Hormones and Behavior 59 (2011) 221–226

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Review

Hypothalamic vasopressin response to stress and various physiological stimuli: Visualization in transgenic animal models

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ARTICLE INFO

Article history:
Received 12 August 2010
Revised 18 November 2010
Accepted 17 December 2010
Available online 23 December 2010

Keywords:
Vasopressin
c-*fos*
Green fluorescent protein
Red fluorescent protein
Homeostasis
Transgenic rat

ABSTRACT

Arginine vasopressin (AVP) is involved in the homeostatic responses numerous life-threatening conditions, for example, the promotion of water conservation during periods of dehydration, and the activation of the hypothalamo-pituitary adrenal axis by emotional stress. Recently, we generated new transgenic animals that faithfully express an AVP–enhanced green fluorescent protein (eGFP) fusion gene in the paraventricular nucleus (PVN), the supraoptic nucleus (SON) and the suprachiasmatic nucleus (SCN) of the hypothalamus. In these transgenic rats, marked increases in eGFP fluorescence and fusion gene expression were observed in the magnocellular division of the PVN and the SON, but not the SCN, after osmotic challenges, such as dehydration and salt loading, and both acute and chronic nociceptive stimuli. In the parvocellular division of the PVN, eGFP expression was increased after acute and chronic pain, bilateral adrenalectomy, endotoxin shock and restraint stress. In the extra-hypothalamic areas of the brain, eGFP expression was induced in the locus coeruleus after the intracerebroventricular administration of colchicine. Next, we generated another transgenic rat that expresses a fusion gene comprised of c-*fos* promoter–enhancer sequences driving the expression of monomeric red fluorescent protein 1 (mRFP1). In these transgenic rats, abundant nuclear fluorescence of mRFP1 was observed in the PVN, the SON and other osmosensitive areas after acute osmotic stimulation. Finally, we generated a double transgenic rat that expresses both the AVP–eGFP and c-*fos*–mRFP1 fusion genes. In this double transgenic rat, we have observed nuclear mRFP1 fluorescence in eGFP-positive neurons after acute osmotic stimulation. These unique transgenic rats provide an exciting new tool to examine neuroendocrine responses to physiological and stressful stimuli in both *in vivo* and *in vitro* preparations.

Contents

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0018-506X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2010.12.007

Introduction

Recent progress in the widespread application of green fluorescent protein (GFP), discovered in 1962 by Shimomura et al. has had a major impact, not only in the field of cell biology, but also in hormone and behavioral studies. By using GFP and other modified fluorescent proteins (Tsien, 1998), "Seeing is believing" has become a reality in the life sciences.

The pleiotropic neuropeptide vasopressin (AVP) is synthesized in specialized neurons principally located in the hypothalamus, although other extra-hypothalamic sites of production are known. Magnocellular neurosecretory cells in the paraventricular (PVN) and the supraoptic nuclei (SON) of the hypothalamus synthesize neuropeptide hormones, AVP and oxytocin, and secrete them in a regulated fashion into the systemic blood flow from axon terminals located in the posterior pituitary (PP) (Brownstein et al., 1980). Circulating AVP acts on the kidney via V2 receptors as an anti-diuretic hormone (Birnbaumer et al., 1992; Lolait et al., 1992).

In contrast, parvocellular neurons in the PVN project to the external layer of the median eminence (ME). The stress-induced secretion of AVP from ME axon terminals into the portal blood results in ACTH release from corticotroph cells of the anterior pituitary, an effect mediated by the V1b receptor (Aguilera and Rabadan-Diehl, 2000). Circulating ACTH stimulates the secretion of glucocorticoids from the adrenal cortex. The activation of the hypothalamo-pituitary adrenal (HPA) axis is known to be an important neuroendocrine response to various kinds of systemic stressors (Viero et al., 2010).

In this review, we focus on recent major advances in the use of AVP–enhanced green fluorescent protein (eGFP) transgenic rats, and the consequent visualization of dynamic changes in AVP expression in these animals subjected to stressful conditions and to various physiological stimuli. The responses of hypothalamic AVP-producing neurons to various stimuli were visualized by green fluorescence in a transgenic animal model that expresses the AVP–eGFP fusion gene. The stressors and consequent dynamic changes in AVP–eGFP fluorescence are summarized in Table 1.

To complement this approach, we engineered rats in which neuronal activation can be readily monitored. The expression of c-*fos* protein has been widely used to detect neuronal activity in the central nervous system (Sagar et al., 1988). In order to easily visualize such activated neurons in the brain, we generated a transgenic animal model that expresses a c-*fos* promoter–monomeric red fluorescent protein 1 (mRFP1) fusion gene. The mRFP1 is one of a number of GFP special variants (Campbell et al., 2002). By combining the two transgenes in a single animal, *activated* AVP neurons can be readily visualized.

Visualization of AVP in AVP–eGFP transgenic rats

A transgenic rat line that expresses eGFP fluorescence in the AVP-producing neurons of the hypothalamus was generated by using an AVP–eGFP fusion gene (Ueta et al., 2005). In these transgenic rats, it is easy to identify AVP neurons in the hypothalamus, AVP and axon terminals in the PP by fluorescence microscopy. AVP neurons expressing eGFP fluorescence were distributed mainly in the magnocellular division of the PVN, the SON and the SCN (Figs. 1A–C) (Ueta et al., 2005, 2008). Axons from the PVN and the SON project into the PP via the internal layer of the ME and are readily observed by virtue of eGFP fluorescence (Fig. 1D).

The response of AVP–eGFP neurons to osmotic challenges and pain

Magnocellular AVP-producing neurons in the hypothalamus are activated by osmotic stimulation such as dehydration (complete fluid deprivation) and chronic salt loading. Dehydration for 2 days and salt loading (2% w/v saline to drink) for 5 days both resulted in marked increases in eGFP fluorescence in the magnocellular division of the PVN and the SON, but not in the SCN (Figs. 1E–G) (Ueta et al., 2005; Fujio et al., 2006). In the internal layer of the ME, eGFP fluorescence was markedly increased after salt loading (Fig. 1H), suggesting increased axonal transport of the AVP–eGFP fusion protein. Interestingly, the up-regulation in the expression of the AVP–eGFP fusion gene after osmotic challenge was exaggerated in comparison with that of endogenous AVP gene expression in the PVN and the SON (Fujio et al., 2006).

AVP release is stimulated not only by osmotic stimuli, but also by non-osmotic stimuli such as hemorrhage and painful insults. The effects of acute and chronic pain on AVP–eGFP fluorescence and the expression of the AVP–eGFP fusion gene were also examined in the PVN and the SON. As an acute pain model, formalin was injected subcutaneously into the bilateral hind paws in AVP–eGFP transgenic rats (Suzuki et al., 2009a). Fifteen minutes after formalin injection, the plasma AVP levels were significantly increased. Over the same time period, AVP heteronuclear (hn) RNA levels in the PVN were increased, particularly in the parvocellular division of the PVN. The level of eGFP mRNA level was also significantly increased after formalin injection, to a greater extent than that of AVP hnRNA levels. A marked increase in eGFP fluorescence was observed in the magnocellular and parvocellular divisions of the PVN, the ME, and the PP. These results suggest that the expression of the AVP–eGFP fusion gene in the PVN and the SON changes rapidly after acute nociceptive stimulation. As a chronic pain model, the adjuvant arthritis (AA) model was used (Suzuki et al., 2009b). AA is often used as a chronic inflammatory/

Table 1
Summary of changes in eGFP fluorescence (eGFP mRNA) in the paraventricular nucleus (PVN), the supraoptic nucleus (SON), the suprachiasmatic nucleus (SCN) and the median eminence (ME) after various stressors. mPVN, magnocellular division of the PVN; pPVN, parvocellular division of the PVN; eME, external layer of the ME; iME, internal layer of the ME.

	Region						
Stressors	mPVN	pPVN	SON	SCN	eME	iME	Refs.
Dehydration	↑(⇧)	→(⇨)	↑(⇧)	→(⇨)	–	–	Ueta et al. (2005)
Salt loading	↑(⇧)	→(⇨)	↑(⇧)	→(⇨)	→	↑	Fujio et al. (2006)
Formalin sc	↑(⇧)	↑(⇧)	↑(⇧)	–(–)	↑	↑	Suzuki et al. (2009a)
Adjuvant arthritis	↑(⇧)	↑(⇧)	↑(⇧)	–(–)	↑	↑	Suzuki et al. (2009b)
Adrenalectomy	→(–)	↑(–)	–(–)	–(–)	↑	→	Shibata et al. (2007)
LPS ip	→(–)	↑(–)	–(–)	–(–)	↑	→	Shibata et al. (2007)
Restraint	→(–)	↑(–)	–(–)	–(–)	–	–	Todoroki et al. (2010)
Colchicine icv	↑(⇧)	↑(⇧)	↑(⇧)	↑(⇨)	↑	↑	Todoroki et al. (2010)

eGFP fluorescence; increase '↑', no change '→', not determined, '–'.
eGFP mRNA; increase '⇧', no change '⇨', not determined, '–'.