Choreographing the Rapid Vascular Effects of Estrogen
Sorting Out the Partners and the Steps
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The rapid effects of steroids (generally) and estrogen (specifically) have emerged from the fringe of vascular biology to the main stage. Based on a series of observations going back more than half a century, scientists have speculated (mostly to small audiences) that a range of steroids, including estrogen, aldosterone, and glucocorticoids, might have rapid effects on smooth muscle. The report by Traupe and colleagues in this issue of *Hypertension* is an important next step in this process of discovery: the transition from identification of the pathway to the delineation of the components of the signaling system/receptor(s) involved.

A model in which classical estrogen receptors (ERs) act as transcription factors adequately explains most of the effects of estrogen. These ERs (ERα, ERβ, and ERγ) are all members of the steroid hormone nuclear receptor subfamily. Estrogen binding mediates a conformational change in these receptors with their consequent dissociation from heat shock protein 90 (hsp90) and ER dimerization. ER receptor dimers then interact with other transcriptional cofactors. The interaction of these receptor complexes with estrogen response elements regulates the target genes mediating the reproductive, developmental, behavioral, skeletal, neural, and cardiovascular effects of steroids. This same signaling pathway has been described for a range of other steroid receptors, including those mediating the effects of mineralocorticoids, glucocorticoids, and androgens.

However, it has become increasingly evident that not all of the effects of estrogen (and other steroids) can be explained by this “genomic” model, especially those processes occurring over a time course too rapid to be explained by transcription factor–based mechanisms. A number of intracellular mechanisms have now been identified to be mediated by these so-called nongenomic effects of estrogen, including activation of mitogen-activated protein, Raf and Src kinases as well as protein kinase A, phosphoinositide 3-kinase (PI3) kinase, NO synthase (NOS), and many others important in cardiovascular regulation. In the vasculature, activation of some of these processes have been shown to parallel the rapid effects of estrogen on vascular tone.

**What Are the Rapid Vascular Effects of Estrogen? Among Steroids, Is This Effect Unique to Estrogen?**

Estrogens primarily mediate vasodilation. This effect appears to be predominantly NOS-dependent, although not necessarily endothelial-dependent. NOS-dependent vasodilation has been reported even in endothelial-denuded preparations. Additionally, other non-NOS dependent mechanisms of estrogen’s vasodilatory effects have also been reported, including the report by Traupe and colleagues. However, the importance of these non-NOS dependent pathways in the overall rapid vascular effects of estrogen and either the physiological importance (see below) or the generalizability of the non-NOS-dependent mechanism of vasodilation across vascular beds is yet to be established.

Another side to the rapid effects of estrogen on vascular tone has more recently been recognized. With NOS inhibition, rapid vasoconstrictor effects of estrogens have been reported in some models. This direct vasoconstrictor effect has also been reported at the single cell level, even in the absence of concurrent NOS inhibition.

Increasingly, it is being appreciated that these Yin and Yang effects of estradiol, reflecting both vasodilator and vasoconstrictor components (Figure), is a recurring theme for a number of vasoactive steroids. The bidirectional rapid vascular effects of steroids have been best characterized for aldosterone. Variably, aldosterone has been reported to be both a vasodilator and a vasoconstrictor. However, regardless of the net effect reported, in those aldosterone studies that have included some means of decreasing endothelial/NOS effects, both vasodilator and vasoconstrictor mechanisms could be teased out. Thus, reported differences in the “net” rapid vascular effects of steroids (ie, whether a specific steroid overall mediates vasodilation or vasoconstriction) may be a function of the strength of these opposing vasodilator and vasoconstrictor components. These net effects appear to vary between steroids as well as between species and between vascular beds. This duality of function of vasoactive steroids follows the pattern of both G-protein-coupled receptor (GPCR) agonists (like catecholamines, endothelins, and acetylcholine) as well as receptor tyrosine kinase agonists (like insulin-like growth factor and insulin) that have both vasoconstrictor and vasodilator actions. We now appreciate that the major determinants of whether these GPCR or receptor tyrosine kinase ligands mediate vasodilation or vasoconstriction relates to variability in receptor
subtypes, in sites of expression (endothelial versus vascular smooth muscle), and in linkage to downstream signaling cascades. We predict that the same situation will apply for the vasoactive steroids.

What Is the Signaling Cascade Linking Estrogen With Its Rapid Vascular Effects

The pathway linking estrogen with its rapid vascular effects remains a black box (or at least, very gray). PI3 kinase activation appears to be central to many of these rapid effects of estrogen as well as of other steroids, including aldosterone and glucocorticoids. Further, PI3 kinase activation may be the common pathway for mediating the dual vasodilator/vasoconstrictor effects of estrogen and other steroids. Beyond its effects on NOS, in vascular smooth muscle PI3 kinase activation enhances contractile responses (via enhancing myosin light chain phosphorylation).

How these vasoactive steroids mediate PI3 kinase activation is less well defined. Interestingly, estradiol has been shown to activate mitogen-activated protein kinase signaling via a Src/epidermal growth factor receptor pathway.4 PI3 kinase activation is a common component of these receptor tyrosine kinase–signaling pathways. Further, a role for both epidermal growth factor receptor and insulin-like growth factor-1 receptor transactivation4 has been recently described as important in mediating the rapid effects of aldosterone on sodium transport and extracellular signal regulated kinase (ERK) activation. Thus, it is feasible to speculate (although yet to be proven) that the effects of estrogen on vascular tone occur via so-called “transactivation” of tyrosine kinase receptors leading to PI3 kinase activation and its consequent effects on NOS and regulation of myosin light chain phosphorylation.

The identity of the receptor(s) mediating the effects of estrogen as well as of the other vasoactive steroids is probably the least understood part of the puzzle. Both classical steroid receptors as well as novel GPCRs have been implicated in mediating the rapid vascular effects of steroids. The present study suggests roles for both Erα and Erβ. However, other candidate receptors have been suggested. GPR30, an “orphan GPCR,” has been shown to contribute to the rapid effects of estrogen in several model systems.6 Further, common to the effects of estrogen in vascular cells, the GPR30 signaling pathway mediates PI3 kinase activation and extracellular signal regulated kinase activation as well as EGFR transactivation.7 These effects have been seen in a range of tissues, but not universally. Notably, in a mouse ER knockout mouse model, no role for GPR30 was evident in mediating the effects of estrogen on endothelial cell extracellular signal regulated kinase and PI3 kinase activity.8 It is anticipated that the recent identification of the GPR30 selective agonist G-1 will help resolve the issue.9

What Can We Conclude From the “Heroic” Concentrations of Estrogens Required To Elicit Vascular Responses

In teasing out the differential effects of Erα- and Erβ-selective agonists, Traupe and colleagues1 have used what would conventionally be viewed as suprapharmacological concentrations of agonists (in the μmol/L range), multiple log units beyond what is achievable in vivo. Superficially it might be suggested that these ER-mediated vascular effects are unlikely to be of biological significance, given the concentrations of ER agonists required to elicit them. However, issues related to the limited aqueous solubility of the ER ligands leading to limited accessibility to their site(s) of action as well as the lack of adequate concentrations of albumin or sex hormone binding globulin could all contribute to the apparent lack of potency of these ER agonists in experimental models. Also, it might be speculated that the high concentrations required to demonstrate estrogen’s effect in the present report are a function of the experimental conditions. It is notable that the effects of estrogens have been shown to be most potent in paradigms where they are used to augment vasodilator responses rather than attenuate vasoconstrictor effects.10 On the other hand, rapid effects were notable with concentrations in the nanomolar range in a number of previous studies assessing rapid biochemical processes regulated by estrogen. Further, in our recent studies of the effects of steroids including estrogen on single-cell contractile responses and myosin light chain phosphorylation effects were evident at nanomolar concentrations.11 Beyond that, much lower concentrations (in the pmoI/L range) can be shown to be effective for several other vasoactive steroids with comparable accessibility issues, including aldosterone.

Thus on net, one could conclude that the “jury is still out” on the biological significance of the rapid vasodilator effects of estrogens. However, notwithstanding the persisting uncertainties relating to mechanisms and significance of the rapid
vascular effects of estrogens (and of other vasoactive steroids) we are at least one step further forward with the current report. Traupe and colleagues do further our appreciation that the rapid vasoactive effects of steroids are complex and are likely mediated by multiple receptor signaling systems. We fully expect that sorting out the ligands, the receptors, and the directionality of effects will keep many of us on the dance floor for the foreseeable future.

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**References**
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