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Short communication

Effect of cocaine on responsiveness of α_1-adrenergic receptors in rat cerebral cortex: modulation by GABA-mimetic drugs

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Abstract:
We investigated the effects of single doses of cocaine (10 mg/kg, *ip*) and the γ-aminobutyric acid (GABA)-mimetics tiagabine (10 mg/kg, *ip*) and vigabatrin (150 mg/kg, *ip*) injected separately or concomitantly with cocaine, on the responsiveness of cerebral cortical α_1-adrenergic receptors. The accumulation of noradrenaline-stimulated inositol phosphates was estimated *in vitro* at 2 and 24 h after the drug injection. Cocaine significantly enhanced α_1-adrenergic receptor responsiveness to noradrenaline. Neither tiagabine nor vigabatrin influenced the accumulation of inositol phosphates. Finally, the cocaine-evoked augmentation of α_1-adrenoceptor responsiveness was counteracted by tiagabine but not by vigabatrin. This effect may represent a characteristic feature of tiagabine, not necessarily shared by other GABA-mimetic drugs.

Key words:
α_1-adrenergic receptor, inositol phosphate, rat cerebral cortex, cocaine, tiagabine, vigabatrin

Abbreviations: GABA – γ-aminobutyric acid, GABA-T – GABA transaminase, *ip* – intraperitoneal, PLC – phospholipase Cβ

Introduction

The noradrenergic system, in concert with other neurotransmitter circuits, is involved in regulation of brain activity and thus modulates various biological phenomena, including general arousal, reactions to stress exposure [7] and memory processes [18]. Noradrenaline governs the system through three main classes of adrenergic receptors: β, α_2 and α_1. The α_1 class consists of three subtypes: α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors. All three subtypes are coupled with Gq/11 protein and phospholipase Cβ (PLC), which stimulates breakdown of phosphoinositides to produce two second messengers: diacylglycerol and inositol trisphosphate [21]. Accumulation of inositol phosphates in response to stimulation can be used as a measure of α_1-adrenoceptor reactivity [14]. The α_1-adrenergic receptors have previously been shown to be engaged in the effects of antidepressant drugs (reviewed in [12]). Moreover, our recent study indicated that these receptors are also involved in the mechanism of action of cocaine, as the phenomenon

of cocaine sensitization was associated with changes in α_1-adrenergic receptor density in certain regions of rat brain [15].

Cocaine alters synaptic transmission by blocking monoamine transporters and inhibiting the uptake of monoamines (dopamine, noradrenaline and serotonin) into neurons. The drug belongs to a class of highly addictive psychostimulants that affect the brain reward circuit, where the mesocorticolimbic dopamine system appears to be the specific anatomical site for the rewarding action of cocaine [11]. Activity of the dopaminergic system is modulated by various neurotransmitters including γ-aminobutyric acid (GABA) (reviewed in [1]). As an enhancement of GABA neurotransmission results in inhibition of dopamine release, GABA-mimetics such as tiagabine and vigabatrin are among the drugs considered for the treatment of cocaine dependence [6, 10]. Moreover, differential manipulation of GABA receptors ($GABA_B$) has been shown to modify behaviors relevant to depression and anxiety [5].

Some evidence exists for an interaction between GABA and the α_1-adrenergic system that occurs at the level of intracellular signaling. Results of *in vitro* experiments from Crawford and Young [2] indicated that GABA was able to modulate the response of cerebral α_1-adrenergic receptors to noradrenaline by means of an augmentation of inositol phosphate accumulation. Hence, this study aimed at examining the effects of GABA-mimetics (tiagabine and vigabatrin), injected separately and concomitantly with cocaine, on the reactivity of cerebral cortical α_1-adrenergic receptors, estimated by measuring noradrenaline-induced inositol phosphate accumulation.

Materials and Methods

Animals and treatment

The experiments were conducted on male Wistar rats weighing 240–280 g that were kept under standard animal house conditions with free access to food and water. Animals were treated with a single dose of cocaine (10 mg/kg, *ip*) that was administered separately or after pretreatment with tiagabine (10 mg/kg, *ip*, administered 30 min before cocaine) or vigabatrin (150 mg/kg, *ip*, 2 h before cocaine). Control groups received a saline injection instead of cocaine. Animals were decapitated at two time-points, 2 and 24 h after the last injection of the drug, and their brains taken out and cerebral cortex dissected.

All experiments were carried out according to the NIH Guidelines for Care and Use of Laboratory Animals and were approved by the 2nd Local Ethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Drugs

Cocaine hydrochloride and noradrenaline (DL-norepinephrine hydrochloride) were purchased from Sigma, Aldrich, USA. Tiagabine hydrochloride (Gabitril, Qualiti Burnley Limited, Great Britain) and vigabatrin (Sabril, Marion Merrell S.A., France) were suspended in a 0.5% solution of Tween 80 (Serva Feinbiochemika, Heidelberg, Germany).

Assessment of [^{3}H]inositol phosphate accumulation

The inositol phosphate assay was carried out according to the protocol described previously [13]. In the present experiment, the washing of Dowex was further modified to completely remove *myo*-[^{3}H]inositol.

In brief, the cerebral cortical tissue was sliced with a McIlwain tissue chopper (0.3 mm prism), and the slices were suspended in glucose-containing modified Krebs-Ringer buffer (118 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 11 mM glucose, pH = 7.4) at 37°C gassed with 95% O_2 : 5% CO_2. After preincubating for 1 h with three changes of buffer, portions of 50 µl of the preincubated, density-packed slices were pipetted into flat-bottom plastic vials containing 200 µl of the buffer, 30 µl of LiCl solution (final concentration of 5 mM) and 20 µl purified *myo*-[^{3}H]-inositol solutions (1 mCi/ml). The vials were gassed with 95% O_2 : 5% CO_2, capped, and shaken at 37°C for 30 min, and then 10 µl of noradrenaline (final concentrations of 1, 5, 10, and 100 µM) was added to the medium. The incubation was continued for 45 min and was stopped by the addition of 1 ml of a mixture of chloroform: methanol (1/2; v/v). To further separate the phases, 530 µl of chloroform and 530 µl of water were added, and the mixture was centrifuged for 10 min at 13,000 × g. A portion of 1 ml of the upper aqueous phase was transferred into vials contain-

ing 2.5 ml of water and 0.5 ml of a 50% (w/v) slurry of Dowex (1X8-400, formate form, Sigma-Aldrich). After four washes with 5-ml of 5 mM aqueous *myo*-inositol solution and three washes with 5 ml borax solution with sodium formate, the phosphates were eluted with 600 μl of a mixture of 1 M ammonium formate in 0.1 M formic acid. The final eluates were tested for radioactivity in a liquid scintillation counter (Beckman LS 6500, [^{3}H]-channel). All samples were tested in triplicate.

Statistical analysis

Data were expressed as the mean stimulation over basal level (fold). EC_{50} values were calculated from a sigmoid curve using GraphPad software. Statistical analyses were performed using: one-way (EC_{50} values) ANOVA, two-way ANOVA with treatment (saline or cocaine treated groups) and noradrenaline concentration as factors, and three-way ANOVA, with pretreatment (tiagabine or vigabatrine) and treatment (saline or cocaine) and time (2 h or 24 h after the cocaine injection) as factors (Statistica 5.0 software). Data were deemed significant at $p < 0.05$.

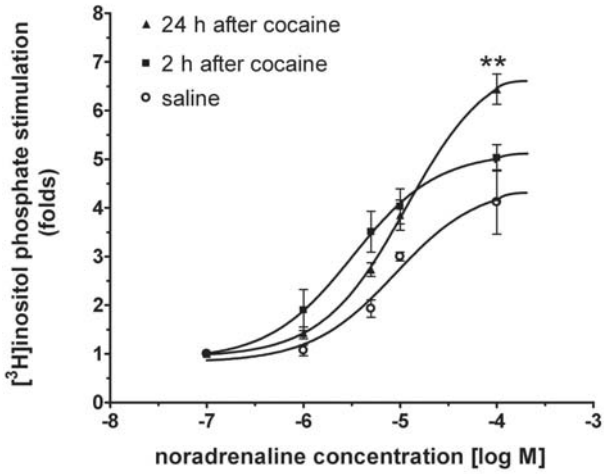

Fig. 1. The effect of cocaine on noradrenaline-induced inositol phosphate accumulation in rat cortical slices, measured 2 and 24 h after drug injection. The data represent net stimulation expressed as fold changes over basal levels, which were 1047 ± 99 d.p.m. 2 h after saline treatment, 917 ± 73 d.p.m. 2 h after cocaine treatment, and 1051 ± 165 24 h after cocaine treatment. The points are the means of 5 experiments performed in triplicate. Two-way ANOVA showed a significant effect of cocaine treatment [$F(2, 48) = 14.88$, $p < 0.001$], a significant main effect of noradrenaline concentration [$F(3, 48) = 70.18$, $p < 0.001$], and a significant interaction (treatment x noradrenaline concentration) [$F(6, 48) = 2.82$, $p < 0.05$]. The asterisks denote a significant difference ($p < 0.01$) between saline and cocaine-treated groups (*post-hoc* LSD test)

Results and Discussion

Injection of cocaine caused an enhancement of inositol phosphate accumulation in response to noradrenergic stimulation *in vitro* (Fig. 1). The response of the α_1-adrenoceptors to a maximal concentration of noradrenaline (100 μM) used in the experiment was higher in the cocaine-treated groups (122% and 156% of the saline group level, respectively) and statistically different in the case of the group assessed 24 h after cocaine administration. Moreover, there was a left shift in the noradrenaline concentration-inositol phosphate response curve for the cocaine-treated group tested 2 h after drug injection, with the EC_{50} approximately 3 times lower than the value for the cocaine-treated animals analyzed 24 h after drug administration (Tab. 1).

The cocaine-evoked augmentation of the α_1-adrenoceptors' responsiveness was counteracted by tiagabine but not by vigabatrin, and the effect was similarly marked at both time-points: 2 h (Fig. 2A) and 24 h (Fig. 2B) after the cocaine injection. Therefore, the accumulation of inositol phosphates induced with 100 μM noradrenaline in cerebral cortical slices of rats pretreated with tiagabine prior to cocaine injection was not different from the saline control. Tiagabine or vigabatrin injections did not substantially influence the responsiveness of α_1-adrenoceptors; there was only a non-significant increase in the accumulation of inositol phosphates (about 15% *vs.* saline group) induced by both drugs. Nonetheless, in the case of vigabatrin, the change was still observed after 24 h.

Tab. 1. EC_{50} values for saline and cocaine-injected groups

Treatment	EC_{50} [μM] ± SEM
Saline	9.15 ± 2.7
2 h after cocaine	3.92 ± 1.7
24 h after cocaine	11.83* ± 1.6

EC_{50} values were calculated from individual noradrenaline concentration-inositol phosphate response curves (n = 5 for each treatment group). Asterisk denotes a significant difference between cocaine groups analyzed 2 and 24 h after the drug injection [$F(1, 2) = 3.65$, $p = 0.057$, one-way ANOVA]; * $p < 0.05$ (Fisher's LSD test)

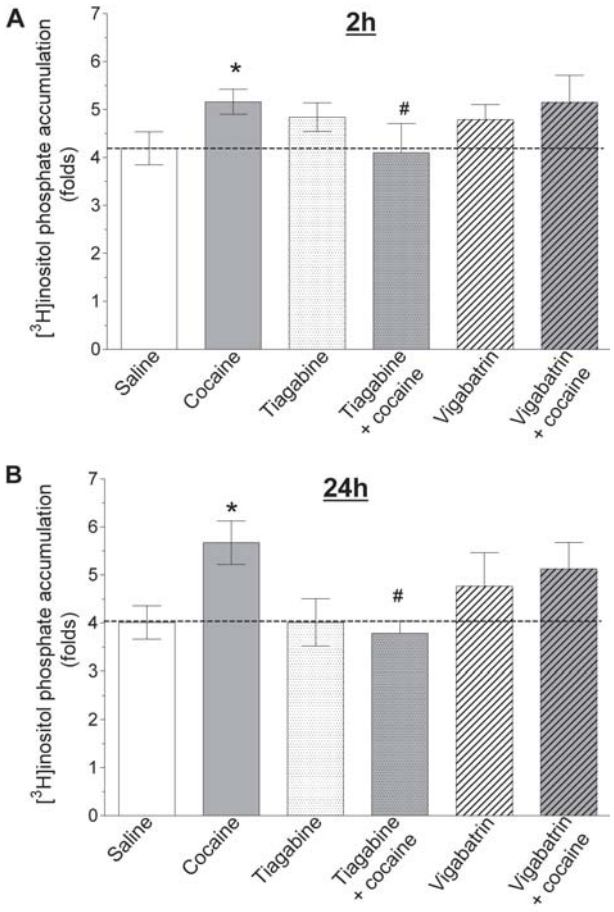

Fig. 2. The effect of pretreatment with tiagabine or vigabatrin on cocaine-evoked changes in the inositol phosphate response stimulated *in vitro* with 100 μM noradrenaline in rat cerebral cortical slices. **(A)** 2 h and **(B)** 24 h after the last drug injection. The bars are the means ± SEM of 4–10 animals. Three-way ANOVA showed a significant pretreatment x treatment interaction [$F(2, 55) = 4.45$, $p < 0.05$]. Individual comparisons were carried out with the *post-hoc* unequal N test of interaction. * $p < 0.05$ *vs.* saline control; # $p = 0.0582$ *vs.* cocaine treated group. The dashed line indicates the level of the saline control

The mechanism responsible for the cocaine-induced augmentation of α_1-adrenoceptor responsiveness may be related to the increase in endogenous noradrenaline levels resulting from the blockade of noradrenergic transporter by cocaine. In fact, the drug was reported to have an approximately equal affinity to all three monoamine (dopamine, noradrenaline and serotonin) transporters (see [8]). Moreover, an interaction between dopamine and noradrenaline circuits was shown in the medial prefrontal cortex of rat, where these neurotransmitters reciprocally increased each other's release [17]. Thus, the increase in dopamine level that results from cocaine-induced inhibition of dopamine reuptake may further enhance the availability of noradrenaline in a synapse and cause stronger stimulation of the α_1-adrenoceptors. However, as the increase in neurotransmitter levels evoked by monoamine transporter blockade is transient (lasting approximately 2–3 h), mechanisms other than noradrenaline-uptake inhibition by cocaine may be involved in the drug-induced potentiation response of inositol phosphates observed after 24 h (present study). Among the possible (adaptive) mechanisms, changes in the activity of PLC or in the coupling of α_1-adrenoceptors to G proteins may be considered. Although the present study does not distinguish between these potential mechanisms, our data corroborate the results of other authors [9].

Our findings showing that tiagabine given prior to cocaine eliminated the cocaine-induced augmentation of the noradrenaline-stimulated inositol phosphate generation suggest that the increase in brain GABA somehow modulates the activity of α_1-adrenoceptors. This could result from GABA action either downstream of α_1-adrenoceptors by reducing accumulation of Ca^{2+} ions in the neuronal cells and decreasing PLC activity, or upstream through inhibition of noradrenergic neuron activity. The latter is prompted by data coming from electrophysiological experiments showing that local GABA application decreased the firing activity of noradrenergic neurons in the locus coeruleus [20].

Tiagabine is a potent and specific inhibitor of GABA uptake into neurons and glial cells [19], whereas vigabatrin inhibits GABA-transaminase (GABA-T), the enzyme that converts GABA to succinate [16]. Although the result of both drugs is an increase in extracellular GABA content, it is important to note that GABA-mimetics have diverse effects on cocaine-evoked self-administration and discriminative stimulus effects in rats [4].

In the present study, vigabatrin did not have the same effect as tigabine, and instead showed no influence on cocaine-evoked potentiation of the noradrenaline-stimulated inositol phosphate response. The reason for the discrepancy in the effects of these two drugs is not clear in light of their GABA-mimetic action. One can only hypothesize that the acute dose of vigabatrin used in this study was insufficient for effective blockade of GABA-T action. In such a case, the resulting increase in synaptic GABA levels (if any) might be too small to enable inhibition of the cocaine-induced effect. On the other hand, the lack of an effect of vigabatrin may instead be related to its

complicated pharmacological properties. It was shown that in addition to inhibitory action on GABA-T, vigabatrin exhibits a complex interaction with the GABA transporter, and, at a certain range of doses, the drug can also be taken up by the transporter as a "false" neurotransmitter, thereby reducing the release of GABA [3].

In summary, we show that although cocaine increases α_1-adrenergic receptor responsiveness to noradrenaline, enhanced GABA-ergic neurotransmission may inhibit this effect. However, this inhibitory influence may be a unique feature of tiagabine, as it was not produced by a different GABA-mimetic drug, vigabatrin.

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