-dependently induced the behavior to a certain extent at doses of 0.21-5 nigikg with significance at 5 mg/kg. The incidence or yawning was potentiated by pretreatment with beta-receptor antagonists and reserpine, as seen also with dopamine D2 receptor agonists. On the other hand, yawning produced by apomorphine was antagonized by aripiprazole. Thus, aripiprazole exerts only antagonistic action on stereotypy and rotation and partial agonistic action on yawning in rats.

Prolactin release from the isolated rat anterior pituitary was dose-dependently decreased by aripiprazole with weaker potency than that of talipexole, a D2 receptor full agonist (74). The decrease by aripiprazole was completely antagonized by haloperidol. Morcover, aripiprazole antagonized the inhibition of prolactin release elicited by talipexole. In in vivo studies (Fig. 6) (74), haloperidol increased serum prolactin levels by 8-fold above the basai level, whereas talipexole decreased them to 49% of the basal level. Aripiprazole dose-dependently increased the levels by 2-fold. Because of increased biosynthesis and release of prolactin in lactotroph cells, estradiol treatment in rats caused elevated serum prolactin levels which stimulated the activities of the tuberoinfundibular dopamine neuron and increased dopamine concentrations in the hypophyseal portal blood (75, 76). Hyperprolactinemia induced by estrogen was inhibited by talipexole and

enhanced by haloperidol and aripiprazole (74). In contrast, reserpine is known to decrease dopamine levels in DA nerve terminals and in the rat pituitarv portal blood and cause supersensitivity of D2 receptor 18 h or more, but not 5 h. after treatment in rats (76). The hyperprolactinernia caused 5 h after reserpine was inhibited by talipexole and aripiprazole and elevated by haloperidol (74). Thus, our results obtained from effects on yawning and prolactin release interestingly indicate that aripiprazole bas a mixed agonist/antagonist profile at D2 receptors and exerts an antagonistic or agonistic action depending on preexisting tone of dopaminergic neuronal activities.

Antipsychotic agents such as haloperidol are known to exert their therapeutic effect on schizophrenia through blockade of dopamine D2 receptors in the mesolimbic and mesocortical dopamine neurons but simultaneously cause undesirable extrapyramidal and endocrinological side effects, e.g., hyperprolactinemia, due to blockade of D2 receptors in the striaturn and anterior pituitary (77). Aripiprazole acts as an antagonist against the excess DA release at over-acting synapses in the brain of psychotic patients, but a low intrinsic activity of the drug can counteract a full blockade of D2 receptors in the striatum and pituitary. Therefore, aripiprazole appears to be a potential antipsycholic drug that does not cause severe side effects, such as extrapyramidal symptoms and hyperprolactinemia. In fact, it has been suggested from a clinical phase II study in Japan that aripiprazole is effective in the treatment of both negative and positive symptoms in schizophrenic patients without causing severe extrapyramidal side effects (78). A large-scale clinical study is now in progress in both Japan and the US.

Partial agonists on dopamine receptors

Several compounds were proposed to be partial agonists on dopamine receptors, since they produced some stereotypy and rotation but antagonized the behavior evoked by dopamine receptor agonists such as apomorphine. However, they induced marked yawning and slightly antagonized apomorphine-induced yawning in our study, indicating that these partial agonists have a relatively strong agonistic and weak antagonistic profile. Clinical trials with these agents have been discontinued because of frequent aggravation of'symptoms in schizophrenic patients, probably because oftheirdominant agonistic effects.

Our experimental results of talipexole, aripiprazole and potential partial agonists obtained from animal studies on awning and prolactin release, but not those seen with studies on locomotion, stereotypy and rotation, coincide with the clinical effects of potential drugs.

Potential nootropic agents acting on cholinergic function

Tacrine, 9-amino-1,2,3,4-tetrahydroacridine, a potent, centrally acting cholinesterase inhibitor (79), is available in the US as an antidementia agent. NIK-247, 9-amino-2,3,5,6,7,8-hexahydro-IH-cyclopenta-(6)-quinoline monohydrate HCI, developed in Japan as acholinesterase inhibitor, improves cognitive functions at different phases of the learning and memory process in rats (80). Both agents dose-dependently induced yawning which was markedly increased by pretreatment with a beta-adrenoceptor antagonist or adrenaline synthesis inhibitor, and the yawning produced by these agents was inhibited by scopolamine without being affected by mecamylamine or spiperone. These agents also increased acetylcholine content in the striatum (Fig. 7)(40).

Muscarinic M1 receptor agonists have also been developed as possible antidementia

agents. A new muscarinic receptor agonist, YM 796, has high affinity for M1 receptor. In our studies (34), YM 796 elicited yawning behavior which was potentiated by beta-adrenoreceptor antagonist and inhibited by scopolamine and pirenzepine, as well as EEDQ, M1 receptor antagonists, but not by spiperone, a dopamine D2 receptor antagonist, and 4-DAMP a muscarinic M3 receptor antagonist (81). Thus, it is possible to assess the cholinergic activation in the brain whether or not these agents cause yawning behavior.

Discrimination between central and peripheral betaadrenoceptor blocking agents

Yawning produced by dopamine receptor agonist was potentiated by central beta-adrenoceptor antagonist which blocked beta-adrenoceptor in the brain after passing through the blood-brain barrier, but not by peripheral antagonists (Fig. 5). The yawning induced by pilocarpine and physostigmine was also increased by the central beta-receptor antagonist (36). The behavior evoked by a neuropeptide, oxytocin and alpha-melanocyte-stimulating hormone was also potentiated after the antagonists (35). Thus, it is possible to functionally discriminate between central and peripheral beta-adrenoceptor antagonists.

CONCLUSIONS

Dopaminergic agents cause yawning at smaller doses and stereotypy at larger doses. The dopamine D2 receptors related to yawning are thus more sensitive to dopamine receptor agonists than those related to stereotypy. In addition, yawning is more selective for D2 receptor activation than stereotypy and rotation. Cholinergic agents also elicit yawning and the doparninergic-cholinergic neuronal link appears to be principally involved. The results obtained from yawning studies, but not those on stereotypy and rotation, are compatible with the clinical effects of potential antipsychotic and antiparkinsonian agents.