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Locomotor activating effects of cocaine and scopolamine combinations in rats: isobolographic analysis

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Abstract

Muscarinic cholinergic receptors are receiving renewed interest as viable targets for treating various psychiatric disorders. Dopaminergic and muscarinic systems interact in complex ways. The goal of this study was to quantify the interaction of a systemically administered psychomotor stimulant and muscarinic antagonist at the behavioral level. Using isobolographic analysis of locomotor activity data, we assessed the effects of three cocaine/scopolamine mixtures in terms of deviation from simple dose addition (additivity), at four effect levels. All three mixtures produced some more-than-additive (synergistic) effects, as lower doses were needed to produce given effects relative to the calculated effect of additive doses. A mixture with comparable contributions from cocaine and scopolamine produced significantly more-than-additive effects at all but the lowest effect level examined. A mostly-cocaine mixture was more-than-additive at low effect levels only, while a mostly-scopolamine mixture produced effects more consistent with additivity, with only the highest effect level barely reaching significant synergism. Our study confirms and quantifies previous findings that suggested synergistic effects of stimulants and muscarinic antagonists. The synergism implies that cocaine and scopolamine stimulate locomotor activity through non-identical pathways, and was most pronounced for a mixture containing cocaine and scopolamine in comparable proportions.

Keywords

Muscarinic receptor; anticholinergic; psychomotor stimulant; rat; drug interactions; synergism; curved isoboles

INTRODUCTION

There has recently been renewed interest in the potential of muscarinic cholinergic ligands in the treatment of central nervous system disorders including Parkinson's disease, Alzheimer's disease and other cognitive disorders, schizophrenia, and drug addiction (Conn et al., 2009; Dencker et al., 2012). Many of those are typically thought of as primarily involving dopamine systems, or are currently treated using dopamine-based approaches

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(e.g., L-DOPA, antipsychotics). The muscarinic and dopaminergic systems are intimately connected. Though they were long regarded as balancing or opposing each other, more recent studies show the relationship to be more complex (Surmeier and Graybiel, 2012). Muscarinic systems modulate dopamine release both positively and negatively, depending on the brain region. For instance, mesolimbic dopamine pathways receive stimulatory muscarinic input at the level of the ventral tegmental area (Gronier and Rasmussen, 1998; Forster and Blaha, 2000). Even within a neuron type, muscarinic input can stimulate or inhibit dopaminergic activity, depending on factors such as stimulus frequency (Threlfell et al., 2010, 2012). The investigation of dopamine/muscarinic interactions thus remains relevant to drug development and our understanding of brain functions.

Cocaine and muscarinic antagonists can produce similar effects on behavioral or neurochemical endpoints such as increasing locomotor activity (Rosenzweig-Lipson et al., 1997; Katz et al., 1999; Sipos et al., 1999; Itzhak and Martin, 2000), disrupting prepulse inhibition of the startle response (Sipos et al., 2001; Jones and Shannon, 2000), and increasing extracellular striatal dopamine levels (Dewey et al., 1993; Chapman et al., 1997). A number of studies have also reported increased effects when psychostimulants and muscarinic antagonists were combined. For example, the non subtype-selective muscarinic antagonist scopolamine and the M₁/M₄-preferring antagonist trihexyphenidyl both induced hyperlocomotion, and both increased cocaine- or methamphetamine-induced hyperlocomotion in mice (Katz et al., 1999; Itzhak and Martin, 2000; Shimosato et al., 2001). Similarly, scopolamine, atropine, trihexyphenidyl, and the M₁-preferring antagonist telenzepine produced leftward shifts in the discriminative stimulus effects of cocaine in rats or mice (Acri et al., 1996; Katz et al., 1999; Tanda and Katz, 2007; Thomsen et al., 2010). Telenzepine and trihexyphenidyl also enhanced cocaine-induced increases in extracellular dopamine in the nucleus accumbens, suggesting a role of striatal dopamine modulation in these effects (Tanda et al., 2007). Our drug discrimination data, using isobolographic analysis of the addition of a fixed dose of scopolamine to various doses of cocaine, indicated a more-than-additive (synergistic) effect, at least at some effect levels (Thomsen et al., 2010).

Despite the number of reports on enhancement of psychomotor stimulant drug effects by a muscarinic antagonist, with the exception of our previous cocaine discrimination report, to our knowledge the interaction has not been quantified in terms of additivity, synergism, or less-than additive effects, beyond. The purpose of this investigation was to provide a systematic, quantitative evaluation of the interaction between cocaine and scopolamine, and how the interaction may vary between different mixture ratios and effect levels. We used locomotor activity as a relatively high throughput behavioral assay to measure the effects of systemically administered cocaine, scopolamine, and three cocaine/scopolamine mixtures in rats, in order to scrutinize deviation from dose additivity by means of isobolographic analysis. This analysis is based on determining equivalent doses of each drug alone, calculated as the dose needed to produce a given effect. An isobologram is a graphical representation of pairs of drug doses expected to produce a given effect if additivity is assumed, to which the actual (experimentally determined) drug mixture values can be compared.

METHODS

Subjects

A separate cohort of 16 male Sprague Dawley rats (Charles River, Wilmington, MA) was used for each drug/mixture, 80 rats in total. Animals were group-housed 4 per cage in a climate-controlled facility, illuminated 07:00–19:00h. Behavioral testing started at approximately 8–10 weeks of age and occurred between 08:00 and 16:00h. Prior to testing, the rats were allowed a week of acclimation during which they were habituated to handling by the experimenter. Food (rodent diet 5001, PMI Feeds, Inc. St. Louis, MO) and water were freely available, and variously flavored treats were given weekly for enrichment. Vivarium conditions were maintained in accordance with the guidelines provided by the National Institutes of Health Committee on Laboratory Animal Resources. Experimental protocols were approved by the McLean Hospital Institutional Animal Care and Use Committee.

Locomotor Activity Testing

Locomotor activity was assessed in clear plastic chambers $41.5 \times 19 \times 28$ cm ($l \times w \times h$) lightly lined with pine shavings and fitted with filter tops, placed within Photobeam Activity System monitoring frames (San Diego Instruments, San Diego, SA). Infrared beams 3 cm above the cage floor were used for assessment of horizontal activity, and additional beams measured vertical activity at 14 cm above the cage floor. The test sessions were 4 h long and data were collected in 10-min bins. Rats were first habituated to the test chamber and procedure during three daily saline sessions. Then, on each test day, the animals were allowed to acclimate to the test chambers for 1 h; then removed for injection with vehicle or drug, and immediately returned to the test chamber for a further 3 h. For brevity and ease of presentation, total beam breaks (horizontal and vertical) were collapsed across time, and total activity counts for the 3 h post-injection were used for isobolographic analysis.

Drugs

Cocaine hydrochloride was supplied by NIDA/NIH, scopolamine hydrobromide was purchased from Sigma/RBI (St. Louis, MO). Both were dissolved in 0.9% saline. The salt form of each drug was used in dose calculations. Injections were given i.p., in a volume of 1 ml/kg. In addition to cocaine alone (1.8, 5.6, 10, 18, 32 mg/kg) and scopolamine alone (0.03, 0.1, 0.32, 1.0, 3.2, 5.6, 10 mg/kg), we tested three fixed ratio mixtures, selected to cover a fairly broad range: 10:1, 1:2, and 1:10 cocaine:scopolamine. These ratios were based on equivalent doses of cocaine and scopolamine, at the effect level of 1000 beam breaks above saline. In other words, cocaine would be expected to contribute mostly in the 10:1 mixture, and scopolamine mostly in the 1:10 mixture. Each mixture was treated as a new drug and tested at five doses (i.e., doses were dilutions of the same ratio within each mixture), with the exception that one lower dilution was added to the 1:10 mixture at the end of testing, to provide a fuller dose-effect curve because effects of the low dose were larger than expected. Doses of cocaine+scopolamine were: 10:1 mixture, 2.0+0.03, 3.6+0.06, 6.3+0.10, 11.2+0.18, 20.2+0.32 mg/kg; 1:2 mixture, 0.63+0.20, 3.6+1.1, 6.3+2.0, 11.2+3.6, 20.1+6.4 mg/kg; and 1:10 mixture, 0.06+0.10, 0.20+0.32, 0.63+1.0, 1.12+1.8, 2.0+3.2, 3.6+5.7 mg/kg. For each drug or mixture, a within-subjects design was employed, with drug assignment

following a Latin-square design (including saline), except 10 mg/kg scopolamine, which was tested last. Sessions were separated by at least 2 days with no testing or drug administration.

Statistical Analysis

The effect of each drug on locomotor activity over 3 h was assessed by repeated-measures one-way ANOVA. Significant main effects of dose were followed by Dunnett's multiple comparisons test comparison of drug doses vs. saline. The significance level was set at $p < 0.05$. Isobolographic analysis used the same methods as in our drug discrimination investigation (Thomsen et al., 2010). The dose-effect curves obtained for each drug were fitted using the GraphPad Prism software to an equation of the form $E = (E_{\max} \cdot A^p) / (A^p + A_{50}^p)$, where E denotes the effect, A the dose, $E_{\max}$ the maximal effect achieved, A_{50} the dose estimated to elicit 50% of this maximal effect, and p ("Hill coefficient") is a factor related to the slope of the curve (Grabovsky and Tallarida, 2004). Individual data, rather than averages, were used for curve-fitting, using the ascending portion of the dose-effect curves obtained for each drug (i.e., all doses of cocaine, and scopolamine doses excluding 10 mg/kg). Confidence intervals were not artificially narrowed by eliminating outliers, constraining curve fitting parameters, or any other means. The lower and upper 95% confidence limits (95%CL) were also obtained from the curve fitting, and these numbers were then used to fit a curve equation for upper and lower 95%CL for cocaine and scopolamine. We examined departure from additivity at four effect levels on the ascending portion of the dose-effect functions of both drugs: 500, 1000, 1500 and 2000 beam breaks above saline levels.

RESULTS

Figure 1 shows dose-effect curves for total beam breaks (Fig.1 A–B), and time courses (C–D), for cocaine and scopolamine. Both drugs dose-dependently increased locomotor activity; ANOVA confirmed a significant effect of dose for cocaine [$F(5,75)=27.6$, $p < 0.001$] and scopolamine [$F(7,105)=13.0$, $p < 0.001$]. Cocaine produced a significant increase in locomotor activity relative to saline at doses of 18 and 32 mg/kg ($p < 0.01$), while scopolamine doses of 3.2 mg/kg and above reached significance ($p < 0.01$). A full characterization of the dose-effect relationship up to a peak was only obtained for scopolamine. For cocaine, higher doses were not tested because previous studies from our and other laboratories have shown that doses above the range used here cause convulsions in rats (Snyder-Keller and Keller, 1995).

The time courses of cocaine- and scopolamine-induced hyperlocomotion are shown in Fig.1 C–D, including the 1-h habituation and 3-h post-injection period. Two-way ANOVA on time (10-min bins) and dose was conducted on the habituation and post-injection data. In the habituation phase, both groups of rats showed a sharp decline in locomotor activity, supported by significant effects of time (cocaine group [$F(5,450)=233$, $p < 0.001$], scopolamine group [$F(5,600)=495$, $p < 0.001$]), with no influence of the dose factor. Following drug or saline administration, analysis confirmed highly significant effects of time and dose, and significant interactions, for both drugs (all $p < 0.001$). Cocaine effects

peaked 20–40min after injection, while scopolamine effects typically peaked in the first 10-min bin. As with the analysis of total beam breaks, 18 and 32 mg/kg cocaine produced significant increases post hoc relative to saline, 18 mg/kg starting after 10 min and lasting 80 min, and 32 mg/kg starting immediately after injection and lasting 120 min. Scopolamine at 1 mg/kg significantly increased locomotor activity only 10 min after injection, while 3.2, 5.6 and 10 mg/kg reached significance immediately after injection and lasting 100–120 min.

The experimentally determined data on the drug mixtures are shown in Figure 2, as beam breaks above saline. All three cocaine/scopolamine mixtures produced dose-dependent increases in total beam breaks, as confirmed by significant effects of dose in each mixture (1:10, $[F(6,90) = 16.6, p < 0.001]$; 1:2, $[F(5,75) = 29.2, p < 0.001]$; 10:1, $[F(5,75) = 26.8, p < 0.001]$). Points statistically significant relative to saline are shown as filled symbols. The mixtures produced time courses with onset intermediate between cocaine and scopolamine, peaking 10–20 min after injection, and lasting up to 150 min (data not shown). The mixtures, especially the more scopolamine-rich ratios, did not produce dose-effect curves resembling a classic sigmoidal shape, and non-linear regression using Equation 1 did not produce a good fit. Therefore, the dose of cocaine and the dose of scopolamine in each mixture estimated to produce the effect levels of 500, 1000, 1500 and 2000 beam breaks above saline were calculated by interpolation in each rat, then group means and 95%CL were calculated from log-transformed data and transformed back to linear doses. Figure 2 also shows these calculated doses.

These doses were then graphed in the isobologram for the respective effect level (Figure 3). Note that where the calculated doses deviated from the experimentally determined dose-effect curve, the dose was overestimated (see Fig. 2, 1:2 mixture), which would lead to an underestimate of the deviation from additivity in the isobologram (type 2 error). Despite this, the 1:2 mixture doses fell below the additive line with non-overlapping 95%CL at effect levels of 1000, 1500, and 2000 beam breaks. The 10:1 mixture fell within the shaded 95%CL area for additivity, although at the 2000 beam break level, the cocaine dose and the scopolamine dose 95%CL were each non-overlapping (scopolamine dose 95%CL bars for the mixture fall within the symbol in Fig. 3). Thus, the 10:1 mixture may be considered to have produced a more-than-additive effect, at the 2000 beam break effect level only. Conversely, the 1:10 mixture fell outside the 95%CL area for additivity only at the lower effect levels, 500 and 1000 beam breaks.

DISCUSSION

Using isobolographic analysis of locomotor activity data, we assessed the effects of three cocaine/scopolamine mixtures in terms of deviation from simple dose additivity. This extends our previous report (Thomsen et al. 2010) from a cocaine discrimination assay to locomotor activity, from mice to rats, and, more importantly, from the examination of adding a single low dose of scopolamine to the evaluation of dose-effect functions of three fixed-ratio drug mixtures at various effect levels. Effect levels up to the peak achieved by the lowest effect drug alone, scopolamine, were examined (Grabovsky and Tallarida, 2004). Consistent with the drug discrimination study, we found that the doses of cocaine and scopolamine needed to produce a given effect were generally lower than those expected

based on additivity, and that this deviation from additivity was significant in some of the conditions. Specifically, a mixture with comparable contributions from cocaine and scopolamine produced more-than-additive (synergistic) effects at all but the lowest effect level examined. The addition of a small amount of cocaine to relatively larger scopolamine doses produced more-than-additive effects at low effect levels, while the addition of a small amount of scopolamine to relatively larger cocaine doses produced effects more consistent with additivity except at the highest effect level examined.

When tested individually, both cocaine and scopolamine dose-dependently increased locomotor activity, showing efficacies, potencies and time courses consistent with previous reports in rats (Campbell et al., 1969; Bauer, 1982; Sipos et al., 1999; Thomsen and Caine, 2011). As expected, the dose-effect functions for cocaine and scopolamine had different slopes, and scopolamine did not induce as high activity levels as cocaine. This precluded the use of simple linear isoboles, which assume a constant ratio between the potency of the two drugs being evaluated, i.e., essentially parallel curves of a comparable shape and peak (Grabovsky and Tallarida, 2004). The locomotor activating effects of scopolamine have been reported to vary significantly between juvenile, young adult, and aged rats (Campbell et al., 1969; Bauer, 1982; Pedigo et al., 1984). This is unlikely to have affected the current findings because testing was completed within a few weeks using age-matched groups of young adult rats. However, the observed cocaine-scopolamine interactions may not necessarily extend to juvenile or aged rats.

Deviation from additivity, especially with synergistic effects, is typically interpreted to imply different modes of action of the two drugs being evaluated. Indeed, there is evidence that cocaine and scopolamine stimulate locomotor activity via distinct mechanisms. 6-OHDA lesion studies have shown that while psychomotor stimulants like cocaine, methylphenidate and d-amphetamine are dependent upon intact mesolimbic (A10) dopaminergic afferents, which arise in the ventral tegmental area and project to the nucleus accumbens, to produce locomotor stimulation, scopolamine is not (Kelly and Iversen, 1976; Joyce and Koob, 1981; French, 1986). Those studies do not exclude the involvement of dopaminergic pathways in the locomotor stimulating effects of scopolamine: interaction with the dopamine systems may occur at the postsynaptic level in the accumbens, or presynaptically at sites outside the lesions (e.g., striatonigral pathways). Postsynaptic modulation of striatal dopaminergic effects by scopolamine has been demonstrated, specifically with D₁ receptor stimulation, while scopolamine had little effect on D₂-stimulated effects (Bordi and Meller, 1989; Morelli et al., 1993). However, scopolamine may not increase effects of mixed D₁/D₂ stimulation, and by extension, of a psychomotor stimulant acting postsynaptically (Morelli et al., 1994; Wirtschafter and Asin, 2001). Interactions – positive or negative – outside the mesolimbic pathway are also plausible. For instance, the infusion of scopolamine into the substantia nigra potentiated the nigral dopamine efflux associated with locomotor activity in rats (Andersson et al., 2010), while scopolamine infusions into the medial prefrontal cortex induced moderate locomotor activity, but decreased cocaine-induced activity (Ikemoto and Goeders, 2000). Striatal dopamine release can be also triggered by cholinergic interneurons, independently from A9 or A10 pathways activity, via stimulation of nicotinic acetylcholine receptors (Threlfell et al., 2012). Scopolamine increases striatal acetylcholine release (Watanabe and Simizu,

1989). This may be one mechanism by which systemic scopolamine increases striatal dopamine efflux, acting independently of mesolimbic afferent activity, providing a convergence of effects of cocaine and scopolamine. Finally, scopolamine may increase striatal dopamine transporter availability (Tsukada et al., 2001; Kilbourn et al., 2004).

In a recent meta-analysis, three main types of interactions were found to result in pharmacodynamically synergistic drug effects: complementary actions, facilitating actions, and anti-counteractive actions (Jia et al., 2009). Complementary actions would be activation of different parts of a pathway, or of different pathways that both modulate locomotor activity. Most of the possible neurobiological mechanisms described above would fall under this category, e.g., a convergence of cocaine and scopolamine effects on dopaminergic pathways. Facilitating actions are seen when one drug enhances the access of the other drug, or an endogenous ligand, to a target molecule. Scopolamine-increased DAT availability would be a facilitating action. Anti-counteractive actions occur if one of the drugs dampens effects that limit or oppose the measured endpoint; in the case of locomotor activity, an example could be stereotypies. Scopolamine can increase cocaine- or amphetamine-induced stereotypies (Klawans et al., 1972; Heidbreder and Shippenberg, 1996; Aliane et al., 2011), an effect that would likely contribute to the descending limb of locomotor activity dose-effect function rather than increasing locomotor activity. This does not exclude the possibility of anti-counteractive action in the cocaine/scopolamine interaction. While the present study was not designed to measure sensitization, synergistic effects on sensitization appear unlikely. Indeed, pretreatment with scopolamine decreased or eliminated conditioned (context-dependent) sensitization to the locomotor stimulant effects of cocaine, an effect attributed to the amnesic effects of scopolamine (Heidbreder and Shippenberg, 1996; Itzhak and Martin, 2000).

Taken together with our previous observations using a drug discrimination assay in mice, the current findings of synergism may extend to other endpoints and species. For instance, enhancement of the effects of cocaine or d-amphetamine have also been observed after the addition of a dose of muscarinic antagonist that did not in itself elicit a significant effect in rats or mice, using assays of locomotor activity, drug discrimination, operant shock avoidance responding, or striatal dopamine microdialysis (Carlton and Didamo, 1961; Carlton, 1961; Galambos et al., 1967; Katz et al., 1999; Tanda and Katz, 2007; Tanda et al., 2007; Thomsen et al., 2010). Scopolamine also increased the toxicity of d-amphetamine in mice (Mennear, 1965). Interestingly, atropine pretreatment also increased cocaine self-administration in rhesus monkeys under a fixed ratio 1 schedule of reinforcement (Wilson and Schuster, 1973), but cocaine/scopolamine mixtures were typically self-administered less than cocaine under higher response requirement schedules (Ranaldi and Woolverton, 2002), perhaps suggesting more complex interactions where reinforcing effects are concerned.

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Figure 1.

Locomotor activity stimulation by cocaine (A, C) and scopolamine (B, D). A, B: Total beam breaks per 3-h session as a function of dose. C, D: time course as total beam breaks per 10-min bin, during the 1-h habituation period and for 3h after injection. To avoid crowding, two doses of scopolamine were omitted in panel D, and the vertical axes in panels C and D have different scales. Data are group means; bars represent one SEM; N=16. * $p < 0.05$, ** $p < 0.01$, Dunnett's multiple comparisons test vs. saline (statistical details are not shown in panels E and F).

Figure 2.

Stimulation of locomotor activity by cocaine/scopolamine mixtures as a function of cocaine dose (upper horizontal axis) and scopolamine dose (lower horizontal axis), as total beam breaks above saline levels. Cross marks show the doses estimated to produce 500, 1000, 1500 and 2000 beam breaks above saline (with 95%CL), calculated by interpolation in each rat as dose of cocaine and as dose of scopolamine. Cocaine/scopolamine mixture ratios are indicated in each panel. Data are group means; bars represent one SEM; N=16. Filled symbols denote $p < 0.05$, Dunnett's multiple comparisons test vs. saline.

Figure 3.

Isobolograms for stimulation of locomotor activity by cocaine/scopolamine mixture ratios, at effect levels 500, 1000, 1500 and 2000 beam breaks above saline. Horizontal axis: scopolamine dose; vertical axis: cocaine dose. Bold lines represent the dose pairs expected to produce the given effect level if additivity is assumed; shaded areas represent doses within the 95%CL of expected additive values. The cocaine/scopolamine dose-pairs estimated to produce each effect level based on the observed drug mixture effects are shown as open circles, with bars representing 95% CL (mixture ratios as indicated).