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Research Report

Increases in preproenkephalin mRNA levels in the Syrian hamster: The influence of glucocorticoids is dependent on age and tissue

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PPenk, preproenkephalin
Penk, proenkephalin
PNMT, phenylethanolamine N-methyltransferase

ABSTRACT

In adult hamsters, basal proenkephalin (Penk) gene expression in adrenals is independent of glucocorticoids and glucocorticoid receptor blockade, by RU 486, increases striatal preproenkephalin (PPenk) mRNA levels. However, glucocorticoids maintain both basal and induced Penk gene expression in rat adrenal (medulla) and striatum. This suggests species and tissue-specific differences in Penk gene regulation. Since studies show temporal coordination in Penk gene expression in developing hamster adrenal and striatum, we tested the hypothesis that increasing PPenk mRNA levels are dependent, while basal levels are independent of glucocorticoids in developing hamsters. To facilitate this study, we examined the influence of glucocorticoids on the temporal increases in developing hamster PPenk mRNA observed in adrenals between postnatal days 0 and 4 and in striatum between postnatal days 12 and 48. PPenk mRNA levels were determined in hamster pups after treatment with increasing doses of metyrapone (an 11β hydroxylase inhibitor) or with the glucocorticoid receptor antagonist RU 486 ± metyrapone between postnatal days 2 and 4. Levels were also determined 36 days after hypophysectomy at age 16–17 days. Although plasma glucocorticoid levels and/or the influence from glucocorticoids were reduced, only developmental increases in PPenk mRNA are influenced by glucocorticoids in hamster adrenals, while basal adrenal mRNA levels are unchanged. However, pituitary influence on striatal PPenk mRNA levels appears complex and may involve steroid and/or non-steroid factors. These results suggest that glucocorticoids regulate hamster Penk gene expression via a mechanism that varies with age and tissue and functions during the induction of the Penk gene and not to maintain basal gene expression. Possible mechanisms and species variation are discussed.

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1. Introduction

The products of the proenkephalin (Penk) gene (the enkephalins and enkephalin-containing peptides) are important to many physiological processes. These processes include normal motor function (Brotchie et al., 1998; Calon and Di Paolo, 2002), the immune response (Salzet et al., 2000), reproduction (Fabbri et al., 1989), and nociception (Drolet et al., 2001; Fontana et al., 1997; Konig et al., 1996; Yamada and Nabeshima, 1995). Furthermore, the enkephalins and the enkephalin-containing peptides are important to the adaptive processes that result from mammalian exposure to physical and psychological stress (Borsook et al., 1994a,b; Drolet et al., 2001; Fontana et al., 1997; Katoh et al., 1990; Konig et al., 1996; Yamada and Nabeshima, 1995); activities associated with fear and anxiety (Hebb et al., 2004; Konig et al., 1996; Ragnauth et al., 2001); and to human diseases such as depression (Bertrand et al., 1997) and post-traumatic stress disorder (PTSD) (Joseph, 1998; Sher, 2004). Many of the functions and properties associated with these enkephalin-containing peptides have also been linked with the tissue-specific distribution of Penk gene expression and with the tissue-specific nature of this gene's regulation. For example, in the adrenal medulla, high levels of Penk gene expression can be induced, and the resulting gene products can be involved in relieving chronic pain when adrenal medullary explants or chromaffin cells of adrenal medullary origin are placed in the central nervous systems of mammals (Czech and Sagen, 1995; Duplan et al., 2000; Hentall et al., 2001; Siegan and Sagen, 1997; Sagen, 2003). In the striatum (caudate putamen), the Penk gene is highly expressed in a subpopulation of medium-sized spiny neurons which also express the D2 dopamine receptor subtype (Gerfen, 2000), and the expression of the Penk gene appears to facilitate stress adaptation (Drolet et al., 2001; Fontana et al., 1997; Konig et al., 1996; Yamada and Nabeshima, 1995).

Steroids, including glucocorticoid, are associated with the regulation the Prenk gene (Ahima et al., 1992; Chao and McEwen, 1990, 1991; Gu et al., 1996; Henion and Landis, 1993; Inturrisi et al., 1988b; La Gamma and Adler, 1987; Lauber et al., 1990; McMillian et al., 1996; Priest et al., 1995; Yoburn et al., 1987; Yoshikawa and Sabol, 1986; Yukhananov and Handa, 1997; Zhu et al., 2001). The secretion of glucocorticoids is a major mammalian response to stress (Turnbull and Rivier, 1999). In addition, a complex set of interactions between the normal function of the hypothalamic pituitary adrenal axis and Penk gene expression in the central nervous system has been described (Drolet et al., 2001). Taken together, these various observations outline the importance of the Penk gene and the influence of glucocorticoids in maintaining a mammal's ability to adapt to stress (Konig et al., 1996). Further studies in this area will enhance our understanding of the potential therapeutic utility of controlling Penk gene expression.

Our knowledge of the role of glucocorticoids in regulating the Penk gene is not complete. Specifically, basal Penk gene expression in the adult hamster adrenal is independent of glucocorticoids and preproenkephalin (PPenk) mRNA levels in the hamster striatum have been shown to increase when glucocorticoid receptors are blocked by the antagonist RU 486 (Jimenez et al., 1999). Meanwhile, glucocorticoids are required for the induction of the Penk gene in the rat adrenal medulla, and Penk gene expression in the rat striatum appears to require the presence of glucocorticoids (Chao and McEwen, 1990, 1991; Inturrisi et al., 1988a,b; Yoburn et al., 1987). In an attempt to reconcile these divergent observations, we considered it possible that only the induction of the Penk gene is dependent on glucocorticoids, while basal gene expression is independent of glucocorticoids. To test this hypothesis, we examined Penk gene expression in the developmental hamster.

The developing hamster adrenal and striatum were examined for a number of reasons. In the adult hamster adrenal medulla, PPenk mRNA levels are more than 90 times those found in the rat. These levels are comparable to those found in the striatum from both rat and hamster (Franklin et al., 1991a). In addition, PPenk mRNA levels in the hamster appear to be more typical of mammals than the rat. Furthermore, the structure and regulatory elements of the Penk gene in the hamster and human are nearly identical and they differ from that in the rat (Zhu et al., 1994). Finally, developmental increases in hamster adrenal PPenk mRNA levels are observed between postnatal days 0 and 4 and in the hamster striatum after postnatal day 12 (Franklin, 1997). Therefore, hamster pups were subjected to treatment with metyrapone, an inhibitor of 11β hydroxylase, which blocks the biosynthesis of glucocorticoids or treatment with the glucocorticoid receptor antagonists (RU 486) with or without metyrapone treatment to insure maximum reduction in the influence from glucocorticoids on gene expression. Treatments began on postnatal day 2 when adrenal PPenk mRNA levels are increasing and striatal levels are not. Finally, hypophysectomy was performed on hamsters on postnatal days 16–17 when striatal PPenk mRNA levels are increasing and adrenal levels are constant at adult level (Franklin, 1997). The data demonstrate that developmental increases in hamster adrenal PPenk mRNA are reduced when the influence from glucocorticoids are reduced, while basal adrenal mRNA levels are unchanged. Furthermore, only the developmental increases in striatal PPenk mRNA levels are reduced when the pituitary is removed. These results suggest that glucocorticoids and pituitary function regulate hamster Penk gene expression via a mechanism that varies with age and tissue.

2. Results

2.1. *Inhibition of glucocorticoid biosynthesis by metyrapone reduces the developmental increases in adrenal PPenk mRNA levels*

Metyrapone inhibits 11β hydroxylase activity and glucocorticoid biosynthesis in the adrenal cortex (Temple and Liddle, 1970). This drug was used, starting on postnatal day 2, to see the effects it would have on the developmental increases in PPenk mRNA levels observed prior to postnatal day 4 (Franklin, 1997). The results show that metyrapone treatment (at 100 mg/kg) produces a significant decline in both plasma