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Differences in basal and morphine-induced FosB/ΔFosB and pCREB immunoreactivities in dopaminergic brain regions of alcohol-preferring AA and alcohol-avoiding ANA rats

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ABSTRACT

Besides alcohol, alcohol-preferring AA and alcohol-avoiding ANA rats differ also with respect to other abused drugs. To study the molecular basis of these differences, we examined the expression of two transcription factors implicated in addiction, ΔFosB and pCREB, in brain dopaminergic regions of AA and ANA rats. The effects of morphine and nicotine were studied to relate the behavioral and molecular changes induced by these drugs. Baseline FosB/ΔFosB immunoreactivity (IR) in the nucleus accumbens core and pCREB IR in the prefrontal cortex (PFC) were elevated in AA rats. Morphine increased ΔFosB-like IR more readily in the caudate-putamen of AA rats than in ANA rats. In the PFC morphine decreased pCREB IR in AA rats, but increased it in ANA rats. In addition to enhanced locomotor response, the development of place preference to morphine was enhanced in AA rats. The enhanced nicotine-induced locomotor sensitization found in AA compared with ANA rats seems to depend in addition to dopamine and ΔFosB on other mechanisms. These findings suggest that enhanced sensitivity of AA rats to morphine is related to augmented morphine-induced expression of FosB/ΔFosB and morphine-induced reduction of pCREB levels. Moreover, altered innate expression of FosB/ΔFosB and pCREB in AA rats is likely to affect the sensitivity of these rats to abused drugs.

1. Introduction

Progressive enhancement of psychomotor responses after repeated exposure to various drugs of abuse, termed behavioral/psychomotor sensitization, is thought to be associated with some critical aspects of drug addiction (Robinson and Berridge, 2003; Vanderschuren and Kalivas, 2000), such as drug-seeking and drug-taking (Robinson and Berridge, 1993; Vezina, 2004). Numerous investigations have shown the involvement of the brain dopaminergic systems in this process, but the molecular mechanisms underlying sensitization are still not well understood. Interesting models for studying these mechanisms are animal lines selected for their drug consumption.

Alcohol-preferring AA and alcohol-avoiding ANA rat lines have been selectively bred on the basis of their alcohol consumption (Eriksson, 1968; Sommer et al., 2006). Previous studies have shown that in addition to alcohol, AA rats consume more etonitazene and cocaine containing solutions than ANA rats (Hyyatia and Sinclair, 1993). Recently, we found that conditioned place preference (CPP) to cocaine develops more readily in AA than in ANA rats (Marttila et al., 2007). Furthermore, repeated cocaine or morphine administration sensitizes the locomotor responses of AA rats more readily than those of ANA rats (Honkanen et al., 1999b; Ojanen et al., 2003; Ojanen et al., 2007). The sensitivity of mesolimbic dopamine system appears to be a critical factor determining the different behavioral effects of cocaine between these rats. This conclusion can be reached after observing an enhanced psychomotor response in AA rats accompanied by sensitized mesolimbic dopamine release (Mikkola et al., 2001a). However, divergent sensitization of mesolimbic dopamine release does not explain the differences between AA and ANA rats in the psychomotor sensitization to morphine (Honkanen et al., 1999a; Mikkola et al., 2000; Ojanen et al., 2003).

Drugs of abuse are known to cause several neuroadaptations in dopaminergic brain areas. One such adaptation is the altered expression of transcription factors, which give rise to changes in gene expression and may possibly lead to alterations in sensitivity to drugs of abuse (Nestler et al., 2001). Several studies have specifically implicated two transcription factors, ΔFosB and CREB (cAMP response element binding protein), in addiction-related neural plasticity. Repeated stimuli, such as repeated exposure to drugs of abuse, lead to a gradual increase in ΔFosB levels, an effect that persists for a relatively long time after the cessation of drug treatment (Hope et al., 1994; Nestler et al., 2001). In contrast, CREB is a constitutively expressed transcription factor, the activity of which is tightly regulated by its phosphorylation at serine 133 (Lonze

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and Ginty, 2002). Plenty of evidence suggests that an increased expression of ΔFosB enhances locomotor-activating and rewarding effects of cocaine (Colby et al., 2003; Kelz et al., 1999; Peakman et al., 2003). Indeed, we recently found that cocaine increases the expression of ΔFosB-like proteins more in the nucleus accumbens (NAc) of AA rats than in that of ANA rats (Marttila et al., 2007). Furthermore, cocaine reward is shown to be accompanied by changes in pCREB (phosphorylated form of CREB) levels (Carlezon et al., 1998). There is also evidence for the role of these transcription factors in morphine sensitization and reward (McDaid et al., 2006; Olson et al., 2005, 2007; Zachariou et al., 2006). Therefore, it is important to find out whether the effects of morphine on these transcription factors differ between AA and ANA rats.

In the present study we examined whether differences in locomotor responses to morphine in AA and ANA rats are associated with differences in the expression of FosB/ΔFosB and pCREB. Further, CPP method was used to assess morphine reward in AA and ANA rats. In addition to those of morphine and cocaine, the addictive properties of nicotine are thought to involve the dopaminergic system. Therefore, we compared the effects of repeated nicotine administration on locomotion, dopamine release and FosB/ΔFosB expression in AA and ANA rats.

2. Materials and methods

2.1. Animals

3–4-month-old male AA and ANA rats (Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland) were used. The rats were housed in groups of 4–5 and kept under a 12:12-h light/dark cycle at an ambient temperature of 20–23 °C. Rat chow and tap water were available ad libitum. Prior to the experiments, the rats were acclimated to handling for 7 days in order to minimize stress during the experiments. All animal experiments were approved by the chief veterinarian of the county administrative board and were conducted according to "the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes".

2.2. Morphine-induced locomotor activity

On the first experimental day, all rats were given saline (0.9% NaCl, 2 ml/kg, s.c.), and on the following day the pretreatment period was started. During that period, the rats were treated with saline or increasing doses of morphine (5, 10, 10, 15, 15 mg/kg; every other day). Fifteen minutes before and immediately after the injections, the rats were placed in locomotor activity boxes. To study the acute locomotor-activating effects of morphine 5 mg/kg, horizontal locomotor activity was measured for 4 h on the first day of the pretreatment period. The locomotor activity of the rats was registered in Plexiglas boxes (43 cm × 43 cm × 30 cm; MED Associates ENV-515, Vermont, GA, USA), and a computer registered the interruptions of infrared photo beams. After the pretreatment period, the rats were abstained from morphine for 72 h prior to the challenge. On the challenge day, all rats received morphine injections. In the first experiment, the rats first received 1 mg/kg of morphine as the challenge, and 48 h after that, a second morphine challenge was given at a dose of 3 mg/kg. In the second experiment, the challenge dose was 5 mg/kg. Locomotor activity was measured for 4 h. 24–29 h after the measurement of locomotor activity, the rats were perfused for FosB/ΔFosB immunohistochemistry. In addition, a separate control group was similarly treated with saline (both pretreated and challenged with saline) and perfused for FosB/ΔFosB immunohistochemistry.

2.3. Morphine-induced conditioned place preference (CPP)

Two place conditioning experiments were carried out with two doses of morphine as the conditioning dose (0.5 mg/kg and 1.0 mg/kg, 2 ml/kg, s.c.). The CPP experiment consisted of three distinct phases on consecutive days: two habituation days, two conditioning days, test day 1 (postconditioning), two conditioning days, test day 2, three conditioning days, and test day 3. Eight rats could be tested simultaneously. The CPP apparatus consisted of two equally sized compartments (41 cm × 21 cm × 28 cm) that were separated by a black wall with a guillotine door (MED Associates ENV-515, Vermont, GA, USA). The compartments had differently colored walls (black or white) and distinct floor textures (a rod floor in the black compartment and a wire mesh floor in the white compartment). White noise was used to cover possible background noise. Computer-registered interruptions of infrared photo beams were used to determine the position of the rat in the apparatus. During the habituation phase, the guillotine door was open, allowing free access to both compartments for 45 min. The time that the rat spent in the non-preferred compartment during the first 20 min on the habituation day 2 was used as the initial preference level (preconditioning time). During the conditioning phase all rats received a saline injection (2 ml/kg s.c.) in the morning before being confined to the vehicle-paired black-walled compartment for 45 min. After an interval of 3–4 h, each rat received a saline or morphine injection immediately before being placed in the drug-paired white-walled compartment for 45 min. Each trial included four AA rats and four ANA rats, with two rats from both lines receiving saline in both compartments and the other two receiving saline in one and morphine in the other compartment. On the postconditioning day, the guillotine door separating the two compartments was open, and the time spent by the rats in either compartment was recorded for 20 min. The change of preference was calculated as the difference in seconds between the times spent in the drug-paired compartment on the postconditioning day and the preconditioning day.

2.4. FosB/ΔFosB and pCREB-immunohistochemistry

24–29 h (FosB/ΔFosB study) or 20 min (pCREB study) after the last injection, the rats were deeply anaesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. The assay time points were based on previous studies showing that 24 h after the stimulus, the observed FosB/ΔFosB IR represents almost solely ΔFosB (Chen et al., 1997; Nestler, 2004; Perrotti et al., 2008; Ulery et al., 2006), and that a peak in the level of pCREB is seen 20 min after the stimulus (Mattson et al., 2005). After perfusion, the brains were post-fixed for 4 h with the same fixative and stored in sodium phosphate buffer containing 20% sucrose until coronal sections (40 μm) were cut on a cryostat. The sections were stored at −20 °C until they were used. Free-floating sections from selected brain areas were stained on 24-well plates. After rinsing the sections in PBS for 3 × 10 min, sections were treated with 0.5% H_2O_2 in PBS for 10 min and rinsed again in PBS 3 × 10 min. Sections were then placed in a blocking solution containing 3% normal goat serum (NGS) and 0.5% Tween 20 in PBS for 1 h. After that, the sections were incubated for 22 h in primary FosB/ΔFosB (1:500 Santa Cruz Biotechnology) or $pCREB^{133}$ (1:5000; Upstate Biotechnologies) antibody diluted in PBS containing 3% NGS, 0.5% Tween 20, and 0.1% sodium azide. Subsequently, the sections were washed in PBS for 3 × 10 min, and incubated for 2 h with biotinylated anti-rabbit antibody (Vectastain® Elite ABC Kit PK-6101, Vector Laboratories, CA, USA) in PBS containing 1.5% NGS. Standard avidin–biotin procedure was then performed using the Vectastain® Elite ABC Peroxidase Kit following the protocol suggested by the manufacturer. After washing the sections in PBS for 3 × 10 min, the immunoreactivity was revealed using 0.06% 3,3-diaminobenzidine and 0.1% H_2O_2 diluted in PBS, followed by washing with phosphate buffer 3 × 5 min. All washes and incubations were carried out at room temperature under gentle shaking. The sections were mounted on gelatin/chrome-alume-coated slides, air-dried and dehydrated through graded ethanols to HistoClear® (National diagnostics, GA, USA), and coverslipped with DePex® (BDH Laboratory supplies, Poole, England).