Journal of Neuroscience Methods 188 (2010) 243–249

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

# Corticosterone urinalysis and nicotinic receptor modulation in rats

Sally Loomis, Gary Gilmour*

*Eli Lilly & Co. Ltd, Psychiatric Disorders Drug Hunting Team, Lilly Research Centre, Erl Wood Manor, Sunninghill Road, Windlesham, Surrey GU20 6PH, UK*

ARTICLE INFO

*Article history:*
Received 7 January 2010
Received in revised form 13 February 2010
Accepted 15 February 2010

*Keywords:*
Corticosterone
Rat
Urine
Nicotine
Nicotinic receptors
Stress
Depression
Forced swim

ABSTRACT

A routine method of measuring circulating corticosterone (CORT) levels in rats involves sampling of plasma from cannulated animals. However, being somewhat invasive, this method can potentially be confounded by its inherently stressful nature. This study investigated the feasibility of measuring corticosterone using a non-invasive sampling method from voided urine of male rats. Reliability was assessed pharmacologically with nicotinic compounds previously demonstrated to modulate plasma glucocorticoid levels. Nicotine (0.1–1 mg/kg sc) dose-dependently increased corticosterone levels in rat urine at 30–70 min following administration. The short-lived nature of this elevation was confirmed as CORT levels measured 6 and 24 h later were shown to have returned to basal levels. Both basal and nicotine-induced (0.5 mg/kg sc) elevations in urinary CORT were consistent between groups of animals with weights ranging from 200 to 400 g. The magnitude of urinary CORT elevation induced by nicotine (0.5 mg/kg sc) was found to be similar to that induced by a forced swim stressor in male Lister Hooded, Sprague Dawley and Fischer 344 rats. The pharmacological specificity of this effect was confirmed as the nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine (0.05–0.5 mg/kg sc) dose-dependently reversed the effects of nicotine (0.5 mg/kg sc) on urinary CORT. Finally, the $\alpha_4\beta_2$-subunit preferring agonist TC-2559 induced a dose-dependent increase in CORT, whereas $\alpha_7$- and $\beta_4$-subunit preferring ligands had no effect, suggestive of the potential for differential involvement of nicotinic receptor subtypes in the mediation of this response. In conclusion, urinary corticosterone sampling in rats represents a robust assay sensitive to experimental manipulations of both pharmacological and behavioural relevances.



## 1. Introduction

A well-documented response to stressful experience in both humans and animals is activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in elevations in levels of circulating glucocorticoids and related hormones (Herman and Cullinan, 1997). While a short-lived elevation in glucocorticoid levels is likely to serve a normal, protective function in most individuals (Munck et al., 1984; Habib et al., 2001), evidence suggests that a prolonged or inappropriately excessive activation of the HPA axis may ultimately prove to be deleterious (Bremner, 1999; Sapolsky, 2000). Maladaptive physiological responses to stress have been postulated to be a precipitating or contributory factor in a number of psychiatric diseases (Brown et al., 1994; Dinan, 1994; van Praag, 2004; Burke et al., 2005; Ilgen and Hutchison, 2005).

The effects of environmental, psychosocial and pharmacological manipulations on HPA axis activity and stress hormone levels (Carrasco and Van de Kar, 2003) are well documented. However, the bulk of this work has focussed primarily on blood plasma measurements of corticosterone (CORT) and related hormones in cannulated animals. This can be problematic, as invasive procedures such as this are likely to be inherently stressful and may therefore interfere with and confound results of these sorts of studies (Fagin et al., 1983). Further, the patency of such preparations is limited and their use would not be viable in longitudinal studies, for example to assess the consequence of neonatal manipulations on HPA axis function across development. Apart from being a considerable ethical refinement of procedure, a non-invasive method of measuring glucocorticoids would allow greater opportunities for research into the relationship between stress, HPA axis function and affective disorders in development.

In the clinic, non-invasive methods of studying HPA axis activation in affective disorder research are routinely used. These include stress hormone analysis of urine and saliva (Rubin et al., 1987; Kirschbaum and Hellhammer, 1994). As increases in urinary cortisol levels in humans are considered to be indicative of increased HPA activity (Plotsky et al., 1998), it follows that the measurement of urinary corticosterone in rats may provide an equivalent readout of stressor-induced HPA activation in this species (Kanda et al., 1993). Clearly, what humans and rats find stressful in the environment can differ considerably (Harkin et al., 2002; Masini et al., 2005). Also, how humans and rats respond to repeated environmental stressful experiences may well be different. Some authors suggest that, in terms of the CORT response, rats do not adapt to a

* Corresponding author. Tel.: +44 01276 483813; fax: +44 01276 474390.
*E-mail address:* gilmour_gary@lilly.com (G. Gilmour).

0165-0270/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2010.02.016

stressor (inescapable shock) over a 3–10-day period (Ottenweller et al., 1992; Brennan et al., 2000). In opposition, it has also been found that habituation may occur following repeated exposure to stressors (inescapable shock, but also restraint stress) in rats, as shown by a reduction in both behavioural and physiological responses (Natelson et al., 1988; Pitman et al., 1990). Such results may indicate that adaptive processes exist which allow animals to cope with some environmental stressors more effectively. It could also suggest that the perception of and reaction to environmental stressors may depend upon the emotionality of individual subjects (Fernandez-Teruel et al., 1991). Importantly, effects similar to those observed with environmental stressors can also be produced via pharmacological manipulations that act indirectly on the HPA axis. For instance, it has been consistently demonstrated that nicotine administration causes elevations in corticosterone levels in rats (Balfour et al., 1975; Bugajski et al., 1998; Porcu et al., 2003). Use of drug-induced CORT responses may offer a considerable advantage in this context, as they would allow for much greater stimulus control than environmental stressors would, and therefore provide a more homogenous sample to study novel drug effects in. Accordingly, this study investigated the feasibility of measuring urinary CORT in rats following nicotine administration, and assessed the potential that this approach may have as a quantitative biomarker of HPA axis activation.

## 2. Methods

### 2.1. Subjects

This work was carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986. All studies, except for the weight study and strain study, used male Lister Hooded rats (Harlan, UK) weighing 225–300 g. The weight study used male Lister Hooded rats (Harlan, UK) ranging from 155 to 495 g. As well as using male Lister Hooded rats, the strain study used weight-matched male Sprague Dawley (Harlan, UK; weight 225–300 g) and male Fischer 344 (Harlan, NL; weight 225–300 g) rats. Animals were housed in groups of 4–8 dependent on their size in individually ventilated cages (Tecniplast, IT) for at least 1 week following delivery. They were kept in a 12 h light–dark cycle (lights on: 07:00–19:00) under standard conditions of constant temperature and humidity. Animals had *ad libitum* access to both food and water. All experiments were carried out between 08:00 and 16:00, in the trough of the circadian rhythm of corticosterone release (Allen-Rowlands et al., 1980; Hausler et al., 1984).

### 2.2. Apparatus

#### 2.2.1. Urine collection

This was conducted in metal holding cages (Le Clair Precision, FR) with grid floors (34 cm × 40 cm), underneath which sat a sample collection tray. These test cages were quite dissimilar from home cages, and rats were not acclimatised to them prior to the collection period. Great care was taken to clean and dry each collection tray between runs.

### 2.3. Drugs

All doses referred to in this work reflect the free base weight of compounds. (−)-Nicotine hydrogen tartrate salt, mecamylamine hydrochloride and hexamethonium chloride were purchased from Sigma Aldrich, UK. TC-2559 hemigalactarate ((E)-N-methyl-4-[3-(5-ethoxypyridin)yl]-3-buten-1-amine), Compound A ((R)-N-(1-azabicyclo[2.2.2]oct-3-yl)(5-(2-pyridyl)thiophene-2-carboxamide (WO 01/60821 A1)) and Compound B (1-methyl-4-(2-chloro-4-hydroxyphenylthio)-piperidine (WO 03/062224)) were synthesised by Lilly Research Laboratories. TC-2559, Compound A and Compound B have been previously described to exhibit selectivity using *in vitro* assays for $\alpha_4\beta_2$, $\alpha_7$- and $\beta_4$-subunit containing nicotinic receptors, respectively (Smith et al., 2007). All compounds were dissolved in 5% (w/v) glucose and pH adjusted with 1 M NaOH as necessary. All drugs were administered subcutaneously (sc) in a volume of 1 ml/kg.

### 2.4. Procedure

#### 2.4.1. Nicotine dose response study

Animals were dosed with either vehicle or nicotine (0.1–1 mg/kg sc) and returned to their home cage for 30 min. Following this pre-treatment period, rats were placed individually into test boxes for 40 min. At the end of this period, clean Pasteur pipettes were used to collect urine samples from each tray beneath the grid floor, which were immediately transferred to Eppendorf tubes and frozen at −20 °C. Any animals that did not void a sufficient quantity of urine (<70 μl) for analysis during this time were excluded from the study.

#### 2.4.2. Nicotine time course study

Nicotine (0.5 mg/kg sc) or vehicle was administered and animals were then placed in test cages for a 40 min collection period at 1, 6 and 24 h post-injection. Rats were returned to their home cage between collection periods.

#### 2.4.3. Weight study

The procedure for this study was similar to that used for the nicotine dose response study described above, except that animals were grouped dependent on their body weight (<200, 200–300, 300–400 and >400 g). Each test session was balanced for treatment and weight, such that an equal number of treatment conditions were tested in any one session.

#### 2.4.4. Swim stress study

This study used a modified version of the original forced swim method devised by Porsolt et al. (1978). For a forced swim stressor, rats were placed individually into a cylinder containing 19 cm of clean water at 21–25 °C for a 10 min period. This depth ensured that only the tail of the animal could touch the bottom of the cylinder. Following forced swim, rats were quickly but thoroughly dried to remove excess water and were immediately placed into test cages for a 40 min collection period. Heat lamps were suspended above test cages to keep animals warm during the post-stress period. Control rats in this experiment were left un-handled in their home cage until the urine collection period commenced, whereupon they were transferred to test cages. As a comparison, nicotine (0.5 mg/kg sc) and vehicle animals tested in a similar manner to the dose response study described above were incorporated into this study in a balanced manner, i.e. each test session has equal amounts of swim stress and dosed animals.

#### 2.4.5. Antagonist studies

Separate studies were conducted assessing the effects of mecamylamine (0.05–0.5 mg/kg sc) and hexamethonium (2.5–10 mg/kg sc) on basal and nicotine-induced (0.5 mg/kg sc) urinary CORT levels. In the basal CORT studies, each antagonist was administered with a 30 min pre-treatment period before the 40 min collection period began. A group of animals receiving a dose of nicotine (0.5 mg/kg sc) acted as a positive control in these studies. In the nicotine-reversal studies, each antagonist was administered 5 min prior to dosing with nicotine (0.5 mg/kg sc). As above, animals were then returned to their home cage for a 30 min pre-treatment period before the 40 min collection period began.