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Tetrabenazine fails to antagonize a behavioral effect of cocaine in rhesus monkeys

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Abstract

For research and therapeutic purposes, a cocaine antagonist is an important drug development goal. The vesicular monoamine transport inhibitor tetrabenazine was tested for interaction with cocaine using food-reinforced responding in rhesus monkeys as an assay. Both tetrabenazine and cocaine suppressed food-maintained behavior individually. However, a low-dose tetrabenazine pretreatment did not alter the rate-suppressing effects of cocaine and cocaine did not alter the rate-suppressing effects of a high dose tetrabenazine pretreatment. Because tetrabenazine interacts with the monoamine oxidase inhibitor deprenyl in this assay, we conclude that cocaine does not produce an effect through vesicular catecholamines in this assay.

Keywords: Tetrabenazine; Cocaine; Operant suppression; Rhesus monkeys; Sodium channels

1. Introduction

Cocaine abuse is a significant medical problem that has stimulated a great deal of research (Johanson and Schuster, 1995). In both research and clinical settings, an antagonist would be very useful. As a research tool, antagonists can be used to map the pharmacology of a drug; in a clinical setting, an antagonist might benefit cocaine users (Johanson and Schuster, 1995).

Cocaine has several effects that an antagonist might need to block in order to be useful; it is known to inhibit monoamine uptake (Graefe and Bönisch, 1988) and also to block sodium channels (Catterall and Mackie, 1996). As an uptake inhibitor, cocaine inhibits the recycling of neurotransmitters. Depolarization of a neuron leads to the release of neurotransmitters that are packaged in vesicles; these neurotransmitters act within the synapse and are inactivated primarily by uptake, or active transport into the nerve terminal (although some are metabolized enzymatically in the extracellular space). Once inside the terminal, the transmitter is either packaged in vesicles by active transport or metabolized by monoamine oxidase enzymes (cf. Graefe and Bönisch, 1988 and Lefkowitz et al., 1996 for reviews). The evidence showing dopamine uptake to mediate behavioral effects of cocaine has led some authors to downplay sodium channel effects and to suggest that therapy be directed at blocking dopamine receptors (Johanson and Schuster, 1995). Indeed, experiments have explored the use of antagonists such as flupenthixol, although more research will be necessary (Johanson and Schuster, 1995).

Tetrabenazine interacts with the same synapses at an earlier step in the neurotransmitter release cycle than cocaine. Tetrabenazine inhibits the accumulation of catecholamines by cells expressing the vesicular monoamine transporter (Gonzalez et al., 1994) and results in a depletion of cerebral stores of monoamines (Pletscher et al., 1962). If the effects of cocaine on behavior are mediated by dopaminergic synapses, tetrabenazine should reduce the amount of dopamine in vesicles, reducing in turn the amount of dopamine released and reducing the substrate available for cocaine's effects. Attempts to demonstrate an interaction between tetrabenazine and stimulants have been published. Tetrabenazine and amphetamine have been shown to have mutually antagonistic effects on operant response rates in pigeons and squirrel monkeys (McMillan, 1968). Although amphetamine and cocaine have different

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molecular mechanisms of action, they both alter the transit of dopamine through the synaptic cycle with similar results (Graefe and Bönisch, 1988). Behavior has also been studied after combinations of cocaine and reserpine, but reserpine either has profound effects on baseline measures (e.g., complete elimination of locomotor activity; Smith, 1964) or baseline measures are not reported (e.g., Sayers and Handley, 1973).

In order to investigate the interaction of tetrabenazine and cocaine, we used food-maintained operant behavior in the rhesus monkey. Stimulants typically decrease the rate of behavior seen after stimulant administration under fixed-ratio (FR) schedules (McMillan et al., 1975). Although this might make fixed-interval (FI) schedules seem more attractive than FR schedules, decreases in behavior are also seen in late FI behavior at the doses of cocaine that cause increases in total and early FI behavior (McMillan et al., 1975). To simplify the analysis, contingencies were programmed on FR schedules. The effects of tetrabenazine and cocaine on operant behavior were determined individually and in combination. Because tetrabenazine prevents catecholamines in the terminal from being packaged in the vesicles (Lefkowitz et al., 1996), and monoamine oxidase reduces the accumulation of extravesicular catecholamines (Graefe and Bönisch, 1988), an inhibitor of monoamine oxidase should potentiate the immediate effects of tetrabenazine. In order to confirm a monoaminergic mechanism of action of tetrabenazine, we also determined its effects in combination with deprenyl.

2. Methods

Five rhesus monkeys (two male and three female) were used in these experiments. Subjects weighed 4.5–9.5 kg and were housed individually with free access to water. They were fed 10–20 high-protein biscuits (Purina, St. Louis, MO) daily after sessions and fresh fruit three times per week. All subjects were drug-naïve at the beginning of the experiment.

Equipment and procedures are adapted from previously published work (Herling et al., 1979). Briefly, the monkeys were placed in Plexiglas and tubular aluminum primate restraint chairs for handling and experimental sessions. The chairs were placed in chambers equipped with one lever on each side of a food cup. A pellet dispenser delivered 300 mg P.J. Noyes (Lancaster, NH) banana-flavored pellets to the cup as reinforcers. The chambers were equipped with light-display panels located above the levers. Ventilation and background-masking noise was provided by an exhaust fan that ran continuously. Responses were recorded and contingencies were programmed with an IBM pcJR microcomputer (Armonk, NY).

Monkeys were trained to press levers by the method of successive approximations. Response requirements were increased through progressively greater FR values. After responding stabilized on an FR30 schedule, contingencies alternated on a cycle of time-out and food availability. Gradually, the number of cycles was increased (to 10 cycles) as the number of pellets delivered and the amount of time per cycle was decreased. Experimental sessions were carried out 5 days per week. Each cycle began with a 5-min time-out period when no stimulus lights were illuminated. All injections were given intramuscularly in the thigh during the first minute of the time-out period. Responses during the time-out period were not recorded or reinforced. The time-out period was followed by a 5-min response period in which two green lights were illuminated. After 30 responses, a reward pellet was delivered; responses on either lever were counted. The response period ended when either the subject received 10 pellets or the maximum time of 5 min was reached.

Control rates of responding were determined from sessions in which an injection of saline was administered prior to the first cycle. Control rates are the average of the response rates for all 10 cycles. Although control rates varied from 1.9 to 4.4 responses per second across subjects, these rates were consistent (within ± 0.5 responses per second) for each individual. Drug trials were scheduled if the control rate was greater than 1 response per second on the day prior to the drug trial. On drug trials, an injection of the vehicle for the drug was administered during the cycle prior to the first drug injection. Drugs were not administered if the response rate following vehicle administration was less than 1 response per second.

Tetrabenazine free base (Hoffmann-LaRoche, Nutley, NJ) was titrated with 0.1 M HCL and diluted with 20% Alkamuls EL-620 (ethoxylated castor oil; Rhone Poulenc Rohrer, Cranbury, NJ); final pH was approximately 5.5. Deprenyl hydrochloride (Somerset Pharmaceuticals, Denville, NJ) and cocaine hydrochloride (NIDA, Research Technology Branch, Rockville, MD) were dissolved in saline. The vehicle control for tetrabenazine was 20% Alkamuls EL-620; for deprenyl and cocaine, it was saline. Initial data suggested that the effect of tetrabenazine increased with repeated administration unless a week or more passed between doses. In order to avoid similar problems, deprenyl was given at 2-week intervals. Cocaine was administered in cumulative doses from 0.03 mg/kg to 1.0 mg/kg in semilogarithmic steps; no injections were given in subsequent cycles. Tetrabenazine was injected as a bolus of 0.3 or 1 mg/kg; either no injections or cumulative doses of cocaine followed. The sequence of cocaine injections was identical to prior cocaine sessions except for starting with a tetrabenzine injection. Deprenyl was also injected as a 1-mg/kg bolus. Bcause deprenyl alone showed an effect after a lag of about 30 min, one cycle without injection was left between deprenyl injection and tetrabenazine vehicle injection. All behavioral cycles were a constant 10 min regardless of drug treatment. Data points for deprenyl alone represent single or duplicate

determinations; all other data points represent duplicate or triplicate determinations.

3. Results

At a dose of 0.3 mg/kg, tetrabenazine reduced rates of food-maintained behavior (Fig. 1). The largest effect of this dose took place from 55 to 65 min after drug administration; a decrease in response rates became apparent 25 min after drug administration. A larger dose (1 mg/kg) eliminated responding from 65 to 75 min after drug administration. Response rates were depressed 5 min after administration of this dose, but showed a substantial recovery after another 10 min; thus, suppression of responding appeared to have two kinetic components. This pattern was not evident in all of the data. Although the dose eliciting this effect was not the same in all cases, all of the five monkeys showed this pattern at least once; of 42 tetrabenazine administrations, 10 showed the pattern. The average of all 10 similar sessions show suppression to 20% of control rates 5 min after drug, recovery to greater than 90% of control rates 15 min after drug and suppression to 40% of control rates 25 min after drug. Higher doses appear to eliminate the recovery at 15 min after drug.

In order to investigate interactions between tetrabenazine and cocaine, tetrabenazine doses producing small and large effects on behavior (0.3 and 1 mg/kg) were administered as pretreatments to cocaine, which was administered in cumulative doses. The dose–response curve for cocaine was not changed by pretreatment with 0.3 mg/kg tetrabenazine (Fig. 2A); a dose of 0.3 mg/kg cocaine suppressed responding with or without tetrabenazine pretreatment. The effect of 1 mg/kg tetrabenazine was not changed by subsequent administration of cocaine; 25 min after tetrabenazine administration, responding was suppressed with or without cocaine administration (Fig. 2B).

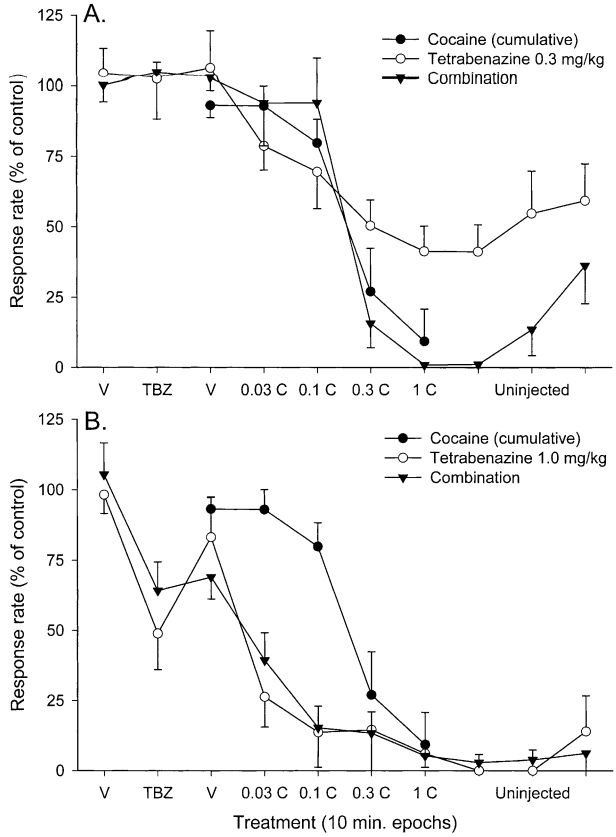

Fig. 2. Combinations of low and high doses of tetrabenazine (single bolus injection) with cocaine (cumulative administration): for tetrabenazine and combination trials, a vehicle injection preceded the first component and a tetrabenazine injection preceded the second component (i.e., 10 min later). For combination trials, injections of the cocaine vehicle and increasing doses of cocaine were made before the next components as shown. For cocaine trials, a vehicle injection preceded the first component and cocaine injections followed; the data are plotted at an *x*-axis offset to correspond to the timing of the combination trial data. (A) 0.3 mg/kg tetrabenazine and cocaine; (B) 1.0 mg/kg tetrabenazine and cocaine.

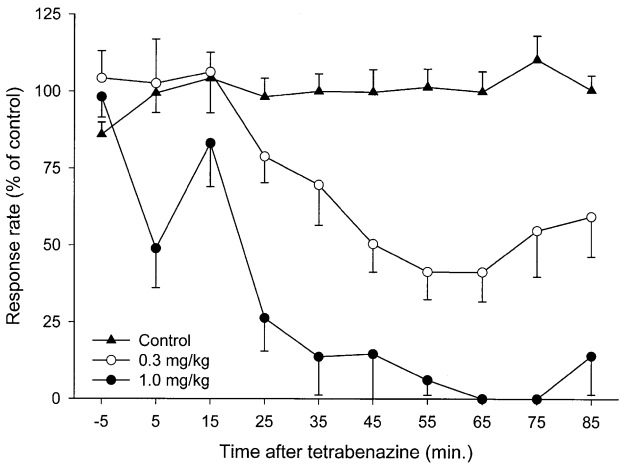

Fig. 1. Effect of tetrabenazine on food-maintained behavior over time: response rates were determined at 10-min intervals, with a vehicle injection preceding the first component (plotted as −5 min) and an injection of vehicle or tetrabenazine preceding the second component (5 min).

Some authors have suggested that tetrabenazine has significant actions that do not involve the vesicular monoamine transporter (Login et al., 1981; Reches et al., 1983). If tetrabenazine alters the distribution of catecholamines (i.e., from vesicles to the synaptic terminal; Lefkowitz et al., 1996), a monoamine oxidase inhibitor should prolong the elevation of cytoplasmic catecholamine levels and potentiate the early effect of tetrabenazine (Graefe and Bönisch, 1988). When 1 mg/kg of the monoamine oxidase B inhibitor deprenyl was administered as a pretreatment to 1 mg/kg tetrabenazine, responding was almost completely suppressed immediately and did not recover during the session (Fig. 3). The response rate suppression at 15 min after tetrabenazine (45 min after deprenyl) is

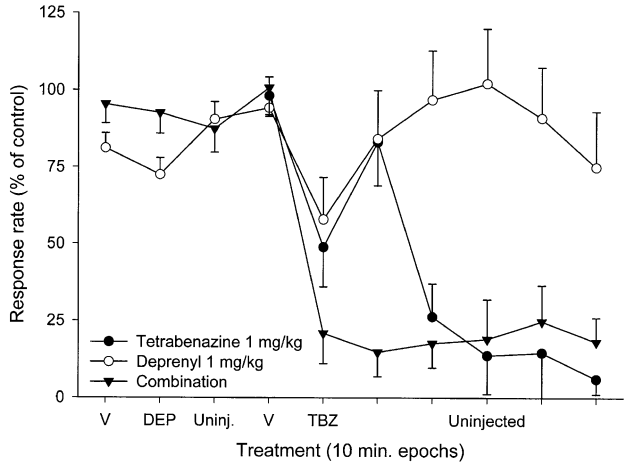

Fig. 3. Combined tetrabenazine and deprenyl administration: for deprenyl and combination trials, a vehicle injection preceded the first component and a deprenyl injection preceded the second component. For combination trials, injections of the tetrabenazine vehicle and tetrabenazine were given after a delay of 10 min (uninjected component). For tetrabenazine trials, a vehicle injection was given before the first component and tetrabenazine before the second; the data are plotted at an *x*-axis offset to correspond to the timing of the combination trial data.

greater than the sum of the effects of either drug alone at those times.

4. Discussion

Despite having mechanisms of action that involve the same synapses, cocaine and tetrabenazine do not interact in operant rate suppression (Fig. 2). Either cocaine or tetrabenazine suppresses responding without action on vesicular stores of catecholamines. Inhibition of vesicular transport results in accumulation of transmitter within the nerve terminal (Lefkowitz et al., 1996); any effect resulting from this will be potentiated by a drug that inhibits intraneuronal metabolism of the transmitter. The monoamine oxidase inhibitor deprenyl potentiated the immediate effect of tetrabenazine (Fig. 3); this is consistent with transporter inhibition, but difficult to explain as a nonspecific effect. Although tetrabenazine has been shown to have dopamine D2 receptor antagonist-like properties and to bind to spiperone-labeled sites (Login et al., 1981; Reches et al., 1983), the affinity cited for displacement of spiperone (e.g., 3–5 μM: Login et al., 1981) is orders of magnitude lower than the affinity for displacement of dihydrotetrabenazine from recombinant vesicular monoamine transporters (2.3 nM, Gonzalez et al., 1994). Similarly, the doses used to demonstrate D2 antagonist effects (e.g., hyperprolactinemia at 30 mg/kg: Login et al., 1981) are an order of magnitude higher than the doses that deplete amines. Using the same species and route of administration, the ED_{50} for depletion of serotonin by tetrabenazine is 3.6 mg/kg (Pletscher et al., 1962). The combination of the present findings with previous work on the effects of tetrabenazine in vitro and in vivo confirm that tetrabenazine, at doses of 0.3 and 1 mg/kg, is an inhibitor of vesicular monoamine transport.

Because tetrabenazine interacts with vesicular amine stores, the lack of interaction with cocaine suggests that cocaine does not produce its effect in this assay through vesicular amines. Cocaine has other mechanisms of action that might produce this behavioral effect. Cocaine can displace batrachotoxinin from sodium channels with an IC_{50} of about 1.3 μM; the IC_{50} for inhibition of dopamine uptake in the same study was 0.69 μM (Andersen, 1989). This blockade of sodium channel activity underlies the clinical utility of cocaine as a local anesthetic (Catterall and Mackie, 1996) and has been implicated in the mechanism of cocaine action on some behaviors. When a series of local anesthetics were tested in rats trained to discriminate procaine from saline, the rats generalized cocaine to procaine. In rats trained to discriminate cocaine from saline, group-average data showed that the rats generalized procaine to cocaine partially (completely in some subjects); dimethocaine was generalized completely (Woolverton and Balster, 1982). The effects of cocaine on food-maintained behavior have been shown to resemble some local anesthetics in pigeons (McMillan et al., 1975) and in rhesus monkeys (Woolverton and Balster, 1983). Although there are no sodium channel agonists that would inhibit a cocaine effect and allow us to prove the hypothesis, we propose that the effects of cocaine on suppression of operant behavior involve inhibition of sodium channels.

The effects of tetrabenazine alone in this assay are also interesting. Previous studies of the effects of tetrabenazine have shown a rapid effect on brain catecholamines or behavior with a subsequent decay of effect loosely corresponding to the time course of the presence of the drug in plasma or brain (e.g., Mehvar and Jamali, 1987; Pletscher et al., 1962; Quinn et al., 1959). No report to date has identified the sort of multiphasic effect described here (recovery between periods of rate suppression; Fig. 1), although there is some indication of this multiphasic time course in one paper (i.e., Fig. 1 of McMillan, 1968). The two separate phases of tetrabenazine effect may correspond to separate actions on a cellular or molecular level: the immediate phase may correspond to release of transmitter from the vesicle into the terminal, with the later phase corresponding to failure of catecholaminergic transmission due to vesicular depletion. Alternately, the combination of intraneuronal release and depletion may be constant, with the two phases corresponding to different populations of vesicles (Moser and Neher, 1997).

Pharmacokinetic studies have shown that tetrabenazine and its active metabolite are nearly eliminated from blood and brain in 12 h (Mehvar and Jamali, 1987). This is consistent with a short duration of action in both biochemical and behavioral terms (Pletscher et al., 1962). Our data show response rates to be normal 1 day after administration of completely disruptive doses (data not shown);

some recovery can even be seen at 85 min after injection (Fig. 1). However, administration of tetrabenazine more often than once per week resulted in a progressive increase in the rate suppressing effects of the drug (data not shown). The reasons for this apparent sensitization with repeated administration are not clear.

Although the data from this study do not describe a cocaine antagonist, they provide a cautionary note. The best cocaine antagonist may not be a drug targeted at cocaine's mechanism of action. The best antagonist may be a pharmacokinetic intervention, such as an anticocaine antibody or a cocaine-metabolizing antibody (Mets et al., 1988).

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