Role of catecholamines and cyclic AMP systems in phencyclidine and morphine dependence. Study of mutant mice*

Toshitaka Nabeshima, Takayoshi Mamiya, and Yukihiro Noda

Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, 65, Tsuruma-cho, Showa-ku, Nagoya 466-8560, Japan

Abstract: To investigate an involvement of catecholamines and/or the cyclic adenosine monophosphate (cAMP) systems in the development of drug dependence, we examined whether phencyclidine (PCP) and morphine dependence were developed in tyrosine hydroxylase (TH) heterozygous (TH+/–) and cAMP response element binding protein (CREB) binding protein (CBP) heterozygous (CBP+/–) mice. PCP (8 mg/kg) induced place preference in wild-type mice pretreated with PCP (10 mg/kg/day for 28 days) and increased the level of cAMP in the striatum, but not in the thalamus/hypothalamus. In TH+/– and CBP+/– mice, however, we could not find PCP-induced place preference. The increased level of cAMP in the striatum was observed in CBP+/–, but not TH+/– mice. When wild-type mice pretreated with morphine (10 mg/kg) twice a day for 5 days were challenged with naloxone (5 mg/kg), they showed increased jumping, rearing, and forepaw tremor counts as a sign of withdrawal and an increased level of cAMP in the thalamus/hypothalamus, but not in the striatum. In TH+/– and CBP+/– mice, however, jumping and forepaw tremor counts were decreased compared to in wild-type mice. An increase in the level of cAMP in the thalamus/hypothalamus in CBP+/–, but not in TH+/– mice was observed. These results suggest that catecholamines and CBP are involved in the development of PCP and morphine dependence, and that changes in catecholaminergic and/or cAMP systems induced by repeated PCP and morphine treatments play an important role in the addiction to PCP and morphine.

INTRODUCTION

The catecholaminergic systems appear to mediate some of the psychological and/or physical dependence on opiates and psychostimulants such as amphetamines, cocaine, and phencyclidine (PCP) [1–4]. For example, several studies have reported that lesions of the dopaminergic systems attenuate the self-administration and conditioned place preference induced by opiates and psychostimulants. In addition, virtually all drugs abused by humans increase dopamine (DA) levels in the brain when administered systemically to rats [5]. On the other hand, clonidine, a drug that decreases noradrenergic activity, prevents behavior induced by morphine withdrawal [6]. An increase in the noradrenergic neuron firing rate in the locus coeruleus (LC) [7] and an increase in the turnover of noradrenaline (NA) have been reported during naloxone-precipitated morphine withdrawal [8].

The failure to account for important aspects of opiate and psychostimulant addiction in terms of the regulation of neurotransmitters and receptors has shifted attention to post receptor mechanisms. Most types of neurotransmitter receptors present in brain produce most of their physiological responses in target neurons through a complex cascade of intracellular messengers. These intracellular messengers include G-proteins [9], which couple the receptors to intracellular effector systems, and the intra-
cellular effector systems themselves, which include second messengers, protein kinases and protein phosphatases, and phosphoproteins [10,11]. Regulation of these intracellular messenger pathways mediates the effects of the neurotransmitter-receptor systems on diverse aspects of neuronal function, including gene expression. Given that many important aspects of drug addiction develop gradually and progressively in response to continued drug exposure, and can persist for a long time after drug withdrawal, it is likely that the regulation of neuronal gene expression is of particular relevance to addiction.

Recently, the increase in knowledge of intracellular messenger pathways has provided an experimental framework for studies of the molecular mechanisms underlying drug addictions. Investigations have demonstrated that changes in the activity of G-protein and cyclic adenosine monophosphate (cAMP) second messenger and the protein phosphorylation pathway mediate important aspects of opiate, and possibly cocaine, addiction in a number of drug-responsive brain regions [12]. Adaptations in the cAMP signal transduction pathway underlie the mechanisms of opioid tolerance and dependence, and up-regulation of these components plays an important role in the onset of the withdrawal syndrome [13–15]. In particular, the activation of the transcription factor cAMP response element binding protein (CREB) is implicated in withdrawal syndrome [16]. However, there is no evidence for the involvement of CREB binding protein (CBP), which is co-activator of CREB.

Abused drugs have profound chronic effects on brain function. It is well known that repeated exposure to opiates such as morphine develops dependence and tolerance. The development of dependence and tolerance has been postulated to be an attempt by the body to compensate for the chronic existence of opiates, however, the exact mechanisms underlying these physiological changes have not been elucidated in vivo.

In the place-conditioning paradigm, PCP produces place aversion in naive mice [17,18], whereas it produces place preference in mice pretreated with PCP repeatedly [18]. These phenomena are similar to those seen in humans; although a single use of PCP produces aversive effects, long-term use of it causes abuse [19]. We have previously found that the PCP-induced aversive and preferred effects on the conditioned place preference in rodents are attributed to interaction with the serotonergic and dopaminergic systems, respectively [18]. Although these findings suggest that the monoaminergic systems are involved in the PCP dependence, the involvement of signal transduction pathways in PCP dependence is not yet clarified.

As stated above, a key role for the catecholaminergic and cAMP signal pathways in the development of drug dependence has been suggested. Therefore, we investigated the involvement of both signal pathways in PCP-induced place preference (psychological dependence) and morphine withdrawal (physical dependence). We used two mutant mice; tyrosine hydroxylase (TH) heterozygous (TH+/–) mice [20] which are impaired in the catecholamine synthetic pathway, and CBP heterozygous (CBP+/–) mice [21] which are impaired in gene transduction through the cAMP pathway. Firstly, we investigated whether PCP and morphine dependence develops in the mutant mice. Secondly, we examined whether cAMP contributes to PCP and morphine dependence by measuring cAMP levels.

MATERIALS AND METHODS

Subjects

Animals
TH+/– [20] and CBP+/– [21] mice and their litter mates, weighing 30–35 g at the beginning of the experiments, were used. TH+ mice were derived from an F1 embryo of a C57BL/6 or ICR female mated with a DBA/2J male [20], and CBP+ mice were derived from an F1 embryo of a C57BL/6 female mated with a CBA male [21]. The animals were housed in plastic cages and kept in a regulated environment (23 ± 1 °C, 50 ± 5% humidity), with a 12/12 h light-dark cycle (lights on at 7:30 am). Food (CE2, Clea Japan Inc. Tokyo, Japan) and tap water were available ad libitum.
All experiments were performed in accordance with the Guidelines for Animal Experiments of the Nagoya University School of Medicine. The procedures involving animals and their care conformed with the international guidelines set out in “Principles of Laboratory Animal Care” (NIH publication no. 85–23, revised 1985).

**Drugs**

Phencyclidine hydrochloride [(1-(1-phenylcyclohexyl)piperidine; PCP] was synthesized by the authors according to the method of Maddox et al. [22] and was checked for purity. PCP, morphine hydrochloride (Shionogi Co., Ltd., Osaka, Japan), and naloxone hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in saline. The injection volume was 0.1 mL per 10 g body weight on each occasion. All doses were expressed in terms of total salts.

**Conditioned place preference test**

**Apparatus**

The apparatus used for the place-conditioning task consisted of two compartments: a black Plexiglas box and a transparent Plexiglas box (both $15 \times 15 \times 15$ cm) with a metal grid floor. To enable the mice to distinguish easily the transparent box from the black one, the floor of the transparent and black boxes were covered with white plastic mesh and with black frosting Plexiglas, respectively. Each box could be divided by a sliding door ($10 \times 15$ cm).

**Drug administration**

Mice were administered PCP (10 mg/kg/day) for 28 days as in a previous report [18]. One day after the last treatment with PCP, the place-conditioning test including pre-conditioning, conditioning, and post-conditioning tests, was commenced.

**Pre-conditioning test**

The place-conditioning paradigm was performed according to the method of Noda et al. [18]. In the pre-conditioning test, the sliding door was opened, and the mouse was allowed to move freely between both boxes for 15 min once a day for 3 days. On the third day of the pre-conditioning test, we measured the time that the mouse spent in the black and transparent boxes using a Scanet SV-10 LD (Toyo Sangyo Co. Ltd., Toyama, Japan). The box in which the mouse spent the most time was referred to as the “preferred side”, and the other box as the “non-preferred side”.

**Conditioning**

Conditioning was performed over six successive days. Mice were given drugs or vehicle in the apparatus with the sliding door closed. That is, a mouse was given PCP and put in its non-preferred side for 20 min. The next day, the mouse was given saline, and placed opposite the drug-conditioning site for 20 min. These treatments were repeated for three cycles (6 days).

**Post-conditioning test**

In the post-conditioning test, the sliding door was opened, and the time that the mice spent in the black and transparent boxes was measured for 15 min using the Scanet SV-10 LD.

**Data analysis**

Place-conditioning behaviors were expressed by post–pre, which was calculated as $[(post \ value) – (pre \ value)]$, where post and pre values were the difference in time spent in the drug-conditioning and the saline-conditioning sites in the post-conditioning and pre-conditioning tests, respectively.
Assay of naloxone-precipitated withdrawal syndrome

Mice received morphine (10 mg/kg sc) twice daily for 5 days as in a previous report [23]. On day 6, mice received morphine and two hours later, naloxone (5 mg/kg ip). Immediately after the naloxone challenge, each mouse was placed in a transparent acrylic cylinder (20 cm diameter, 35 cm high), and then, the signs of naloxone-precipitated withdrawal (escape jumping, rearing and forepaw tremor) were counted for 15 min. The control group was administered with saline. The scoring of the behavioral syndrome was done by an observer “blind” to the treatment.

Measurement of cAMP level in the mouse brain

The PCP- and morphine-dependent animals were killed by focused microwave irradiation (Toshiba Microwave Applicator TMW-6402A, Tokyo, Japan) for 1.5 s at 5 kW 24 h after the final PCP (10 mg/kg) treatment and 5 min after the naloxone challenge, respectively. For the measurement of cAMP, the brain tissue of the striatum or thalamus/hypothalamus was dissected. The tissue was homogenized with 6% trichloroacetic acid to give a 10% homogenate, and centrifuged at 15000 g for 10 min. The supernatant was extracted with water-saturated diethyl ether to remove the acid. Cyclic AMP in the aqueous extract was quantified with enzyme immunoassay kits (BIOTRAK, Amersham, UK).

Statistical analysis

The statistical difference between groups was assessed with Student’s t-test. Differences were considered significant at a level of p < 0.05.

RESULTS AND DISCUSSION

PCP dependence

In the present study, PCP (8 mg/kg sc) produced place preference in the wild-type mice pretreated with PCP (10 mg/kg/day sc) for 28 days (Table 1), as reported previously [17,18]. Although the single use of PCP produces aversive effects, long-term use was shown to cause abuse in animal experiments and a clinical study [18,19]. Thus, it appears that functional changes induced by repeated PCP treatment play an important role in PCP-induced place preference.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Post–Pre value (% of saline-treated, wild-type mice)</th>
<th>Saline</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type¹</td>
<td>100.0 ± 56.2</td>
<td>ns</td>
<td>346.2 ± 89.0 *</td>
</tr>
<tr>
<td>TH⁺/⁻</td>
<td>102.2 ± 43.6</td>
<td>105.4 ± 63.3</td>
<td></td>
</tr>
<tr>
<td>Wild-type²</td>
<td>100.0 ± 101.3</td>
<td>ns</td>
<td>464.5 ± 118.7 *</td>
</tr>
<tr>
<td>CBP⁻/⁻</td>
<td>218.8 ± 112.4</td>
<td>125.3 ± 111.8</td>
<td></td>
</tr>
</tbody>
</table>

Post–Pre value is expressed as a percentage of that of the saline-treated, wild-type mice (Post–Pre value: wild-type¹ 99.8 ± 56.1 sec, wild-type² 76.0 ± 77.0 sec). Values are the means ± S.E.M. (n = 5–20 each group). *p < 0.05 vs. corresponding salinetreated group. #p < 0.05 vs. corresponding wild-type mice. ns: not significant.

© 2000 IUPAC, Pure and Applied Chemistry 72, 1035–1044
This preferred effect of PCP (8 mg/kg) was not observed in the TH+− mice pretreated with PCP (10 mg/kg/day) repeatedly (Table 1), suggesting that catecholaminergic systems are involved in the development of PCP-induced place preference, since TH is the first and the rate-limiting enzyme in the catecholamine biosynthetic pathway. In this mouse, the level of catecholamine, NA and DA, was decreased 20–30% and 30–40%, respectively, compared to the control. We have reported that PCP-induced place preference was blocked by coadministration of a TH inhibitor, α-methyl-p-tyrosine (AMPT) and lesion of the catecholaminergic systems by 6-hydroxydopamine (6-OHDA) [18]. Analysis of the neurochemical effects of AMPT and 6-OHDA treatment revealed a marked decrease of the DA level and no reduction of NA and serotonin (5-HT) levels in the brain [18]. Further, DSP-4, a NA neurotoxin, which caused significant depletion of NA, but not of DA and 5-HT, in the brain, did not affect the PCP-induced place preference [18]. The dopaminergic systems have been demonstrated to play an important role in rewarding and abuse properties of drugs in the place-conditioning paradigm [24]. Thus, dopaminergic systems may be involved in PCP-induced place preference in mice pretreated with PCP repeatedly, and it can be hypothesized that the activity of DA, but not of NA and 5-HT, neurons may be necessary for the expression of the rewarding effects of PCP.

Recent studies employing selective DA-D1 and DA-D2 receptor antagonists have demonstrated that the selective DA-D1 receptor antagonist (+) SCH-23390, but not the selective DA-D2 receptor antagonist, (−) sulpiride, blocked the PCP-induced place preference [18]. DA-D1 receptors are generally thought to exert their effects via the G-protein Gs and the subsequent activation of the cAMP pathway. In the present biochemical study, the cAMP levels in the striatum of wild-typed mice pretreated with PCP were higher than in mice pretreated with saline repeatedly (Table 2). Taken together, these findings suggest that PCP can sensitize DA-D1 receptors to DA, enabling them to act independently from DA-D2 receptors, as is observed in some cases of sensitization [25], and that DA-D1 receptors are involved in the conditioning of the rewarding effect of PCP. Further, the elevation of cAMP levels seen in the wild-type mice pretreated with PCP repeatedly was not observed in the TH−+ mice (Table 2). Based on these results, we speculate that the functional changes in the dopaminergic systems, particularly in DA-D1 receptors, were produced during repeated PCP treatment for 28 days and then, the rewarding effect of PCP resulted from an increase in cAMP levels, secondary to a DA-D1 receptor activation.

**Table 2** cAMP levels in TH+− and CBP+− mice pretreated with PCP repeatedly.

<table>
<thead>
<tr>
<th>Mice</th>
<th>cAMP levels (% of saline-treated, wild-type group)</th>
<th>Striation</th>
<th>Thalamus/hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>PCP</td>
<td>Saline</td>
</tr>
<tr>
<td>Wild-type1</td>
<td>100.0 ± 5.1</td>
<td>ns</td>
<td>134.1 ± 14.4 *</td>
</tr>
<tr>
<td>TH−+</td>
<td>105.2 ± 7.7</td>
<td>119.4 ± 12.6</td>
<td>71.1 ± 9.9</td>
</tr>
<tr>
<td>Wild-type2</td>
<td>100.0 ± 7.7</td>
<td>ns</td>
<td>134.1 ± 14.4 *</td>
</tr>
<tr>
<td>CBP+−</td>
<td>105.5 ± 9.4</td>
<td>182.9 ± 31.1 *</td>
<td>95.4 ± 6.2</td>
</tr>
</tbody>
</table>

cAMP levels are expressed as a percentage of those of the saline-treated, wild-type mice (cAMP levels in the striatum and thalamus/hypothalamus: wild-type1 314.1 ± 16.2 and 287.9 ± 31.5 pmol/g tissue, wild-type2 1267.9 ± 161.3 and 1159.9 ± 74.3 pmol/g tissue). Values are the means ± S.E.M. (n = 5–8 each group). *p < 0.05 vs. corresponding saline-treated group. ##p < 0.01 vs. corresponding PCP-treated, wild-type group. ns: not significant.

Intracellular messenger pathways, including the cAMP pathway, could regulate gene expression. It has been reported that amphetamine regulates the expression of several genes, including c-fos, via
DA-D$_1$ receptor activation in the rat brain [26]. Thus, the cAMP signal transduction pathway has been demonstrated to play an important role in drug addiction [12]. Activation of CREB stimulates the expression of a family of genes encoding transcription factors, referred to as immediate-early genes, such as $c$-fos, $c$-jun and zif268. Since CBP is a co-activator of CREB, CBP-mutant mice would not show the expression of genes mediating the activation of CREB. In the CBP$^{+/–}$ mice pretreated with PCP repeatedly, in the present study, PCP-induced place preference did not develop, but an elevation of cAMP levels in the striatum was observed as in wild-type mice (Table 2). Thus, in the CBP$^{+/–}$ mice pretreated with PCP, CREB can not be transcriptionally activated by stimulating gene expression. CBP may be involved in the development of PCP-induced place preference.

In summary, the present results indicated that dopaminergic system, are involved in PCP-induced place preference and that changes in these systems induced by repeated PCP treatment play an important role in the addiction of this drug. Further, the present study demonstrates an involvement of the cAMP signal transduction cascade via DA-D$_1$ receptors, in the conditioning of the rewarding effect of PCP (Fig. 1).

Fig. 1 Schematic illustration of the possible mechanisms of phencyclidine (PCP) and morphine dependence. Some functional changes in catecholaminergic and cyclic AMP (cAMP) signal pathways are potentiated by repeated PCP and/or morphine treatment. An upregulated cAMP signal transduction cascade expresses the transcription factors in the Fos/Jun family mediated by phosphorylation of cAMP response element binding protein (CREB). However, since the tyrosine hydroxylase (TH)$^{+/–}$ and CREB binding protein (CBP)$^{+/–}$ mice have an impaired catecholamine synthetic pathway and are impaired in gene transduction through the cAMP pathway, respectively, PCP and morphine dependence does not develop in either mutant. Try: tyrosine, TH: tyrosine hydroxylase, DOPA: 3,4-dihydroxyphenyalanine, AADC: aromatic L-amino acid decarboxylase, DA: dopamine, DBH: dopamine beta-hydroxylase, G: G-protein, ATP: adenosine triphosphate, PKA: protein kinase A, CRE: cAMP response element.

© 2000 IUPAC, Pure and Applied Chemistry 72, 1035–1044
Morphine dependence

Physical dependence on opiate drugs is characterized by a withdrawal syndrome following either the abrupt termination of morphine intake or precipitated by the administration of a narcotic antagonist. In the present study, when the wild-type mice pretreated with morphine (10 mg/kg sc) twice a day for five days were challenged with naloxone (5 mg/kg), they showed increased jumping, rearing, and forepaw tremor counts as signs of withdrawal (Table 3). In the TH+/– mice pretreated with morphine, however, the frequency of jumping and forepaw tremor was significantly lower than in wild-type mice after naloxone challenge (Table 3). These results suggest that the catecholaminergic signal pathway is involved in the naloxone-precipitated morphine withdrawal syndrome.

Table 3 Naloxone-induced precipitated morphine withdrawal syndrome in TH+/– and CBP+/– mice pretreated with morphine.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Naloxone-precipitated withdrawal syndrome (％of wild-type mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jumping</td>
</tr>
<tr>
<td>Wild-type1</td>
<td>100.0 ± 13.3</td>
</tr>
<tr>
<td>TH+/–</td>
<td>50.2 ± 10.6</td>
</tr>
<tr>
<td>Wild-type2</td>
<td>100.0 ± 11.8</td>
</tr>
<tr>
<td>CBP+/–</td>
<td>9.9 ± 5.6</td>
</tr>
</tbody>
</table>

Jumping and forepaw tremor counts are expressed as a percentage of those of the wild-type mice (jumping and forepaw tremor: wild-type 1 64.9 ± 8.6 and 18.9 ± 2.2, respectively, wild-type 2 68.4 ± 8.1 and 28.7 ± 5.3, respectively). Values are the means ± S.E.M. (n = 10–16 each group). ##p < 0.01 vs. corresponding wild-type mice.

The LC represents the largest cluster of noradrenergic neurons in the brain [27]. This nucleus possesses a high density of opioid receptors, particularly of the mu and kappa subtypes [28]. Experimental evidence suggests functional interactions in the LC between opioid and noradrenergic systems during opiate withdrawal. A noradrenergic hyperactivity in the LC has been hypothesized to mediate the expression of some components of the morphine withdrawal syndrome. Supporting this hypothesis, an increase in the noradrenergic neuron firing rate in the LC [7] and an increase in the turnover of NA have been reported during naloxone-precipitated morphine withdrawal [8]. Further, clonidine, a drug that decreases noradrenergic activity, prevents behaviors induced by morphine withdrawal [6]. On the other hand, although the dopaminergic systems are a neuronal substrate of opiate-induced reward [29], it is debatable whether withdrawal shares common neuronal mechanisms with opiate reward. Bozarth and Wise [30] proposed that opiate dependence and withdrawal are mediated by the periaqueductal gray, and Koob et al. [31] and Stinus et al. [32] have suggested that the nucleus accumbens is a substrate for the aversiveness of opiate withdrawal. In mice lacking DA-D2 receptors, the behavioral expression of morphine withdrawal was unchanged [33]. There is very little information to date on the specific effects of opiate withdrawal on dopaminergic systems. Thus, noradrenergic, rather than dopaminergic, systems may play an important role in the behavioral expression of morphine withdrawal.

Several lines of evidence indicate that an up-regulation of intracellular cAMP systems is responsible for the development of morphine dependence. If a compensatory up-regulation of cAMP systems following chronic morphine exposure is essential for the development of dependence, morphine dependence may be ensured by inhibiting alterations of the cAMP systems induced by chronic morphine exposure. In the present biochemical study, the wild-type mice experiencing the naloxone-precipitated morphine withdrawal syndrome showed an elevation of cAMP levels in the thalamus/hypothalamus (Table 4). However, the TH+/– mice which did not undergo the naloxone-precipitated withdrawal showed
no such change of cAMP levels in the thalamus/hypothalamus (Table 4). Therefore, our results suggest that naloxone-precipitated morphine withdrawal syndrome is mediated by the up-regulation of intracellular cAMP, and there is no increase in the cAMP level in the TH+/– mice because they lack catecholaminergic-cAMP signal pathways.

Table 4 cAMP levels in TH+/– and CBP+/– mice pretreated with morphine and naloxone.

<table>
<thead>
<tr>
<th>Mice</th>
<th>cAMP levels (% of saline-treated, wild-type group)</th>
<th>Striatum</th>
<th>Thalamus/hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Saline</td>
<td>Morphine</td>
</tr>
<tr>
<td>Wild-type1</td>
<td>100.0 ± 5.1</td>
<td>ns</td>
<td>100.1 ± 15.0</td>
</tr>
<tr>
<td>TH+/–</td>
<td>105.2 ± 7.7</td>
<td>112.6 ± 14.0</td>
<td>ns</td>
</tr>
<tr>
<td>Wild-type2</td>
<td>100.0 ± 12.1</td>
<td>ns</td>
<td>96.5 ± 8.3</td>
</tr>
<tr>
<td>CBP+/–</td>
<td>91.2 ± 10.7</td>
<td>119.7 ± 17.5</td>
<td>ns</td>
</tr>
</tbody>
</table>

cAMP levels are expressed as a percentage of those of the saline-treated, wild-type mice (cAMP levels: wild-type1 99.8 ± 56.1 nmol/g protein, wild-type2 76.0 ± 77.0 nmol/g protein). Values are the means ± S.E.M. (n = 5–8 each group). *p < 0.05 vs. corresponding saline-treated group. ##p < 0.01 vs. corresponding (morphine and naloxone) - treated, wild-type group. ns: not significant.

Recently, Nestler [34] suggested that the regulation of gene expression is involved in the up-regulation of cAMP systems in the development of morphine dependence. The CREB is a transcription factor, which is activated in response to a second messenger stimulus and mediates many effects of cAMP on gene expression [35]. It has been demonstrated that acute morphine treatment decreases CREB phosphorylation and withdrawal of morphine after chronic exposure increases it [16]. Furthermore, development of morphine dependence is attenuated in CREB-deficient mice [36]. More recently, direct evidence of a role for CREB in mediating up-regulation of cAMP systems in the morphine-dependent state was obtained by Lane-Ladd et al. [37]. In the present study, although the CBP+/– mice pretreated with morphine following naloxone challenge had elevated levels of cAMP in the thalamus/hypothalamus (Table 4), the frequency of the symptoms of naloxone-precipitated morphine withdrawal was significantly low compared to in wild-type mice (Table 3). Thus, these results suggest that the expression of transcription factors in the Fos/Jun family mediated by phosphorylation of CREB is not high enough in the CBP+/– mouse. Further, it can be considered that CBP+/– mice developed fewer symptoms of morphine withdrawal due to inhibition of the alteration of CREB phosphorylation resulting from a continuous reduction in the expression level of related genes. The cAMP signal pathway including the expression of a family of genes encoding transcription factors may play an important role in the expression of morphine withdrawal. The mechanisms for transcriptional activation/repression are not fully understood, but likely involve the direct or indirect interaction of transcription factors with the RNA polymerase II transcription complex [38]. Further studies are needed to elucidate the role of CBP in the morphine withdrawal.

In conclusion, our findings suggest that the alteration of catecholaminergic biosynthetic and cAMP signal pathways plays a key role in the development of morphine dependence and, further, that the expression of genes mediated by phosphorylation CREB is involved in the development of morphine withdrawal (Fig. 1).
ACKNOWLEDGMENTS

The TH+/– and CBP+/– mice were established by Drs. K. Kobayashi and T. Nagatsu and their collaborators (Fujita Health University) and by Drs. Y. Oike and K. Yamamura and their collaborators (Kumamoto University), respectively. This study was supported in part by grants (No. 10044260, 10897005, COE) from the Ministry of Education, Science and Culture of Japan, and an SRF Grant for Biomedical Research.

REFERENCES