Effects of phosphodiesterase 10 inhibition on striatal cyclic AMP and peripheral physiology in rats

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Phosphodiesterases (PDEs) form a family of enzymes involved in the hydrolysis of cyclic adenosine and guanosine monophosphate (cAMP and cGMP). PDE10A is a member of this family that is almost exclusively expressed in the striatum. Increasing cAMP/cGMP levels *via* inhibition of PDE10A is under consideration as a novel therapeutic avenue in the discovery of antipsychotics. Papaverine has been used as a pharmacological tool to establish the possible clinical use of PDE10A inhibitors as antipsychotics. Papaverine is known to increase cAMP levels in striatum and to decrease blood pressure, body temperature and locomotor activity after systemic administration. In this study, the effects of papaverine are compared to those of a more specific PDE10A inhibitor MP10. Papaverine raised striatal cAMP levels with hypothermia, hypoactivity and decreased cardiovascular responses. The more selective MP10 had significantly less effects on body temperature and cardiovascular functions, but reduced locomotor activity to a similar extend as papaverine.

Key words: PDE10, papaverine, MP10, cAMP

INTRODUCTION

Phosphodiesterases (PDE) form a family of enzymes involved in the hydrolysis of cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) into nucleotide monophosphates, which play a central role in signal transduction and regulation of physiological responses (Essayan 2001). PDE10A is a dual substrate PDE which is abundantly expressed in the striatum (Fujishige et al. 1999). The dopaminergic (DA) D2 receptor is a G-protein-coupled receptor, linked to inhibition of adenylyl cyclase; activation of D2 receptors results in a decrease of cAMP.

DA D2 receptors are the main target of currently used antipsychotic drugs and blockade of D2 receptors in striatum is known to increase cAMP (Guillin et al. 2007). Therefore, increasing striatal cAMP levels *via* intracellular targets might provide a novel therapeutic avenue in the development of drugs to treat schizophrenia (Maxwell et al. 2004, Menniti et al. 2007). A role for PDE10A in regulating striatal activity within both striatonigral and striatopallidal pathways was suggested (Seeger et al. 2003).

Papaverine, an inhibitor of PDE10A, has been extensively used as a pharmacological tool to study this target and preclinical evidence was obtained for efficacy of PDE10A inhibition in improving cognitive as well as positive symptoms in schizophrenia (Rodefer et al. 2005, Siuciak et al. 2006). Later, a new class of PDE10A inhibitors, comprising MP10 (2-(4-1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)-phenoxymethyl)-quinoline succinic acid), with improved potency and selectivity, were described (Kehler et al. 2007, Schmidt et al. 2008). In the present study, we confirmed the effect of papaverine on basal levels of cAMP in striatum using microdialysis in freely moving rats and compared the effects of papaverine and the more selective PDE10A inhibitor MP10 after systemic administration using telemetry in order to define whether any of the observed effects is related to central activity of PDE10A inhibitors.

METHODS

Animals

Male Sprague-Dawley rats (Harlan, The Netherlands), weighing 200–300 g at the time of surgery, were used. Rats were singly housed in individually ventilated cages (25 × 33 × 18 cm). The animals were allowed to acclimate at least seven days after receipt from the supplier prior to surgery. Animals were provided with a microchip for identification purposes and maintained under controlled environmental conditions throughout the study: 22 ± 2°C ambient temperature, relative humidity at 60%, with a 12/12h light/dark cycle. Food and water were available *ad libitum*. All experiments were performed in the light phase. All experimental protocols were carried out in strict accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) and were approved by the animal care and use committee of Johnson & Johnson Pharmaceutical Research and Development and local ethical committee.

Fig. 1. Effect of s.c. administration of papaverine (A) and MP10 (B) on body temperature (°C) of rats (10 s epochs averaged over 30 min SEM; *n*=8 per treatment group). The arrow indicates time of injection of vehicle or drugs.

Surgery

Telemetry

Under isoflurane and O_2 30% / N_2O 70% mixture inhalation anesthesia, on an isothermic heating blanket that maintained their core body temperature at 37°C, 32 rats were instrumented with radiotelemetric transmitters (TL11M2-C50-PXT, Data Sciences International). The biopotential probes were placed in the peritoneal cavity and catheters were inserted in the femoral arteries.

Microdialysis

Under isoflurane and O_2 30% / N_2O 70% mixture inhalation anesthesia, the rats were mounted in a stereotactic apparatus on an isothermic heating blanket that maintained their core body temperature at 37°C. Microdialysis guide cannulas (CMA/12; CMA/Microdialysis, Stockholm, Sweden) with inserted dummy probe were placed in left and right striatum following coordinates taken from Paxinos and Watson atlas (Paxinos and Watson 1997): 2.5 mm lateral (left) and 0.5 mm anterior to bregma, lowered into the brain ventral at −3.25 mm from dura (incisor bar set at −3.5 mm).

After surgery, the animals were allowed to recover for at least 10 days prior to experimentation.

Telemetry experiments

Body temperature (BT), locomotor activity (LMA), mean arterial blood pressure (BP), and heart rate (HR) were continuously monitored by means of radio telemetry using a transmitter (Data Sciences International, USA). This transmitter produced temperature dependent frequency modulated signals, which were received with an antenna board (model RA1010, DataSciences) underneath the cage. Locomotor activity was obtained by monitoring changes in the received signal strength that resulted from movement of the animals. Changes in signal strength beyond a predetermined threshold generated a pulse that was counted by the acquisition system.

Data were sampled in 10 seconds epochs and averaged over 30 minutes. For each treatment group, recordings were performed 1 hour before treatment for baseline levels and for 4 hours following subcutaneous administration of vehicle, papaverine (50, 100 mg/kg)
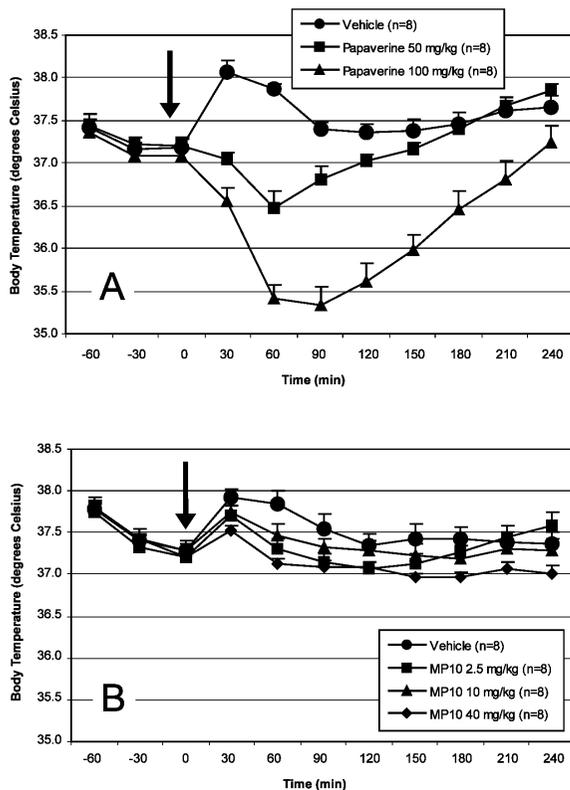
or MP10 (2.5, 10, 40 mg/kg) in a fully randomised order (*n*=8 for each condition). A minimal washout period of 2 weeks was included after every treatment.

Dialysate sampling

Ten days after surgery, the animals were habituated to the recording procedure that took place in their home cages with free access to food and water ad libitum. On the afternoon prior to drug administration, probes (CMA/12 probes with a 20 000 MW cut-off membrane of 4 mm; CMA/Microdialysis, Stockholm, Sweden) were perfused continuously with MilliQ water at a rate of 1 µl/min. The animals were then briefly anesthetized with an isoflurane/O_2/N_2O mixture to slowly insert the probes. The animals were left undisturbed overnight with a flow rate of 0.1 µl/min Ringer solution (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$ $4H_2O$) and the following day 3 hours before the start of sampling, the

flow rate was brought to 1 µl/min. First, three baseline samples of 60 minutes were sampled. Then, animals were administered with papaverine and four 60-minute samples were collected over a total experimental duration of 480 minutes. During the sampling period all dialysate samples were cooled at 4°C. cAMP levels were assessed in 45 animals, which were randomly assigned to the 3 treatment conditions (*n*=15 rats per condition).

cAMP dialysate analysis

cAMP levels were determined in 50 µl dialysate samples using a radioactive immunoassay kit ([^{125}I] cAMP Biotrak assay kit RPA 509, GE Healthcare) with acetylating protocol to increase sensitivity of the assay. One standard curve in duplo was prepared for each assay from 0.02–1.28 nM cAMP. Data were analyzed with Graph Pad Prism software. The data are presented as real concentrations measured in the samples without adjustments for diffusion of cAMP.

Histological verification of microdialysis probe positions

After sampling, animals were sacrificed for histological verification of the probe placement. The brains were stored in 10% buffered formaldehyde solution until coronal sections were taken. The location of the probes was determined using the atlas of Paxinos and Watson (1997) and were located within the region 1.0−0.0 mm anterior to bregma for the striatum. Lesions found with more than 0.5 mm divergence to target coordinates, or lesions found to affect other brain structures caused the animal to be discarded from analysis. Considering this, 2 striatal probes were excluded from the study.

Drugs

Papaverine was purchased from Sigma-Aldrich (St Louis, MO) and MP10 was synthesized at Johnson & Johnson Research and Development Janssen Pharmaceutica laboratories. As a vehicle and as the solute of papaverine (50, 100 mg/kg) and MP10 (2.5, 10, 40 mg/kg) 10% cyclodextrine + NaCl was used. Animals were injected subcutaneous (s.c.) at a volume of 5 ml/kg.
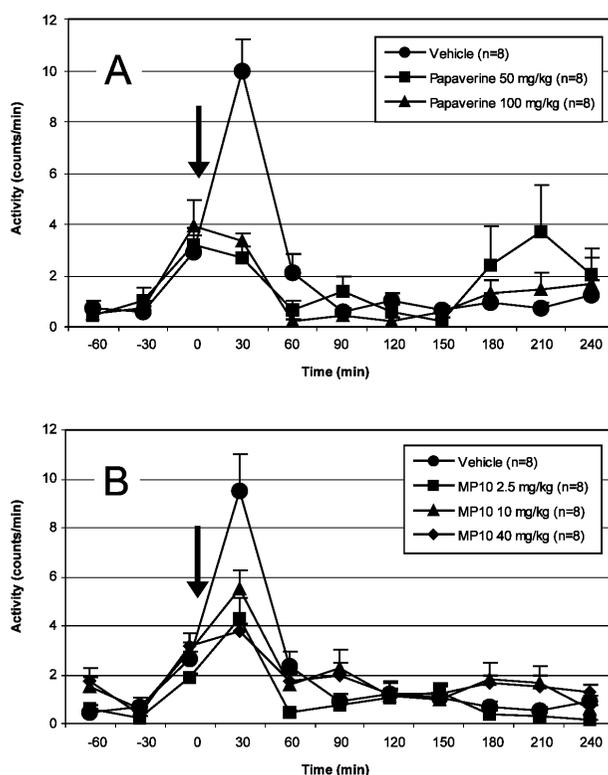
Fig. 2. Effect of s.c. administration of papaverine (A) and MP10 (B) on spontaneous locomotor activity (counts per min) of rats (10 s epochs averaged over 30 min SEM; *n*=8 per treatment group). The arrow indicates time of injection of vehicle or drugs.

Statistical analysis

Telemetric variables

Telemetric variables were presented as mean value ± SEM and evaluated using ANOVA for repeated measures in consecutive 30 minutes intervals for 4 hours post administration on 2 variables (doses and time) with correction for multiple comparisons. A value of $P<0.05$ was considered to be significant.

Cyclic AMP in striatum

For each animal the cAMP concentrations at the different measured time points were scaled to the animal specific median baseline value, which resulted in the percent change response variable. Next, an Area Under the Curve (AUC) value of the percent change response variable between 0 and 240 minutes after drug administration was calculated. Finally the animal specific

calculated AUC were $\log_e$ transformed and analysed using a one-way ANOVA with treatment, existing of the levels vehicle, 50 and 100 mg/kg of papaverine, as factor.

RESULTS

Telemetric variables

For papaverine, body temperature was different between treatment groups ($P<0.0001$). Systemic administration of 50 and 100 mg/kg papaverine significantly and dose-dependently decreased body temperature from 30 min after treatment (both $P<0.0001$ compared to vehicle group; $P=0.014$ comparing 50 and 100 mg/kg group), with a maximum decrease at 60 minutes after administration (both $P<0.0001$ compared to vehicle group; $P<0.0001$ comparing 50 and 100 mg/kg group). In the 50 mg/kg group body temperature returned to baseline level approximately at 120 minutes, while body temperature of the highest dosed group was decreased until 210 minutes after administration of papaverine (Fig. 1A).

A difference in body temperature was observed after administration of MP10 ($P=0.0004$). No significant effect on body temperature was observed after injection of lower doses of MP10. Injection of the highest dose slightly decreased temperature at 60 min after treatment ($P=0.009$ compared to vehicle group) (Fig. 1B).

A significant difference in locomotor activity was observed after papaverine injection ($P<0.0001$). Both doses produced a significant but not dose-dependent decrease in activity the first 30 min following the administration (both $P<0.0001$ compared to vehicle group) (Fig. 2A). Also for MP10, a difference in activity between treatment groups was observed ($P=0.004$). All doses of MP10 significantly but not dose-dependently decreased activity the first 30 min following administration ($P=0.007$; 0.04; 0.004 compared to vehicle group, respectively) (Fig. 2B).

A decrease in heart rate was observed ($P=0.004$) during the first 30 min after administration of the highest dose of papaverine ($P=0.017$ compared to vehicle group) (Fig. 3A). A similar decrease in mean blood pressure was observed ($P<0.0001$) at 30 min ($P=0.013$ compared to vehicle group) up to 60 min ($P=0.026$ compared to vehicle group) after treatment.

No significant effects on heart rate and blood pressure were observed after administration of 2.5 and 10
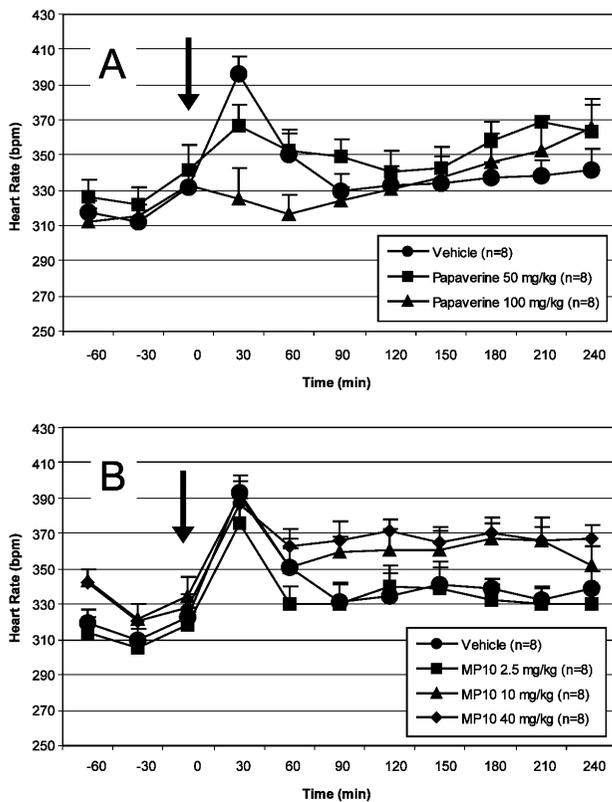

Fig. 3. Effect of s.c. administration of papaverine (A) and MP10 (B) on heart rate (beats per min) of rats (10 s epochs averaged over 30 min SEM; $n=8$ per treatment group). The arrow indicates time of injection of vehicle or drugs.

mg/kg MP10. Mildly increased heart rate (Fig. 3B) and blood pressure was observed from 90 min after injection of the highest doses MP10 (P=0.003/P=0.0004, respectively).

Handling and injection procedures can explain the increase in measured parameters observed in all vehicle-injected groups.

Cyclic AMP in striatum

The mean percent change from baseline cAMP levels by treatment with papaverine was studied (Fig. 4). From the ANOVA analysis it followed that there is a significant effect on cAMP levels (P=0.011). The AUC was estimated to be 46% higher in the 100 mg/kg dose group and 40% in the 50 mg/kg dose group compared to vehicle. These estimates suggest a dose dependant effect, although not significant (P=0.74).

Mean ± STDEV baseline levels of cAMP were 0.11 ± 0.06 nM (n=35); mean ± STDEV levels 1 hour post administration of 50 and 100 mg/kg papaverine were respectively 0.19 ± 0.13 nM (n=11) and 0.19 ± 0.09 nM (n=13).

DISCUSSION

Recently, PDE10A inhibition was proposed to have therapeutic effectiveness on positive and cognitive symptoms of schizophrenia and preclinical evidence for these effects were obtained using the PDE10A
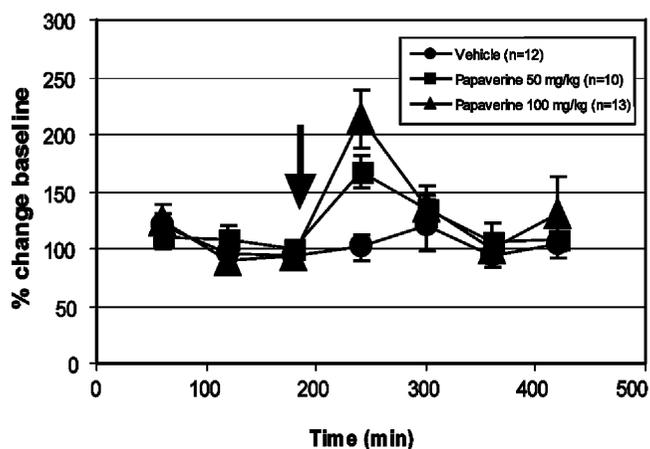

Fig. 4. Concentration of cAMP in striatum of rats (% change from baseline concentrations ± SEM) treated with vehicle (n=12), 50 mg/kg papaverine (n=10) and 100 mg/kg papaverine (n=13). The arrow indicates time of injection of vehicle or drugs.

inhibitors papaverine and MP10 (Rodefer et al. 2005, Menniti et al. 2007, Schmidt et al. 2008). The psychoactive effects were related to the enhancement in striatal cAMP and cGMP levels resulting in increased medium spiny neuron activity (Siuciak et al. 2006). It is known that currently used antipsychotics act as dopamine receptor D2 antagonists (Kapur and Mamo 2003, Guillin et al. 2007) believed to result in an increase of cAMP in striatal tissue. However, data on effects of antipsychotics on cAMP are conflicting (Santiago and Westerink 1990, Muly 2002, Kelly et al. 2007).

Increase in brain cAMP levels have been shown to be associated with changes in physiological parameters and characteristic behavioral effects such as hypothermia and hypoactivity (Wachtel 1982).

In order to establish whether these effects can be used to distinguish central activity of specific PDE10A inhibitors, the effects of papaverine were compared to those of the more selective PDE10A inhibitor MP10 in this study. The doses of 50–100 mg/kg subcutaneous (s.c.) papaverine and 2.5–10 mg/kg (s.c.) MP10 used are in the same range of potency for antipsychotic activity. In the conditioned avoidance response, a test which is characterized as a good measure for antipsychotic activity (Wadenberg and Hicks 1999), the ED50 values for papaverine and MP10 are, respectively, 74 mg/kg (s.c.) and 1.8 mg/kg (s.c.) in rats (unpublished results). These data are in line with the ED50 values reported after intra-peritoneal dosing for papaverine and for MP10 (Grauer et al. 2009). In this study, it was confirmed that systemic administration of the PDE10A inhibitor papaverine increased striatal cAMP concentration (Fig. 4). Iyo and coauthors (1996) observed 40–50% increased cAMP levels while no change in dopamine levels in rat striatum after administration of rolipram. Increased cGMP release in the rat frontal cortex after local PDE inhibition with IBMX was reported (Laitinen et al. 1997). Siuciak and others (2006) showed that papaverine (56 mg/kg i.p.) resulted in a 3-fold increase in striatal cAMP measured by *in vivo* microdialysis while in this study, a 60 and 100% increase of striatal cAMP was observed one hour after subcutaneous injection of 50 and 100 mg/kg papaverine, respectively. In line with baseline cAMP dialysate values in this study of Siuciak and coworkers (2006) (0.17 ± 0.04 nM; n=5), our study showed similar baseline values of cAMP (0.11 ± 0.06 nM; n=35). Recently, Schmidt and col-

leagues (2008) reported a 150–200% increase of cAMP and cGMP levels in striatum of MP10 treated rats (3.2 mg/kg) with baseline values for cAMP of 0.049 nM. Using telemetry measurement in freely moving rats, this study confirmed that elevations in brain c AMP levels after papaverine administration coincide with a significant decrease in body temperature, hypoactivity and mild decrease of heart rate and blood pressure in rats (Figs 1–3). The more selective PDE10A inhibitor MP10 was shown to have significantly less pronounced effects on body temperature, heart rate and blood pressure but caused a significant decrease in activity at all doses, similar to papaverine.

In line with our findings, forskolin, an activator of adenylate cyclase, shown to increase cAMP (Hutson and Suman-Chauhan 1990), was previously shown to lower rectal temperature and decrease locomotor activity of rats. These effects were enhanced by an additional injection of the PDE4 inhibitor rolipram (Wachtel et al. 1987). Similarly, administration of dBcAMP, a cAMP analog resistant to hydrolysis by PDE, also results in hypothermia and hypoactivity (Wachtel 1982).

CONCLUSION

These findings suggest that PDE10A inhibition raises central cAMP levels in correlation with hypoactivity. The cardiovascular responses observed after administration of papaverine are not directly related to cAMP increase after inhibition of PDE10A since the more specific PDE10A inhibitor MP10 does not induce the same effects at doses that have been shown to increase cAMP in the brain. A decrease in body temperature, previously described as a direct consequence of cAMP elevation in brain, could not be directly related to PDE10A inhibition in this study. Measurement of hypoactivity however, seems to be an easy approach for the determination of *in vivo* activity of PDE10A inhibitors, and is most likely related to the capacity of the inhibitors to increase cAMP levels in the brain.

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