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Endocrine consequences of an acute stress under different thermal conditions: A study of corticosterone, prolactin, and thyroid hormones in the pigeon (*Columbia livia*)

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ABSTRACT

In the context of global change, the physiological and hormonal stress responses have received much attention because of their implications in terms of allostasis. However, most studies have focused on glucocorticoids only as the "common" response to stressors while neglecting other endocrine axes and hormones (e.g. prolactin, thyroid hormones) that play a crucial role in metabolic adjustments. Interestingly, the responsiveness of all these endocrine axes to stress may depend on the energetic context and this context-dependent stress response has been overlooked so far. In the wild, temperature can vary to a large extent within a short time window and ambient temperature may affect these metabolic-related endocrine axes, and potentially, their responsiveness to an acute stressor. Here, we explicitly tested this hypothesis by examining the effect of a standardized stress protocol on multiple hormonal responses in the rock pigeon (*Columbia livia*). We tested the effect of an acute restraint stress on (1) corticosterone levels, (2) prolactin levels, and (3) thyroid hormone levels (triiodothyronine, thyroxine) in pigeons that were held either at cool temperature (experimental birds) or at room temperature (control birds) during the stress protocol. Although we found a significant influence of restraint stress on most hormone levels (corticosterone, prolactin, and thyroxine), triiodothyronine levels were not affected by the restraint stress. This demonstrates that stressors can have significant impact on multiple endocrine mechanisms. Importantly, all of these hormonal responses to stress were not affected by temperature, demonstrating that the exposure to cold temperature does not affect the way these hormone levels change in response to handling stress. This suggests that some endocrine responses to temperature decreases may be overridden by the endocrine responses to an acute restraint stress.

1. Introduction

In the context of global change, there is growing interest in understanding how free-living vertebrates can cope with environmental stressors. In that respect, stress-related physiological mechanisms have received much attention because they are thought to both facilitate and to constrain the ability of individuals, populations, and species to adjust to our changing world (Wingfield, 2008; Wingfield et al., 2011). Specifically, the hypothalamo–pituitary–adrenal (HPA) axis appears relevant because this endocrine axis is thought to help vertebrates to cope with stressors in general (Wingfield et al., 1998, Wingfield, 2003; Romero et al., 2009). When a stressor is perceived by a vertebrate, this axis is activated, which results in a rapid increase of glucocorticosteroid secretion (corticosterone in birds, Sapolsky et al., 2000, Romero, 2004). This increase is important because it allows the individual to restore homeostasis through behavioural and physiological adjustments (McEwen and Wingfield, 2003, Romero et al., 2009). Importantly, this glucocorticoid (GC) stress response varies between individuals, populations and species (Hau et al., 2010; Addis et al., 2011; Lendvai et al., 2013; Cockrem, 2013) and investigating the proximate basis of this variability is essential to understanding whether vertebrates will be able to adjust to new stressful situations or not (Angelier and Wingfield, 2013; Wingfield et al., 2015).

Research on the endocrine response to stress in wild vertebrates has been mainly limited to GC although other hormonal axes are certainly essential to the ability of individuals to cope with stress. Indeed, multiple stressors can occur in the wild (ex: food shortage, inclement weather, predator attack) and they may require specific behavioral and physiological adjustments that are under control of not only the HPA axis but also other endocrine mechanisms (Romero et al., 2009). Other hormones are known to regulate specific physiological or behavioural processes that may help an individual to cope with stress. For example, prolactin, a pleiotropic hormone, is involved in the regulation of parental behavior (Angelier and Chastel, 2009; Angelier et al., 2016), food intake (Buntin and Tesch, 1985; Koch et al., 2004), body mass regulation (Boswell et al., 1995; Holberton et al., 2008) and lipid

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metabolism (Ben-Jonathan et al., 2006). Similarly, thyroid hormones (triiodothyronine and thyroxine) are involved in metabolic processes (Vézina et al., 2009; Welcker et al., 2013; Elliot et al., 2013) and thermoregulation (McNabb, 2007) in birds. Modifications of all these physiological and behavioural processes are classically involved in the organismal response to stress in vertebrates (Sapolsky et al., 2000), and therefore, studying how prolactin and thyroid hormones levels change in response to stress could help us better understand how individuals cope with stress. A few studies have found that these hormones can be affected by stressors in captive laboratory animals but the results often appear inconsistent (prolactin: Angelier and Chastel, 2009; Angelier et al., 2016; thyroid hormones: Wodzicka-Tomaszewska et al., 1982; Williamson et al., 1985; Hangalapura et al., 2004; Etches et al., 2008). Moreover, we currently lack detailed data on these hormonal stress responses in wild vertebrates because most studies have been limited to only a few species (Angelier et al., 2013, 2016). Finally, the potential functional connection between the GC stress response and the responses of these other endocrine axes to stress remain overlooked in wild vertebrates. Therefore, it appears crucial to further investigate how these endocrine axes may be concomitantly affected by an acute stressor in wild vertebrates.

Interestingly, there is recent evidence that the levels of circulating prolactin and thyroid hormones may change in response to stressors under some circumstances but not others (prolactin: Angelier and Chastel, 2009; Angelier et al., 2016; thyroid hormones, Wodzicka-Tomaszewska et al., 1982; Williamson et al., 1985; Hangalapura et al., 2004; Etches et al., 2008). This suggests that the responsiveness of all these endocrine axes (GC, prolactin and thyroid hormones) to stress may depend on the environmental or the energetic context. For instance, the GC response to an acute stressor is known to be magnified when individuals are in poor body condition (Angelier et al., 2015), and similarly, several environmental factors can affect the intensity of the GC stress response (e.g., risk of predation, Pakkala et al., 2013). In the wild, temperature can vary to a large extent within a short time window (ex: a drop of several °C within a few hours) and such changes in temperature are probably among the most important factors affecting the energetic balance of wild vertebrates because individuals need to adjust their activity and metabolism to the ambient temperature. Several endocrine pathways are thought to govern the metabolic adjustments to cold temperatures (Hulbert, 2000; Sapolsky et al., 2000; Rozenboim et al., 2004), and importantly these endocrine pathways may also be involved in the metabolic responses to stress (Wingfield et al., 1998; McNabb, 2007; Angelier and Chastel, 2009). First, GC levels increase not only in response to stress (Wingfield et al., 1998, 2015; Romero, 2004; Landys et al., 2006; Angelier and Wingfield, 2013) but also in response to temperature changes in endotherms (de Bruijn and Romero, 2011, 2013; Lynn and Kern, 2014), probably because such increase promotes protein and lipid catabolism that sustain increased metabolism and heat production (Astheimer et al., 1992; Sapolsky et al., 2000; Landys et al., 2006; DuRant et al., 2008; Wack et al., 2012). Second, prolactin levels are also affected by stressors (Angelier and Chastel, 2009; Angelier et al., 2016) and by acute and chronic changes in ambient temperatures (Gahali et al., 2001; Rozenboim et al., 2004; Dawson and Sharp, 2010). The metabolic effects of prolactin remain overlooked in birds but changes in prolactin levels may help individuals coping with cold temperature because prolactin is involved in food intake (Buntin and Tesch, 1985; Koch et al., 2004), body mass regulation (Boswell et al., 1995; Holberton et al., 2008) and lipid metabolism (Ben-Jonathan et al., 2006). Finally, the hypothalamus–pituitary–thyroid (HPT) axis is not only affected by exposure to stressors (such as long-term fast or handling stress, e.g. Groscolas and Leloup, 1989) but also involved in the ability of vertebrates to cope with a change in ambient temperature (McNabb, 2007). Thus, triiodothyronine and thyroxine are known to activate heat production in poultry (Klandorf et al., 1981, Williamson et al., 1985, Collin et al., 2003, McNabb, 2007) and elevated thyroid hormone levels have been associated with increased metabolic expenditures in wild birds (Chastel et al., 2003; Criscuolo et al., 2003; Vézina et al., 2009; Welcker et al., 2013; Elliot et al., 2013). Although the influence of temperature on the GC stress response has been investigated in a few studies (de Bruijn and Romero, 2011, 2013; Narayan et al., 2013; Dupoué et al., 2013; Lynn and Kern, 2014; Narayan and Hero, 2014), no study has, to our knowledge, examined this question on multiple endocrine pathways simultaneously in wild vertebrates.

In this study, our objective was to better understand how these endocrine pathways (corticosterone, prolactin, and thyroid hormones) are affected by a restraint stress under contrasting thermal conditions in a wild bird species, the rock pigeon (*Columbia livia*). Specifically, we used the standardized restraint stress protocol established by Wingfield et al. (1992) and, concomitantly, we experimentally reduced the ambient temperature during the restraint period (experimental birds) or not (controls). Therefore, the experimental group had to cope with an acute stressor in a moderately cold environment (10 °C) whereas the control group had to cope with an acute stressor at ambient temperature (21 °C). In temperate areas, such drops of 11 °C are quite common and are part of the daily or seasonal routines of pigeons. We focused on three endocrine pathways, which are thought to govern the metabolic adjustments that allow individuals to cope with stressors (Wingfield et al., 1998; McNabb, 2007; Angelier and Chastel, 2009), and decreases in temperature (Hulbert, 2000; Sapolsky et al., 2000; Rozenboim et al., 2004). As in many other vertebrate species and as previously found in pigeons, we expected corticosterone levels to increase in response to the standardized stress protocol (Wingfield et al., 1992, 1995; Hau et al., 2010; Lendvai et al., 2013; Pakkala et al., 2013). We also predicted that the corticosterone stress response of experimental birds will be stronger than that of controls (prediction 1) because experimental birds may need to additionally activate corticosterone-related metabolic and behavioral adjustments to cold temperature (de Bruijn and Romero, 2011, 2013; Lynn and Kern, 2014). In addition, we expected prolactin levels to decrease in response to the restraint protocol as previously reported in several bird species (Angelier and Chastel, 2009; Angelier et al., 2016). Although the metabolic role of prolactin has been overlooked in birds, previous studies have reported that prolactin levels usually increase in response to changes in ambient temperatures in birds and laboratory rodents (Fregly, 1989; Gahali et al., 2001; Rozenboim et al., 2004; Dawson and Sharp, 2010). This suggests that increased prolactin levels may mediate a physiological and behavioral adjustment to cold temperature, and therefore, we predict that elevated prolactin levels will be maintained in experimental birds that are exposed to moderately cold temperatures despite the restraint stress (prediction 2). As previously reported in other studies (e.g. Wodzicka-Tomaszewska et al., 1982), we also expected that triiodothyronine and thyroxine levels will decrease in experimental and control pigeons in response to handling stress. Because elevated thyroid hormone levels trigger metabolic activities and heat production (McNabb, 2007), elevated thyroid hormone levels should be maintained when individuals have to cope with cold temperatures (Kühn and Nouwen, 1978; Bobek et al., 1980; Collin et al., 2003). Therefore, we predicted that thyroid hormone stress responses will be attenuated in experimental birds relative to controls (prediction 3). Finally, we also investigated whether corticosterone, prolactin and thyroid hormones levels were correlated in order to shed some light on the potential functional connections between these hormonal responses to stressors.

2. Materials & methods

2.1. Study model

Fieldwork was carried out in 2013 (1–9 May 2013) in Vallauris, France (43°34′N, 7°02′E). Rock pigeons are medium size birds (from 250 to 350 g) and their usual lifespan is about 4–5 years in the wild. This gregarious species is widespread all over the globe and originated from Europe, Asia, and North Africa. This species initially used cliffs to breed and build their nests but they are now very common in urban