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Short communication

The anesthetics propofol and ketamine inhibit cocaine-induced *egr-1* gene expression in rat forebrain

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Abstract

Acute cocaine injection to rats is known to induce the expression of immediate early genes in the forebrain, the effect being primarily mediated by the dopaminergic system. We examined the effect of the anesthetics ketamine and propofol on cocaine-induced *egr-1* mRNA expression. Using in situ hybridization, we show that both compounds did not induce *egr-1* gene by themselves, but were able to dose-dependently reduce cocaine-induced *egr-1* mRNA synthesis in the nucleus accumbens, caudate-putamen and cingulate cortex. Our data suggest that in addition to glutamate NMDA receptors, propofol may act via $GABA_A$ receptors or ion channels.

Keywords: Cocaine; Ketamine; Propofol; Immediate early gene; *egr-1*; Dopamine; Forebrain, rat

1. Introduction

Cocaine inhibits the uptake of dopamine, serotonin and noradrenaline into presynaptic neurons (Ritz et al., 1990), which results in the over-stimulation of the corresponding receptors. The psychostimulant and locomotor effects of cocaine are primarily mediated by the dopaminergic system. Dopaminergic pathways consist of neurons in the substantia nigra compacta and in the ventral tegmental area, which project to the caudate-putamen, the nucleus accumbens, the frontal cortex and other limbic structures. The reinforcing properties of cocaine have been associated with elevated dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988; Koob and Bloom, 1988).

More recent studies indicate that the glutamatergic system is also involved in the stimulant effect of cocaine in rodents. Glutamatergic projections from the prefrontal cortex have been shown to regulate the activity of dopaminergic neurons in the ventral tegmental area (Karreman and Moghaddam, 1996). They are therefore, critically involved in basal and evoked dopamine release in the nucleus accumbens. Besides, stimulation of glutamatergic afferences from the hippocampus and amygdala produces strong excitatory responses in neurons of the nucleus accumbens (Yang and Mogenson, 1984; Yim and Mogenson, 1982). Regulation by glutamate of the behavioral effects produced by cocaine is mainly through NMDA glutamate receptors, whereas, metabotropic glutamate receptors negatively regulate the dopaminergic neurons (Karler et al., 1994).

Ketamine [2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone hydrochloride, Imalgene®] is a phencyclidine analog which displays non-competitive NMDA receptor antagonistic properties (O'Shaughnessy and Lodge, 1988). It is still used in human anesthesia, despite the fact that it has been reported to cause psychotic-like reactions. The anesthetic agent propofol (2,6-diisopropylphenol, Diprivan®) is used in human medicine. Patients anesthetized with propofol report positive changes in mood like euphoria or elation upon recovery. Interestingly, propofol at subanesthetic dose may have abuse potential (Zacny et al., 1993). Neurobiologically, propofol was shown to exhibit properties of an NMDA receptor antagonist (Yamakura et al., 1995) and of a $GABA_A$ receptor agonist (Sanna et al., 1995). It was also found to regulate voltage-sensitive Ca^{2+} channels (Hirota et

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al., 2000) and Na^+ channels (Ratnakumari and Hemmings, 1996).

Acute cocaine administration has been shown to elicit rapid and transient induction of several immediate early genes in brain neurons, including *egr-1*, c-*fos*, *jun*B and *h*VH5 (Hope et al., 1992; Moratalla et al., 1992; Thiriet et al., 1998). Inducibility of immediate early genes like *egr-1* can be regarded as a tool to study neuronal activation in different brain systems. In the striatum, early gene induction occurs in medium-spiny GABAergic neurons that express the dopaminergic D1 receptor. In the present study, we examined the effect of propofol on cocaine-induced *egr-1* mRNA expression in rat forebrain, by comparing its effect to that of another anesthetic agent, ketamine.

2. Materials and methods

2.1. Animals and tissue preparation

Male Sprague–Dawley rats (Janvier, France) that weighed 250–300 g, were housed with a fixed 12 h light–dark cycle and free access to food and water. All experiments were conducted in conformity with the European Community Council guidelines. Rats were injected i.p. with propofol (Zeneca, UK), ketamine (Merial, France) or with equivalent volume of vehicle (intralipid emulsion, Braun Médicale, France). Fifteen minutes later, rats were given an i.p. injection of either 20 mg/kg cocaine hydrochloride (Sigma) or NaCl 0.9% solution. At 45 min after cocaine injection, animals were given an overdose of pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 1% paraformaldehyde in phosphate-buffered saline (pH 7.2; 250 ml). The brains were then processed as described earlier (Thiriet et al., 1998).

2.2. In situ hybridization

In situ hybridization with [^{35}S] (UTP)-labeled RNA probe was performed as previously described (Thiriet et al., 1998). Briefly, 30 μl of labeled *egr-1* probe, diluted to 60,000 dpm/μl with hybridization buffer (50% formamide, 0.6 M NaCl, 60 μM sodium citrate, pH 7.0, 10% dextran sulfate, 10 mM dithiothreitol) was placed on tissue section and covered with coverslips. Hybridization was carried out overnight at 52 °C. The sections were then washed and exposed to X-ray film (Kodak Biomax-MR) for 6 days. For quantitative analysis, densitometry was performed with an image analyzer and Samba software (Alcatel TITN, France). Optical density was converted into kBq/g tissue, using [^{14}C]micro-scales (Amersham) for calibration, and results were expressed as percentages of the corresponding control.

3. Results

As shown in Fig. 1, in situ hybridization for *egr-1* in coronal sections taken at the level of the nucleus accumbens showed basal expression in two layers of the cortex and in olfactory tubercles. No significant induction was

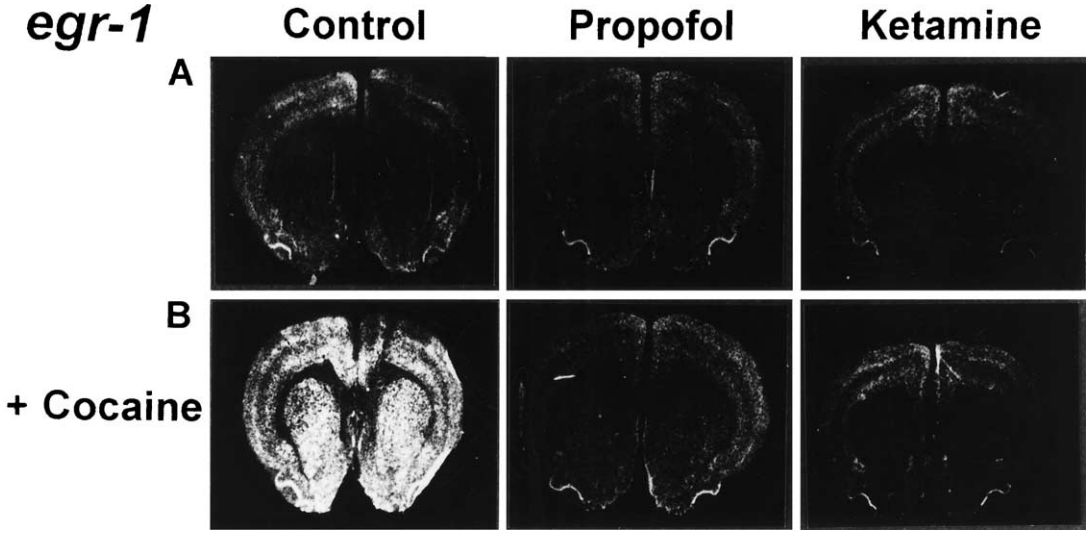

Fig. 1. Effects of propofol and ketamine on cocaine-induced *egr-1* gene expression. Negative prints of in situ hybridization autoradiograms showing mRNA expression of *egr-1* in rat brain coronal sections at the level of striatum (approximately 1.2 mm anterior to bregma) and probed with [^{35}S] antisense riboprobe. Rats were injected i.p. with 100 mg/kg propofol, ketamine or equivalent volume of intralipid emulsion (Control). Fifteen minutes later, they were injected with 0.9% NaCl (A) or 20 mg/kg cocaine (B), and sacrificed 45 min after cocaine injection.

observed in animals injected i.p. with 100 mg/kg propofol or ketamine for 60 min (Fig. 1A). Prominent signals were observed 45 min after an acute cocaine injection in the nucleus accumbens, caudate-putamen, as well as in frontal and piriform cortices (Fig. 1B). This high *egr-1* expression was however, abolished when propofol or ketamine were injected 15 min before cocaine.

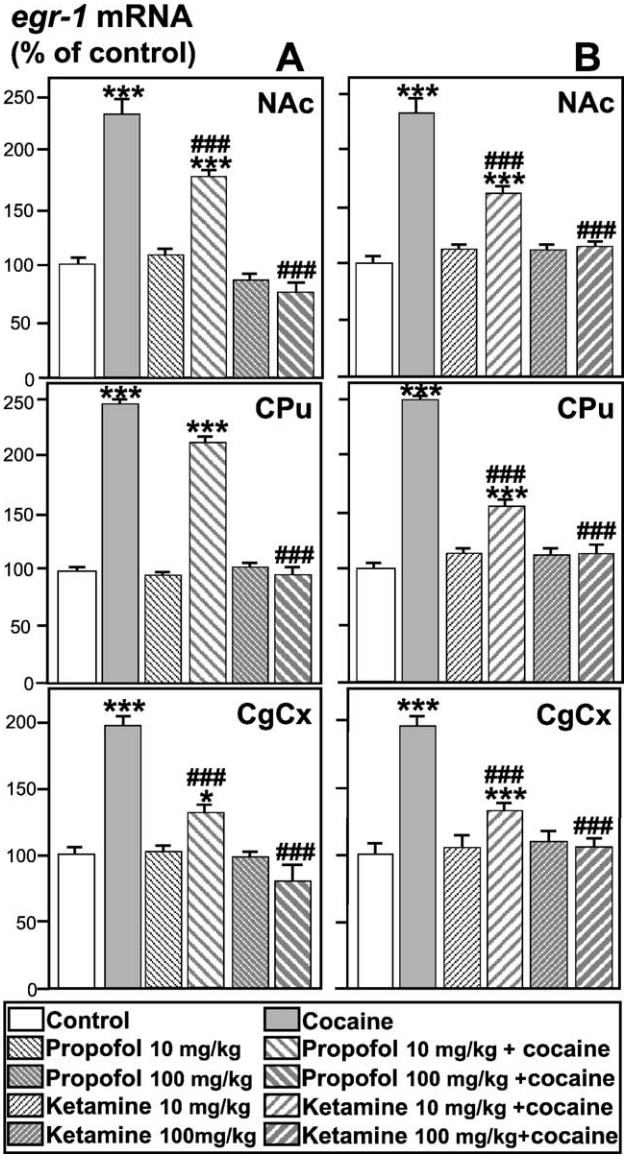

Fig. 2. Densitometric analysis of the effects of propofol (A) and ketamine (B) on cocaine-induced *egr-1* gene expression. Comparable areas of the nucleus accumbens shell (NAc), the dorsal region of caudate-putamen (CPu) and the cingulate cortex (CgCx) were quantified from autoradiograms of sections from rats treated as described in legend to Fig. 1. Rats were injected i.p. with 10 or 100 mg/kg propofol, ketamine, or vehicle, and 15 min later with 20 mg/kg cocaine or 0.9% NaCl (Control). They were sacrificed 45 min after cocaine injection. Results are expressed as % of control, as mean ± S.E.M. ($n = 12$ sections from three animals). * $p < 0.05$; *** or ###$p < 0.001$. *, significant when comparing treatment vs. control group and #, significant when comparing (anesthetic agent + cocaine) vs. cocaine group (ANOVA followed by Student–Newman–Keuls multiple comparison test).

Quantitative densitometric analysis of *egr-1* mRNA synthesis in the shell part of nucleus accumbens, dorsal region of caudate-putamen and cingulate cortex is shown in Fig. 2. Injection of propofol (Fig. 2A) or ketamine (Fig. 2B) alone was not found to induce *egr-1* expression in the brain structures examined, even at the dose of 100 mg/kg. Acute cocaine administration caused an approximately 2.3-, 2.4- and 1.9-fold increase in *egr-1* induction in the nucleus accumbens shell, caudate-putamen and cingulate cortex, respectively. This expression was significantly reduced in the nucleus accumbens and cingulate cortex, but not in caudate–putamen, when rats were given an injection of 10 mg/kg propofol 15 min before cocaine (Fig. 2A). Similarly, injection of 10 mg/kg ketamine before cocaine reduced *egr-1* expression in the three structures examined (Fig. 2B). Moreover, injection of the higher dose of 100 mg/kg propofol or ketamine was found to completely abolish cocaine-induced *egr-1* expression in the three brain areas.

4. Discussion

We show in the present study that acute administration of the anesthetics ketamine or propofol did not trigger *egr-1* immediate early gene transcription in rat forebrain. This result confirms previous reports describing the action of anesthetic agents on immediate early gene expression. In general, the NMDA receptor antagonist ketamine was found to be without effect, or to suppress early gene expression in rat brain (Huang and Simpson, 1999; Nakao et al., 1993; Torres and Rivier, 1993). A report by Nakao et al. (2002), showed that ketamine induced c-Fos in mouse cortex, but this induction involved sigma receptors together with NMDA receptors. On the opposite, stimulation of the NMDA receptor is well described for triggering early gene expression, via calcium influx and mitogen-activated protein kinase activation (Xia et al., 1996). Concerning the anesthetic propofol, it was shown to induce transiently c-*fos* but not c-*jun* expression in rat brain (Hamaya et al., 2000).

We found that both anesthetic compounds were able to dose-dependently reduces cocaine-induced *egr-1* mRNA synthesis. The effect of ketamine is most likely due to its NMDA receptor-suppressing activity, since antagonists of this receptor have already been shown to reduce early gene expression induced by cocaine in the striatum (Torres and Rivier, 1993). Even constitutive expression of *egr-1* was almost abolished in neocortical neurons by the NMDA receptor antagonist dizocilpine (MK-801) (Gass et al., 1993). Striatal gene regulation by glutamatergic agents may result from the glutamatergic projection originating in the prefrontal cortex that is known to regulate positively the activity of dopaminergic neurons (Karreman and Moghad-

dam, 1996). In this way of thinking, inhibition of glutamatergic neuronal activity by the NMDA receptor antagonist ketamine would reduce the activity of dopaminergic neurons, which would be accompanied by a lesser *egr-1* expression in their projection fields. A similar gene regulation mechanism in the nucleus accumbens may result from the inhibition of glutamatergic projections originating from hippocampus or amygdala (Yang and Mogenson, 1984; Yim and Mogenson, 1982). Alternatively, striatal medium-spiny neurons may be directly inhibited by NMDA receptor antagonists, thus, rendering ineffective the permissive role of dopamine on these neurons.

Since both ketamine and propofol showed very similar effects on cocaine-induced gene expression and that ketamine is a well-characterized NMDA receptor antagonist, it is tempting to attribute the effects of propofol to NMDA receptor antagonistic properties. NMDA receptor-suppressing activity for propofol has indeed been proposed (Yamakura et al., 1995). Other reports, however, have indicated additional targets for propofol, in particular $GABA_A$ receptors. Low concentrations of propofol increase GABA-evoked chloride currents and higher concentrations activate the $GABA_A$ receptor in the absence of GABA (Sanna et al., 1995). Propofol-induced inhibition of glutamate release in cortical slices may be mediated by activation of $GABA_A$ receptors, revealing a subtle interplay between GABAergic and glutamatergic transmission in anesthetic action (Buggy et al., 2000). This mechanism may ultimately be responsible for a lesser dopamine release in the striatum by propofol, hence a decreased cocaine-induced *egr-1* gene expression. Stimulated dopamine release was indeed shown to be reduced by propofol, using fast cyclic voltammetry (Schulte et al., 2000). In addition, propofol has been shown to regulate voltage-sensitive Ca^{2+} channels (Hirota et al., 2000) and Na^{+} channels (Ratnakumari and Hemmings, 1996), which contributes to its anesthetic, amnesic and anti-convulsant properties. Our results may therefore, also be explained by propofol inhibiting ion channel activities, leading to the inhibition of dopamine release and reduced gene transcription in dopaminergic projection areas.

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