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BRIEF COMMUNICATION

The Effects of In Vivo Cocaine on Norepinephrine-Stimulated Phosphoinositide Hydrolysis in Rat Brain

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JAVAID, J. I., S. C. PANDEY AND J. M. DAVIS. *The effects of in vivo cocaine on norepinephrine-stimulated phosphoinositide hydrolysis in rat brain.* PHARMACOL BIOCHEM BEHAV 47(4) 989–992, 1994. – We examined the effects of a cocaine challenge on behavioral stimulation and NE-stimulated [^{3}H]inositol-1-phosphate (IP_1) formation in rat cerebral cortex after single (high dose) or repeated (low dose) cocaine administration. As previously reported, single high dose (40 mg/kg, IP) and repeated low dose (10 mg/kg, 8 IP injections) administrations of cocaine resulted in behavioral sensitization to a challenge injection of cocaine (10 mg/kg). In saline-pretreated animals, the acute cocaine challenge significantly potentiated the NE-stimulated [^{3}H]IP_1 formation as compared with the saline challenge, while in cocaine-pretreated animals, NE-stimulated phosphoinositide (PI) turnover was not significantly altered. These results suggest that although some of the acute effects of cocaine may be mediated by enhanced α_1-adrenergic receptor-linked PI hydrolysis, behavioral sensitization does not involve the α_1-adrenergic receptor-linked PI signal transduction system.

Cocaine Behavioral sensitization Phosphoinositide hydrolysis α_1-Adrenergic receptor Rat

COCAINE is a central nervous system (CNS) stimulant that produces several behavioral and physiological effects in mammals. In humans, it is a powerful euphoric agent and a widely abused drug. In rodents, a single high dose or repeated low doses of cocaine result in enhanced locomotor stimulation and stereotypic behavior in response to a cocaine challenge (behavioral sensitization). However, the underlying neurochemical mechanisms associated with the effects of cocaine are not well understood.

Although enhanced dopaminergic function plays a role in several effects of cocaine (5,10), there is evidence which suggests that the α_1-adrenergic system may be involved in some of the effects of cocaine. For example, inhibition of spontaneously firing locus coeruleus neurons by low doses (0.25–1 mg/kg, IV) of cocaine can be reversed by the α-antagonist piperoxan (16). Also, cocaine-induced pressor effects are antagonized by phentolamine (a nonselective α-adrenoceptor antagonist) and by prazosin (an α_1-selective antagonist), whereas yohimbine (an α_2-selective antagonist) does not alter the pressor effect of cocaine (20). Recently, it was shown that prazosin also attenuates cocaine-induced locomotor activity (2). These results suggest that the activation of α_1-adrenoceptor mechanisms may mediate some of the physiological and behavioral effects of cocaine.

Because α_1-adrenergic receptors are linked with the phosphoinositide (PI) signalling system, functional aspects of this receptor have been studied by norepinephrine (NE)-stimulated PI-turnover determination (4,11). Although it was recently reported that repeated administration of cocaine reduced NE-stimulated inositol accumulation in rat thoracic aorta (25), the effects of in vivo cocaine administration on brain PI hydrolysis have not been investigated. The α_1-adrenergic receptor-linked PI hydrolysis in cerebral cortex has been well characterized. It has also been shown that in vitro cocaine potentiates NE-stimulated PI hydrolysis in rat cortical slices (12). In the present studies we, therefore, examined the effects of acute

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and chronic administrations of cocaine on the formation of [^{3}H]inositol-1-phosphate (IP_1) in rat cortical slices as a measure of PI hydrolysis.

METHOD

Animals and Drug Treatment

Male Sprague-Dawley rats (225-250 g, Harlan, Indianapolis, IN) were housed in groups of three with food and water available ad lib. Cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in a sterile physiological saline vehicle (10 and 40 mg/ml, free base).

For the acute study, animals were pretreated with a single injection of physiological saline (1 ml/kg) or cocaine (40 mg/kg) and 6 days later, either cocaine (10 mg/kg) or saline (1 mg/kg) challenge was administered. Twenty-four hours after the challenge injection, animals were killed by decapitation and cortices were removed for the determination of inositol-phosphate formation as described below. Four groups of animals were used: a) saline pretreatment/saline challenge, b) saline pretreatment/cocaine challenge, c) cocaine pretreatment/cocaine challenge, and d) cocaine pretreatment/saline challenge. In this experiment, NE-stimulated IP_1 formation was determined at an NE concentration (10^{-4} M) which gives maximal response. In another acute experiment, two groups of animals (saline pretreatment/cocaine challenge, and cocaine pretreatment/cocaine challenge) were treated as above and IP_1 formation was determined at various concentrations of NE.

For the chronic study, two groups of animals were treated with cocaine (10 mg/kg, IP) or saline (1 mg/ml) for a total of nine injections (five daily injections, 2 days off, followed by another four daily injections). Locomotor activity and stereotypyic behaviors were measured after each injection. Animals were sacrificed by decapitation 24 h after the last injection for inositol phosphate formation studies.

Behavioral Studies

Stereotypy was assessed as described by Rebec and Segal (19). Each animal was observed for a 1-min period at 10-min intervals after the injection. Individual components of stereotypy, including sniffing, repetitive movements of the head and limbs, licking, and biting were rated according to their duration (1 = discontinuous, 2 = continuous) and intensity (1 = mild, 2 = moderate, 3 = intense) during the 1-min observation period. For analysis of results, the duration and intensity ratings were multiplied to yield a single value. A score of 6 (duration × intensity) was the maximum possible score for a given behavior at each 10-min interval.

For locomotor activity measurement, each animal was transferred to an individual locomotor activity recording unit (50 × 50 × 30 cm acrylic monitor cage, Digiscan Model RXYZCM 8, Omnitech Electronics, Inc., Columbus, OH) and the activity was monitored for 30 min preinjection and for up to 60 min postinjection. The monitor system was set to automatically print out the activity of each animal in 10-min intervals. Total distance travelled (cm) was used to determine the ambulatory behavior (animal movement from one location to another).

Determination of [^{3}H]Inositol-1-Phosphate (IP_1) Formation

The formation of IP_1 in cortical slices was determined as previously described (15). Briefly, rats were killed by decapitation 24 h after the challenge injection, brains were immediately removed, and rinsed with Krebs-Ringer bicarbonate (KR) buffer saturated with O_2/CO_2 mixture (95 : 5). Cerebral cortices were dissected, crosschopped (350 μm), washed, and incubated at 37°C for 1 h in 5.0 ml of KR buffer and 20 μCi of [^{3}H]*myo*-inositol (specific activity, 15.6 Ci/mmol). To remove the unincorporated [^{3}H]*myo*-inositol, the slices were washed with 4 × 20 ml of KR buffer. The final two washings included KR buffer containing 10 mM LiCl, isotonically substituted for NaCl. After the final wash, 100 μl aliquots of gravity-packed slices were transferred to tubes containing 50 μM pargyline and KR buffer. Samples were preincubated for 10 min at 37°C in the incubator chamber saturated with O_2/CO_2. Norepinephrine was added in a total incubation volume of 0.5 ml and tubes were incubated for another 35 min at 37°C. The reaction was terminated by the addition of 1.88 ml of $CHCl_3$/MeOH (1 : 2 v/v) and then 0.62 ml $CHCl_3$ and 0.62 ml H_2O were added to the mixture. The samples were placed in a shaker for 10 min with vigorous shaking and then centrifuged at 4000 × *g* for 15 min to separate the chloroform and aqueous phases. An aliquot (1.5 ml) of the upper phase was removed and applied to a 2.0 ml Dowax (AG 1 × 8) column (formate form, 100-200 mesh). The columns were washed with 4.0 ml of 5 mM sodium tetraborate solution to elute the free [^{3}H]inositol and [^{3}H]glycerophospho-inositol. [^{3}H]Inositol-1-phosphate (IP_1), [^{3}H]inositol-1,4-diphosphate (IP_2), and [^{3}H]inositol-1,4,5-triphosphate (IP_3) were then eluted stepwise from the column with 2 × 8.0 ml of 0.2 M ammonium formate/0.1 M formic acid , 2 × 8 ml of 0.4 M ammonium formate/0.1 M formic acid, and 2 × 8 ml of 1 M ammonium formate/0.1 M formic acid, respectively. For the determination of radioactivity in the phospholipids, a portion of the organic (lower) phase (0.5 ml) was transferred into scintillation vials. The radioactivity in various fractions was then determined by adding 15 ml of scintillation cocktail (3a 70b, RPI) and subsequent counting in a liquid scintillation counter. The PI hydrolysis was expressed as percent of [^{3}H]IP_1 released from total [^{3}H]-inositol incorporated (i.e., dpm from [^{3}H]IP_1 fraction × 100/total dpm from column + dpm in chloroform).

Statistical Analyses

The main effect was assessed by an analysis of variance (ANOVA) using R × C design with R factors (i.e., pretreatment, challenge), each at C levels (e.g., in one experiment

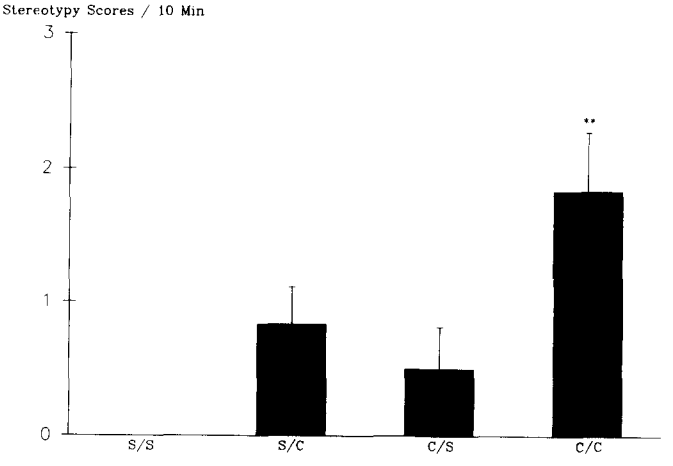

FIG. 1. Repetitive movement scores with a challenge injection. Animals were pretreated with saline (S) or cocaine (C) as described in the Method section and stereotypy was scored after a challenge injection.

there were two levels, i.e., saline challenge, cocaine challenge). In the absence of factor interaction when main effect was significant, the Tukey method was used for multiple comparisons. Adjusted student *t*-test was used where appropriate.

RESULTS

Behavioral Studies

Behavioral sensitization was observed with the cocaine challenge (10 mg/kg) in animals pretreated with a single high dose (40 mg/kg) of cocaine (Fig. 1). In this experiment, we also observed that the saline challenge injection produced higher stereotypy in cocaine-pretreated rats than in the saline group. This probably represents a conditioning phenomenon with a high dose of cocaine which produces behavioral sensitization (18). Although a single low dose of cocaine (10 mg/kg, IP) does not result in behavioral sensitization, a single high dose (40 mg/kg, IP) of cocaine produces behavioral sensitization which has been shown to be context dependent, implicating conditioning (18). These results are similar to those observed with repeated cocaine administration (9). As previously reported (9,17), repeated administration of cocaine resulted in a progressive enhancement of locomotor activity and stereotypic behaviors (repetitive movements, and sniffing).

Effects of Cocaine on NE-Stimulated PI Hydrolysis

The effects of acute cocaine administration on NE (10^{-4} M)-stimulated formation of [^{3}H]IP$_1$ in cortical slices are shown in Table 1. The magnitude of 10^{-4} M NE-stimulated formation of [^{3}H]IP$_1$ observed in the control animals was similar to that previously reported (15). After acute cocaine challenge, the net NE-stimulated (the value in the presence of NE minus the basal value) [^{3}H]IP$_1$ formation was significantly ($p < 0.05$) higher in saline-pretreated animals as compared to other groups. As a percent of the basal formation, acute cocaine enhanced the NE-stimulated [^{3}H]IP$_1$ formation by 38% (mean ± SEM; 136.1 ± 8.3% in saline/cocaine group vs. 98.4 ± 6.2% for saline/saline controls). We then studied the [^{3}H]IP$_1$ formation induced by various concentrations of NE in two groups (saline pretreatment/cocaine challenge and cocaine pretreatment/cocaine challenge) and observed that the single high dose cocaine pretreatment (40 mg/kg) attenuated the enhancement of NE-stimulated [^{3}H]IP$_1$ formation caused by a single low dose (10 mg/kg) cocaine challenge at all NE concentrations used (10^{-6} to 10^{-4} M NE).

To examine whether the effect of chronic low dose cocaine treatment was similar to acute treatment, the effects of repeated low dose (10 mg/kg) cocaine administration on [^{3}H]IP$_1$ formation induced by different concentrations of NE were determined. There were no differences in NE-stimulated [^{3}H]IP$_1$ formation in the chronic cocaine-treated group as compared with saline control animals (percent of basal at 10^{-4} M NE, 104 ± 15 vs. 101 ± 8, respectively). Neither acute nor chronic cocaine treatment altered the basal [^{3}H]IP$_1$ formation in rat cortical slices.

DISCUSSION

The results of the present investigation show that the acute administration of cocaine potentiates α_1-adrenergic receptor function, as determined by NE-stimulated PI hydrolysis in rat brain. However, pretreatment with a single high dose of cocaine as well as with repeated cocaine administration attenuates the augmentation of NE-stimulated PI hydrolysis produced by acute cocaine injection.

As previously reported (9,17,18), behavioral sensitization was observed with repeated cocaine administration, as well as with a single high dose injection of cocaine. The acute cocaine challenge produced significantly greater stereotypic behavior in cocaine-pretreated rats than in saline controls. The slight increase in stereotypic scores observed in the cocaine-pretreated animals with the saline challenge most likely represents a conditioning phenomenon (18). Similar results with the saline challenge were obtained in rats after chronic treatment (9).

The underlying mechanisms of cocaine-induced behavioral sensitization are still not well understood. Because repeated cocaine administration results in dopamine depletion in some brain regions (24), it has been hypothesized that cocaine-induced behavioral sensitization may be associated with supersensitive postsynaptic dopamine receptors as a compensatory mechanism. To explore this hypothesis, several investigators have examined the role of the D_2 receptor subtype using membrane binding or autoradiographic techniques with [^{3}H]spiroperidol or [^{3}H]sulpride as ligands. The number of D_2 receptors has been shown to be increased (7,22), decreased (7), or not changed (6) in different brain areas with repeated cocaine injections. In spite of these inconsistencies in DA receptors, behavioral sensitization was reported in all the studies. Because these results suggest that changes in dopamine receptors are not associated with cocaine-induced behavioral sensitization, it is possible that other aminergic systems may be involved.

Cocaine has sympathomimetic effects and is known to inhibit the uptake of NE in a variety of tissues including brain.

TABLE 1

THE EFFECTS OF ACUTE COCAINE ADMINISTRATION ON NOREPINEPHRINE-STIMULATED [^{3}H]INOSITOL-1-PHOSPHATE FORMATION IN RAT CORTEX

	Saline/Saline	Saline/Cocaine	Cocaine/Saline	Cocaine/Cocaine
Basal	4.13 ± 0.11	3.87 ± 0.28	4.05 ± 0.16	4.32 ± 0.34
+10^{-4} M NE	8.18 ± 0.22	9.00 ± 0.42	8:59 ± 0.35	8.71 ± 0.19
Net NE stimulation	4.04 ± 0.23	5.14 ± 0.21*	4.54 ± 0.19	4.39 ± 0.18

Rat were pretreated with saline (1 ml/kg) or cocaine (40 mg/kg). Six days later, a saline (1 ml/kg) or cocaine (10 ml/kg) challenge was administered. Twenty-four hours after the injection, animals were killed by decapitation. Cortical slices were labelled with [^{3}H]*myo*-inositol, incubated in the absence (basal) or presence of 10^{-4} M NE and inositol phosphates were separated as described in the Method section. The values represent the radioactivity (dpm) in the [^{3}H]IP$_1$ fraction as the percent of the total [^{3}H]*myo*-inositol incorporated (mean ± SEM, $n = 6$).

*$p < 0.05$, saline/cocaine vs. saline/saline.

The involvement of the α_1-adrenergic receptors in the effects of cocaine has been suggested by recent behavioral and physiological studies (2,20). To examine the role of α_1-adrenergic receptors in cocaine-induced behavioral sensitization, we studied the effects of acute and chronic cocaine treatment on NE-stimulated PI hydrolysis in rat brain. Our results suggest that although acute cocaine enhances NE-stimulated [^{3}H]IP$_1$ formation, this is not associated with cocaine-induced behavioral sensitization.

The mechanism by which acute cocaine causes the enhancement of NE-stimulated PI turnover is not clear but may involve NE-uptake inhibition by cocaine as suggested by Mossadaghi et al. (12). As reported by these investigators, we also observed that the NE dose-response curve was shifted to the left by in vitro cocaine (results not shown). However, in vivo cocaine has a very short half-life in rats (14) and was not detectible in brain even at 4 h after 10 mg/kg, IP injection [8]. Because we observed the potentiation of NE-stimulated PI hydrolysis by in vivo cocaine 24 h after a single injection, mechanisms other than NE-uptake inhibition by cocaine may be involved in this potentiation. Billman (3) has suggested that cardiotoxic effects of cocaine may be mediated by stimulation of phospholipase C and mobilization of Ca^{+2} due to enhanced inositol phosphate formation by the hydrolysis of phosphoinositides. It is possible that acute cocaine treatment enhances NE-stimulated PI hydrolysis by stimulating the phospholipase C activity or, by affecting the coupling of α_1-adrenergic receptors to the G-protein or phospholipase C enzyme without affecting the α_1-adrenergic receptor number. However, the present studies do not distinguish between these potential mechanisms.

The attenuation of acute cocaine-induced NE-stimulated PI hydrolysis in cortical slices by pretreatment with a single high dose or repeated administration of cocaine is in agreement with a recent study in peripheral tissue; specifically, it was reported that repeated administration of cocaine reduced the NE-stimulated inositol accumulation in rat thoracic aorta (25). These authors also reported that aortic contraction was also reduced in rats chronically treated with cocaine. These effects of cocaine may involve desensitization of α_1-receptor function. While a decrease in α_1-receptor number could result in attenuation of PI hydrolysis, repeated administration of cocaine has been reported to either increase (1) or not affect (21) adrenergic receptors. Also, cocaine does not affect the affinity constant of prazosin binding for α_1-adrenoceptors (25), suggesting that this may not be the primary mechanism. On the other hand, chronic administration of cocaine has been shown to decrease G-protein in rat brain (13). Because α-receptor-mediated PI hydrolysis is G-protein dependent (23), it is possible that pretreatment with a single high dose or repeated low dose cocaine results in attenuation of acute cocaine-induced PI hydrolysis through a decrease in G-protein coupling. However, further studies are needed to resolve these possibilities.

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