The Antidepressant and Antiinflammatory Effects of Rolipram in the Central Nervous System

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ABSTRACT

Rolipram is a selective inhibitor of phosphodiesterases (PDE) IV, especially of the subtype PDE IVB. These phosphodiesterases are responsible for hydrolysis of the cyclic nucleotides cAMP and cGMP, particularly in nerve and immune cells. Consequences of rolipram-induced elevation of intracellular cAMP are increased synthesis and release of norepinephrine, which enhance central noradrenergic transmission, and suppress expression of proinflammatory cytokines and other mediators of inflammation. In humans and animals rolipram produces thereby a variety of biological effects. These effects include attenuation of endogenous depression and inflammation in the central nervous system (CNS), both effects are of potential clinical relevance. There are some discrepancies between *in vitro* and *in vivo* effects of rolipram, as well as between results obtained in animal models and clinical studies. The clinical use of rolipram is limited because of its behavioral and other side effects. Newly developed selective PDE IV inhibitors with presumably higher potency and lower toxicity are currently under investigation.

INTRODUCTION

3',5'-cyclic nucleotide phosphodiesterases (CN PDE) were discovered nearly 40 years ago (64). They are specific for purine cyclic nucleotide substrates and constitute the only known pathway for the catalysis of cyclic AMP (cAMP) and cyclic GMP (cGMP) hydrolysis. These processes result in decreased intracellular levels of the cyclic nucleotides. CN PDEs are, therefore, important regulators of the intensity and duration of intracellular

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messenger mediated signal transduction (73). The enzymology of CN PDE has proven to be complex. There are at least 8 isotypes of CN PDE (18), which have distinct CN specificity. They are activated by different signal pathways and show different subcellular localization (e.g., in cortical nerve cells) (21). Type I CN PDE is activated by calcium/calmodulin and found primarily in the cytosol. Type II is cGMP-activated and predominantly membrane associated, while the cGMP-insensitive type IV is equally distributed between cytosol and membranes (80). In synaptosomes PDE V has the highest, whereas PDE IV has the lowest hydrolytic activity for cGMP. Four isoforms of PDE IV (PDE IVA-D) show distinct expression patterns in human brain (52). Generally, PDE IV is regarded as cAMP-specific and PDE V as cGMP-specific, however, high concentrations of cGMP are also hydrolyzed by PDE IV (6).

The second messenger cAMP is produced by adenylyl cyclases in response to numerous extracellular stimuli such as neurotransmitters via binding to β -adrenergic, A_2 -adenosine (12,72), and H_2 -histamine (5) receptors. Therefore, the PDE IV family is a major modulator of cAMP signalling by controlling free intracellular cAMP concentrations through its enzymatic activity (17). Levels of cAMP regulate PDE IV activity through a rapid process involving phosphorylation (42) and a longer term process involving increased PDE IV gene expression (44). PDE IV isozymes have been shown to be the predominant PDE expressed in lymphocytes and monocytes (13,54). Elevation of intracellular cAMP downregulates a wide variety of immune cell functions, including tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukins (IL-2 and IL-12), production, IL-2 receptor (IL-2R) expression in lymphocytes, IgE production, oxidative burst in granulocytes, NO production in macrophages and lymphokine-activation of NK cells (8,14,16,38,43,50,54,72). Disturbances in cAMP-mediated signaling have been implicated also in several neuropsychiatric disorders, such as major depressive disorder (67), Alzheimer's disease (20), multi-infarct dementia (61) and tardive dyskinesia (62). An increase in cAMP levels through inhibition of PDE IV appears to improve the symptoms of these disorders.

CN PDE can be inhibited not only by nonselective drugs like pentoxifylline, but also by highly selective drugs for single PDE isozymes (76). Rolipram is a prototype inhibitor of PDE IV. It produces a variety of biological effects, which are characteristic of a potentially useful clinical agent. A beneficial effect of rolipram on ischemic neuronal damage favors its use in the treatment of CNS disorders (10). Furthermore, rolipram inhibits methamphetamine- and morphine-induced hyperlocomotion. This effect probably occurs via increase of cAMP levels at dopamine D_2 -receptors (46). In addition, rolipram reduces morphine withdrawal symptoms probably via decrease of c-Fos expression (28).

This article will review the pharmacology, toxicology, and clinical effects on the CNS and the immune system. It will also consider its adverse effects in experimental and clinical situations.

***IN VIVO* ANIMAL NEUROPHARMACOLOGY**

Rolipram causes a typical pattern of behavior when administered to rats at single doses in the range of 0.02–400 mg/kg (55,77). It was found to elicit a head twitch reaction in rats, but not in mice. It also stimulated the flexor reflex of the hind limb in the rat spinal

cord. This effect was not blocked by cyproheptadine, pizotifen, and phenoxybenzamine. In rats kept at a high ambient temperature, rolipram induced slight hypothermia but did not affect hyperthermia induced by fenfluramine. It also produced slight hypothermia in rabbits. These results indicate that rolipram does not affect central serotonergic transmission. However, some of its central effects (e.g., the head twitch reaction) seem to involve the noradrenergic system and the opiate receptors (55). Symptomatology (hypothermia, hypoactivity, forepaw shaking, grooming, head twitches) associated with the enhanced availability of brain cAMP was observed in rats following systemic administration of rolipram (77). Rolipram-induced behavior is characterized by an inhibition of spontaneous motility, increased maintenance activity (forepaw shaking, grooming, head twitches), vocalization on touch, salivation, and an increased rate of respiration (40). This behavioral syndrome is mimicked by dibutyryladenosine cAMP, an analogue of cAMP, indicating that the syndrome may be the result of increased amounts of cAMP in target tissues (77). The behavioral effects produced by rolipram are associated with enhanced central adrenergic signal transduction (77).

ANTIDEPRESSANT EFFECTS OF ROLIPRAM

Rolipram was first discovered as an enhancer of neurotransmitter-stimulated cAMP accumulation in brain cortical slices. This effect is due to inhibition of PDE IV-mediated Ca^{2+} -dependent cAMP hydrolysis (64). Rolipram also enhanced the excitability of neurons, providing a functional basis for cognition enhancement (4). Unlike conventional antidepressants, rolipram stimulates both the presynaptic and the postsynaptic components of monoaminergic transmission (11). Rolipram did not affect adenosine uptake, but it stereospecifically reduced β -adrenoceptor sensitivity in rat cerebral cortex. Presynaptic denervation using intracerebroventricular injections of 6-hydroxydopamine (6-OHDA) prior to or during rolipram treatment did not completely block the effect of rolipram on downregulation of cerebral cortical β -adrenoceptors. The data favor a pre- and postsynaptic action of rolipram that is different from all other antidepressants studied under similar experimental conditions (63).

A potent antidepressant effect of rolipram was first found in 1983 in animal experiments (78). Rolipram was regarded as an atypical antidepressant, since it lacked anticholinergic and monoamine oxidase inhibiting properties. Its antidepressant action was similar to that of tricyclic antidepressants. However, it demonstrated a unique mechanism of action by stimulating tyrosine hydroxylase, increasing neuronal activity, accumulating DOPA, slowing the disappearance of dopamine, and increasing the synthesis and release of norepinephrine (37). In animal studies rolipram was compared with traditional antidepressants, e.g., imipramine, for its effectiveness as a potential antidepressant. It antagonized reserpine-induced hypothermia or hypokinesia, and potentiated yohimbine lethality. As an antagonist of reserpine-induced hypothermia rolipram was approximately 15 times more potent than imipramine and approximately as potent as ICI 63 197. The antihypothermic effect of rolipram occurred at a lower dose than that of imipramine. In contrast to imipramine, rolipram reversed reserpine-induced hypokinesia. Rolipram was approximately as potent as imipramine and ICI 63 197 in potentiating the lethality of yohimbine. These data indicated that the antidepressant action of rolipram was due to enhancement of

central noradrenergic transmission. The hypothesis is that the increase of norepinephrine turnover, induced by rolipram in conjunction with the inhibitory action on cAMP metabolism, induces adaptive changes in central synapses more efficiently, resulting in a rapid onset of antidepressant activity. Preliminary results with rolipram in patients with endogenous depression seem to support this assumption (78).

Overstreet et al. (51) studied the effects of rolipram on selectively bred Flinders Sensitive Line (FSL) rats, a genetic animal model of depression. Rolipram produced comparable decreases in temperature and activity in the FSL and the Flinders Resistant Line (FRL) rats. Upon chronic treatment there was a trend for rolipram to counteract the shock-induced suppression of activity in the FSL rats. This finding suggested an antidepressant-like effect. However, both groups gained a significant amount of body weight, which appeared to be associated with polydipsia and polyuria. In addition, both groups were significantly more affected by the muscarinic agonist, oxotremorine, than their vehicle-treated counterparts. Thus, the FSL rats, which are genetically supersensitive to cholinergic agonists, are even more sensitive to cholinergic agonists following chronic treatment with rolipram. The increase in the sensitivity to cholinergic agonists suggested a potential clinical side effect.

Rolipram represents a new approach to antidepressant therapy. Initial clinical studies suggested that rolipram may be an effective antidepressant with few side effects. The place of this drug in antidepressant therapy is, however, still to be established (49).

A double-blind trial compared the antidepressant efficacy and adverse effects of rolipram with those of amitriptyline in patients with depression (65). Fifty patients meeting *DSM-III* criteria for major depression, whose scores on the Hamilton Rating Scale for depression (HRSD) remained above 17 after 5 to 7 days on placebo, were randomly allocated to either treatment. The rate of recovery in patients treated with amitriptyline was substantially higher than in patients treated with rolipram. Twice as many patients dropped out of the rolipram group because of lack of efficacy or adverse effects as compared to patients treated with amitriptyline. Rolipram produced fewer adverse effects attributable to cholinergic blockade than amitriptyline, but more nausea. The results indicated that amitriptyline is more effective than rolipram in the treatment of depressed hospitalized patients.

However, Bobon et al. conducted several double blind trials to assess the clinical efficacy and safety of rolipram. One study was a randomized, 4-week interindividual, double-blind, double-dummy comparison of rolipram with desipramine in inpatients with major (*DSM-III*) and/or endogenous (ICD-9) depression. The data showed no differences between the two drugs in regard to efficacy, but a definite trend in favor of rolipram in regard to side effects and, in particular, anticholinergic effects (11).

An open phase II study evaluated rolipram in 10 depressed patients, most of whom had been refractory to previous antidepressant therapy. Five patients displayed a good to very good improvement of their depression. Four patients failed to show any substantial improvement, and therapy had to be withdrawn in one case due to deterioration of the condition. In most cases, the antidepressant effect of rolipram became noticeable after only 2 to 4 days of treatment. Based on the presented cases, the tolerance can be described as excellent compared to other antidepressants (81).

More recently Fujimaki et al. (26) reported that coadministration of two selective PDE IV inhibitors, rolipram and Ro 20-1724, can shorten the time required for the upregulation of BDNF mRNA by antidepressant treatment, which supports the possibility that this

treatment may provide an effective therapy for major depression. In situ hybridization analysis demonstrated that the induction of BDNF mRNA in response to the repeated co-administration paradigm occurs in the dentate gyrus granule and CA1 and CA3 pyramidal cell layers of the hippocampus (26).

ANTIINFLAMMATORY EFFECTS OF ROLIPRAM

Rolipram also plays a major role in modulating the activity of virtually all cells involved in the inflammatory process. It could be active at inflammatory sites where multiple factors are likely to be involved. Inhibitors of the PDE family display impressive antiinflammatory effects in a variety of experimental models and human cell cultures (1,9,19,27,33,36,41,56,69,70,83).

TNF- α is a potent cytokine released primarily by lipopolysaccharide-activated macrophages and monocytes (54), but also by activated T cells (36). There is considerable evidence suggesting that TNF- α plays an important role in a number of pathological conditions. Agents blocking TNF- α effects have an antiinflammatory action. Rolipram displays antiinflammatory effects, which are linked at least partly with the inhibition of TNF- α . Rolipram-induced TNF- α inhibition occurs both at the mRNA and protein levels (48,83). The absolute increase in intracellular cAMP observed with rolipram treatment is rather modest, regardless of the time point at which the cyclic nucleotide is measured (54). The antiinflammatory effects of rolipram can be mediated also by downregulation of IL-13, which is a proinflammatory cytokine of T cell origin. Both IL-13 gene expression and protein secretion into culture supernatants are downregulated by rolipram (24). Furthermore, the antiinflammatory effects of rolipram are linked with downregulation of antigen-driven proliferation and cytokine gene expression for IL-5 and IFN- γ in human peripheral blood mononuclear cells (22,23). IL-5 and IFN- γ are regarded as proinflammatory cytokines, which are involved in several neuropathological conditions. Interestingly, despite rolipram's antiinflammatory properties, it appeared ineffective in downregulating IL-4 gene expression.

Rolipram has been tested in several animal models of autoimmune disorders, such as experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS) (27,69,70), and experimental autoimmune neuritis (EAN), an animal model for Guillain-Barré syndrome (GBS) (1,83), as well as in HIV infections (3). It can suppress the acute and chronic clinical symptoms of EAE (2,25,27,36,45,69,70). Agnello et al. (2) studied the mRNA expression of pentraxin 3 (PTX3) and the binding of the peripheral-type benzodiazepine receptor (PBR) ligand [3 H]PK11195 in the spinal cord of Lewis rats with actively induced EAE. They found two types of inflammatory changes: acute, short-term changes (decrease of TNF- α and IL-6) that correlated with the disease; and effects, such as PTX3 expression and PK11195 binding, that lasted longer after recovery from the disease. Rolipram delayed the onset of the disease and decreased EAE severity. However, the peak levels of TNF- α , IL-6, PBR, and PTX3 in spinal cord were only delayed, but not reduced, by rolipram treatment.

Rolipram-increased intracellular cAMP levels also enhanced the electrical resistance of endothelial monolayers by stabilizing intercellular junctional complexes (25). The effect on vascular endothelium in CNS has the potential to reduce severity of EAE because both

inflammatory cells and humoral factors readily cross a disrupted blood-brain barrier (BBB). Rolipram significantly reduced BBB permeability in the spinal cord of mice with EAE by reducing tissue edema, as well as entry of inflammatory cells and humoral factors.

In the relapsing-remitting EAE model of the SJL mouse, there was marked reduction of demyelination and also less inflammation throughout the CNS of rolipram-treated animals (69,70). Gene expression of proinflammatory cytokines, such as TNF- α , in the CNS was reduced in most of the rolipram-treated animals. Additional experiments demonstrated that rolipram acted principally by inhibiting the secretion of Th1 cytokines.

However, the encephalitogenic potential of myelin basic protein-specific T cells was not impaired. These findings suggested that rolipram opens a promising cytokine-directed therapy in chronic autoimmune-demyelinating diseases. However, Martinez et al. (45) demonstrated that the cytokine gene expression profile was similar to that of untreated rats, although delayed, with no evidence of IL-10 upregulation during the observation period. The delayed inflammation was not associated with changes in the expression of VCAM-1 and ICAM-1. In peripheral blood mononuclear cells, TNF- α mRNA levels were decreased and IL-10 mRNA was unchanged. The therapy did not alter the proliferative activity of T lymphocytes in response to myelin basic protein. The encephalitogenic potential of splenocytes from treated animals was also unaffected. The high levels of both, induced NO synthase (iNOS) mRNA and nitric oxide (NO) found before the appearance of clinical signs of EAE, suggest that suppression of NO generation might be a contributing factor to the therapeutic benefit achieved by rolipram in the rat (45).

Histological data suggest that relevant immunosuppressive mechanisms of rolipram may also be the inhibition of the migration of leukocytes into the CNS and to some extent its inhibitory effect on T cell proliferation and macrophage activity (TNF- α production) (36). However, rolipram did not suppress the TNF- α production of activated T cells. It had only a preventive effect on the clinical course of actively induced EAE and a therapeutic effect on adoptive transfer EAE. Therefore, it may not be a more effective immunomodulator of actively induced EAE than the nonspecific PDE inhibitor pentoxifylline (60), which shifted the Th1 response to the Th2 response (57).

Constantinescu et al. (19) found that EAN, a demyelinating disease of the peripheral nervous system (PNS), could be effectively suppressed by pentoxifylline. We induced EAN in Lewis rats by inoculation with the peripheral nervous myelin P2 protein peptide 57–81 and found a strong suppression by rolipram administered twice daily i.p. from day 9 after immunization (i.e., after the onset of clinical signs of EAN) (1,83). The suppression of EAN was associated with downregulated myelin antigen-induced T cell responses, as well as downregulated IFN- γ and TNF- α production. A relapse of clinical symptoms of EAN occurred upon treatment of a short duration (7 days), while prolongation of treatment prevented the return of the clinical signs of EAN relapse. There was no relationship between clinical signs of EAN relapse and high levels of TNF- α (83). The beneficial clinical effect of rolipram was associated with dose-dependent downregulated production of IFN- γ and of the chemokines macrophage inflammatory protein-1 α (MIP-1 α), MIP-2, and monocyte chemotactic protein-1 (MCP-1). In addition, IL-4 production was upregulated in sciatic nerve sections from rolipram-treated rats on day 14 after immunization (when clinical symptoms of EAN were maximal) (1). These findings suggest that rolipram may be useful in T cell-dependent autoimmune diseases of the PNS.

Preclinical and clinical studies on the antiinflammatory effects of rolipram have also been conducted in humans. *In vitro* rolipram reduced the numbers of myelin-basic protein-reactive blood mononuclear cells of MS patients that expressed IFN- γ and TNF- α mRNA (48). It also reduced early side effects of IFN- β treatment in MS patients (58). In a pilot trial, 20 MS patients were treated with pentoxifylline (75). The outcome parameter was the effect of treatment on levels of various cytokines and adhesion molecules in cerebrospinal fluid and serum, on production of Th1 and Th2 cytokines using cell stimulation assays, as well as on measures of T-cell activation and proliferation. Kurtzke's EDSS was a secondary efficacy parameter. A convincing and consistent effect of pentoxifylline could not be demonstrated. In addition, rolipram treatment had no convincing beneficial influence on immune variables and clinical course in chronic progressive MS (unpublished data).

Another important pathogenic effect of TNF- α is the enhancement of HIV-1 replication. Blocking TNF- α , and thereby inhibition of HIV-1 replication may potentially delay progression of HIV disease (35, 54). Pentoxifylline has been shown in preliminary clinical studies to decrease viral replication in HIV-1-infected patients (68,82). Rolipram, with its selective inhibitory effect on the predominant PDE IV isoenzyme of monocytes, inhibits lipopolysaccharide-induced TNF- α with 500-fold greater potency than pentoxifylline (54). Additionally, rolipram inhibits HIV-Tat-activity, which is mainly responsible for CD4⁺ T cell hyperactivation and increased susceptibility to HIV-infection (66). Therefore, it is hypothesized that rolipram would be a powerful inhibitor of HIV-1 replication and deserves further investigation as a potential therapeutic agent in the treatment of HIV-1-infected patients (3).

TOXICOLOGY AND SIDE EFFECTS OF ROLIPRAM

Rolipram has been actively pursued as a treatment for several animal disease models (1,19,27,33,36,69,70,83). In the study of Larson et al. (40) rolipram was administered to female rats at 0, 10, 30, or 100 mg/kg/day orally for up to 2 weeks. All rats given rolipram 100 mg/kg/day became moribund and were euthanized on day 5. Clinical signs observed in treated rats included increased salivation, slight distension of the abdomen, emaciated appearance, and ataxia. After 14 days of treatment, the rats were necropsied and their tissues were examined microscopically. A number of drug-related histopathological changes were observed in rats receiving rolipram 30 or 100 mg/kg/day. Myocardial degeneration and necrosis, as well as arteritis/periarteritis of intramural and extramural coronary arteries, were seen in the heart. Necrotizing vasculitis and inflammation were observed in the mesentery and interstitial areas of the liver, which affected medium-sized portal arteries and veins. Squamous hyperplasia and hyperkeratosis, with or without ulceration in the nonglandular stomach, were seen in at least one animal in each treatment group. Enlarged salivary glands were noted at necropsy in animals treated with rolipram, 100 mg/kg/day. These data indicate that rolipram can cause effects on the heart and vasculature of rats. The cardiac effects of rolipram in dogs resulted obviously from enhancement of cardiac responses to sympathetic (cAMP-dependent) stimulation (79). In addition, rolipram has been demonstrated to produce respiratory stimulation in monkeys (34).

Rolipram could potentiate multiple proemetic neurotransmitters in the brain. Heaslip and Evans (30) demonstrated the involvement of the CNS in rolipram-induced emesis after i.v. administration. The emesis was associated with the induction of a variety of behavioral responses. Interestingly, however, rolipram also activates the emetic reflex via a gastrointestinal mechanism in dogs (30). The other systemic effects of rolipram were reduced following intragastric administration. The failure to induce behavioral side effects, when administered orally, presumably reflects the poor systemic bioavailability of rolipram in most species (39).

Rolipram also induced anxiety in conscious dogs (30). Anxiety responses, which are commonly associated with increased adrenergic tone, might also result from a potentiation of adrenergic signal transduction in dogs (32).

The initial therapeutic application for rolipram, at doses of 0.25 to 3.0 mg/day, was in the treatment of depression, where the compound had a success rate comparable to that of the traditional tricyclic antidepressants (81). The clinical application of rolipram was discontinued for a period of time due to its side effects (74). However, only a few studies on the toxicology and side effects of rolipram in the clinical trial were reported. For instance, nausea emerged as a typical side effect of rolipram in clinical trials (7,31). This effect is probably triggered via a noradrenergic pathway mimicking presynaptic α_2 -adrenoceptor inhibition (59).

Heaslip and Evans (30) reported that emesis in man and animals is a consistent side effect of acute rolipram administration. Such an effect might be triggered, in part, by an effect of rolipram on the area postrema in the brain; emetic reflexes in the area postrema are known to be controlled by cAMP-dependent signal transduction pathways. In addition, systemically administered Ro 20-1724 (another PDE IV inhibitor) has been previously demonstrated to render the area postrema hypersensitive to drugs inducing emesis through cAMP-dependent mechanisms (15).

Finally, rolipram has been reported to have toxic effects on plasma osmolality that might limit its clinical use (71). New selective PDE IV inhibitors with higher potency and lower toxicity than rolipram are being currently developed (29,47). They may have improved immunomodulatory and antidepressant effects.

SUMMARY

Phosphodiesterases (PDE) increase intracellular levels of cAMP and cGMP in various tissues. The selective tissue and cellular distribution of at least eight isotypes and several subtypes of PDE opens up the possibility to modulate cAMP-dependent cell functions by applying selective PDE inhibitors. Rolipram preferentially blocks the enzyme activity of PDE IVB. This enzyme is mainly expressed in monocytes, macrophages, granulocytes, and T lymphocytes, but also in dopaminergic and adrenergic nerve cells of the CNS. Therefore, rolipram has been successfully applied as an antidepressant drug. It enhances the central adrenergic transmission and induces adaptive changes at central synapses. Due to its ability to suppress proinflammatory cytokines (predominantly TNF- α) and chemokines, reactive oxygen and nitrogen species, as well as the migration of inflammatory cells into the CNS and the PNS, rolipram has also been tested in the treatment of EAE, MS, and EAN. However, the *in vitro* and *in vivo* data on the immunosuppressant effects of rolipram

were inconsistent and partly contradictory depending on the experimental models used, whereas studies in EAN are most promising. Toxic and adverse effects of rolipram in animal experiments involve mainly myocardial and liver degeneration, arteriitis and hypothermia. In addition disturbed behavioral activity and head twitches, as well as nausea have been reported. Therefore, initially expected widespread clinical use of rolipram in the treatment of depression and inflammatory disorders of the CNS did not materialize. Newly derived selective, more potent and hopefully less toxic PDE IV inhibitors could be more promising.

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