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Effects of acrolein on the production of corticosterone in male rats

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ABSTRACT

Acrolein, an α, β-unsaturated aldehyde, exists in a wide range of sources. Acrolein can be not only generated from all types of smoke but also produced endogenously from the metabolism by lipid peroxidation. The cellular influence of acrolein is due to its electrophilic character via binding to and depleting cellular nucleophiles. Although the toxicity of acrolein has been extensively studied, there is relatively little information about its impact on hormone release. This study aimed at the effect of acrolein on hypothalamic–pituitary–adrenal (H–P–A) axis. In an in vivo study, male rats were administrated with acrolein for 1 or 3 days. The plasma corticosterone in response to a single injection of adrenocorticotropic hormone (ACTH) increased slowly in acrolein-pretreated rats than in control rats. Further investigating the steroidogenic pathway, the protein expressions of steroidogenic acute regulatory protein (StAR) and the upper receptor-melanocortin 2 receptor (MC2R) were attenuated in acrolein-treated groups. Another experiment using trilostane showed less activity of P450scc in zona fasciculata-reticularis (ZFR) in acrolein-treated groups. In addition to the suppressed ability of corticosterone production in ZFR cells, acrolein even had extended influence at higher concentrations. The lower ACTH was observed in the plasma from acrolein-pretreated rats. In an in vitro study, ZFR cells were incubated with acrolein and the results showed that corticosterone concentrations in media were decreased in a dose-dependent manner. Acrolein also desensitized the response of the ZFR cells to ACTH. These results suggested that acrolein decreased the releasing ability of corticosterone via an inhibition on the response of ZFR cells to ACTH and the reduction of protein expressions of StAR and MC2R as well as the activity of P450scc in rat ZFR cells. The present evidences showed that the H–P–A axis was affected by the administration of acrolein.

1. Introduction

In modern society, air pollution is getting a more and more serious concern in public. People exposed to high enough levels of air pollutants may experience irritated sensory organs and even diseases [1–4]. Acrolein is one of many toxic pollutants defined and investigated in the past years [5]. This compound is a highly electrophilic, α, β-unsaturated aldehyde [6]. Due to its high vapor pressure and water solubility, acrolein is expected to be highly

Abbreviations: HPA axis, hypothalamic–pituitary–adrenal axis; ACTH, adrenocorticotropic hormone; MC2R, melanocortin 2 receptor; ZFR, zona fasciculate-reticularis; StAR, steroidogenic acute regulatory protein.

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mobile when it is released into air. People would be unaware of exposure to acrolein sources. The sources include all types of smoke (e.g. cigarette smoke), materials of industry, fried food or even come from the metabolic products of anticancer drugs (e.g. cyclophosphamide) [7–9].

So far adverse effects of acrolein have been thought to occur only in tissues directly exposed to the toxin like the oral cavity or the respiratory tract. However, abundant literature suggests that acrolein is capable of rapidly reacting at many nucleophilic sites in a cell. Acrolein may not only rapidly bind to but also deplete cellular nucleophiles, particularly glutathione (GSH) [10]. In addition, many in vitro or in vivo studies have reported that acrolein is highly cytotoxic [11,12]. Besides these toxic effects, some studies also indicated that acrolein can inhibit the production of proinflammatory cytokines [13]. The inhibitory effect of acrolein may be related to suppressed NFκB activation resulting in GSH depletion (GSH can induce NFκB) or modification of the DNA-binding domain of NFκB [14]. These findings revealed that acrolein may have an impact on transcription factor and our immunology.

Moreover, previous evidences also found that long-term exposure to cigarette smoke, where a major source of acrolein can alter plasma testosterone level, but didn't change gonadotropin levels. This result also revealed the histology of testes decreased in the portion of Leydig cells [15]. As the result, the harmful environmental factor has a profound influence on endocrine system and the balanced physiological process [16]. Glucocorticoid is one of the essential hormones that released from zona fasciculata of adrenal and regulated by hypothalamus–pituitary–adrenal (H–P–A) axis. It releases in response to stress and the low level of blood sugar, which relates to some life threatening situations. Moreover, glucocorticoid is a vital hormone majorly involved in metabolism and immunologic responses [16]. It plays an important role in immunosuppression, therefore, glucocorticoids have been commonly applied in pharmacological treatment with the effect of reducing lymphocytes count, and cytokines produced from lymphocytes [17].

Cigarette smoking has also been reported to be a major cause of disturbance in adrenal hormone levels. This can be associated with a smoking-induced decrease in the activity of the enzymes including 21-and 11-beta-hydroxylase in the adrenal cortex. In order to describe completely about the change of stress-responsive activities after the exposure to acrolein, one of principal components in cigarette, this study focus on glucocorticoid, which is also a crucial mediator taken part in stress response.

2. Experimental

2.1. Animal

Male Sprague–Dawley rats (8-week old) from Laboratory Animal Center of National Yang-Ming University were housed in a temperature-controlled room (22 ± 1 °C) with 14 h of automatic illumination daily (0600-2000), food and water were given *ad libitum*. Rats were cared in accordance with the National Yang-Ming University's Guidelines for Animal Care.

2.2. Preparation of zona fasciculata-reticularis (ZFR) cells for cell culture

An adrenocortical preparation enriched with zona-fasciculata-reticularis (ZFR) cells for culture was performed based on previous method [18] with a few modifications [19]. Inner zone (zona fasciculata-reticularis) fraction from rat adrenal glands was obtained by decapsuling outer zone (zona of glomerulosa) with forceps. After removing outer glomerulosa zone completely, cells from inner zones were incubated in Krebs–Ringer bicarbonate buffer (3.6 mM K^+, 11.1 mM glucose) with 0.2% BSA medium (KRBGA), pH 7.4 and collagenase (10 mg/ml, Sigma, St. Louis, MO, USA) at 37 °C in a shaking water bath (50 cycles/min) for 1 h. ZFR cells were dispersed mechanically by being repeatedly pipetted and filtered by using a 70 μm filter (BD Falcon, Franklin Lakes, NJ, USA). After centrifugating at 200×*g* for 10 min, ZFR cells were resuspended and washed in KRBGA medium. Furthermore, erythrocytes were eliminated from ZFR cells by washing with 9 ml distilled water for a few seconds, and then quickly mixed with 1 ml of 10× Hanks' balanced salt solution (HBSS pH 7.4, Sigma). Then, cells were spun down and resuspended cells in KRBGA again. Aliquots (50 μl each) were used for performing the cell counting in a hemocytometer after staining with 0.15% trypan blue. Cells in culture medium were further diluted to an equal concentration of 5×10^4 cells/ml in each tube.

2.3. Effect of acrolein on the concentration of plasma corticosterone and ACTH

Male rats at the age of 8-week were intraperitoneal injected with or without acrolein (2 mg/ml/kg, Sigma) once daily for 1 or 3 days. The control rats were injected with normal saline (1 ml/kg/day). To avoid the variation of adrenal rhythm, rats were sacrificed between 09:00 and 10:00 AM, the trunk blood was collected at the same time, and plasma samples were collected and stored at −20 °C until measurement of the concentrations of corticosterone and ACTH by radioimmunoassay (RIA).

2.4. Effects of acrolein on corticosterone release in vivo

Male rats were pretreated with acrolein as previous procedure and undergone a right jugular vein catheterization based on previous method [20] with minor modifications [21]. After one night for recovery, conscious rats received a single injection of stimulant ACTH (5 μg/ml/kg, Sigma) through the jugular catheter. Blood samples (0.5 ml each) were collected immediately before or at 10, 30, 60, 120 min after the injection of ACTH. Plasma was separated by centrifugation and stored at −20 °C until analysis of the concentration of corticosterone.

2.5. Effects of acrolein on corticosterone release in vitro

In order to study the effects of acrolein on corticosterone release, ZFR cells were preincubated with KRBGA medium for 1 h and then incubated with different concentrations of acrolein (10^{-11}–10^{-7} M). For further studying the effect of ACTH on corticosterone release in acrolein-treated cells, cultured ZFR cells were incubated with the stimulant ACTH (10^{-9} M) in the presence or absence of acrolein (10^{-9}–10^{-6} M).

To examine the effect of acrolein on corticosterone release in rat ZFR cells, rats were sacrificed after exposure to acrolein for 1-day or 3-day. ZFR cells were prepared from adrenal glands as previous description and then incubated with ACTH (10^{-9} M), 8-Br-cAMP (5×10^{-5} M), forskolin (10^{-5} M) or vehicle.

2.6. RIA of corticosterone

The levels of corticosterone in plasma were extracted by diethyl ether (10× volume, Merck, Whitehouse Station, NJ, USA). After generous mixing for 30 min, samples were stayed at room temperature for 15 min to extract the steroid hormone from inorganic phase, and then frozen inorganic phase in a mixture of acetone and dry ice. The upper organic phase was collected, dried, and reconstituted in assay buffer before RIA.

The corticosterone in plasma or media was measured by RIA, which has been well established [14]. The standard of corticosterone

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