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Research Report

Involvement of urocortinergic neurons below the midbrain central gray in the physiological response to restraint stress in pigeons

Roberta P. Cunha[a], *Anton Reiner*[b], *Claudio A.B. Toledo*[a,*]

[a]*Laboratório de Neurociências, Universidade Cidade de São Paulo, UNICID, Rua Cesário Galeno, 448, 03071-000, São Paulo, S. P., Brazil*
[b]*Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38163, USA*

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ABSTRACT

The present study was carried out to identify the diencephalic and midbrain neurons in pigeons that respond to stress (using restraint as the stressor) and determine if the urocortinergic neurons (expressing urocortin 1, Ucn1) below the midbrain central gray are among those activated. Immunolabeling for the immediate early gene *Egr-1* was used to identity stress-responsive neurons, following 1–3 h of restraint. A large increase in nuclear Egr-1 immunolabeling was observed in several dorsomedial thalamic nuclei, and in a stream of neurons extending from below the mesencephalic central gray (overlapping the nucleus of Darkschewitsch at these levels) to just anterior to the nucleus of Edinger–Westphal. A more modest increase in neuronal nuclear Egr-1 was observed in the medial posterior hypothalamic area, the mesencephalic periventricular area, the ventral tegmental area, the inferior colliculus, the nucleus paramedianus of the midbrain, and the intercollicular nucleus. The distribution and abundance of urocortin-immunolabeled neurons coincided with that of the stress-responsive neurons below the mesencephalic periaqueductal gray, and about 50% of these urocortin neurons were activated by stress. These results suggest that, as in some mammals, the urocortinergic neurons of the paramedian subgriseal mesencephalon respond to stress. In those mammals, in which the boundaries of the nucleus of Edinger–Westphal are indistinct, the caudal part of the homologous field of

* *Corresponding author*. Fax: +55 11 2178 1415.
E-mail address: ctoledo@cidadesp.edu.br (C.A.B. Toledo).

Abbreviations: ACTH, adrenocorticotrophic hormone; AVT, area ventralis tegmenti (or ventral tegmental area); CRF, corticotrophin-releasing factor; CRH, corticotrophin-releasing hormone; DIP, nucleus dorsointermedius posterior thalami; DLA, nucleus dorsolateralis anterior thalami; DLL, nucleus dorsolateralis anterior thalami, pars lateralis; DLM, nucleus dorsolateralis anterior thalami, pars medialis; DLP, nucleus dorsolateralis posterior thalami; DMA, nucleus dorsomedialis anterior thalami; DMP, nucleus dorsomedialis posterior thalami; EW, nucleus of Edinger–Westphal; FLM, fasciculus longitudinalis medialis; GLv, nucleus geniculatus lateralis, pars ventralis; H, habenula; ICo, nucleus intercollicularis; IP, nucleus interpeduncularis; MLD, nucleus mesencephalicus lateralis, pars dorsalis; OM, nucleus nervi oculomotorii; PaM, nucleus paramedianus; PeV, periventricular area; PMH, nucleus medialis hypothalami posterioris; PMI, nucleus paramedianus internus thalami; Ru, nucleus ruber; SPC, nucleus superficialis parvocellularis (also called the nucleus tractus septomesencephalici); Sub-G, subgriseal paramedian midbrain neuronal stream (partly overlapping the nucleus of Darkschewitsch); TeO, tectum opticum; TrO, nucleus nervi trochearis; TU, nucleus tuberis; Ucn1, urocortin 1; V, ventriculus

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urocortinergic neurons has been referred to as the nucleus of Edinger–Westphal. In pigeons, in which the nucleus of Edinger–Westphal is cytoarchitectonically well-defined, the caudal part of this urocortinergic field clearly does not include the nucleus of Edinger–Westphal.

1. Introduction

Urocortin 1 (Ucn1) is a 40-amino-acid peptide belonging to the same neuropeptide family as corticotrophin-releasing hormone (CRH, also know as CRF, corticotrophin-releasing factor) (Skelton et al., 2000). Ucn1 was cloned by Vaughan et al. (1995) from mesencephalic extracts of rat brain, and it stimulates the release of both corticotrophin and glucocorticoid hormones when injected into the brain ventricle. Ucn1 has a strong affinity for CRH receptors, especially the type II (Moreau et al., 1997; Kozicz et al., 1998; Morin et al., 1999), for which Ucn1 has an affinity reportedly 10 times greater than CRH itself (Vaughan et al., 1995). CRH receptor activation in the anterior pituitary stimulates adenocorticotrophic hormone (ACTH) release, and regulation of systemic ACTH levels was the first action proposed for Ucn1. CRH receptors have also been found in diverse brain regions (Vaughan et al., 1995; Sánchez et al., 1999; Van Pett et al., 2000). Since Ucn1-positive fibers are abundant in many of the regions rich in CRH receptors, such as the amygdala, septum, periventricular hypothalamus, the supraoptic and paraventricular nuclei, the superior olive, the cerebellum, and the vestibular nuclei complex (Kozicz et al., 1998, 2002; Vasconcelos et al., 2003), it is likely that Ucn1 plays a role in interneuronal communication in the central nervous system, in addition to its role in pituitary ACTH release (Bachtell et al., 2003; Kozicz et al., 2004).

Studies of the effects of intracerebral Ucn1 injections on behavior suggest that at least some Ucn1 circuits of the brain play a role in anxiogenic responses and behavioral stress (Koob and Heinrichs, 1999; De Fanti and Martinez, 2002; Hammack et al., 2003), and these appear to be part of the hypothalamic–pituitary–adrenal axis (HPA) (Chung et al., 1987; Koob and Heinrichs, 1999; Goodson et al., 2005). In mammals, a few Ucn1-positive neurons have been found in the supraoptic nucleus (Vasconcelos et al., 2003), ventral tegmental area, and substantia nigra (Yamamoto et al., 1998), but the most prominent population of Ucn1+ neurons occupies a bilateral midline stream that extends from below the rostral central periaqueductal mesencephalic gray (PAG) to the level of the rostral part of the oculomotor nuclear complex (Vaughan et al., 1995; Yamamoto et al., 1998; Morin et al., 1999; Toledo et al., 2002). Some authors have suggested that the caudal part of this Ucn1+ field, in fact, includes the nucleus of Edinger–Westphal of the oculomotor complex (Vasconcelos et al., 2003; Spina et al., 2004).

The distribution of Ucn1 neurons and their possible involvement in stress responses is not well characterized in birds. Restraint is a simple manipulation that can be used, in conjunction with immunolabeling for immediate early genes, such as *Egr-1*, to identify stress-responsive neurons in both rats and pigeons (Chan et al., 1993; Melia et al., 1994; Remage-Healey and Romero, 2001; Djordjevic et al., 2003; Gaszner et al., 2004). We used this approach to define stress-responsive neurons in pigeon diencephalon and midbrain and assess their overlap with Ucn1 neurons.

2. Results

While Egr-1 labeling occurred in the telencephalon of both control and restrained birds, because of our interest in Ucn1+ neurons we focus here on labeling in the diencephalon and midbrain. Control pigeons showed labeling for Egr-1 in the nuclei of many neurons of the nucleus tractus septomesencephalicus of the thalamus (SPC), the periventricular area of the hypothalamus (PeV), the nucleus tuberis of the hypothalamus (TU), the nucleus medialis hypothalami posterioris (PMH), the tectum opticum (TeO, mostly in the tectal layers 10 and 11), the nucleus intercollicularis (ICo), the ventral tegmental area (also called the area ventralis tegmenti, AVT), and the nucleus mesencephali lateralis pars dorsalis (MLD, the homologue of the mammalian inferior colliculus). Some neurons were also labeled in the trochlear nucleus (TrO), in the fasciculus mesencephalicus lateralis (FLM), and scattered just dorsal to the nucleus paramedianus (PaM) of the midbrain nucleus raphe (Table 1; Figs. 1, 2).

Table 1 – Comparison between normal and restrained pigeons in the number of neurons with EGR-1 immunolabeled nuclei for each of several major cell groups of the diencephalon, pretectum and midbrain

Brain subdivision	Cell group	No restraint control	Combined restraint
Diencephalon	TU	189.89±26.60	215.46±150.33
Diencephalon	PMH	45.00±14.79	74.07±61.77
Diencephalon	GLv	67.48±16.19	46.45±39.13
Diencephalon	DMA	11.20±03.65 *	137.43±78.58 *
Diencephalon	DLM	11.30±03.43 *	201.59±146.74 *
Diencephalon	DLL	8.09±04.82 *	148.35±100.63 *
Diencephalon	DLA	10.54±04.29 *	89.50±13.43 *
Diencephalon	DIP	10.45±05.31 *	61.25±30.05 *
Diencephalon	DLP	7.27±02.96 *	25.83±14.60 *
Diencephalon	PMI	12.40±03.56 *	67.50±32.02 *
Diencephalon	SPC	40.37±22.73 *	199.92±125.76 *
Diencephalon	PeV	68.56±17.47 *	30.01±15.55 *
Pretectum	Sub-G	3.27±02.22 *	30.23±22.46 *
Pretectum	ICo	266.09±37.93	325.94±175.38
Pretectum	OM	6.87±04.96 *	46.10±24.36 *
Mesencephalon	PaM	41.49±11.63	28.09±23.31
Mesencephalon	AVT	54.29±24.80	15.10±0.85
Mesencephalon	TrO	12.75±05.46	14.41±03.41
Mesencephalon	FLM	17.33±15.57	13.77±07.71
Mesencephalon	MLD	372.67±54.0	388.39±156.0
Mesencephalon	IP	4.61±04.57 *	111.85±70.33 *
Mesencephalon	TeO	2467.47±267.43 *	1211.10±974.41 *

The data shown are the mean and standard deviation (±SD) for a nucleus per section (single side) to the control group ($n=8$) and the combined restraint groups ($n=8$).
* Significant difference between control and combined restraint groups ($p<0.05$).