

Early Cerebral Activities of the Environmental Estrogen Bisphenol A Appear to Act via the Somatostatin Receptor Subtype sst_2

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Recently, considerable interest has been aroused by the specific actions of bisphenol A (BPA). The present investigation represents a first study dealing with the interaction of BPA with the biologically more active somatostatin receptor subtype (sst_2) in the rat limbic circuit. After treating pregnant female Sprague-Dawley rats with two doses (400 $\mu\text{g}/\text{kg}/\text{day}$; 40 $\mu\text{g}/\text{kg}/\text{day}$) of BPA, the binding activity of the above receptor subtype was evaluated in some limbic regions of the offspring. The higher dose proved to be the more effective one, as demonstrated by the elevated affinity of sst_2 with its specific radioligand, [¹²⁵I]-Tyr⁰somatostatin-14. The most dramatic effects of BPA on sst_2 levels occurred at the low-affinity states of such a subtype in some telencephalic limbic areas of postnatal rats (10 days of age; postnatal day [PND] 10). These included lower ($p < 0.05$) sst_2 levels in the gyrus dentate of the hippocampus and basomedial nucleus of the amygdala; significantly higher ($p < 0.01$) levels were observed only for the high-affinity states of the periventricular nucleus of the hypothalamus. A similar trend was maintained in PND 23 rats with the exception of much lower levels of the high-affinity sst_2 receptor subtype in the amygdala nucleus and ventromedial hypothalamic nucleus. However, greater changes produced by this environmental estrogen were reported when the binding activity of sst_2 was checked in the presence of the two more important selective agonists (zolpidem and Ro 15-4513) specific for the α -containing γ -aminobutyric acid (GABA) type A receptor complex. In this case, an even greater potentiating effect ($p < 0.001$) was mainly obtained for the low-affinity sst_2 receptor subtype in PND 10 animals, with the exception of the high-affinity type in the ventromedial hypothalamic nucleus and gyrus dentate. These results support the contention that an sst_2 subtype α -containing GABA type A receptor system might represent an important neuromediating station capable of promoting estrogenlike mechanisms of BPA, especially during the early developmental phases. **Key words:** autoradiography, brain, development, GABA, rats, somatostatin receptors, xenoestrogen. *Environ Health Perspect* 110(suppl 3):397–402 (2002). <http://ehpnet1.niehs.nih.gov/docs/2002/suppl-3/397-402facciolo/abstract.html>

It has been widely accepted that estrogen activities are mediated either genomically through binding of specific intracellular receptors located within target cells or through local membrane types of mechanisms at the cell surface (1,2). A number of environmental agents recognized as environmental estrogens or simply xenoestrogens interact at the estrogen receptor level. As a result, public and scientific interests regarding estrogenic functions have focused their attention on both the toxic and biologically beneficial actions of some classes of environmental chemicals. A member of this class of environmental estrogen is the industrial phenolic chemical bisphenol A (BPA). Such a xenoestrogen is widely used in the manufacture of polycarbonate plastics, epoxy resins for lining food cans, and dental sealants, and as a stabilizing agent in plastics such as polyvinyl chloride (3,4). These man-made chemicals, because of their leaching from numerous reservoirs, can enter the body by ingestion or adsorption and mimic the actions of estrogens. Earlier studies revealed that when derivatives of BPA, such as the well-known diglycidyl ether of BPA, commonly used in food packaging, come into

contact with food products, the residual monomer solvents and/or additives in the polymer may migrate to the nourishing component (3,5). Thus, major attention has been directed toward the potentially toxic steroidal actions of this environmental estrogen as displayed by some reproductive malformations of offspring after maternal exposure to BPA (6,7).

Despite the 1,000-fold less potent activity of BPA with respect to that of estradiol (E_2), it is still able to mimic E_2 biological actions such as vaginal cornification (7) and growth and differentiation of the mammary gland (8). Consequently, it appears that not only estrogens but also xenoestrogen derivatives have important and diverse pleiotropic actions in both reproductive and nonreproductive tissues. In this context, the growth hormone system, and above all somatostatin (SRIF) play a crucial role in neurosecretory function (9) at the encephalic level, especially in relation to tissue content (10), which is still an unresolved facet of BPA effects. SRIF is widely distributed in the mammalian brain and is contained in short interneurons plus projecting neuronal pathways. To date, five distinct receptor

subtypes, designated as sst_{1-5} , have been identified as a family of G protein-coupled receptors (11). Of these five receptor subtypes, sst_2 , which is densely distributed in the various brain regions, is considered to be, from a functional point of view, one of the most important cerebral subtypes (12,13). This subtype also promotes neurotransmission actions, probably through the involvement of both the high and low receptor-binding states (14), as revealed by the successful neurobiological functions of sst_2 , especially in the presence of E_2 (15,16).

Recently, the subtype sst_2 has provided a specific analgesic type of behavioral response similar to that noted for another major neuronal inhibitory system, the heterooligomeric γ -aminobutyric acid (GABA) type A receptor (17). This receptor system, which constitutes nearly 60% of neuronal synapses, consists of at least seven classes of genes that encode for the following subunits: α , β , γ , δ , ϵ , π , ρ . Among this long list of subunits, the first, which is involved in the assembly of the other sequences and also determines the overall biophysical and pharmacological properties of the GABA type A receptor (18), has been chosen for this study. On the basis of the specific interactions between sex steroids and sst_2 receptor subtype, as well as the colocalization of estrogen receptors and GABA type A neurons (19), we investigated whether the binding activity of sst_2 alone or in the presence of some α GABA type A receptor subunit isoforms occurs in an estrogenic fashion in the presence of BPA. These relationships may bring us closer to identifying molecular receptor activities operating at the brain level after exposure to BPA.

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Materials and Methods

Animals

Sexually mature Sprague-Dawley female rats (200–250 g) were purchased from Charles River (Como, Italy), caged individually, housed in the Cellular Biology Department (University of Calabria, Cosenza, Italy) stabularium, and maintained on a 12-hr dark/12-hr light schedule (lights on 14:00–2:00 hr). Animal maintenance and experimental procedures were in accordance with the *Guide for Care and Use of Laboratory Animals* (20). Efforts were made to minimize animal suffering and reduce the number of specimens used.

Bisphenol A Administration

Thirty-two 60-day-old female rats were subdivided into three experimental groups in which four females were housed per cage with stainless steel wire lids for 3 days for acclimation purposes. Afterward these rats received either 40 $\mu\text{g}/\text{kg}/\text{day}$ BPA (IBPA; Sigma Chemical, Milan, Italy) po or 400 $\mu\text{g}/\text{kg}/\text{day}$ BPA (hBPA) po dissolved in arachis oil and were compared with controls that consisted of only arachis oil (OIL)-treated rats for 10 days. BPA was given orally because this route of exposure is more relevant to human exposure. These two doses of BPA were chosen on the basis of their capability to promote evident morphometrical changes in offspring (21,22), as well as to represent the concentrations that may be released when resins for lining food cans and dental sealants come into contact with food products. During this treatment period, a sexually mature Sprague-Dawley male was introduced into each cage and left there for 5 days. Pregnant females were isolated in single cages; at birth, litters were culled to eight, and pups (one per litter) were randomly assigned to dams of the same treatment. For the next 23 days, while the dam reared her foster pups within the same cage, she continued to receive the same treatment until the weaning of the pups at postnatal day (PND) 23, to assure exposure both via the placenta and lactation. During this period PND 10 rats from the treatment groups IBPA ($n = 6$), hBPA ($n = 7$), and OIL ($n = 4$) were decapitated and their brains quickly removed for sst_2 receptor autoradiography. Others were decapitated at PND 23 (IBPA, $n = 7$; hBPA, $n = 7$; OIL, $n = 5$).

Effects of Bisphenol A on Interaction between sst_2 and α -Containing GABA Type A Receptor

Receptor autoradiography approaches were used to identify the relationship between BPA, sst_2 , and GABA type A receptor activity. For this part, the brain of

BPA-treated rats at PND 10 (hBPA, $n = 5$; IBPA, $n = 6$) and PND 23 (hBPA, $n = 6$; IBPA, $n = 4$), along with their respective controls ($n = 4$; $n = 5$), were used. The autoradiographic analyses of the SRIF subtype sst_2 were conducted on posterior limbic brain sections (12 mm) according to previously described methods (23), plus modifications. In such a trial it was important that brain tissue was prewashed 3 times at room temperature in 50 mM Tris-HCl buffer, pH 7.4, with one of these washes (30 min) performed in the presence of 10^{-5} M guanosine 5'-triphosphate (GTP) (Sigma Chemical) to allow the dissociation of endogenous ligand as well as the binding specificity of this class of G-coupled receptor subtype (sst_2) with respect to those considered the deglycosylated type (24). Subsequently, slices were incubated for 1 hr at the same temperature and in the same buffer containing 0.5% bovine serum albumin and varying concentrations (5–500 pM) of [^{125}I]-Tyr 0 -SRIF14 (81.4 TBq/mmol; New England Nuclear Division, Milan, Italy) \pm 1 mM cold SRIF14 (nonspecific binding). The selection of this radioligand was based on its preferential affinity toward sst_2 receptor subtypes (13). After an exposure period of 18 days at room temperature, autoradiographic films (Hyperfilm; Amersham, Milan, Italy) of dried sections plus relative standards were evaluated with a Zeiss VIDAS image analyzer (Zeiss, Milan, Italy). Labeled sections were stained with cresyl violet acetate to identify the different posterior limbic areas.

To evaluate interaction of the sst_2 receptor subtypes with certain α GABA type A receptor subunits, adjacent brain slices were incubated in a fashion similar to that used in the saturation study. This time incubation was handled, as determined in a previous study (14), in the presence of the best [^{125}I]-Tyr 0 -SRIF14 affinity state (25 pM) and different concentrations (5 nM–500 μM) of the selective agonists (the imidazopyridine zolpidem [Synthelabo Recherche, Paris, France] or the imidazobenzodiazepinone Ro 15-4513 [Hoffmann-LaRoche, Basel, Switzerland]), or a selective agonist of the GABA type A complex, isoguvacine (ICN Biomedicals, Milan, Italy). The former two drugs, specific for the α subunit, are noted for their strong analgesic actions, whereas the latter, which is specific for the β subunit, was included as a means of receptor subunit specificity (25). The levels of sst_2 induced by the higher dose of BPA with respect to arachis-treated animals in rats of both 10 ($n = 12$) and 23 days of age ($n = 13$) in the presence of the GABA type A selective agonists were expressed as a ratio with respect to the same treatment groups in

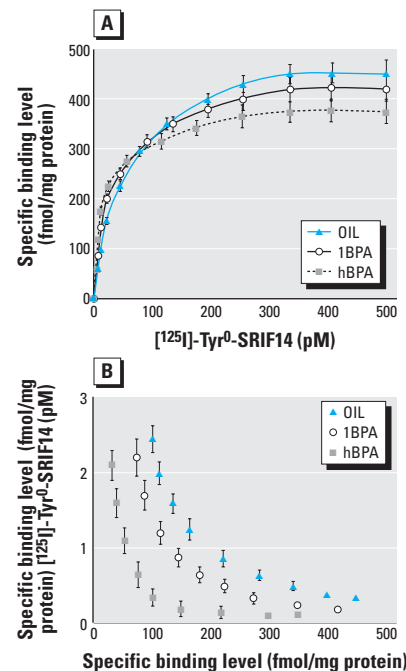


Figure 1. Ligand-binding assay. (A) Saturation curves of [^{125}I]-Tyr 0 -SRIF14 (pM) versus specific binding level (fmol/mg protein) \pm SEM in posterior brain regions of rats treated with IBPA ($n = 6$) and hBPA ($n = 5$) compared with those treated with OIL ($n = 5$) were obtained by wipe assay procedures using different concentrations (5–500 pM) of the radioligand, as reported in “Materials and Methods.” (B) The linear Scatchard plots of specific binding level (fmol/mg protein) versus specific binding levels/[^{125}I]-Tyr 0 -SRIF14 (fmol/mg protein/pM) \pm SEM enabled us to calculate the negative slope that provided the mean dissociation constant, while the intercept of the curve at the abscissa provided the maximal number of binding sites. Evaluation of saturation-binding study supplied similar results in five separate tests.

the absence of the selective agonists. The choice of hBPA treatment group and the concentration of [^{125}I]-Tyr 0 -SRIF14 to apply in the *in vitro* autoradiographic evaluation were determined by both saturation binding and wipe assay evaluations (26) of posterior brain regions.

Statistical Analysis

Results were reported as means \pm SEM for all trials. For the receptor binding study, Scatchard analysis of saturation binding data, which were fitted by a one-site and/or two-site model [based on the significance of extra-sum square using LIGAND program (27)], supplied relative affinity states and maximal receptor binding densities. A two-tailed Student's t -test was applied for BPA effects on sst_2 receptor activity. One-way analysis of variance (ANOVA) was also used for a GABA type A- sst_2 receptor differences, followed where necessary by Newman-Keuls multiple range test. Significance was checked when the p value was less than 0.05.

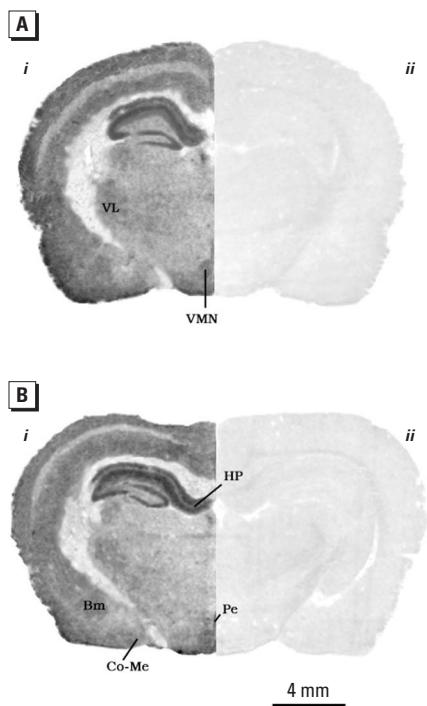


Figure 2. Representative binding autoradiograms of posterior brain regions. Abbreviations: HP, hippocampus; VL, ventral lateral posterior thalamic nucleus. (i) A heterogeneous binding activity of the sst_2 receptor subtype was obtained in some limbic areas of PND-23 rats when the posterior brain regions of (A) OIL-treated and (B) hBPA-treated animals were incubated in the presence of 25 pM [125 I]-Tyr⁰-SRIF14, as described in "Materials and Methods." This binding pattern was compared with that of their respective (ii) nonspecific binding autoradiograms. Bar = 4 mm.

Results

Effects of BPA on Interaction between sst_2 and α -Containing GABA Type A Receptor

This study dealing with early cerebral activities of the environmental estrogen BPA via the sst_2 receptor subtypes in some limbic areas of the rat displayed a fairly specific and stable binding activity, as shown by the representative saturation curve of posterior limbic regions of rats 23 days of age (Figure 1). Additionally, comparison of the two BPA doses (40 and 400 μ g/kg/day) permitted us to ameliorate the heterogeneous and stable binding activity of [125 I]-Tyr⁰-SRIF14 to its preferred receptor subtype (sst_2), especially in the case of the latter dose, which is regarded as the most effective dose capable of disrupting any endocrine function (28). For this purpose, and because of the weaker affinity of the radioligand [125 I]-Tyr⁰-SRIF14 toward sst_2 subtype in the presence of IBPA, only hBPA was tested for binding activities with sst_2 in the posterior limbic areas of animals 10 and 23 days of age. The

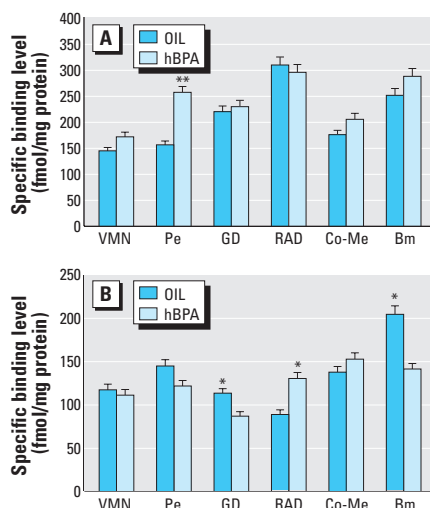


Figure 3. BPA-dependent binding activity variations of the sst_2 receptor subtype in PND-10 rats. [125 I]-Tyr⁰-SRIF14 binding levels (fmol/mg protein; $\bar{x} \pm$ SEM) to the high (A) and low (B) affinity state of sst_2 receptor subtypes were evaluated in some limbic areas of PND-10 rats ($n = 10$) treated with either hBPA or OIL, as described in "Materials and Methods." Mean values in each brain region of the same treatment group were analyzed using a two-tailed Student's t -test. * $p < 0.05$; ** $p < 0.01$.

labeling of this receptor subtype with its specific radioligand provided a heterogeneous and uniform type of binding in the different posterior limbic areas, as shown in the representative autoradiograms (Figure 2). When the binding parameters of the sst_2 subtype were identified by Scatchard analysis, it was possible to observe two different binding affinities under the influence of hBPA (high affinity ≤ 75 pM; low affinity $\geq 75 \leq 500$ pM), as reported in previous work for another rodent study (14).

The variations of sst_2 binding parameters after treatment with this xenoestrogen proved to be primarily of a mixed nature in the telencephalic regions of both PND 10 and PND 23 rats. In the former animals, hBPA was responsible for the diminished levels of the low-affinity sst_2 receptors ($p < 0.05$) in the gyrus dentate (GD) of the hippocampus and basomedial nucleus of the amygdala (Bm) (Figure 3B). A similar variation, this time of the enhanced type, was obtained for the stratum radiatum lacunosum CA1 layer of the hippocampus (RAD). As far as the high-affinity type of sst_2 receptors were concerned, BPA significantly enhanced ($p < 0.01$) the levels of this affinity type in the hypothalamus and specifically the periventricular nucleus (Pe) (Figure 3A). A comparable trend was reported for mainly the same limbic areas of PND 23 animals (Figure 4). Diminished ($p < 0.05$) and even significantly ($p < 0.001$) lower levels of the low-affinity type of sst_2 receptor were

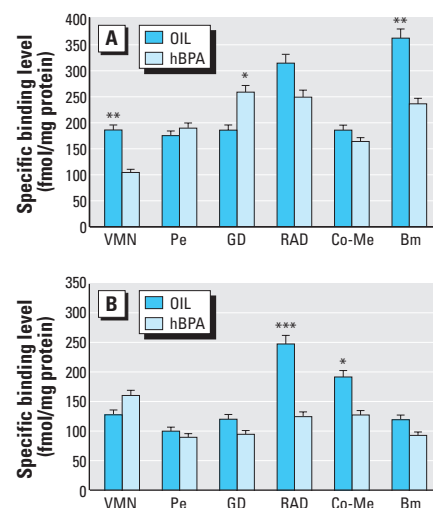


Figure 4. BPA-dependent binding activity variations of the sst_2 receptor subtype in PND-23 rats. [125 I]-Tyr⁰-SRIF14 binding (fmol/mg protein; $\bar{x} \pm$ SEM) to the (A) high-affinity and (B) low-affinity state of sst_2 receptor subtypes were evaluated in some limbic areas of PND-23 rats ($n = 11$) treated with either hBPA or OIL, as described in "Materials and Methods." Mean values in each brain region of the same treatment group were analyzed using a two-tailed Student's t -test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

registered in the cortico-medial (Co-Me) nucleus of the amygdala and RAD, respectively (Figure 4B). In the high-affinity type, significantly diminished levels were found in Bm and the ventromedial hypothalamic nucleus (VMN), whereas higher levels were observed only in the GD (Figure 4A) of the same animals.

Next, the influence of BPA on cerebral sst_2 binding activity in the presence of the selective agonists of the α -containing GABA type A receptor system was examined to determine the role of this other major neuronal system on BPA-dependent effects. Displacement activities of [125 I]-Tyr⁰-SRIF14 binding by these two agonists (zolpidem and Ro 15-4513) resulted in the shifting of the curve to the left, which corresponds to a greater affinity for sst_2 receptor subtype compared with the other GABA type A agonist (Figure 5). Consequently, incubation of the same brain regions with these two selective agonists accounted for even greater potentiating activities of BPA on the two sst_2 affinity states, especially the low-affinity type in postnatal animals. When the effects of BPA in the presence of the two α -containing GABA type A agonists were compared with those in the absence of the two agonists (Figures 6, 7), higher levels of the low-affinity sst_2 receptor occurred in the Pe and GD of PND 10 animals, whereas significantly higher Ro 15-4513-induced levels were reported for both the Bm and VMN (Figure 6B). In the same biological stage, a

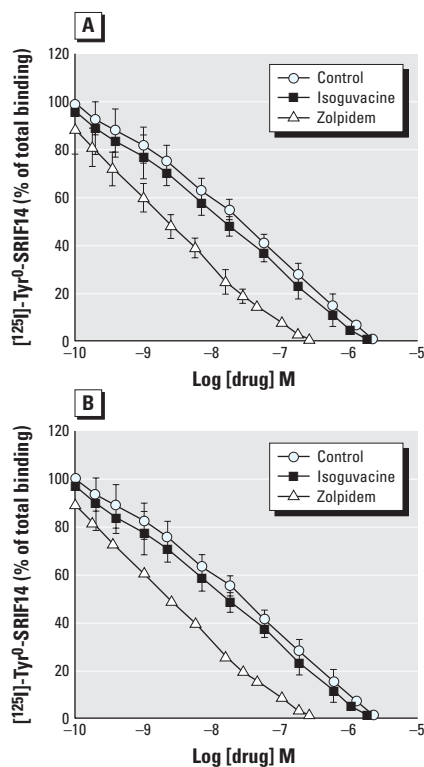


Figure 5. Competition assay. Displacement curves of [125 I]-Tyr 0 -SRIF14 (% of total binding) in some limbic areas of animals treated with (A) OIL ($n = 5$) and (B) hBPA ($n = 5$) were generated in the presence of different concentrations (5 nM–500 μ M) of selective benzodiazepine (zolpidem) and GABA type A (isoguvacine) agonists, as described in “Materials and Methods.” Each point represents the mean of five separate tests.

notable BPA influence was registered, this time for the high-affinity type of ss_2 receptor in the presence of Ro 15-4513 (Figure 6A). When the effects of GABA type A agonists were checked in animals 23 days of age, variations of low-affinity receptors were similar to those of PND 10 animals (Figure 7B), whereas a marked reduction of the changes in the high-affinity type was observed again, mostly in the presence of Ro 15-4513 (Figure 7B).

Discussion

Investigation of the specific SRIF receptor subtype (ss_2) under the influence of the environmental estrogen BPA made it feasible to discern, for the first time, the importance of this xenoestrogen on cerebral-dependent mechanisms that might be considered critical for morphological aspects and neuroendocrine activities in the developing rat. In this study, the binding activity of the ss_2 subtype was compared with a somewhat low dose and a sufficiently high BPA dose that accounted for, in the case of the latter dose, the alteration of both body weight and reproductive tract morphology in rodents (29,30). These

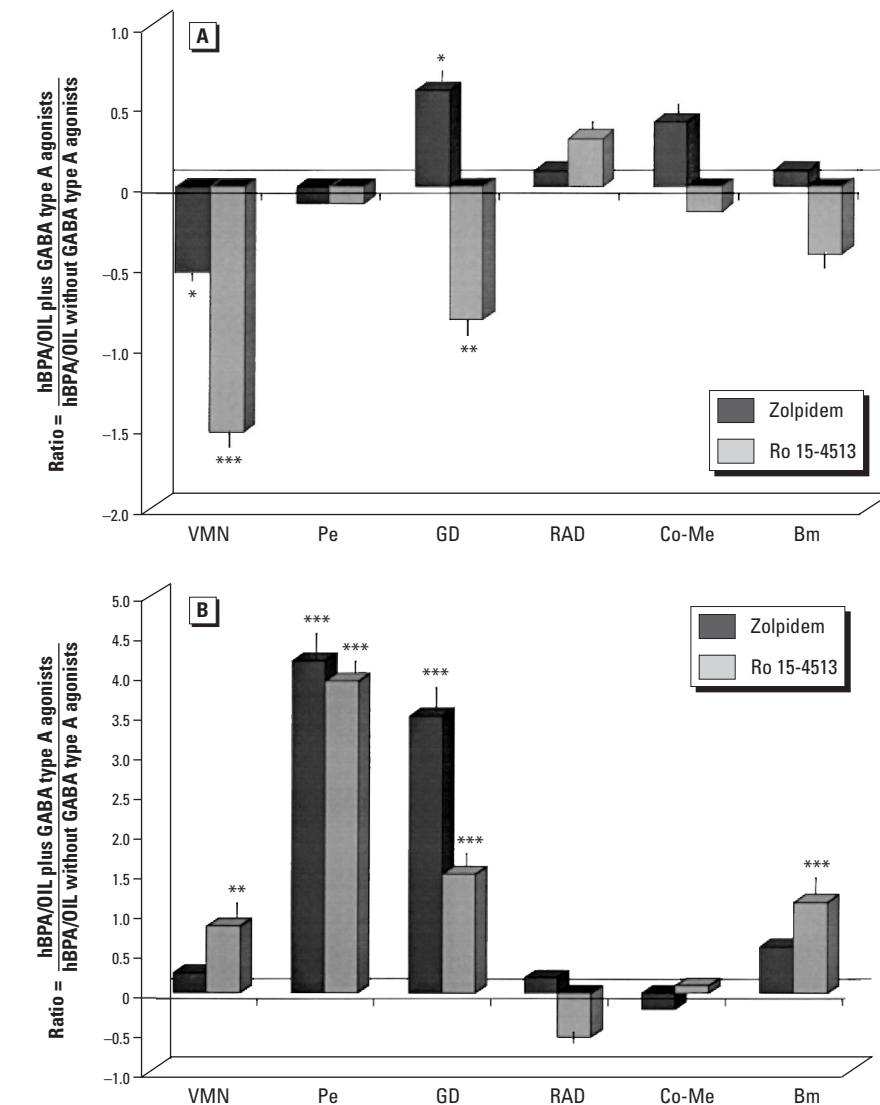


Figure 6. BPA-dependent changes of ss_2 in presence of GABA type A selective agonists in PND-10 rats. Effects of 20 nM of the selective benzodiazepine agonists, the imidazopyridine zolpidem, or the imidazobenzodiazepine Ro 15-4513 on [125 I]-Tyr 0 -SRIF14 binding to both (A) high high-affinity and (B) low-affinity ss_2 receptor binding states were tested in some limbic areas of PND 10 rats ($n = 12$) treated with hBPA (400 μ g/kg/day) compared with OIL-treated rats, as described in “Materials and Methods.” Values of ss_2 induced by the hBPA dose compared with OIL-treated animals in the presence of the GABA type A selective agonists were expressed as a ratio to the same treatment groups in the absence of the selective agonists. The ratios ($\bar{x} \pm$ SEM) were compared using ANOVA, and differences, where necessary, were obtained by Newman-Keuls multiple range test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

morpho-functional aspects have led researchers to consider doses greater than 200 μ g as a selective developmental toxicant (16,28). At the brain level, the actions of both BPA doses appeared to behave in an estrogenic fashion, as demonstrated by the similar Scatchard curves of [125 I]-Tyr 0 -SRIF14 binding in the presence of 17 β -estradiol (16,31). However, it was the high dose that was associated with the shifting of the curve toward a greater affinity of the ss_2 receptor subtype. Indeed, application of the higher BPA dose was responsible for the evident binding capacities of both affinity states of the ss_2 subtype in some areas of the posterior limbic regions.

This distinction was made possible by the binding conditions adopted in our study, specifically a radioligand exhibiting a strong affinity for the ss_2 subtype along with an elevated concentration of GTP. This concentration, higher than that used in the past (22), allowed us to explore the more extreme saturation ranges of the ss_2 receptor subtype. The identification of both high- and low-affinity states as probable targets of xenoestrogen interactions, as shown in a previous work (14), was also facilitated by the greater preference of the [125 I]-Tyr 0 -SRIF14 toward the deglycosylated form of ss_2 receptors, a condition typical for the ss_2 subtype (13,32).

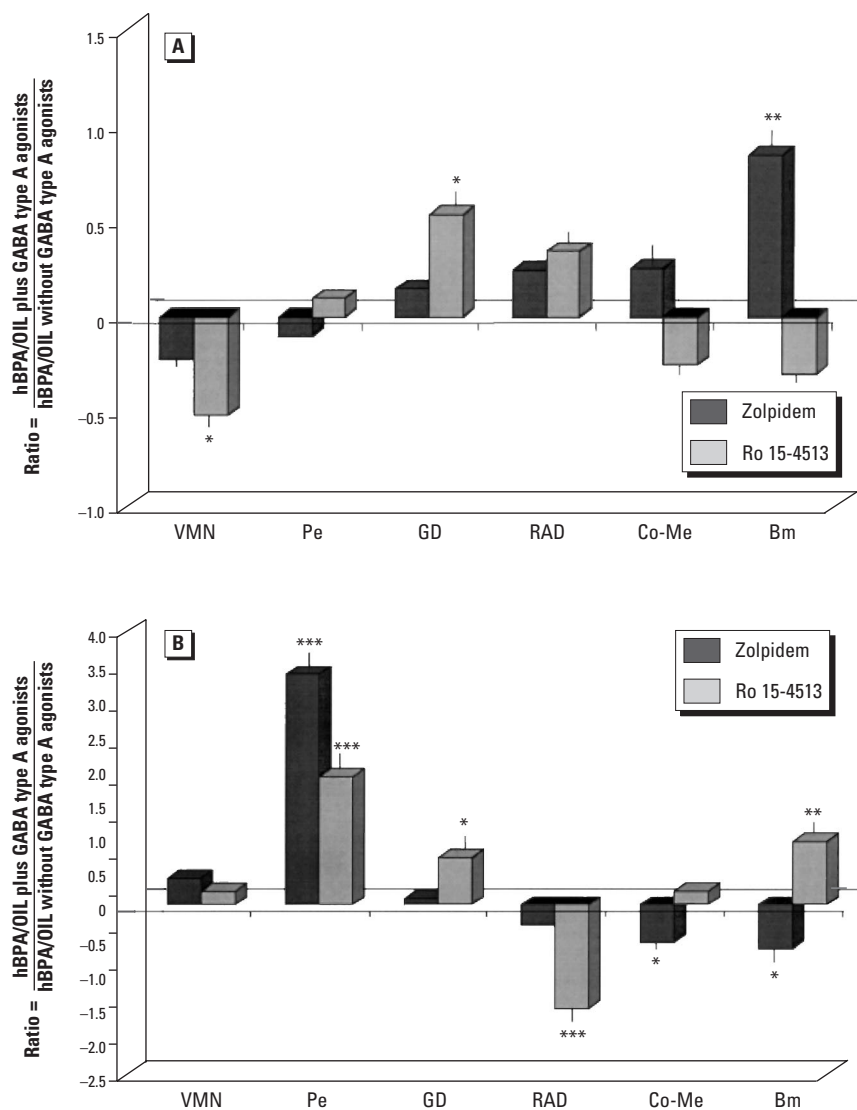


Figure 7. BPA-dependent changes of sst₂ in presence of GABA type A selective agonists in PND-23 rats. Effects of 20 nM of the selective benzodiazepine agonists, the imidazopyridine zolpidem or the imidazobenzodiazepinone Ro 15-4513 on [¹²⁵I]-Tyr⁰-SRIF14 binding to both (A) high-affinity and (B) low-affinity sst₂ receptor binding states were tested in some limbic areas of PND-23 rats (*n* = 13) treated with hBPA compared with OIL-treated rats, as described in "Materials and Methods." Values of sst₂ induced by the hBPA dose compared with OIL-treated animals in the presence of the GABA type A selective agonists were expressed as a ratio to the same treatment groups in the absence of the selective agonists. The ratios ($\bar{x} \pm \text{SEM}$) were compared using ANOVA, and differences, where necessary, were obtained by Newman-Keuls multiple range test. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

The evaluation of the two affinity states of the sst₂ subtype in some posterior limbic areas of postnatal rats treated with a high BPA dose showed that mainly the low-affinity state was the preferential target of this environmental estrogen. In fact, the greater changes occurred in hypothalamic, hippocampal, and amygdalar areas that are not only noted for a marked binding density of the sst₂ receptor subtype (22,33,34) but also for the E₂-dependent regulation of this receptor subtype (35,36), though at different concentrations. This was particularly evident by the higher levels of the sst₂ receptor subtype in the Pe and RAD of PND 10 rats,

whereas an inverse trend was provided by the VMN, Bm, and Co-Me of PND 23 animals. Whereas the major effects seemed to occur in steroid-enriched brain regions, studies have demonstrated that this female sex steroid does not always require the colocalization of their specific steroids as in the case of the Pe (37), indicating that perhaps the estrogenic effects in similar areas are accomplished by indirect means such as a local membrane type of mechanism (2) or through the sharing of colocalized E₂ receptors on GABAergic terminals of neighboring areas (19). This relationship appears to support the strong E₂-dependent modulatory

release of SRIF by GABA agonists, with the consequent alteration of the growth hormone secretion levels (38) in areas that lack E₂ receptors. Moreover, the prevalence of the low-affinity type of sst₂ receptor as a target of the xenoestrogen, plus the major changes occurring in the PND 10 stage, tend to point to either a predominance of this type of affinity state at the early developmental stages responsible for neuronal communicating functions (39) or simply that this represents the preferred targets of BPA in an age-independent manner.

The differences of BPA-induced actions on the sst₂ subtype were even greater in the presence of major agonists zolpidem and Ro 15-4513, which are specific for α_1 and α_4 GABA type A receptor subunits (17), further emphasizing the cruciality of such receptor binding conditions for the success of this environmental E₂. That GABAergic components are involved in the enhancement of BPA-dependent sst₂ levels, with the exception of the VMN and GD, is not surprising if we consider that these two receptor systems are both widely distributed. Moreover, the two receptor systems are not only colocalized functionally (40,41) but also structurally, as revealed by the identification of a novel site for sst₂ on GABA type A complex (42). However, it is worth noting that although the two α isoforms are implicated, in a similar fashion, on steroidal regulatory interactions (2,43), it is the latter isoform that exerts greater BPA-induced sst₂ receptor subtype changes. The lack of a consistent zolpidem-dependent modulatory action could be due to the GABA type A receptor complex not being assembled by this subunit isoform, as some hypothalamic stations do not contain the α_1 subunit (44). On the other hand, it is specifically the α_4 isoform that seems to be the preferred target of steroids involved in the restoration of anxiolytic and analgesic states (45,46). Thus, in line with these observations, our results seem to further extend the participation of α_4 -dependent GABAergic functions.

Taken together, these results provide for the first time direct evidence of BPA activities being regulated in a heterogeneous fashion at the cerebral level through the interaction of the sst₂ receptor subtype at the early developmental phases. In most cases, BPA was responsible for greater diminished levels of the sst₂ receptor subtype. This accounts for the lower inhibitory activities of this subtype, with the exception of Pe, in which case the higher quantity of sst₂ is probably linked to the hypothalamic area being the major site of SRIF mRNA expression (36).

However, the sensitivity of this interaction appears to rely heavily on participation

of some α GABA type A receptor subunits, in particular the α_4 isoform. Observations of the present study could provide further insights into phenomena such as the acceleration of puberty after treatment with this environmental estrogen (47), as well as contribute to the understanding of the steroidal influence on hypothalamic circadian pacemakers under stress and estrous cycle (48). In the latter case, BPA could assume a prominent role, especially because of its more recently recognized feature: the capability of promoting gene transcriptional activities necessary for the synthesis of progesterone receptors in hypothalamic neurons of ovariectomized animals (49). Indeed it is obvious that we are still at the beginning, but interests concerning the type of models for studying this endocrine disruptor are rapidly emerging. Perhaps the exploitation of its estrogenic-like activity might represent a potential value for the screening of environmental E_2 as agents of congenital neural problems and memory loss that are linked to glutamate-induced neuronal cell death (50).

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