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EFFECT OF MONOAMINE UPTAKE INHIBITORS ON NOREPINEPHRINE-STIMULATED PHOSPHATIDYLINOSITOL HYDROLYSIS IN RAT CORTEX

MAHMOOD MOSADDEGHI, JOSEPH M. MOERSCHBAECHER and RUEBEN A. GONZALES*
Department of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112, U.S.A.

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Abstract—The effects of the monoamine uptake inhibitors cocaine, nisoxetine, and desipramine (DMI) on norepinephrine (NE) stimulated phosphatidylinositol (PI) hydrolysis were investigated. Rat cortical tissue slices were labeled with [^{3}H]inositol. Slices were then stimulated, *in vitro*, with NE in LiCl containing buffer in the presence and absence of monoamine uptake inhibitors. Cocaine and nisoxetine, but not DMI, potentiated NE-stimulated PI hydrolysis with a significant decrease in the EC_{50}. Nisoxetine appeared to be more potent than cocaine with respect to the potentiation of NE-stimulated PI hydrolysis. The potentiating effect of cocaine was biphasic and dependent upon the concentrations of cocaine and NE. The NE concentration–effect curve was shifted to the right 100-fold in the presence of 0.1 μM prazosin. Cocaine at 10 μM did not potentiate NE-stimulated PI hydrolysis in the presence of 0.1 μM prazosin. Cocaine at 10 μM did not affect significantly the binding of [^{3}H]prazosin or the NE–[^{3}H]prazosin competition binding to cortical membranes. The results suggest that NE-uptake inhibition by cocaine and nisoxetine is the mechanism for the enhancement of NE-stimulated PI hydrolysis.

Recent studies in a variety of cell types have indicated that receptor-activated hydrolysis of phosphoinositides (PI†) is an early event in the transduction of hormone and neurotransmitter signals [1]. Hydrolysis of these lipids by a PI specific phospholipase C (PLC) is coupled to the activation of neuronal receptors such as alpha-1 adrenergic and muscarinic cholinergic receptors. The products of PLC hydrolysis, namely, diacylglycerol (DG) and inositol trisphosphate (IP_3), may serve as intracellular second messengers [2].

Monoamine uptake inhibitors may exert their effect in the central nervous system (CNS) by increasing the concentration of neurotransmitter in the synaptic cleft, therefore increasing the duration of action of the neurotransmitter as well as the intensity of post-synaptic receptor stimulation. Various pharmacological effects are thought to depend on the inhibition of the uptake of specific monoamine neurotransmitters. For example, uptake inhibition of norepinephrine (NE) may contribute to the antidepressant effect of desipramine (DMI), whereas uptake inhibition of dopamine (DA) by cocaine may be responsible for the reinforcing effect of the drug [3]. It has been documented that NE has agonist activity at the alpha-1 adrenoceptor and that the activation of this receptor is coupled to the hydrolysis of phosphoinositides [4]. However, the effect of monoamine uptake inhibitors on NE-stimulated PI hydrolysis has not been well characterized. The present study was designed to investigate the effects of cocaine, nisoxetine, and DMI on NE-stimulated PI hydrolysis. Previous data have shown that prazosin, a selective alpha-1 adrenergic receptor blocker, inhibits the locomotor stimulant effect of cocaine [5]. Therefore, the possible interaction between prazosin and cocaine *in vitro* was also investigated.

The results of our experiments indicate that cocaine and nisoxetine, but not desipramine, potentiated NE-stimulated PI hydrolysis in the rat cerebral cortical tissue slices. The enhancement of NE-stimulated PI hydrolysis by cocaine was dependent not only on the NE concentration but also on the cocaine concentration. This enhancement of alpha-1 adrenergic stimulation linked to PI hydrolysis may be relevant to understanding the locomotor stimulant activity and/or abuse potential of cocaine.

MATERIALS AND METHODS

PI hydrolysis assay. The procedure of Berridge *et al.* [6] was followed as described by Gonzales and Crews [4]. Male Sprague–Dawley rats of 180–250 *g* were decapitated, and their brains were excised. The cerebral cortex was isolated immediately and placed on the platform for slice preparation. The brain tissue slices were then prepared with a McIlwain tissue slicer set at 350 μm. The plate was rotated 90° and the tissue was sliced again. The slices were separated and washed at 37° for 15–20 min with Krebs–Ringer bicarbonate buffer (KRB) (118 mM NaCl, 4.7 mM KCl, 0.75 mM $CaCl_2$, 1.18 mM

* Address correspondence to: Dr. Rueben A. Gonzales, Department of Pharmacology, College of Pharmacy, University of Texas at Austin, Austin, TX 78712.

† Abbreviations: PI, phosphoinositide; NE, norepinephrine; KRB, Krebs–Ringer bicarbonate buffer; IP_3, inositol triphosphate; DG, diacylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; and DMI, desipramine.

KH_2PO_4, 1.18 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 10 mM glucose) which had been oxygenated with O_2/CO_2 (95:5) to give a final pH of 7.4.

A volume of 5 ml of the brain tissue slices in KRB was gassed, capped, and incubated with 20 μCi of *myo*-[2-^{3}H]inositol (sp. act. 17–19 Ci/mmol) in a shaking water bath (37° and 70 oscillations/min) in a conical tube. Pargyline, a non-selective monoamine oxidase inhibitor, was added in the last 30 min of the labeling period at a final concentration of 50 μM. The labeling was terminated after 1 hr by removing excess KRB, and suspending the slices three times in 30 ml KRB for 5 min each. The slices were allowed to settle by gravity and the buffer was decanted.

Aliquots (50 μl) of the tissue slices were added to 12 × 75 mm polypropylene tubes which contained 180–200 μl KRB including 10 mM LiCl isotonically substituted for NaCl. Norepinephrine, prazosin and/or cocaine solutions were added in volumes of 10 μl to yield final volumes of 250 μl. The tubes were preincubated with monoamine uptake inhibitor or antagonist for 10 min prior to addition of the agonist. The tubes were then gassed, capped, and incubated at 37° for another 45 min. The phospholipid hydrolysis was stopped by adding 1.0 ml of chloroform/methanol (1:2 v/v), 0.35 ml chloroform, and 0.35 ml deionized water. The lipids were extracted by shaking for 10 min, and the phases were separated by centrifugation for 10 min at 2000 rpm in an IEC tabletop centrifuge.

An aliquot (750 μl) of the upper aqueous phase, which contained [^{3}H]inositol and its water-soluble metabolites, was removed. The inositol phosphates were separated by ion exchange chromatography. Bound [^{3}H]inositol phosphates were eluted from Dowex 1-X8 (formate form) with 6 ml of 1.0 M ammonium formate/0.1 M formic acid, and the radioactivity was determined. A portion (200 μl) of the lower, lipid-containing phase was also removed, the chloroform was allowed to evaporate, and radioactivity was determined by liquid scintillation spectrometry. Data are expressed as a ratio of [^{3}H]inositol phosphates released (dpm from column) to the total [^{3}H]inositol incorporated into the phospholipids (dpm from column + dpm in chloroform).

[^{3}H]*Prazosin binding.* Rats were decapitated and the cerebrums were removed and placed in the ice-cold 50 mM Tris–hydrochloride buffer. The cerebral cortex was dissected on ice and homogenized in 40 vol. (w/v) of the ice-cold Tris buffer for 30 sec using a Tekmar Tissumizer set at 50 output. The homogenate was centrifuged at 39,200 *g* for 10 min at 4°. The pellet was resuspended in Tris buffer to give a final protein concentration of 0.1 to 0.2 mg/ml. Aliquots (50 μl) of the tissue suspension were added to 13 × 100 mm glass tubes that contained 10 μl cocaine (10 μM, final concentration) and/or phentolamine solution (10 μM final concentration, non-specific binding). The competition binding reaction, performed in triplicate, was initiated by adding NE and [7-*methoxy*-^{3}H]prazosin (83 Ci/mmol), and the mixture was incubated in a shaking water bath for 60 min at 37°. Specific binding of [^{3}H]prazosin was shown initially to have reached equilibrium by 60 min and was linear with protein over the concentration range used. The reaction was terminated by adding 4 ml of cold buffer and filtering under vacuum followed by two washes of 4 ml each through Whatman GF/B glass microfiber filters. Filters were placed in plastic scintillation vials containing 6 ml of Beckman Ready-Solv EP scintillation fluid. The vials were shaken for 1 hr and counted in a Beckman LS 7500 scintillation spectrometer. Specific binding was determined by subtracting binding in the presence of phentolamine from total binding. Equilibrium dissociation constants were obtained by linear regression of Scatchard plots [7]. Protein content was determined by the method of Lowry *et al* [8].

Drugs and chemicals. [^{3}H]Inositol and [^{3}H]prazosin were purchased from Amersham (Arlington Heights, IL). Norepinephrine and pargyline were purchased from the Sigma Chemical Co. (St. Louis, MO). The authors would like to thank The Research Technology Branch of NIDA (Rockville, MD), Ciba-Geigy (Suffern, NY), and Eli Lilly & Co. (Indianapolis, IN) for providing some of the drugs used in these studies.

Statistical procedures. The significance of the differences between control and monoamine uptake inhibitor treated dose–effect curves was assessed initially by performing one-way or two-way ANOVA. Where a significant difference was demonstrated, further analysis was undertaken using the Newman–Keuls multiple comparison procedure. The significance of differences between EC_{50} values was compared using a paired *t*-test or ANOVA when appropriate.

RESULTS

Cortical tissue slices were incubated with 3 μM NE for various times, and the agonist-stimulated PI hydrolysis was examined in the absence and presence of 10 μM cocaine. The accumulation of inositol phosphates stimulated by NE was linear for at least 45 min (Fig. 1). Cocaine (10 μM) *in vitro* significantly potentiated NE-stimulated PI hydrolysis between 40 and 60 min (two-way ANOVA, $F = 22.9$, $df = 1,30$; $P < 0.05$). Based upon these data a 45-min stimulation period was chosen for all the experiments, unless otherwise indicated.

Further experiments were performed to determine the effects of cocaine *in vitro* on the NE concentration–effect curve for the stimulation of PI hydrolysis. Slices of cortex incubated with 1 and 10 μM cocaine in the presence of various NE concentrations caused an increase in the maximum PI hydrolysis by 15 and 30%, respectively, in addition to a shift to the left of the NE dose–response curve (Fig. 2). The EC_{50} for NE-stimulated PI hydrolysis was shifted from 3.93 ± 0.42 μM for control to 2.39 ± 0.24 and 1.91 ± 0.31 μM in the presence of 1 and 10 μM cocaine respectively ($P < 0.01$, two-way ANOVA, $F = 10.3$, $df = 2,11$; $P < 0.01$ for 10 μM cocaine, Newman–Keuls test). The effects of various concentrations of cocaine with 0.3 and 3 μM NE were also investigated. The data indicate that the enhancement of NE-stimulated PI hydrolysis by cocaine was dependent on the concentrations of both NE and cocaine (Fig. 3). In the presence of 0.3 μM NE, concentrations from 0.1 to 100 μM of cocaine

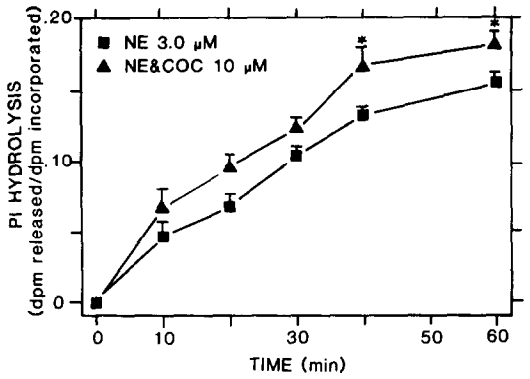

Fig. 1. Effect of cocaine on the time course of NE-stimulated PI hydrolysis. Labeled cortical tissue slices were incubated with NE (3.0 μM) and cocaine for various times at 37° in a lithium-containing buffer. Accumulation of [^{3}H]inositol phosphates was determined by chromatography of aqueous extracts on Dowex-1 columns (see Materials and Methods). Results are means ± SEM of triplicate incubations from three experiments. Asterisks indicate $P < 0.05$ by Newman–Keuls test.

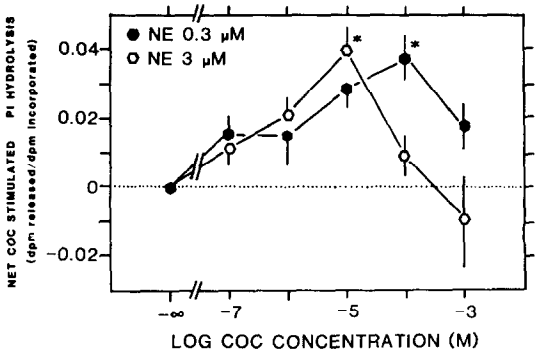

Fig. 3. Concentration–effect curve for cocaine on NE-stimulated PI hydrolysis in rat cortical slices. PI hydrolysis was determined as described in the legend of Fig. 1. Net potentiation was calculated as the difference between NE responses in the presence and absence of cocaine. Results are means ± SEM of six experiments. Asterisks indicate $P < 0.05$ by Newman–Keuls test.

significantly potentiated the PI hydrolysis response *in vitro* (0.04 ± 0.007 dpm released/dpm incorporated above control at 100 μM cocaine). Concentrations above 100 μM reversed the enhancement of the PI response in the presence of 0.3 μM NE. When the level of stimulation of PI hydrolysis was increased by addition of 3 μM NE, a similar concentration-dependent biphasic effect of cocaine was observed except that the maximal effect of cocaine was obtained at 10 μM. Thus, the concentration-dependence of cocaine-induced potentiation of NE-stimulated PI hydrolysis is influenced by the level of stimulation by NE.

To determine if other NE uptake inhibitors [nisoxetine and desmethylimipramine (DMI)] may potentiate NE-stimulated PI hydrolysis in cortical slices, various concentrations of NE were added to slices in the presence or absence of the uptake inhibitor, and concentration–effect curves were constructed. Figure 4 indicates that, like cocaine, at 0.1 μM nisoxetine also potentiated NE-stimulated PI hydrolysis. Nisoxetine caused a significant shift to the left of the concentration–effect curve for NE-stimulated PI hydrolysis with a decrease in the EC_{50} from 2.54 ± 0.72 μM for control to 1.04 ± 0.35 μM ($P < 0.05$, paired *t*-test, N = 3) and a 12% increase in the maximum response. In contrast to the effects of cocaine and nisoxetine, DMI at concentrations of 0.1 and 1.0 μM did not affect significantly the concentration–effect curve for NE-stimulated PI hydrolysis (data not shown).

Further experiments were undertaken to determine if prazosin, an alpha-1 adrenergic selective

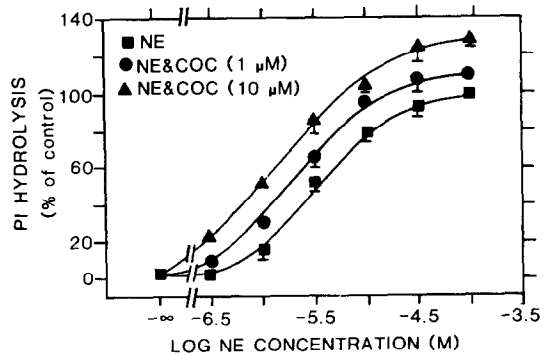

Fig. 2. Effect of cocaine on the concentration–effect curve for NE-stimulated PI hydrolysis in rat cortical slices. PI hydrolysis in the absence or presence of 1 or 10 μM cocaine was determined as described in the legend of Fig. 1. Results are means ± SEM of four experiments. The control curve was significantly different from the 10 μM cocaine curve by two-way ANOVA, $F = 7.22$, $df = 2, 63$; $P < 0.05$. The basal and the maximum NE-stimuated PI hydrolysis values, in the absence of cocaine, were 0.04 ± 0.01 and 0.17 ± 0.02 dpm released/dpm incorporated respectively.

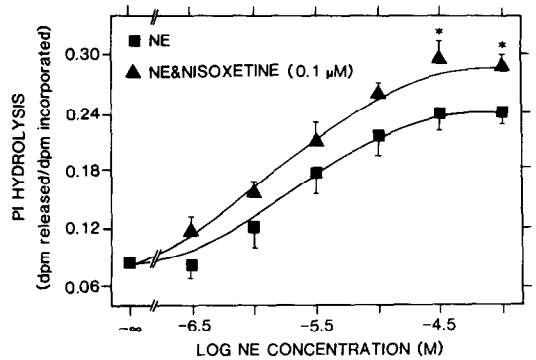

Fig. 4. Effect of nisoxetine on the concentration–effect curve for NE-stimulated PI hydrolysis in cortical tissue slices. PI hydrolysis in the absence and presence of 0.1 μM nisoxetine was determined as described in the legend of Fig. 1. Results are means ± SEM of four experiments. The control curve was significantly different from the 0.1 μM nisoxetine curve by two-way ANOVA, $F = 14.5$, $df = 1, 28$: $P < 0.01$. Asterisks indicate $P < 0.05$ by Newman–Keuls test.

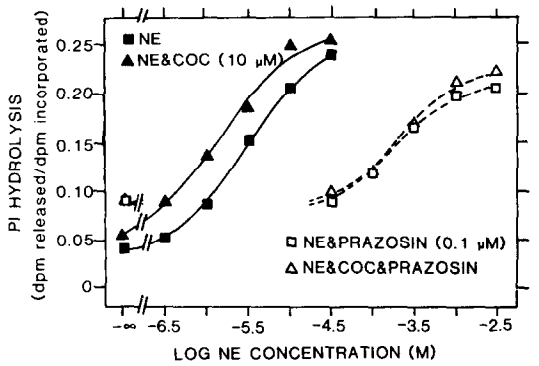

Fig. 5. Effect of prazosin on NE-stimulated PI hydrolysis in the absence and presence of cocaine. Prazosin and cocaine were incubated with tissue slices for 10 min before adding NE. Results were obtained as described in the legend of Fig. 1. Means from three experiments are presented.

antagonist, could alter the ability of cocaine to potentiate NE-stimulated PI hydrolysis. In agreement with previous findings, 0.1 μM prazosin shifted the NE concentration–effect curve for NE-stimulated PI hydrolysis to the right by 100-fold (Fig. 5). The dose-ratio analysis showed that the equilibrium dissociation constant (K_d) of prazosin was 0.33 nM. Cocaine (10 μM) shifted the NE concentration curve up and to the left as indicated in Fig. 5. However, in the presence of prazosin, cocaine was not able to shift significantly the NE concentration–effect curve to the left.

To determine whether the local anesthetic properties of cocaine may contribute to the potentiation

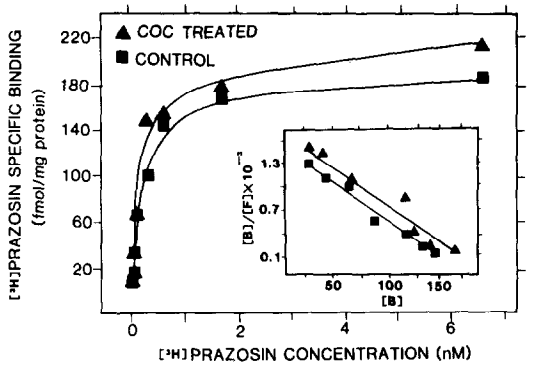

Fig. 6. Effect of cocaine on [^{3}H]prazosin binding to crude membrane preparations. Cortical membrane homogenates were prepared as described in Materials and Methods. Membranes (160–200 μg protein) were incubated with [^{3}H]prazosin in the absence and presence of 10 μM cocaine and Tris–hydrochloride buffer, pH 7.4, at 37° for 60 min. Radioactivity bound to the membranes was separated from ligand from ligand by filtration as described in Materials and Methods. Data are presented as femtomoles of [^{3}H]prazosin specifically bound per milligram of protein. Insert: Scatchard analysis of binding of [^{3}H]prazosin to membranes. [B] is represented as femtomoles [^{3}H]prazosin bound per milligram of protein, and [B]/[F] is the bound-to-free concentration ratio.

Table 1. Effects of cocaine on [^{3}H]prazosin binding to rat cerebral cortical membranes

Treatment	K_d (nM)	B_{max} (fmol/mg protein)
Control	0.21 ± 0.17	168 ± 39
Cocaine (10 μM)	0.16 ± 0.09	204 ± 22

Membrane homogenates were incubated with drugs as described in Materials and Methods. The data are the means ± SEM of three experiments, each performed in triplicate.

of NE-stimulated PI hydrolysis, the effects of procaine, a local anesthetic that does not block NE uptake, were investigated. Procaine at concentrations of 0.1 to 1000 μM did not affect the basal or NE-stimulated PI hydrolysis (data not shown).

To investigate the possibility that the effect of cocaine on NE-stimulated PI hydrolysis may be mediated through a direct interaction with alpha-1 adrenergic receptors, the binding of [^{3}H]prazosin to cortical membranes in the presence and absence of cocaine *in vitro* was assayed. Scatchard analysis of saturation experiments for [^{3}H]prazosin binding to cortical membranes indicated that [^{3}H]prazosin bound to a single class of binding site with a K_d of 0.21 nM and a B_{max} of 168 fmol/mg protein in agreement with previously published values and the K_d of prazosin inhibition of NE-stimulated PI hydrolysis data (Fig. 6, Table 1) [9]. Cocaine (10 μM) *in vitro* did not affect significantly the binding of [^{3}H]prazosin to rat cortical membranes (Table 1). In addition, the effect of cocaine on NE competition for [^{3}H]prazosin binding to cortical membranes was studied to determine if cocaine interfered with agonist binding to alpha-1 adrenergic receptors. Control competition curves indicated that the K_i for NE inhibition of [^{3}H]prazosin binding to cortical membranes was 4.6 μM with a Hill slope of 0.74 (Fig. 7, Table 2). The presence of 10 μM cocaine did not

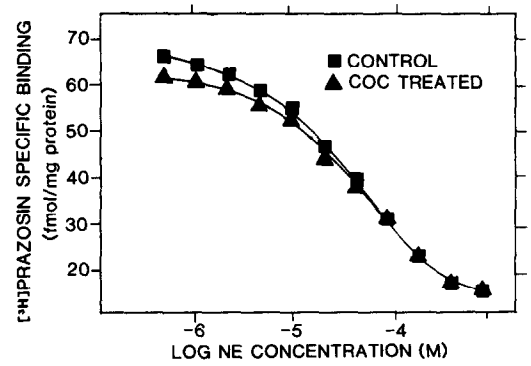

Fig. 7. Effect of cocaine on [^{3}H]prazosin–NE competition curve. The crude membranes were incubated with various concentrations of NE and 0.5 nM [^{3}H]prazosin in the absence or presence of 10 μM cocaine under experimental conditions described in the legend of Fig. 6. The data presented are the means of four experiments, each performed in triplicate.

Table 2. Effects of cocaine on norepinephrine competition for [^{3}H]prazosin binding to rat cortical membranes

Treatment	IC_{50} (μM)	Hill slope
Control	4.6 ± 0.3	0.74 ± 0.17
Cocaine (10 μM)	4.7 ± 1.4	0.74 ± 0.13

[^{3}H]Prazosin was incubated with various concentrations of NE in the absence (control) or presence of cocaine (cocaine). The data are the means ± SEM of at least four experiments, each performed in triplicate.

affect significantly either the K_i or the Hill slope for NE inhibition of [^{3}H]prazosin binding to cortical membranes (Table 2). These results suggest that cocaine did not exert its effects on NE-stimulated PI hydrolysis through a direct interaction with an alpha-1 adrenergic receptor.

DISCUSSION

Previous *in vitro* studies provide substantial evidence that exogenous NE can stimulate the metabolism of inositol phospholipids and that this effect is mediated through alpha-1 adrenoceptors [10]. The purpose of our study was to investigate the effect of monoamine uptake inhibitors, namely cocaine, nisoxetine, and DMI, on NE-stimulated PI hydrolysis. Results of *in vitro* experiments indicate that cocaine and nisoxetine indeed potentiated NE-stimulated PI hydrolysis. This suggests that the active uptake mechanism may limit the ability of NE to stimulate inositol phosphate (IP) accumulation in cerebral cortical slices. This may also indicate that the central effects of cocaine may be mediated, in part, through an interaction with NE-stimulated PI hydrolysis.

The maximum response to NE-stimulated PI hydrolysis was increased in the presence of cocaine or nisoxetine. The increased maximal responses after the blockade of neuronal uptake by either cocaine or nisoxetine may be explained in terms of the concentration-gradient hypothesis [11]. According to this hypothesis, there is an NE concentration gradient present in a tissue slice with the minimal NE concentration in the central regions of a cortical tissue slice compared to the maximal concentration in the external regions. Depending on the regional concentration of NE, the active presynaptic uptake system may limit the stimulant activity of the agonist at the postsynaptic alpha-1 adrenoceptors. Therefore, the inhibition of the NE uptake system by an uptake inhibitor at the central regions of tissue slices may contribute to the increase in the maximal response (as compared to the regions with high NE concentrations in which the uptake system does not limit NE-stimulated PI hydrolysis).

The aim of the present study was also to compare the potencies of cocaine, nisoxetine, and DMI on NE-stimulated PI hydrolysis. Previous work has demonstrated that nisoxetine is a potent and selective inhibitor of NE uptake in rat brain slices and synaptosomes [12, 13]. Aside from the ability of DMI to inhibit neuronal uptake of monoamines, the drug also acts as an antagonist at alpha-1 receptors [14, 15]. Nisoxetine lacks the marked alpha-1 adrenoceptor blocking activity of DMI [16]. Our finding that both cocaine and nisoxetine, but not DMI, were able to potentiate NE-stimulated PI hydrolysis correlates well with observations that these drugs are more selective monoamine uptake inhibitors and lack alpha adrenoceptor blocking effects [17]. Nisoxetine appeared to be 10-fold more potent than cocaine with respect to potentiation of NE-stimulated PI hydrolysis (compare Fig. 2 to Fig. 5). However, DMI at 0.1 μM did not potentiate NE-stimulated PI hydrolysis. Higher concentrations of DMI (10 and 100 μM) caused depression of NE-stimulated PI hydrolysis in a concentration-dependent manner (data not shown). The negative effect of DMI may be due to its alpha-1 adrenergic blocking properties.

The effects of cocaine on NE-stimulated PI hydrolysis were dependent on both the level of stimulation by exogenous NE and the concentration of cocaine added *in vitro*. Approximately 10 μM cocaine was the maximally effective concentration for the potentiation of NE-stimulated PI hydrolysis in the presence of either 0.3 or 3 μM NE. These concentrations of NE represent the threshold and EC_{50} values for the stimulation of PI hydrolysis by NE alone. However, concentrations of cocaine above 10 μM had no significant effect on PI hydrolysis stimulated by 3 μM NE. In contrast, 100 μM cocaine was able to enhance the PI response in the presence of 0.3 μM NE. Further increases in the cocaine concentration at this level of NE stimulation were less effective. This loss of effectiveness of cocaine to potentiate NE-stimulated PI hydrolysis is not due to the local anesthetic effect of cocaine. This conclusion is supported by experiments which show that procaine, a local anesthetic with no NE uptake inhibitory properties, did not affect NE-stimulated PI hydrolysis in either the presence or absence of cocaine. In addition, cocaine alone at concentrations up to 1 mM had no effect on the basal PI hydrolysis. PI hydrolysis expressed as dpm released/dpm incorporated in the presence of buffer compared to 100 μM and 1000 μM cocaine was 0.03 ± 0.01, 0.04 ± 0.01, and 0.02 ± 0.01 respectively. It is possible, however, that concentrations of cocaine above 10 μM may directly inhibit events that occur distal to the activation of the alpha-1 receptor by NE. Further experiments are necessary to clarify the mechanism of the biphasic effects of cocaine on NE-stimulated PI hydrolysis.

Cocaine did not potentiate NE-stimulated PI hydrolysis in the presence of 0.1 μM prazosin. This may be due to the use of high concentrations of NE at which the monoamine uptake system is saturated. The B_{max} value for the control membranes was not significantly different from cocaine-treated membranes, indicating that cocaine did not alter the number of [^{3}H]prazosin binding sites. This finding is in agreement with the results of Ritz *et al.* [3]. Cocaine did not have any effect on agonist binding sites (Fig. 7). These data provide indirect evidence that NE uptake inhibition by cocaine is probably the mechanism for the enhancement of NE-stimulated PI hydrolysis.

The results of experiments presented here suggest that some of the central effects of cocaine may be mediated, in part, through the enhancement of NE-stimulated PI hydrolysis. The *in vitro* concentrations of cocaine which cause potentiation of NE-stimulated PI hydrolysis correspond to those seen after *in vivo* administration in doses that cause behavioral changes [18]. There is evidence that stimulation of alpha-1 adrenoceptors may be involved in mediating the locomotor stimulant effect of cocaine and that the stimulant effect of the drug is attenuated or blocked by prazosin [5, 18]. The mechanisms underlying the ability of prazosin to antagonize the locomotor-stimulant effect of cocaine are not understood completely. Furthermore, it has been demonstrated repeatedly that central administration of direct alpha-1 adrenergic agonist can stimulate locomotor activity in rodents [19, 20]. The possible mechanisms and sites of alpha-1 adrenergic receptors which may be involved in the locomotor stimulant effect of cocaine are unknown. It is interesting to note, however, that the effects of cocaine on schedule-controlled behavior in the rat are not antagonized by prazosin.

In summary the results of our experiments indicate that cocaine and nisoxetine, probably through NE uptake inhibition, potentiated the alpha-1 adrenoceptor stimulation of PI hydrolysis in cortical slices. The correlation between these biochemical changes and ultimate behavioral changes due to cocaine abuse remains to be clarified.

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