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Hexachlorocyclohexanes Inhibit Steroidogenesis in Y1 Cells

ABSENCE OF CORRELATION WITH BINDING TO THE PERIPHERAL-TYPE BENZODIAZEPINE BINDING SITE

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ABSTRACT. Lindane, the γ-isomer of hexachlorocyclohexane (HCH), and two other HCH-isomers, α and δ-HCH, inhibit steroidogenesis in a Y1 adrenocortical cell line. In determining the mechanism by which HCH isomers inhibit steroidogenesis, they were found not to act directly on the mitochondrial Cyt P-450scc enzyme, making it likely that they act, instead, on intramitochondrial transport of cholesterol. γ-HCH, but not α or δ-HCH, is a potent and selective inhibitor of ligand binding to the peripheral-type benzodiazepine binding site (PBBS) which, in turn, is reported to regulate the rate-limiting step in steroidogenesis. Although these results demonstrate that α and δ-HCH do not inhibit steroid production through the PBBS, the possibility that the interaction of γ-HCH with the PBBS is responsible for its inhibitory effect on steroidogenesis could not be excluded. BIOCHEM PHARMACOL 51;10:1303–1308, 1996.

KEY WORDS. lindane; hexachlorocyclohexane isomers; peripheral-type benzodiazepine binding site; steroidogenesis; Y1 cells

Lindane, the gamma isomer of the compound HCH,‡ has been used since the 1940s for a variety of insecticidal purposes, including protection of crops, prevention of insect-borne diseases such as malaria, and removal of ectoparasites. It is well documented that, in mammals, high-dose exposure to lindane results in neuronal excitability, tremors, and convulsions [1, 2]. The other HCH isomers have negligible insecticidal activity [3] and do not exhibit the convulsant activity displayed by γ-HCH. The exact mechanism by which γ-HCH induces convulsions still remains unclear, although electrophysiological studies have demonstrated that lindane interacts directly with the $GABA_A$ receptor to prevent activation [4]. These workers have suggested that this is the explanation for both the insecticide and the convulsant properties of lindane. γ-HCH has also been shown to induce hepatotoxicity [5] and has been implicated in inducing aplastic anaemia [6].

In *in vivo* studies, γ-HCH has been reported to inhibit steroidogenesis [7]. Cholesterol side-chain cleavage to pregnenolone in ovarian mitochondria, the first step in steroid biosynthesis and catalysed by the enzyme Cyt P-450scc, was studied in mice fed with γ-HCH at various doses and over various periods. The insecticide adversely affected cholesterol side-chain cleavage in the ovary as judged by decreased conversion of cholesterol to pregnenolone and, subsequently, to progesterone in a dose-dependent manner. γ-HCH has also been reported to suppress adrenocortical function in mice fed with this insecticide [8]. However, the actual mechanism by which γ-HCH influences steroidogenesis still remains unclear.

There is much evidence that the PBBS is involved in regulating the rate-limiting step of steroid biosynthesis [9, 10]. The PBBS is distinct from the central-type or $GABA_A$/benzodiazepine receptor and, as the name suggests, is found in many peripheral tissues and also in the brain in glial cells. The PBBS is sometimes called the peripheral-type benzodiazepine receptor or the mitochondrial benzodiazepine receptor although, despite its being called 'receptor', no direct role in signal transduction has been demonstrated. The PBBS, once occupied by a specific ligand such as Ro5-4864 or PK 11195, has been reported to regulate the transfer of cholesterol from the mitochondrial outer membrane to the mitochondrial inner membrane, where the Cyt P-450scc enzyme is located [10]. This intramitochondrial cholesterol transfer is the rate-limiting step in steroidogenesis [11]. It may be possible that γ-HCH inhibits steroid production through an interaction with the PBBS.

This suppressing of steroidogenesis by γ-HCH in peripheral tissues may result in further effects, such as hormone deficiency and related reproductive disorders. It may also result in effects in the brain either indirectly by suppression of peripheral steroidogenesis or directly by suppression of

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‡ *Abbreviations:* HCH, hexachlorocyclohexane; PBBS, peripheral-type benzodiazepine binding site; Cyt P-450scc, cytochrome P-450 side-chain cleavage; RIA, radioimmunoassay.

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brain steroidogenesis. It has been shown that glial cells of the brain synthesize steroids [12], and that some of these 'neurosteroids' (pregnenolone sulphate and dehydroprogesterone) can act at the the $GABA_A$ receptor/Cl^- ionophore complex. This receptor complex is modulated by multiple drug-binding sites, including a site for steroids [13]. Neurosteroids have been shown to modulate, in a positive or negative manner, the $GABA_A$ receptor-mediated chloride flux [14]; thus, in turn, controlling neuronal excitability. Therefore, γ-HCH may act on $GABA_A$ receptors indirectly, *via* an inhibition of neurosteroid synthesis and release from glial cells.

The aims of this study were first to determine the effect of γ-HCH on steroid production by using a model Y1 adrenocortical steroid-producing cell line, and second to examine the mechanism by which γ-HCH affects steroidogenesis in these Y1 cells.

MATERIALS AND METHODS

Chemicals

The γ- and δ-isomers of HCH were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.), the α-isomer from Aldrich Chemical Company (Dorset, U.K.) and the β-isomer was a gift from Prof. E. Rodríguez Farré (C.S.I.C., Barcelona, Spain). Pregnenolone, 20α-hydroxycholesterol and 22(R)-hydroxycholesterol were obtained from Sigma. Ro5-4864 was obtained from Fluka Chemical Company (Dorset, U.K.). SU 10603 was a gift from A. Sedlacek (Ciba-Geigy Ltd., Basel, Switzerland). Trilostane was a gift from Sterling-Winthrop (Newcastle Upon Tyne, U.K.). PK 11195 was a gift from Dr. A. Doble (Rhône-Poulenc Rorer, Vitry Sur Seine, France), and [^{3}H]PK 11195 and [^{3}H]pregnenolone were obtained from NEN Research Products (Hertfordshire, U.K.).

Cells

The Y1 mouse adrenal tumor cell line was obtained from the European Collection of Animal Cell Culture. Stock cultures were grown in Dulbecco's Modified Eagles Medium supplemented with fetal calf serum (10%; v/v), L-glutamine (2 mM) and gentamicin (100 mg/mL). Mouse cerebellar and cortical astrocytes were a gift from Prof. A. Schousboe (Royal Danish School of Pharmacy, Copenhagen, Denmark).

Radioligand Binding Assays

Mitochondrial membranes were isolated as previously described [15]. Cell homogenates or mitochondrial membranes (35 μg of protein) were incubated in Tris-HCl buffer, pH 7.4, final volume 0.5 mL, in the presence of [^{3}H]PK 11195 (5 nM), and in the absence and presence of increasing concentrations of HCH-isomer (10 nM–1 μM). Control binding was defined as specific binding in the absence of the HCH-isomer. Nonspecific binding was determined in the presence of 10 μM PK 11195. After 60 min, the incubations were stopped by filtration through Whatman GF/B filters and washed with 10 mL of ice-cold incubation buffer. Radioactivity trapped on the filters was determined by liquid scintillation counting. K_i values were generated by the use of the computer program LIGAND [16].

Steroid Biosynthesis

Y1 cells were seeded in 24-well plates at a density of 3×10^5 cells/well in a final volume of 1 mL. Prior to measurement of pregnenolone production, the cells were washed 3 times with a simple salts medium consisting of 140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgSO_4$, 10 mM glucose, 10 mM HEPES/NaOH, pH 7.4, plus 0.1% BSA. During experiments, cells were incubated with this simple salts medium in an air incubator at 37°C. To measure pregnenolone secreted into the medium, its further metabolism was blocked by the addition of trilostane (5 μM) and SU 10603 (20 μM) (inhibitors of 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase, respectively) to the simple salts medium. The addition of HCH isomers, PK 11195 and Ro5-4864 and 22(R)- or 22α-hydroxycholesterol to the Y1 cells were made by the complete change of the simple salts medium to medium containing the appropriate concentration of the compounds as indicated in the figure legends. The final concentration of DMSO was constant for all the wells within each experiment and did not exceed 0.5% (v/v), a concentration which, on its own, had no effect on steroid production. At the end of the incubation period, the cell medium was saved and centrifuged at $1500 \times g$ for 10 min. The amount of pregnenolone secreted into the medium was quantified by RIA using an antibody obtained from ICN Biomedicals Inc. (Costa Mesa, CA, U.S.A.), under the conditions described by the manufacturer. Analysis of RIA data was performed using the Apple Macintosh ASSAYZAP program obtained from Biosoft (Cambridge, U.K.).

Protein Determination

Protein was quantitated by the method of Markwell *et al.* [17] using BSA as a standard.

Statistics

Statistical analysis of differences between control values and various treatment values of pregnenolone production were performed with a Student's *t*-test.

RESULTS

To use pregnenolone secreted by Y1 cells as a measure of steroidogenesis, preliminary experiments were performed to set up a standard protocol. The effect of the Cyt P-450scc inhibitor, aminoglutethimide, on pregnenolone production was examined. Aminoglutethimide significantly inhibited the conversion of cholesterol to pregnenolone and, thus,

the amount of this latter steroid secreted into the medium (Fig. 1). Y1 cells were also incubated with trilostane and SU 10603, inhibitors of 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase, respectively. In the presence of these inhibitors, the levels of this steroid in the medium significantly increased, apparently as a result of blocking the activities of different metabolic pathways.

The effect of a range of concentrations of γ-HCH on steroid production by Y1 cells was determined. The amount of pregnenolone secreted into the medium of Y1 cells was used as a measure of steroid production, and was quantified by radioimmunoassay. γ-HCH caused a dose-dependent inhibition of pregnenolone production (Fig. 2), resulting in 50% inhibition at the highest concentration examined (50 μM).

To determine whether or not other nonconvulsant HCH isomers also inhibit steroidogenesis, the effect of the α-HCH and δ-HCH isomers on pregnenolone production in Y1 cells was examined. At a concentration of 25 μM, both α-HCH and δ-HCH caused the same extent of inhibition of pregnenolone production as γ-HCH (Fig. 3).

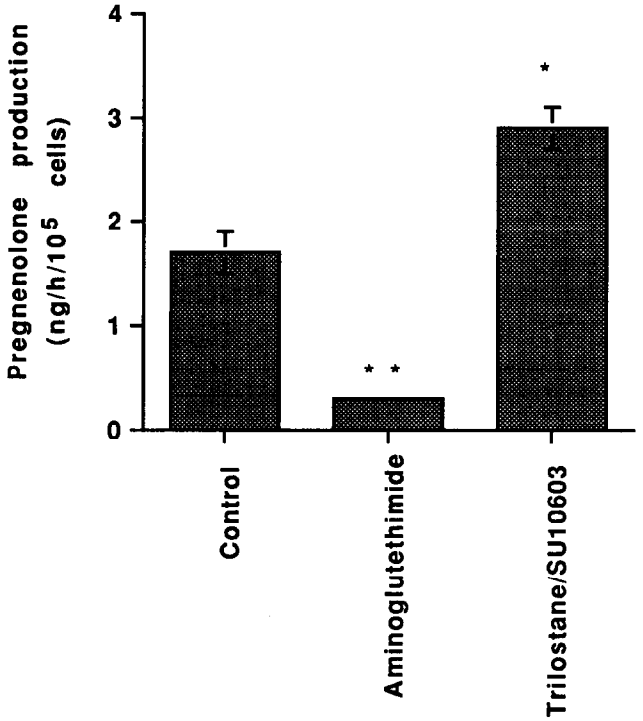

FIG. 1. Inhibitors of steroidogenic enzymes affect pregnenolone production in Y1 cells. Y1 cells, grown in 24-well dishes to a density of 3 x 10^5 cells/well, were incubated in a simple salts medium, either in the presence or absence of aminoglutethimide (0.76 mM), the Cyt P-450scc inhibitor, or a combination of trilostane (5 μM) and SU 10603 (20 μM), inhibitors of 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase, respectively. The volume of solvent in each well remained constant and did not exceed 0.3% (v/v). After 2 hr, pregnenolone secreted into the medium was quantified by RIA. The values for pregnenolone production are the mean ± SEM for 3 experiments, each performed in triplicate. *$P < 0.05$; **$P < 0.01$ (vs control value).

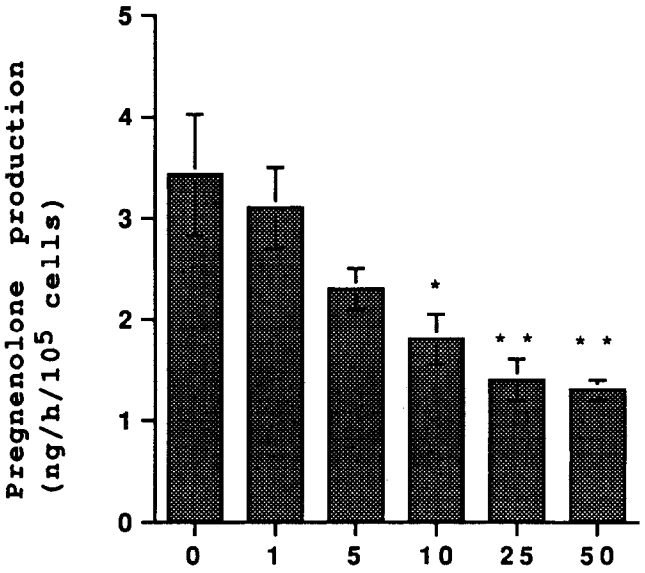

FIG. 2. Dose-dependent inhibition of pregnenolone production in Y1 cells by γ-HCH. Y1 cells, grown in 24-well dishes to a density of 3 x 10^5 cells/well, were incubated in a simple salts medium with trilostane (5 μM) and SU 10603 (20 μM), as described in Materials and Methods, either in the presence or absence of the indicated concentrations of γ-HCH. The volume of solvent in each well remained constant and did not exceed 0.5% (v/v). After 2 hr, pregnenolone secreted into the medium was quantified by RIA. The values for pregnenolone production are the mean ± SEM for 3 experiments, each performed in triplicate. *$P < 0.05$; **$P < 0.01$ (vs control value).

The possibility that HCH-isomers reduce cholesterol conversion to pregnenolone, either by inhibiting the transfer of cholesterol to the mitochondrial Cyt P-450scc enzyme, or by directly acting on this enzyme, was examined. The metabolism to pregnenolone of 22(R)-hydroxycholesterol and 20α-hydroxycholesterol were used as indices for Cyt P-450scc activity. These soluble cholesterol analogues have direct access to the enzyme; thus, bypassing the rate-limiting step [10]. 20α-hydroxycholesterol and 22(R)-hydroxycholesterol showed approximately 6-fold and 40-fold increased rates, respectively, to the endogenous rate of pregnenolone production in these intact Y1 cells (Table 1). Aminoglutethimide, the Cyt P-450scc inhibitor, dramatically reduced pregnenolone formation from both of these soluble cholesterol analogues. The HCH isomers, however, under conditions where they caused approximately a 50% inhibition of the endogenous rate of pregnenolone production, did not inhibit pregnenolone formation from either 20α-hydroxycholesterol or 22(R)-hydroxycholesterol.

There is much evidence that the PBBS is involved in regulating steroid biosynthesis. This binding site, once occupied by a specific ligand, such as Ro5-4864 or PK 11195, is reported to regulate the transfer of cholesterol from the mitochondrial outer membrane to the mitochondrial inner membrane, where the Cyt P-450scc enzyme is located [10]. In these Y1 adrenocortical cells, PK 11195 and Ro5-4864 caused a 2-fold stimulation of pregnenolone production

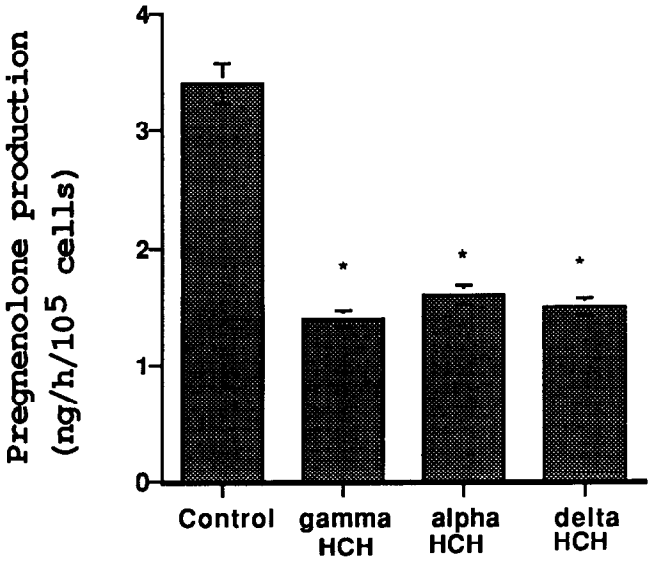

FIG. 3. Hexachlorocyclohexane isomers inhibit pregnenolone production in Y1 cells. Y1 cells, grown in 24-well dishes to a density of 3 × 10^5 cells/well, were incubated in a simple slats medium with trilostane (5 μM) and SU 10603 (20 μM), as described in Materials and Methods, either in the presence or absence of α-HCH (25 μM), δ-HCH (25 μM), or γ-HCH (25 μM). The volume of solvent in each well remained constant and did not exceed 0.5% (v/v). After 2 hr, pregnenolone secreted into the medium was quantified by RIA. The values for pregnenolone production are the mean ± SEM for 3 experiments, each performed in triplicate. *$P < 0.01$ (vs control value).

(data not shown) that is similar in extent to the stimulation originally reported for Y1 cells by Kreuger and Papadopoulos (1990) [10].

The possible interaction of γ-HCH with the PBBS in Y1 adrenocortical cells was then evaluated, by examining the effect of γ-HCH and of three other nonconvulsant isomers α, β and δ-HCH on [^{3}H]PK 11195 (a specific high-affinity PBBS ligand) binding to homogenates of Y1 cells. γ-HCH was shown to inhibit specific [^{3}H]PK 11195 binding, over a concentration range of 10 nM to 40 μM (limits of solubility), with a K_i value of 1.8 ± 0.7 μM (Fig. 4). The α, β, and δ isomers of HCH did not exhibit any inhibition of binding up to the highest concentration tested. Identical results were obtained when these binding studies were repeated with tissue from a variety of sources, ranging from rat brain mitochondrial membranes to mouse cerebellar astrocytes (Table 2), and also in Y1 cells when the benzodiazepine [^{3}H]Ro5-4864 was used as an alternative ligand (results not shown).

DISCUSSION

The first aim of this work was to establish the effect of γ-HCH and other HCH isomers on steroid production. The Y1 cell line is a well-characterised *in vitro* model system for studying steroidogenesis, and it exhibits a high density of PBBS [10]. Thus, the Y1 cell line was chosen for studying the effect of HCH isomers on steroidogenesis. In agreement with the *in vivo* studies of Lahiri and Sicar [8], who reported a suppression of adrenocortical function in mice fed with γ-HCH, this HCH isomer, at a concentration of 50 μM, caused a 50% inhibition of pregnenolone production by Y1 adrenocortical cells. This effect is obtained within the range of concentrations at which lindane is reported to elicit toxic effects *in vivo* [18]. However, when the effects of two of the nonconvulsant isomers of HCH were examined, they also affected steroid production in Y1 cells in a manner quantitatively similar to γ-HCH. At the same concentration (25 μM), both α-HCH and δ-HCH caused a 50% inhibition of pregnenolone production.

The second aim of the present study was to investigate the mode of action by which HCH-isomers inhibit steroid synthesis by Y1 adrenocortical cells. Two possible mechanisms could be envisaged to explain this inhibitory effect. Either the HCH-isomers acted directly on the mitochondrial Cyt P-450scc, which is involved in the activity of the 20 and 22 cholesterol hydroxylases [11] and which, ultimately, converts cholesterol to pregnenolone and/or HCH-isomers, inhibited the transfer of cholesterol from the mitochondrial outer membrane to the mitochondrial inner membrane where the Cyt P-450scc enzyme is located. The PBBS has been considered by some authors to modulate this intramitochondrial cholesterol transfer [10].

In examining the first of these possible mechanisms, the conversion of the soluble cholesterol analogues, 22(R)-hydroxycholesterol and 20α-hydroxycholesterol, to pregnenolone by Y1 cells were used as indices for Cyt P-450scc activity. The classical hydroxylase activity of the Cyt P-450scc is used to hydroxylate two adjacent C atoms in the side-chain of cholesterol and the intervening bond (C_{20-22}) is then cleaved to release a six carbon atom fragment (isocacapraldehyde) and pregnenolone [11]. None of the HCH-isomers inhibited pregnenolone formation from either 22(R)-hydroxycholesterol or 20α-hydroxycholesterol), implying that its mechanism of inhibition is not by direct inhibition of either the 20 or 22 cholesterol hydroxylations of the Cyt P-450scc enzyme, but is more likely to be by affecting cholesterol delivery to the enzyme by acting on the transfer of cholesterol from the outer to the mitochondrial inner membrane (the rate-limiting step). Studies by Bernstein and Gut [19] have shown that the order of hydroxylation is either not random or predominantly sequential with hydroxylation at C22 preceding that at C20. The rates found when the C22 hydroxylated substrate is used are approximately 8–10 times faster than with the C20 hydroxylated substrate. Although it is difficult to extrapolate from intact cells to the individual enzyme, this result is consistent with a predominantly, but not totally, sequential order of hydroxylation; that being C22 followed by C20.

There is much evidence that the PBBS is involved in regulating steroid biosynthesis through the rate-limiting step. This binding site, once occupied by a specific ligand, such as Ro5-4864 or PK 11195, is reported to regulate the transfer of cholesterol from the mitochondrial outer mem-

TABLE 1. Hexachlorocyclohexane isomers do not directly affect Cyt P-450scc activity

	Pregnenolone production (ng/h/10^5 cells)		
	Control	22(R)-hydroxycholesterol	20α-hydroxycholesterol
Endogenous	2.8 ± 0.1	118 ± 13	16.8 ± 1.5
Aminoglutethimide	0.3 ± 0.01	5.8 ± 1.7	3.2 ± 0.5
γ-HCH	1.4 ± 0.2	114 ± 20	16.8 ± 3
α-HCH	1.6 ± 0.03	112 ± 15	19.0 ± 7
δ-HCH	1.6 ± 0.1	114 ± 20	18.2 ± 0.2

Y1 cells, grown in 24-well dishes to a density of 3 × 10^5 cells/well, were incubated in a simple salts medium with trilostane (5 μM) and SU 10603 (20 μM), either in the presence or absence of aminoglutethimide (0.76 mM), HCH-isomers, (25 μM), 22(R)-hydroxycholesterol (50 μM), or 20α-hydroxycholesterol (50 μM) or the combinations of these compounds indicated. The volume of solvent in each well remained constant and did not exceed 0.5% (v/v). After 2 hr, pregnenolone secreted into the medium was quantified by RIA. The values for pregnenolone production are the mean ± SEM for 3 experiments, each performed in triplicate.

brane to the mitochondrial inner membrane, where the Cyt P-450scc enzyme is located [10]. The effect of γ-HCH on the specific binding of [^{3}H]PK 11195 to homogenates of Y1 cells was carried out to investigate whether or not γ-HCH specifically interacts with the PBBS in this Y1 cell line. At the same time, the effect of three other nonconvulsant isomers α, β, and δ-HCH on [^{3}H]PK 11195 binding was also determined. Only the γ-isomer inhibited binding to the receptor, whereas the other HCH isomers showed no inhibition at concentrations up to 40 μM. The K_i value for inhibiting binding to the PBBS in Y1 cells was found to be within the range of concentrations at which γ-HCH caused an inhibition of steroid production in these cells. This suggested a possible role for the PBBS in mediating the inhibitory action of γ-HCH on steroid production.

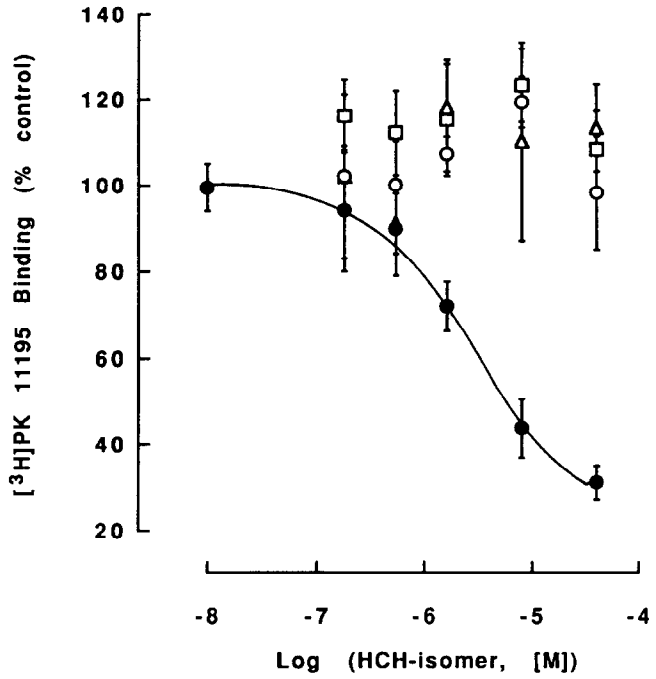

FIG. 4. γ-Hexachlorocyclohexane inhibits the specific binding of [^{3}H]PK 11195 to the PBBS in Y1 cells. Homogenates of Y1 cells (35 μg of protein) were assayed for specific binding of [^{3}H]PK 11195 (5 nM; 86.9 Ci/mmol) to the PBBS, as described in Materials and Methods, in the absence and presence of various concentrations of the α(△), β(○), δ(□) and γ(●) isomers of HCH. Control binding is defined as specific binding in the absence of HCH isomers. The displacement curve was generated for inhibition by γ-HCH, assuming mutually exclusive binding of γ-HCH and PK 11195. The K_i value of 1.8 ± 0.7 μM for inhibition of binding by γ-HCH represents the mean ± SEM of triplicate determinations.

The exact mechanism by which γ-HCH induces convulsions still remains unclear, although electrophysiological studies have demonstrated that lindane interacts directly with the $GABA_A$ receptor [4]. These workers have suggested that this is the explanation for both the insecticide and the convulsant properties of γ-HCH. However, an inhibitory action of γ-HCH on steroidogenesis mediated by the PBBS may have relevance in the brain where an involvement with convulsions could also be proposed. The selective inhibitory effect of only γ-HCH on PBBS ligand binding, described in this work, correlates well with the convulsive activity of only the γ-HCH isomer [18]. Also, its potency for inhibition of binding to the PBBS (K_i values of 1–10 μM) is well within the range of concentrations at which γ-HCH causes convulsions in mammals [18].

TABLE 2. γ-HCH inhibits the specific binding of [^{3}H]PK 11195 to the PBBS in a variety of tissues

	K_i values for HCH isomers (μM)			
Source	α-HCH	β-HCH	γ-HCH	δ-HCH
Rat adrenal mitochondria	NI*	NI	5.0 ± 0.9	NI
Rat brain mitochondria	NI	NI	2.1 ± 0.3	NI
C6 glioma cells	NI	NI	9.2 ± 4.0	NI
Mouse cortical astrocytes	NI	NI	4.0 ± 0.3	NI
Mouse cerebellar astrocyes	NI	NI	3.1 ± 0.2	NI

Tissues from a variety of sources (35 μg of protein) were assayed for specific binding of [^{3}H]PK 11195 (5 nM; 86.9 Ci/mmol) to the PBBS, as described in Materials and Methods, in the absence and presence of a range of concentrations (10 nM to 40 μM) of the α, β, δ, and γ isomers of HCH. The K_i values for inhibition of binding by γ-HCH represent the mean ± SEM of triplicate determinations. *NI, no inhibition.

The PBBS is thought to regulate the production of steroids in the glial cells of the brain, these steroids being termed neurosteroids. Some of these neurosteroids have been shown to down- or upregulate the $GABA_A$ receptor by binding to the steroid binding site of the $GABA_A$ receptor complex, thereby controlling neuronal excitability [20]. It could be hypothesized that γ-HCH, by binding to the PBBS in the brain, could inhibit the biosynthesis of certain neurosteroids; thus, indirectly affecting $GABA_A$ receptor activity.

However, when the effects of two of the nonconvulsant isomers of HCH that do not inhibit binding to the receptor were examined, they also affected steroid production in Y1 cells in a manner quantitatively similar to γ-HCH. At the same concentration (25 μM), both α and δ-HCH caused a 50% inhibition of pregnenolone production. This result suggests that the inhibitory action of the α and δ-HCH isomers on steroid production is not mediated through the PBBS.

The total specificity of the PBBS in several tissues for the γ-isomer and not the α, β, and δ-isomers is surprising. However, it may be the case that the γ-isomer, being different in conformation to the other isomers, could bind to the PBBS in a mutually exclusive manner to PK 11195. The other isomers, although binding to PBBS and, thus, still able to interfere with cholesterol transport, may bind in a manner that does not cause mutually exclusive binding. It is still, however, possible that γ-HCH and the other isomers inhibit cholesterol transport independently of any interaction with PBBS or, alternatively, that γ-HCH inhibits cholesterol transport through the PBBS and the other isomers inhibit cholesterol transport independently of the PBBS.

The results presented here could neither affirm nor deny the possibility that γ-HCH acts as a convulsant by inhibiting neurosteroid synthesis through glial PBSSs. The selective and potent inhibition by γ-HCH of ligand binding to the PBSS in many tissues and cells including brain may, indeed, suggest that the PBSS is somehow involved in the convulsant activity of γ-HCH either through its action on steroidogenesis or through some other mechanism. Indeed, this possibility has previously been suggested by other workers in the field [21], whose study involved a comparison of the toxicokinetics of γ-HCH and Ro5-4864. However, these proposals require further investigation.

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References

1. Stein K, Portig J and Noack G, Neuropharmacological effects of isomers of hexachlorocyclohexane. III. An account of the convulsant activity of the γ-isomer in rats. *Neuropharmacol* **20:** 1017–1024, 1981.
2. Joy IM, Mode of action of lindane, dieldren and related compounds in the central nervous system. *Neurobehav Toxicol Teratol* **4:** 813–823, 1982.
3. Demozay D and Maréchal G, *Lindane*. Schillinger, Freiburg im Breisgau, 1972.
4. Zhang H-G, ffrench-Constant RH and Meyer JB, A unique amino acid of the Drosophila GABA receptor with drug sensitivity by two mechanisms. *J Physiol* **479:** 65–76, 1994.
5. Videla LA, Simizu K, Barros SBM and Junqueira VBC, Mechanisms of lindane-induced hepatotoxicity. *Xenobiotica* **21:** 1023–1032, 1991.
6. Rauch AE, Kowalsky SF, Lesar TS, Sauerbier GA, Burkart PT and Scharfmann WB, Lindane (kwell)-induced aplastic anemia. *Arch Intern Med* **150:** 2393–2395, 1990.
7. Sircar S and Lahiri P, Effect of lindane on mitochondrial side-chain cleavage of cholesterol in mice. *Toxicology* **61:** 41–46, 1990.
8. Lahiri P and Sircar S, Suppression of adrenocortical function in female mice by lindane (γ-HCH). *Toxicology* **66:** 75–79, 1991.
9. Yanagabashi K, Ohno Y, Naamichi N, Matsui T, Hayashida K, Takamura M, Yamada N, Tou S and Kawamura M, Peripheral-type benzodiazepine receptors are involved in the regulation of cholesterol side-chain cleavage in adrenocortical mitochondria. *J Biochem* **106:** 1026–1029, 1989.
10. Kreuger KE and Papadopoulos V, Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells. *J Biol Chem* **265:** 15015–15022, 1990.
11. Hall PF, Cellular organisation for steroidogenesis. *Int Rev Cyto* **86:** 53–95, 1984.
12. Hu ZY, Bourreau E, Jung-Testas I, Robel P and Baulieu E, Neurosteroids: Oligodendrocyte mitochondria convert cholesterol to pregnenolone. *Proc Natl Acad Sci U.S.A.* **84:** 8215–8219, 1987.
13. Sieghart W, $GABA_A$ receptors: ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. *Trends Pharmacol Sci* **13:** 446–450, 1992.
14. Majewska MD and Schwartz RD, Pregnenolone sulfate: an endogenous antagonist of the γ-aminobutyric acid receptor complex in brain? *Brain Res* **404:** 335–360, 1987.
15. Moynagh PN, Bailey CJ, Boyce SJ and Williams DC, Immunological studies on the rat peripheral-type benzodiazepine acceptor. *Biochem J* **275:** 419–425, 1991.
16. Munson PJ and Robard D, LIGAND: a versatile computerised approach for the characterisation of ligand binding systems. *Anal Biochem* **107:** 220–239, 1980.
17. Markwell M, Haas SM, Bieker L and Tolbert NA, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* **87:** 206–210, 1978.
18. Tusell JM, Suñol C, Gelpî E and Rodrîguez-Farré E, Relationship between lindane concentration in blood and brain and convulsant response in rats after oral or intraperitoneal administration. *Arch Toxicol* **60:** 432–437, 1987.
19. Burstein S and Gut M, Intermediates in the conversion of cholesterol to pregnenolone: kinetics and mechanism. *Steroids* **28:** 115–131, 1976.
20. Romeo E, Auta J, Kozikowski A, Ma A, Papadopoulos V, Puia G, Costa E and Guidotti A, 2-Aryl-3-indoleacetamides (FGIN-1): A new class of potent and specific ligands for the mitochondrial DBI receptor (MDR). *J Pharmacol Exp Ther* **262:** 971–978, 1992.
21. Drummer ML and Woolley DE, Toxicokinetics of Ro5-4864, lindane and picrotoxin compared. *Pharmacol Biochem Behav* **28:** 235–242, 1991.