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BRIEF COMMUNICATION

One Injection of Cocaine Produces a Long-Lasting Increase in [^{3}H]-Dopamine Release

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PERIS, J AND N R ZAHNISER *One injection of cocaine produces a long-lasting increase in [^{3}H]-dopamine release* PHARMACOL BIOCHEM BEHAV 27(3) 533–535, 1987 — A single cocaine exposure has been reported to sensitize animals to the behavioral effect of subsequent cocaine administration for up to one week We now report that a single injection of cocaine results in an augmentation in amphetamine-induced release of tritium from rat striatal slices preloaded with [^{3}H]-dopamine The augmentation appears within 24 hr and persists for at least 2 weeks after injection This increase in release may result in increased synaptic concentrations of dopamine possibly caused by a change in the membrane transporter for dopamine Increased dopaminergic synaptic transmission could explain behavioral sensitization

Dopamine release Amphetamine Cocaine Sensitization Striatum

COCAINE is a widely abused drug that results in increasing degrees of stereotypy and locomotor behavior when administered repeatedly to rats [12,15], a phenomenon termed behavioral sensitization Sensitization to the stereotypic effect of cocaine has also been shown to occur one week after a single cocaine injection [7] d-Amphetamine, another stimulant, also results in behavioral sensitization when given repeatedly or singly [14] Sensitization to both of these stimulants is thought to have a neurochemical basis via enhanced dopamine (DA) neurotransmission since d-amphetamine and cocaine act to block DA uptake and d-amphetamine also induces DA release [6] A single exposure to d-amphetamine capable of eliciting behavioral sensitization also increases d-amphetamine-stimulated DA release [14] The magnitude and duration of both behavioral sensitization and augmented amphetamine-stimulated DA release are increased by repeated exposure to d-amphetamine while other indicators of dopaminergic function (e g , K^+-stimulated release, pre- or post-synaptic receptors, metabolism, synthesis) are not changed (see [13])

It is possible that the basis for behavioral sensitization may be similar for both cocaine and amphetamine Therefore, we examined the effects of an acute dose of cocaine on amphetamine- or electrically-stimulated release of [^{3}H]-DA from superfused rat striatal slices

METHOD

Male Sprague-Dawley rats (130–150 g, Sasco, Omaha, NE) were injected with either saline or 10 mg/kg cocaine hydrochloride (Mallinckrodt, Inc , St Louis, MO), intraperitoneally, at various times (24, 48, 72 hr or 7 or 14 days) prior to sacrifice for dopamine release experiments

The method for [^{3}H]-dopamine release has been described in detail elsewhere [3]. Briefly, fresh corpus striatum was sliced 0 4 mm thick, placed in Krebs' solution (pH=7 4) saturated with 95% O_2–5% CO_2 and incubated for 30 min in a shaking water bath at 35°C The composition of the Krebs' buffer was (in mM) NaCl, 118, glucose, 11 1, $NaHCO_3$, 25, KCl, 4 7, NaH_2PO_4, 1 0, $MgCl_2$, 1 2, $CaCl_2$, 1 3, EDTA, 0 004, ascorbate, 0 11 The medium was replaced with fresh buffer containing 5 μl of [^{3}H]-DA (dihydroxyphenylethylamine 3,4-ethyl-2-[N-^{3}H], 31 6 Ci/mmol, New England Nuclear, Boston, MA) such that the final concentration was 0 1 μM and the incubation was continued for another 30 min Rinsed slices were then placed into separate glass chambers maintained at 33°C and superfused with oxygenated Krebs' buffer at a rate of 1 ml/min The superfusate was collected at 5-min intervals beginning 50 min after the start of superfusion At 70 min, slices were superfused with either 2, 6 or 20 μM d-amphetamine (Sigma Chemical

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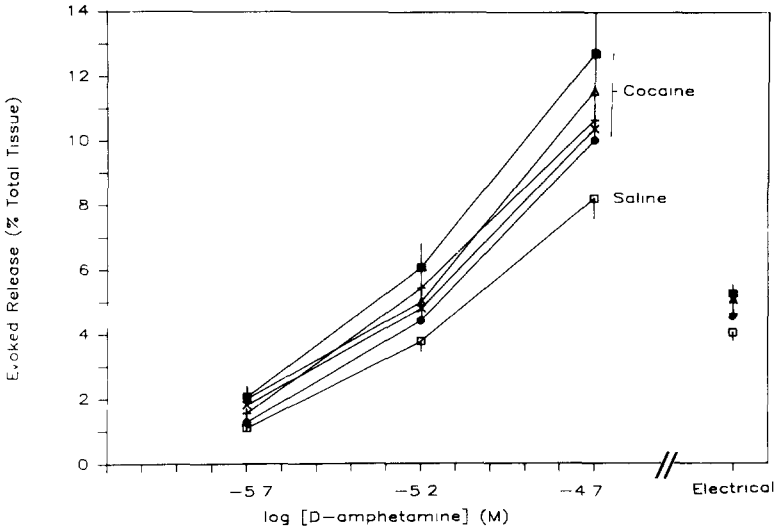

FIG 1 d-Amphetamine-induced [^{3}H]-DA release is augmented up to 14 days after a single cocaine injection Treatment of rats with a single injection of cocaine (10 mg/kg, IP) either 24 hr, 48 hr, 72 hr, 7 days or 14 days earlier results in an augmented amphetamine-induced but not electrically-evoked, tritium release from striatal slices preloaded with [^{3}H]-DA Spontaneous release was allowed to stabilize for 70 min before the various concentrations of d-amphetamine were superfused for 2 5 min Electrically-stimulated release was evoked 40 min after the amphetamine stimulation by 300 pulses (5 Hz, 20 mA, 2 msec duration) Shown are mean values±SEM for N=13 animals for the saline (□), N=5 for the 24 hr (+), N=8 for the 48 hr (●), N=4 for the 72 hr (△), N=5 for the 7 day (×) and N=3 for the 14 day (■)

Co , St Louis, MO) for 2 5 min as the first stimulation At 115 min, slices were exposed to 5 Hz electric pulses (20 mA, 2 msec duration) for 60 sec Radioactivity in both superfusate and solubilized tissue was determined by liquid scintillation counting

Neither uptake blockers nor monoamine oxidase (MAO) inhibitors were included in our superfusion buffer, therefore the tritium collected in the superfusate was comprised primarily of [^{3}H]-dihydroxyphenylacetic acid ([^{3}H]-DOPAC) rather than [^{3}H]-DA [10] It has been shown, however, that [^{3}H]-DOPAC is a reliable indicator of DA release since DOPAC is produced from DA that has been released, taken back up into the terminal and then metabolized [2,10] We henceforth refer to release as measured in our system as tritium release indicating the release of both [^{3}H]-DA and [^{3}H]-DOPAC

The amount of tritium released in each fraction was expressed as a precentage of total tritium content present in each slice at the time of sample collection An estimate of spontaneous release was averaged from the two 5-min fractions preceeding each stimulus plus the first fraction after each stimulus in which release was either equal to or below pre-stimulation (spontaneous) levels These values were used as estimates of the spontaneous release occurring in each fraction The evoked tritium release was then calculated by subtracting existing spontaneous release from the tritium efflux collected in each fraction beginning immediately after the stimulus and continuing until the efflux again equalled spontaneous release These values were summed for the first and second periods of stimulation Each dose of d-amphetamine was tested in at least duplicate slices from the same animal Results are expressed as mean± S E M , n indicates the number of animals tested Two-factor ANOVAs were used to analyze the data

RESULTS

A dose-related increase in tritium overflow in response to d-amphetamine exposure (2, 6, or 20 μM) and an increase in tritium overflow in response to electrical stimulation (5 Hz) was observed (Fig 1) While there was no difference in the amount of electrically-stimulated tritium release between cocaine-treated and saline-treated animals, the amount of amphetamine-induced overflow was greater at all doses for animals injected once with cocaine as compared to those injected with saline (Fig 1) Analysis of variance revealed a significant effect of d-amphetamine dose, $F(2,66)=506$, $p<0\ 001$, and a significant effect of treatment group, $F(5,33)=3\ 4$, $p<0\ 05$, but no interaction between these two factors Subsequent Neuman-Keuls comparisons between treatment groups revealed significant differences between the saline group and each cocaine group ($p<0\ 5$) and no major differences between the 5 cocaine groups (except between the 48 hr and 14 day comparison) implying that the difference was completely developed at 24 hr and persisted fully up to 14 days Also, no differences were observed in amphetamine-induced tritium release from slices from animals that had received saline injections at different times before sacrifice, therefore, these animals were treated as a single group There was no significant difference between the saline or cocaine pretreatment on the amount of electrically-stimulated release, $F(5,33)=1\ 57$, $p>0\ 1$, when all the data were analyzed by ANOVA or when paired observations (11 out of 13 controls) were analyzed using a paired t-test

DISCUSSION

These results show that a single injection of cocaine augments d-amphetamine-stimulated striatal [^{3}H]-DA release up to two weeks after injection These data agree both with reports of behavioral sensitization to a single injection of cocaine [7] and with reports of long-lasting augmentation of amphetamine-induced DA release after a single injection of amphetamine [14] The mechanism of such long-lasting actions of both cocaine and amphetamine on DA release is not known, although the direct effects of these drugs on DA function are well-described At concentrations lower than 100 μM, d-amphetamine releases [^{3}H]-DA primarily by accelerated exchange diffusion due to its use of the DA uptake carrier to enter into neurons thereby blocking uptake as well [6,11] Cocaine, on the other hand, blocks the DA uptake carrier directly and does not promote release until concentrations are greater than 10 μM [4]

It should be emphasized that tritium release measured in our system is actually a net overflow of [^{3}H]-DA and [^{3}H]-DOPAC which can be affected by a number of parameters including changes in release, uptake or metabolism of DA Since the $t_{1/2}$ of cocaine in rat brain after an 8 mg/kg injection is approximately 20 min [8], even in the 24-hr treatment group, a negligible amount of cocaine would be present at sacrifice Therefore, the difference between the cocaine- and saline-treated animals cannot be due to blockade of DA reuptake by residual cocaine Inhibition of oxidative deamination potentiates the DA-releasing effects of amphetamine [9] However, it is unlikely that cocaine caused an increase in amphetamine-stimulated release in these studies by long-term inhibition of MAO activity since electrically-stimulated DA release was not affected either in this experiment (Fig 1) or in experiments in which two periods of only electrical stimulation were used (L P Dwoskin and N R Zahniser, manuscript in preparation) There is also evidence that DA metabolite levels are unchanged after single doses of 15–40 mg/kg cocaine [1]

In these studies, we used [^{3}H]-DA to detect changes in release caused by cocaine treatment It has been argued that this method does not detect release from the same intraneuronal pools as endogenous DA [5,9] but this may depend on the stimulation parameters used [10] Robinson and colleagues (see [13]) have measured endogenous DA release when studying the effects of *in vivo* d-amphetamine treatment, and their results are similar to ours with cocaine treatment in the extent of augmentation and the long-lasting nature of these changes In addition, amphetamine induces a 3-fold greater increase in endogenous DA release compared to [^{3}H]-DA [5] Therefore, it is likely that similar, if not greater, differences would be found between cocaine and saline-treated animals if endogenous DA release had been measured instead of [^{3}H]-DA release

It is possible that sensitization occurs via uncoupling of DA uptake sites and/or changing of the DA carrier protein such that DA release is favored over uptake thereby increasing DA concentration in the synapse and at the post-synaptic site This is currently being investigated In summary, our finding of persistent augmentation of amphetamine-induced [^{3}H]-DA release in animals exposed only once to cocaine confirms other reports of long-lasting behavioral sensitization to the stereotypic effect of cocaine Such persistent changes in both behavior and DA neurotransmission, especially after such limited exposure to the drug, may play a role in the social problems produced by cocaine

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