Published in final edited form as:

Anesth Analg. 2011 August ; 113(2): 259–264. doi:10.1213/ANE.0b013e318222af17.

Upregulation of DeltaFosB by Propofol in Rat Nucleus Accumbens

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Abstract

BACKGROUND—It is well established that all drugs of abuse converge onto common circuitry and induce chronic addiction by modulating the addictive signaling molecules such as DeltaFosB in the mesocorticolimbic system. Recent case reports suggest that propofol may have abuse potential. However, there is no direct evidence showing that propofol has an effect on the key addictive signaling molecules in the mesocorticolimbic system. In this study, we determined the effect of propofol on the expression of DeltaFosB in rat nucleus accumbens (NAc) and the potential mechanism involved.

METHODS—To determine the effect of propofol on the expression of DeltaFosB in rat NAc, 2 well-known addictive agents, ethanol and nicotine, were used as positive controls. Experiments were conducted on 36 male Sprague–Dawley rats (150 to 200 g). These animals were divided into 4 treatment groups: vehicle (saline), propofol (10 mg/kg), ethanol (1 g/kg), and nicotine (0.5 mg/kg). All drugs were administered by intraperitoneal injection twice per day for 7 days. The animals were then killed and their NAc were isolated for DeltaFosB measurements.

RESULTS—As expected, both ethanol and nicotine significantly increased DeltaFosB expression. Intriguingly, propofol elicited a robust increase in DeltaFosB expression similar to that of ethanol and nicotine. Moreover, the dopamine receptor D1, an upstream molecule of DeltaFosB, was also significantly upregulated by propofol.

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The authors declare no conflicts of interest.

This report was previously presented, in part, at the American Society of Anesthesiologists' Annual Meeting, October 18, 2010.

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CONCLUSIONS—In the current study, we have identified, for the first time, that propofol is able to induce the addictive signaling molecule DeltaFosB in NAc via dopamine receptor D1. This new evidence at the molecular level suggests that propofol may have abuse potential.

Drug abuse is a major social and health concern. Propofol is the most widely used IV drug in anesthesia and intensive care. Although propofol has not traditionally been considered a drug of abuse, growing evidence suggests that it may have an abuse potential.¹ Patients anesthetized with propofol report experiencing euphoria during recovery.² Several case reports have described propofol abuse in nurses, anesthesiologists, and laypersons.³ In a study in which the rewarding effects of propofol were assessed in humans, using a discrete-trials choice procedure, normal healthy volunteers without a drug abuse history ($n = 12$) were exposed in a blind fashion to acute bolus injections of 0.6 mg/kg of propofol twice and to a similar volume of Intralipid® twice. Then, for the next 3 sessions, subjects chose which drug (identified by a color code) they wished to receive. Six subjects were choosers: 4 subjects chose propofol on all 3 choice occasions and 2 subjects chose the drug on 2 out of 3 occasions. Six subjects were nonchoosers: 5 subjects chose Intralipid® on all 3 choice occasions, and 1 subject chose Intralipid® twice (referred to hereinafter as *propofol nonchoosers*). Propofol choosers reported pleasant acute effects and no unpleasant residual effects, whereas propofol nonchoosers reported either unpleasant acute subjective effects or residual effects from propofol.^{4,5} Propofol abuse potential has also been demonstrated in animal studies. Propofol was self-administered by baboons⁶ and rats.⁷ Conditioned place preference for propofol has been established in rats.^{8,9} However, all the above evidence is only from case reports and simple behavior animal observations. More recently, we have found, via an electrophysiological method, that nanomolar propofol is able to stimulate glutamate transmission to dopamine neurons and that exogenous propofol facilitates glutamatergic transmission to neurons of the ventrolateral preoptic nucleus. This electrophysiological evidence has provided additional support that propofol might be an addictive drug.^{10,11}

Although the original abuse drugs, reagents, or factors are diverse, in their molecular mechanisms, they converge on a common circuitry and finally induce addiction by modulating gene expression in this circuitry such as Delta-FosB in the nucleus accumbens (NAc).^{12–14} Indeed, the transcription factor DeltaFosB in the NAc is the critical addictive signaling molecule in drug abuse induced by cocaine, morphine, ethanol, and marijuana.¹⁵

Although propofol might have abuse potential, there is a lack of direct evidence showing that propofol could affect addictive gene expression in the common abuse circuitry. In the current study, we have identified for the first time that propofol is able to elicit a robust increase in DeltaFosB expression similar to that induced by ethanol and nicotine in rat brain NAc. We further demonstrated that propofol induced the expression of DeltaFosB, which is associated with the upregulation of the dopamine receptor D1 (DRD1).

METHODS

Animal Groups, Administration of Propofol, Ethanol, and Nicotine, and Tissue Isolation

Male Sprague–Dawley rats were used for this experiment. All animals were housed in separate cages in 12-hour-light and 12-hour-dark periods with free access to water and food. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Medicine and Dentistry New Jersey and was consistent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85 to 23, revised 1985).

To determine whether propofol is addictive, we examined the effect of propofol on the expression of DeltaFosB in rat NAc. Two well-known addictive agents, ethanol and nicotine, were used as positive controls. Vehicle-treated animals were used as the negative controls. Experiments were conducted on 36 male Sprague–Dawley rats (150 to 200 g). These animals were treated with vehicle (saline), propofol (10 mg/kg), ethanol (1 g/kg), and nicotine (0.5 mg/kg). All drugs were administered by intraperitoneal injection twice per day for 7 days. In addition, 6 animals given no injections were used to provide an additional control group for the basal level of DeltaFosB expression. Moreover, to test and to avoid any possible effects of Intralipid, the vehicle of propofol, on the expression of DeltaFosB in rat NAc, 6 rats were injected with Intralipid.

The animals were then killed by decapitation, and their brains were rapidly removed and immediately frozen in liquid nitrogen. Coronal brain slices (500 μ m thick) were cut with a Vibratome (Campden Instruments Ltd., Loughborough, Leicestershire, UK), and NAc were identified and isolated under an Olympus dissecting microscope according to the atlas of the rat brain for protein and mRNA measurements.

RNA Levels Were Determined by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Briefly, RNAs from NAc were isolated with an RNA isolation kit (Ambion, Inc., Austin, TX). Quantitative real-time polymerase chain reaction (qRT-PCR) for DeltaFosB and DRD1 was performed on cDNA generated from 200 ng of total RNA using the protocol of a qRT-PCR mRNA detection kit (Roche, Indianapolis, IN). Amplification and detection of specific products were performed with a Roche Lightcycler 480 Detection System as described in our previous studies.^{16,17} As an internal control, GAPDH was used for template normalization. Fluorescent signals were normalized to an internal reference, and the threshold cycle (Ct) was set within the exponential phase of the PCR. The relative gene expression was calculated by comparing cycle times for each target PCR. The target PCR Ct values were normalized by subtracting the GAPDH Ct value, which provided the Δ Ct value. The relative expression level among treatments was then calculated using the following equation: relative gene expression = $2^{-(\Delta\text{Ct} - \text{sample} - \Delta\text{Ct} - \text{control})}$.^{16,17}

Western Blot Analysis

Proteins isolated from NAc were determined by Western blot analysis.^{16,17} Equal amounts of protein were subjected to SDS-PAGE. A standard Western blot analysis was conducted using DeltaFosB rabbit monoclonal antibody (1:100 dilution; Cell Signaling Technology, Danvers, MA) and DRD1 rabbit monoclonal antibody (1:1000 dilution; Sigma) GAPDH antibody (1:5000 dilution; Cell Signaling) was used as a loading control.

Statistical Analysis

All data are presented as mean $\pm$ SE. Power analysis was used to determine the animal numbers in each group on the basis of the propofol-induced increase in DeltaFosB expression being similar to that induced by ethanol or nicotine. For relative gene expression, the mean value of the control group was defined as 100% or 1. Two-tailed unpaired Student *t* tests and analysis of variance (ANOVA) were used for statistical evaluation of the data. Sigma Stat Statistical Analysis Program was used for data analysis. A *P* value <0.05 was considered significant.

RESULTS

Both Ethanol and Nicotine Increase DeltaFosB Expression in Rat NAc

As expected, after 1 week of treatment, both ethanol and nicotine significantly increased DeltaFosB expression in rat NAc at both protein (Fig. 1A) and mRNA levels (Fig. 1C). Representative Western blots from vehicle and ethanol-and nicotine-treated rats NAc are shown in Figure 1B. The results were consistent with previous studies^{15,18} and suggested that our animal model was successful.

Administration of Propofol Elicits DeltaFosB Expression in Rat NAc

As shown in Figure 2, A and C, administration of propofol resulted in a robust increase in DeltaFosB expression in rat NAc at both protein and mRNA levels. At 7 days after propofol injection, the level of DeltaFosB protein level in rat NAc was increased by about 40%. The robust increase in DeltaFosB expression induced by propofol was similar to that induced by 2 addictive regents, ethanol and nicotine (Fig. 1). Representative Western blots from vehicle and propofol-treated rat NAc are shown in Figure 2B. No significant effect of saline injection or Intralipid injection was found on the expression of DeltaFosB, in comparison with that in rats not receiving injections (Fig. 2D).

Propofol Induces the Expression DeltaFosB and Is Associated with the Upregulation of DRD1

DRD1 is an upstream signal molecule of DeltaFosB that is related to addictive gene expression in common addictive circuitry including NAc. To determine the potential involvement of DRD1 in propofol-induced DeltaFosB expression, the expression levels of DRD1 in NAc from vehicle or propofol-treated rats were measured. Indeed, the robust increase in DeltaFosB expression induced by propofol was associated with DRD1, because the expression level of DRD1 in propofol-treated animals was much higher than that in vehicle-treated animals (Fig. 3).

DISCUSSION

Drug addiction is a chronic relapsing disorder characterized by persistent drug-seeking and drug-taking behaviors. Although drugs of abuse possess diverse neuropharmacological profiles, recent studies have revealed that activation of the mesocorticolimbic system—particularly the ventral tegmental area, NAc, amygdale, and prefrontal cortex via dopaminergic and glutamatergic pathways—constitutes a common pathway by which various drugs of abuse mediate their acute and chronic addictive effects.^{13,14,19} Delta-FosB plays a central role in addiction of all drugs of abuse within the common addictive circuitry.^{13,14,20}

In the current study, we identified that propofol elicited a robust increase in DeltaFosB expression similar to that induced by ethanol and nicotine in rat brain NAc. This novel finding provides strong support at the molecular level that propofol might be an addictive drug. Operating room staff may have contact with small amounts of propofol via skin contact or inhalation, whereas patients have a much greater exposure during IV administration. Determining the dose response of propofol-induced upregulation of DeltaFosB should be considered for future study. In one study we found that nanomolar propofol is sufficient to stimulate glutamate transmission to dopamine neurons, suggesting that a small amount of propofol exposure might also have some effect on addictive gene expression.¹⁰

The detailed molecular mechanisms responsible for propofol-induced upregulation of DeltaFosB are still unclear. Dopamine and its receptor DRD1 are the upstream molecules of DeltaFosB within the addictive circuitry of the mesolimbic dopamine system. In previous rat studies, propofol has been found to alter dopamine levels in NAc,²¹ suggesting that propofol may modify the activity of ventral tegmental area dopamine neurons. Using an electrophysiological method, we found that propofol was able to facilitate glutamatergic transmission neurons of the ventrolateral preoptic nucleus.^{10,11} We thus hypothesized that DRD1 might be an upstream molecule in propofol-induced upregulation of DeltaFosB. To test the hypothesis, the expression levels of DRD1 in NAc from vehicle or propofol rats were measured. Interestingly, propofol indeed increased DRD1 expression both at mRNA and protein levels. The result indicates that dopamine and its receptor DRD1 could be upstream molecules of DeltaFosB associated with propofol-induced upregulation of DeltaFosB in the NAc. The potential molecular mechanisms of propofol-induced addiction are displayed in Figure 4 on the basis of these results.

In summary, we have found that propofol is able to induce the addictive signaling molecule DeltaFosB in the NAc via DRD1. The new evidence at the molecular level suggests that propofol has abuse potential. Although the study is in its initial stage, the final confirmation that propofol is an addictive drug will have a huge impact on the entire biomedical and social community. First, propofol is widely used in and out of the operating rooms. Moreover, the air in the operating rooms contains propofol that may be harmful to the health care staff. New administrative regulations and methods to monitor and control the use of propofol might be warranted as new addictive evidence is accumulated.

Acknowledgments

Funding: This work was supported by National Institutes of Health grants HL080133 and HL095707.

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Figure 1.

Upregulation of DeltaFosB expression in rat NAc by ethanol or nicotine. Experiments were conducted on 27 male Sprague–Dawley rats. These animals were treated with vehicle (saline), ethanol (1 g/kg), and nicotine (0.5 mg/kg). All drugs were administered by intraperitoneal injection twice per day for 7 days. The animals were then killed and brain nucleus accumbens (NAc) were isolated for DeltaFosB measurements. A, DeltaFosB expression determined by Western blot at the protein level. B, Representative Western blots of DeltaFosB. C, DeltaFosB expression determined by quantitative real-time polymerase chain reaction (qRT-PCR) at the mRNA level. Note: $n = 9$. * $P < 0.05$ in comparison with vehicle control.

Figure 2.

Propofol elicits DeltaFosB expression in rat NAc. Experiments were conducted on 18 male Sprague–Dawley rats. These animals were treated with vehicle (saline) or propofol (10 mg/kg). Saline and propofol were administered by intraperitoneal injection twice per day for 7 days. The animals were then killed and brain nucleus accumbens (NAc) were isolated for DeltaFosB measurements. A, DeltaFosB expression determined by Western blot at the protein level. B, Representative Western blots of DeltaFosB. C, DeltaFosB expression determined by quantitative real-time polymerase chain reaction (qRT-PCR) at the mRNA level. D, DeltaFosB expression determined by Western blot at the protein level. Note: $n = 9$ in A–C, $n = 6$ in D. * $P < 0.05$ in comparison with vehicle control.

Figure 3.

Propofol increased the expression of dopamine receptor D1 (DRD1) in rat NAc.

Experiments were conducted on 18 male Sprague–Dawley rats. These animals were treated with vehicle (saline) or propofol (10 mg/kg). Saline and propofol were administered by intraperitoneal injection twice per day for 7 days. The animals were then killed and brain nucleus accumbens (NAc) were isolated for DRD1 measurements. A, DRD1 expression determined by Western blot at the protein level. B, Representative Western blots of DeltaFosB. C, DRD1 expression determined by quantitative real-time polymerase chain reaction (qRT-PCR) at the mRNA level. Note: $n = 9$. * $P < 0.05$ in comparison with vehicle control.

Figure 4.

The potential molecular mechanisms of propofol-induced addiction. Propofol increases the levels of dopamine (DA) and dopamine receptor D1 (DRD1) expression within the common addictive circuitry, including the ventral tegmental area (VTA) and nucleus accumbens (NAc). The increased dopamine and DRD1 upregulates the key addiction-inducing signal molecule, DeltaFosB in NAc, although the directly upregulatory effect of propofol on DeltaFosB expression in NAc cannot be excluded. The increased expression of DeltaFosB will finally induce the propofol addiction.