Published in final edited form as:

Psychopharmacology (Berl). 2008 April ; 197(3): 443–448.

The role of beta-endorphin in the acute motor stimulatory and rewarding actions of cocaine in mice

Paul Marquez, Ramkumarie Baliram, Ibrahim Dabaja, Nagaraju Gajawada, and Kabirullah Lutfy

Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, 309 East 2nd Street, Pomona, CA 91766, USA e-mail: klutfy@westernu.edu

Abstract

Rationale—Opioid receptor antagonists have been shown to attenuate the rewarding and addictive effects of cocaine. Furthermore, cocaine has been shown to cause the release of beta-endorphin, an endogenous opioid peptide.

Objective—We assessed whether this neuropeptide would play a functional role in cocaine-induced motor stimulation and conditioned place preference (CPP).

Materials and methods—Mice lacking beta-endorphin and their wild-type littermates were habituated to motor activity chambers for 1 h, then injected with cocaine (0, 15, 30, or 60 mg/kg, intraperitoneally) or morphine (0, 5, or 10 mg/kg, subcutaneously), and motor activity was recorded for 1 h. In the CPP paradigm, mice were tested for baseline place preference on day 1. On days 2 and 3, mice received an alternate-day saline/cocaine (15, 30, or 60 mg/kg) or saline/ morphine (10 mg/kg) conditioning session and then tested for postconditioning place preference on day 4.

Results—Cocaine-induced motor stimulation and CPP were both reduced in mice lacking beta-endorphin. On the other hand, motor stimulation and CPP induced by morphine were not altered in mutant mice.

Conclusion—The present results demonstrate that the endogenous opioid peptide beta-endorphin plays a modulatory role in the motor stimulatory and rewarding actions of acute cocaine.

Keywords

Cocaine; Morphine; Motor activity; Conditioned place preference; Reward; beta-Endorphin; Proopiomelanocortin; Knockout mouse

Introduction

The endogenous opioid system has long been known as one of the principal modulators of the mesolimbic dopaminergic reward circuitry (Belluzzi and Stein 1977; Kosterlitz and Hughes 1975). This system has also been implicated in the rewarding and addictive effects of cocaine and other drugs of abuse. For example, opioid receptor antagonists have been shown to attenuate cocaine-induced motor stimulation (Houdi et al. 1989; Kim et al. 1997). Likewise, opioid receptor antagonists have been shown to reduce the rewarding action of cocaine (Houdi et al. 1989; Rademacher and Steinpreis 2002), raising the possibility that cocaine may cause the release of endogenous opioid peptides and these neuropeptides play a functional role in the actions of cocaine. However, it is not known which one of the endogenous opioid peptides may be important in this regard.

Intracerebroventricular beta-endorphin administration has been shown to increase motor activity and to induce conditioned place preference (CPP) in rats (Amalric et al. 1987). Furthermore, cocaine has been shown to cause the release of beta-endorphin in the nucleus accumbens (Olive et al. 2001), raising the possibility that this neuropeptide may be important in these actions of cocaine. Mice lacking beta-endorphin (Rubinstein et al. 1996) have been generated by the placement of a premature translational stop codon instead of the tyrosin codon at position 179 of the proopiomelanocortin (POMC) gene. Previous studies have shown that the hypothalamic–pituitary–adrenal axis functions normally in these mice. Diurnal corticosterone levels, hypothalamic expression of corticotropin-releasing hormone messenger ribonucleic acid, as determined by in situ hybridization, and adrenal gland weight, an indicator of chronic integrated adrenocorticotrophic hormone secretion, appeared normal in these mice (Rubinstein et al. 1996). Likewise, the distribution and level of enkephalins and dynorphins appeared to be normal in homozygous mice as determined by immunohistochemical analysis (Rubinstein et al. 1996). Moreover, the density of mu-, delta-, or kappa-opioid receptors in the amygdala, nucleus accumbens, thalamus, periaqueductal gray, and dorsal horn of the spinal cord is not altered in these mutant mice (Mogil et al. 2000). Total brain mu-opioid receptor level was not different between wild-type mice and mice lacking beta-endorphin (Slugg et al. 2000). Furthermore, electrophysiological techniques show a normal coupling of the mu-opioid receptors to the potassium channels (Slugg et al. 2000). Thus, using these mutant mice and their wild-type littermates, the present study determined the role of beta-endorphin in cocaine-induced motor stimulation and CPP. For comparison, we also assessed the role of this neuropeptide in the motor stimulatory and rewarding actions of morphine.

Materials and methods

Subjects

Mice lacking beta-endorphin (Rubinstein et al. 1996), backcrossed for at least ten generations to the C57BL/6J background, and wild-type mice were obtained from Jackson Laboratories (Bar Harbor, ME) and used to generate a heterozygous breeding colony. These mutant mice are named POMCX*4– or betaend– in the Jackson Laboratories search engine. Male offspring (2–6 months old) of these mice were used for all experiments. Mice were maintained in humidity- (30–70%) and temperature- ($21 \pm 3^\circ\text{C}$) controlled room with free access to water and food. All experiments were carried out during the light phase of a 12-h light/12-h dark cycle and approved by the Institutional Animal Care and Use Committee at Western University of Health Sciences (Pomona, CA).

Drugs

Cocaine hydrochloride and morphine sulfate, obtained from Sigma (St. Louis, MO), were dissolved in normal saline and injected intraperitoneally (i.p.) and subcutaneously, respectively, in a volume of 0.1 ml/10 g of body weight.

Experimental procedures

The role of beta-endorphin in cocaine-induced motor stimulation—Distance traveled (cm) was recorded using a Videomex-V system (Columbus Instruments, Columbus, OH) and used as a measure of motor activity. To determine the role of beta-endorphin in acute motor stimulatory action of cocaine, mice lacking beta-endorphin and their wild-type littermates were habituated to locomotor activity chambers for 1 h, then injected with saline or cocaine (15, 30, or 60 mg/kg), and motor activity was recorded for 1 h. For comparison, in a separate set of experiments, the role of endogenous beta-endorphin in morphine-induced locomotor stimulation was also assessed. Naïve groups of wild-type and beta-endorphin-deficient mice were habituated to the testing chambers for 1 h, then injected with saline or morphine (5 or 10 mg/kg), and motor activity was recorded for 1 h.

The role of beta-endorphin in cocaine-induced conditioned place preference—

The description of the CPP apparatus is provided elsewhere (Marquez et al. 2006). The CPP paradigm consisted of preconditioning, conditioning, and postconditioning session(s) and was carried out over a 4-day period. On day 1 (preconditioning session), basal place preferences of the mice toward the CPP chambers were measured. On this day, each mouse was placed in the neutral chamber of the CPP apparatus and allowed to explore the conditioning chambers via this central neutral chamber for 15 min. The amount of time that the mouse spent in each chamber was recorded and used for analysis of preconditioning data. The conditioning session was carried out on days 2 and 3. On the first conditioning day (day 2), mice were treated with either saline or cocaine (15, 30, or 60 mg/kg, i.p.) and confined, respectively, to the vehicle- or drug-paired chamber for 30 min. On the second conditioning day (day 3), mice were injected with the alternate treatment and confined to the opposite conditioning chamber for 30 min. The assignment of the mice to the treatments (saline or cocaine) and conditioning chambers (decorated with 2.54 cm black and white horizontal or vertical stripes paired with olfactory cues: almond or orange scent) was balanced. Thus, some mice received cocaine conditioning on day 2 and some on day 3. On day 4, each mouse was placed in the neutral chamber and allowed to freely explore the CPP chambers for 15 min. The amount of time that the mouse spent in each chamber was recorded and used for analysis of postconditioning data. CPP was defined as a significant increase in the amount of time that the mice spent in the drug-paired over the vehicle-paired chamber after the conditioning session. In a separate set of experiments, we also assessed the role of beta-endorphin in morphine-induced CPP. Naïve wild-type and beta-endorphin-deficient mice were tested before and after a single alternate-day conditioning session, as described above, except morphine (10 mg/kg) was used instead of cocaine and the conditioning duration was 60 min. The choice of the dose of cocaine and morphine was based on our pilot dose–response studies (data not shown).

Genotyping procedure—The genotyping of offsprings into wild-type, heterozygous, and beta-endorphin-deficient mice were conducted by established polymerase chain reaction with annealing temperature of 56°C and the following primers: primer 1 for wild type: ATGACCTCCGAGAA GAGCCAG, primer 2 for knockout: GAGGATTGGGAA GACAATAGC, and primer 3 for common: GCTGGGG CAAGGAGGTTGAGA. The primers were purchased from SigmaGenosys (The Woodlands, TX).

Data analysis—Data are presented as mean ($\pm$ SEM). Motor activity data were analyzed by two-factor analysis of variance (ANOVA). The factors were dose and genotype. The CPP data were also analyzed by two-factor ANOVA, in which the factors were genotype and treatment (conditioning in the presence of saline vs cocaine or saline vs morphine). The least squares of means analysis was used to reveal significant differences between the groups. A $p < 0.05$ was considered significant.

Results

Mice lacking beta-endorphin displayed reduced cocaine-induced motor stimulation

Figure 1 demonstrates the dose–response relationship of cocaine-induced motor stimulation in mice lacking beta-endorphin (betaend $^{-}$) and their wild-type (betaend $^{+}$) littermates. A two-way ANOVA (genotype and dose of cocaine) revealed no significant effect of genotype ($F_{1,41}=0.01$; $p > 0.05$), but there was a significant effect of cocaine dose ($F_{3,41}=27.19$; $p < 0.001$) and a significant interaction between cocaine dose and genotype ($F_{3,41}=9.83$; $p < 0.001$). Further analysis of the data showed that cocaine dose-dependently increased motor activity in both wild-type and beta-endorphin-deficient mice, but the magnitude of this response was significantly reduced in mutant mice ($p < 0.05$). At the highest dose of cocaine (60 mg/kg), however, the motor stimulatory action of cocaine declined in wild-type mice but not in mutant

mice, suggesting for a rightward shift in the dose–response curve of cocaine. We also tested the effect of a higher dose of cocaine (90 mg/kg) on motor activity in both genotypes. However, this dose of cocaine induced lethality in all mice ($n=6$ mice/genotype).

The motor stimulatory action of morphine was not altered in mice lacking beta-endorphin

Figure 2 shows the motor stimulatory action of morphine in beta-endorphin-deficient mice and their wild-type litter-mates. A two-factor ANOVA revealed a significant effect of dose ($F_{2, 42}=84.78$; $p<0.001$) but no significant effect of genotype ($F_{1, 42}=0.01$; $p>0.05$) and no significant interaction between dose and genotype ($F_{2, 42}=0.09$; $p>0.05$), showing that morphine dose-dependently increased motor activity in both wild-type and beta-endorphin-deficient mice, but the magnitude of this response was not altered in the mutant mice.

Cocaine-induced CPP was attenuated in beta-endorphin-deficient mice

Figure 3 illustrates the amount of time that the mice spent in the conditioning chambers after a single alternate-day conditioning session with various doses of cocaine (15, 30, or 60 mg/kg). Analysis of the postconditioning data showed that cocaine at the lowest dose tested (15 mg/kg) failed to induce CPP in either genotype (Fig. 3a; compare VPCh vs DPCh for each genotype; $p>0.05$). In contrast, conditioning with a higher dose of cocaine (30 mg/kg) induced CPP in wild-type mice (Fig. 3b). Analysis of the data revealed a significant effect of treatment ($F_{1, 28}=29.20$; $p<0.001$) and a significant interaction between genotype and treatment ($F_{1, 28}=22.22$; $p<0.001$). Further analysis of the data revealed that wild-type mice spent more time in the drug-paired as compared to vehicle-paired chamber ($p<0.05$). However, this response was blunted in beta-endorphin-deficient mice ($p>0.05$). Our motor activity data suggested that the dose–response curve of cocaine became bell shaped in wild-type mice and shifted to the right in beta-endorphin-deficient mice. Thus, we also assessed the effect of the highest dose of cocaine (60 mg/kg) to determine whether the dose–response curve of cocaine-induced CPP would become bell shaped in wild-type mice and shifted to the right in mutant mice (Fig. 3c). A two-way ANOVA of the data revealed a significant effect of treatment ($F_{1, 30}=15.17$; $p<0.0001$) and a significant interaction between genotype and treatment ($F_{1, 30}=4.05$; $p<0.05$). Further analysis of the data revealed that beta-endorphin-deficient mice spent more time in the drug-paired as compared to vehicle-paired chamber ($p<0.05$), showing that mutant mice expressed a robust CPP after conditioning in the presence of this dose of cocaine (60 mg/kg). These results suggest that the dose–response curve of cocaine was shifted to the right in mutant mice.

The rewarding action of morphine was not altered in beta-endorphin-deficient mice

Figure 4 illustrates the amount of time that wild-type (betaend+) and mutant (betaend–) mice spent in the conditioning chambers after a single morphine (10 mg/kg) conditioning session (day 4). A two-factor ANOVA of the postconditioning data revealed a significant effect of treatment ($F_{1, 26}=9.36$; $p<0.005$) but no significant effect of genotype ($F_{1, 26}=0.89$; $p>0.05$) or genotype×treatment interaction ($F_{1, 26}=0.97$; $p>0.05$), showing that both genotypes expressed CPP. Although the mutant mice appeared to show smaller CPP than their wild-type littermates, the difference was not significantly different ($p>0.05$). These results suggest that CPP induced after a single conditioning with morphine (10 mg/kg) was not altered in beta-endorphin-deficient mice.

Discussion

The main findings of the present study are that motor stimulation and CPP induced by acute cocaine were attenuated in beta-endorphin-deficient mice. On the other hand, these actions of morphine were not altered in mutant mice. Together, the present results suggest that

endogenous beta-endorphin may function as a modulator of the motor stimulatory and rewarding actions of cocaine.

Accumulating evidence implicates the endogenous opioid system in the motor stimulatory, rewarding, and addictive effects of cocaine. For example, the motor stimulatory action of cocaine has been shown to be reduced in the presence of opioid receptor antagonists (Houdi et al. 1989; Kim et al. 1997) or in mice lacking the mu-opioid receptor (Hummel et al. 2004). Importantly, cocaine has been reported to cause the release of beta-endorphin in the nucleus accumbens (Olive et al. 2001), a response that could play a functional role in the motor stimulatory and rewarding actions of cocaine. Thus, in the present study, we tested the hypothesis that endogenous beta-endorphin might be involved in the motor stimulatory action of cocaine. Consistent with our hypothesis, we discovered that cocaine-stimulated motor activity was significantly reduced in mice lacking beta-endorphin. The reduced cocaine-induced motor stimulation in beta-endorphin-deficient mice was not due to a decrease in basal motor activity in these mutant mice because they showed comparable exploratory behaviors to that of their wild-type littermates during the habituation period and in response to saline injection. Furthermore, the reduction in cocaine-induced motor stimulation in beta-endorphin-deficient mice was selective because the motor stimulatory action of morphine was not altered in these mice. Thus, the present data clearly show that the endogenous opioid peptide beta-endorphin plays a modulatory role in the ability of cocaine to increase motor activity.

Analysis of cocaine dose–response curves in wild-type and beta-endorphin-deficient mice revealed that the decrease in cocaine-induced motor stimulation in mutant mice was a shift to the right of the dose–response curve of cocaine without reduction in its maximal effect. Furthermore, we observed that cocaine-stimulated motor activity became bell-shaped in wild-type mice which we attributed to the emergence of stereotypy (informal observations). It is interesting to note that cocaine failed to induce a bell-shaped dose–response curve in mutant mice, suggesting that beta-endorphin may also be important in stereotypy induced by high doses of cocaine. Alternatively, a more parsimonious interpretation of this result would be that a simple rightward shift would be observed, but not high enough doses were tested to show the descending limb in the mutant mice. Thus, we tested the effect of a higher dose of cocaine (90 mg/kg) in mutant mice and their wild-type littermates. However, this dose of cocaine produced lethality in all mice of both genotypes ($n=6$ mice/genotype), which made us unable to test these possibilities.

CPP has been used widely to study the rewarding action of cocaine and other drugs of abuse (Bardo and Bevins 2000). As central administration of beta-endorphin induces CPP (Amalric et al. 1987) and cocaine causes the release of beta-endorphin in the nucleus accumbens (Olive et al. 2001), a brain region where local administration of opiates induces reward (Kelsey et al. 1989; Wise 1989; Wise and Hoffman 1992), we then tested the possibility that the endogenous opioid peptide beta-endorphin could also be important in the cocaine-induced reward. A single conditioning with cocaine (15 mg/kg) failed to induce CPP (Fig. 3a), raising the possibility that either the neurobiological substrates for cocaine-induced motor stimulation and CPP are different or the amount of dopamine elevated in response to this dose of cocaine is not robust enough to induce CPP. A higher dose of cocaine (30 mg/kg) induced a significant CPP in wild-type mice, a response that was blunted in beta-endorphin-deficient mice (Fig. 3b). The decrease in CPP response was not due to the inability of mutant mice to express CPP because conditioning with a higher dose of cocaine (60 mg/kg) induced a robust CPP in these mice (Fig. 3c), suggesting a rightward shift in the dose–response curve of cocaine.

The observation that the rewarding action of cocaine was shifted to the right in mice lacking beta-endorphin strongly suggests that this neuropeptide functions as a facilitator of rewarding action of acute cocaine. We then determined whether this modulatory role of beta-endorphin

is selective to the action of cocaine or can be generalized to the rewarding action of other drugs of abuse. Thus, we determined whether CPP induced by a single morphine conditioning would be altered in beta-endorphin-deficient mice as compared to their wild-type littermates. Our results showed that the rewarding action of morphine was not altered in mutant mice, indicating for a selective involvement of endogenous beta-endorphin as a facilitator of the rewarding action of cocaine.

Currently, it is unclear how beta-endorphin contributes to the acute motor stimulatory and rewarding actions of cocaine. For example, it is not known whether beta-endorphin facilitates dopaminergic neurotransmission along the mesolimbic neurons by acting in the ventral tegmental area or in the nucleus accumbens or elsewhere in the brain. As stated above, cocaine has been shown to cause the release of beta-endorphin in the nucleus accumbens (Olive et al. 2001; Roth-Deri et al. 2003), a brain region where opioid can induce CPP (Kelsey et al. 1989; Wise 1989; Wise and Hoffman 1992). Thus, we propose that the release of beta-endorphin in the nucleus accumbens in response to cocaine in wild-type mice facilitates the rewarding action of cocaine. However, because of the lack of beta-endorphin in mutant mice, this response may not occur, thereby leading to a rightward shift in the dose–response curve of cocaine in these mice. It is of interest to note that intra-accumbal administration of a selective antibody against beta-endorphin has been shown to alter cocaine-seeking behaviors (Roth-Deri et al. 2004). However, further studies are needed to identify the underlying mechanism and the exact neuroanatomical site of modulatory action of endogenous beta-endorphin in this regard.

In summary, the motor stimulatory and rewarding actions of cocaine were both attenuated in beta-endorphin-deficient mice. In contrast, the actions of morphine were not altered in mutant mice. Collectively, the present results demonstrate that the endogenous beta-endorphin plays a modulatory role in the acute motor stimulatory and rewarding actions of cocaine.

Acknowledgments

The author would like to thank Drs. Rajan Radhakrishnan and Charles Young for reviewing the article. We also express our gratitude to Alexander T. Nguyen and Abdul Hamid for technical support. The present study was supported in part by an intramural grant from the Western University of Health Sciences and in part by a MIDARP Grant R24 DA017298-02 to Dr. Theodore C. Friedman and in part by a NIDA Grant DA016682-03 to KL.

References

- Amalric M, Cline EJ, Martinez JL Jr, Bloom FE, Koob GF. Rewarding properties of beta-endorphin as measured by conditioned place preference. *Psychopharmacology (Berl)* 1987;91:14–19. [PubMed: 2950541]
- Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* 2000;153:31–43. [PubMed: 11255927]
- Belluzzi JD, Stein L. Enkephaline may mediate euphoria and drive-reduction reward. *Nature* 1977;266:556–558. [PubMed: 193035]
- Houdi AA, Bardo MT, Van Loon GR. Opioid mediation of cocaine-induced hyperactivity and reinforcement. *Brain Res* 1989;497:95–198.
- Hummel M, Ansonoff MA, Pintar JE, Unterwald EM. Genetic and pharmacological manipulation of mu opioid receptors in mice reveals a differential effect on behavioral sensitization to cocaine. *Neuroscience* 2004;125:211–220. [PubMed: 15051160]
- Kelsey JE, Carlezon WA Jr, Falls WA. Lesions of the nucleus accumbens in rats reduce opiate reward but do not alter context-specific opiate tolerance. *Behav Neurosci* 1989;103:1327–1334. [PubMed: 2558677]
- Kim HS, Park WK, Jang CG, Oh KW, Kong JY, Oh S, Rheu HM, Cho DH, Kang SY. Blockade by naloxone of cocaine-induced hyperactivity, reverse tolerance and conditioned place preference in mice. *Behav Brain Res* 1997;85:37–46. [PubMed: 9095340]

- Kosterlitz HW, Hughes J. Some thoughts on the significance of enkephalin, the endogenous ligand. *Life Sci* 1975;17:91–96. [PubMed: 806763]
- Marquez P, Baliram R, Gajawada N, Friedman TC, Lutfy K. Differential involvement of enkephalins in analgesic tolerance, locomotor sensitization, and conditioned place preference induced by morphine. *Behav Neurosci* 2006;120:10–15. [PubMed: 16492112]
- Mogil JS, Grisel JE, Hayward MD, Bales JR, Rubinstein M, Belknap JK, Low MJ. Disparate spinal and supraspinal opioid anti-nociceptive responses in beta-endorphin-deficient mutant mice. *Neuroscience* 2000;101:709–717. [PubMed: 11113319]
- Olive MF, Koenig HN, Nannini MA, Hodge CW. Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. *J Neurosci* 2001;21:RC184. [PubMed: 11717387]
- Rademacher DJ, Steinpreis RE. Effects of the selective mu(1)-opioid receptor antagonist, naloxonazine, on cocaine-induced conditioned place preference and locomotor behavior in rats. *Neurosci Lett* 2002;332:159–162. [PubMed: 12399005]
- Roth-Deri I, Schindler CJ, Yadid G. A critical role for beta-endorphin in cocaine-seeking behavior. *Neuroreport* 2004;15:519–521. [PubMed: 15094515]
- Roth-Deri I, Zangen A, Aleli M, Goelman RG, Pelled G, Nakash R, Gispan-Herman I, Green T, Shaham Y, Yadid G. Effect of experimenter-delivered and self-administered cocaine on extracellular beta-endorphin levels in the nucleus accumbens. *J Neurochem* 2003;84:930–938. [PubMed: 12603818]
- Rubinstein M, Mogil JS, Japon M, Chan EC, Allen RG, Low MJ. Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. *Proc Natl Acad Sci USA* 1996;93:3995–4000. [PubMed: 8633004]
- Slugg RM, Hayward MD, Ronnekleiv OK, Low MJ, Kelly MJ. Effect of the mu-opioid agonist DAMGO on medial basal hypothalamic neurons in beta-endorphin knockout mice. *Neuroendocrinology* 2000;72:208–217. [PubMed: 11070424]
- Wise RA. Opiate reward: sites and substrates. *Neurosci Biobehav Rev* 1989;13:129–133. [PubMed: 2573023]
- Wise RA, Hoffman DC. Localization of drug reward mechanisms by intracranial injections. *Synapse* 1992;10:247–263. [PubMed: 1557697]

Fig. 1.

The motor stimulatory action of cocaine was attenuated in beta-endorphin-deficient mice (*betaend*⁻) as compared to their wild-type (*betaend*⁺) littermates. Mice were habituated to motor activity chambers for 1 h, then injected with saline or cocaine (15, 30, or 60 mg/kg, i.p.), and motor activity was recorded for 1 h. Data are presented as mean ($\pm$ SEM) of five to eight mice per dose for each genotype

Fig. 2.

The motor stimulatory action of morphine was not altered in beta-endorphin-deficient mice. Mice lacking beta-endorphin (*betaend*⁻) and their wild-type (*betaend*⁺) littermates were habituated to the test chambers for 1 h, then injected with saline or morphine (5 or 10 mg/kg, s.c.), and motor activity recorded for 1 h. Data are mean ($\pm$ SEM) of six to nine mice per dose for each genotype

Fig. 3.

The acute rewarding action of cocaine was shifted to the right in mice lacking beta-endorphin. Wild-type mice (*betaend+*) and mice lacking beta-endorphin (*betaend-*) were tested for baseline place preference on day 1 (data not shown). On days 2–3, mice received alternate-day saline/cocaine (15, 30, or 60 mg/kg, i.p.) or cocaine/saline conditionings. Mice were then tested for postconditioning place preference on day 4. Data are presented as mean ($\pm$ SEM) of six to nine mice per genotype. **a**, **b**, and **c** illustrate the reinforcing action of cocaine at doses of 15, 30, and 60 mg/kg, respectively

Fig. 4.

The rewarding action of morphine was not altered in mice lacking beta-endorphin. Wild-type mice (*betaend+*) and mice lacking beta-endorphin (*betaend-*) were tested for baseline place preference on day 1 (data not shown). On days 2–3, mice received alternate-day saline/morphine (10 mg/kg, s.c.) or morphine/saline conditioning trainings. Mice were then tested for postconditioning place preference on day 4. Data are presented as mean ($\pm$ SEM) of seven to eight mice per genotype