Central but Not Peripheral Glucocorticoid Infusion in Adrenalectomized Male Rats Increases Basal and Substrate-Induced Insulinemia through a Parasympathetic Pathway

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Abstract

Objective: Glucocorticoids acting through the central nervous system are postulated to play a role in the hyperinsulinemia and increased adiposity of obesity. We investigated the role of parasympathetic activation in glucocorticoid- induced hyperinsulinemia.

Research Methods and Procedures: Plasma pancreatic polypeptide (PP) levels were used as an index of parasympathetic output. Insulinemia and plasma PP levels were measured basally and after intravenous glucose injection (300 mg/kg) in adrenalectomized male rats infused with dexamethasone (7.5 μg/kg per day) intracerebroventricularly (ICV) or subcutaneously (SC) for 3 to 6 days in the presence or absence of acute atropine blockade (1.0 mg/kg). Food intake was controlled between groups.

Results: Compared with normal rats, adrenalectomy decreased white adipose tissue depot weights and leptinemia, and these were restored to normal values by ICV but not SC dexamethasone infusion. Adrenalectomy significantly reduced insulinemia below normal levels, which was restored by SC dexamethasone replacement. However, ICV dexamethasone replacement increased insulinemia of adrenalectomized rats to levels higher than normal control values (basal, 500 ± 40 pM vs. 280 ± 40 pM; 1-minute postglucose, 2500 ± 180 pM vs. 1240 ± 260 pM; $p < 0.0001$) and increased plasma PP levels, which were correlated with insulinemia. Atropine significantly reduced plasma insulin and PP to levels similar to normal controls but had no effect in any other group.

Discussion: These data show that glucocorticoids act within the brain to increase insulinemia, most likely through activation of parasympathetic efferent fibers. Such an affect would contribute to the adipogenic effects of central glucocorticoids.

Introduction

Glucocorticoids have been implicated in the etiology of obesity because of their "permissive" role in many rodent obesity syndromes. Indeed, adrenalectomy prevents or attenuates obesity of genetic (1–5), dietary (6), or hypothalamic (7–9) origin in rodents, and also prevents the obesity syndrome caused by chronic central neuropeptide Y (NPY) infusion in rats (10). Moreover, all of these obesity syndromes can be restored by administration of replacement glucocorticoids (1,2,4 –7,9). In some studies, glucocorticoids were effective after specific microinjection into the brain (3,8,9), suggesting a central site of glucocorticoid action in the regulation of adiposity.

  More recent data emphasize that glucocorticoids per se can cause obesity-like effects in non-obese animals, suggesting that glucocorticoids are not simply permissive to other primary causes of obesity. For example, some studies showed that peripheral corticosterone administration dose-dependently increased food intake, fat depot weight, adipocyte size, lipoprotein lipase activity, and insulinemia of normal or adrenalectomized (ADX) rats, while decreasing food efficiency (11,12). These effects may have been mediated through the brain, because another study using lower doses found that intracerebroventricular (ICV) but not sub cutaneous (SC) corticosterone injections increased weight gain of ADX rats (13). Furthermore, 3-day low-dose ICV

dexamethasone infusion in normal rats increased food in- take, body weight gain, and plasma triglyceride, leptin, and insulin levels, and decreased brown adipose tissue thermogenin expression (14). When given intraperitoneally, the same dose of dexamethasone decreased food intake and body weight gain and had much less marked effects on leptinemia and insulinemia (14).

Hyperinsulinemia observed after in vivo glucocorticoid administration (11–16) is likely to mediate the glucocorticoid-induced obesity syndrome, because the ability of corticosterone to increase fat depot weight in ADX rats was shown to be prevented by prior streptozotocin destruction of beta cells (12). Furthermore, attenuation of the hyperinsulinemia of genetically obese *ob/ob* mice or *fa/fa* rats by streptozotocin or insulin antibodies (17), or by inhibition of glucose-mediated insulin secretion, reduced the obese phenotype in the absence of significant effects on feeding (18). This further illustrates the importance of hyperinsulinemia in the etiology of obesity.

This study aimed to further understand the mechanisms of central glucocorticoid-induced hyperinsulinemia. To this end, we measured basal and intravenous glucose-induced insulin levels in ADX male rats after 3 to 6 days of ICV dexamethasone infusion, in comparison with normal or ADX vehicle-infused control rats. An additional group of ADX rats received SC dexamethasone infusion, which controlled for possible peripheral effects of the steroid. All animals were fed the same amount to prevent confounding effects of differences in food intake on insulinemia. The contribution of parasympathetic activation to hyperinsulinemia was investigated by two means: First, by administration of atropine methyl nitrate, which blocks muscarinic receptors in the peripheral but not the central nervous system (19); and second, by measurement of plasma levels of pancreatic polypeptide (PP) in the different groups of rats as a relative index of parasympathetic activity. This index was used because PP secretion from islet F cells in several species is controlled by vagal input to the pancreas (20–22). Indeed, plasma PP levels are increased by vagal stimulation, cholinergic agonists, meal feeding, hypoglycemia, or neuroglucopenia, and the increase is largely prevented by vagotomy or acute muscarinic blockade (20 –22). Furthermore, basal PP secretion is under control of oscillating cholinergic tone (22). In this study we also investigated whether central dexamethasone infusion could increase fat mass and leptinemia in ADX rats when the hyperphagic effect of dexamethasone was prevented by pair-feeding with control rats.

Research Methods and Procedures

Animals
Procedures were approved by the Animal Experimentation Ethics Committee of the Garvan Institute and St. Vincent's Hospital and follow the National Health and Medical Research Council of Australia's guidelines on animal experimentation. Male Wistar rats (Animal Resources Centre, Perth, Australia) were housed under conditions of con- trolled temperature (23 °C) and illumination (6:00 AM to 6:00 PM) on pelleted paper bedding. They were allowed ad libitum access to standard laboratory chow (Norco Stock- feeds, South Lismore, Australia) and water, unless otherwise stated.

Surgery
To avoid possible differential effects of central vs. peripheral dexamethasone administration on endogenous corticosterone production, ADX rats were used in this study to standardize glucocorticoid dose. Rats were anesthetized with intraperitoneal ketamine (60 mg/kg; Park-Davis- Pfizer, Sydney, Australia) and xylazine (10 mg/kg; Bayer AG, Leverkusen, Switzerland) and were bilaterally ADX- or sham-operated at 10 to 11 weeks of age (body weight, 387 ± 5 g; *n* = 29). Drinking water of ADX rats was supplemented with 0.9% NaCl. At recovery (7 to 10 days), the right lateral cerebral ventricle (23) and jugular vein were cannulated. Animals were left to recover presurgery weights (7 to 10 days) in individual cages and were handled daily. The drinking response to ICV injection of angiotensin II (25 ng in 5 µl saline; Auspep,

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Melbourne, Australia) was tested. Only rats that drank ~8 mL or more in the 30 minutes after injection (~90% of the animals) were used for further studies. Blood samples were taken from ADX animals 10 days postsurgery, and only rats with negligible plasma corticosterone levels (<25 ng/mL) were used.

Chronic ICV Infusions

Osmotic minipumps (model 2001; Alza Corp., Palo Alto, CA) were SC implanted (23) under halothane anesthesia for ICV infusion of dexamethasone (7.5 μg/kg per day) or saline vehicle. One group of ADX rats (ICV vehicle-infused) received an additional minipump for SC dexamethasone infusion (7.5 μg/kg per day). The four groups of rats were abbreviated as normal (sham-operated) + ICV vehicle, ADX + ICV vehicle, ADX + ICV dexamethasone, and ADX + ICV vehicle + SC dexamethasone. All animals consumed 32 g/d of chow, an amount equivalent to their spontaneous food intake measured before infusion.

Determination of Basal and Glucose-stimulated Insulin and Glucose Levels

Experiments were commenced after 3 to 4 days of ICV infusion, between 10:30 AM and 12:00 PM, 2 to 3 hours after removal of food. A basal blood sample was taken from the jugular catheter, and an intravenous glucose bolus was injected (300 mg/kg), which was followed by removal of blood samples at 1 and 5 minutes postglucose. These time points were chosen because data from our laboratories showed that insulinemia reached a peak 1 minute after glucose injection and returned toward baseline values at 5 minutes (24). After 2 days of recovery, the above procedure was repeated, but atropine methyl nitrate (1.0 mg/kg; Sigma, St. Louis, MO) was intravenously injected as a bolus 20 minutes before glucose. An additional basal sample was taken immediately before atropine injection. All plasma samples were stored at -20 °C until assay.

Appetite Test

In this study, all animals were fed the same amount of food to avoid possible effects of variations in spontaneous food intake with the different treatments on measures of insulinemia and adiposity. However, the following appetite test was used as an indication of how much the animals would have eaten if they were allowed to eat ad libitum. After 4 to 5 days of infusion (on a day when intravenous glucose tests were not performed), rats were fasted from 8:00 AM to 12:00 PM and then presented with chow for measurement of 1-hour ad libitum food intake. The amount consumed was subtracted from their daily food ration.

White Adipose Tissue Weights

After 6 to 7 days of infusion, food was removed from cages, and 2 to 3 hours later (10:30 AM to 12:00 PM) a blood sample was taken from the jugular catheter for plasma leptin measurement. Rats were anesthetized with ketamine and xylazine and the right epididymal and retroperitoneal white adipose tissue depots were removed and weighed. Two different fat depots were investigated to determine whether there were any site-specific effects of glucocorticoid administration. Animals were killed by anesthetic overdose followed by cardiac puncture.

Plasma Hormone and Metabolite Measurements

Plasma glucose concentrations were determined with the Trace Scientific (Melbourne, Australia) glucose oxidase method, and plasma insulin and leptin levels were assayed with Linco Research Inc. (St. Louis, MO) radioimmunoassay kits. Plasma PP radioimmunoassay was performed using guinea pig antirat PP serum (Linco Research Inc.), rat ^{125}I-PP (NEN Life Science Products Inc, Boston, MA), and Linco's 2nd Antibody

Precipitating System (Linco Research Inc.). Plasma corticosterone levels were measured with a radioimmunoassay kit from ICN Biomedicals (Costa Mesa, CA).

Statistical Analysis

Results were assessed by ANOVA followed by Fisher's post hoc tests, using StatView version 4.5 (Abacus Concepts Inc., Berkeley, CA). For all statistical analyses, $p < 0.05$ was accepted as being statistically significant.

Results

There was no significant difference in body weight or body weight gain among the four groups of rats during the 6 to 7 days of infusion. Body weight after 6 days of ICV infusion was 406 ± 7 g (normal + ICV vehicle), 392 ± 15g(ADX + ICV vehicle), 434 ± 18g(ADX + ICV dexamethasone), and 411 ± 7 g (ADX + ICV vehicle + SC dexamethasone); n = 4 to 8 rats per group.

All rats were presented with and consumed 32 g/d of chow. When allowed to eat ad libitum for 1 hour (4 hours after removal of food from cages, Figure 1), ADX + ICV dexamethasone-infused rats ate 3- to 5-fold more than ICV vehicle- or SC dexamethasone-infused normal or ADX rats.
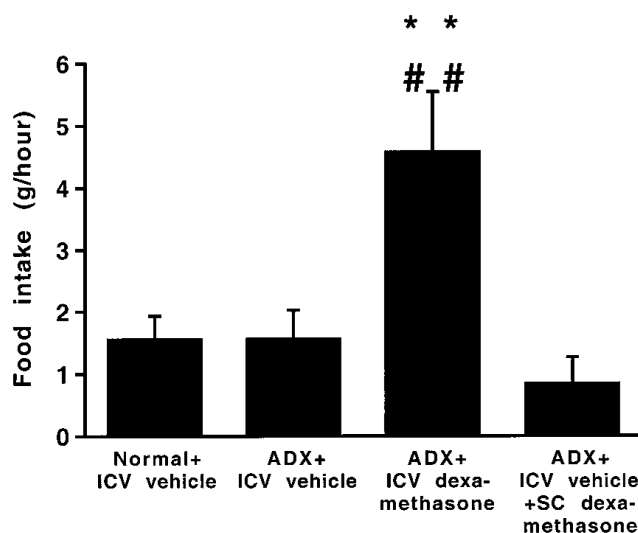

Figure 1. 1-hour ad libitum food intake measured after fasting from 8:00 AM to 12:00 PM in normal rats or in ADX rats. ADX rats received dexamethasone at 7.5 μg/kg per day for 4 to 5 days either through the ICV or SC route. Apart from during the feeding test, all animals were fed the same amount, which was equivalent to their spontaneous food intake before infusion. Plotted values are means $\pm$ SEM of 4 to 8 rats per group. **$p < 0.01$ vs. normal + ICV vehicle. ##$p < 0.01$ vs. ADX + ICV vehicle.

Compared with normal control rats, adrenalectomy reduced the weight of epididymal and retroperitoneal white adipose tissue depots and reduced plasma leptin levels (Figure 2). ICV dexamethasone infusion in ADX rats significantly increased white adipose tissue depot weights and plasma leptin levels (compared with ICV vehicle-infused ADX rats), to values not significantly different from normal controls (Figure 2). SC dexamethasone infusion had no such stimulatory effect on adiposity and plasma leptin concentrations of ADX rats (Figure 2).

Basal and stimulated (1 minute postglucose injection) plasma insulin levels of the four groups of rats are shown in Figure 3, and values of insulinemia at 5 minutes postglucose are shown in Table 1 in the presence or absence of muscarinic blockade by intravenous atropine injection. Under control conditions (Figure 3, black columns; Table 1), basal but
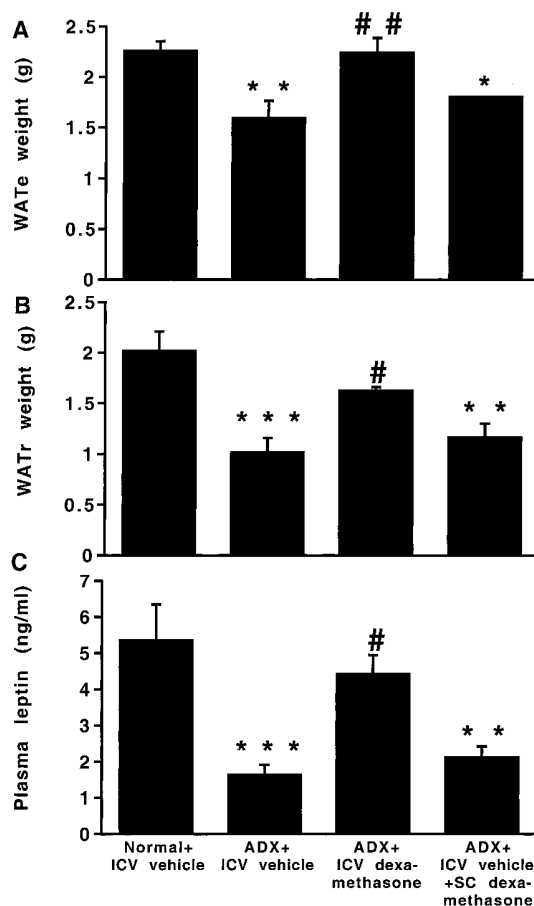
Figure 2. Weight of (A) epididymal and (B) retroperitoneal white adipose tissue depots (WATe and WATr) and (C) leptinemia of normal or ADX rats. ADX rats received dexamethasone at 7.5 μg/kg per day for 6 to 7 days either through the ICV or SC route. All animals were fed the same amount, which was equivalent to their spontaneous food intake before infusion. Plotted values are means $\pm$ SEM of 4 to 8 rats per group. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ vs. normal $+$ ICV vehicle; $\#p < 0.05$; $\#\#p < 0.01$ vs. ADX $+$ ICV vehicle.

not glucose-induced insulinemia was significantly reduced in ADX + ICV vehicle-infused rats compared with normal controls. 3- to 6-day ICV dexamethasone infusion in ADX rats significantly increased basal and glucose-stimulated insulinemia compared with normal and ADX vehicle- infused controls. This basal hyperinsulinemia was significantly reduced by atropine pretreatment to values no different from normal and ADX control rats (Figure 3A), and glucose-stimulated hyperinsulinemia was reduced to values similar to normal controls, although levels still remained significantly greater than ADX control values (Figure 3B; Table 1). The basal and 1-minute glucose-stimulated insulinemia of ADX + SC dexamethasone-infused rats were significantly greater than corresponding values of ADX + ICV vehicle-infused controls, but were no different from values of normal control rats (Figure 3; Table 1).

 Plasma PP levels of the four animal groups are shown in Figure 4; basally (Figure 4A), and 1 minute after intravenous glucose injection (Figure 4B). A glucose bolus did not significantly alter plasma PP from respective basal levels in any group. ICV (but not SC) dexamethasone infusion significantly increased plasma PP levels of ADX rats when measured basally or 1 minute after glucose injection, in comparison with normal or ADX ICV vehicle-infused controls (Figure 4). These effects of ICV dexamethasone were
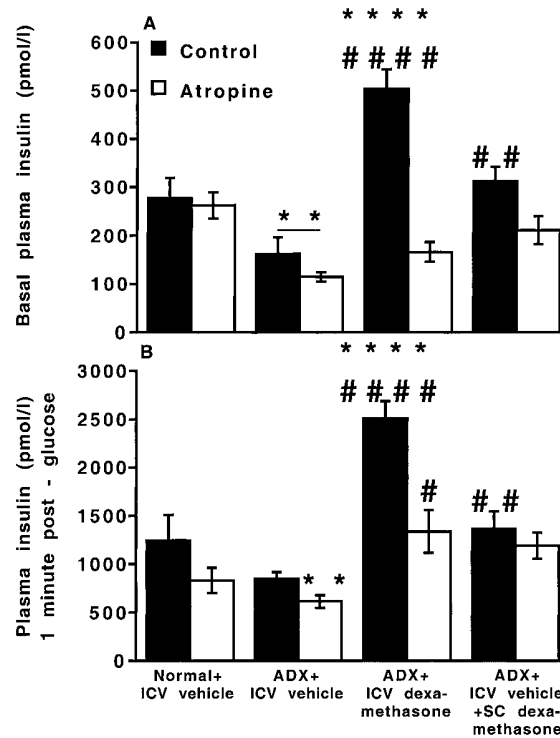
Figure 3. (A) Basal and (B) intravenous glucose-stimulated (300 mg/kg) insulinemia, measured under control conditions or 20 minutes after intravenous injection of atropine methyl nitrate (1.0 mg/kg) in normal and ADX rats. ADX rats received dexamethasone at 7.5 μg/kg per day for 3 to 6 days either through the ICV or SC route. All animals were fed the same amount, which was equivalent to their spontaneous food intake before infusion. Plotted values are means $\pm$ SEM of 4 to 8 rats per group. $**p < 0.01$; $****p < 0.0001$ vs. normal + ICV vehicle-infused controls; $\#p < 0.05$; $\#\#p < 0.01$; $\#\#\#\#p < 0.0001$ vs. ADX + ICV vehicle-infused controls.

Table 1. Plasma glucose levels measured basally, as well as 1 and 5 minutes after intravenous glucose injection (300 mg/kg), and insulinemia at 5 minutes postglucose†‡

Treatment	Atropine	Basal (mM)	1 min postglucose (mM)	5 min postglucose (mM)	Insulinemia at 5 min postglucose (PM)
Norm + ICV vehicle	−	6.7 ± 0.1	17.7 ± 0.1	14.4 ± 0.6	680 ± 90
	+	7.9 ± 0.2	17.3 ± 0.4	13.5 ± 0.7	700 ± 140
ADX + ICV vehicle	−	7.2 ± 0.3	20.4 ± 1.0	14.0 ± 1.0	440 ± 40
	+	6.9 ± 0.3	18.6 ± 1.6	12.2 ± 0.9	340 ± 30*
ADX + ICV DEX	−	7.1 ± 0.6	19.2 ± 2.2	12.1 ± 1.3	1320 ± 200†‡
	+	6.6 ± 0.4	20.5 ± 2.5	12.8 ± 0.6	910 ± 20§
ADX + SC DEX	−	6.9 ± 0.4	20.3 ± 1.7	11.1 ± 1.2	500 ± 40
	+	6.7 ± 0.6	19.9 ± 0.3	12.7 ± 1.1	630 ± 30

Values are means $\pm$ SEM of 4 to 8 rats per group. No significant differences between treatment groups in each category of glycemia. ADX, adrenalectomized rats; ICV DEX and SC DEX, intracerebroventricular or subcutaneous dexamethasone infusion (7.5 μg/kg per day for 3 to 6 days). Atropine (1.0 mg/kg) or saline was injected intravenously 20 minutes before basal blood sampling or glucose injection.
* $p < 0.05$; † $p < 0.001$ vs. normal control (no atropine).
‡ $p < 0.001$; § $p < 0.0001$ vs. ADX control (no atropine).

reversed by prior muscarinic blockade with atropine to values no different from normal and ADX control rats. Apart from ADX + ICV dexamethasone-infused rats, atropine did not significantly affect basal or glucose-induced plasma levels of insulin or PP in any other experimental group.

There was a significant linear correlation between plasma insulin and PP concentrations, both basally ($r^2 = 0.26$, $p < 0.001$, $n = 42$) and 1 minute after glucose injection ($r^2 = 0.42$, $p < 0.0001$, $n = 34$).

No significant differences in glycemia were observed among the four experimental groups of rats at any stage of the experiment, either under basal conditions, 1 or 5 minutes postglucose injection, or in the presence or absence of atropine (Table 1).

Discussion

This study showed that 3- to 6-day ICV but not SC dexamethasone infusion in ADX male rats increased basal and intravenous glucose-induced insulinemia to levels greater than normal rats, in parallel with increases in plasma PP levels, which is a marker of parasympathetic output to the pancreas (20–22). Furthermore, these central glucocorticoid-induced changes were completely normalized by atropine. Therefore, our data strongly support a central action of glucocorticoids to increase parasympathetic, muscarinic output to the pancreas, and that this is the major cause of the observed increases in insulinemia.

As part of this study, we compared the effects of central and peripheral glucocorticoid replacement on adiposity of ADX rats. ICV but not SC dexamethasone infusion increased white adipose tissue depot weights and plasma leptin levels, even though food intake was controlled between the different groups of animals by pair-feeding. It is likely that the hyperinsulinemia we observed in ICV dexamethasone-infused ADX rats contributed to the associated increase in adiposity and plasma leptin levels, because insulin administration is known to cause increased fat accumulation in animals (25,26) and in patients with type 2 diabetes (27).

Our finding of central glucocorticoid-induced increases in parasympathetic output to the pancreas may have been mediated through the effects of hypothalamic NPY and corticotropin releasing hormone (CRH) activities, which are thought to influence autonomic outflow. Glucocorticoids increase NPY mRNA and peptide levels and NPY receptor expression in the hypothalamus in vivo (14,28–36) and in vitro (33,37), whereas adrenalectomy reduces hypothalamic NPY and NPY receptor expression (28,29,31,32,36,38). In contrast to their effects on NPYergic pathways, glucocorticoids decrease hypothalamic CRH expression (39,40). NPY and CRH seem to have reciprocal effects on autonomic outflow. Evidence suggests that central NPY administration to rats increases parasympathetic output (24,41,42) and de- creases sympathetic nervous activity (43– 45), whereas ICV CRH injection inhibited parasympathetically mediated functions and induced sympathetically mediated events (46). Therefore, glucocorticoid-induced changes in hypothalamic NPYergic and CRHergic activity would increase the ratio of parasympathetic to sympathetic outflow, thereby explaining the muscarinically antagonized increases in insulinemia and plasma PP levels in this study.

In contrast to our present data using ADX male Wistar rats, a previous study with ADX female Sprague–Dawley rats showed no effect of ICV dexamethasone infusion (at the same dose presently used) on food intake, body weight gain, or basal plasma insulin, leptin, or triglyceride levels (47). These discrepancies suggest a sex or species difference in sensitivity or responsiveness to the obesity-like effects of central glucocorticoids in rats. Sexual dimorphism in hypothalamo-pituitary-adrenal function has been observed in several rat strains, with females showing higher plasma adrenocorticotropic hormone

and corticosterone concentrations than males (48). Male rats were also more responsive than females to the catabolic effects of chronic ICV CRH administration (46).

In conclusion, glucocorticoids increase basal and glucose-induced insulinemia in male rats by specific action within the central nervous system to activate parasympathetic, muscarinic activity in the endocrine pancreas. This hyperinsulinemia occurs even when central glucocorticoid- induced hyperphagia is prevented by pair-feeding and may contribute to the lipogenic effects of central glucocorticoids.

Acknowledgments

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