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Behavioral cross-sensitization between cocaine and ethanol is accompanied by parallel changes in the activity of AMPK system

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ABSTRACT

Behavioral sensitization is thought to be relevant to the psychopathology of drug addiction. A previous study from our research group demonstrated cross-sensitization between cocaine and ethanol. Although these findings suggest a common mechanism of action between these two drugs, little is known about the molecular or cellular aspects of this commonality. The AMPK pathway functions as an intracellular energy sensor and plays a critical role in maintaining cellular energy homeostasis. Thus, the present study examined AMPK signaling following reciprocal cross-sensitization between cocaine and ethanol in the rat prefrontal cortex and dorsal striatum. Male Sprague–Dawley rats were repeatedly treated with either cocaine (15 mg/kg, 5 times) or ethanol (0.5 g/kg, 15 times) and then challenged reciprocally with the other drug. When sensitized to either cocaine or ethanol, the phosphorylation in response to additional challenges with the same drug was enhanced, indicating the development of sensitization. However, responses to the cocaine challenge were enhanced in the ethanol-sensitized state, whereas the responses to the ethanol challenge were not apparently enhanced in the cocaine-sensitized state. This was likely due to the ceiling effect of cocaine sensitization, which suggested that cocaine had more robust effects than ethanol. Although the same changes were found for two upstream kinases of AMPK (LKB1 and CaMK4), TAK1 responded differently and was not affected by acute challenges from either cocaine or ethanol. In the prefrontal cortex, there was an increase in activity, whereas there was a decrease in activity in the dorsal striatum. This difference might be due to dopamine D_1 receptor dominance in the prefrontal cortex and D_2 receptor dominance in the dorsal striatum. Taken together, these results suggest that both cocaine and ethanol may share overlapping molecular pathways in the process of behavioral sensitization. However, the action of cocaine was stronger than that of ethanol.

1. Introduction

Repeated treatment with drugs of abuse can induce behavioral sensitization, which is a process relevant to the psychopathology of drug addiction (Hunt and Lands, 1992; Robinson and Berridge, 1993). This common effect has led to the proposal that various drugs of abuse may share some common neural mechanisms regardless of their neurochemical properties (Robinson and Berridge, 1993; Wise and Bozarth, 1987; Wolf, 1998). Studies investigating cross-sensitization have demonstrated some overlaps in the mechanisms that mediate sensitization (Itzhak and Martin, 1999; Lessov and Phillips, 2003; Manley and Little, 1997) and we have reported that cross-sensitization occurs between cocaine and ethanol (Xu and Kang, 2017). Although cocaine and ethanol exert their initial effects at different sites in the brain, these drugs produce similar changes in the dopamine signaling pathway (Koob et al., 1998; Kuhar et al., 1991; Ortiz et al., 1995; Wise and Bozarth, 1987), which may represent the mechanism leading to cross-sensitization. However, little is known about the cellular mechanisms underlying cross-sensitization between ethanol and cocaine.

Thus, the present study focused on changes in the 5′ adenosine monophosphate-activated protein kinase (AMP)-activated protein kinase (AMPK) system following the induction of sensitization. AMPK functions as a major metabolic sensor that is activated in response to numerous environmental stressors to restore cellular and whole-body

Abbreviations: ANOVA, analysis of variance; AMP, 5′ adenosine monophosphoate; AMPK, AMP-activated protein kinase; CaMK4, Ca^{2+}/calmodulin-dependent protein kinase 4; CaMKKβ, Ca^{2+}/calmodulin-dependent protein kinase kinaseβ; DA, dopamine; LKB1, liver kinase B1; PKA, protein kinase A; TAK1, transforming growth-factor-β-activated kinase 1; OD, optical density

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energy balance levels (Hardie et al., 2006; Young et al., 2005). In mammals, AMPK can be activated by three kinases: liver kinase B1 (LKB1), TGF-β activated kinase-1 (TAK1), and Ca^{2+}/calmodulin-dependent protein kinase kinaseβ (CaMKKβ) (Hawley et al., 2003; Woods et al., 2005; Xu et al., 2011). In the hypothalamus, AMPK controls food intake by altering the expression of melanin concentrating hormone and neuropeptide Y (Minokoshi et al., 2004; Oh et al., 2016). These neuropeptides and their receptors play important roles in the rewarding and reinforcing effects of cocaine and alcohol (Chung et al., 2009; Cippitelli et al., 2010; Sorensen et al., 2013). AMPK activity regulates fasting-induced excitatory synaptic activity in agouti-related peptide (AgRP)-expressing neurons which, in turn, influences the dopamine reward circuitry and cocaine-associated behaviors (Dietrich et al., 2012; Kong et al., 2016; Yang et al., 2011). Furthermore, a recent study demonstrated that AMPK activity in the nucleus accumbens core plays an important role in cocaine-seeking behaviors (Gao et al., 2017).

To determine whether abused drugs could share a common cellular mechanism in terms of behavioral sensitization, the present study investigated the AMPK signaling pathway in the prefrontal cortex and dorsal striatum after the induction of reciprocal cross-sensitization between cocaine and ethanol. These regions were chosen because the mesocorticostriatal dopamine system plays significant roles in drug-induced sensitization (Di Chiara and Imperato, 1988; Steketee, 2005; Wise, 2009 #32).

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley rats weighing 150–200 g (KoaTech; Gyeonssi-do, Korea) were maintained on a 12/12-h light/dark cycle (lights on at 06:00) with water and food available ad libitum. Cocaine hydrochloride (15 mg/kg, Belgopia; Louvain-La-Neuve, Belgium) and ethanol (0.5 g/kg, Merck; Whitehouse Station, NJ, USA, cat. #1.00983.1011) were dissolved in 0.9% saline and administered via the intraperitoneal (IP) route. This study was approved by the Institutional Animal Care and Use Committee at Seoul National University Hospital (SNUH-IACUC) and the animals were maintained in a facility accredited by the Association for Assessment and Accreditation or Laboratory Animal Care (AAALAC) International (#001169) in accordance with the Guide for the Care and Use of Laboratory Animals 8th edition, National Research Council (NRC, 2010).

2.2. Drug treatment schedules

Two sets of experiments were performed in the present study (Fig. 1). In the first set of experiments, the rats were randomly divided into two groups: saline (S-) or cocaine (C-) were administered daily for 5 days; these chronic treatments were designated as “sensitization”. Following the sensitization, each group was further divided into three subgroups, which were acutely administered with saline (subgroup S−S and C−S), cocaine (subgroup S−C and C−C), or ethanol (subgroup S-E and C-E); these treatments were designated as “challenge”. In the second set of experiments, the rats randomly assigned to two groups: saline (S-) or ethanol (*E*-) were administered for 3 weeks (administered 15 times, 5 times per week from Monday to Friday between 10:00 and 16:00). After the last treatment, the rats were further divided into three subgroups and challenged with saline (subgroup S−S and E-S), cocaine (subgroup S−C and E-C), or ethanol (subgroup S-E and E-E). Locomotor activity was measured after the challenges, and the rats were dissected 30 min thereafter ($n = 6$ or 8 for each subgroup). The treatment regimen for inducing the sensitization with ethanol, cocaine, and reciprocal cross-sensitization were based on a previous study from our research group (Xu and Kang, 2017).

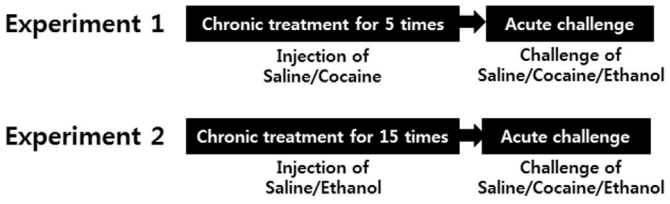

Fig. 1. Experimental schedule: chronic treatment with cocaine (15 mg/kg) 5 times (Experiment 1) or ethanol (0.5 g/kg) 15 times (Experiment 2), and then acute challenges with saline, cocaine, or ethanol.

2.3. Measurement of locomotor activity

Locomotor activity was measured using a video-tracking apparatus in a transparent acrylic box (42 × 42 × 30 cm) located in a sound-attenuated test room (Activity Monitor Ver. 5.0, Med-Associates; St. Albans, VT, USA). Each rat was placed in the box and measured during a 30-min habituation period. Next, each rat was challenged with a drug and measured for an additional 30-min period that was divided into 5-min bins.

2.4. Western blot analysis

The rats were decapitated 30 min after the final challenge, and the prefrontal cortex and dorsal striatum of each animal were dissected on ice and immediately frozen in liquid nitrogen. The frozen tissues were then homogenized in lysis buffer and centrifuged, as previously described (Xu and Kang, 2014). The lysis buffer was composed of 50 mM Tris buffer (pH 7.4), 150 mM NaCl, 1 mM DTT, 4 mM EGTA, 10 mM EDTA, 100 mM β-glycerophosphate, 40 mM NaF, 4 mM sodium vanadate, 15 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich; St. Louis, MO, USA), 0.2% NP40, and a protease inhibitor cocktail (Roche; Mannheim, Germany). Following centrifugation at 20,000 rpm for 20 min at 4 °C, the supernatants were quantified using the Bradford protein assay method (Bio-Rad Laboratories; Hercules, CA, USA) and then boiled with Laemmli sample buffer. The protein was separated using 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membranes (Whatman; Dassel, Germany). The membranes were blocked with 5% skim milk in Tris-buffered saline (pH 7.4) containing 0.1% Tween 20 for 1 h at room temperature, incubated with a primary antibody overnight at 4 °C, and then incubated with a secondary antibody for 1 h at room temperature. Primary antibodies against AMPKα, p-Thr-172-AMPKα, TAK1, p-Thr-184/187-TAK1, CaMK4, LKB1 (Cell Signaling Technology; Danvers, Ma, USA), p-Thr-196-CaMK4, and p-Ser-431-LKB1 (Santa Cruz Biotechnology; Santa Cruz, CA, USA) were used at dilutions of 1:750 to 1:8000 prior to incubation with anti-rabbit or mouse IgG secondary antibodies (Jackson Immuno Research Laboratories Inc.; USA). Signals were detected using an enhanced chemiluminescence system (Pierce Biotechnology; Rockford, IL, USA), and the X-ray films were quantified with a TINA 2.10G system (Raytest; Straubenhardt, Germany).

2.5. Statistical analysis

The results were quantitated using optical densitometry (OD) and normalized against the signals from the total forms of the corresponding proteins. Statistical analyses were performed using two-way analysis of variance (ANOVA) tests followed by a post hoc Tukey tests. All analyses were performed using the version 21.0SPSS software package for Windows (SPSS Inc.; Chicago, Illinois, USA), and *P*-values < 0.05 were considered to indicate statistical significance.

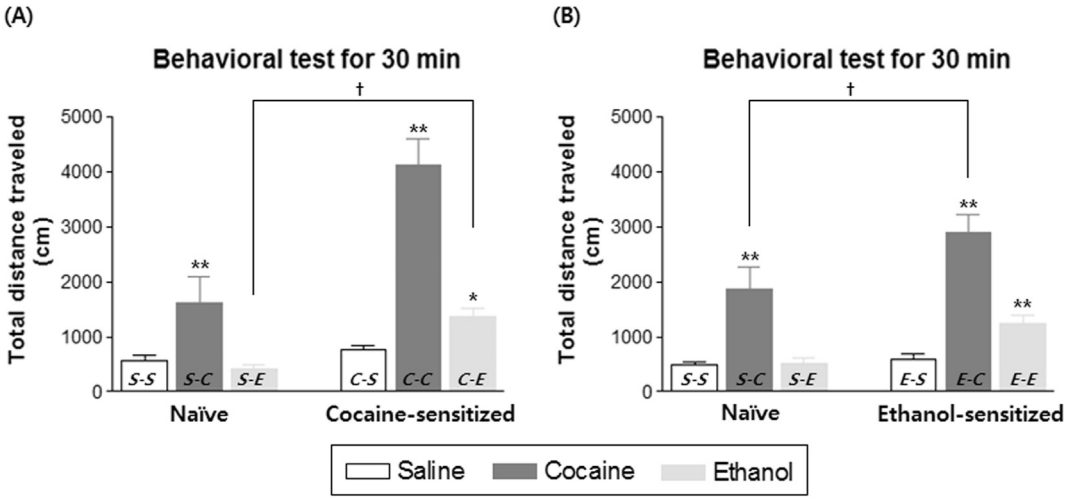

Fig. 2. Mean activity counts following challenge injections with saline (0.9% NaCl, -S), cocaine (15 mg/kg, -C), or ethanol (0.5 g/kg, -E) in the animals sensitized with cocaine (5 times, C-, Panel A) or ethanol (15 times, E-, Panel B). Locomotor activity was summed over 30 min. Data are presented as means ± *SE* (n = 6 or 8 rats/subgroup). *, $P < 0.05$; **, $P < 0.01$, significant differences compared to the S—S subgroup. †, significant differences between S—C vs. E-C and S-E vs. C-E.

3. Results

3.1. Behavioral cross-sensitization between ethanol and cocaine

Fig. 2 shows the locomotor effects of an acute cocaine or ethanol challenge in cocaine- or ethanol-sensitized animals. Locomotor activity was increased significantly by both chronic treatment and acute challenges with cocaine or ethanol, and these responses were enhanced by previous sensitization with either cocaine or ethanol [$F(2, 42) = 7.272$, $P = 0.002$, Fig. 2A; $F(2, 42) = 4.902$, $P = 0.012$, Fig. 2B]. Subsequent pair-wise comparisons revealed that acute treatment with cocaine increased locomotor activity ($P = 0.002$, for S—S vs. S—C, Fig. 2A), whereas a single injection of ethanol did not elicit locomotor activation ($P = 0.440$, for S—S vs. S-E, Fig. 2A) in non-sensitized naïve animals. The results were similar in the samples from the second experimental set ($P = 0.002$, for S—S vs. S-C; $P = 0.891$, for S—S vs. S-E, Fig. 2B). When sensitization was induced by repeated treatment with either cocaine (C-) or ethanol (*E*-), non-stimulated baseline (challenge with saline, —S) locomotor activity did not significantly differ compared to that of the control (S—S) group ($P = 0.223$, for S—S vs. C—S, Fig. 2A; $P = 0.353$, for S—S vs. *E*-S, Fig. 2B). On the other hand, when sensitized animals (C- or *E*-) were challenged with either cocaine (—C) or ethanol (-E), behavioral sensitization became evident. This effect was observed in cross-treated animals ($P < 0.001$, for S-E vs. C-E, Fig. 2A; $P = 0.012$, for S—C vs. E-C, Fig. 2B) as well as animals treated with the same drug ($P = 0.003$, for S—C vs. C—C, Fig. 2A; $P = 0.001$, for S-E vs. E-E, Fig. 2B).

3.2. Phosphorylation of AMPK signaling pathways in the prefrontal cortex following cross-sensitization between cocaine and ethanol

p-Thr-172-AMPK levels changed significantly after both chronic treatment and acute challenge in the prefrontal cortex [$F(2, 40) = 6.745$, $P = 0.003$, Fig. 3A; $F(2, 40) = 9.093$, $P = 0.001$, Fig. 3B]. Follow-up pair-wise comparisons revealed that p-AMPK levels were higher in the cocaine-sensitized state ($P < 0.001$, for S—S vs. C—S) but not in the ethanol-sensitized state ($P = 0.116$, for S—S vs. *E*-S) compared to that of control group (saline-treated). Ethanol sensitization resulted in greater phosphorylation after a further challenge with ethanol ($P = 0.018$, for E-S vs. E-E, Fig. 3B-1) and augmented the phosphorylation response to cocaine relative to the control group ($P = 0.039$, for S—C vs. *E*-C, Fig. 3B-2). In the cocaine-sensitized state, p-AMPK levels were further increased by a cocaine challenge ($P = 0.014$, for C—S vs. C—C, Fig. 3A-1) but not by a challenge with ethanol ($P = 0.269$, for C—S vs. C-E, Fig. 3A-2). Although the ethanol challenge increased p-AMPK levels in the cocaine-sensitized state compared to the control ($P = 0.011$, for S-E vs. C-E, Fig. 3A-3), this increase was due to the effects of cocaine sensitization rather than the acute effects of ethanol.

The changes in the phosphorylation levels of the upstream regulators CaMK4 [$F(2, 40) = 7.174$, $P = 0.002$, Fig. 3C; $F(2, 40) = 11.814$, $P < 0.001$, Fig. 3D] and LKB1 [$F(2, 40) = 7.330$, $P = 0.002$, Fig. 3E; $F(2, 40) = 13.605$, $P < 0.001$, Fig. 3F] were similar to those of AMPK itself in the prefrontal cortex under the present experimental paradigm. Subsequent pair-wise comparisons revealed that p-CaMK4 and p-LKB1 levels were not affected by ethanol sensitization ($P = 0.232$ and $P = 0.971$, respectively, for S—S vs. *E*-S) but were increased by cocaine sensitization ($P = 0.001$ and $P = 0.003$, respectively, for S—S vs. C—S) in the prefrontal cortex. However, in the ethanol-sensitized state, an additional challenge with ethanol increased p-CaMK4 and p-LKB1 levels ($P = 0.027$ and $P = 0.013$, respectively, for *E*-S vs. E-E, Fig. 3D-1 and F-1). Additionally, p-CaMK4 and p-LKB1 levels were further enhanced by a cocaine challenge in the ethanol-sensitized state ($P = 0.025$ and $P = 0.034$, respectively, for S—C vs. E-C, Fig. 3D-2 and F-2). In the cocaine-sensitized state, a cocaine challenge further increased p-CaMK4 and p-LKB1 levels ($P = 0.047$ and $P = 0.033$, respectively, for C—S vs. C—C, Fig. 3C-1 and E-1), whereas an ethanol challenge did not alter phosphorylation levels ($P = 0.406$ and $P = 0.437$, respectively, for C—S vs. C-E, Fig. 3C-2 and E-2). The increased phosphorylation levels of both kinases after the ethanol challenge in the cocaine-sensitized state compared to the control group ($P = 0.005$ and $P = 0.018$, respectively, for S-E vs. C-E, Fig. 3C-3 and E-3) reflected the effects of cocaine sensitization rather than the acute effects of the ethanol challenge. The phosphorylation of another upstream kinase, p-Thr-184/197-TAK1, was affected only by ethanol sensitization regardless of any subsequent cocaine or ethanol challenge ($P = 0.011$, for *E*-S; $P = 0.019$, for E-C; and $P = 0.029$, for E-E, Fig. 3H).

3.3. Phosphorylation of AMPK signaling pathways in the dorsal striatum following cross-sensitization between cocaine and ethanol

p-Thr-172-AMPK levels in the dorsal striatum changed significantly following both chronic treatment and acute challenge [$F(2, 40) = 10.129$, $P < 0.001$, Fig. 4A; $F(2, 40) = 21.184$, $P < 0.001$, Fig. 4B]. Follow-up pair-wise comparisons revealed that p-AMPK levels

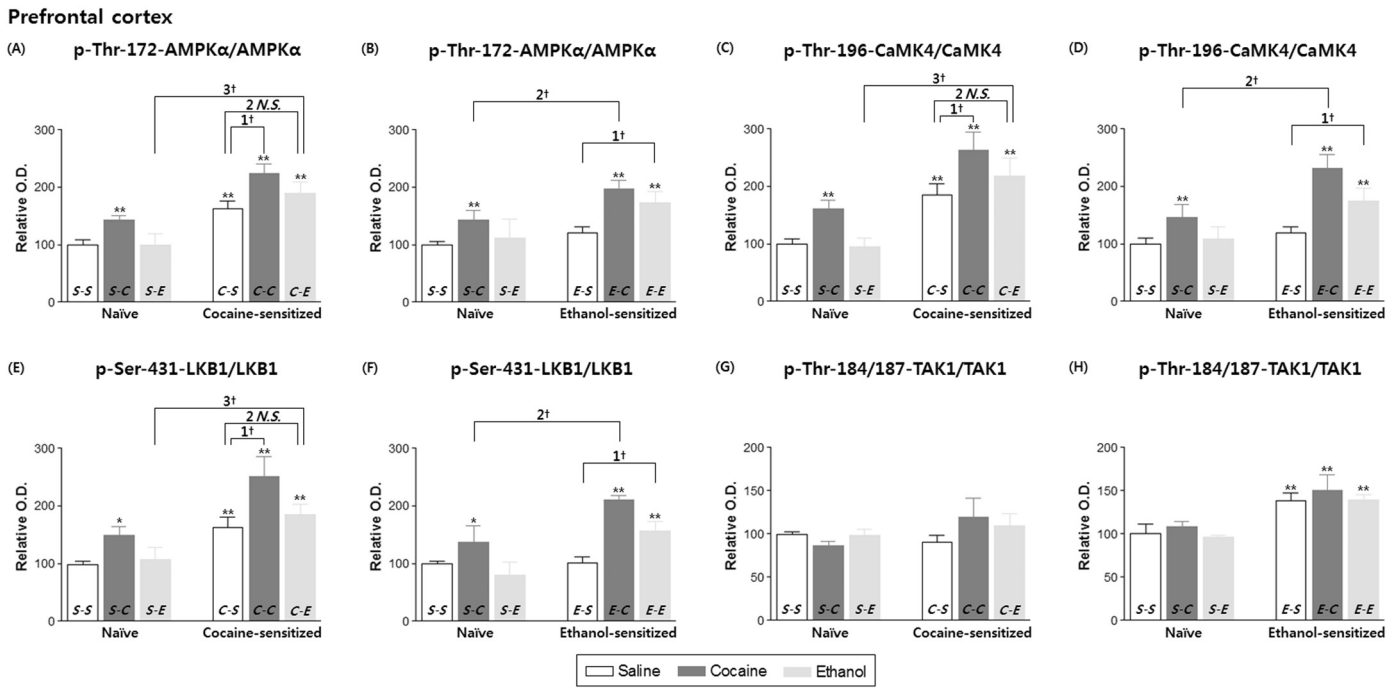

Fig. 3. Drug effects on the phosphorylation levels of AMPKα (A, B) and its upstream kinases CaMK4 (C, D), LKB1 (E, F), and TAK1 (G, H) in the prefrontal cortex. Optical densities (ODs) normalized to the ODs of the corresponding total protein levels. Data are presented as means ± *SE* (n = 6 or 8 rats/subgroup). *, $P < 0.05$; **, $P < 0.01$, significant differences compared to the S—S subgroup. †, $P < 0.05$, indicates significant difference and number of figure-ground represented by number of results.

were significantly reduced by cocaine sensitization ($P < 0.001$, for S—S vs. C—S) but not by ethanol sensitization ($P = 0.853$, for S—S vs. E-S). However, in the ethanol-sensitized state, p-AMPK levels decreased following a challenge with ethanol ($P = 0.016$, for E-S vs. E-E, Fig. 4B-1) and were further reduced by a cocaine challenge ($P < 0.001$, for S—C vs. E-C, Fig. 4B-2); this enhanced cocaine response was also observed in the cocaine-sensitized state ($P = 0.029$, for C—S vs. C—C, Fig. 4A-1). An additional challenge with ethanol did not alter p-AMPK levels in the cocaine-sensitized state ($P = 0.118$, for C—S vs. C-E, Fig. 4A-2) but the reduction of p-AMPK following an ethanol challenge in the cocaine-sensitized state compared to the control group ($P < 0.001$, for S-E vs. C-E, Fig. 4A-3) likely reflected the effects of

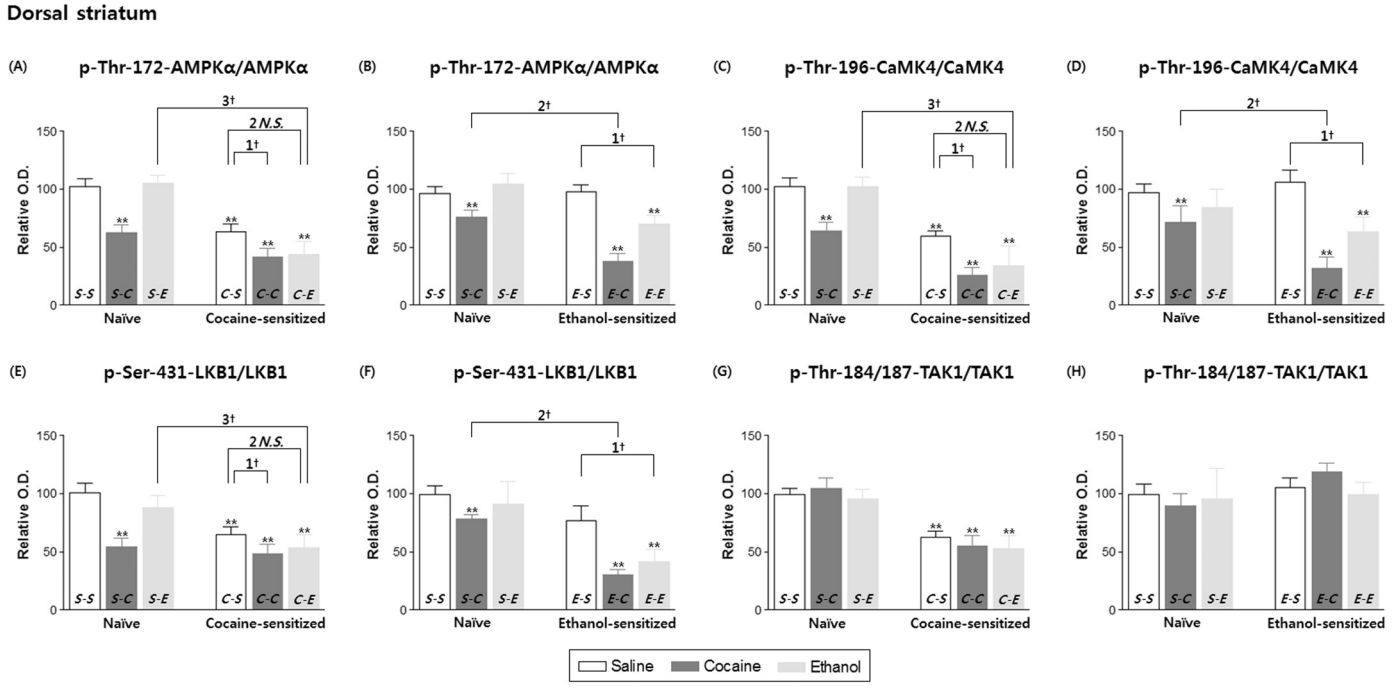

Fig. 4. Drug effects on the phosphorylation levels of AMPKα (A, B) and its upstream kinases CaMK4 (C, D), LKB1 (E, F), and TAK1 (G, H) in the dorsal striatum. ODs were normalized against their corresponding protein levels. Data are presented as means ± *SE* (n = 6 or 8 rats/subgroup). *, $P < 0.05$; **, $P < 0.01$, significant differences compared to the S—S subgroup. †, $P < 0.05$, indicates significant differences and number of figure-ground corresponded to the number of results.

cocaine sensitization. In short, the effects in the dorsal striatum were the same as those in the prefrontal cortex except that the change was in the opposite direction.

The levels of p-Thr-196-CaMK4 [$F(2, 40) = 10.301$, $P < 0.001$, Fig. 4C; $F(2, 40) = 11.828$, $P < 0.001$, Fig. 4D] and p-Ser-431-LKB1 [$F(2, 40) = 11.627$, $P < 0.001$, Fig. 4E; $F(2, 40) = 5.906$, $P = 0.006$, Fig. 4F] in the dorsal striatum were altered in exactly the same manner as those of p-AMPK after cocaine or ethanol sensitization. Subsequent pair-wise comparisons that p-CaMK4 and p-LKB1 levels were not affected by ethanol sensitization ($P = 0.597$ and $P = 0.146$, respectively, for S−S vs. E-S), although they were reduced by cocaine sensitization ($P < 0.001$ and $P < 0.001$, respectively, for S−S vs. C−S). In the ethanol-sensitized state, ethanol decreased p-CaMK4 and p-LKB1 levels ($P = 0.038$ and $P = 0.036$, respectively, for E-S vs. E-E; Fig. 4D-1 and F-1), and the responses to cocaine were augmented ($P = 0.040$ and $P < 0.001$, respectively, for S−C vs. E-C; Fig. 4D-2 and F-2). In the cocaine-sensitized state, p-CaMK4 and p-LKB1 levels were further attenuated by cocaine treatment ($P < 0.001$ and $P = 0.036$, respectively, for C−S vs. C-C; Fig. 4C-1 and E-1) but not significantly changed by ethanol treatment ($P = 0.200$ and $P = 0.365$, respectively, for C−S vs. C-E; Fig. 4C-2 and E-2). Although ethanol decreased p-CaMK4 and p-LKB1 levels in the cocaine-sensitized state compared to the control group ($P = 0.007$ and $P = 0.020$, respectively, S-E vs. C-E; Figs. 4C-3 and 3E-3), this effect also likely reflected the effects of cocaine sensitization rather than acute ethanol treatment. On the other hand, p-TAK1 levels were affected by only cocaine sensitization regardless of the other treatments ($P < 0.001$, for C-S; $P < 0.001$, for C-C; and $P < 0.001$, for C-E; Fig. 4G).

4. Discussion

One of the most consistent findings in the present study was that the phosphorylation levels of AMPK, CaMK4, and LKB1 in the prefrontal cortex and dorsal striatum changed in opposite directions regardless of whether they were induced by sensitization or an acute challenge. Dopamine affects cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling through D_1 and D_2 receptors (Glatt and Snyder, 1993; Neve et al., 2004) such that D_1 receptors are linked to the G_s class of G-proteins, which activates the cAMP-PKA pathway, and D_2 receptors are linked to the G_i class, which inhibits the cAMP-PKA pathway (Kebabian et al., 1972). D_1 receptors are dominant in the prefrontal cortex, but D_2 receptors are dominant in the dorsal striatum (Boyson et al., 1986). A previous study from our research group showed the different effects of cocaine on the phosphorylation of AMPK in the prefrontal cortex and dorsal striatum, and it was suggested that these differential effects are associated with the relative abundances of dopamine D_1 and D_2 receptors in these regions (Xu and Kang, 2016). Thus, the present findings support the hypothesis that dopamine receptor-mediated signaling underlies the effects of both the cocaine- and ethanol-induced sensitization paradigms.

Although AMPK is not a direct substrate of cAMP/PKA, its upstream kinase LKB1 is regulated by PKA (Sapkota et al., 2001). Additionally, another upstream AMPK activator, CaMKKβ, may be activated by cAMP-dependent pathways (Bateup et al., 2008; Rushlow et al., 2009; Swulius and Waxham, 2008). However, in the present study, the phosphorylation of TAK1 was induced only by ethanol sensitization in the prefrontal cortex, and it was altered only by cocaine sensitization in the dorsal striatum. This pattern of activation was incompatible with that of AMPK in terms of behavioral sensitization; thus, the phosphorylation of TAK1 did not seem to be related to dopamine or the cAMP-PKA signaling pathway.

Another previous study from our research group demonstrated that behavioral cross-sensitization occurs between ethanol and cocaine, which suggests that commonalities exist between these drugs (Xu and Kang, 2017). In the present study, the regulation of AMPK signaling by cocaine or ethanol challenge was enhanced by the establishment of behavioral sensitization, which indicates that sensitization occurred at a neurochemical level as well as a behavioral level. Moreover, cross-sensitization occurred; That is, rats in an ethanol-sensitized state (*E*-) exhibited an increased response to the cocaine (−C) challenge (E-C vs. S−C) compared to rats in a naïve state (S-). Conversely, rats in a cocaine-sensitized state (C-) exhibited an increased response to an ethanol (−E) challenge (C-E vs. S-E) compared to rats in a naïve state (S-). Although acute ethanol treatment of rats in a cocaine-sensitized state did not significantly alter the phosphorylation status of AMPK (C-E vs. C−S), it is possible that the cocaine-induced sensitization was strong enough to reach a ceiling effect that the relatively weak influence of ethanol could not break through. However, the changes induced by ethanol sensitization were relatively weak and allowed for further changes following acute treatments with either ethanol or cocaine.

At a behavioral level, the magnitude of the change in locomotion was greater for cocaine than for ethanol. For example, in the present study, ethanol did not have a locomotor-activating effect when given to non-sensitized animals whereas cocaine did (Fig. 2). Also, in the case of conditioned locomotion, chronic treatment with cocaine was effective, whereas the same treatment with ethanol was not (Marinho et al., 2017; Oliveira-Lima et al., 2015). Moreover, the period required for the establishment of sensitization to each drug differed. Whereas locomotor sensitization to cocaine was developed within five daily treatments, the same responses to ethanol manifested only after 15 treatments (Xu and Kang, 2017). It has been suggested that behavioral sensitization results from the accumulated effects of repeated treatments on the mesolimbic dopaminergic pathway and that the weaker effects of ethanol produce discernable neurochemical and/or behavioral changes only after long-term treatment. This accumulated effect seems to be the core of behavioral sensitization. Because cocaine acts directly on the dopaminergic system, whereas ethanol exerts only indirect action (Boileau et al., 2003; Ritz et al., 1987), the “sensitization threshold” may be achieved more easily for cocaine than ethanol.

In the present study, baseline locomotor activity did not increase after the establishment of sensitization to cocaine or ethanol (for S−S vs. C−S or S−S vs. E-S; Fig. 2), and the expression of sensitization required further challenges with the sensitizing drugs. However, in the case of cocaine, while the locomotor effect was latent, the neurochemical effects manifested even in the absence of a drug challenge. This phenomenon might be related to the postsynaptic mechanisms associated with the dopamine receptor in that the proportions of high-affinity dopamine D_1 and D_2 receptors increase following cocaine sensitization (Seeman et al., 2005; Shuto et al., 2008). In the case of ethanol, both effects were latent and required further drug challenges to be observable. Previous studies have demonstrated that repeated treatment with ethanol enhances the responsiveness to dopamine neurotransmission without causing changes in dopamine concentrations in alcohol-preferring rats (Engleman et al., 2000; Sahr et al., 2004).

5. Conclusions

In conclusion, the present study demonstrated that the CaMK4/LKB1-AMPK signaling pathway appears to be involved in the behavioral sensitization effects commonly induced by both cocaine and ethanol and that the effects of cocaine were stronger than those of ethanol. Furthermore, the upstream effects seemed to require activation of the dopaminergic system. The cross-sensitization between cocaine and ethanol at the neurochemical and behavioral levels suggests that both drugs have common neural effects, which supports the “common cellular and molecular mechanisms” hypothesis regarding various addictions.

Declaration of Competing Interest

There are no conflicts of interest.

Acknowledgements

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