ELSEVIER

European Journal of Pharmacology 367 (1999) 125–130

ejp

Cocaine decreases the glycine-induced Cl^- current of acutely dissociated rat hippocampal neurons

Jun Ren [a], Jiang Hong Ye [a,b,*], Philip L. Liu [a,b], Krešimir Krnjević [c], Joseph J. McArdle [a,b]

[a] *Department of Anesthesiology, New Jersey Medical School (UMDNJ), 185 South Orange Avenue, Newark, NJ 07103-2714, USA*
[b] *Department of Pharmacology and Physiology, New Jersey Medical School (UMDNJ), 185 South Orange Avenue Newark, NJ 07103-2714, USA*
[c] *Anaesthesia Research Department, McGill University, McIntyre Medical Building, 3655 Drummond Street, Montréal, QC Canada H3G 1Y6*

Received 2 November 1998; accepted 18 December 1998

Abstract

The effects of cocaine on glycine-induced Cl^- current (I_{GLY}) of single neurons, freshly isolated from the rat hippocampal CA1 area, were studied with conventional whole-cell recording under voltage-clamp conditions. Cocaine depressed I_{GLY} in a concentration-dependent manner, with an IC_{50} of 0.78 mM. Preincubation with 1 mM cocaine alone had no effect on I_{GLY}, suggesting that resting glycine channels are insensitive to cocaine. The depression of I_{GLY} by cocaine was independent of membrane voltage. Internal cell dialysis with 1 mM cocaine failed to modify I_{GLY}. Because the depression of I_{GLY} was noncompetitive, cocaine may act on the glycine receptor–chloride ionophore complex at a site distinct from that to which glycine binds. The cocaine suppression of I_{GLY} was unaffected by 1 μM tetrodotoxin and 1 μM strychnine. Blockers of protein kinase C (Chelerythrine), kinase A (*N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide HCl, (H-89)) and Ca-calmodulin-dependent kinase (1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62)) were also ineffective, which suggests that these phosphorylating mechanisms do not modulate cocaine-induced suppressant action on I_{GLY}. This extracellular, strychnine-independent depression of I_{GLY} may contribute to cocaine-induced seizures.

Keywords: Cocaine-induced seizure; Whole-cell recording; Glycine receptor; Strychnine-insensitive depression; Voltage-insensitive depression; Phosphorylation-insensitive depression

1. Introduction

Despite many investigations, the mechanisms underlying the toxic and sometimes fatal consequences of cocaine abuse remain unclear. Because the psychostimulant and euphoric effects of cocaine are associated with blockade of dopamine uptake in the central nervous system, several investigators have examined the possible role of dopaminergic mechanisms in cocaine-induced seizures and death though it is known that cocaine can induce convulsions even after severe depletion of dopamine from rat brain (Mason and Corcoran, 1979).

Besides its depressant effect on dopamine uptake, cocaine is a local anesthetic, which blocks voltage-dependent Na channels (Khodorov et al., 1976; Wang, 1988). At high blood concentrations, other local anesthetics, including lidocaine, also produce seizures (Post and Weiss, 1989). Accordingly, the convulsant actions of cocaine could be related to its local anesthetic properties (Post and Weiss, 1989). However, in low doses, cocaine and other local anesthetics are *anticonvulsant* (Fischer et al., 1992; Zhu et al., 1992, 1993). Though high concentrations of local anesthetics are neurotoxic, equivalent doses of tetrodotoxin (a particularly selective Na channel blocker), are not. These data suggest that local anesthetic neurotoxicity does *not* result from blockade of voltage-gated Na channels (Sakura et al., 1995). Cocaine's local anesthetic properties therefore cannot account for its ability to induce seizures.

Inhibitory neurotransmission may be a relevant target of cocaine. For example, cocaine inhibits γ-aminobutyric acid (GABA) release in the ventral tegmental area (Cameron and Williams, 1994). It also depresses $GABA_A$-induced inhibitory postsynaptic potentials of CA1 pyramidal neu-

* Corresponding author. Department of Anesthesiology, New Jersey Medical School (UMDNJ), 185 South Orange Avenue, Newark, NJ 07103-2714, USA. Tel.: +1-973-972-4399; Fax: +1-973-972-4172; E-mail: ye@umdnj.edu

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00954-6

rons (Dunwiddie et al., 1988), and directly depresses postsynaptic GABA-induced current of hippocampal neurons (Ye et al., 1997a,b,c). Repeated injections of cocaine decrease the function of striatal $GABA_A$ receptors (Peris, 1996). Furthermore, central nervous system depressants, such as benzodiazepines and barbiturates can prevent and treat seizures induced by local anesthetics, including cocaine (Derlet and Albertson, 1990; Barat and Abdel-Rahman, 1996).

Glycine is another inhibitory neurotransmitter. Immunological and molecular cloning studies have revealed that the glycine receptor is widely distributed not only in the spinal cord and brain stem, but throughout the mammalian central nervous system (Betz, 1991). For example, glycine receptor mRNA has been found in several brain regions, including the cerebral cortex and the hippocampus (Malosio et al., 1991). According to Hara et al. (1995), another local anesthetic, lidocaine, reduces currents evoked by exogenous inhibitory neurotransmitters applied to dissociated hippocampal neurons; I_{GLY} was more sensitive than I_{GABA}.

The aim of the present study is to determine whether cocaine decreases I_{GLY}, whether any such effect is direct or mediated by blocking Na channels and whether it is modulated by phosphorylation. A possible interaction between cocaine and strychnine was also investigated in order to explore the site of cocaine's action on glycine receptors.

2. Methods

2.1. Isolation of neurons

Hippocampal pyramidal neurons were prepared as described previously (Ye et al., 1997a). The 7- to 17-day old Sprague–Dawley rats were decapitated and their brains quickly excised and placed into iced 'standard external solution' containing (in mM) NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and HEPES 10 (pH was adjusted to 7.4 with Tris base, and the osmolarity to 320 mosM with sucrose). To avoid possible interference by a general anesthetic, no anesthetic was given prior to decapitation. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

The isolated brain was glued to the chilled stage of a vibratome (Campden Instrument, England) and sliced to a thickness of 400 μm. The slices were then incubated at 31°C in buffer containing 1 mg pronase/6 ml for 20 min, before incubation in 1 mg thermolysine/6 ml for an additional 20 min. Micro-punches of the hippocampus CA1 area were then isolated, transferred to a 35-mm culture dish and subjected to mild trituration through heat polished pipettes of progressively smaller tip diameter, in order to isolate single neurons. Within 20 min of this procedure, isolated neurons had attached to the bottom of the culture dish and were ready for electrical recording.

2.2. Whole-cell recording

Membrane currents were recorded (Axopatch 1D, Axon Instruments, Foster City, CA, USA) with conventional whole-cell technique. pCLAMP software (Axon Instruments) executed voltage clamp protocols and acquired data. The junction potential was nulled immediately before each seal was made in whole-cell recording. Routinely, 80% of the series resistance was compensated, resulting in approximately 3 mV error for a 1 nA current. The patch electrode had a resistance of 3–5 MΩ when filled with the standard internal solution, containing (in mM): CsCl 120, tetraethylammonium chloride (TEACl) 21, $MgCl_2$ 4, $CaCl_2$ 1, EGTA 11, HEPES 10, ATP 2, and GTP 0.1; pH was 7.2. Given these internal and external solutions, the calculated equilibrium potential for chloride ions was 0 mV. All experiments were performed at room temperature (20–23°C).

2.3. Drugs

Solutions of glycine, strychnine, tetrodotoxin, protease (type XIV, P5147), thermolysine (Type X, P1512) (Sigma, St. Louis, MO), *N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide HCl (H-89), chelerythrine, and 1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62) (Calbiochem, San Diego, CA, USA) were prepared on the day of recording. Drug solutions were applied to dissociated neurons via a superfusion system having a multi-barreled pipette (as described previously by Ye et al. (1997a)). The tip of the superfusion pipette was usually 50 to 100 μm away from the cell, a position which allowed rapid and uniform drug application, without loss of the cell's mechanical stability. Complete exchange of solutions in the vicinity of the recorded neuron, took less than 20 ms. Throughout all experimental procedures, the bath was perfused with the standard external solution.

2.4. Statistics

Experimental values are presented as mean ± standard error of the mean (SEM). Means were compared by Student's *t*-test, $p < 0.05$ being considered significant. To evaluate the half-maximal effective concentration of glycine (EC_{50}) and the Hill coefficient (n), the Michaelis–Menten equation ($I/I_{max} = 1/(1 + (EC_{50}/C)^n)$) was fit to the data by the method of least-squares. In the equation, I is the observed I_{GLY}, I_{max} the maximum current, C is the glycine concentration and n is the Hill coefficient. To evaluate the cocaine concentration giving half-maximal inhibition of I_{GLY} (IC_{50}) and the Hill coefficient (n), the mirror image of the Michaelis–Menten equation ($I/I_{max} = 1/(1 + (C/IC_{50})^n)$) was fit to the data by

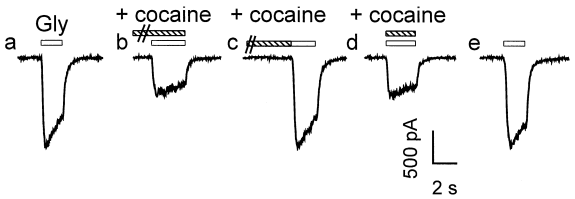

Fig. 1. Co-application of cocaine and glycine inhibited glycine-induced current, while preincubation with cocaine had no effect on I_{GLY}. (a) Current in response to 30 μM glycine alone. (b) Co-application of 1 mM cocaine and glycine depressed I_{GLY} after 1 min preincubation with 1 mM cocaine. (c) The 1-min preincubation with 1 mM cocaine had no effect on I_{GLY} in response to glycine applied alone. (d) Co-application of 1 mM cocaine and glycine, without cocaine preincubation, depressed I_{GLY}. (e) I_{GLY} recovered after 2 min wash. Note: All records are from the same neuron. Holding potential was −50 mV.

the least-squares method. In this equation, C is the antagonist (cocaine) concentration.

3. Results

3.1. Cocaine decreases glycine-induced currents of hippocampal neurons

Glycine (1–1000 μM) elicited a current (I_{GLY}) in about 85% of the hippocampal neurons examined, with a threshold concentration of 3 to 10 μM. I_{GLY} reversed direction near 0 mV, the calculated equilibrium potential for chloride ions. When applied alone, cocaine (0.01–6 mM) had no detectable effect on membrane current. When co-applied with glycine, cocaine depressed I_{GLY} (Fig. 1). In contrast, when co-applied with kainate (200 μM), 1 mM cocaine had no appreciable effect on kainate-induced current, though it depressed I_{GLY} in the same neurons (data not shown). As illustrated in Fig. 2, cocaine decreased I_{GLY} in a concentration-dependent manner, with an IC_{50} of 0.78 mM.

Cocaine (1 mM) effectively suppressed I_{GLY} evoked by subsaturating (Fig. 3A-a) and saturating (Fig. 3A-b) concentrations of glycine (Fig. 3). This is further demonstrated by the concentration–response plots obtained in the ab-

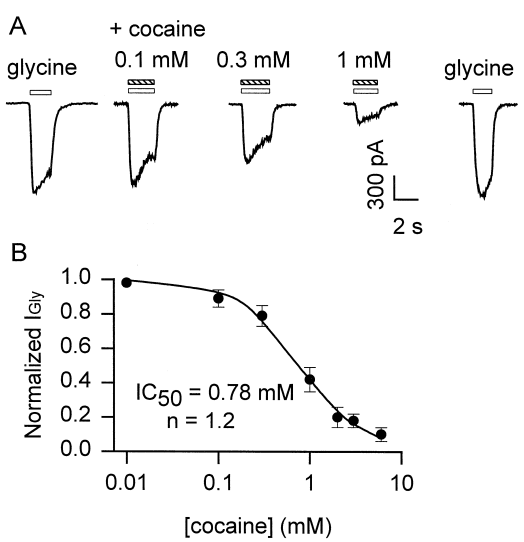

Fig. 2. Cocaine decreases glycine-induced current in a concentration-dependent manner. (A) Representative whole-cell current response of a hippocampal neuron to 30 μM glycine (empty bars), or in combination with 0.1 mM, 0.3 mM, or 1 mM cocaine (stippled bars). Holding potential was −50 mV. (B) Concentration–response relation for cocaine suppression of I_{GLY}. Normalized peak current in response to 30 μM glycine plus various concentrations of cocaine is plotted as a function of the cocaine concentration; peak current was first normalized to the value obtained in response to 30 μM glycine alone. Each data point is the mean ± SEM of five to six neurons. The IC_{50} and Hill coefficient were 0.78 mM and 1.2, respectively.

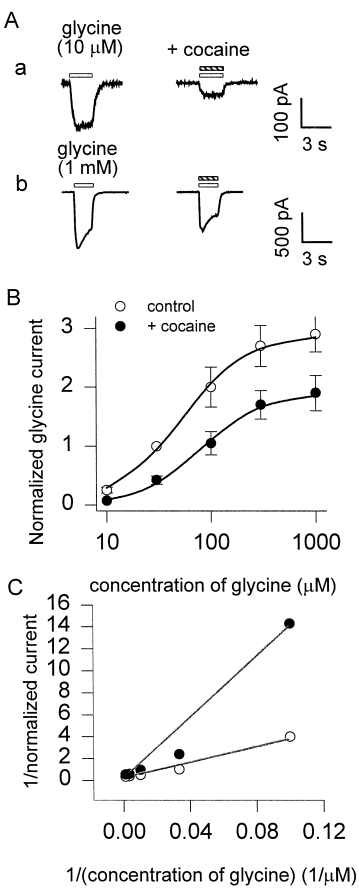

Fig. 3. Cocaine decreased glycine-induced current over wide range of glycine concentrations. (A) The records are representative current traces in response to 10 μM (A-a) and 1 mM (A-b) glycine recorded from a hippocampal neuron in the absence (empty bars) and presence (stippled bars) of 1 mM cocaine. All records are from the same cell. Holding potential was −50 mV. (B) Concentration–response relationships for glycine (10–1000 μM) in the absence (○) and in the presence of 1 mM cocaine (●). Peak I_{GLY} was plotted as a function of glycine concentration. Each data point represents the mean of five to seven neurons. For each cell, the data were normalized to the current evoked by 30 μM glycine in the absence of cocaine. The EC_{50} and Hill coefficient were 51 μM and 1.4, respectively, for glycine alone; 80 μM and 1.4, respectively, for glycine plus 1 mM cocaine. (C) Double-reciprocal plot of cocaine suppression of I_{GLY}, suggesting a noncompetitive inhibition.

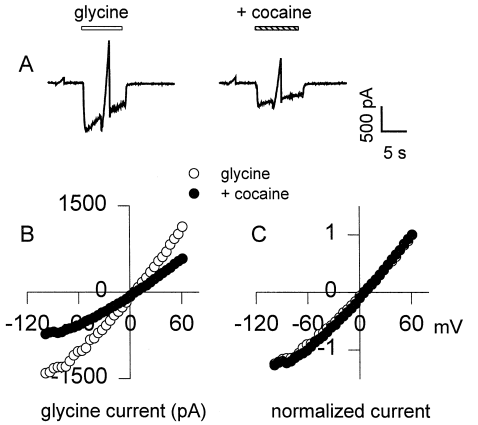

Fig. 4. Cocaine suppression of glycine-induced current is voltage-independent. The suppressant effect of cocaine on the current–voltage relationship of I_{GLY} was studied with voltage ramps (1 mV/ms) ranging from −110 to +60 mV. Drugs were applied to the cell and covered the second ramp of each pair. Traces obtained from the first ramp served as background (or leakage current). The difference current–voltage curves were obtained by subtracting the current trace produced by the first ramp from that produced by the second ramp. (A) Typical I_{GLY} recorded from a neuron exposed to 30 μM glycine alone (left) and in combination with 1 mM cocaine (right). (B) Current–voltage curves derived from A, show that cocaine suppressed I_{GLY} at all potentials without changing its reversal potential. (C) To determine any voltage dependence, currents recorded in control (○) and in the presence of cocaine (●) were first normalized to the values obtained at 60 mV. The superimposed normalized current–voltage relations from the same experiment of (B) show that suppression of I_{GLY} by cocaine is voltage-independent.

sence and presence of cocaine (Fig. 3B). The EC_{50} for glycine in the absence and presence of 1 mM cocaine was 51 and 80 μM ($n = 4$), respectively. The Lineweaver–Burke double reciprocal plot in Fig. 3C suggests that cocaine does not compete with glycine.

To explore the voltage-dependence of cocaine's suppressant effect, we studied the current–voltage relations of I_{GLY} in the absence and presence of cocaine. Fig. 4 shows cocaine's effect was not altered over a wide range of

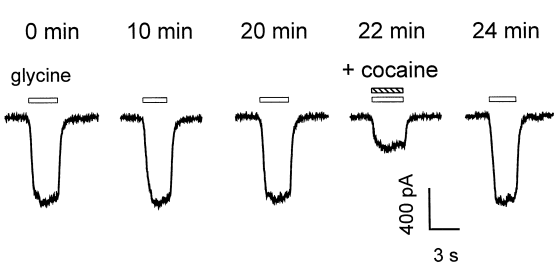

Fig. 5. Effect of internal cell dialysis with cocaine on glycine-induced current. 30 μM glycine (empty bars) was repeatedly applied at 2 min intervals over a period of 30 min immediately after gaining the whole-cell configuration. The patch pipette contained 1 mM cocaine, holding potential was −50 mV. As can be seen, internal cell dialysis with cocaine did not affect I_{GLY}. After 20 min recording of the cocaine-loaded cell, an additional *extracellular* application of 1 mM cocaine (stippled bar) resulted in the inhibition of I_{GLY}. The current recovered to control level after 2 min washout of cocaine containing superfusate.

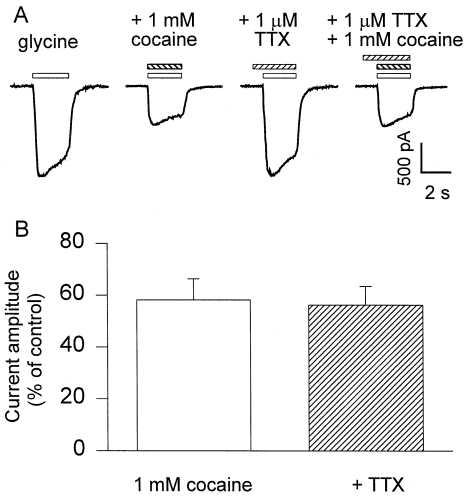

Fig. 6. Tetrodotoxin (TTX) effect on cocaine inhibition of glycine-induced current. (A) Representative whole-cell current responses to 30 μM glycine alone (empty bars), or in combination with 1 mM cocaine (stippled bars). External application of 1 μM tetrodotoxin (stippled bars) had no effect on I_{GLY}; cocaine still depressed I_{GLY} in the presence of TTX (stippled bars). Holding potential was −50 mV. (B) Cocaine depressed I_{GLY} to the same extent in the absence and the presence of 1 μM tetrodotoxin. Each column represents the mean ± SEM ($n = 5$).

membrane voltage. In addition, the reversal potential of I_{GLY} remained close to equilibrium potential for chloride ions, indicating that the glycine-activated channels remained selectively permeable to Cl^-. Similar results were obtained from four other neurons.

To further determine whether the site of action of cocaine is intracellular or extracellular, experiments were carried out with cocaine-containing microelectrodes. Immediately after the start of whole-cell recording, I_{GLY} was repeatedly elicited by brief pulses of glycine (30 μM), applied at 2-min intervals (Fig. 5). After 20 min of cell dialysis with 1 mM cocaine, the amplitude of I_{GLY} did not significantly differ from that observed just after rupturing the membrane ($n = 5$). By contrast, an additional *extracellular* application of 1 mM cocaine to the cocaine-containing neurons decreased I_{GLY} by 46 ± 8% ($n = 5$), an amount similar to the 44 ± 7% ($n = 5$, $p > 0.10$) observed in neurons recorded with the standard pipette solution.

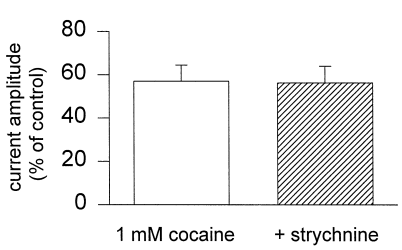

Fig. 7. Strychnine effect on cocaine inhibition of glycine-induced current. Cocaine depressed I_{GLY} to the same extent in the absence and in the presence of 1 μM strychnine. Each column represents the mean ± SEM ($n = 5$).

3.2. Interactions between cocaine and tetrodotoxin or strychnine

To explore whether cocaine's effect on I_{GLY} is related to its local anesthetic properties (Khodorov et al., 1976; Wang, 1988), we studied the effect of tetrodotoxin (a more selective Na channel blocker) on the cocaine-mediated suppression of I_{GLY}. As can be seen in Fig. 6, in the presence of 1 μM tetrodotoxin, 1 mM cocaine decreased I_{GLY} by $42 \pm 6\%$ ($n = 4$), which is *not* significantly different from the $45 \pm 7\%$ ($p > 0.10$, $n = 4$) depression seen in the control tests.

Strychnine is a glycine receptor antagonist and is a convulsant (Curtis, 1967). To explore whether cocaine acts at the strychnine-binding site on the glycine receptor–Cl^- channel complex, we compared cocaine's action in the absence and presence of strychnine. As illustrated by Fig. 7, in the presence of 1 μM strychnine, cocaine decreased I_{GLY} by $44 \pm 6\%$ ($n = 5$), an amount *not* significantly different from the $42 \pm 6\%$ ($p > 0.10$, $n = 5$) observed in the control tests.

3.3. PKC-, PKA- or CaMKII-mediated phosphorylation is not involved in the cocaine-induced depression of I_{GLY}

To explore a possible role of glycine receptor phosphorylation, we studied the effects of chelerythrine (a protein kinase C inhibitor), H-89 (a cAMP-dependent protein kinase inhibitor), and KN-62 (a Ca^{2+}/calmodulin-dependent protein kinase inhibitor) on the cocaine-induced reduction of I_{GLY}. There are numerous examples of modulation of receptor or ion channel function via mechanisms that are unrelated to their effects on cellular pathways regulating receptor phosphorylation and dephosphorylation, e.g., low affinity interactions with various external binding sites on the receptor (Lambert and Harrison, 1990). To circumvent this, we took advantage of conventional whole-cell recording, which allowed us to directly apply agents inside the cells, near the sites where phosphorylation or dephosphorylation takes place. As shown in Fig. 8, after the neurons were dialyzed with 1 μM chelerythrine, 1 μM H-89, or 20 μM KN-62 for 30 min, I_{GLY} remained stable. In cells dialyzed with chelerythrine, H-89, and KN-62, respectively, external applications of 1 mM cocaine resulted in decreases of I_{GLY} by $42 \pm 7\%$ ($n = 5$), $45 \pm 6\%$ ($n = 4$), $46 \pm 7\%$ ($n = 5$)—amounts *not* significantly different from the $41 \pm 6\%$ depressions observed in the control tests ($n = 7$).

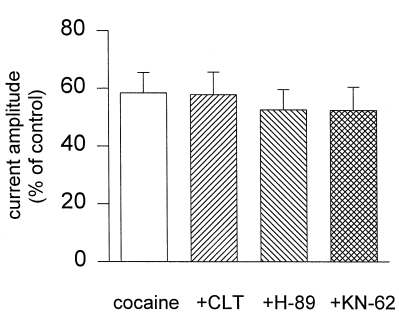

Fig. 8. Effects of intracellular application of chelerythrine (CLT, 1 μM), H-89 (1 μM), and KN-62 (20 μM) on cocaine inhibition of glycine (30 μM)-activated currents. Each column represents the mean ± SEM of five to seven neurons.

4. Discussion

In this study of isolated hippocampal neurons, we examined the depressant effect of cocaine on I_{GLY} evoked by exogenous glycine. We observed that the depression of I_{GLY} by cocaine is concentration-dependent, with an IC_{50} of 0.78 mM. This is similar to the 0.67 mM IC_{50} for cocaine inhibition of 10 μM GABA-induced currents of hippocampal neurons, recorded with conventional whole-cell technique (Ye et al., 1999). Both GABA and glycine are inhibitory neurotransmitters in the central nervous system (Betz, 1991). Other local anesthetics, such as lidocaine, also reduce currents elicited by inhibitory transmitters which can induce seizures (Post and Weiss, 1989). It is therefore likely that reduction of glycine- and GABA-induced currents contributes to the mechanism of cocaine-induced seizures.

In their resting state, the glycine-controlled channels were not affected by preincubation in the presence of cocaine. This contrasts with our recent report that preincubation with cocaine caused much stronger suppression of I_{GABA} (in nystatin-perforated whole-cell recordings) than when cocaine was co-applied with GABA (Ye et al., 1997a). This effect of cocaine on resting GABA receptor-channels warrants further study. Some factors, such as protein kinases mediating cocaine's action on resting channels may be washed out in conventional whole-cell recording. On the other hand, there may be a significant difference in this regard between receptors for GABA and for glycine.

As in the case of GABA receptors, the effect of cocaine on glycine receptors was voltage-independent (Fig. 4). This suggests that the site of cocaine's action is not inside the channel or near its inner opening, in keeping with the lack of effect of internally-applied cocaine. Cocaine must therefore act at an extracellular site, by a mechanism different from its voltage-dependent block of Na channels having relevant binding sites which are probably inside the channel or close to the cytoplasm (Khodorov et al., 1976; Wang, 1988). The lack of interaction between tetrodotoxin and cocaine is further evidence that cocaine acts directly on I_{GLY}, by a mechanism distinct from its local anesthetic site of action.

Since 1 mM cocaine suppresses the maximal I_{GLY}, cocaine inhibition of I_{GLY} is noncompetitive, probably affecting the glycine receptor–chloride ionophore complex

at a site distinct from that to which glycine binds. According to a previous study (Vandenberg et al., 1992), the binding sites for glycine and strychnine are mutually interactive and possibly overlapping. In our study, however, cocaine decreased I_{GLY} to the same extent in the absence and the presence of strychnine, which suggests that cocaine and strychnine bind to independent sites on the glycine receptor/channel complex.

Changes in phosphorylation provide an important means of regulating the function of ligand-gated ion channels (Walaas and Greengard, 1991; Raymond et al., 1993). PKC, PKA and CaMKII modulate the phosphorylation of glycine receptors (Vaello et al., 1994; Wang and Randic, 1996). In a previous study (Ye et al., 1997b), we observed that phosphorylation can modulate the cocaine-induced suppression of I_{GABA}. In the present experiments, the depressant effect of cocaine on I_{GLY} was not altered by chelerythrine, H-89, or KN-62, drugs that block PKC, PKA and CaMKII activity, respectively. Thus, the cocaine-induced suppression of I_{GLY} may be independent of the phosphorylation state of glycine receptors.

In conclusion, cocaine has a direct inhibitory action on postsynaptic glycine receptors, at an extracellular site distinct from the binding site for glycine and strychnine. The suppression of I_{GLY}, a current activated by an important inhibitory synaptic transmitter, may contribute to the mechanism of cocaine-induced seizures.

Acknowledgements

This research was supported by a UMDNJ Foundation Grant to J.H.Y. The authors gratefully acknowledge Rebecca Schaefer's assistance in editing the text.

References

Barat, S.A., Abdel-Rahman, M.S., 1996. Cocaine and lidocaine in combination are synergistic convulsants. Brain Res. 742, 157–162.

Betz, H., 1991. Glycine receptors: heterogeneous and widespread in the mammalian brain. Trends Neurosci. 14, 458–461.

Cameron, D.L., Williams, J.T., 1994. Cocaine inhibits GABA release in the VTA through endogenous 5-HT. J. Neurosci. 14 (11), 6763–6767.

Curtis, D.R., 1967. Acetylcholine, strychnine and spinal inhibition. Nature 215, 1503.

Derlet, R.W., Albertson, T.E., 1990. Anticonvulsant modification of cocaine-induced toxicity in the rat. Neuropharmacology 29, 255–259.

Dunwiddie, T.V., Proctor, W.R., Tyma, J., 1988. Local anesthetic actions of cocaine: effects on excitatory and inhibitory synaptic responses in hippocampus in vitro. Brit. J. Pharmacol. 95, 1117–1124.

Fischer, W., Bodewei, R., Satzinger, G., 1992. Anticonvulsant and sodium channel blocking effects of ralitoline in different screening models. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 442–452.

Hara, M., Kai, Y., Ikemoto, Y., 1995. Local anesthetics reduce the inhibitory neurotransmitter-induced current in dissociated hippocampal neurons of the rat. Eur. J. Pharmacol. 283, 83–89.

Khodorov, B., Shishikova, L., Peganov, E., Revenko, S., 1976. Inhibition of Na currents in frog Ranvier node treated with local anesthetics: role of slow Na inactivation. Biochem. Biophys. Acta 433, 409–435.

Lambert, N.A., Harrison, N.L., 1990. Analogs of cyclic AMP decrease γ-aminobutyric acidA receptor-mediated chloride current in cultured rat hippocampal neurons via an extracellular site. J. Pharmacol. Exp. Ther. 255, 90–94.

Malosio, M.L., Marqueze-Pouey, B., Kuhse, J., Betz, H., 1991. Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. EMBO J. 10, 2401–2409.

Mason, S.T., Corcoran, M.E., 1979. Catecholamines and convulsions. Brain Res. 170, 497–507.

Peris, J., 1996. Repeated cocaine injections decrease the function of striatal γ-aminobutyric acid A receptors. J. Pharmacol. Exp. Ther. 276 (3), 1002–1008.

Post, R.M., Weiss, S.R.B., 1989. Psychomotor stimulant vs. local anesthetic effects of cocaine: role of behavioral sensitization and kindling. In: Clouet, D., Asghat, K., Brown, R. (Eds.), Mechanisms of Cocaine Abuse and Toxicity. US Dept. of Health and Human Services, Washington, DC, pp. 217–238.

Raymond, L.A., Blackstone, C.D., Huganir, R.L., 1993. Phosphorylation of amino acid neurotransmitter receptors in synaptic plasticity. Trends Neurosci. 16, 147–153.

Sakura, S., Bollen, A.W., Ciriales, R., Drasner, K., 1995. Local anesthetic neurotoxicity does not result from blockade of voltage-gated Na channels. Anesth. Analg. 81, 338–346.

Vaello, M.L., Ruiz-Gomez, A., Lerma, J., Mayor, F. Jr., 1994. Modulation of inhibitory glycine receptors by phosphorylation by protein kinase C and cAMP-dependent protein kinase. J. Biol. Chem. 269, 2002–2008.

Vandenberg, R.J., Handford, C.A., Schofield, P.R., 1992. Distinct agonist- and antagonist-binding sites on the glycine receptor. Neuron 9, 491–496.

Wallas, S.I., Greengard, P., 1991. Protein phosphorylation and neuronal function. Pharmacol. Rev. 43, 299–349.

Wang, G.K., 1988. Cocaine-induced closures of single batrachotoxin-activated Na^+ channels in planar lipid bilayers. J. Gen. Physiol. 92, 747–765.

Wang, R.A., Randic, M., 1996. α-Subunit of CaM-KII increases I_{GLY}s in acutely isolated rat spinal neurons. J. Neurophysiol. 75, 2651–2653.

Ye, J.H., Liu, P.L., Wu, W.-H., McArdle, J.J., 1997a. Cocaine depresses $GABA_A$ current of hippocampal neurons. Brain Res. 770, 169–175.

Ye, J.H., Ren, J., Krnjević, K., Liu, P.L., 1997b. Phosphorylation mediates cocaine's depressant effect on $GABA_A$ current. Soc. Neurosci. Abstr. 23, 2411.

Ye, J.H., Ren, J., Liu, P.L., 1997c. Cocaine suppresses the inhibitory neurotransmitter-activated current of rat central nervous system neurons. Anesthesiology V 87 (3A), A736.

Ye, J.H., Ren, J., Krnjević, K., Liu, P.L., McArdle, J.J., 1999. Cocaine and lidocaine have additive inhibitory effects on the $GABA_A$ currents of acutely dissociated hippocampal pyramidal neurons. Brain Res., in press.

Zhu, Y., Im, W.-B., Lewis, R.A., Von Voigtlander, P.F., 1992. Comparison of pyrrolidinyl and piperidinyl benzamides for their anticonvulsant activity and inhibitory action on sodium channel. Br. J. Pharmacol. 106, 49–54.

Zhu, Y., Im, W.-B., Lewis, R.A., Althaus, J.S., Cazers, A.R., Nielsen, J.W., Palmer, J.R., Von Voigtlander, P.F., 1993. Two metabolites of anticonvulsant U-54494A: their anticonvulsant activity and interaction with Na channel. Brain Res. 606, 50–55.