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Commentary

Endogenous hormones matters in evaluation of endocrine disruptive effects mediated by nuclear receptors

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In recent decades, endocrine-disrupting chemicals (EDCs) have emerged as a prominent focus on environmental science and policy. Notably, extensive use of *in vitro* bioassays founded on ligand-receptor binding has facilitated the identification of a substantial number of potential EDCs, particularly agonists/antagonists of nuclear receptors such as estrogen receptors and thyroid hormone receptors [1]. These datasets have significantly augmented the discovery of potential EDCs. However, when subjecting these compounds to *in vivo* exposures, spanning from teleost fish to rodents and humans, observations frequently deviated from *in vitro* anticipation [2,3]. This discrepancy and the apparent lack of prognostic value are shared with the difficulties of *in vitro-in vivo* extrapolations in classical toxicological endpoints. While the utility of *in vitro* identification is without doubt, its potential EDCs require *in vivo* confirmation. The resulting complexity in EDCs outcomes, nevertheless, has not yet received adequate attention and thorough interpretation.

One crucial factor often neglected is the substantial presence of endogenous hormones during *in vivo* exposures, which interfere with the action of exogenous EDCs and result in erroneous interpretation of the outcomes on endocrine disruption. We compiled and quantified the blood concentrations of eleven endogenous hormones (act as ligands) for nineteen nuclear receptors in mice and/or zebrafish, representing those of primary interest (Fig. 1A). The concentrations of these hormones in mice's blood plasma show a large range, spanning from 10.9 ng/L to 44.7 μg/L. Notably, the highest concentration (44.7 μg/L) occurred for the hormone cortisol that binds to the glucocorticoid receptor (GR). This was followed by progesterone (24.9 μg/L) that binds to the progesterone receptor (PR), testosterone (5.1 μg/L) binding to the androgen receptor (AR), and 15-deoxy-Δ-12,14-prostaglandin J2 (2.4 μg/L) binding to the peroxisome proliferator-activated receptors (PPARs). The lowest blood hormone concentration of 10.9 ng/L was 17β-estradiol (E_2), which binds to estrogen receptors (ERs).

Moreover, noteworthy gender differences exist, particularly in the concentrations of steroid hormones. For instance, the blood concentrations of E_2, a classic endogenous ligand of ERα and ERβ were significantly different between sexes. In male mice, the E_2 concentration is approximately 10.9 ng/L, whereas in female mice, it reaches as high as 36 ng/L, nearly 3.3 times the concentration observed in males. Similarly, in female mice, the concentration of testosterone, an endogenous ligand of the AR, is approximately 171 ng/L, while in male mice, it reaches 5,128 ng/L. The significant presence and gender differences of endogenous hormones also occur in fish (Fig. 1A). Variations in endogenous hormone concentrations differentially affect the outcomes of EDC exposure between females and males. This is considered when performing exposure to only female or male experimental animals. However, this carries broader implications.

In vitro bioassays help to discover a significant number of potential EDCs that function as agonists of nuclear receptors. These compounds typically manifest moderate or weak receptor binding activities, which is in contrast to the highly active endogenous hormones. In animal cells, the substantial presence of endogenous ligands would remarkably weaken the agonistic effects of compounds via competitive binding (Fig. 1B). Hence, considerably high concentrations of the compounds are required to displace endogenous ligands and elicit an activity. This clarifies why, besides differences in metabolic activity *in vitro* and *in vivo*, some compounds with relatively low potencies towards nuclear receptors have not exhibited agonistic effects *in vivo* in animals and humans, for instance, the effect of UV filters [4]. The concentrations of EDCs within the human body as well as in wild animals are generally low. Their activities often operate at levels several orders of magnitude lower than those of circulating endogenous hormones. As a result, the likelihood of EDCs interacting with the corresponding receptors with causal physiological consequences is generally low, with the exception of highly active hormones also present in the environment. This finds further support in a series of epidemiological investigations, where factors such as paternal age and maternal age at first pregnancy emerged as significant contributors to the reported growth of endocrine-related diseases rather than the direct influence of EDCs [5,6].

In addition, the abundant expression of endogenous hormones influences the antagonistic action on the same receptor. Antagonists block

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	Nuclear Receptor	Abb.	Endogenous Hormone	Conc. in Female (ng/L)	Conc. in Male (ng/L)
Mice	Thyroid hormone receptor-α	TRα	Triiodothyronine (T3)	1,205	1,009
	Thyroid hormone receptor-β	TRβ	Thyroxine (T4)	45	51
	Retinoic acid receptor-α	RARα	All-Trans-retinoic acid (ATRA)	941	1440
	Retinoic acid receptor-β	RARβ			
	Retinoic acid receptor-γ	RARγ			
	Peroxisome proliferator-activated receptor-α	PPARα	7(S)-Hydroxydocosahexaenoic acid	—[a]	—
	Peroxisome proliferator-activated receptor-γ	PPARγ	15d-PGJ2	—	2,358
	Vitamin D receptor	VDR	Calcitriol (1,25-D3)	77	73
	Estrogen receptor-α	ERα	17β-Estradiol (17β-E_2)	36	11
	Estrogen receptor-β	ERβ			
	Androgen receptor	AR	Testosterone (TTR)	171	5,128
	Progesterone receptor	PR	Progesterone (P4)	24,907	426
	Glucocorticoid receptor	GR	Cortisol (CRL)	23,125	44,693
Zebrafish	Estrogen receptor-α	ERα	17β-Estradiol (17β-E_2)	863	355
	Estrogen receptor-β1	ERβ1			
	Estrogen receptor-β2	ERβ2			
	Androgen receptor	AR	11-ketotestosterone (11-KT)	928	6,593
	Progesterone receptor	PR	Progesterone (P4)	4,700	—
	Glucocorticoid receptor	GR	Cortisol (CRL)	37,869	62,350

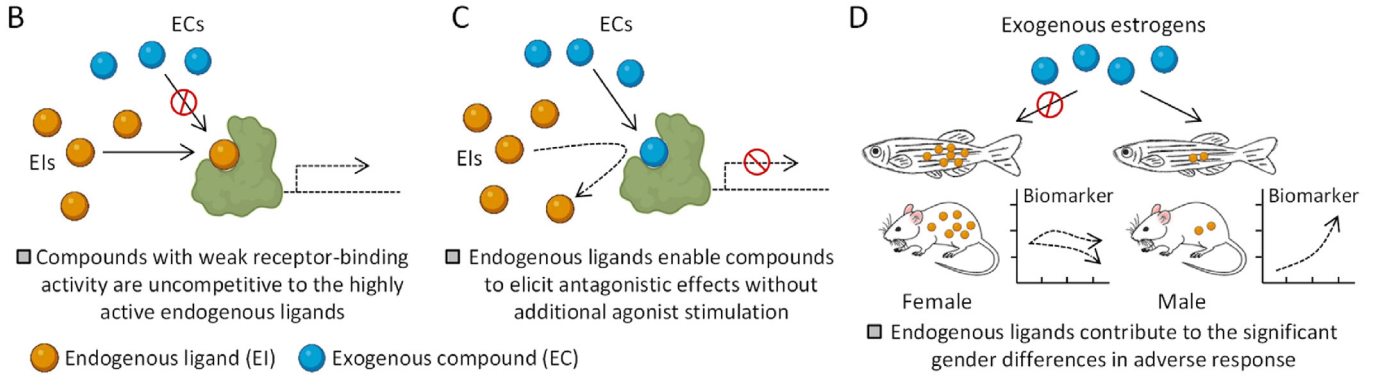

Fig. 1. The presence of endogenous ligands influences the action of agonists and/or antagonists in mice and zebrafish. (A) The incorporated 19 types of frequently studied nuclear receptors and the mean blood concentrations of their respective endogenous ligands in mice and/or zebrafish (ng/L). Data for the concentrations of each endogenous ligands were retrieved and compiled from more than thirty reports, except for the ligands of PPARs in mice and the ligands of PR in zebrafish, where limited reference data were available. [a]dash line indicates that no available data can be obtained. (B) Endogenous ligands attenuate the agonistic activity of compounds. (C) Endogenous ligands modulate receptor-dependent antagonist effects compounds. (D) Gender differences of endogenous ligands concentrations contribute to the gender difference in the responses of the compounds, taking exogenous estrogens as an example. 15d-PGJ2, 15-Deoxy-Δ-12,14-Prostaglandin J2.

the binding of an agonist at a receptor molecule, thereby inhibiting the signal produced by the receptor–agonist interaction. High concentrations of a competitive agonist (endogenous ligand) increase the proportion of receptors it occupies. As a result, introducing an additional agonist as a positive control to test the antagonistic effects, a practice commonly employed in *in vitro* tests, may be deemed redundant for *in vivo* exposures. The presence of endogenous ligands in physiological settings can adequately elicit and modulate receptor responses, rendering supplementary agonists unnecessary to achieve the desired experimental outcomes. On the contrary, when the endogenous ligand concentrations are notably low, the addition of an extra agonist stimulation becomes essential to accurately reflect antagonistic effects. This is particularly important for embryonic exposures, as embryos lack the capability to produce sufficient endogenous ligands, especially hormones [7]. Therefore, the interactions between endogenous ligands and exogenous antagonists within the physiological context should be considered during *in vivo* experimental designing and interpreting (Fig. 1C).

Furthermore, differences in endogenous ligand concentrations significantly contribute to the gender differences in responses to environmental compounds. A notable illustration pertains to the actions of agonists and antagonists to steroid hormone receptors, such as ERα, ERβ, AR, and PR. Xenoestrogens, mimicking endogenous E_2 action, typically exhibit more pronounced effects in males, with a comparatively lesser impact in females. A case in this point is vitellogenin (VTG), a sensitive and reliable biomarker indicative of estrogenic activity in animals [8]. Investigations consistently reveal a substantial increase in VTG expression in males for effective estrogens, spanning diverse species such as mollusks, amphibians, and teleost fish, reaching magnitudes of hundreds to thousands of times

the baseline. Conversely, in females, the observed changes may be lower or minimal [9]. We conducted a systematic analysis of 26 reports that documented variations in VTG levels in adult female fish following exposure to the well-known xenoestrogen, bisphenol A (BPA). The findings revealed that 12 studies (46.2%) reported no statistically significant alterations in VTG expression, while 7 studies (26.9%) even demonstrated a notable inhibitory effect on female VTG expression resulting from BPA exposure (Fig. 1D). These effects, thus, may be attributed to the competitive binding to ERα/ERβ, as described above.

Therefore, experimental animals for *in vivo* exposure are not consistently flawless models devoid of background noise. To mitigate the potential false interpretation arising from endogenous hormones/ligands, opting for appropriate species, gender, or reproduction cycles exhibiting low endogenous hormone levels is an optimal strategy. For instance, it is advisable to select males for research on estrogenic effects and females for research on androgenic and progesterone effects. Moreover, embryonic organisms are commonly recommended due to their typically low hormone levels. An alternative approach involves the application of advanced genome editing tools. They facilitate the selective inhibition of endogenous hormone synthesis, consequently leading to a significant reduction in their overall contents. For instance, the aromatase knockout (ArKO) mouse model through targeted mutation, characterized by estradiol insufficiency [10], may serve as a useful model for ERα and ERβ agonists or antagonists screening and risk assessment. These approaches are anticipated to reduce the occurrence of false negative or positive results in experimental outcomes.

In conclusion, the presence of endogenous hormones is frequently underestimated in *in vivo* exposures, while in fact, it constitutes a pivotal factor capable of complicating the interpretation of endocrine disruptive effects. This is particularly relevant for the EDCs with low potency. Hence, adopting appropriate strategies to mitigate the potential influence of endogenous hormones, including the reproductive cycle of test animals, would enhance data reliability and reproducibility. Meanwhile, gender differences and developmental stage variations also influence the expression of endogenous receptors. Endocrine disruption may be modulated by both endogenous hormone levels and relevant receptor levels, which are also worthy of our attention. Furthermore, the limitations of *in vitro* testing in EDC research should also be considered, as they neglect the potential interference of EDCs with endogenous hormones. Consequently, the popular evaluation of EDCs potential of environmental samples by use of *in vitro* bioassays has its limitation for predictive value, adding to the difficulties arising from species differences and differences in metabolic activity between *in vitro* and *in vivo*. The consideration of these facts, in turn, would improve the (eco)toxicological assessment of environmental contaminants.

CRediT authorship contribution statement

Y.B.Z.: conceptualization, investigation, writing–original draft and funding acquisition; K.F.: writing–review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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