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Increased analgesic tolerance to acute morphine in fosB knock-out mice: A gender study

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ABSTRACT

The proteins of Fos family are a potential candidate to link molecular mechanisms of morphine action with behavioural effects such as morphine-induced reward, dependence and tolerance. We used both male and female mice lacking fosB gene to study its contribution to morphine effects. Morphine analgesia (tail-flick test) and hypothermia were studied using morphine at cumulative doses in morphine-naïve and morphine-tolerant (tolerance induced by 24 h prior 100 mg/kg morphine administration) mice. FosB −/− mice, as compared to fosB +/+ mice, developed enhanced tolerance to morphine-induced analgesia. No effects of genotype or gender on tolerance to morphine-induced hypothermia were observed. These results suggest that fosB may be involved in the development of tolerance to morphine analgesia but not hypothermia. The gender study implicates that lack of FosB proteins in female fosB −/− mice enhanced morphine analgesic potency. In conclusion, we show that fosB gene is important to analgesia but not hypothermia phenotype indicating its role in morphine effects.

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1. Introduction

Mu opioid receptor activation by morphine triggers the signal transduction cascade, including induction of immediate early genes (c-*fos*, junB, fosB), transcription factors (e.g. CREB) and AP-1 binding activity (Bilecki et al., 2004; Liu et al., 1994; Nye and Nestler, 1996) which are potential candidates to link morphine-regulated signal transduction pathways, gene expression and long-lasting behavioural effects. One of such lasting morphine behaviours, being a consequence of repeated morphine use, is tolerance to morphine effects which might greatly limit its clinical use. Tolerance to analgesic effects of opiates results from complex changes of various molecular and biochemical pathways (Harrison et al., 1998; Labuz et al., 2002; Przewłocka et al., 2002; Starowicz et al., 2005). It has been shown that Fos protein is implicated in the development of analgesic tolerance (Baptista et al., 1998; Ren et al., 2004; Rohde et al., 1997). There are, however, only a few data evidencing the role of other fos family genes: *fosB*, *fra*-1, and *fra*-2. The fosB gene is of special interest since FosB proteins are known to be robustly induced in different brain structures after morphine treatment (McDaid et al., 2006; Nye and Nestler, 1996; Xu et al., 2007). An involvement of delta FosB (a truncated variant of FosB) in morphine phenotype has been demonstrated (Muller et al., 2005; Wang et al., 2005; Zachariou et al., 2006). A recent study showed that mouse overexpressing delta FosB in the nucleus accumbens and the dorsal striatum displayed reduced analgesic responses to acute morphine administration as well as faster development of morphine tolerance (Zachariou et al., 2006). Still, there are no direct studies investigating the role of fosB gene in either morphine analgesia or in the development of morphine tolerance.

Here we investigated the function of fosB in morphine analgesia and hypothermia, in morphine morphine-naïve and treated male and female fosB knock-out (−/−), heterozygotic (+/−) and wild type (+/+) mice. We showed that lack of fosB had specific behavioural and gender specific effects since we observed enhanced analgesic but not hypothermic properties of morphine only in female KO mice. In addition, our results suggest involvement of fosB gene in acute morphine analgesia but not hypothermic tolerance. We hypothesize that fosB gene is important in mediating morphine analgesia but not hypothermia indicating its specific, gender-dependent role in morphine-induced phenotype.

2. Methods

2.1. Subjects

Male and female fosB knock out (−/−), heterozygotic (+/−) and wild type (+/+), age- and litter-matched mice (25–30 g) were housed under standard laboratory conditions. The subjects had free access to standard lab chow (Labofeed H, WPiK, Kcynia, Poland) and tap water

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during the entire experiment. The fosB mutation was originally bred into 129SvxBALB/c background mice in the Nencki Institute Animal House as described in more detail in the paper of Korkosz et al. (2004). Genotypes of all animals were verified in each generation. All experiments were conducted during the light cycle (8:00–20:00, lights on at 8.00) and performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Local Bioethics Committee (Krakow, Poland). Experimenters were blind to the mouse genotype and drug treatment.

2.2. Drugs and solutions

Morphine hydrochloride (Polfa, Kutno, Poland) was dissolved in sterile saline (0.9%NaCl, Polfa, Kutno) and injected subcutaneously (s.c.) in a volume of 0.1 ml/10 g. Vehicle-treated animals were s.c. injected with saline in a volume of 0.1 ml/10 g.

2.3. Tail-flick test

Each mouse was placed in tail-flick apparatus (Ugo Basile, Italy) with the tail extended from one end. A focused light (60 beams) from a projection bulb was applied directly to the one third of the tail from the tip, and a digital timer measured the tail tail-flick latency. To avoid the possibility of tissue injury the cut-off latency was set at 9 s. Latency to reflexive withdrawal of the tail was measured twice with each determination separated by a minimum of 15 s. The two determinations were later averaged.

2.4. Body temperature assessment

Rectal temperature was measured using Elab thermometer sensor (Elab, Denmark). Immediately after tail tail-flick test, each animal was carefully handled and a lubricated 0.5 mm diameter rectal probe was inserted to 2 cm and temperature was recorded 5–10 s later.

2.5. Nociceptive threshold and basal body temperature determination

Nociceptive thresholds and basal body temperature were evaluated with tail-flick test and rectal temperature measurement (respectively) as described above, 30 min after s.c. injection of saline in tested mice.

2.6. Morphine analgesic potency determination

Morphine analgesic potency was determined using cumulative dose–response curves. Immediately following baseline latency assessment, subjects were injected s.c. every 30 min with a rank of morphine doses (1.0, 2.0, 3.6, 6.5, 11.7, 21.0 mg/kg). In female fosB −/− mice, effects of additional morphine doses (0.5, 1.5 mg/kg) were studied. Tail flick and body temperature were tested 30 min after each dose, and a subsequent dose of morphine was injected immediately thereafter.

2.7. Morphine treatment: induction of analgesic tolerance to acute morphine

Acute tolerance was induced by s.c. injection of morphine at the dose of 100 mg/kg 24 h prior to morphine dose–response curves determination (Bilsky et al., 1996). Morphine-induced analgesia in morphine-treated mice was measured as described above.

2.8. Data analysis

Analgesia was expressed as a percentage of the maximum possible analgesic effect (%MPE) as calculated by the formula: % analgesia = [(post-morphine latency − baseline latency)/(cut-off latency − baseline latency)] × 100. Two-way ANOVA was used to examine the main effects of genotype, treatment, gender, and their interaction, on tail-flick baseline latencies and temperature. Bonferroni test was employed for individual post hoc comparisons. Half-maximal analgesic doses (AD_{50}) and associated 95% confidence intervals (CI) were calculated for dose–response data using the method of Tallarida and Murray (1987). Two-way ANOVA was used to compare morphine AD_{50}'s (±95% CI) between morphine-naive and morphine-treated male and female mice. *P* values less than 0.05 were considered significant.

Table 1
Pain thresholds in fosB −/− mice

fosB genotype	Male		Female	
	Morphine-naïve mice [s]	Morphine treated mice [s]	Morphine Morphine-naïve mice [s]	Morphine treated mice [s]
+/+	3.07±0.79	3.25±0.17	3.3±0.9	3.27±0.19
+/−	3.1±0.12	3.45±0.1	3.0±0.19	3.0±0.18
−/−	2.8±0.2	3.43±0.19	2.9±0.48	3.23±0.16

Pain threshold in fosB wild type (+/+), heterozygote (+/−) and knock-out (−/−) morphine-naïve (no prior morphine treatment) and morphine-treated (24 h prior 100 mg/kg morphine administration) male and female mice (n=8 per group). Data are shown as mean latencies (±SEM) in seconds to flick the tail in mice tail-flick test.

3. Results

3.1. Pain thresholds in fosB −/− mice

Nociceptive sensitivity in fosB +/+, +/− and −/− male and female mice is shown in Table 1. There was no significant genotype [$F_{2,42}$=0.11; p=0.8975 for male and $F_{2,42}$=0.23; p=0.7966 for female], treatment [$F_{1,42}$=1.79; p=0.1886 for male and $F_{1,42}$=0.08; p=0.7829 for female] or genotype × treatment [$F_{2,42}$=0.21; p=0.815 for male and $F_{2,42}$=0.1; p=0.903 for female] effects on pain threshold expressed as baseline tail-flick latencies in morphine-naïve and morphine-treated mice. Additionally, there were no significant effects of sex and genotype × sex on pain threshold in morphine-naïve [$F_{1,42}$=0.19; p=0.8242 for sex, $F_{2,42}$=0.05; p=0.9539 for genotype × sex] and morphine-treated mice [$F_{1,42}$=2.35; p=0.133 for sex, $F_{2,42}$=0.98; p=0.383 for genotype × sex].

3.2. Morphine analgesia and hypothermia in morphine morphine-naïve fosB −/− mice

Cumulative dose–response curves for morphine in the tail-flick test in male and female fosB +/+, +/− and −/− mice are shown in Fig. 1. Results of morphine administration at a dose of 21 mg/kg produced 100% of maximal possible effect and are not shown in Fig. 1. Morphine in a dose dose-dependent manner produced analgesia in both male [$F_{5,126}$=102; p<0.001] and female [$F_{5,126}$=94.27; p<0.001] mice. There were no significant genotype or genotype × treatment interaction effects in male mice. However, in female mice, morphine AD_{50} in fosB −/− mice (Table 2) was significantly lower in comparison to +/+ and +/−, revealing genotype effect [$F_{2,126}$=22.08; p<0.001]. Moreover, comparison of morphine AD_{50} of fosB −/− between male and female mice, showed a gender effect [$F_{4,16}$=24.84; p<0.001] indicating enhanced morphine analgesic properties in female fosB −/− mice.

Cumulative dose–response curves for morphine hypothermia in male and female fosB +/+, +/− and −/− mice are shown in Fig. 1. Morphine administration in cumulative doses (1.0, 2.0, 3.6, 6.5, 11.7, 21.0 mg/kg and additionally 0.5, 1.5 mg/kg in female −/− mice) caused a dose-dependent decrease in body temperature in both male [treatment effect $F_{5,126}$=81.91; p<0.001] and female [treatment effect $F_{5,126}$=76.39; p<0.001] mice. Results of morphine administration at a dose of 21 mg/kg produced the strongest decrease in body temperature in both male (−4.74±0.35 in fosB +/+; −4.95±0.32 in fosB +/−

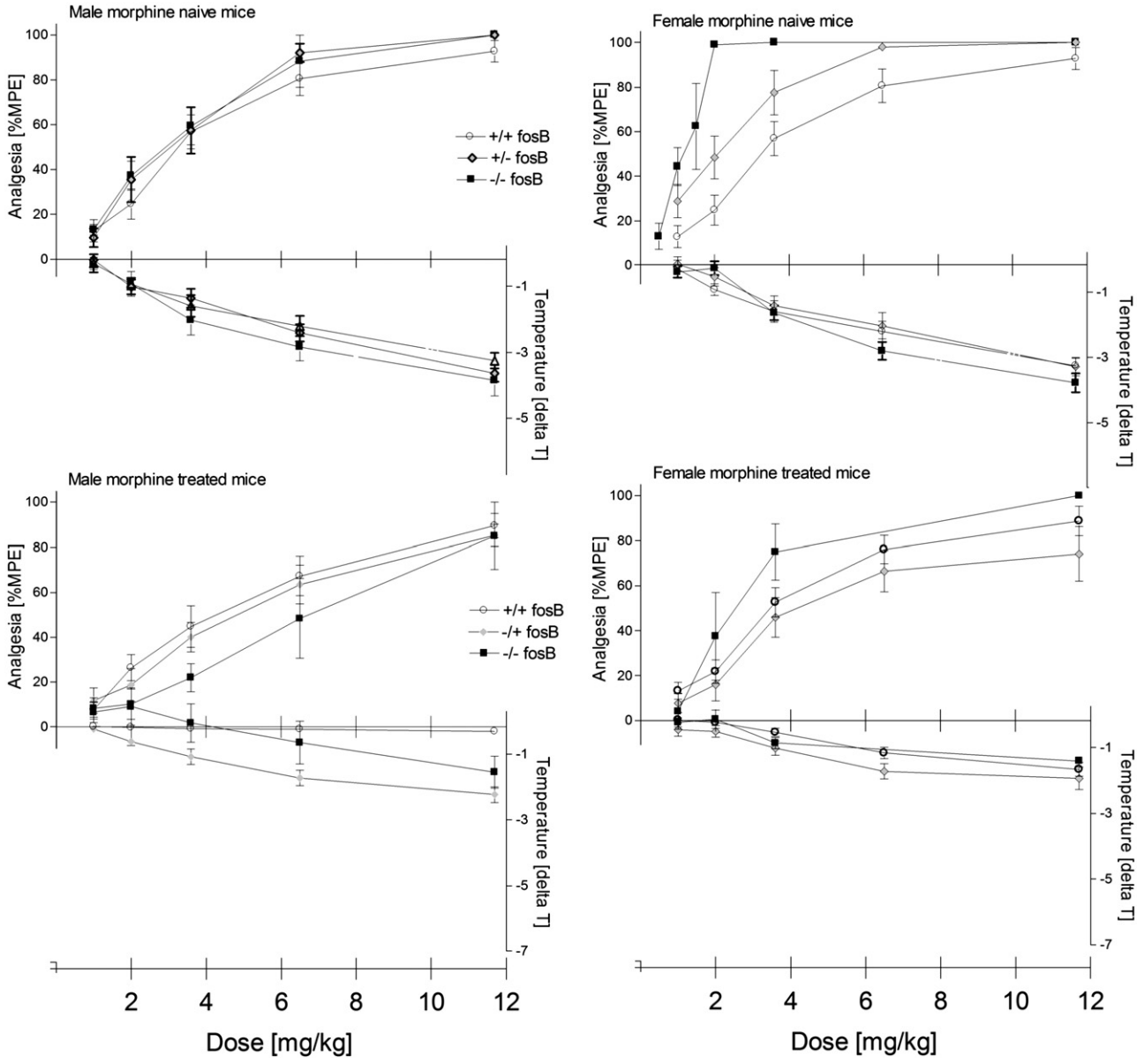

Fig. 1. Cumulative dose–response curves for morphine (1, 2, 3.6, 6.5, 11.7 mg/kg, s.c. 0.5 and 1.5 mg/kg doses were additionally studied in female mice) analgesia in the tail-flick test (left *Y* axis) and hypothermia (right *Y* axis) in male (left panels) and female (right panels) fosB wild type (+/+), heterozygotic (+/−) and knock-out (−/−) morphine-naive (upper panels) and morphine-treated (lower panels) mice (n=8 per group). %MPE and delta *T* are expressed as the mean±SEM.

and −5.14±0.62 in fosB −/−) and female (−4.24±0.37 in fosB +/+; −3.7±0.4 in fosB +/− and −4.46±0.7 in fosB −/−) mice, but these data are not shown in Fig. 1. Hypothermic action of morphine did not differ between genotype as well as no genotype×treatment interaction effect was seen in both male and female mice.

3.3. Morphine analgesia and hypothermia in morphine-treated fosB −/− mice

Morphine analgesic dose–response curves in morphine-treated mice are illustrated in Fig. 1. Morphine administration at a 21 mg/kg

Table 2
Morphine analgesic potency in fosB −/− mice

fosB genotype	Male		Female	
	Morphine-naïve mice	Morphine treated mice	Morphine-naïve mice	Morphine treated mice
+/+	3.06 (2.6–3.5)	3.7 (3.2–4.2)	2.04 (1.3–3.1)	3.3 (2.8–3.8)
+/−	2.7 (2.3–3.1)	4.01 (3.4–4.6)***	1.7 (1.4–1.9)$^{\#,+++}$	4.45 (3.06–6.3) ***
−/−	2.5 (2.2–2.9)	4.9 (4.6–5.2)***,$^{\#\#\#}$	1.02 (0.5–1.5)$^{\#,+++}$	2.43 (2.1–2.6)***,$^{\#\#\#,+++}$

Morphine analgesic potency in morphine-naïve and morphine-treated fosB wild type (+/+), heterozygote (+/−) and knock-out (−/−) male and female mice (n=8 per group). Values are expressed as morphine half-maximal analgesic dose (AD_{50} in mg/kg)±95% confidence intervals. ***$p<0.001$ vs morphine AD_{50} in the same sex and genotype; $^{\#\#\#}p<0.001$ vs wild type morphine AD_{50} after morphine treatment; $^{+++}p<0.05$ vs male mice in the same genotype.

dose produced 100% of maximal possible effect and these results are not shown in Fig. 1.

Morphine (100 mg/kg) administration 24 h prior to the measurements produced a rightward shift of morphine dose–response curve in both male [treatment effect: $F_{1,84}=17.21$; $p<0.001$] and female [treatment effect: $F_{1,84}=23.76$; $p<0.001$] fosB −/− mice (Fig. 1). In morphine-treated fosB −/− male and female mice, morphine AD_{50} was significantly different in comparison to morphine AD_{50} in morphine-naive fosB −/− mice [genotype effects: $F_{1,84}=11.97$; $p<0.001$ in male, $F_{1,84}=32.25$; $p<0.001$; in female; Table 2]. Moreover, comparison of morphine AD_{50} of fosB −/− between male and female morphine-treated mice, showed a gender effect [$F_{4,16}=24.84$; $p<0.001$; Table 2], namely, enhanced morphine analgesic properties in female fosB −/− mice after morphine tolerance induction.

Morphine administration in cumulative doses after prior morphine experience caused a dose-dependent decrease in body temperature in male and female fosB +/+, +/− and −/− mice (Fig. 1). Morphine administration at a dose of 21 mg/kg produced the strongest decrease in body temperature in both male (−2.54±0.3 in fosB +/+; −2.82±0.18 in fosB +/− and −1.65±0.35 in fosB −/−) and female (−1.93±0.27 in fosB +/+; −2.2±0.48 in fosB +/− and −1.4±0.05 in fosB −/−) mice, but these results are not shown in Fig. 1. Morphine hypothermia in morphine-treated mice was significantly weaker in comparison to measurements in morphine morphine-naïve both male fosB +/+ [treatment effect: $F_{1,84}=65.31$; $p<0.001$], +/− [treatment effect: $F_{1,84}=41.84$; $p<0.001$] and −/− [treatment effect: $F_{1,84}=51.91$; $p<0.001$] as well as female fosB +/+ [treatment effect: $F_{1,84}=90.39$; $p<0.001$], +/− [treatment effect: $F_{1,84}=9.18$; $p<0.001$] and −/− [treatment effect: $F_{1,84}=65.15$; $p<0.001$] mice.

4. Discussion

The results of our study evidenced the role of fosB in morphine analgesia in female but not male mice (lower morphine AD_{50} in female fosB −/− mice) as well as no effect of fosB mutation on morphine-induced hypothermia in neither male or female mice. Furthermore, the results indicate that the analgesic tolerance developed in fosB male and female −/− mice. Such results suggest that lack of fosB enhances the analgesic tolerance. Interestingly, both male and female fosB +/+ did develop slight, if any, tolerance to morphine analgesia 24 h after prior morphine administration. Therefore, a higher dose of morphine is required to induce analgesic tolerance in naive mice, however, a clear induction of tolerance to hypothermic effects of morphine was observed. This observation is in agreement with other reports that mice of 129 inbred strain — a part of fosB +/+ genetic background (129SvxBALB/c chimera) — are resistant to morphine analgesic tolerance (Kolesnikov et al., 1998), whereas BALB/c mice develop only average morphine tolerance (Kest et al., 2002).

It has been previously proposed that fosB might be critical for adaptive neuronal responses (Brown et al., 1996; Nestler et al., 1999; Nestler et al., 2001). It has been shown that fosB gene products may be involved in brain responses to both acute and chronic chemical and environmental stimuli in mice. It has also been postulated that protein products of the fosB are critically involved in neural adaptations induced by chronic treatment with various drugs of abuse, including morphine (Muller et al., 2005; Nestler et al., 1999; Nestler et al., 2001; Wang et al., 2005; Zachariou et al., 2006).

Mice overexpressing delta FosB within the nucleus accumbens and dorsal striatum show increased vulnerability to behavioural effects of morphine as measured by conditioned place preference, locomotor activation, physical dependence syndrome, analgesia and tolerance (Zachariou et al., 2006). Such results could indicate that fosB mediate morphine behaviours in mice in such a way that the fosB products are positively correlated with vulnerability to behavioural effects of morphine. In the present study however, we show that mice lacking fosB gene display greater morphine-induced analgesia and tolerance.

Several explanations for such discrepancy between results of morphine analgesia and tolerance studies can be proposed. First of all, we used fosB −/− mice, therefore, the observed differences in morphine phenotype were due to the lack of all fosB protein products, as opposed to effects of only delta FosB reported by Zachariou et al. (2006). Secondly, we observed functional effects of fosB −/− on morphine analgesia and tolerance induction, which most probably are due to differences in its expression in the central or peripheral nervous system structures implicated in pain transmission (Inturrisi, 2002; Willis and Westlund, 1997), as opposed to differences linked specifically to NAc or dorsal striatum (Zachariou et al., 2006). Moreover, in order to measure morphine-induced analgesia, we used tail-flick assay in contrast to the hot plate test used by Zachariou et al. (2006). These two assays were reported to involve different regions of the nervous system — the tail flick relaying on spinal, and hot plate on spinal and supraspinal mechanisms of animal reaction to noxious stimuli (Caggiula et al., 1995).

Finally, knock out of fosB could produce several neural adaptations, which might be responsible for our results. However, such observations indicate that induction of morphine analgesia and tolerance might be mediated by FosB and/or delta FosB, or their interaction in different brain structures especially considering that FosB and delta FosB proteins have been shown to form complexes with differential activity at AP-1 binding site (Nakabeppu and Nathans, 1991). Taken together these results suggest that fosB-derived proteins are involved in morphine phenotype and their interaction may play a crucial role in induction of acute morphine tolerance.

In conclusion, our results show that fosB gene is implicated in morphine analgesia in morphine-naive female mice, and more importantly, in induction of acute morphine tolerance in both male and female mice. In contrast, morphine hypothermia as well as development of tolerance to morphine hypothermia are independent of fosB gene and/or gender.

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