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Chemical interference with genomic and nongenomic actions of steroids in fishes: role of receptor binding

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Abstract

The characteristics of steroid nuclear and membrane receptors and their interactions with xenobiotic chemicals in two marine perciform species, Atlantic croaker (*Micropogonias undulatus*) and spotted seatrout (*Cynoscion nebulosus*) are briefly reviewed. Several organochlorines that bind to the nuclear progesterone receptor in mammals show negligible binding to the nuclear progestogen receptor in seatrout ovaries. Two distinct nuclear androgen receptors with different tissue distributions have been identified in croaker, but only one of them binds xenobiotic anti-androgens previously identified in mammals. Multiple forms of the nuclear estrogen receptor (ER) have been identified in fishes. The ER in croaker testis has a higher affinity than the croaker liver ER for estrogens and xenoestrogens and may be more susceptible to chemical interference. In addition, differences in the feedback effects of estrogens and xenoestrogens on gonadotropin secretion in croaker are observed, depending on the stage of the reproductive cycle. Finally, the first clear evidence in any vertebrate for xenobiotic chemical interference with the nongenomic actions of steroids by binding to steroid membrane receptors was obtained with the seatrout ovarian progestogen membrane receptor and since has been confirmed with progestogen and estrogen membrane receptors in croaker sperm and testes. These various factors that influence chemical/steroid receptor interactions are likely to significantly modify steroid hormone actions at target tissues and consequently the toxicological effects of chemical exposure.

Keywords: Organochlorines; Steroids receptor binding; Atlantic croaker; Spotted seatrout; Nongenomic steriod actions

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1. Introduction

In general, the magnitude of the cellular response to hormones is dependent upon the number of receptors occupied by the hormone which in turn is related to hormone concentration. Therefore, chemicals could potentially alter endocrine function by influencing the concentration of a hormone through changes in the rates of its secretion or metabolism, or by interfering with hormone action at the receptor or at other sites along the hormone signal transduction pathway. There is now substantial evidence that a variety of environmental contaminants can interfere with hormone-dependent processes during sexual development and the reproductive cycle in vertebrates by binding to nuclear steroid receptors and mimicking or antagonizing steroid hormone actions. The degree of endocrine disruption by this mechanism is in general related to the proportion of receptors binding the chemical which depends on the binding affinity of the chemical for the steroid receptor as well as chemical and steroid concentrations at the target tissues. However, certain features of the molecular actions of steroids can greatly affect the activities of these chemicals, including species differences in receptor-binding affinity, the presence of multiple receptor subtypes with different binding affinities, differential tissue distributions of the receptor subtypes, reproductive stage-dependent changes in steroid action, and whether the steroid action is genomic or nongenomic. The potential influences of these factors on the endocrine-disrupting activities of xenobiotic chemicals is briefly discussed with reference to studies in our laboratory on nuclear and membrane steroid receptors in Atlantic croaker and spotted seatrout.

2. Species differences

Extensive information is available on xenobiotic binding to mammalian nuclear steroid receptors which potentially could be useful for extrapolation to other vertebrates, including the distantly related teleost fishes. Overall, the binding of xenobiotics to croaker nuclear estrogen receptors (ERs) and their affinities are broadly similar to those in mammals (Gray, Monosson & Kelce, 1996; Loomis & Thomas, 1999). In contrast, it is not possible to predict from binding studies with the progesterone receptor (PR) in birds and mammals the affinities of xenobiotics for the teleostean progestogen receptor (Prog R; Pinter & Thomas, 1997). The ovarian Prog R most likely controls 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) induction of ovulation in seatrout (Pinter & Thomas, 1997) and displays different binding affinities for steroids and organochlorines than those observed with tetrapod PRs. For example, DDT derivatives, which have been shown to bind to PRs and antagonize the actions of progesterone in tetrapods (Gray et al., 1996), showed no affinity for the seatrout Prog R at concentrations up to 100 μM and therefore are unlikely to interfere with ovulation in this species (Pinter & Thomas, 1997; Table 1). Significant binding to the seatrout receptor was observed with Aroclor 1242, several hydroxylated polychlorinated biphenyls (PCBs), chlordane and lindane at these concentrations, however, which suggests that progestogen induction of

Table 1
Summary of binding affinities of xenobiotics for croaker and seatrout progestogen (Prog R), androgen (AR) and estrogen nuclear receptors (ER)[a]

Chemical	Seatrout progestogen receptor[b]	Croaker estrogen receptor[c]		Croaker androgen receptor[d]		
	Ovary Prog R	Testis ER	Liver ER	Brain AR1	Ovary AR2	Testis AR2
o,p′-DDT derivative	0	++	+	0	+	++
p,p′-DDT derivative	0	L	L	0	+	++
4,4′-PCB-3-OH	–	L	0	L	+	++
2,2′,5′-PCB-4-OH	L	++	L	L	+	++
2′,3′,4′,5′-PCB-4-OH	L	+++	+++	L	++	L
Kepone	L	++	+	–	–	–
Nonylphenol	–	+++	++	–	–	–
Chlordane	L	++	L	–	–	–
Aroclors	L	L	L	0	+	+
Vinclozolin metab. M1	–	–	–	0	++	++
Vinclozolin metab. M2	–	–	–	0	+++	+++

[a] –, not tested; 0, no displacement from receptor at 100 μM (PR), 500 μM (AR); L, < 50% binding at 100–1000 μM; +, 50% binding at 100–1000 μM; ++, 50% binding at 10–100 μM; +++, 50% binding at < 10 μM; for DDT derivatives and Aroclors maximum affinities shown.
[b] From Pinter and Thomas (1997).
[c] From Loomis and Thomas, (1999).
[d] From Sperry and Thomas (1999b).

ovulation in fishes may be susceptible to interference by certain types of xenobiotic chemicals.

3. Receptor subtypes and tissue differences

Multiplicity of receptor subtypes is widespread among members of the nuclear steroid receptor superfamily. These receptor subtypes display differences in their ligand-binding specificities and tissue distributions and therefore could profoundly influence the potencies and actions of steroids and endocrine-disrupting chemicals at different target tissues. Two ER subtypes, ERα and ERβ, with different steroid-binding affinities and tissue distributions have been described in mammals. In addition a third form (ERγ) has been identified in Atlantic croaker (Hawkins, Skipper, Crews & Thomas, 1998). Competition studies show that binding to the ER in croaker testes is saturated at much lower concentrations of estrogens and xenoestrogens (five- to 10-fold lower) than those required to saturate binding to the hepatic ER in this species (Loomis & Thomas, 1999; Table 1). This suggests that estrogen actions in the testis may be more susceptible to interference by xenoestrogens than those in the liver. Multiple forms of the androgen receptor (AR) have also been identified in croaker tissues (Sperry & Thomas, 1999a). One AR, termed AR1, is present in the brain and shows specific binding to testosterone but little affinity for a variety of

chemicals which bind to the AR in mammals and have anti-androgenic actions in these species (Gray et al., 1996; Sperry & Thomas, 1999a, b; Table 1). The other receptor, AR2, is present in gonadal tissues and displays a broader specificity for androgens and xenobiotic anti-androgens (Table 1). The receptor-binding studies suggest, therefore, that steroid actions in teleost gonads mediated by binding to ERs and ARs may be particularly susceptible to endocrine disruption by xenobiotic chemicals.

4. Reproductive stage-dependent effects

Marked changes in the concentrations, actions and potencies of sex steroids and other reproductive hormones occur during the reproductive life history cycle. Therefore, the pattern and degree of reproductive dysfunction induced by a hormone mimic or antagonist will depend upon the developmental or reproductive stage of the animal when chemical exposure occurs. For example, the feedback effects of steroids on gonadotropin secretion in vertebrates vary considerably with the stage of the reproductive cycle. In croaker estrogens exert positive effects on basal and stimulated (luteinizing hormone-releasing hormone-induced gonadotropin). (Luteinizing hormone, LH) secretion at the beginning of gonadal recrudescence. Exposure to *o,p′*-DDT mimics the stimulatory effects of estradiol on gonadotropin secretion at this time (Khan, Hawkins & Thomas, 1999; Khan & Thomas, 1998). The positive influence of estradiol switches to that of a negative feedback effect at the end of the reproductive cycle when the gonads are fully developed (Khan et al., 1999; Table 2). Currently, the effects of xenoestrogens on gonadotropin secretion at this stage of the reproductive cycle are unknown, but one would predict that they could also have negative feedback effects.

5. Nongenomic actions

The classic genomic mechanism of steroid action involves diffusion of steroids into the cell where they bind to intracellular nuclear receptors. The activated nuclear

Table 2
Summary of gonadal stage-dependent effects of estradiol on gonadotropin secretion in Atlantic croaker[a]

Gonadal stage	LH[b] secretion	
	Basal	LHRH[c]-stimulated
Early recrudescence	↑	↑
Late recrudescence	↑	0
Fully recrudesced	0	↓

[a] From Khan et al., (1999). ↑, positive effect; 0, no effect; ↓, negative effect.
[b] LH-Luteinizing hormone.
[c] LHRH-Luteinizing hormone-releasing hormone.

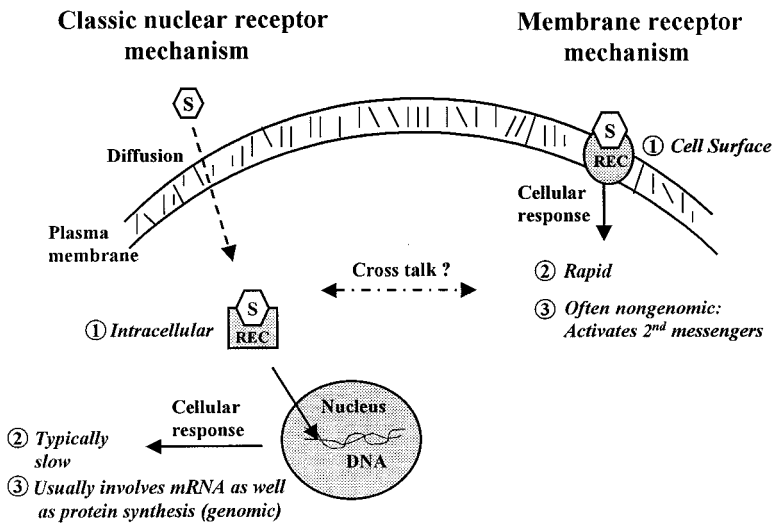

Fig. 1. Characteristics of nuclear and membrane receptor-mediated mechanisms of steroid hormone action. Numbers refer to defining characteristics of the two steroid mechanisms.

receptor binds to hormone response elements on genes and alters their rates of transcription and translation. These actions of steroids are typically slow (Fig. 1). However, there is now convincing evidence that steroids can also exert rapid, nongenomic actions by binding to receptors on the surface of the target cell. Rapid steroid actions and steroid membrane receptors have recently been identified in a wide variety of target tissues including the brain, pituitary, bone, kidney, liver, gonads and gametes (Revelli, Massobrio & Tesarik, 1998; Watson & Gametchu, 1999). Ligand binding to steroid membrane receptors results in activation of intracellular signal transduction pathways leading to a biological response (Fig. 1).

Although interference with the genomic actions of steroid hormones, especially estrogens, is considered to be the principal mechanism of endocrine disruption by many xenobiotics, chemicals could also potentially interfere with the nongenomic actions of steroids mediated by binding to steroid membrane receptors (Thomas, 1999a, b). Preliminary evidence for this was obtained from studies showing xenobiotic chemicals could interfere with progestogen induction of meiotic maturation of Atlantic croaker and *Xenopus* oocytes and mimic estradiol stimulation of rat smooth muscle relaxation (Ghosh & Thomas, 1995; Pickford & Morris, 1999; Ruehlmann, Steinert, Vlaverde, Jacob & Mann, 1998; Thomas & Budiantara, 1995). However, binding of the chemicals to the membrane receptors which mediated these steroid actions was not determined or could not be demonstrated, so the mechanism of chemical action remained uncertain. Recently a study showing that several estrogenic chemicals, including Kepone and *o,p′*-DDD, bind to the ovarian maturation-inducing steroid (17,20β,21-trihydroxy-4-pregnen-3-one; 20β-S) membrane receptor in spotted seatrout (Fig. 2) and antagonize 20β-S-induced meiotic maturation of oocytes in vitro in this species has provided the first clear demonstration of

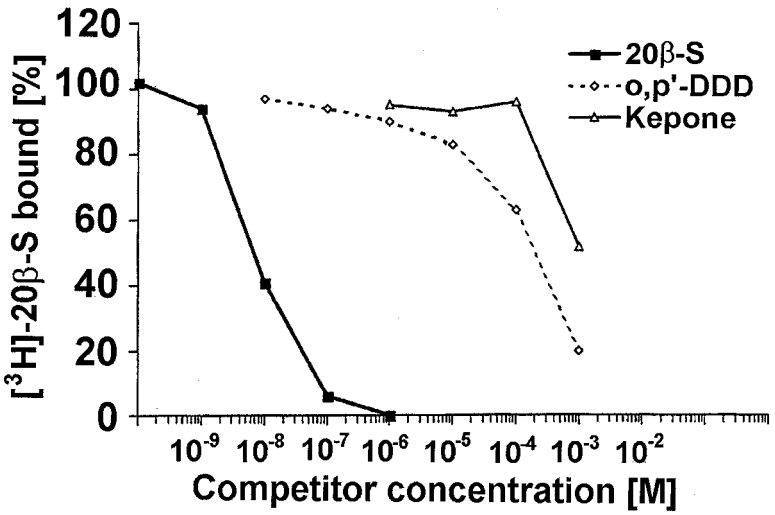

Fig. 2. Competition by the xenoestrogens *o,p′*-DDD and kepone for [^{3}H]-20β-S binding to the spotted seatrout ovarian MIS membrane receptor. Binding is expressed as a percentage of total binding (binding suppressed by 200 nM 20β-S). MIS-Maturation inducing steroid.

endocrine disruption via this mechanism of steroid action in any vertebrate species (Das & Thomas, 1999). Previously, a close correlation had been shown between 20β-S membrane receptor binding of a broad range of steroids and their agonist or antagonist activities in the in vitro oocyte maturation bioassay (Thomas & Das, 1997). Kepone and *o,p′*-DDD showed competitive inhibition of 20β-S binding, causing concentration-dependent displacement of 20β-S over the range of 10^{-4} to 10^{-6} or 10^{-7} M, and also caused concentration-dependent inhibition of 20β-S-induced final oocyte maturation over the same concentration range (lowest effective concentration 20–40 ppb) (Das & Thomas, 1999). Initial studies indicate that binding of these and other organochlorines (methoxychlor, hydroxylated PCBs and other DDT derivatives) is dependent upon localization of the receptor in the plasma membrane and is related to their lipophilicity, which suggests that the nature of xenobiotic interactions with steroid membrane receptors differs from that with steroid nuclear receptors (Thomas, 1999a). Steroid membrane receptors showing specific 20β-S binding have also been characterized on sperm from spotted seatrout and Atlantic croaker (Thomas, Breckenridge-Miller & Detweiler, 1997) and recent studies suggest that the sperm 20β-S receptor is an intermediary in 20β-S stimulation of sperm motility. Interestingly, preliminary experiments show that xenoestrogens (Kepone, a hydroxylated PCB, *o,p′*-DDE) bind to the 20β-S membrane receptor on Atlantic croaker sperm and inhibit 20β-S stimulation of sperm motility (Thomas et al., 1998). Recently an estrogen membrane receptor has been identified in croaker testes which most likely mediates estrogen down-regulation of androgen production by a rapid, nongenomic, cell surface-mediated mechanism (Loomis & Thomas, 2000). A broad range of xenoestrogens bind to the estrogen membrane receptor with

relative binding affinities similar to those observed with the nuclear estrogen receptor in this species (Loomis & Thomas, 2000). Moreover, several of the xenobiotics display estrogenic activities in an in vitro testicular androgen production bioassay. Thus, there is now clear evidence for xenobiotic interference with three nongenomic, cell surface-mediated steroid actions in vertebrates, progestogen induction of final maturation of oocytes and sperm where they act as antagonists, and estrogen down-regulation of testicular androgen production where the xenoestrogens have agonist actions. Taken together, these studies suggest that membrane receptor-mediated steroid actions may be as susceptible to disruption by a variety of xenobiotics as those mediated by nuclear steroid receptors and therefore warrant additional study.

6. Conclusions

Investigations of xenobiotic chemical interactions with steroid receptors can provide mechanistic explanations for the endocrine-disruption observed in vivo after whole animal exposure and also identify chemicals with high binding affinities for further evaluation of their endocrine-disrupting activities. For example, the relatively high affinity of *ortho*, *para* derivatives of DDT and several hydroxylated PCBs for the AR in croaker testes (Table 1) suggests that, in addition to their well-known estrogenic actions in vertebrates, they also probably influence androgen actions. Recent studies on the identification of steroid receptors in reproductive tissues and the actions they mediate provides basic information for interpreting toxicology studies with hormonally active chemicals. The identification of an ER in croaker testes and an AR in the ovary indicates that the gonadal actions of estrogens and androgens are not sex-specific and both steroids are likely to have important physiological functions in the gonads of both sexes. Therefore, compounds with high affinities for both receptors such as the *ortho*, *para* DDT derivatives probably influence both estrogenic and androgenic actions in teleost gonads under certain exposure conditions. Moreover, as discussed previously, a broad variety of factors that influence chemical/steroid receptor interactions are likely to dramatically alter the degree and type of endocrine dysfunction after xenobiotic chemical exposure. Thus, a complex pattern of chemical/steroid receptor interactions in teleosts has emerged from these recent studies. It is important, therefore, to consider these factors and their complexity when interpreting the results of studies using chemicals which may have steroid receptor-mediated endocrine actions.

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References

Das, S., & Thomas, P. (1999). *Endocrinology, 140*(4), 1953–1956.
Ghosh, S., & Thomas, P. (1995). *Marine Environmental Research, 39,* 159–163.
Gray, L. E., Monosson, E., & Kelce, W. R. (1996). Chapter 4. In R. T. Di Giulio, & E. Monosson, *Interconnections between human and ecosystem health* (pp. 44–82). London: Chapman and Hall.
Hawkins, M. B., Skipper, J. K., Crews, D., & Thomas, P. (1998). *Cloning of three estrogen receptor mRNAs from Atlantic croaker*. Abstracts Xth Intl. Congr. Hormonal Steroids, Quebec.
Khan, I. A., & Thomas, P. (1998). *Marine Environmental Research, 46,* 149–152.
Khan, I. A., Hawkins, M. B., & Thomas, P. (1999). *Biology of Reproduction, 61,* 834–841.
Loomis, A. K., & Thomas, P. (1999). *Biology of Reproduction, 61,* 51–60.
Loomis, A. K., & Thomas, P. (2000). *Biology of Reproduction, 62,* 995–1004.
Pickford, D. B., & Morris, I. D. (1999). *Environmental Health Perspectives, 107,* 285–292.
Pinter, J., & Thomas, P. (1997). *Journal of Steroid Biochemistry and Molecular Biology, 60*(1–2), 113–119.
Revelli, A., Massobrio, M., & Tesarik, J. (1998). *Endocrine Reviews, 19*(1), 3–17.
Ruehlmann, D. O., Steinert, J. R., Vlaverde, M. A., Jacob, R., & Mann, G. E. (1998). *FASEB Journal, 12,* 613–619.
Sperry, T., & Thomas, P. (1999a). *Endocrinology, 140*(4), 1602–1611.
Sperry, T., & Thomas, P. (1999b). *Biologiy of Reproduction, 61,* 1152–1161.
Thomas, P. (1999a). In R. K. Naz, *Endocrine disruptors: effects on male and female reproductive systems* (pp. 3–38). Boca Raton, FL: CRC Press.
Thomas, P. (1999b). In D. Henshel, M. C. Black, & M. C. Harrass, *Environmental Toxicology and Risk Assessment: Eighth Volume* (ASTM STP 1364). West Conshohocken, PA: American Society for Testing and Materials.
Thomas, P., Breckridge-Miller, D., & Detweiler, C. (1997). *Fish Physiology and Biochemistry, 17,* 109–116.
Thomas, P., & Das, S. (1997). *Biology of Reproduction, 57,* 999–1007.
Thomas, P., & Budiantara, L. (1995). *Marine Environmental Research, 39,* 147–150.
Thomas, P., Breckenridge-Miller, D., & Detweiler, C. (1998). *Marine Environmental Research, 46,* 163–167.
Watson, C. S., & Gametchu, B. (1999). *Proceedings of the Society for Experimental Biology and Medicine, 220,* 9–19.