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Lewis and Fischer 344 strain differences in α_2-adrenoceptors and tyrosine hydroxylase expression

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Abstract

Lewis and Fischer 344 (F344) rats differ in their pharmacological responses to a variety of drugs such as opioids, which has been partially attributed to differences in the endogenous opioid tone. Since opioid and α_2-adrenergic mechanisms closely interact in nociception and substance abuse, a comparative study of the endogenous α_2-adrenergic system in both inbred strains is of interest. Alpha-2 adrenoceptor subtypes and tyrosine hydroxylase, the rate-limiting enzyme of the catecholamine biosynthesis, were studied by Taqman RT-PCR analysis of gene expression in four brain areas of F344 and Lewis rats: hypothalamus, hippocampus, striatum and cortex. No differences were found in the mRNA levels of α_{2A}- and α_{2C}-adrenoceptors in any of the areas examined, however F344 rats exhibited lower levels of α_{2B}-adrenoceptor transcripts in the hippocampus and higher levels in the hypothalamus. Tyrosine hydroxylase gene expression was found to be higher in hippocampus and striatum of F344 rats compared to Lewis, and a consistent 2-fold increase of the protein levels was detected by Western blots only in the case of the hippocampus. These results together with previous studies strongly suggest that the hippocampal noradrenergic activity of Lewis and F344 rats could be involved in their different responses to pain, stress and drug addiction.

Keywords: F344; Lewis; α_2-adrenoceptors; Tyrosine hydroxylase; Catecholamines

Introduction

Fischer 344 (F344) and Lewis rats are two inbred strains that have been comparatively studied in the field of drug addiction, since the latter seems to be more sensitive to the addictive effects of drugs of abuse such as opioids (Guitart et al., 1992; Suzuki et al., 1992; Martin et al., 1999). Furthermore, differences between F344 and Lewis rats have been also reported concerning opioid analgesia (Herradon et al., 2003a). It is known that the degree of activation of the Hypothalamic Pituitary Adrenal (HPA) axis is much higher in F344 rats (Chaouloff et al., 1995; Chisari et al., 1995; see review by Kosten and Ambrosio, 2002), which may contribute to the above differences since a link between stress and its concomitant HPA axis responses with behavioural sensitivity to psychoactive drugs is known (Kosten and Ambrosio, 2002). Recent evidences suggest that at least part of these differences can be directly attributed to heterogeneous function of the endogenous opioid system, since Lewis rats show a reduced endogenous opioid activity (Sternberg et al., 1989a,b; Nylander et al., 1995; Martin et al., 1999), including a less effective activation pathway in the case of the μ-opioid receptor (Selley et al., 2003; Herradon et al., 2003b).

Extensive basic and clinical data are now available to affirm that opioid receptors and α_2-adrenoceptors are closely interrelated, which apply both to addiction and pain control (see review by Alguacil and Morales, 2004). It is widely known that α_2-adrenergic agonists limit opioid withdrawal symptoms (Tseng et al., 1975; Gold et al., 1978; Gowing et al., 2004; Bailey, 2004), an effect related to reduction of central

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noradrenergic hyperactivity (Williams et al., 2001). Conversely, a single administration of α_2-adrenergic antagonists increases opioid withdrawal symptoms (Stine et al., 2002), while chronic coadministration decreases opioid dependence both in rodent models (Iglesias et al., 1992, 1998; Laorden et al., 2000) and humans (Hameedi et al., 1997). These interactions are extendable to antinociception where additive or synergistic interactions between opioids and α_2-adrenoceptor agonists have been described in rodents (Harada et al., 1995; Ossipov et al., 1997) and have been used to manage pain in humans specially when high levels of opioid tolerance are present (Walker et al., 2002).

Although the endogenous opioid system has been widely studied in F344 and Lewis rats, a deep comparative study of the noradrenergic system in these two inbred strains is lacking, even when there is some evidence to think that there could be some differences. For example, the activity of the rate-limiting enzyme of catecholamine biosynthesis, tyrosine hydroxylase, is much lower in the locus coeruleus of F344 rats, whose neurons also exhibit less spontaneous firing rate (Beitner-johnson et al., 1991; Guitart et al., 1993). We have recently reported that the acute antinociceptive effect of the α_2-adrenoceptor agonist clonidine is significantly higher in F344 rats (Herradon et al., 2003a). However, direct evidence of strain-related differences concerning the α_2-adrenergic system is not available at the molecular level. In this work we have combined gene and protein expression studies to determine possible strain differences concerning α_2-adrenergic system in the brain of Lewis and F344 rats, mainly focusing on some brain areas clearly involved in drug reward and nociception according to the literature (Di Chiara and Imperato, 1988; McEwen, 2001; Millan, 2002; Kosten and Ambrosio, 2002; Maihofner et al., 2004).

Methods

Animals and drugs

Male Lewis and F344 rats (7 weeks old; Harlan) were used. The animals had free access to water and standard diet and were maintained in a controlled environment (20–22 °C, 12 h/12 h dark/light cycle) until the day of the experiment. The assays were carried out in accordance with the NIH guidelines for care and use of laboratory animals.

Analysis of gene expression

Tissue acquisition and RNA isolation

After sacrifice four brain areas (cortex, striatum, hypothalamus and hippocampus) were rapidly dissected in all animals (5/strain), frozen in dry ice and stored to − 80 °C until the RNA isolation procedure. Frozen tissues were directly homogenized in 1 ml TRIZOL reagent (Invitrogen, Carlsbad, CA) per 50–100 mg tissue and total RNA was extracted following manufacturer's suggested protocol. The concentration of each sample was obtained from A_{260} measurements. RNA integrity was confirmed using agarose gel electrophoresis. RNA samples were purified with DNAses enzymes (Ambion, Austin, TX) following manufacturer's protocol.

cDNA synthesis

One microgram of total RNA was mixed with 10× buffer RT, 25 mM $MgCl_2$, 10 mM dNTPs, 50 μM oligodT, 50 μM random hexamers, 20 U/μl RNAses inhibitor and 50 U/μl Multiscribe. All the reagents were purchased to Applied Biosystems (Foster City, CA). The reaction conditions were 10 min/25 °C, 60 min/42 °C, 5 min/95 °C.

Taqman RT-PCR

Probe and primer sequences of α_2-adrenoceptors and tyrosine hydroxylase genes were designed using Primer Express software (Applied Biosystems, Foster City, CA) as close as possible to the 3′-coding region of target gene sequences obtained from Genbank and are presented in Table 1.

Primers and probes were purchased from PE Applied Biosystems and each probe was synthesized with the fluorescent reporter dye FAM (6-carboxy-fluorescein) attached to the 5′-end and a quencher dye TAMRA (6-carboxy-tetramethyl-rhodamine) to the 3′-end.

We performed Taqman RT-PCR assays to measure the relative expression of our target genes following the protocol previously described (Medhurst et al., 2000). As housekeeping gene expression we used 18S (human rRNA, Applied Biosystems, Foster City, CA). Briefly, the results quantification is obtained through standard curves for each primer/probe set that were plotted showing the threshold cycle (C_t) vs. log initial copy number of genomic DNA in order to extrapolate our target genes C_t and generate an estimate copies/ng RNA (Wang et al., 1998). To correct for both RNA quality and quantity, data were also normalized by dividing target genes copies/ng by the copies/ng of an assay-dependent 18S gene, and expressed as a percentage (relative expression). We performed three experimental determinations for each gene.

Table 1
Taqman probes and primers sequences for every target gene

Gene	Taqman probe
α_{2A}-Adrenoceptor	TTTCAACCACGACTTCCGCCGC
α_{2B}-Adrenoceptor	TTGTGCTGGCCGTGGTCATTGG
α_{2C}-Adrenoceptor	ACGGTCTTCAACCAGGATTTCCGGC
Tyrosine hydroxylase	TGTCCGAGAGCTTCAATGACGCCAA
Gene	Forward primer
α_{2A}-Adrenoceptor	AGCTCGCTGAACCCTGTTATCT
α_{2B}-Adrenoceptor	GCCGGGAGAAGAGGTTCAC
α_{2C}-Adrenoceptor	CTCGCTCAACCCGGTCAT
Tyrosine hydroxylase	ATCAAACCTACCAGCCTGTGTACT
Gene	Reverse primer
α_{2A}-Adrenoceptor	CACGGCAGAGGATCTTCTTGA
α_{2B}-Adrenoceptor	GAAACCAGCAGACCACGAAAA
α_{2C}-Adrenoceptor	CTCCGTCGGAAGAGGATGTG
Tyrosine hydroxylase	GAGGCATAGTTCCTGAGCTTGTC