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Short communication

Stress triggered rise in plasma aldosterone is lessened by chronic nicotine infusion

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Abstract

The ability of nicotine infusion to modulate plasma aldosterone levels in response to different stressors was investigated. Sprague–Dawley rats given nicotine (5 mg/kg/day) or saline for 14 days were subjected to stress. Baseline plasma aldosterone (86 ± 17 pmol/l) was unaffected by nicotine. Aldosterone was significantly elevated by restraint (450 ± 72 pmol/l) and especially with cold (1249 ± 172 pmol/l) or immobilization (1779 ± 247 pmol/l) stress. Nicotine infusion attenuated the rise in aldosterone with restraint and cold stress, but not immobilization. These results reveal that nicotine infusion can attenuate the aldosterone response, depending on the type of stress.

Keywords: Aldosterone; Nicotine; Stress

1. Introduction

Acute stress triggers important neuroendocrine responses that enable the organism to survive and restore homeostasis. Primary among these is the rapid acute activation of the hypothalamic–pituitary–adrenocortical axis and the sympathetic nervous and adrenomedullary systems, leading to release of adrenocorticotropic hormone (ACTH), glucocorticoids, catecholamines and co-stored neuropeptides into the circulation. When these systems are activated repeatedly over a long period of time, the response is not only adaptive but also maladaptive. Stress is a major player in increased incidence of a number of common life-threatening disorders (McEwen, 1998) including myocardial infarction and hypertension (Goldstein, 1995).

Many causes influence individual susceptibility to stress: prior experience with stress, genetic factors and drugs such as nicotine. Nicotine has paradoxical effects on stress; it triggers some of the same physiological changes stress elicits, such as activation of the hypothalamic–pituitary–adrenocortical axis and release of catecholamines (Morse, 1989) yet smokers often report that cigarettes alleviate feelings of stress. To understand these diverse effects of nicotine, we have previously shown that administration of nicotine to rats by infusion with osmotic pumps did not activate the hypothalamic–pituitary–adrenocortical axis and actually lessened the elevation of gene expression for catecholamine biosynthetic enzymes by acute immobilization stress (Serova et al., 1999).

The renin–angiotensin–aldosterone system also plays an important role in the response to stress (Aguilera et al., 1995; Goldstein, 1995). It has been suggested that under stressful situations, aldosterone, in addition to producing salt and water retention, may serve as a restore and repair hormone in view of its activities to promote thrombosis (Funder, 2001) and fibrosis (Weber, 2001). Recent findings indicate that aldosterone plays a much larger role than was once thought, inclusive of cardiovascular disease states such as congestive heart failure, kidney damage, stroke and hypertension (Stier et al., 2003). The present studies were performed to compare the effects of various forms of acute stress on plasma aldosterone levels. We also examined whether chronic nicotine infusion, which mimics the clinical situation of transdermal administration by using a nicotine patch, would alter stress-induced changes in aldosterone levels, as we previously found that this modifies the effect

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of acute stress on the gene expression of catecholamine biosynthetic enzymes (Serova et al., 1999).

2. Materials and methods

2.1. Experimental animals

Pathogen-free adult male Sprague–Dawley rats (250–300 g) were purchased from Taconic Farms (Germantown, NY). Animals were housed three to four per cage in a room at an ambient temperature of 23 ± 2 °C with a 12-h light/12-h dark cycle and given standard rodent diet (Purina Lab Chow # 5001; Ralston-Purina, St. Louis, MO) and allowed tap water ad libitum. All experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23).

2.2. Procedures

Animals were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.) and osmotic minipumps (model 2002, Alzet, Palo Alto, CA) were implanted subcutaneously at the nape of the neck to release 5 mg (0.03 mmol) of (−) nicotine ditartrate (calculated as the free base) (Sigma, St. Louis, MO) per kg body weight per day for 14 days or saline as previously described (Serova et al., 1999). On day 12, rats were either unstressed or exposed to one of the following types of stress: (1) restraint for 2 h; (2) immobilization for 2 h; or (3) cold for 24 h. The restraint stress was performed by placing each rat in a wire mesh cylinder with a diameter of 5.5 cm. The immobilization stress was performed by taping the forelimbs and hindlimbs with surgical tape to metal mounts attached to a board as previously described (Kvetnansky and Mikulaj, 1970; Serova et al., 1999). The cold stress was performed by placing the rats, two per cage, in a pre-cooled metal cage in a cold room at 4 °C for 24 h. Animals were euthanized by rapid decapitation immediately after the stress at the same time of day (between the hours of 0800 and 1300) and blood was collected into chilled polyethylene tubes containing disodium ethylenediaminetetraacetate, centrifuged, and the supernatant stored at −70 °C for later determination of aldosterone levels.

2.3. Assays and analyses

Plasma aldosterone concentration was determined by standard radioimmunoassay (Diagnostic Products, Los Angeles, CA), the sensitivity of which was approximately 31 pmol/l. The within- and between-assay coefficients of variation were 3% and 8%, respectively.

2.4. Statistical analysis

Data were analyzed for the overall effects of treatment and stress by two-way analysis of variance followed by post hoc analysis using one-way analysis of variance and unpaired *t*-tests as appropriate (BMDP Statistical Software, Los Angeles, CA). Differences between means were considered statistically significant at $P < 0.05$.

3. Results

All three types of stress significantly increased plasma aldosterone, but to varying degrees (Fig. 1). The highest level (21-fold above baseline) was observed with immobilization stress. There was a 14.5-fold increase in plasma aldosterone with cold stress. Restraint stress triggered a more modest (5 fold) but significant rise in plasma aldosterone. Nicotine infusion did not change basal plasma aldosterone levels in unstressed rats. The infusion of nicotine significantly attenuated the rise in plasma aldosterone by 69.1% in response to restraint stress and by 45.5% in response to cold stress. In contrast, chronic nicotine infusion did not alter the rise in plasma aldosterone in response to immobilization stress.

4. Discussion

This study demonstrates varying increases in plasma aldosterone levels in response to restraint, cold and immobilization stress. The results reveal that chronic nicotine infusion can differentially modulate the elevation in aldosterone levels to the stressors. Stressor-specific variations in the response of the hypothalamic–pituitary–adrenocortical

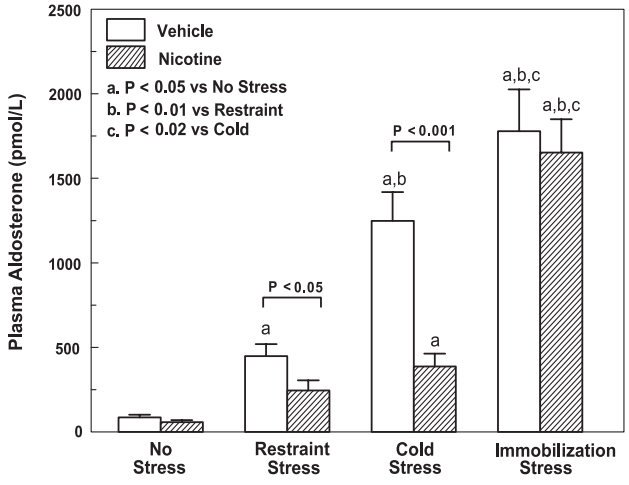

Fig. 1. Bar graph showing plasma aldosterone levels in male Sprague–Dawley rats. Animals were infused subcutaneously with either vehicle (saline; unfilled bars) or nicotine [5 mg (0.03 mol) of (−) nicotine ditartrate, calculated as the free base; filled bars] by osmotic minipump for 14 days and then either unstressed ($N = 14$ to 15 rats per group) or exposed to one of the following types of stress ($N = 7$ to 8 rats per group): (1) restraint for 2 h (2) immobilization for 2 h or (3) cold for 24 h. (A) $P < 0.05$ compared with no stress; (B) $P < 0.01$ compared with restraint stress; (C) $P < 0.02$ compared with cold stress. Values are mean ± S.E.M.

axis as well as peripheral and central catecholaminergic systems have been described (Pacak et al., 1998; Palkovits, 2002). In the present study, immobilization stress was found to produce the greatest increase in plasma aldosterone, which reached levels similar to those previously reported (Moncek et al., 2003). This increase in plasma aldosterone was nearly four times greater than with restraint stress although the duration of stress was identical in these two situations. This observation is consistent with previous findings that greater rises are seen in plasma ACTH, catecholamines and catecholamine biosynthesis with immobilization, compared with restraint stress. Cold stress also elicited a robust aldosterone response. The factors responsible for the observed changes in aldosterone by specific stressors remain to be determined. A variety of stimuli, several of which are known to be altered by stress, stimulate aldosterone secretion. These include angiotensins, potassium, ACTH, neurotransmitters and other factors (Quinn and Williams, 1988). Immobilization and restraint stress can markedly elevate plasma ACTH while cold stress has little effect (Pacak et al., 1998). Although ACTH can stimulate aldosterone release from isolated zona glomerulosa cells, production of aldosterone is, mostly independent of ACTH in vivo (Foster et al., 1997). Cold stress strongly stimulates the sympathetic nervous system and markedly elevates plasma norepinephrine (Pacak et al., 1998). Cold stress was found to elevate α-bungarotoxin sensitive nicotinic receptors in the adrenal gland (Miner et al., 1989). Whereas plasma angiotensin II is elevated 10 fold by 4 h and returns to baseline at 1 day of cold stress (Cassis et al., 1998), activation of the sympathetic nervous system remains high as long as cold exposure lasts (Fukuhara et al., 1996) and thus may be involved in mediating the observed rise in aldosterone.

In the present study, there was no effect of chronic nicotine administration on plasma aldosterone levels under unstressed conditions. Blood was obtained following rapid decapitation of animals as plasma aldosterone can increase markedly following induction of anesthesia. Consistent with our precautions to avoid stress in control animals, extremely low levels of plasma aldosterone were obtained. Similar to the absence of an effect of nicotine on basal aldosterone seen here, chronic transdermal infusion of nicotine to humans failed to alter urinary aldosterone excretion (Zevin et al., 1998). In contrast, when nicotine was administered by injection, plasma aldosterone increased markedly by 10 min but returned to levels near or below baseline by 1 to 2 h later (Andersson et al., 1993). However, in adrenal cell cultures, nicotine and its metabolite, cotinine, inhibited aldosterone synthesis (Skowronski and Feldman, 1994).

Nicotine infusion was found to attenuate the rise in aldosterone in response to cold or restraint stress. This effect may be due to receptor desensitization. Tachyphylaxis to nicotine is a well-known phenomenon and is also thought to involve depolarization block (Bowman and Rand, 1980). It remains to be determined at what site the inhibition by nicotine infusion occurs. It might occur by a central mechanism or at the level of sympathetic ganglia, which innervate the adrenal cortex, or in zona glomerulosa cells of the adrenal cortex. In this regard, repeated intraarterial injections of nicotine diminished the rise in plasma catecholamines in response to restraint stress (Kiritsy-Roy et al., 1990). However, in the case of immobilization stress, nicotine infusion failed to diminish the rise in plasma aldosterone, despite our previous finding that chronic nicotine infusion could inhibit the immobilization stress triggered rise in mRNA for adrenomedullary catecholamine biosynthetic enzymes (Serova et al., 1999). This suggests that the mechanism by which immobilization stress increases plasma aldosterone levels may be distinct from that which modulates catecholamine biosynthesis. Indeed, immobilization stress is one of the strongest experimental stress situations and is considered to be a combination of physical and psychological stressors; it activates many neuronal input and output circuits (Palkovits, 2002). Therefore, a higher dose of nicotine may be required to attenuate this effect. In addition, since aldosterone release is affected by a myriad of factors (Quinn and Williams, 1988), additional stimulatory factors may come into play during immobilization stress that override an inhibitory effect of nicotine in this situation.

In conclusion, these results reveal for the first time that aldosterone, an important hormonal regulator of cardiovascular function and dysfunction, is differentially affected by various stressors and that chronic exposure to nicotine can modulate some of these responses.

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