

Effects of some endocrine disruptors on the secretory and proliferative activity of the regenerating rat adrenal cortex

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Abstract. The effects of some endocrine disruptors that possess estrogen-like activity on the secretion and growth of regenerating rat adrenal cortex have been investigated in ovariectomized (OVX) and sham-OVX rats. As reference groups, dexamethasone (Dx)-administered sham-OVX and 17 β -estradiol-administered OVX animals were used. Dx, estradiol and endocrine disruptors were subcutaneously injected daily at a dose of 3 nmoles/100 g for 10 consecutive days after surgery, and adrenal enucleation was performed on day 5 of the experiment. Dx and genistein significantly decreased corticosterone plasma concentration (as measured by RIA) in sham-OVX rats with regenerating adrenals, while other disruptors (eusolex, procymidone, linurone, resveratrol, bisphenol-A and and silymarin) were ineffective. Mitotic index (as assayed by the stachmokinetic method with vincristine) was not changed by either Dx or disruptors. Estradiol significantly increased and genistein significantly lowered corticosterone blood level in OVX rats; similar effects were induced in the mitotic index of regenerating adrenals, but the changes were not significant. Eusolex increased the mitotic index, without altering the level of circulating corticosterone. Collectively, our findings allow us to conclude that, of the endocrine disruptors tested, only genistein is able to suppress the secretory activity of regenerating adrenal cortex, this Dx-like effect being apparently unrelated to its estrogen-like activity, and only eusolex enhances the proliferation rate of regenerating adrenal, the effect being conceivably connected with its estrogen-like activity.

Introduction

The best recognized endocrine disrupting properties of

chemicals are those connected with their estrogenic activity. Unexpectedly, the current testing strategies to assess the effects of the endocrine disruptors have omitted adrenal glands, as far as either steroidogenesis or growth are concerned (1).

One of the classic *in vivo* models to study adrenocortical cell proliferation and secretory function is enucleation-induced regeneration (for review, see refs. 2-6), which not only primarily depends on pituitary ACTH release (7-11), but is also influenced by several neural and endocrine signals (12-17). Hence, we investigated the effects of several endocrine disruptors on glucocorticoid (corticosterone) secretion and the proliferative activity of the regenerating rat adrenal cortex.

Materials and methods

Animals and reagents. Adult female Wistar rats (100-120 g body weight), bred in our laboratory facilities, were kept under a 14/10-h light/dark cycle at 23°C, and maintained on a standard diet and tap water *ad libitum*. The study protocol was approved by the local ethics committee for animal studies. Dexamethasone (Dx), 17 β -estradiol, diphenylpropane (bisphenol-A; BSP) and benzophenone-3 (eusolex-4360) were purchased from Merck & Co. (Whitehouse Station, NJ). Resveratrol was obtained from Nabio Biotech. Co. (Shanghai, China), and vincristine was from Gedeon-Richter (Budapest, Hungary). Genistein, procymidone, linurone, silymarin and all other reagents were provided by Sigma-Aldrich Corp. (St. Louis, MO).

Surgical procedures. Bilateral ovariectomy (OVX), and left adrenal enucleation and contralateral gland removal (adrenal cortex regeneration) were carried out via dorsal approach and under ether anesthesia, as previously described (14). Operated animals were given 0.9% NaCl to drink, and were sacrificed 5 days after adrenal enucleation.

Experimental protocol. Groups of sham-OVX rats (n=8) were given daily subcutaneous (sc) injections of 3 nmoles/100 g of Dx, genistein, eusolex, procymidon, linuron, resveratrol, BSP or silymarin dissolved in 0.2 ml 0.9% NaCl for 10 consecutive days. Groups of OVX rats (n=8) were treated with estradiol, genistein or eusolex, as detailed above. Control rats received sc injections of vehicle. The treatment started immediately after surgery and, on day 5 of the experiment, all rats underwent

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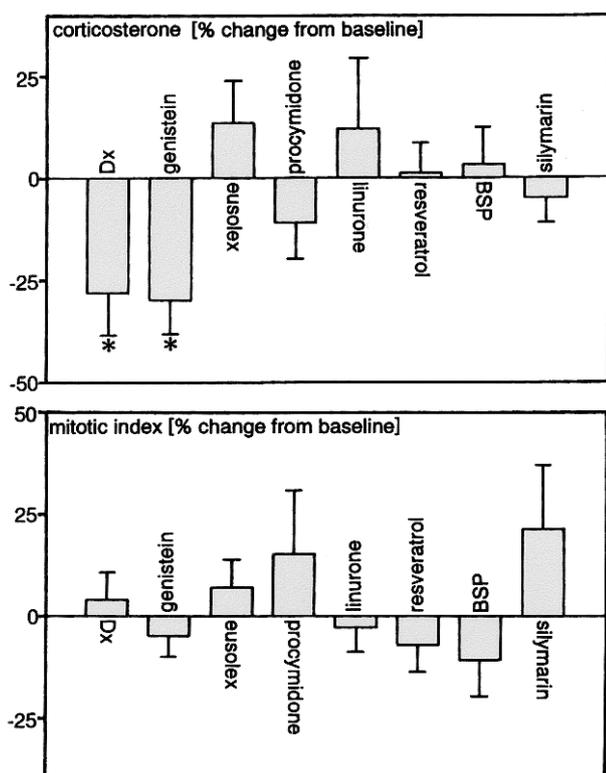


Figure 1. Effects of Dx and endocrine disruptors on the plasma corticosterone concentration (upper panel) and mitotic index of regenerating adrenal (lower panel) of sham-OVX rats. Results, expressed as percent change from controls are the mean \pm SEM of 8 separate experiments. * p <0.05 from the respective control value.

adrenal enucleation. All animals were given an intraperitoneal injection of 0.1 mg/100 g of vincristine 3 h before being sacrificed. Rats were decapitated at 11:00 am; their trunk blood was collected in the presence of EDTA (1 mg/ml), and plasma was separated and stored at -36°C . Regenerating adrenals were promptly removed.

Corticosterone assay. Corticosterone was extracted from plasma and its concentration measured by RIA, as previously detailed (18). RIA sensitivity was 50 pg/ml. Intra- and inter-assay CVs were 7% and 9%, respectively.

Cell proliferation. Regenerating adrenals were fixed in Bouin's solution for 24 h, embedded in paraffin and sectioned at 5–6 μm . Sections were stained with hematoxylin and eosin. The mitotic index (% of metaphase-arrested cells) was calculated at 400x, by counting 5,000 cells in the regenerating adrenal parenchyma (14).

Statistics. Data, expressed as percent change from control value, were the mean \pm SEM of 8 separate experiments. Statistical comparison was performed by ANOVA followed by Student's *t*-test.

Results

Dx and genistein significantly decreased corticosterone plasma concentration in sham-OVX rats with regenerating adrenals,

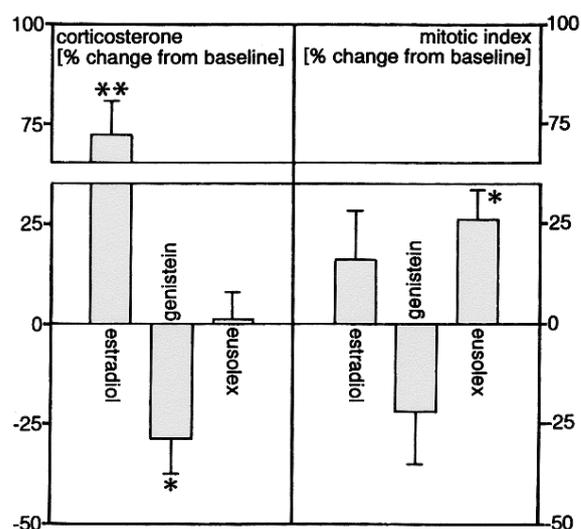


Figure 2. Effects of estradiol, genistein and eusolex on the plasma corticosterone concentration (left panel) and mitotic index of regenerating adrenal (right panel) of OVX rats. Results, expressed as percent change from controls, are the mean \pm SEM of 8 separate experiments. * p <0.05 and ** p <0.01 from the respective control value.

while other chemicals were ineffective (Fig. 1, upper panel). In these animals, mitotic index was not significantly changed by any disruptor (Fig. 2, lower panel).

Estradiol markedly increased and genistein lowered corticosterone plasma concentration in OVX rats with regenerating adrenals. Similar effects were induced in the mitotic index, but the changes were not significant. Conversely, eusolex did not change corticosterone blood level, but evoked a significant rise in the mitotic index (Fig. 2).

Discussion

The effects of various endocrine disruptors on the glucocorticoid secretion and growth of regenerating adrenal cortex were investigated. As reference groups, DX-administered sham-OVX and estradiol-administered OVX animals were used. As expected, Dx lowered corticosterone secretion but did not affect proliferative activity. This last finding is at variance with those reported previously (2,4,7,8) but is in keeping with the earlier observation of the lack of effect of both hydrocortisone and Dx on the growth of regenerating rat adrenal cortex (19). In the other reference group, estradiol was found to enhance corticosterone secretion without affecting the regenerating-adrenal growth rate. This finding is in partial disagreement with the reported stimulating affect of estradiol on both secretion and growth of rat adrenals (reviewed in ref. 20). However, it must be emphasized that the effects of estradiol on the adrenal cortex are known to depend on either the experimental model used (e.g. intact versus gonadectomized animal) or the dose (physiological versus supraphysiological doses) and the chemical nature (natural versus synthetic estradiol) (referenced in ref. 20).

Our present findings show that, of the endocrine disruptors tested, only genistein and, to a less extent, eusolex affect the function of regenerating rat adrenals, despite the fact that all these chemicals are reported to exert sex hormone-like effects.

 SPANDIDOS PUBLICATIONS and functional characteristics of genistein and will be briefly discussed in relation to their adrenocortical effects.

The isoflavone, genistein, possesses structural characteristics similar to that of 17 β -estradiol, which enables it to exert estrogenic and antiestrogenic effects (21,23). Genistein was found to affect cell proliferation and differentiation, apoptosis, angiogenesis, and cell adhesion and migration. Evidence has been provided that isoflavones can also exert biological effects independent of their estrogenic properties (24), which seems to be the case for its action on the regenerating rat adrenal cortex. In fact, in contrast with estradiol, genistein was found to lower corticosterone secretion from regenerating adrenals. This effect resembles that of Dx, thereby suggesting that genistein exerts a glucocorticoid-like action. Genistein is known to be a potent inhibitor of tyrosine kinase (TK) (25,26), an enzyme that seems to play a role in steroid synthesis (27-29). However, TK also activates MAPK, whose involvement in the stimulation of adrenocortical growth is well documented (30-34). Genistein was not found to affect the growth of regenerating adrenals; therefore, it is unlikely that the inhibitory action of this isoflavone is connected with its anti-TK activity.

The benzophenone, eusolex, is a UV absorber that displays a certain structural relationship with steroid hormones (35). It was shown to exert variable estrogen-like uterotrophic effects (36) and to enhance the proliferative activity of the MCF-7 breast cancer cell line (37). The presently observed stimulation of the regenerating adrenal growth in OVX rats may be related to the estradiol-like action of eusolex. However, this contention does not agree with the lack of effect of this benzophenone on corticosterone secretion.

For the reader's convenience, we wish to spend few words on the other endocrine disruptors studied. Procymidone and linurone are commonly used pesticides that are reported to interact with steroid-hormone receptors to variously affect sex-hormone synthesis and to exert different effects on sex hormone-target organs and tissues (38-43). BSP and some alkylphenols (p-nonylphenol and p-tert-octylphenol) have been found to possess estrogenic activity, as they display uterotrophic effects, disrupt the estrus cycle in rodents (44) and cause testis atrophy in rats (45). The phytoalexin, resveratrol, structurally resembles 17 β -estradiol, and possesses estrogen-receptor modulating activity, thereby affecting numerous biological functions (22,45). Finally, silymarin, a plant flavonoid, has been shown to have preventive effects against carcinogenesis and cell damage in various animal models, as well as anti-oxidant, anti-inflammatory and immunomodulatory activity (47). None of these chemicals was found to affect the secretion and growth of regenerating rat adrenals. However, this does not rule out the possibility that, due to their estrogen-like properties, these chemicals may affect the function of normal adrenocortical cells, as preliminary *in vitro* results seem to suggest.

To conclude, our study shows that, among the various endocrine disruptors tested, only genistein exerts inhibitory effects on glucocorticoid secretion from regenerating rat adrenals *in vivo*. This observation stresses that great caution must be used in interpreting the results of experiments on adrenal cortex where genistein is used as a TK inhibitor.

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