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# Emetic, central nervous system and pulmonary activities of rolipram in the dog

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**Abstract**

Rolipram was characterized for its emetic, behavioral, cardiovascular and pulmonary activities in dogs, to assess its systemic pharmacology and potential bronchodilatory selectivity. At doses $\geq 0.1$ mg/kg i.v., rolipram induced emesis, anxiety, and stepping behaviors in conscious dogs, and increased heart rate and cardiac contractility in anesthetized instrumented dogs not treated with a $\beta$-adrenoceptor antagonist. Cardiovascular effects in anesthetized dogs were apparently related to rolipram's central nervous system activities, in that they were associated with a reversal pentobarbital-induced anesthesia and could be ablated by pentobarbital supplementation. Rolipram's reversal of anesthesia was confirmed in uninstrumented dogs, where rolipram shortened pentobarbital sleep time while increasing heart and respiratory rates. After intragastric administration, rolipram exhibited greater emetic potency (100% emesis at 0.1 mg/kg p.o.) and lesser bronchodilatory potency ($ED_{50} = 0.04$ mg/kg i.d.) than after i.v. administration. The data demonstrate that rolipram is a potent bronchodilator that produces central nervous system effects only at higher doses when administered i.v. to the dog. Administered intragastrically, however, the bronchodilatory selectivity of rolipram is reduced, presumably as a result of the activation of emetic reflexes at sites within the gastrointestinal tract.

*Keywords:* Asthma; Bronchodilation; Cardiovascular; Central nervous system, dog; Emesis; Phosphodiesterase; Rolipram

## 1. Introduction

In recent years, with the discovery and cloning of multiple molecular forms of cyclic nucleotide phosphodiesterases, there has been a resurgence of research investigating the potential use of isozyme-specific phosphodiesterase inhibitors as therapeutic agents (Beavo et al., 1994; Beavo and Reifsnyder, 1990; Nicholson et al., 1991). In particular, the potential use of selective phosphodiesterase 4 inhibitors in the treatment of asthma has been extensively researched, based on observations that phosphodiesterase 4 is a principal regulator of cAMP concentrations in inflammatory cells and respiratory smooth muscle (Giembycz, 1992; Nicholson et al., 1991; Nicholson and Shahid, 1994; Torphy and Undem, 1991). In vitro, selective phosphodiesterase 4 inhibitors effectively suppress inflammatory mediator release from a variety of inflammatory cell types (Chan et al., 1993; Dent et al., 1994; Giembycz, 1992; Molnar-Kimber et al., 1992), and are potent relaxants of respiratory smooth muscle (Harris et al., 1991; Heaslip et al., 1994; Torphy, 1988). In vivo, phosphodiesterase 4 inhibitors block antigen-induced pulmonary inflammation and hyperreactivity in guinea pigs and monkeys (Howell et al., 1993; Sturm et al., 1990; Turner et al., 1994; Underwood et al., 1993), and produce bronchodilation in guinea pigs, rats and dogs (Harris et al., 1991; Heaslip et al., 1991,1994; Heaslip and Sickels, 1993). Thus, the available preclinical evidence suggests that selective phosphodiesterase 4 inhibitors would be uniquely capable of treating both the bronchoconstrictive and inflammatory symptoms of asthma.

Ultimately, however, the utility of phosphodiesterase 4 inhibitors as antiasthma drugs is likely to

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depend upon their pulmonary selectivity. Preclinical and clinical studies have generally suggested that selective phosphodiesterase 4 inhibitors do not produce profound effects on the cardiovascular systems of most species (Bertolino et al., 1988; Heaslip et al., 1991; Heaslip and Sickels, 1993; Ho et al., 1990). However, centrally mediated side effects could limit the utility of phosphodiesterase 4 inhibitors in the treatment of asthma. Phosphodiesterase 4 is abundantly distributed in the brain, and low (10–100 μg/kg i.p.) doses of selective phosphodiesterase 4 inhibitors such as rolipram have been demonstrated to produce behavioral and thermoregulatory effects in mice and rats (Griebel et al., 1991; Koe et al., 1990; Wachtel, 1982), and respiratory stimulation in monkeys (Howell, 1993). Thus far, the predominant side effect reported for phosphodiesterase 4 inhibitors in clinical trials has been nausea (Bertolino et al., 1988; Hebenstreit et al., 1989; O'Connolly et al., 1988).

This study was performed to explore the emetic, behavioral and cardiovascular effects of rolipram in the dog, and to compare the doses of rolipram required to produce these effects with doses of rolipram reported to produce bronchodilation ($\geq$ 0.0001 mg/kg i.v.; $ED_{50}$ = 0.007 mg/kg i.v.). Canine models were chosen for these studies based upon the apparent similarity of canine emetic responses to those of humans (Carpenter et al., 1988), and the demonstrated potency and efficacy of phosphodiesterase 4 inhibitors as bronchodilators in the dog (Heaslip et al., 1991,1994).

## 2. Materials and methods

### 2.1. Emetic and behavioral effects in conscious dogs

The effects of rolipram in fasted (24 h) conscious dogs were assessed using a colony of mongrels (7–12 kg) that had previously been conditioned to stand quietly in a sling for several hours. On the day of experimentation, a dog was placed in a sling and a 1.25″ teflon angiocatheter was secured in either a cephalic or saphenous vein for drug administration. 15 min later, rolipram (0.1 μg/kg) was administered by infusion over a 5 min period, and its effects were recorded during the infusion and for the next 25 min. Additional doses of rolipram were similarly administered at 30 min intervals in a cumulative manner, to achieve 10-fold increases in dosage, until rolipram's limit of solubility or the dog's limit of tolerability (based upon the incidence or intensity of emetic or behavioral responses) was reached. After each dose, the absence or presence of the following effects were noted: emesis/retching, muscle tremors, stepping behaviors (repetitious forepaw treading, swimming motions, hindlimb side-stepping, or leg retraction), anxiety (efforts to depart sling), and sedation. For the purposes of this study, retching was considered as equivalent to an emetic episode. Salivation, which is commonly associated with the emetic reflex (Borison and Wang, 1953), was observed in all dogs administered emetic doses of rolipram and was not scored as a separable pharmacological effect. Dogs exhibiting a moderate frequency of emesis or intensity of behavioral effects were considered to have reached their limit of tolerability, and were exempted from further dosing. At the end of each experiment, dogs were returned to their cages, the i.v. catheter was removed, and each dog was observed for the next 24 h. All effects of rolipram observed during these studies were transient in nature, lasting for $< 20$ min after dosing had been discontinued. Combiotic penicillin was administered i.m. upon completion of the experiment and daily for 2 days thereafter, according to manufacturer's directions. At least a 2 week washout period was allowed between experiments.

Dogs exhibiting a specific behavioral effect after receiving a given dose of rolipram were scored as 'positive responders' for that effect at the given dose and at all subsequently administered (higher) doses. Dose-response curves for each effect were then constructed by expressing the number of 'positive responders' for that effect as a percentage of the total number of dogs that had received each dose of rolipram.

The effects of orally administered rolipram were also assessed in uninstrumented conscious dogs. Fasted (24 h) dogs were administered gelatin capsules containing either dextrose alone (controls) or dextrose to which rolipram had been adsorbed (0.01, 0.03, or 0.1 mg/kg), and the effects of rolipram were monitored for 4 h. In a limited additional study, the ability of ondansetron to inhibit rolipram-induced emesis was also explored, by pretreating dogs (30 min) with the clinically recommended antiemetic dose of ondansetron (0.15 mg/kg i.v.), and then orally administering a capsule containing 0.1 mg/kg rolipram.

### 2.2. Bronchodilatory and cardiovascular effects in anesthetized serotonin-infused β-adrenoceptor antagonist-treated dogs

The bronchodilatory effects of rolipram following intragastric administration were assessed using an anesthetized serotonin-infused nadolol-treated dog model described previously (Heaslip et al., 1991), except that rolipram was administered as a single intraduodenal (i.d.) dose using a cannula that had been secured in the duodenum surgically. Lung inflation back-pressure was measured from a side arm of the endotracheal tube, mean arterial blood pressure and heart rate were measured through a cannula in the left femoral artery, and cardiac contractility was assessed as the first derivative of left ventricular pressure

$(+dP/dt)$ via a pressure-tip catheter inserted in the second femoral artery and advanced into the left ventricle. Bronchodilation was measured as a reduction in lung inflation back-pressure and was expressed as a percentage of maximum (Heaslip et al., 1991); cardiovascular effects were measured as a percentage change from basal values determined immediately prior to rolipram administration.

### 2.3. Effects in anesthetized dogs

To assess the cardiovascular and pulmonary effects of rolipram in anesthetized dogs not treated with nadolol, fasted mongrels (10–20 kg) of either sex were anesthetized with pentobarbital (33 mg/kg i.v. bolus). Each dog was ventilated with 15 ml/kg air at 2 breaths/min as previously described (Heaslip et al., 1991). Anesthesia was maintained by pentobarbital infusion (0.08 mg/kg per min) through a cannula in a cephalic vein. After each dog had been allowed to stabilize (~ 90 min), rolipram was administered i.v. through a femoral vein catheter in doses that increased in a cumulative manner. Succeeding doses were administered after peak cardiovascular effects of the previous dose had been achieved (~ 5 min). Cardiovascular and pulmonary responses were measured as described above, and expressed as percentage change from baseline parameters determined immediately prior to the administration of the first dose of rolipram. At the end of these experiments, dogs were killed via an overdose of Beuthanasia.

### 2.4. Effects of pentobarbital supplementation on cardiovascular system responses to rolipram

Fasted mongrels of either sex were anesthetized with pentobarbital (33 mg/kg i.v. bolus plus 0.08 mg/kg per min infusion), ventilated, and instrumented as described above. After a 30–60 min stabilization period, each dog was administered an additional bolus dose of pentobarbital (15 or 33 mg/kg, i.v.), the rate of pentobarbital infusion was increased to 0.24 mg/kg per min, and an additional 60 min stabilization period was allowed. A 1 mg/kg dose of rolipram or its vehicle (0.1 ml/kg dimethylsulfoxide, DMSO) was then administered i.v. over a 1 min period, and its effects were monitored for 60 min. Cardiovascular and pulmonary responses were measured and expressed as described above. At the end of these experiments, dogs were killed with Beuthanasia.

### 2.5. Effects of rolipram on sleep time, respiratory rate and heart rate in anesthetized dogs

The effects of rolipram on pentobarbital-induced sleep were assessed using a colony of eight mongrel dogs (three male and five female) randomized in a crossover experimental design. Dogs that had been fasted overnight were anesthetized with pentobarbital (33 mg/kg i.v.), and a 1.25″ teflon angiocatheter was secured in either a cephalic or saphenous vein to allow for the administration of drugs. After a 60 min stabilization period, 1 mg/kg rolipram or its vehicle (0.1 ml/kg DMSO) was administered. Pentobarbital sleep time was assessed by observing each dog every 10 min for the next 6 h, and recording the time that elapsed between rolipram or vehicle administration and the following endpoints: reflex muscular responses (to touch, limb extension, or paw pressure), voluntary skeletal muscle movements (ability to lift head), and ability to focus attention (visually track a moving object). Respiratory rate and heart rate were measured by palpitation 10 and 30 min after rolipram administration, and thereafter every 30 min for 3 h. Accurate measurements of respiratory and heart rates could not be made beyond 3 h because of motor activities exhibited by the dogs as they recovered from anesthesia. At the end of the experiment, the i.v. catheter was removed, and each dog was allowed to recover under observation. Combiotic penicillin was administered i.m. upon completion of the experiment, and daily for 2 days thereafter. Dogs initially receiving rolipram or vehicle were retested with vehicle or rolipram, respectively, 8–14 days later. Thus, each dog served as its own control, and the significance of the differences in sleep time after each treatment were determined using paired $t$-tests.

### 2.6. Protocol approvals

All experimental protocols were reviewed and approved by Wyeth-Ayerst Research Animal Protocol Review Committees.

### 2.7. Drugs and solutions

Rolipram (provided by Dr. Thomas Caggiano Wyeth-Ayerst Research, Princeton, NJ) was dissolved in DMSO to allow administration of the 1 or 3 mg/kg doses in a final volume of 0.1 ml/kg or 0.3 ml/kg. Lower doses of rolipram were prepared by serial dilution in saline. Nadolol (provided by Bristol-Meyers Squibb, Princeton, NJ) was administered dissolved in saline. Serotonin, indomethacin (both Sigma Chemicals, St. Louis, MO), ondansetron (Glaxo, Research Triangle Park, NC), pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, IL), gallamine triethiodide (Flaxedil, American Cyanamid Co., Pearl River, NY), Beuthanasia (Schering-Plough, Kenilworth, NJ), and Combiotic (Vedco, Overland Park, KS) were purchased from their respective suppliers.

## 3. Results

### 3.1. Emetic and behavioral effects in conscious dogs

When administered i.v. to conscious dogs, rolipram produced emesis, stepping behaviors, tremors and anxiety at doses ≥ 0.1 mg/kg (Fig. 1). Emesis was the most consistent of the effects produced by rolipram, being induced in 40% of the dogs receiving 0.1 mg/kg rolipram, and in 100% of dogs reaching a 3 mg/kg dose. In general, rolipram-induced emesis was produced within 2–5 min of dose administration and had subsided within 7–15 min of dosing. Upon receiving an emetic dose of rolipram, dogs averaged 2.5 ± 0.3 episodes of emesis or retching. Emesis was not generally considered to be the dose-limiting side effect of i.v. dosing, however, because of the transience and low frequency of the emetic episodes observed.

Anxiety was induced in 60% of dogs administered rolipram (Fig. 1), as evidenced by uncharacteristic attempts of the dogs to leave their restraining slings, avoid handling, and return to their cages. Behavioral anxiety was associated with elevations in heart rate, as determined by palpitation. Anxiety was considered to be the dose-limiting side effect of rolipram during this study, and dogs exhibiting a moderate degree of anxiety (1/10 after 0.1 mg/kg rolipram, 6/10 after 1.0 mg/kg rolipram) were excluded from further rolipram dosing. Rolipram-induced anxiety responses were not necessarily associated with emetic responses, in that three dogs administered rolipram displayed anxiety without exhibiting emesis or the associated salivation reflex, and one dog exhibiting emesis (and administered up to 3 mg/kg rolipram) showed no signs of anxiety. Rolipram-induced anxiety was short lived, and generally subsided within 30 min of returning each dog to its colony cage.

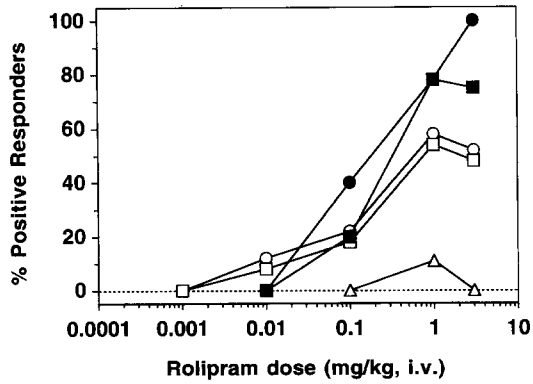


Fig. 1. Emetic and behavioral effects of rolipram in conscious dogs. Rolipram was administered at 0.5 h intervals in cumulative i.v. doses to conscious dogs as described in Methods, and the absence or presence of emesis (●), anxiety (■), stepping behaviors (○), tremors (□) and sedation (△) were noted. Each dog exhibiting one of these effects was scored as a 'positive responder' for that effect at the administered rolipram dose and at all subsequent doses. Dose response curves were constructed by expressing the number of positive responders for each effect as a percentage of the total number of dogs receiving a given rolipram dose.

Stepping behaviors and tremors were induced in a dose-dependent manner by rolipram, and were commonly observed at i.v. doses ≥ 0.1 mg/kg. A single transient episode of hindlimb stepping and tremor occurred in one dog administered 0.01 mg/kg. Sedation, as evidenced by lethargy and inactivity, was observed in only one dog receiving a high dose of rolipram (1 mg/kg), and lasted approximately 20 min.

Administered p.o. in gelatin capsules, rolipram produced no observable effects at doses of 0.01 or 0.03 mg/kg ($n = 4$ each). Administration of 0.1 mg/kg rolipram p.o. resulted in a 100% incidence of emesis, beginning an average 16 ± 4 min after dosing and lasting < 15 min ($n = 8$). No other behavioral effects were noted. Emesis was considered the dose-limiting side effect of p.o. rolipram administration, inasmuch as emptying of gastric contents was presumed to limit further drug exposure. No emesis was observed in dogs administered control capsules ($n = 12$). Pretreatment of dogs with ondansetron (0.15 mg/kg i.v.) apparently reduced the emetic potential of rolipram: only half the dogs pretreated with ondansetron and administered 0.1 mg/kg rolipram p.o. (two of four) exhibited an emetic response.

### 3.2. Bronchodilatory and cardiovascular effects in anesthetized serotonin-infused dogs pretreated with nadolol

Administered i.d., rolipram induced dose-dependent bronchodilation with an estimated $ED_{50}$ of 0.04 mg/kg (Fig. 2). The bronchodilatory effects of rolipram were produced rapidly following i.d. administration, with peak bronchodilation generally occurring within 15–20 min of i.d. dosing. Bronchodilatory dosages of rolipram did not produce marked effects on heart rate or cardiac contractility. However, decreases in blood pressure were observed at higher doses (0.1 and 1 mg/kg) (Fig. 2).

### 3.3. Effects in anesthetized dogs not treated with a β-adrenoceptor antagonist

When administered i.v. to anesthetized dogs not treated with nadolol, rolipram doses ≥ 0.01 mg/kg produced dose-dependent increases in heart rate and cardiac contractility without affecting blood pressure or pulmonary inflation pressure (Fig. 3). A large increase in heart rate was produced by the 0.1 mg/kg dose of rolipram, and further increases were apparent following 1 and 3 mg/kg (i.v.). Maximal increases in $+dP/dt$ (~ 70%) occurred at doses of 0.1–3 mg/kg. In separate vehicle control studies, 0.1 ml/kg of 10% DMSO

in saline (the vehicle used for the 0.1 mg/kg dose of rolipram) did not affect the cardiovascular or pulmonary parameters being measured. Administration of 0.1 ml/kg and 0.3 ml/kg DMSO (the vehicle used for the 1 mg/kg and 3 mg/kg doses of rolipram) reduced blood pressure by 18% and 28%, respectively, but did not produce significant cardiac or pulmonary effects.

Unexpectedly, two of the three dogs investigated during these studies exhibited abdominal movements and swallowing reflexes after receiving rolipram at dosages $\geq 0.1$ mg/kg. These dogs were administered several 50 mg supplements of pentobarbital during the experiment. Despite the administration of supplemental pentobarbital, all three dogs administered rolipram during these studies became responsive to touch at the end of the experiment, as evidenced by a return of the eye blink reflex. A similar responsiveness to touch was not observed in the vehicle control dogs administered DMSO.

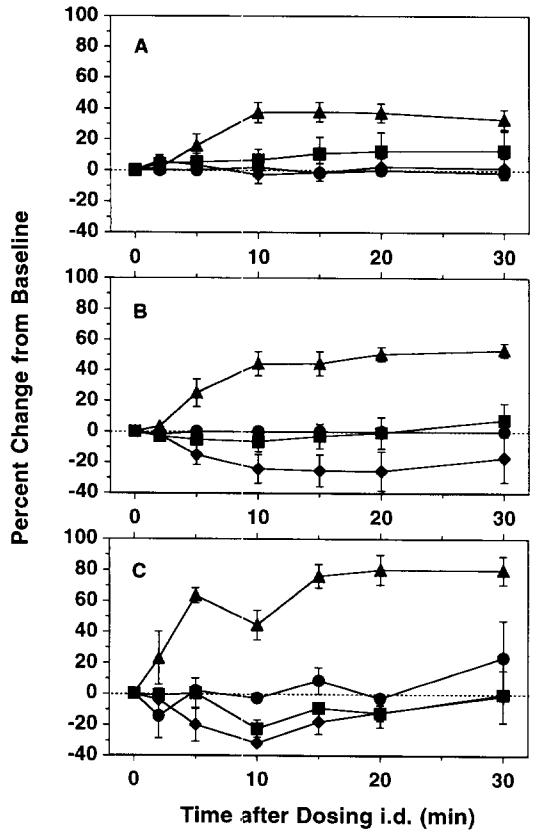


Fig. 2. Bronchodilatory and cardiovascular effects of rolipram in anesthetized serotonin-infused dogs. The bronchodilatory and cardiovascular effects of rolipram were determined in anesthetized β-adrenoceptor antagonist-treated serotonin-infused dogs, following i.d. dosing at 0.01 mg/kg (A), 0.1 mg/kg (B), or 1.0 mg/kg (C). Bronchodilation (▲) was measured as a reduction in pulmonary inflation pressure, and expressed as a percentage of maximum inducible bronchodilation. Changes in heart rate (●), $+dP/dt$ (■) and blood pressure (♦) were expressed as a percentage change in values determined immediately prior to rolipram administration. All data are expressed as means ± S.E. ($n = 3$), whenever S.E.s were larger than the symbol size.

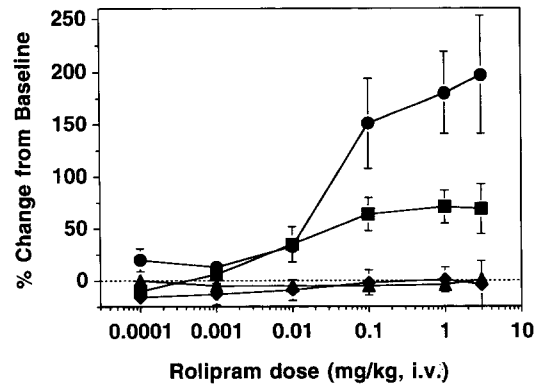


Fig. 3. Effects of rolipram in anesthetized dogs not treated with a β-adrenoceptor antagonist. The effects of rolipram on pulmonary inflation pressure (▲), heart rate (●), $+dP/dt$ (■) and blood pressure (♦) were determined in pentobarbital-anesthetized dogs, following cumulative i.v. dosing. Cardiovascular and pulmonary effects were measured as a percentage change from values determined immediately prior to rolipram administration. All data are expressed as means ± S.E. ($n = 3$), whenever S.E.s were larger than the symbol size.

### 3.4. Effects of pentobarbital supplementation on responses to rolipram

In previously anesthetized dogs, administration of an i.v. bolus dose of 15 mg/kg pentobarbital and tripling the pentobarbital infusion rate from 0.08 to 0.24 mg/kg per min did not affect the heart rate but did decrease $+dP/dt$ (25%, to 1650 mm Hg/s) and mean arterial blood pressure (21%, to 95 mm Hg) (Fig. 4). Subsequent administration of rolipram (1 mg/kg i.v.) resulted in an immediate elevation of heart rate and $+dP/dt$, and a more gradual elevation in mean arterial blood pressure. Peak elevations in heart rate, $+dP/dt$, and mean arterial blood pressure occurred approximately 30 min after rolipram administration, and averaged 48%, 35%, and 30%, respectively. No drug-related changes in lung inflation pressure were noted. 60 min after rolipram administration, cardiovascular parameters remained elevated, and all three dogs were observed to exhibit strong eye-blink reflexes and to breathe freely when removed from the respirator.

Administration of a supplemental dose of 33 mg/kg pentobarbital (i.v. bolus) plus tripling the pentobarbital infusion rate to 0.24 mg/kg per min resulted in a 22% decrease in mean heart rate (to 120 beats/min), a 47% decrease in mean $+dP/dt$ (to 1130 mm Hg/s), and a 51% decrease in mean arterial blood pressure (to 61 mm Hg) in three previously anesthetized dogs (Fig. 3). Subsequent administration of rolipram (1 mg/kg i.v.) to these dogs resulted in an average 11% increase in

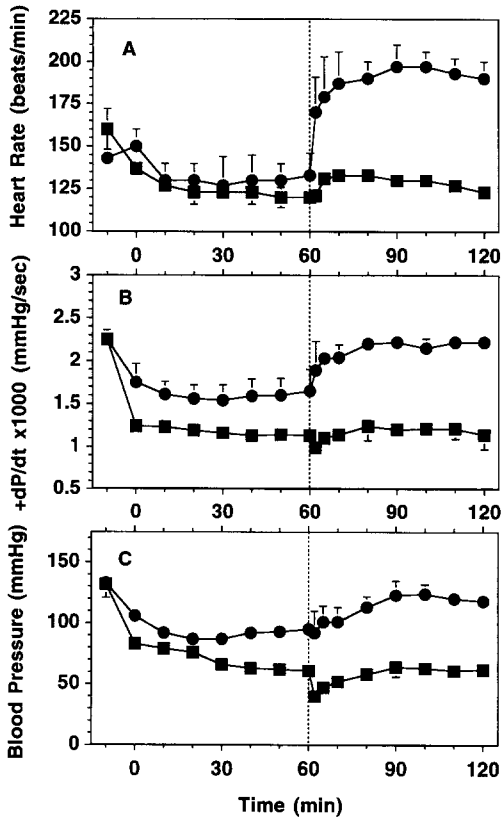


Fig. 4. Effects of pentobarbital on cardiovascular system responses to rolipram. Dogs previously stabilized under pentobarbital anesthesia (33 mg/kg i.v. bolus, 0.08 mg/kg per min infusion) were placed under deep pentobarbital anesthesia by increasing the pentobarbital infusion rate to 0.24 mg/kg per min, and administering a supplemental i.v. bolus dose of either 15 mg/kg (●) or 33 mg/kg (■) pentobarbital at −2 min ($n = 3$ each). 60 min later, each dog was administered an i.v. bolus dose of 1 mg/kg rolipram. Heart rate (A), cardiac contractility ($+dP/dt$; B) and blood pressure (C) were measured and expressed as their means ± S.E., whenever the S.E. was larger than the symbol size.

heart rate, and no significant changes in $+dP/dt$ or mean arterial blood pressure (Fig. 4). No drug-related changes in lung inflation pressure were noted. 60 min after rolipram administration, dogs in this study did not demonstrate eye-blink reflexes. These dogs did not breathe independently when removed from the respirator.

### 3.5. Effects of rolipram on sleep time, heart rate, and respiratory rate in uninstrumented anesthetized dogs

Administration of 1 mg/kg rolipram (i.v.) significantly reduced the duration of anesthesia in uninstrumented dogs administered a 33 mg/kg i.v. bolus of pentobarbital (Table 1). Using response to touch (reflex muscular responses) as the earliest sign of anesthesia reversal, it was observed that rolipram reduced the average duration of anesthesia significantly, from 50 min to 20 min during this study (Table 1). Five dogs administered rolipram responded to touch within 10 min, whereas only one dog administered vehicle responded to touch this quickly. Average times to lift the head (voluntary muscle movement) and track moving objects (focus attention) were also significantly shorter in dogs administered rolipram than those administered

Table 1
Effect of rolipram on pentobarbital-induced anesthesia in dogs

| Endpoint | Time to indicated endpoint (min) | |
|---|---|---|
| | Vehicle (DMSO) | Rolipram (1 mg/kg) |
| Reflex muscular movements (sensitivity to touch, pressure) | 50 ± 11 | 20 ± 6 [a] |
| Voluntary muscular movements (head lifting, changes in position) | 250 ± 32 | 188 ± 19 [a] |
| Focus of attention (movement tracking) | 283 ± 24 | 220 ± 20 [a] |

Dogs that had been anesthetized with nembutal (33 mg/kg i.v. bolus) 60 min previously were administered rolipram (1 mg/kg i.v.) or its vehicle (DMSO), and times for each dog to reach the indicated post-anesthesia endpoints were determined. Data represent the mean results (± S.E.) from eight dogs administered rolipram or its vehicle (DMSO) in a randomized crossover design. [a] Significantly different from vehicle control ($P < 0.05$) as determined by paired $t$-test.

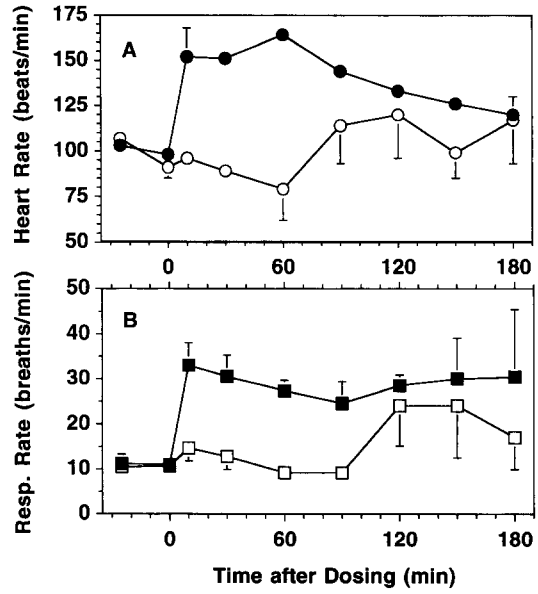


Fig. 5. Effects of rolipram on heart rate and respiratory rate in uninstrumented anesthetized dogs. Using a crossover experimental design, rolipram (1 mg/kg) (solid symbols) or its vehicle (DMSO) (open symbols) were administered i.v. to each of eight dogs that had been anesthetized 60 min earlier with pentobarbital (33 mg/kg i.v.). Heart rates (A) and respiratory rates (B) were respectively monitored by palpitation and visually, and plotted as a function of time after rolipram dosing. All data are plotted as means ± S.E., whenever S.E.s were larger than the symbol size.

vehicle (Table 1). Of the dogs administered vehicle, one failed to exhibit a head lift response, and two failed to visually track moving objects within the 6 h post-dosing observation period used in these experiments. In contrast, every dog administered rolipram was capable of lifting its head and visually tracking movement within 5 h of dosing.

Administration of 1 mg/kg rolipram to anesthetized uninstrumented dogs produced a significant increase in both heart rate and respiratory rate within 10 min of dosing (Fig. 5). Among the three dogs that had not demonstrated sensitivity to touch within 10 min of rolipram dosing, all three exhibited elevated respiratory rates and one exhibited an elevated heart rate within this time. Rapid increases in heart and respiratory rates were not observed following the administration of DMSO vehicle to anesthetized dogs. Instead, these dogs exhibited apparent increases in heart rate an average 60–90 min after vehicle dosing, and increases in respiratory rate an average 90–120 min after vehicle (Fig. 5). In sharp contrast to the results with rolipram, none of the dogs administered DMSO in these studies exhibited elevations in heart rate or respiratory rate prior to or coincident with their becoming responsive to touch.

## 4. Discussion

The therapeutic utility of a drug that acts via cAMP-dependent mechanisms is likely to depend not only on the efficacy of that drug in modulating cAMP, but also on its selectivity for the target tissue. Recent studies investigating the pharmacological profile of phosphodiesterase 4 inhibitors suggest that these inhibitors would be useful for the treatment of asthma, inasmuch as they are effective in suppressing the activation of both respiratory smooth muscle and inflammatory cells from various species (see Introduction). However, a primary concern when considering this use of phosphodiesterase 4 inhibitors relates to the role of phosphodiesterase 4 as a regulator of central nervous system function. The data from this study demonstrate that canine models might be useful for assessing the emetic or central nervous system activities of phosphodiesterase 4 inhibitors being investigated for antiasthmatic or other activities.

The results of this study demonstrate that in dogs, as in man, emesis is a consistent side effect of acute rolipram administration. Such an effect might be presumed to 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, and systemically administered Ro 20-1724 (another phosphodiesterase 4 inhibitor) has been previously demonstrated to render the area postrema hypersensitive to drugs inducing emesis through cAMP-dependent mechanisms (Borison and Wang, 1953; Carpenter et al., 1988; Harding et al., 1987). Thus, rolipram might be expected to potentiate multiple proemetic neurotransmitters in the brain (Borison and Wang, 1953). The data from this study also implicate the central nervous system's involvement in rolipram-induced emesis by demonstrating that emesis induced by i.v. rolipram is associated with the induction of a variety of behavioral responses (Fig. 1). These effects are reminiscent of the behavioral effects produced by rolipram in other species, and associated with enhanced central adrenergic signal transduction (Wachtel, 1982). The induction of stepping behaviors and tremors by rolipram at doses $\geq 0.1$ mg/kg in dogs is similar to the induction of stepping behaviors and head twitches induced by rolipram and other cAMP-phosphodiesterase inhibitors in rats (Wachtel, 1982). Similar doses of rolipram potentiate central adrenergic signal transduction in mice, as evidenced in their abilities to reverse reserpine-induced hypothermia (Griebel et al., 1991; Koe et al., 1990; Wachtel, 1983). Anxiety responses, which are commonly associated with increased adrenergic tonus, might also be suggested to result from a potentiation of adrenergic signal transduction in the dog (Hoehn-Saric, 1982).

Interestingly, however, the data also suggest that p.o. rolipram also activates the emetic reflex via a gastrointestinal mechanism, because dogs were more sensitive to the emetic effects of rolipram following intragastric administration ($ED_{100} = 0.1$ mg/kg p.o.) than following i.v. administration ($ED_{100} = 3$ mg/kg), and because the emetic response to p.o. rolipram could be blunted by ondansetron (Borison and Wang, 1953). In contrast, the other systemic effects of rolipram were reduced following intragastric administration: rolipram exhibited lesser potency as a bronchodilator when administered i.d. (Fig. 2) versus i.v. (Heaslip et al., 1991), and failed to induce behavioral side effects when administered p.o. These results presumably reflect rolipram's poor systemic bioavailability in most species (Krause and Kuhne, 1988). Thus, results in conscious canine models appear to reflect clinical experience in man, where nausea and emesis were the limiting side effects of p.o. dosing with rolipram (Bertolino et al., 1988; Hebenstreit et al., 1989; Laux et al., 1988). As a result of rolipram's greater emetic potency and lesser bronchodilatory potency when administered intragastrically (versus i.v.), the bronchodilatory selectivity of rolipram was substantially lower after intragastric administration: the separation between bronchodilatory and emetic doses of rolipram was over 100-fold after i.v. dosing (0.007 mg/kg vs. ~ 1 mg/kg, respectively) (Heaslip et al., 1991), but only approximately 2.5-fold after intragastric dosing (0.04 mg/kg i.d. vs. 0.1 mg/kg

p.o.). The bronchodilatory versus emetic selectivity of rolipram, and comparisons of the emetic potential of i.v. versus p.o. rolipram in man have not been reported.

The observation that rolipram increased heart rate and $+dP/dt$ in anesthetized dogs not treated with nadolol (Fig. 1) is in sharp contrast with results from nadolol-treated anesthetized dog models, where rolipram produced few cardiac effects (Fig. 2) (Heaslip et al., 1991; Weishaar et al., 1987). Such results have been taken to suggest that the cardiac effects of rolipram result from its enhancement of cardiac responses to sympathetic (cAMP-dependent) stimulation (Weishaar et al., 1987). However, the data from this study suggest that the cardiac effects of rolipram may also result from rolipram's effects in the canine central nervous system, inasmuch as these effects occurred over the same i.v. dose range as was associated with rolipram-induced behavioral effects in conscious dogs (0.01–3 mg/kg, Figs. 1 and 3) and reversal of pentobarbital-induced anesthesia (Table 1; Fig. 4), but not at the lower i.v. doses that induce bronchodilation (0.0001 mg/kg and up) (Heaslip et al., 1991).

Rolipram has not been demonstrated to reverse anesthesia in studies published to date on other species. Nonetheless, the reversal of pentobarbital-induced anesthesia by i.v. rolipram is consistent with results from rats, where agents that potentiate or block central nervous system adrenergic signal transduction were demonstrated to reduce or increase barbiturate-induced sleep times, respectively (Louie et al., 1986; Mason and Angel, 1983a,b; Mason et al., 1983). In the present study, an association was established between rolipram's induction of cardiovascular effects and its reversal of pentobarbital-induced anesthesia, inasmuch as the appearance of cardiovascular symptoms in anesthetized dogs occurred coincidentally with the return of eye blink reflexes during experiments using pentobarbital supplements (Fig. 4). It is unlikely that the cardiovascular effects of rolipram observed during these studies were a separable consequence of the stress associated with emergence from anesthesia, because elevations in heart rate occurred prior to the return of 'responsiveness to touch' in rolipram-treated dogs during sleep studies, whereas cardiovascular changes occurred well after 'responsiveness to touch' in vehicle-treated dogs (Fig. 5). It is also unlikely that the reversal of pentobarbital anesthesia resulted non-specifically from a pH-dependent repartitioning of barbiturates from the central nervous system or increased pentobarbital metabolism, inasmuch as this reversal occurred within minutes of rolipram dosing in uninstrumented dogs (Fig. 5, Table 1) and maintenance of a constant respiratory frequency and volume (especially in the absence of pulmonary function changes) would minimize changes in blood pH in instrumented dogs ( Figs. 3 and 4). Thus, rolipram's reversal of pentobarbital-induced anesthesia is likely to occur also as a result of it's effects on the central nervous system.

Rolipram has been previously demonstrated to potentiate cAMP-dependent signal transduction in the cerebral cortex and hippocampus of both rats and guinea pigs in vitro (Donaldson et al., 1988; Gaal et al., 1991; Schwabe et al., 1976; Stanley et al., 1989), and autoradiography studies have shown that phosphodiesterase 4 is abundantly distributed in the several specific regions of the rat brain, including the cerebellum, frontal cortex and hippocampus (Kaulen et al., 1989). Results from the present study suggest that a 1 mg/kg i.v. dose of rolipram stimulates medullar centers in the canine brain, inasmuch as rolipram induced an immediate increase in respiratory frequency in anesthetized uninstrumented dogs (Fig. 4), and the cardiovascular and 'antianesthetic' effects of rolipram were completely suppressed only at doses of pentobarbital that completely suppressed normal respiratory drive (Fig. 3). Rolipram has also been reported to have respiratory-stimulant activity in conscious rhesus monkeys (Howell, 1993).

In considering literature suggestions that phosphodiesterase 4 inhibitors may be useful in the treatment of asthma, it is interesting to note that i.v. doses of rolipram inducing bronchodilation in the nadolol-treated dog ($\geq 0.0001$ mg/kg; $ED_{50} = 0.007$ mg/kg) (Heaslip et al., 1991) are much lower than i.v. doses that induced emetic, behavioral, cardiovascular or 'antianesthetic' activities in the absence of nadolol during these studies ($\geq 0.1$ mg/kg). The blockade of $\beta$-adrenoceptors during bronchodilation studies, if anything, might be expected to reduce the bronchodilatory potency and cause underestimation of the therapeutic selectivity of rolipram (Heaslip et al., 1991, 1994; Heaslip and Sickels, 1993). Thus, the data suggest that systemically administered phosphodiesterase 4 inhibitors may have bronchodilatory selectivity suitable for use in the treatment of asthma. However, the more similar potencies of rolipram as a bronchodilator and as an emetic agent following intragastric administration highlight a potential need to avoid local triggering of the emetic reflex in the gastrointestinal tract, if anti-asthmatic phosphodiesterase 4 inhibitors are to be administered orally.

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