Dopamine D₃ Receptor Antagonist (GSK598809) Potentiates the Hypertensive Effects of Cocaine in Conscious, Freely-Moving Dogs

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Received March 2, 2015; accepted July 14, 2015

ABSTRACT

The chronic and relapsing nature of addiction presents unique challenges for ensuring the safety of a potential medication. A patient may use cocaine, for example, while taking the medication or take more medication than prescribed. Thus, a potential medication must be safe *and* not exacerbate the effects of cocaine. Multiple published studies support antagonism of brain dopamine D₃ receptor function as a potential mechanism of action for an anti-addiction medication. Dopamine D₃ receptors are widely distributed outside the central nervous system, however; for example, dopamine D₃ receptors in the kidneys are implicated in regulating blood pressure. The selective dopamine D₃ receptor antagonist GSK598809 [1-(2-fluoro-4-trifluoromethyl-phenyl)-3-{3-[4-methyl-5-(4-methyl-oxazol-5-yl)-4H-[1,2,4]triazol-3-ylsulfanyl]-propyl}-3-aza-bicyclo[3.1.0]hexane] has been proposed as a medication to treat cocaine and

other substance use disorders. The US Food and Drug Administration has established guidelines recommending safety studies to investigate potential undesirable pharmacodynamic effects of a substance in relation to exposure in the therapeutic range and above. Hence, we assessed the interaction between this selective dopamine D₃ receptor antagonist and cocaine on hemodynamics and cardiac function in freely-moving, telemetered dogs before conducting a clinical trial. GSK598809 increased the hemodynamic effect of cocaine in this model. Thus, the increase in blood pressure after intravenous cocaine was greater in animals that had been pretreated with GSK598809 compared with vehicle. This finding suggests that GSK598809 in particular, and perhaps dopamine D₃ receptor antagonists as a class, may produce unacceptable cardiovascular risks as medications to treat cocaine use disorder.

Introduction

Dopamine has been implicated in the rewarding actions of drugs of abuse, in part through circuitry in the mesocorticolimbic system (Wise and Bozarth, 1985). These circuits originate in the ventral tegmental area and project to limbic and cortical structures, especially the nucleus accumbens, prefrontal cortex, and amygdala (Swanson, 1982; Walsh and Han, 2014). Elevation of dopamine levels in the nucleus accumbens has been proposed as a final common pathway in the actions of drugs of abuse, including cocaine, amphetamine, opiates, nicotine, and ethanol (Di Chiara and Imperato, 1988).

The dopamine D₃ receptor subtype has long been viewed as a potential target for medications to treat cocaine use disorders (Sokoloff et al., 1990), and interest in dopamine D₃ receptor antagonists as drug abuse medications has been driven by behavioral data suggesting the potential efficacy of compounds

that selectively block dopamine D₃ receptors. These findings have been reported by multiple laboratories, using varied compounds and species. For example, the selective and potent dopamine D₃ receptor antagonist SB277011A blocks cocaine enhancement of electrical brain-stimulation reward, attenuates cocaine-induced conditioned place preference, and attenuates cocaine priming-induced reinstatement of cocaine seeking (Vorel et al., 2002). Further, this compound reduced cue-induced reinstatement (Gilbert et al., 2005; Cervo et al., 2007) and cocaine self-administration under certain conditions (Xi et al., 2005). Similar results have been generated using other dopamine D₃ receptor antagonists. The dopamine D₃ receptor antagonist NGB 2904 attenuated cocaine self-administration, reduced cocaine-induced extracellular dopamine, and inhibited both cue- and prime-induced relapse (Gilbert et al., 2005; Xi and Gardner, 2007). The dopamine D₃ receptor antagonist S33138 attenuated cocaine-enhanced brain stimulation reward and priming-induced relapse (Peng et al., 2009), and the dopamine D₃ receptor antagonist SR 21502 reduced cocaine-conditioned place preference (Hachimine et al., 2014), cue-induced relapse, and cocaine self-administration (Galaj et al., 2014). The dopamine D₃ receptor antagonist PG01037 reduced cocaine-primed relapse in

This work was supported by the National Institutes of Health, National Institute on Drug Abuse [Contract N01DA-8-8880 to Avanza Laboratories, LLC]. T.H.H. received partial salary support for this study from the National Institutes of Health, National Institute on Drug Abuse through the Department of Veterans Affairs Cooperative Studies Program [Interagency Agreement Y01DA-4-0032].
dx.doi.org/10.1124/jpet.115.224121.

ABBREVIATIONS: ECG, electrocardiogram; GSK598809, 1-(2-fluoro-4-trifluoromethyl-phenyl)-3-{3-[4-methyl-5-(4-methyl-oxazol-5-yl)-4H-[1,2,4]triazol-3-ylsulfanyl]-propyl}-3-aza-bicyclo[3.1.0]hexane; FDA, Food and Drug Administration; NIDA, National Institute on Drug Abuse.

squirrel monkeys (Achat-Mendes et al., 2010), and the dopamine D₃ receptor antagonist YQA14 reduced cocaine-enhanced brain stimulation reward and attenuated cue-induced relapse (Song et al., 2014), as well as cocaine self-administration (Song et al., 2012) in rats. There is anatomic evidence for the dopamine D₃ receptor as a target also. Dopamine D₃ receptors are concentrated in projection regions of the mesolimbic dopamine system and islands of Calleja (Sokoloff et al., 1990; Levant et al., 1993). In addition, dopamine D₃ receptors have been reported to be upregulated in postmortem brains of cocaine-overdose victims (Staley and Mash, 1996; Mash, 1997) and with positron emission tomographic imaging in cocaine-dependent subjects (Payer et al., 2014). Thus, convergent lines of evidence indicate that blockade of the dopamine D₃ receptor may prevent abuse-related effects of cocaine.

These and other experimental findings have resulted in a proliferation of reviews suggesting that the dopamine D₃ receptor is a promising target for a cocaine abuse medication (Le Foll et al., 2000, 2005, 2007; Heidbreder et al., 2005; Micheli and Heidbreder, 2006; Heidbreder and Newman, 2010; Heidbreder 2013). The abundance of these reviews stoked enthusiasm for evaluating a dopamine D₃ receptor antagonist in clinical studies.

As a result of the interest in this target for a cocaine abuse therapeutic, the National Institute on Drug Abuse (NIDA) evaluated GSK598809 [1-(2-fluoro-4-trifluoromethyl-phenyl)-3-{3-[4-methyl-5-(4-methyl-oxazol-5-yl)-4H-[1,2,4]triazol-3-ylsulfanyl]-propyl}-3-aza-bicyclo[3.1.0]hexane] as a potential medication to treat cocaine use disorders. GSK598809 is a selective dopamine D₃ receptor antagonist (Micheli et al., 2010; Searle et al., 2010) that has been tested in clinical trials for other indications (see <http://clinicaltrials.gov/ct2/results?term=598809&Search=Search>). One of these clinical studies, which focused on craving in smokers, reported that a single dose of GSK598809 that produced 72% to 89% dopamine D₃ receptor occupancy could transiently alleviate craving after overnight abstinence (Mugnaini et al., 2013). Hence, there are data on GSK598809 safety and kinetics in animals and in humans, and human plasma levels at potential intent-to-treat dosages.

The US Food and Drug Administration (FDA) Guidance for Industry S7A (FDA, 2001) recommends conducting safety pharmacology studies for undesirable pharmacodynamic effects of a substance on physiologic functions related to therapeutic exposure. The FDA previously required NIDA to test the safety of potential cocaine abuse medications before initiating clinical trials. Thus, before conducting a clinical trial with GSK598809 for cocaine abuse in human subjects, we performed a preclinical cardiovascular safety interaction study with GSK598809 and cocaine in dogs to help predict whether the two compounds would be safe if taken together. We report here the results of this study, and discuss the feasibility of using GSK598809 or other dopamine D₃ receptor antagonists as medications to treat cocaine use disorders.

Materials and Methods

The study was reviewed by an Institutional Animal Care and Use Committee for compliance with provisions of the US Department of Agriculture Animal Welfare Act, US Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the US Interagency Research Animal Committee Principles for the Utilization

and Care of Research Animals before execution. It was conducted in compliance with current FDA Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies (21 CFR Part 58) in a research facility accredited by Association for Assessment and Accreditation of Laboratory Animal Care International.

Drugs. GSK598809 was generously provided as a tartrate salt by GlaxoSmithKline (Research Triangle Park, NC). It was formulated in a vehicle comprising 0.5% w/v hydroxypropylmethylcellulose (Sigma-Aldrich, St. Louis, MO) and 0.1% v/v Tween 80 (Fisher Scientific, Fairlawn, NJ) buffered to pH 5 with 25 mM citrate in sterile water for injection, USP (Baxter Healthcare, Marion, NC). Cocaine hydrochloride (Mallinckrodt, St. Louis, MO) was acquired through the NIDA Drug Supply Program. It was formulated in 0.9% sodium chloride USP vehicle (Baxter Healthcare, Jayuya, Puerto Rico). All dosing formulations were analyzed for homogeneity and to verify identity and concentration.

Animals and Surgery. Male beagle dogs were obtained from Marshall BioSciences (North Rose, NY). They weighed (mean $\pm$ S.D.) 9.4 ± 0.26 kg at the beginning of the study and 10.9 ± 0.50 kg at the end of the study. Each dog was surgically implanted in its left flank with a sterile telemetry transmitter unit (model TL11M2-D70-PCT; Data Sciences International, St. Paul, MN) and in its right lateral thoracic region with a titanium vascular access port (model CP4; Access Technologies, Skokie, IL). The telemetry unit measures body temperature via a sensor in the telemetry unit body itself; systolic, diastolic, and mean arterial blood pressures via a femoral artery catheter; and heart rate and electrocardiogram (ECG; modified lead II) via an electrode in the left lateral thoracic region and wirelessly transmits the data to a receiver connected to a computer that records the telemetry. The dogs were unrestrained and freely moving. The vascular access port was connected to a catheter in the right jugular vein. After recovery from surgery, the dogs were acclimated to wearing a jacket and backpack system (model DJ04H; Lormir Biomedical, Malone, NY) that would be worn on study days. The backpack carried a programmable peristaltic infusion pump (model Pegasus Light; Pegasus GmbH, Kiel, Germany) to administer either cocaine or its vehicle.

Experimental Design. The experiment was conducted in phases. In the first (hemodynamics) phase, the dogs were dosed at weekly intervals over a period of 9 weeks with combinations of either GSK598809 or vehicle, followed by either cocaine or vehicle. GSK598809 (or vehicle) was administered by oral gavage, and cocaine (or vehicle) was administered intravenously by an infusion pump connected to the vascular access port. The interval between the GSK598809 and cocaine doses was 45 minutes, corresponding to the T_{max} of formulated oral GSK598809 in dogs (GlaxoSmithKline, unpublished data). The dosing day study design is summarized in Fig. 1. In brief, on study days, animals were jacketed and connected to their infusion pumps. The technical staff left the animal room, and then baseline body temperature, blood pressure, and ECG measurements were recorded for at least an hour. After the baseline measurements were recorded, technical staff briefly returned to the animal room to administer GSK598809 (or vehicle) to all the dogs via oral gavage and activate the timers on the preloaded and preprogrammed infusion pumps. The infusion pumps were programmed to start 45 minutes after GSK598809 (or vehicle) and deliver the cocaine (or vehicle) over 30 seconds, followed by saline for 1.5 minutes to ensure completeness of dosing. Parameters were recorded continuously for at least 6 hours. A technician reentered the animal room at 60, 120, and 360 minutes after the cocaine infusion to conduct postdose observations of potential physical and behavioral effects, and those events were noted on the recordings. Each infusion pump created an electronic event log. After recording sessions, pump event logs were downloaded and reviewed to confirm that each pump did, in fact, operate; that each pump started at the appropriate time; and that each pump turned off at the appropriate time. In addition, the infusion lines were examined to confirm that drug was delivered.

Fig. 1. Study design. Male beagle dogs were dosed with combinations of oral GSK598809 or its vehicle (first arrow) and then with intravenous doses of cocaine or its vehicle (second arrow) 45 minutes later (the T_{max} of oral GSK598809) over a period of 9 weeks. The interval between successive study dates was at least 6 days to ensure completeness of drug washout.

Blood pressure parameters, ECG, and body temperature measurements were recorded for at least 6 hours to establish the baseline one day before the dosing day during the study phase. On study days, telemetry recording began at least 60 minutes before the GSK598809 (or vehicle) dose. These measurements were not recorded while staff was in the room to administer GSK598809. Recording resumed after the GSK598809 dose and continued at least 5 hours after the last animal was dosed with cocaine or vehicle, except during the 2 minutes when the infusion pumps that delivered cocaine or vehicle were running. ECG recordings were sampled the day before each dosing and on study days at 45 ± 5 minutes before GSK598809 or its vehicle was administered, at 10 ± 5 minutes before cocaine or its vehicle was administered, at the time of maximum hemodynamic effect (based on mean arterial blood pressure) within the 5-minute interval after cocaine or its vehicle were infused, and at 30 ± 5 minutes, 60 ± 5 minutes, and at 1-hour intervals (± 5 minutes) thereafter for 6 more hours.

In the second (pharmacokinetic) phase, the dogs were dosed a week apart at each dose level of GSK598809. Blood for bioanalysis was collected via jugular venipuncture before and at 0.25 hour ± 1 minute, 0.50 hour ± 5 minutes, 1.0 hour ± 5 minutes, 2.0 hours ± 5 minutes, 4.0 hours ± 10 minutes, 6.0 hours ± 10 minutes, 12.0 hours ± 15 minutes, and 24.0 hours ± 15 minutes after dosing. GSK598809 levels were assayed in 0.05-ml aliquots of plasma using a validated liquid chromatography/tandem mass spectrometry method in a Good Laboratory Practice-compliant facility. The lower limit of quantitation was 5 ng/ml.

Dose Selection. The GSK598809 dose levels were selected to reach estimated human plasma levels of the intent-to-treat dose of 60 mg daily for a substance use indication. Modeling from single-dose studies suggested this dose would result in a steady state median C_{max} of 664 ng/ml and trough of 125 ng/ml (GlaxoSmithKline, unpublished data). A 3 mg/kg oral dose would yield exposure in dogs similar to human exposure at 60 mg/day. To provide a safety margin in the event of increased exposure in patients attributable to individual differences in metabolism or excess drug taking, 9 mg/kg was tested also. GSK598809 was administered by oral gavage because oral administration is the expected route for human therapeutic drugs. The cocaine dose levels for the present study, 0.56 and 1.7 mg/kg, were informed by results from previous unpublished studies examining the effects of intravenous cocaine on hemodynamics in unrestrained, freely moving telemetered male beagle dogs. In those studies, a 0.56 mg/kg cocaine dose yielded relatively small peak changes in mean arterial blood pressure from baseline, and a 1.7 mg/kg dose yielded profound peak increases in mean arterial blood pressure. A 3 mg/kg dose yielded only slightly higher increases in mean arterial blood pressure, but the animals dosed with 3 mg/kg cocaine manifested central nervous system stimulation and petechial hemorrhages in internal organs. Thus 0.56 mg/kg would provide a sensitive range to detect potential hemodynamic changes, and a 1.7 mg/kg dose would avoid ceiling effects but still allow sufficient sensitivity to detect hemodynamic changes at high cocaine plasma levels.

Data Analysis. Measures of hemodynamics maximum effects were regressed on dosages of each drug (GSK598809 and cocaine) and their interaction, all as fixed coefficients, plus random coefficients (intercepts) for each dog to account for random variation from dog to dog. Although carryover across repeated measurements on each dog was unlikely due to the washout periods (Fig. 1), a first-order residual autocorrelation structure was included to mitigate bias from any

unforeseen carryover effects. Model fit was verified by examining Studentized residuals. Four planned (a priori) means comparisons were conducted to probe the safety concern for which this study was designed, which was the potentiation of cocaine effects on peak mean arterial blood pressure by GSK598809 dosages. Because each of these four means comparisons is of interest in itself and planned a priori, attained significance levels (P values) are reported without adjustment for multiple comparisons (Sokal and Rohlf, 1981). The remaining means comparisons are exploratory in nature so their P values were adjusted per the Tukey-Kramer method (Sokal and Rohlf, 1981) and are denoted by " P (adjusted)" in *Results*. In addition, peak mean arterial blood pressure during the period before cocaine was administered was regressed on GSK598809 dosage, session, and their interaction, all as fixed coefficients, plus random coefficients (intercepts) for each dog and a first-order residual autocorrelation structure. Throughout, means comparisons were based on estimated least-squares means (Milliken and Johnson, 1992) from the fitted regression models due to the presence of a missing value in the analysis of peak mean arterial blood pressure during the period of cocaine administration. All regression analyses were performed in SAS v.9.3 (SAS Institute Inc., Cary, NC).

ECG recordings of 10 to 30 seconds duration were collected at 45 ± 5 minutes before test article or vehicle dosing, 10 ± 5 minutes before interaction article or vehicle dosing, at the time of maximum pressor effect (mean arterial pressure) in the 5-minute interval after interaction article infusion, and at 30 ± 5 minutes, 60 ± 5 minutes and at 1-hour intervals (± 5 minutes) thereafter for 6 additional hours and evaluated by a veterinary cardiologist. The cardiologist analyzed five complexes per animal per time point. These values were averaged to give individual animal averages at each time point. This qualitative analysis included an evaluation of any abnormalities in rhythm and conduction, waveform morphology (for P, QRS, and T waves), or apparent functional changes. The Van de Water correction was applied to the QT interval to calculate QTc (Van de Water et al., 1989).

Results

Time-course effects of oral GSK598809 and intravenous cocaine on mean arterial blood pressure are depicted in Fig. 2. The peak pressor effect of cocaine occurred within 5 minutes after the end of its infusion. Thus, we analyzed peak mean arterial blood pressure within that time interval.

Cocaine and GSK598809 each increased peak mean arterial blood pressure [overall $F_{(2,39)} = 31.02$; $P < 0.0001$ and overall $F_{(2,39)} = 5.49$; $P = 0.008$, respectively]. Least square mean differences were significant for both doses of cocaine compared with its vehicle [$t_{(39)} = 4.78$; P (adjusted) < 0.0001 and $t_{(39)} = 7.80$; P (adjusted) < 0.0001 , respectively]. Likewise, least square mean differences were significant for both doses of GSK598809 as compared with its vehicle [$t_{(39)} = 2.52$; P (adjusted) < 0.04 and $t_{(39)} = 3.15$; P (adjusted) < 0.009 , respectively]. The experimental question of interest, that is, whether GSK598809 significantly increased cocaine effects on peak mean arterial blood pressure, was probed by a priori t tests on the means of the fitted regression model. The dose of 3 mg/kg GSK598809 significantly increased the pressor effects

Fig. 2. Time courses of mean arterial blood pressure in unrestrained dogs treated with combinations of oral GSK598809 and intravenous cocaine. Data were collected in 30-second bins via telemetry. Two sequential 30-second bins were averaged to produce 1-minute values that were defined as the beginning of the first 30-second bin. Male beagle dogs were dosed with either vehicle or GSK598809 by oral gavage (time -47 minutes), and then the technicians left the room. Forty-five minutes later, infusion pumps automatically dosed the animals with either intravenous vehicle or cocaine. Pumps started at time point -2 minutes and shut off at time point 0 on the graph. Results are depicted at 1-minute intervals for the 30-minute period beginning after the infusion pumps shut off (0 on abscissa) and at 5-minute intervals otherwise. One person entered the room at times 60 and 120 minutes to conduct postdose observations. (A) Mean arterial blood pressure time courses after GSK598809 vehicle and cocaine ($n = 6$ in all treatment groups except the 0.56 mg/kg cocaine treatment group, where $n = 5$ as the result of a telemetry malfunction). (B) Mean arterial blood pressure time courses after 3 mg/kg GSK598809 and cocaine ($n = 6$ in all treatment groups). (C) Mean arterial blood pressure time courses after 9 mg/kg GSK598809 and cocaine ($n = 6$ in all treatment groups).

Fig. 3. Effects of oral GSK598809 and intravenous cocaine on peak mean arterial blood pressure in dogs. Six male beagle dogs were dosed with GSK598809 vehicle (white bars), 3 mg/kg GSK598809 (gray bars) or 9 mg/kg GSK598809 (black bars), followed by saline, 0.56 mg/kg cocaine, or 1.7 mg/kg cocaine 45 minutes later. Data represent averaged peak mean arterial blood pressures ($\pm$ S.E.M.) during the 5-minute interval immediately after the end of the cocaine infusion. Dosing sessions were separated by at least 6 days. * $P < 0.05$ compared with cocaine alone.

of 5.6 mg/kg cocaine and of 1.7 mg/kg cocaine [$t_{(39)} = 2.26$; $P < 0.03$ and $t_{39} = 2.46$; $P < 0.02$, respectively]; and, although the 9 mg/kg dose of GSK598809 is estimated to increase the pressor effect of 1.7 mg/kg cocaine, this result did not achieve statistical significance [$P > 0.07$] (Fig. 3). The data were also analyzed to determine whether GSK598809 affected blood pressure in the period of time before cocaine infusion and whether responses to GSK598809 were stable over repeated sessions (Table 1). This analysis revealed significant effects of GSK598809 dose level [$F_{(2,40)} = 43.11$; $P < 0.001$], session [$F_{(2,40)} = 3.84$; $P = 0.03$], and an interaction of session and dose level [$F_{(4,40)} = 2.76$; $P = 0.04$]. Analysis by session indicated that mean arterial blood pressure responses to 9 mg/kg GSK598809 were greater than vehicle and greater than 3 mg/kg GSK598809 consistently across all three sessions: session 1 [$t_{(40)} = 5.64$; P (adjusted) < 0.0001 and $t_{(40)} = 7.05$; P (adjusted) < 0.0001 , respectively], session 2 [$t_{(40)} = 7.55$; P (adjusted) < 0.0001 and $t_{(40)} = 5.98$; P (adjusted) < 0.0001 , respectively], and session 3 [$t_{(40)} = 5.74$; P (adjusted) < 0.0001 and $t_{(40)} = 4.63$; P (adjusted) < 0.0001 , respectively]. The

interaction effect arises because the effect of session is limited to a difference in means between sessions 2 and 3 and only at 9 mg/kg GSK598809 [$t_{(40)} = 2.81$; P (adjusted) < 0.03].

Time course effects of oral GSK598809 and intravenous cocaine on heart rate are depicted in Fig. 4. Heart rate increased in a dose-related manner after cocaine in dogs treated with 3 mg/kg GSK598809 that paralleled the pressor effect. In contrast, heart rate decreased after cocaine in animals treated with the 9 mg/kg dose of GSK598809. The data were analyzed to determine whether GSK598809 also affected heart rate in the period of time before cocaine infusion and whether responses to GSK598809 were stable over repeated sessions (Table 1). GSK598809 affected heart rate on its own [$F_{(2,40)} = 48.60$; $P < 0.001$], and there was an interaction of session and dose level [$F_{(4,40)} = 3.40$; $P = 0.02$]. Analysis by session indicated that heart rate responses to 9 mg/kg GSK598809 were greater than vehicle and greater than 3 mg/kg GSK598809 consistently across all three sessions: session 1 [$t_{40} = 6.48$; P (adjusted) < 0.0001 and $t_{(40)} = 5.46$; P (adjusted) < 0.0001 , respectively], session 2 [$t_{(40)} = 8.84$; P (adjusted) < 0.0001 and $t_{(40)} = 7.23$; P (adjusted) < 0.0001 , respectively], and session 3 [$t_{(40)} = 5.77$; P (adjusted) < 0.0001 and $t_{(40)} = 4.08$; P (adjusted) < 0.0001 , respectively]. As with blood pressure, an interaction of session and dose level [$F_{(4,40)} = 3.40$; $P = 0.02$] was noted. The interaction effect arises because the effect of session is limited to a difference in means between sessions 2 and 3 and only at 9 mg/kg GSK598809 [$t_{(40)} = 3.29$; P (adjusted) < 0.002].

All the ECGs evaluated in this study were considered normal. There were no effects on PR, QRS, and QTc intervals at the sampled time points. Rare and intermittent cases of sinus arrhythmia and atrial premature complexes were noted over the 9-week course of the study, but they were also seen in baseline recordings and with vehicle treatments and therefore could not be attributed to drug effects.

There was no mortality in this study. Moreover, there were no noteworthy clinical observations of physical or behavioral effects (stimulation, convulsions, stereotyped or bizarre behaviors) after either dose of GSK598809 or cocaine or when the drugs were administered in combination when the animals were observed 60, 120, and 360 minutes after the cocaine infusion.

GSK598809 was rapidly absorbed after oral administration. Peak GSK598809 plasma concentrations occurred between 15 and 60 minutes after dosing, which encompassed the time

TABLE 1

Effects of oral GSK598809 alone on mean arterial blood pressure and on heart rate in male beagle dogs. Animals ($n = 6$) received GSK598809 vehicle in 3 sessions (weeks 1, 2, and 3), 3 mg/kg GSK598809 in three sessions (weeks 4, 5, and 6) and 9 mg/kg GSK598809 in three sessions (weeks 7, 8, and 9). Data represent average mean arterial blood pressure and average heart rate in the sessions from 30 to 40 minutes after the GSK598809 dose (its C_{max}), that is, before any subsequent cocaine treatment.

Parameter	GSK598809 Dosage (mg/kg)	Treatment Session			
		1	2	3	Grand Mean
Mean arterial blood pressure (mmHg $\pm$ S.E.M.)	0	103 $\pm$ 6	94 $\pm$ 3	93 $\pm$ 4	96 $\pm$ 3
	3	97 $\pm$ 2	104 $\pm$ 3	100 $\pm$ 5	100 $\pm$ 2
	9	133 $\pm$ 4	135 $\pm$ 4	124 $\pm$ 5*	131 $\pm$ 3
Heart rate (bpm $\pm$ S.E.M.)	0	95 $\pm$ 5	83 $\pm$ 7	90 $\pm$ 6	89 $\pm$ 4
	3	109 $\pm$ 7	104 $\pm$ 8	110 $\pm$ 6	108 $\pm$ 4
	9	167 $\pm$ 7	181 $\pm$ 7	154 $\pm$ 13*	167 $\pm$ 6

* $P < 0.05$ compared with session 2.

Fig. 4. Time courses of heart rate in unrestrained dogs treated with combinations of oral GSK598809 and intravenous cocaine. Data were collected in 30-second bins via telemetry. Two sequential 30-second bins were averaged to produce 1-minute values that were defined as the beginning of the first 30-second bin. Male beagle dogs were dosed with either vehicle or GSK598809 by oral gavage (time -47 minutes), and then the technicians left the room. Forty-five minutes later, infusion pumps automatically dosed the animals with either intravenous vehicle or cocaine. Pumps started at time point -2 minutes and shutoff at time point 0 on the graph. Results are depicted at 1-minute intervals for the 30-minute period beginning after the infusion pumps shut off (0 on abscissa) and at 5-minute intervals otherwise. One person entered the room at times 60 and 120 minutes to conduct postdose observations. (A) Heart rate time courses after GSK598809 vehicle and cocaine ($n = 6$ in all treatment groups except the 0.56 mg/kg cocaine treatment group, where $n = 5$ as the result of a telemetry malfunction). (B) Heart rate time courses after 3 mg/kg GSK598809 and cocaine ($n = 6$ in all treatment groups). (C) Heart rate time courses after 9 mg/kg GSK598809 and cocaine ($n = 6$ in all treatment groups).

cocaine was administered (45 minutes after GSK598809), and its half-life was approximately 6 hours. GSK598809 exposure was dose-proportional in the tested range. Inter-animal variability was low, with the exception of one dog treated with 3 mg/kg. Plasma levels of GSK598809 in that dog were much lower than the levels in the other dogs treated at the same dose level and were not detectable after 6 hours. In contrast, when that same dog was treated with the 9 mg/kg dose, its plasma levels were similar to the other dogs dosed at 9 mg/kg. The data are summarized in Table 2.

Discussion

The selective dopamine D₃ receptor antagonist GSK598809 potentiated the hemodynamic effects of cocaine in dogs. Thus, after i.v. cocaine, blood pressure was significantly higher in animals pretreated with GSK598809 compared with animals treated with cocaine alone. The cocaine doses increased blood pressure and were in a range sufficiently sensitive to reveal the potentiating effect of GSK598809. Plasma levels of GSK598809 in the animals were comparable to those observed in clinical trials using 75 and 175 mg GSK598809 to study its effects on cigarette craving (Mugnaini et al., 2013) and on interaction with alcohol (te Beek et al., 2012).

The pressor effect was most pronounced in animals treated with the combination of the low dose of GSK598809 and cocaine, perhaps because the high dose of GSK598809 had sufficiently increased blood pressure and heart rate before the cocaine dose such that the combined pressor effects of GSK598809 and cocaine stimulated a baroreceptor reflex that dampened the overall pressor effect. This hypothesis is supported by the heart rate data. Heart rate increased in a dose-dependent manner after cocaine administration in dogs that had received the low dose of GSK598809, an effect similar to that seen with blood pressure (see Figs. 2B and 4B). In contrast, heart rate, which was already significantly elevated by the high dose of GSK598809, *decreased* after cocaine in a dose-dependent manner (see Fig. 4C). In addition, rate pressure product was similarly elevated in dogs that received the high dose of GSK598809, regardless of cocaine treatment (data not shown). These data suggest that therapeutic doses of GSK598809 could potentiate the effects of cocaine on blood pressure in patients undergoing treatment for substance use disorders. Given the role of cardiovascular consequences in cocaine-induced lethality, exposing cocaine-abusing patients to GSK598809 might be harmful.

The chronic, relapsing nature of addiction presents unique challenges for ensuring the safety of a potential anti-addiction medication. The patient population is characterized by uncontrolled drug use, drug seeking, and relapse. Thus, in the case of a medication intended to reduce cocaine use, maintain abstinence, or prevent relapse, there is likelihood that a patient may relapse and resume use. This requires understanding how a potential treatment interacts with cocaine to determine whether exposure to the combination is a potential hazard and understanding any risks associated with concomitant exposure. Cocaine has well documented hypertensive effects in humans (Goldstein et al., 2009; Zimmerman, 2012), so that a drug that increases blood pressure may be a potential hazard when used in combination with cocaine. In addition, a patient may take more drug than prescribed because of a missed a dose, seeking further relief from craving, or to accelerate recovery. In this study, doses of GSK598809 that resulted in exposure levels comparable to those reported in clinical trials (te Beek et al., 2012; Mugnaini et al., 2013) potentiated the hypertensive effects of cocaine. In addition, GSK598809 increased mean arterial blood pressure and heart rate at exposure levels encompassed by intent to treat dosages.

Dopamine receptors are distributed throughout the body, and peripheral dopamine is involved in many aspects of mammalian homeostasis, including regulation of blood pressure. Thus, dopaminergic tone affects or modulates vascular beds, cardiac contractility, and diuresis (reviewed in Amenta et al., 2002). In the kidneys, dopamine is synthesized independently of renal nerves. It is not converted to norepinephrine and it plays a role in regulating fluid and electrolyte balance and systemic blood pressure (reviewed in Armando et al., 2011). Multiple dopamine receptor subtypes are expressed in the kidneys, and dopamine D₃ receptors are localized in both the proximal and distal convoluted tubules, the collecting ducts, and the macula densa of the nephron (reviewed in Zeng et al., 2004). Reports that renal dopamine D₃ receptors may play a significant role in the regulation of systemic blood pressure first appeared in the literature more than 15 years ago. Pharmacology and gene-deletion studies demonstrated that interfering with dopamine D₃ receptor function resulted in renin-dependent hypertension and changed responsiveness to amino acids (Asico et al., 1998; Luippold et al., 1998, 2006). Subsequent studies have linked dopamine D₃ receptors in the kidneys with angiotensin II receptor expression and endothelin B in renal proximal tubule

TABLE 2
Kinetics of oral GSK598809 in male beagle dogs
Data are mean $\pm$ S.E.M.

Dose (mg/kg)	C _{max} (ng/ml)	T _{max} (h)	AUC _{0-t} (h $\times$ ng/ml)	AUC _{0-inf}	DN_C _{max} (ng/ml)	DN_AUC _{0-inf} (h $\times$ ng/ml)	t _{1/2} (h)
3 (n = 5) ^a	1199 $\pm$ 173	0.7 $\pm$ 0.15	5980 823	6543 $\pm$ 1,010	399 $\pm$ 59	2181 $\pm$ 337	5.7 $\pm$ 0.91
9 (n = 6)	3,120 $\pm$ 289	0.6 $\pm$ 0.08	19,465 $\pm$ 2086	22,050 $\pm$ 2848	347 $\pm$ 32	2,450 $\pm$ 316	5.9 $\pm$ 0.47

AUC_{0-t}, area under plasma drug concentration versus time curve from time of dose administration (time zero) to the last time point with measureable drug concentration; AUC_{0-inf}, AUC from time of drug administration with extrapolation to infinity; DN_C_{max}, dose-normalized C_{max}; DN_AUC_{0-inf}, dose-normalized AUC_{0-inf}.

^aOne animal with levels much lower than the other dogs and not detectable after 6 hours was excluded. At 9 mg/kg, the same dog had plasma levels that were similar to those of the other dogs at the 9 mg/kg dose.

cells (Zeng et al., 2008; Yu et al., 2009). Recent evidence suggests dopamine D₃ receptor activation in the kidneys ultimately stimulates secretion of a sodium load (to reduce hypertension) by promoting degradation of the Na⁺-H⁺ exchanger in renal proximal tubules (Armando et al., 2014). The pressor effect of the dopamine D₃ receptor antagonist in this study and its augmentation of cocaine's pressor effects are consistent with this body of evidence.

In the context of our evolving understanding of the role of renal dopamine D₃ receptor activity in regulating blood pressure, the present findings with GSK598809 and cocaine indicate that this interaction might not be unique to the molecule itself but rather may represent a class effect of dopamine D₃ receptor antagonists when administered with cocaine. We intend to conduct systematic studies with structurally distinct dopamine D₃ antagonists to determine whether this is indeed a class effect as opposed to a molecule-specific effect of GSK598809.

Whereas the potential hypertensive effect of a dopamine D₃ receptor antagonist may preclude using this approach to treat stimulant use disorders, it may not preclude treating other substance use disorders such as nicotine (Mugnaini et al., 2013), alcohol (te Beek et al., 2012), and opiates (Ashby et al., 2003; Rice et al., 2012). Because of the chronic and relapsing nature of substance abuse, any therapy would likely be repetitive and prolonged. Johnson et al. (2013) recently reported that dopamine D₃ receptor knockout mice manifested age-related hypertension and interstitial cardiac fibrosis earlier than wild-type controls. This finding suggests that long-term treatment with a dopamine D₃ receptor antagonist drug may ultimately affect cardiac muscle itself, but the possibility that these findings are unique to dopamine D₃ receptor knockout mice cannot be excluded. This would, presumably, be revealed in chronic toxicology studies (1 year or longer) as recommended by the FDA. Such studies are typically not conducted until late in a drug development program, however.

A meta-analysis of one million adults suggested that lowering systolic blood pressure by as little as 2 mm Hg could conceivably reduce mortality from stroke by 10% and ischemic heart disease or other vascular causes by 7% (Lewington et al., 2002). By extension, a medication that has a slight hypertensive effect, alone or in combination with another substance, may present a potential risk for patients. A recent systematic review of epidemiologic studies suggests that cocaine use increases the risk of stroke (Sordo et al., 2014). Thus, a treatment that increases blood pressure in combination with cocaine might increase further the risk of stroke or ischemic heart disease. Such a risk would have to be weighed against the potential benefit of a new medicine for substance abuse.

Numerous reports in the literature describe the hemodynamic effects of cocaine in dogs, as well as the effects of dopamine D₃ receptor antagonists (and agonists) in dogs. To date, no studies on the effects of a dopamine D₃ receptor antagonist and cocaine in dogs have appeared. Dogs are a mainstay of cardiovascular safety evaluation of drugs (Pugsley et al., 2008; Ewart et al., 2013). Although it is uncertain whether the interaction between a dopamine D₃ receptor antagonist and cocaine we are reporting in dogs is predictive of a similar effect in humans, this finding would likely preclude further development of such a compound as a treatment for cocaine use disorder.

Acknowledgments

The authors thank GlaxoSmithKline, Research Triangle Park, NC, for generously providing GSK598809, and Drs. Jay A. Graham and Keith T. Muir for helpful discussions and sharing formulation advice, unpublished kinetics data, and dosing solution and bioanalytical assay methods. The authors also thank members of the NIDA Division of Pharmacotherapies and Medical Consequences of Drug Abuse Protocol Review Team, Drs. Naresh Chand, Nora Chiang, Rik Kline, David J. McCann, and Mr. Robert Walsh for helpful discussions regarding study design and dose selection, and Dr. Phil Skolnick for reviewing the manuscript. The authors thank Dr. C. Steven Godin and Ms. Grace Gesswein of Smithers Avanza for diligent work and professionalism in conducting the studies, compiling and managing the massive amount of telemetry data, and invaluable discussions.

Authorship Contributions

Participated in research design: Appel, Aciri.

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Wrote or contributed to the writing of the manuscript: Appel, Aciri, Holmes.

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